Torradoviruses

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Abstract

Torradoviruses are an example of a group of recently discovered plant viruses. The first description of *Tomato torrado virus*, now the type member of the newly established genus *Torradovirus* within the family *Secoviridae*, was published in 2007 and was quickly followed by findings of other torradoviruses, initially all on tomato. Their characterization led to the development of tools that allowed recognition of still other torradoviruses, only very recently found on non-tomato crops, which indicates these viruses have a much wider host range and diversity than previously believed. This review describes the characteristics of this newly emerged group of plant viruses. It looks in detail at taxonomic relationships and specific characteristics in their genomes and encoded proteins. Furthermore, it discusses their epidemiology, including host range, semipersistent transmission by whitefly vectors, and impact on diverse cropping systems.

INTRODUCTION

Recent years have seen a marked increase in the number of newly described plant viruses. More than 300 new viruses were recognized by the International Committee on the Taxonomy of Viruses (ICTV) between the years 2009 and 2012. The latest ICTV report from 2012 (35) and Master Species list (V2, 2013) at the ICTV website (**http://talk.ictvonline.org/files/ictv_documents/m/msl/default.aspx**) already list more than 1,200 distinct species. Apart from the (re)grouping of viruses over already established genera, new plant virus genera were also recognized by the ICTV in the 2012 report, including the genus *Torradovirus*, a clear example of a group of newly emerging plant viruses. Since 2007, several new torradoviruses have been described. Although initially all were isolated from tomato, recently several new species have been isolated from other host plants, including lettuce, carrot, cassava, and motherwort.

Tomato Torrado Virus

The first symptoms caused by *Tomato torrado virus* (ToTV), the type member of the genus *Torradovirus*, were seen around 2004 on tomato (*Solanum lycopersicum* L.) plants in the region of Murcia in southern Spain (63). Typically, symptoms initiate at the base of the leaf and consist of necrotic spots surrounded by light green or yellow areas. These later develop into a severe necrosis of leaves and fruit (see **Figure 1*a***) and an overall reduction in plant growth. Because of the associated necrosis, this disease was referred to by local farmers as torrado, meaning burnt or roasted.

Upon initial analysis, plants collected from Murcia and showing typical torrado symptoms proved to be infected with *Pepino mosaic virus* (PepMV). By inoculation on filter hosts insusceptible to PepMV (e.g., *Physalis floridana*), a spherical virus could be isolated and further propagated. A purification method was developed for this virus, which allowed subsequent analyses of its physical properties. Moreover, Koch's postulates could be fulfilled, proving that the isolated virus was indeed the causal agent of the observed disease. The complete genome of the virus was elucidated, and the virus was named after the disease it causes: *Tomato torrado virus* (ToTV) (63).

Since its first description, ToTV isolates have been described in Australia (26), Colombia (61), France (68), Hungary (2), Italy (24), Panama (32), and Poland (50) as well as Spain (5, 6).

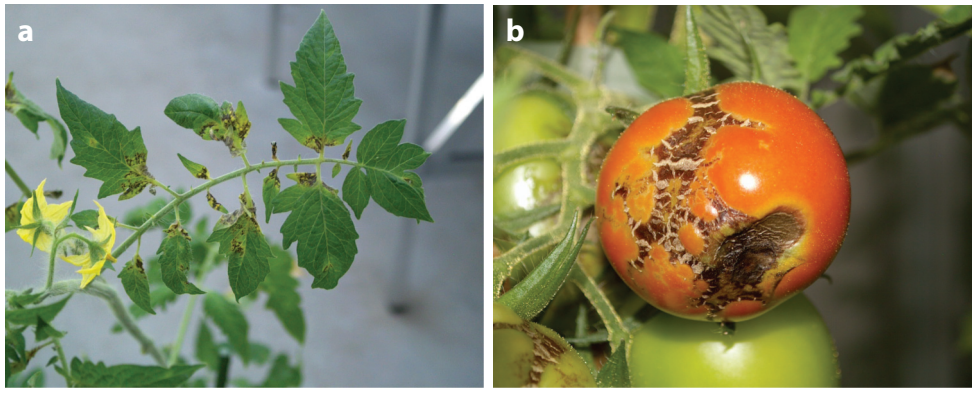


Figure 1

(*a*) Typical initial symptoms of *Tomato torrado virus* infection on tomato leaves with necrotic spots surrounded by a light green or yellow area beginning at the base of a leaflet. Reprinted from Verbeek 2007 (63). (*b*) Typical necrosis on tomato fruit as a result of infection with *Tomato marchitez virus*.

Full-length sequences from three ToTV isolates are available. In addition to the original isolate PRI-ToTv0301 from Murcia (RNA1, NC_009013 = DQ388879; RNA2, NC_009032 = DQ388880; we refer to this isolate as ToTV-PRI), there are two isolates from Poland: ToTV-Wal'03 (18) (RNA1, EU563948; RNA2, EU563947) and ToTV-Kra (51) (RNA1, KJ940975; RNA2, KJ940974).

ToTV infections are frequently associated with whitefly infestations. Two studies showed the transmission of ToTV by the whitefly species *Trialeurodes vaporariorum* (Westwood) (51) and *Bemisia tabaci* (Gennadius) (8). A subsequent study (67) not only identified *Trialeurodes abutilonea* (Haldeman) as a third whitefly vector but also showed a semipersistent stylet-borne mode of transmission for ToTV [as well as for *Tomato marchitez virus* (ToMarV) and Tomato chocolàte virus (ToChV)].

Tomato Marchitez Virus

Shortly after the first description of ToTV, a disease with symptoms very similar to those caused by ToTV was observed on tomato plants in Mexico (**Figure 1*b***). The causal agent of the Mexican disease, locally called "Marchitez," proved to be a virus, which clearly resembled ToTV in its particle morphology and in its number and sizes of coat proteins and RNAs. However, sequence comparisons showed that this new virus was distinct from ToTV. Both RNA1 (NC_010987 = EF681764) and RNA2 (NC_010988 = EF681765) of isolate PRI-TMarV0601 differed in length from ToTV, and the amino acid (aa) identities between the two viruses were clearly below the ICTV *Secoviridae* species demarcation thresholds. In the same vein as ToTV, this virus was named after the local term for the disease it causes: *Tomato marchitez virus* (ToMarV) (64).

During the same period, an Italian research group was also investigating tomato samples from Mexico showing marchitez disease. They identified a virus that they named Tomato apex necrosis virus (ToANV) (58). The RNA 1 and RNA2 sequences of ToANV as deposited in the National Center for Biotechnology (NCBI) database (EF063641 and EF063642, respectively) apparently lack the 5′ ends, including the torradovirus-characteristic RNA2 open reading frame 1 (RNA2-ORF1). Later, ToANV was proposed as a separate torradovirus species, but nucleotide (nt) and aa sequence comparisons showed levels of identity with ToMarV well within the ICTV species demarcation thresholds. ToANV is now considered a member of the species ToMarV, but until the full genome sequence is determined for this virus, it remains unclear whether ToANV represents an isolate or a strain of ToMarV.

Tomato Chocolate Spot Virus and Tomato Chocolàte Virus

A disease in tomato with symptoms similar to torrado disease, and locally known as mancha de chocolate (or chocolate spot), was observed in Guatemala. Two independent research groups described torradovirus-like viruses from these chocolate spot diseased tomatoes. Batuman et al. (15) found a putative new torradovirus species that they named Tomato chocolate spot virus (ToChSV). Its RNA sequences are available under NC_013075 (RNA1) and NC_013076 (RNA2).

A second virus was described as Tomato chocolàte virus (ToChV isolate G01) by Verbeek et al. (65), and its RNA sequences are published under FJ560489 (RNA1) and FJ560490 (RNA2). Based on overall sequence characteristics, both ToChV-G01 and ToChSV were tentatively designated as members of the genus *Torradovirus*.

Remarkably, both 3′ untranslated regions (3′ UTRs) of RNA1 of ToChV-G01 and ToChSV are nearly identical, whereas their RNA2 3′ UTRs share only 45.6% sequence identity. The 3′ UTR of both RNAs of all reported torradoviruses share high homologies with each other in the

3′ end of the 3′ UTR, but this was not observed in ToChSV. Sequences of amplicons obtained with generic primers for torradoviruses from the original ToChSV isolate, and clones thereof, share high homologies with both ToChSV and ToChV (66). These observations suggest that the original ToChSV isolate was mix-infected with ToChV.

Tomato Necrotic Dwarf Virus

Tomato necrotic dwarf virus (ToNDV), a virus originally collected from Imperial County, California, United States, was initially described by Larsen et al. in 1984 (38) as a whitefly-transmitted spherical virus infecting tomato, pepper, eggplant, tomatillo, and several weeds. It caused significant losses for the tomato industry in southern California during the 1980s and was largely responsible for the displacement of commercial tomato production from the region; however, the virus was never fully characterized. An antiserum against ToNDV reacted positive in ImmunoSorbent Electron Microscopy (ISEM) with the Polish Wal′03 isolate of ToTV (50), indicating a possible relationship with torradoviruses. An isolate of ToNDV was maintained through whitefly transmission and grafting, and its genome was recently characterized (69), along with evaluation of transmission by relevant whitefly species.

Three whitefly species were found to be effective vectors of ToNDV, with efficient transmission by *T. abutilonea* and the *B. tabaci* biotypes but a lower rate of transmission by *T. vaporariorum* (W.M. Wintermantel & A.A. Cortez, unpublished results). ToNDV is also transmitted efficiently by grafting (W.M. Wintermantel & A.A. Cortez, unpublished results), and it can be transmitted mechanically (38), although the latter is not an efficient mode of transmission for this virus. Recently, the complete sequences of both ToNDV genomic RNAs were determined (RNA1, KC999058; RNA2, KC999059) (69), and sequence comparisons demonstrated ToNDV is a member of the genus *Torradovirus*.

Lettuce Necrotic Leaf Curl Virus

The availability of full genome sequences of tomato-infecting (TI) torradoviruses, generic reverse-transcriptase polymerase chain reaction (RT-PCR) primers, and next-generation sequencing (NGS) facilitated the detection of other torradoviruses in crops other than tomato. The first non-tomato-infecting (NTI) torradovirus was found in lettuce (*Lactuca sativa* L.) in the Netherlands. In a sample from a field-grown necrotic lettuce plant, a virus sequence with a typical torradovirus genome organization was detected using NGS. However, viral sequences obtained showed low identities with the known TI torradoviruses. The virus was isolated and proven to be the causal agent of the disease symptoms observed in lettuce, comprising severe necrosis and leaf curling (62). The virus was named *Lettuce necrotic leaf curl virus* (LNLCV) and proposed as a new member of the genus *Torradovirus*. The full-length sequence has been determined and deposited in the NCBI database (RNA1, KC855266; RNA2, KC855267).

Motherwort Yellow Mottle Virus

Motherwort (*Leonurus sibiricus* L.) is an herbaceous biennial plant in the mint family (*Lamiaceae*). It is a native weed plant in Asia and is grown commercially in Korea because of its traditional medicinal use. Plants showing conspicuous virus-like symptoms, i.e., yellow mottle, mild mosaic, and stunting, were collected from a South Korean field and subjected to Illumina NGS sequencing (55). Read assembly revealed two large contigs of viral origin that showed homology to LNLCV. NGS results were confirmed by Sanger sequencing of full-length RT-PCR products, and 5′ and 3′

ends of both RNAs were confirmed by rapid amplification of cDNA ends (RACE). The genome organization of the virus, named Motherwort yellow mottle virus (MYMoV), was very similar to those of the other torradoviruses. Phylogenetic analyses of both RNAs (RNA1, KM229700; RNA2, KM229701) and the encoded ORFs showed that MYMoV showed only limited homology to the other torradoviruses and was most closely related to LNLCV.

Carrot Torrado Virus

In the United Kingdom, an investigation into the potential cause of carrot root necrosis in field-grown carrots, employing NGS, yielded large fragments (6,900 and 4,700 nts) of a bipartite viral genome (1). Further analysis suggested that the 6,900-nt fragment was the genomic RNA1 of a novel torradovirus. The smaller fragment contained two ORFs. ORF1 encoded a putative 202 aa (22-kDa) protein with 43% homology to the RNA2 ORF1 of LNLCV. The second ORF encoded a putative 130-kDa polyprotein that appeared to contain movement-protein and coat-protein domains and have 35% homology to the RNA2-ORF2 of ToTV. These observations supported the idea that this was a novel member of the genus *Torradovirus*, tentatively named Carrot torrado virus 1 (CaTV1). To avoid possible confusion with *Citrus tristeza virus* (CTV), we propose CaTV1 as the new acronym for Carrot torrado virus. Sequences of CaTV1 RNA1 and RNA2 have been published (KF533719 and KF533720, respectively), but their 5′ and 3′ ends have not been confirmed by 5′ and 3′ RACE and thus the CaTV1 sequence should not be considered complete.

Cassava Torrado-Like Virus

The application of NGS on small interfering RNA (siRNA) isolated from cassava plants showing cassava frogskin disease, collected in different regions of Colombia, revealed the presence of several viruses in symptomatic material. One of the viruses had a typical torradovirus genome organization and was able to induce leaf symptoms associated with frogskin disease on cassava (*Manihot esculenta* Crantz) and indicator plants (22). This virus, which showed only limited sequence similarity (50–60% aa similarity) to other torradoviruses, was tentatively named Cassava torrado-like virus (CsTLV). Partial sequences of CsLTV RNA1 and RNA2 have been published (KC505250 and KF533720, respectively).

Initial screenings for CsTLV were carried out using generic primers designed to recognize members of the genus *Torradovirus* (66). Characterization of several isolates has revealed wide sequence diversity for CsTLV in Colombia and suggested the presence of at least two different strains that share 80–88% aa identity in the replicase region (W.J. Cuellar, unpublished results). Following the initial description of CsTLV, additional sequence information has been obtained (W.J. Cuellar, unpublished results), which is included in the analyses described in this review. After Cassava frogskin-associated virus (CsFSaV) (21), CsTLV is the second most frequently found virus infecting cassava plants that show leaf and root symptoms in Colombia, and though not confirmed yet, CsTLV is likely to be present in other countries where similar symptoms have been described, such as Costa Rica, Brazil, Peru, Panama, and Venezuela (20).

GENERAL CHARACTERISTICS OF TORRADOVIRUSES

Torradoviruses possess small spherical virions, approximately 30 nm in diameter (see **Figure 2**), composed of three coat proteins of approximately 23, 26, and 35 kDa. Buoyant density gradient centrifugation yielded two types of particles that differed only in density. The bottom band

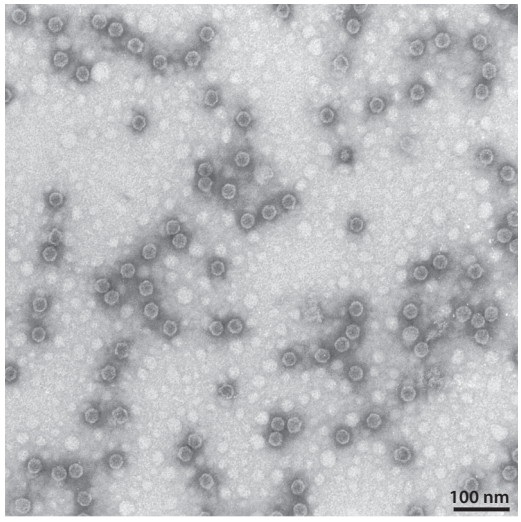


Figure 2

Electron micrograph of purified particles of *Tomato torrado virus*.

contained the largest RNA molecule (RNA1) and the top band the smallest (RNA2). These two single-stranded positive-sense RNA molecules range in size from 6,911 to 7,802 nts for RNA1 and 5,695 to 4,701 nts for RNA2 (excluding their poly-A tail) (see **Table 1**).

RNA1 contains a single large ORF, ranging in size from 2,151 to 2,224 aa, that encodes replication domains including a protease cofactor (PRO-co), a helicase (HEL), a 3C-like protease (PRO), and an RNA-dependent RNA polymerase (RdRp) (see **Figure 3**).

Unlike the RNA2 of all other known genera of the *Secoviridae*, the RNA2 of torradoviruses contains two ORFs. The first, RNA2-ORF1, encodes a hypothetical protein of unknown function that ranges in size from 185 to 212 aa. The second, RNA2-ORF2, partially overlaps with the first and encodes a polyprotein of 1,168–1,223 aa, of which the N-terminal region contains domains for a putative movement protein (MP) and the remainder encodes the three coat proteins (CPs).

Another characteristic typical of torradoviruses is the presence of a long 3′ UTR in both RNAs. Within the TI torradoviruses, these regions range in size from 625 to 1,230 nts for RNA1 and from 647 to 1,406 nts for RNA2. The NTI torradoviruses generally have somewhat shorter 3′ UTRs, ranging in length from 734 to 87 nts for RNA1 and 766 to 33 nts for RNA2. The 3′ UTRs of RNA1 and RNA2 are highly conserved within each torradovirus species, and most of the 3′-terminal sequences directly preceding the poly(A)-tail are shared by both RNAs. Only a relatively short 5′-terminal region of the 3′ UTR shows clear differences, both in length and in sequence.

TAXONOMIC POSITION OF TORRADOVIRUSES

Following a revision of the plant-infecting picornaviruses by the ICTV working group on plant-infecting members of the order *Picornavirales*, the torradoviruses are now grouped within the *Secoviridae* family, one of five families within the order *Picornavirales* and the only one containing plant-infecting viruses (35). The *Secoviridae* are further divided into the subfamily *Comovirinae*, which contains the genera *Comovirus*, *Fabavirus*, and *Nepovirus*, and five unassigned genera: *Cheravirus*, *Sadwavirus*, *Torradovirus*, *Sequivirus*, and *Waikavirus* (53, 54).

Table 1 Lengths in nucleotides (and in amino acids in parentheses for the respective encoded proteins) of RNA1 and RNA2 and their different regions of the recognized and tentative torradoviruses

Virus		RNA1				RNA2				
	Isolate	Length	5′ UTR	ORF1	3′ UTR	Length	5′ UTR	ORF1	ORF2	3′ UTR
ToTV	PRI	7,793	106	6,477 (2,158)	1,210	5,389	181	564 (187)	3,597 (1,198)	1,091
	Wal'03	7,814	107	6,477 (2,158)	1,230	5,390	181	564 (187)	3,597 (1,198)	1,092
	Kra	7,802	107	6,477 (2,158)	1,218	5,390	181	564 (187)	3,597 (1,198)	1,092
ToMarV	PRI	7,322	60	6,456 (2,151)	625	4,898	138	573 (191)	3,576 (1,191)	652
	ToANV		ND	5,833[a] (1,943)	627	ND	ND	ND	2,344[a] (780)	647
ToChV	G01	7,474	135	6,468 (2,156)	871	5,695	181	570 (190)	3,579 (1,192)	1,406
	G02	ND	ND	ND	ND	5,366	181	570 (190)	3,579 (1,192)	1,077
ToChSV		7,473	138	6,468 (2,156)	867	5,093	91	555 (185)	3,594 (1,198)	814
ToNDV		7,239	150	6,453 (2,151)	636	4,896	138	570 (190)	3,573 (1,191)	659
LNLCV		7,406	170	6,672 (2,224)	734	5,290	326	636 (212)	3,669 (1,223)	766
MYMoV		7,203	135	6,711 (2,191)	357	4,963	406	618 (206)	3,588 (1,196)	461
CaTV1		6,911	127[b]	6,579 (2,193)	205[b]	4,701	611[b]	609 (203)	3,504 (1,168)	33[b]
CsTLV		4,395[a]	ND	4,301 (1,433)	87	ND	ND	ND	3,015[a] (1,005)	154

[a]Only partial sequence information available.

[b]5′ and 3′ UTR sequences were not verified through rapid amplification of cDNA ends (RACE).

Abbreviations: CaTV1, Carrot torrado virus 1; CsTLV, Cassava torrado-like virus; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ND, not determined; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*; UTR, untranslated region.

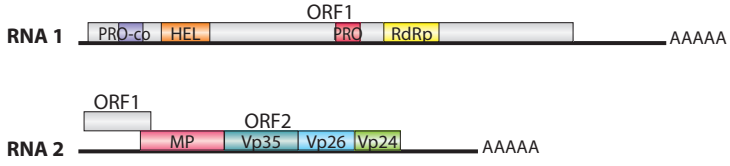


Figure 3

Genomic organization of torradoviruses. Regions containing typical motifs of a protease cofactor (PRO-co), helicase (HEL), a 3C-like protease (PRO), and the RNA-dependent RNA-polymerase (RdRp) in RNA1-ORF1 are indicated. Regions containing typical motifs of the movement protein (MP) and the three coat proteins (Vp35, Vp26, and Vp24) on RNA2-ORF2 are also shown. Adapted from Verbeek 2007 (63).

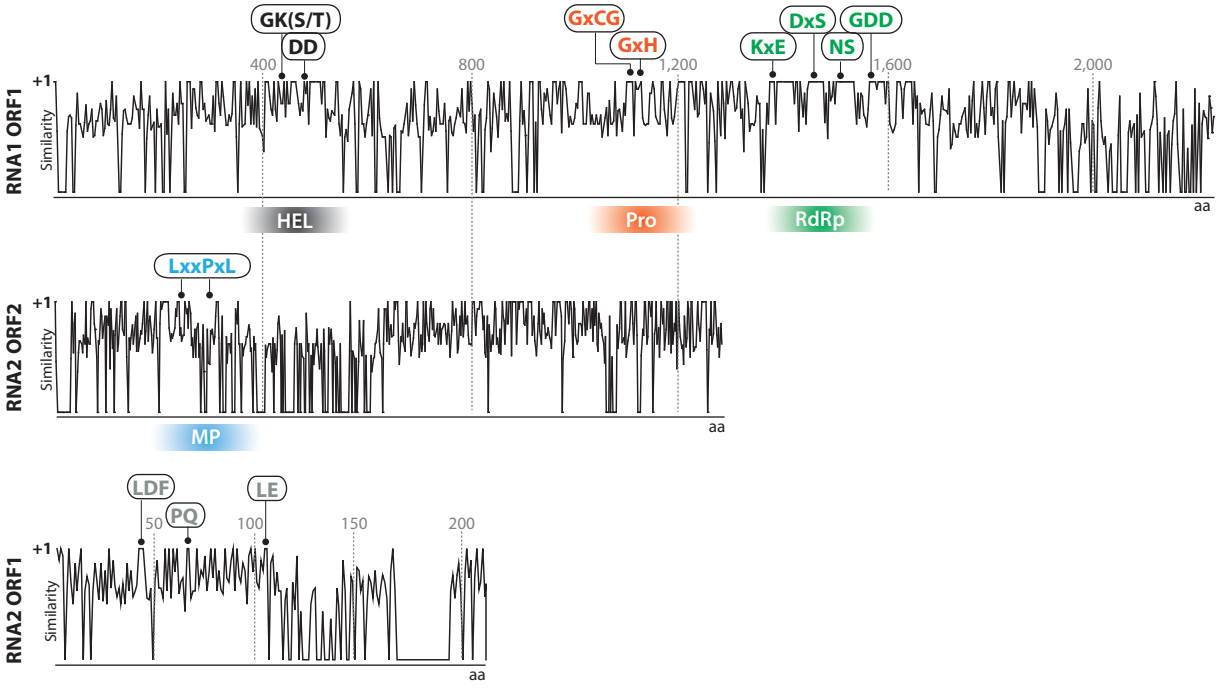


Figure 4

Similarity plots of the three open-reading frames of eight torradoviruses (CaTV1, LNLCV, MYMoV, ToChSV, ToChV, ToMarV, ToTV, and ToNDV). Y-axes show the degree of similarity, X-axes the amino acid (aa) position. The positions and amino acid composition of virus-wide conserved motifs (29, 30, 37, 43) are indicated above the plots. Colored boxes below show the location of the corresponding functional domains. The motif has a variable position depending on virus. Abbreviations: HEL, helicase; MP, movement protein; PRO, protease; RdRp, RNA-dependent RNA polymerase.

The genome organization of torradoviruses is highly similar to that of other genera within the *Secoviridae*, in particular the genus *Sadwavirus*; however, the genus *Torradovirus* has a putative, unique 5′ ORF on RNA2 (RNA2-ORF1) in addition to the typical MP-CP ORF (RNA2-ORF2). Although the existence of the unique RNA2-ORF1 has yet to be proven experimentally, it is consistently present in all torradovirus species and strains analyzed so far.

Similarity plots of the three ORFs of eight torradoviruses (ToTV, ToMarV, ToChV, ToChSV, ToNDV, LNLCV, MYMoV, and CaTV1) are shown in **Figure 4**. These plots demonstrate that a number of virus-wide conserved motifs are present in all torradoviruses. Generally the aa sequences in the ORFs are quite diverse; however, there are significant differences between TI and NTI torradoviruses (see also **Tables 2–5**). Neighbor joining–derived unrooted phylogenetic trees for the three full-length ORFs of torradoviruses, based on alignments of predicted aa sequences, clearly confirm a distinction between the TI and NTI torradoviruses (results not shown).

Le Gall et al. (39, 40) used the Pro-Pol region, an aa region between the CG protease motif (16) and the GDD RdRp site (13) in the RNA1-ORF1, as an indicator of phylogenetic relationships within the picorna-like viruses. Levels of aa identity resulting from an alignment of this region among the different torradoviruses are depicted in **Table 2**.

Two of the current species demarcation criteria for members of the *Secoviridae* are (*a*) a <80% aa sequence identity in the Pro-Pol region of RNA1-ORF1 and (*b*) a <75% aa sequence identity in the CP region (53). Based on the 80% species demarcation threshold in the Pro-Pol region alone,

Table 2 Levels (%) of amino acid identity between the different torradoviruses in the 3C-like proteinase RNA–dependent RNA polymerase (Pro-Pol) region of RNA1-ORF1

	ToTV-PRI	ToTV-Wal'03	ToTv-Kra	ToMarV	ToANV	ToChV	ToChSV	ToNDV	LNLCV	MYMoV	CaTV1	CsTLV
ToTV-PRI	•	99.8	99.8	78.4	78.8	79.0	78.6	77.1	54.5	54.8	56.3	50.1
ToTV-Wal'03		•	99.6	78.4	78.8	79.0	78.6	77.1	54.3	54.8	56.5	50.1
ToTV-Kra			•	78.2	78.6	78.8	78.6	77.3	54.3	55.0	56.0	49.9
ToMarV				•	99.6	84.9	90.0	91.7	57.1	58.0	59.9	53.6
ToANV					•	85.4	90.2	92.1	57.3	58.2	60.1	53.8
ToChV						•	82.1	82.5	56.3	55.8	59.3	51.8
ToChSV							•	88.9	55.4	57.1	58.4	52.7
ToNDV								•	56.3	58.0	59.1	52.7
LNLCV									•	70.4	68.4	57.9
MYMoV										•	71.4	54.9
CaTV1											•	56.6
CsTLV												•

Abbreviations: CaTV1, Carrot torrado virus 1; CsTLV, Cassava torrado-like virus; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ToANV, *Tomato apex necrosis virus*; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

ToTV and ToMarV, the two currently recognized species, can be considered distinct viruses, as can the newly described NTI torradoviruses LNLCV, MYMoV, CaTV1, and CsTLV. ToANV, sharing nearly 100% aa identity in the Pro-Pol region, can be considered a strain or isolate of ToMarV. The taxonomy of the three other TI torradoviruses, ToChV, ToChSV, and ToNDV, is far less certain. These viruses share 82% to nearly 89% aa identity in their Pro-Pol region. In this region, all three also share over 84% identity with ToMarV, 85% with ToChV, 90% with

Table 3 Overall levels (%) of amino acid identity between the different torradoviruses in their ORF2 on RNA2

	ToTV-PRI	ToTV-Wal'03	ToTv-Kra	ToMarV	ToChV	ToChSV	ToNDV	LNLCV	MYMoV	CaTV1
ToTV-PRI	•	99.1	99.0	69.0	68.0	69.2	68.9	31.3	31.3	32.3
ToTV-Wal'03		•	99.3	69.0	67.9	69.1	68.8	31.3	31.4	32.2
ToTV-Kra			•	68.8	67.8	69.0	68.6	31.2	31.3	32.2
ToMarV				•	77.6	83.7	90.9	31.6	31.5	32.5
ToChV					•	77.9	77.9	30.3	30.9	31.9
ToChSV						•	83.8	31.7	31.7	33.1
ToNDV							•	32.4	31.8	33.5
LNLCV								•	56.1	48.2
MYMoV									•	49.4
CaTV1										•

Abbreviations: CaTV1, Carrot torrado virus 1; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

Table 4 Overall levels of amino acid identity between the different tomato-infecting torradoviruses in the coat-protein domain of ORF2 on RNA2

	ToTV-PRI	ToTV-Wal'03	ToTv-Kra	ToMarV	ToANV	ToChV-G01	ToChV-G02	ToChSV	ToNDV	LNLCV	MYMoV	CaTV1
ToTV-PRI	•	99.4	99.2	71.4	72.3	70.6	70.6	72.0	71.4	34.7	34.5	35.6
ToTV-Wal'03		•	99.6	71.5	72.4	70.7	70.7	72.1	71.5	34.6	34.7	35.4
ToTV-Kra			•	71.5	72.4	70.7	70.7	72.1	71.5	34.6	34.7	35.4
ToMarV				•	90.3	79.5	79.4	83.6	89.6	35.3	34.1	35.1
ToANV						78.3	78.3	82.9	88.9	35.4	34.8	35.6
ToChV-G01						•	97.1	79.6	79.0	33.9	33.3	34.4
ToChV-G02								79.6	78.8	34.1	33.2	34.1
ToChSV								•	83.5	34.8	34.1	35.9
ToNDV									•	35.4	34.8	36.2
LNLCV										•	68.5	57.7
MYMoV											•	59.8
CaTV1												•

Abbreviations: CaTV1, Carrot torrado virus 1; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ToANV, *Tomato apex necrosis virus*; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

Table 5 Overall levels (%) of amino acid identity between the torradoviruses in their putative movement-protein regions on RNA2-ORF2

	ToTV-PRI	ToTV-Wal'03	ToTv-Kra	ToMarV	ToChV-G01	ToChV-G02	ToChSV	ToNDV	LNLCV	MYMoV	CaTV1
ToTV-PRI	•	98.6	98.6	64.0	63.4	63.9	62.9	64.3	19.5	21.3	22.5
ToTV-Wal'03		•	98.6	63.6	62.9	63.5	62.5	63.8	20.0	21.3	22.7
ToTV-Kra			•	63.1	62.7	63.2	62.0	63.3	19.7	21.3	22.7
ToMarV				•	74.1	73.9	83.9	93.4	20.1	21.9	22.0
ToChV-G01					•	95.0	74.6	74.6	18.8	20.1	21.1
ToChV-G02						•	74.0	73.7	18.4	20.4	21.1
ToChSV							•	84.3	20.4	21.7	21.7
ToNDV								•	20.3	21.7	21.9
LNLCV									•	39.0	36.1
MYMoV										•	38.0
CaTV1											•

Abbreviations: CaTV1, Carrot torrado virus 1; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

ToChSV, and 92% with ToNDV (**Table 2**), suggesting these three viruses could be regarded as strains or isolates of ToMarV. **Figure 5** shows an unrooted neighbor-joining tree of selected members of the order *Picornavirales*, including nine torradoviruses, based on the aa sequences of the conserved domains of the Pro-Pol region.

TORRADOVIRUS RNA2-ORF2

ORF2 on RNA2 encodes a polyprotein that is thought to contain the putative viral MP at its N terminus followed by the three viral CPs. Tandem mass spectrometer analyses of the three sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separated CPs of ToTV (63) revealed small peptides whose aa sequences could be aligned with the deduced aa sequence of ORF2 on RNA2. This alignment identified the order of the three viral coat proteins Vp35, Vp26, and Vp23 (see **Figure 3**) as well as likely regions in which the cleavages between the MP and Vp35 (upstream aa position 486), between Vp35 and Vp26 (aa positions 729–732), and between Vp26 and Vp23 (aa positions 970–981) could possibly occur (63).

A significant number of dipeptide sequences that could serve as the possible substrate-binding pocket of the 3C-like proteinase of viruses in the family *Secoviridae* have been identified, either experimentally or by inference from sequence alignments (35, 54). A more detailed analysis of an alignment of the RNA2-ORF2 polyprotein sequences of TI torradoviruses, focusing particularly on the likely cleavage regions between the individual proteins, as identified earlier in ToTV, harbored a number of these potential protease cleavage sites.

The cleavage between the putative MP and largest CP (Vp35) of ToTV must occur upstream of aa position 486 of RNA2-ORF2 (63). All TI torradoviruses contained several of these dipeptide sequences in the region up to 70 aa upstream of residue 486 (in ToTV). However, none were fully conserved among all TI torradoviruses except for one Q/R dipeptide sequence at aa position 427/428 in the ToTV RNA2-ORF2 sequence, which remarkably was part of a motif (VxxLxxQ/RDx) present in all TI torradoviruses (see **Figure 6**).

The likely cleavage site between Vp35 and Vp26 of ToTV was identified to be at aa positions 729–732. All TI torradoviruses contain a glutamine (Q) residue in this region (aa position 732 of ToTV) followed by an A (ToTV, ToMarV, and ToNDV), a T (ToChV), or an S residue (ToChSV). The potential cleavage site between Vp26 and Vp23 of ToTV is likely to be located between aa positions 970 and 981. This region also contains a conserved Q residue in all TI torradoviruses (position 973 in ToTV) followed by a V, an S, or a T residue.

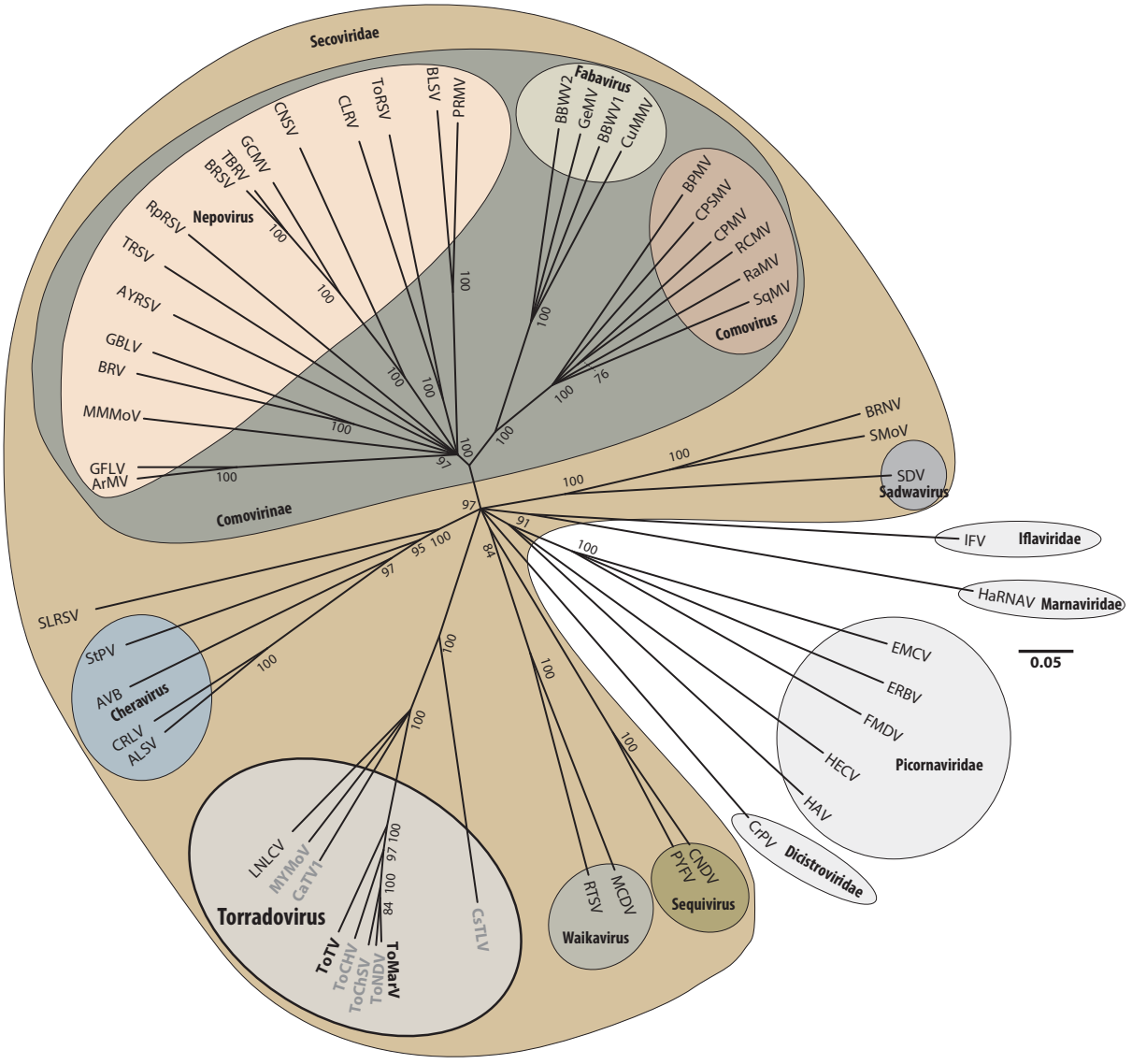
All dipeptide sequences identified in the RNA2-ORF2 as potential cleavage sites between the individual proteins are in line with previously identified or suggested proteinase substrate-binding pocket sequences within the *Secoviridae* (35). Only for ToTV, ToMarV, ToANV, and ToChSV were the sizes of the three CPs determined experimentally. Whether the suggested dipeptide sequences are indeed the exact locations for the cleavage sites between the putative MP and each of the three CPs therefore remains to be established experimentally.

Identification of potential dipeptide cleavage sites in the full RNA2-ORF2 aa sequences of the three NTI torradoviruses available is less obvious. Not only do these ORF2s differ significantly from the TI torradoviruses (see **Table 3**) but they also share limited overall aa sequence identity (48.2–56.1%) among themselves. The conserved aa motif VxxLxxQ/RDx observed in the TI torradoviruses is absent, and numerous potential dipeptide substrate-binding pockets of the 3C-like proteinase of viruses in the family *Secoviridae* are dispersed throughout the polyprotein sequences, making the identification of possible dipeptide cleavage sites impossible. None of the NTI torradoviruses have been purified so far, and the sizes of their CPs remain to be determined experimentally.

Viral Coat Proteins Encoded on RNA2-ORF2

On the assumption that the putative cleavage site between the MP and the CPs in RNA2-ORF2 of the TI torradoviruses is located at the Q/R dipeptide sequence, as suggested above, an amino acid alignment was made between the CP-encoding regions of the different torradoviruses. The levels of aa identity between isolates of the same virus (available for ToTV and ToChV) are all clearly above 97%. Among different viruses, they range from 70.6% to 89.6% (see **Table 4**). ToMarV and ToANV share just over 90% aa identity in their CPs. This confirmed the earlier suggestion that ToMarV and ToANV are likely to be strains or isolates of the same virus species (64).

The CP regions in RNA2-ORF2 of ToChV, ToChSV, and ToNDV display levels of aa identity with those of ToMarV and ToANV (79%, 83%, and 89%, respectively) that are all above the 75% species demarcation criterion (53). In view of the similarly high levels of aa identity to ToMarV



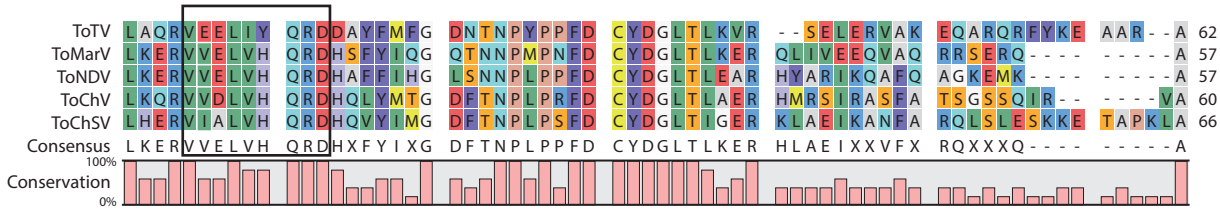


Figure 6

The amino acid sequence in the likely cleavage region between the putative movement protein and largest coat protein (Vp35) of the tomato-infecting torradoviruses, showing the conserved VxxLxxQ/RD proteinase recognition motif (boxed). Abbreviations: ToTV, *Tomato torrado virus*; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToChV, Tomato chocolàte virus; ToChSV, Tomato chocolate spot virus.

in the Pro-Pol region in RNA1-ORF1 (see **Table 2**), ToChV, ToChSV and ToNDV could be regarded as strains or isolates of ToMarV too.

When it becomes available, additional information on the other species demarcation criteria for viruses from the *Secoviridae* (i.e., differences in antigenic reactions, distinct host ranges, distinct vector specificity, and absence of cross-protection) may shed more light on the taxonomic position of ToChV, ToChSV, and ToNDV within the torradoviruses.

Figure 5

Unrooted neighbor-joining tree of selected members of the order *Picornavirales* based on the amino acid sequences of the conserved domains between the CG motif of the 3C-like proteinase and the GDD motif of the polymerase (Pro-Pol region). The alignment was produced by the program T-Coffee (46), and the tree was generated using CLUSTALX (56). Representative sequences were included for the family *Secoviridae*; only representative members of other families within the order *Picornavirales* were included. Numbers on nodes show bootstrap values (1,000 replicates) above 70%. The bar represents the number of substitutions per site. The GenBank accession numbers used for each virus are as follows: *Infectious flacherie virus* (IFV, NC_003781 = AB000906), *Hepatitis A virus* (HAV, NC_001489 = M14707), *Human enterovirus C* (HECV, NC_002058.3 = V01149), *Foot-and-mouth disease virus* type C (FMDV, NC_002554 = AF274010), *Encephalomyocarditis virus* (EMCV, NC_001479 = M81861), *Equine rhinitis B virus 1* (ERBV, NC_003983 = X96871), *Cricket paralysis virus* (CrPV, NC_003924 = AF218039), *Heterosigma akashiwo RNA virus* (HaRNAV, NC_005281 = AY337486), *Parsnip yellow fleck virus* (PYFV, NC_003628 = D14066), *Carrot necrotic dieback virus* (CNDV, EU980442), *Maize chlorotic dwarf virus* (MCDV, NC_003626 = U67839), *Rice tungro spherical virus* (RTSV, NC_001632 = M95497), *Tomato torrado virus* (ToTV, NC_009013 = DQ388879), *Tomato marchitez virus* (ToMarV, NC_010987 = EF681764), *Strawberry latent ringspot virus* (SLRSV, NC_006964 = AY860978), *Stocky prune virus* (StPV, DQ143874), *Apple latent spherical virus* (ALSV, NC_003787 = AB030940), *Cherry rasp leaf virus* (CRLV, NC_006271 = AJ621357), *Arracacha virus B* (AVB, JQ437415), *Satsuma dwarf virus* (SDV, NC_003785 = AB009958), *Strawberry mottle virus* (SMoV, NC_003445 = AJ311875), *Black raspberry necrosis virus* (BRNV, NC_008182 = DQ344639), *Raspberry ringspot virus* (RpRSV, NC_005266 = AY303787), *Peach rosette mosaic virus* (PRMV, AF016626), *Tobacco ringspot virus* (TRSV, NC_005097 = U50869), *Melon mild mottle virus* (MMMoV, AB518485), *Arabis mosaic virus* (ArMV, NC_006057 = AY303786), *Grapevine fanleaf virus* (GFLV, NC_003615 = D00915), *Artichoke yellow ringspot virus* (AYRSV, AM087671), *Blackcurrant reversion virus* (BRV, NC_003509 = AF368272), *Grapevine Bulgarian latent virus* (GBLV, NC_015492 = FN691934), *Cycas necrosis stunt virus* (CNSV, NC_003791 = AB073147), *Grapevine chrome mosaic virus* (GCMV, NC 003622 = X15346), *Beet ringspot virus* (BRSV, NC_003693 = D00322), *Tomato black ring virus* (TBRV, NC_004439 = AY157993), *Tomato ringspot virus* (ToRSV, NC_003840 = L19655), *Cherry leaf roll virus* (CLRV, NC_015414 = FR851461), *Squash mosaic virus* (SqMV, NC_003799 = AB054688), *Radish mosaic virus* (RaMV, NC_010709 = AB295643), *Cowpea mosaic virus* (CPMV, NC_003549 = X00206), *Red clover mottle virus* (RCMV, NC_003741 = X64886), *Bean pod mottle virus* (BPMV, NC_003496 = U70866), *Cowpea severe mosaic virus* (CPSMV, NC_003545 = M83830), *Broad bean wilt virus* 1 (BBWV1, NC_005289 = AB084450), BBWV2 (NC_003003 = AF225953), *Gentian mosaic virus* (GeMV, BAD99001), *Cucurbit mild mosaic virus* (CuMMV, FJ194941), Tomato chocolàte virus (ToChV, FJ560489), Tomato chocolate spot virus (ToChSV, NC_013075 = GQ305131), Tomato necrotic dwarf virus (ToNDV, KC999058), *Lettuce necrotic leaf curl virus* (LNLCV, KC8552566), Motherwort yellow mottle virus (MYMoV, KM229700), Carrot torrado virus 1 (CaTV1, KF533719), and Cassava torrado-like virus (CsTLV, KC505250). Genera, subfamilies, and families are indicated in bold within each colored shape. Viruses within the genus *Torradovirus* are shown in gray as they are presently not recognized as species by the International Committee on the Taxonomy of Viruses.

DIFFERENT ORIGINS OF TORRADOVIRUS MOVEMENT PROTEINS

BLAST (Basic Local Alignment Search Tool) searches of the RNA2-ORF2 predicted movement protein (MP) at the National Center for Biotechnology used the nonredundant (nr) or UniProtKB/SwissProt (swissprot) databases and either the position-specific iterated (PSI) (7) or domain-enhanced lookup time accelerated (DELTA) algorithm (17). These searches yielded hits with several plant virus MPs (see **Table 6**). Interestingly, the TI torradovirus MPs gave hits with MPs from the genus *Umbravirus*, whereas the NTI torradoviruses appear more closely related to viruses from the *Bromoviridae*, *Tombusviridae*, and *Bunyaviridae* families. Additionally, the difference in location of the typical LxxPxL MP motif between the TI and NTI torradoviruses may indicate diverse origins of the MPs as acquired by these two groups (suggested previously in Reference 57).

Putative Movement-Protein Region in RNA2-ORF2

If a cleavage site between the putative MP and the CPs of the TI torradoviruses is located between aa residues 427 and 428 in the ToTV RNA2-ORF2 polyprotein, this would result in proteins ranging in size from 418 to 426 aa (47.6–47.3 kDa). Alignment of these proteins confirmed the status of ToTV and ToMarV as distinct species, as they each share less than 65% aa identity with any of the other torradoviruses (see **Table 4**). With aa identities between 74% and 84%, ToChV and ToChSV appear more closely related to ToMarV and to each other; however, ToNDV is clearly more closely related to ToMarV, with a shared aa identity in the putative MP region of over 93% (see **Table 5**).

The alignment of the putative MP region showed little sequence identity between NTI torradoviruses (36.1–38.9%). The overall levels of identity with the MPs of the TI torradoviruses are even lower, ranging between 18.8% and 22.7% (see **Table 5** and sidebar, Different Origins of Torradovirus Movement Proteins).

The proposed MP consensus sequence LxxPxL (44) is found in all of the putative MPs except MYMoV. Within the TI torradoviruses, the LxxPxL motif is located at aa 262–267 (in ToTV) and aa 263–268 (in ToMarV, ToChV, ToChSV, and ToNDV). For the NTI torradoviruses, the LxxPxL motif is located in a different position in RNA2-ORF2 (aa 226–231 in LNLCV; aa 211–216 in CaTV1). Interestingly, in the putative MYMoV MP, although no LxxPxL motif is present, the similar motif LxxPxI is located in a region similar to that for LNLCV and CaTV1, at aa positions 227–232. Whether this difference indicates a truly different motif remains to be established.

RNA2-ORF1 Hypothetical Protein

One distinctive feature of torradoviruses in comparison to other species within the *Picornavirales* is the presence of a unique small ORF at the 5′ end of RNA2, upstream of and partially overlapping the large ORF2. This small ORF (RNA2-ORF1) could encode a putative protein of approximately 20 kDa. None of the putative torradovirus RNA2-ORF1 proteins shows any significant homology to any known protein in the NCBI database, and to date there is no experimental proof for translation of a functional protein from this RNA2-ORF1.

The overall level of identity in the hypothetical RNA2-ORF1 protein is generally low (see **Table 7**). Within the TI torradoviruses, it ranges from 59.8% to 74.1% over a length of 184–190 aa. Within the three NTI torradoviruses for which this sequence was determined (LNLCV, MYMoV, and CaTV1), the levels of identity vary between 41.4% and 50.9% over a length

Table 6 BLAST results obtained for the ORF1-RNA2 domains of different torradoviruses

e-Value[a]	Hit	Virus[b]	Accession number	Database[c]	Method[d]
2.00E-95	Movement protein 3A [*Cucumber mosaic virus*, *Bromoviridae* (strain FT)]	NTI	Q66139.1	swissprot	PSI
2.00E-77	Movement protein 3A [*Peanut stunt virus*, *Bromoviridae* (strain J)]	NTI	P22117.1	swissprot	PSI
3.00E-77	Cell-to-cell movement protein [*Carrot mottle mimic virus*, *Umbravirus*]	TI	ACJ03577.1	nr	PSI
7.00E-75	ORF4 [*Groundnut rosette virus*, *Umbravirus*]	NTI	AAF74551.1	nr	PSI
3.00E-70	Movement protein 3A [*Pelargonium zonate spot virus*, *Bromoviridae*]	NTI	Q9DUT1.1	swissprot	PSI
8.00E-48	Movement protein [*Red clover necrotic mosaic virus*, *Tombusviridae*]	NTI	P10838.1	swissprot	DELTA
1.00E-44	Hypothetical protein [*Tobacco bushy top virus*, *Umbravirus*]	TI	CBH71323.1	nr	PSI
5.00E-43	Movement protein [*Tomato spotted wilt virus* (strain Brazilian BR-01), *Bunyaviridae*]	NTI	P36292.1	swissprot	DELTA
8.00E-43	ORF4 [*Groundnut rosette virus*, *Umbravirus*]	TI	AAF74551.1	nr	PSI
4.00E-35	Cell-to-cell movement protein [*Pea enation mosaic virus-2*, *Umbravirus*]	TI	AEM45996.1	nr	PSI
9.00E-35	Movement protein [*Impatiens necrotic spot virus*, *Bunyaviridae*]	NTI	Q01268.1	swissprot	DELTA
8.00E-33	Putative movement protein [*Citrus leprosis virus*, *Cilevirus*]	NTI	Q1KZ55.1	swissprot	DELTA
6.00E-09	3A protein [*Tomato aspermy virus*, *Bromoviridae*]	NTI	AAA67088.1	nr	PSI
1.00E-07	3A protein [*Brome mosaic virus*, *Bromoviridae*]	NTI	BAD83844.1	nr	PSI

[a]The single most significant hits (lowest e-values) per molecule per species are listed (i.e., less significant hits of the same molecule and species with a different search approach were not included).
[b]Searches were made using sequences specific to either tomato-infecting (TI) or non-tomato-infecting (NTI) torradovirus.
[c]BLAST searches were done using either the nonredundant (nr) or UniProtKB/SwissProt (swissprot) databases.
[d]BLAST methods used were either the position-specific iterated (PSI) or domain-enhanced lookup time accelerated (DELTA) algorithm. In all searches, iterations were done three times.

of 202–211 aa. Between the two groups of torradoviruses, the levels of identity within this RNA2-ORF1 are significantly lower (21.4%–30.7%).

Remarkably, the RNA2-ORF1 of ToNDV and ToMarV share a higher level of identity (80.5%) with each other than with the other TI torradoviruses. Their similarity supports their grouping as strains or isolates of the same virus.

As seen for the other ORFs on both RNA1 and RNA2 of the TI torradoviruses and NTI torradoviruses, an alignment of the hypothetical protein encoded by RNA2-ORF1 also results in two distinct groups with little to no homology between them (see **Table 7** and **Figure 7**). In this overall alignment, very few aa residues are fully conserved between the two groups (see **Figure 8**).

A comparison of separate alignments of this region for the two virus groups, however, identified two motifs that are fully conserved among all RNA2-ORF1s. Motif 1 consists of the aa-triplet LDF at positions 41–43. It is present in all the torradoviruses sequenced to date and was already identified in the similarity plot of RNA2-ORF1 (see **Figure 5**). A BLASTp search in the nonredundant protein sequences at NCBI returned no significant similarities for this LDF motif.

Table 7 Overall levels of amino acid identity between the different torradoviruses in their ORF1 on RNA2

	ToTV-PRI	ToTV-Wal'03	ToTv-Kra	ToMarV	ToChV	ToChSV	ToNDV	LNLCV	MYMoV	CaTV1
ToTV-PRI	•	99.5	99.5	61.1	59.8	61.2	61.4	21.4	29.2	27.2
ToTV-Wal'03		•	100.0	61.1	60.3	61.7	60.9	21.4	29.2	27.2
ToTV-Kra			•	61.1	60.3	61.7	60.9	21.4	29.2	27.2
ToMarV				•	63.7	71.6	80.5	22.3	30.7	25.6
ToChV					•	63.2	63.2	20.0	27.7	24.8
ToChSV						•	74.1	22.6	28.2	26.1
ToNDV							•	23.2	29.5	26.6
LNLCV								•	41.4	41.6
MYMoV									•	51.0
CaTV1										•

Abbreviations: CaTV1, Carrot torrado virus 1; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

A similar BLASTp search with the second, more diffuse motif GxxxSxLxVxWxxxxPQxxxxH, located between positions 51 and 77, returned a large number of hits. Most hits comprised the motifs LxxWxxxxPQ, VxWxxxxPQ, or WxxxPQ and were found in a wide variety of prokaryotic and eukaryotic organisms, mainly bacterial and mammalian. Exceptionally few hits with known viral sequence were found, but this could be a result of sample bias in the databases. At this moment, it remains to be confirmed whether RNA2-ORF1 indeed encodes a protein, and what function such a protein could have remains unclear (see sidebar A Biological Function for RNA2-ORF1?).

HOMOLOGIES, DIFFERENCES, AND REPEATS IN THE VIRAL 3′ UNTRANSLATED REGIONS

Torradoviruses, in comparison to many other RNA plant viruses, have long 3′ UTRs on both RNAs. For the TI torradoviruses, these UTRs vary in length between 1,230 and 625 nts for RNA1 and between 1,406 and 647 nts for RNA2. The 3′ UTRs of the NTI viruses vary in length between 734 and 91 nts for RNA1 and between 766 and 33 nts for RNA2.

Analogous to the subgroups B and C of the nepoviruses (35), both 3′ UTRs of RNA1 and RNA2 of a given torradovirus are nearly identical for most of the 3′ UTR sequences directly preceding

A BIOLOGICAL FUNCTION FOR RNA2-ORF1?

The observed levels of homology of amino acid sequence encoded by the first open reading frame (ORF) on RNA2 (RNA2-ORF1) of the torradoviruses with bacterial and mammalian proteins and the (near) absence of any significant hits with known viral sequences are intriguing. It is tempting to speculate that this ORF was acquired from an unknown source, possibly even in a number of unrelated events. Nevertheless, as the ORF was apparently maintained upon acquisition, it is likely to have some important biological function. Given the low levels of homology between the tomato-infecting and non-tomato-infecting torradoviruses, this function need not be the same in these two groups.

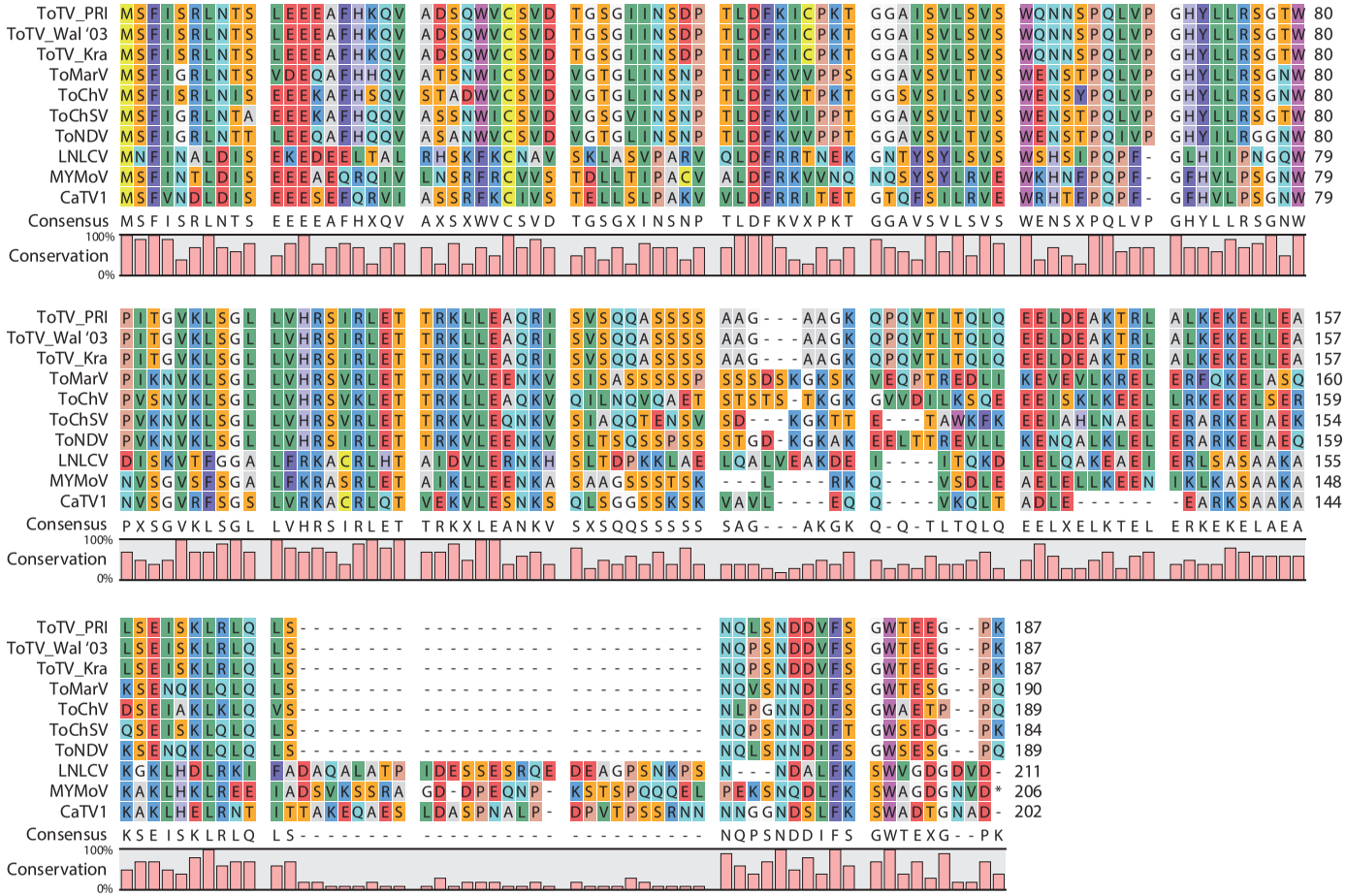


Figure 7

An overall alignment of the amino acid residues encoded by ORF1 on RNA2 of the torradoviruses. Abbreviations: CaTV1, Carrot torrado virus 1; LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

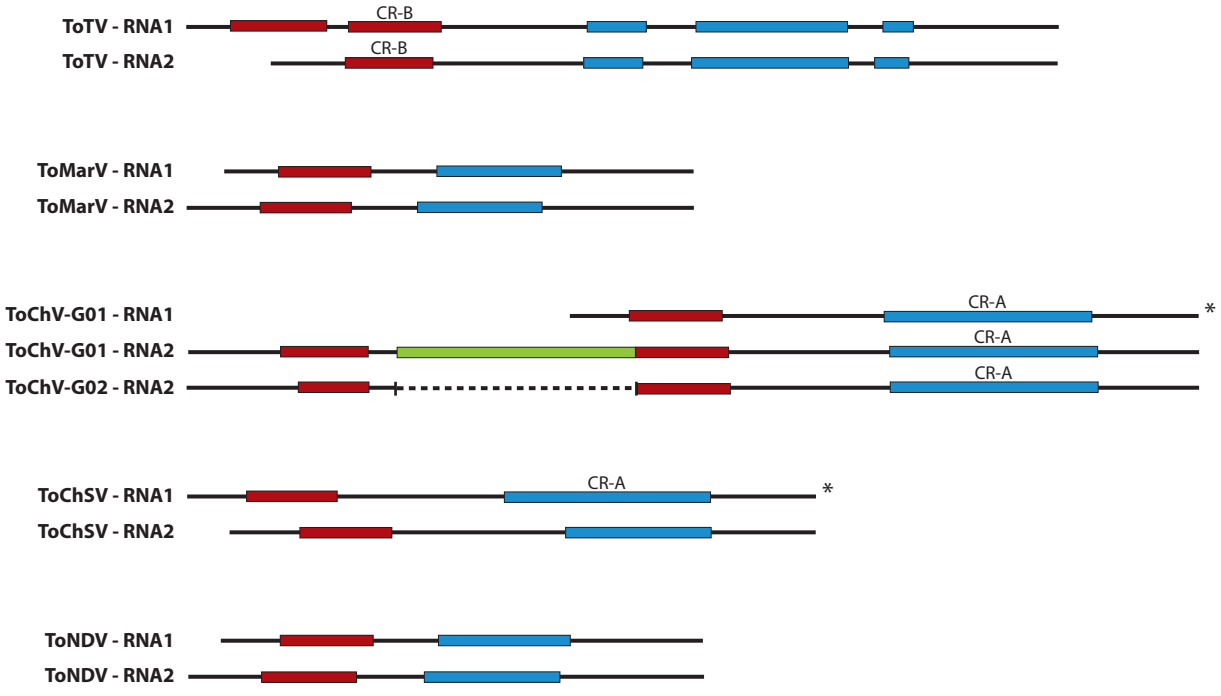


Figure 8

Schematic presentation indicating the locations of the conserved regions (CR-A shown in blue; CR-B in red) in the 3′ untranslated regions (UTRs), aligned at their 3′ termini, of RNA1 and RNA2 of the different tomato-infecting torradoviruses. Duplications of these regions, with levels of sequence identity varying between 77–83% for Cr-A and 74–83% for CR-B, are indicated in each virus with the same-colored rectangles. Within one virus, the CR-A and CR-B regions on RNA1 and RNA2 are nearly identical. The 329-nucleotide insertion in the 3′ UTR of RNA2 of ToChV-G01 (see text) is indicated in green, and the gap (*dashed line*) was introduced in RNA2 of ToChV-G02 to visually align corresponding regions. *RNA1 3′ UTRs of ToChV-G01 and ToChSV are identical. Abbreviations: ToChSV, Tomato chocolate spot virus (NC_013075 and NC_013076); ToChV, Tomato chocolàte virus (FJ560489 and FJ560490); ToMarV, *Tomato marchitez virus* (NC_010987 and NC_010988); ToNDV, Tomato necrotic dwarf virus (KC999058 and KC999059); ToTV, *Tomato torrado virus* (NC_009013 and NC_009032).

the poly(A)-tail; the common region, though only a relatively short 5′-terminal variable region, shows clear differences, both in length and in sequence (see **Table 8**).

Remarkably, of all TI torradoviruses, only the variable regions of the 3′ UTRs of RNA2 of ToChV isolates G01 (65) and G02 (GU071087, unpublished) differ significantly in length. A more detailed analysis of these two genetic variants (isolated from the same plant; A.M. Dullemans, M. Verbeek, R.A.A. van der Vlugt, unpublished results) identified a 329-nt insertion at positions 291–619 in the RNA2 3′ UTR of ToChV-G01. Apart from this insertion, both 3′ UTRs are identical in length (1,077 nts) and share 98% sequence identity.

Interestingly, a BLASTn search using the 329-nt insertion from the RNA2 3′ UTR of ToChV-G01 identified a 293-nt duplication (with 74% sequence identity) of this insertion sequence between positions 969 and 1,261 in the RNA2 3′ UTR of ToChV-G01. The same 293-nt sequence (with 100% sequence identity) was present in the RNA2 3′ UTR of ToChV-G02 between positions 640 and 930.

When the 293-nt sequence was used as a query sequence in a local BLASTn search in CLC-Bio (Aarhus, Denmark) Main Workbench V7.5 against the entirety of the RNA1 and RNA2 torradovirus sequences available, a 100% duplication of this 293-nt conserved region (which we

Table 8 A comparison of the lengths in nucleotides [excluding the poly-(A) tail] and percentages of sequence identity of the variable regions and common regions in the 3′ untranslated regions (3′ UTRs) of RNA1 and RNA2 of the recognized and tentative torradoviruses

Virus		Variable regions			Common regions		
	Isolate	RNA1	RNA2	% Identity	RNA1	RNA2	% Identity
ToTV	PRI	222	103	27.9	988	988	99.0
	Wal'03	241	103	31.2	989	989	100
	Kra	229	103	26.2	989	989	100
ToMarV	PRI	74	101	42.0	553	553	98.9
	ToANV	74	94	41.5	553	553	99.3
ToChV	G01	84	619	11.0	787	787	100
	G02	ND	290	ND	ND	787	100
ToChSV	ND	78	96	40.6	789	718	74.1
ToNDV	ND	80	102	46.7	556	557	93.5
LNLCV	ND	0	32	0	734	734	95.5
MYMoV	ND	143	247	41.4	214	214	95.3

Abbreviations: LNLCV, *Lettuce necrotic leaf curl virus*; MYMoV, Motherwort yellow mottle virus; ND, not determined; ToChSV, Tomato chocolate spot virus; ToChV, Tomato chocolàte virus; ToMarV, *Tomato marchitez virus*; ToNDV, Tomato necrotic dwarf virus; ToTV, *Tomato torrado virus*.

refer to as CR-A) was identified in the 3′ UTR of RNA1 of ToChV-G01 (positions 434–726) as well as in the 3′ UTR of RNA1 of ToChSV (positions 428–720). Moreover, significant parts of the 3′-terminal part of CR-A (varying in size from 172 to 210 nts) were conserved (75–83%) as single regions in both the RNA1 and RNA2 3′ UTRs of ToMarV and ToNDV and the RNA2 3′ UTR of ToChSV. Between the 3′ UTRs of one virus, this CR-A was nearly identical. Interestingly, different parts of this 293-nt CR-A region were duplicated at three positions in both the 3′ UTRs of ToTV (see **Figure 8**).

The high level of sequence homology between the 3′ UTRs of both genomic RNAs was also observed for LNLCV (91.5%) but remarkably not for MYMoV (66.3%) and CsTLV (40.3%). All these 3′ UTR sequences have been confirmed by RACE. For CsTLV, alignments with the 3′ UTRs of a second isolate showed high levels of homology for RNA1 (92%) and RNA2 (96%) (W.J. Cuellar, unpublished results).

Budziszewska et al. (19) also showed sequence heterogeneity and duplications in the 3′ UTRs of ToTV isolates. By high-resolution melting curve analyses, they observed a number of unique genetic variants that each showed a distinct deletion in the variable region of the 3′ UTR on RNA1 of ToTV-Kra. They identified a 127-nt conserved region (D-CR_{RNA1}) that, with only some nt differences and deletions, was present in the 5′ end of the common region of the 3′ UTR of RNA1 of ToTV, ToMarV, ToChV-G01 and ToChSV. A duplication of this region (D-VR_{RNA1}, with 82% sequence identity) was identified in the variable region of RNA1 3′ UTR of ToTV but not in the variable regions of the 3′ UTRs of RNA1 of the other torradoviruses. Sequences similar to this conserved region were also observed in the 3′ UTRs of RNA2 of ToTV-Wal'03, ToMarV, ToChV, and ToChSV.

A BLASTn search at NCBI with a 130-nt fragment containing the D-CR_{RNA1} sequence showed that this sequence was present in both 3′ UTRs of all TI torradoviruses (see **Figure 8**). Levels of identity for this conserved region (which we refer to as CR-B) between the 3′ UTRs of a given virus are (nearly) 100% and are in the range of 74–83% between different viruses.

SECONDARY STRUCTURES IN 5′ AND 3′ UTRS OF TORRADOVIRUS RNAS

Although they do not contain any obvious open reading frames (ORFs), and therefore are termed untranslated regions (UTRs), viral 3′ UTRs (as well as 5′ UTRs) sometimes harbor extensive secondary RNA structures, which are (or are suspected to be) correlated with specific biological functions.

Initial RNA secondary structure analyses of the TI torradoviruses using PFold (36) identified several conserved stem-loop structures in both 5′ and 3′ UTRs of RNA1 and RNA2. In particular, a region of 62 nts in conserved region B (CR-B) of the 3′ UTRs of all tomato-infecting torradoviruses contained three distinct stem loops (see **Figure 9**) that are highly conserved in sequence as well as in structure through appropriate co-variation on stem base pairing.

Although not extensive, these and other studies (65) already clearly indicate that torradoviruses share not only a very substantial and nearly identical common region in their 3′ UTRs but also significant stretches of homologous conserved regions between distinct virus species, including distinct secondary RNA structures (see sidebar Secondary Structures in 5′ and 3′ UTRs of Torradovirus RNAs). More detailed analyses will probably identify conserved regions additional to CR-A and CR-B. The reasons for these extensive sequence duplications in the torradovirus 3′ UTRs remain to be established.

Despite their significantly shorter length and the absence of any significant sequence homologies, the 5′ UTRs of the TI torradoviruses also contained two distinct stem-loop structures, which were highly conserved between viral RNAs as well as between the different viruses (results not shown). Remarkably, the same stem-loop structure was also present in a similar position in the 5′ UTRs of the NTI torradoviruses. Although the existence of these secondary structures needs experimental confirmation, their positional and structural conservation strongly suggests a function in torradovirus replication and/or translation.

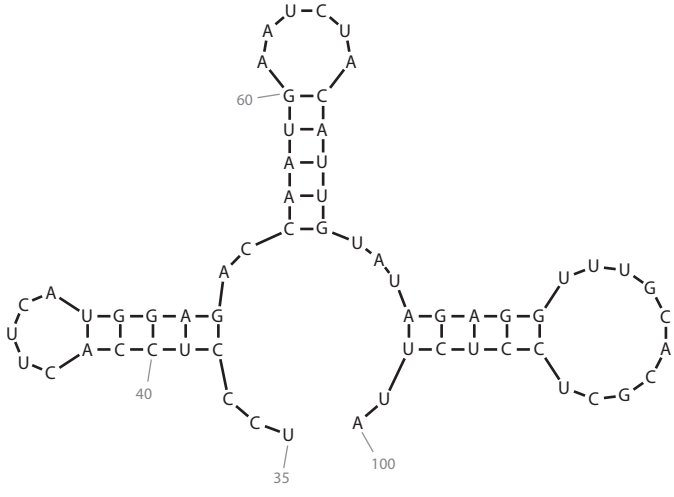


Figure 9

Highly conserved stem-loop structures in the 5′ region of the 3′ UTR of RNA2 of ToMarV as predicted by PFold (36). Although variable in sequence, the structures shown are highly conserved among all 3′ UTRs of TI torradoviruses.

EPIDEMIOLOGY OF TORRADOVIRUSES

Torradovirus Host Range

Initially, natural torradovirus infections were all described from tomato (*Solanum lycopersicum* L.). Experimental host range studies have shown, however, that ToTV also systemically infects eggplant (*Solanum melongena* cv. Black Beauty) and pepper (*Capsicum annuum* cv. Italian Long Sweet) (8). ToNDV can also infect these host species, as well as tomatillo (*Physalis philadelphica*) (38). In addition, torradoviruses can infect different experimental hosts, including several tobacco species and other non-tobacco indicator plants (27, 31 and references therein).

Alfaro-Fernández et al. (4) surveyed several weed species present in Spanish tomato-growing areas. ToTV was detected in 22 weed samples belonging to the families *Amaranthaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Cruciferae*, *Malvaceae*, *Polygonaceae*, and *Solanaceae*. This indicates that natural infection occurred in these weed species, which in turn could act as ToTV reservoirs and sources for transmission to tomato.

Of the NTI torradoviruses, LNLCV was successfully inoculated on *Nicotiana occidentalis* "P1," *Nicotiana benthamiana*, *Nicotiana hesperis* "67A," *Physalis floridana*, and *Physalis acutifolia*, as well as on several commercial lettuce cultivars (62). LNLCV could not infect tomato cv. "Realeza," and, in the same experiment, ToTV, ToMarV, and ToChV could not infect lettuce (62). Mechanical inoculation of CsTLV to *N. benthamiana* and *Nicotiana tabacum* did not result in visible symptoms, and no virus could be detected by RT-PCR in the inoculated plants (22). No information is available on the host range of MYMoV. During 2014, field surveillance was conducted to collect CaTV1 isolates for biological characterization, CaTV1 was not detected from the limited range of Apiaceous field weeds collected during these field visits (A. Fox, personal communication).

Vector Transmission

The torrado disease in Spain and Poland was generally observed in greenhouses or fields that were heavily infested with whiteflies (6), which led to the suspicion that whiteflies were the insect vectors. Pospieszny (47) demonstrated transmission of a spherical virus associated with torrado disease (at that time not yet identified as ToTV) by the greenhouse whitefly *T. vaporariorum* (Westwood). In 2008, Amari et al. (8) reported that ToTV was also transmitted by the sweet potato whitefly *B. tabaci* (Sulzer). Verbeek et al. (67) showed that three TI torradoviruses (ToTV, ToMarV, and ToChV) are transmitted by three whitefly species, *T. vaporariorum*, *B. tabaci*, and *T. abutilonea* (Haldeman).

Transmission of ToNDV was evaluated for two *B. tabaci* biotypes—New World 1 (formerly known as biotype A) and *B. tabaci* MEAM1 (formerly known as biotype B)—as well as for *T. abutilonea* and *T. vaporariorum*, the latter of which is common in greenhouses and temperate regions throughout the world. All four species were found to be effective vectors of ToNDV, with efficient transmission by *T. abutilonea* and the *B. tabaci* biotypes but a lower rate of transmission by *T. vaporariorum* (W.M. Wintermantel, unpublished results).

Previously, only members of the genus *Crinivirus* were reported with certainty to be transmitted by more than one whitefly species. *Tomato chlorosis virus* (ToCV) is transmitted by three whitefly species in two genera: *B. tabaci*, *T. vaporariorum*, and *T. abutilonea* (70). Similarly, *Sweet potato chlorotic stunt virus* (SPCSV) is transmitted by *B. tabaci* and *Bemisia afer* sensu lato (Priesner & Hosny), as well as by *T. abutilonea* (25, 34). In addition, *Diodia vein chlorosis virus* (DVCV) and *Blackberry yellow vein-associated virus* (BYVaV) can be transmitted by both *T. abutilonea* and *T. vaporariorum* (52, 59). This phenomenon has been described for two other viruses outside the genus *Crinivirus*, but these observations could not be confirmed by other

researchers. *Tomato yellow leaf curl virus* (TYLCV, family *Geminiviridae*, genus *Begomovirus*) is transmitted not only by *B. tabaci* but also by *Trialeurodes ricini* (Misra) (33, 34), and *Cassava brown streak virus* (CBSV, family *Potyviridae*, genus *Ipomovirus*) is transmitted by both *B. tabaci* and *B. afer* (34, 41, 42).

Torradoviruses represent the first spherical viruses reported to be transmitted by whiteflies. Formerly, only viruses with geminate particles (genus *Begomovirus*) or filamentous particles (genera *Crinivirus*, *Ipomovirus*, and *Carlavirus*) (34, 45) were known to be transmitted by these insects. However, so far, there is evidence of whitefly transmission only for the TI torradoviruses ToTV, ToMarV, ToChV (67), ToANV (14), and ToNDV (38, 69). For the recently discovered NTI torradoviruses of cassava (CsTLV), lettuce (LNLCV), motherwort (MYMoV), and carrot (CaTV1), vectors have not yet been identified. Early field studies on cassava diseases in Colombia indicated that the transmission of leaf symptoms from diseased cassava plants to cassava landrace "Secundina" plants coincided with elevated numbers of whiteflies (9, 10).

At the moment, it seems that the spread of LNLCV and CaTV1 in the open field in The Netherlands and Great Britain cannot be explained by transmission by the known whitefly vector species for the TI torradoviruses, as these vectors are generally considered to be greenhouse pests and are usually not found in northern European fields. Whether these viruses are transmitted by other whitefly species, whether they represent a new group of torradoviruses whose members are transmitted by aphids or other unidentified vectors, or whether they are spread by some other route remains to be investigated.

A better understanding of the epidemiology of torradoviruses requires a better understanding of their mode of transmission, e.g., whether they are transmitted in a nonpersistent manner (with short periods of acquisition and inoculation) or in a semipersistent or persistent manner (in which longer acquisition and inoculation periods are needed, and dispersion may occur over longer distances). From previous studies demonstrating whitefly transmission of ToTV (8, 47, 51), the mode of transmission could not be identified. Two studies have been published investigating the mode of transmission of TI torradoviruses. Barajas-Ortiz et al. (14) described transmission studies with ToANV, using the vector *B. tabaci*. They determined a minimal acquisition access period (AAP) of 12 h and a minimal inoculation access period (IAP) of 9 h with a retention time of ToANV in *B. tabaci* up to 7 days. The authors concluded that ToANV is transmitted in a persistent manner. Verbeek et al. (67) determined an optimal AAP between 8 and 16 h and an optimal IAP of 8 h for transmission of ToMarV by *T. vaporariorum*. These results were in the same range as found for ToANV and *B. tabaci* (14). However, much shorter retention periods were measured when the whiteflies, before gaining access to the receptor plant for inoculation, were held on a plant that is a nonhost for the virus during various retention periods. When this transmission strategy was used, *T. vaporariorum* remained viruliferous for less than 24 h. Therefore, Verbeek et al. (67) concluded that torradoviruses are transmitted in a semipersistent manner. The authors also described experiments to localize the retention site of torradoviruses in the vector insect. Persistently transmitted viruses normally are translocated throughout the body of the insect until they reach the salivary glands, from which they can be transmitted, and detection of these viruses in several body parts (head, thorax, and abdomen) is to be expected. In contrast, semipersistent viruses have their retention site in the foregut of the insect (and sometimes within the stylet) and can be transmitted for several hours. Nonpersistent viruses are retained only within the stylet and for short periods of a few hours. Verbeek et al. (67) showed that particles of ToMarV and ToTV are retained within the stylets of the whitefly vectors *T. vaporariorum*, *T. abutilonea*, and *B. tabaci*. Based on the measured AAP, IAP, retention times, and retention site within the vector, it was concluded that the TI torradoviruses are transmitted in a semipersistent stylet-borne manner. At

this time, no studies comparing the transmission efficiency of the three reported whitefly vectors have been published.

Seed Transmission

When ToTV was found in Australia (26), seed transmission of this virus was suspected because of the strict import regulations on plant material into this country. The transmission of Polish ToTV isolates through seeds of tomato and pepper has been reported at a rate of 0.5–0.8% after mechanical inoculation of pepper plants and growing out the seeds from the infected pepper plants (48, 49). In a single, preliminary test, no seed transmission of CsTLV was detected in a sample of 140 botanical seeds obtained from infected cassava (W.J. Cuellar, unpublished results). Similarly, a preliminary test in which 169 seeds collected from six ToNDV-infected tomato plants were grown also failed to yield infected plants (W.M. Wintermantel, unpublished results). These results suggest that seed transmission of ToNDV, if it occurs, is quite rare. Furthermore, many tomatoes infected with ToNDV produce few or no seeds, and most produce very few fruit owing to the severe necrosis and stunting (W.M. Wintermantel, unpublished results). Conclusive studies will require larger numbers of samples from multiple plants to clarify if any seed transmission of CsTLV or ToNDV occurs. No information is available on possible levels of seed transmission for any of the other torradoviruses.

Detection and Identification of Torradoviruses

Efficient plant virus control depends heavily on the availability of healthy planting material and seeds as well as on timely recognition of infections. Detection methods play an important role in ensuring healthy crops. Currently, most available detection methods for the known torradoviruses are RNA-based (e.g., RT-PCR). Antisera against torradoviruses are scarce because it is quite laborious to purify their virions to the extent required to produce ELISA-grade antisera. Nevertheless, antisera have been produced against ToNDV, ToTV, and ToANV (38, 51, 58). Currently, only the antiserum against ToANV (ToMarV) is commercially available. The ToNDV antiserum reacted in double antibody sandwich (DAS)-ELISA with the Wal'03 isolate of ToTV (51), and the ToANV antiserum reacted with the PRI-TMarV0601 isolate of ToMarV (M. Verbeek, unpublished results), indicating that the currently available antisera might be useful to confirm a torradovirus infection, but serological differentiation of distinct torradovirus species or strains might be problematic.

On the basis of published sequences, different research groups constructed specific RT-PCR primer sets for the detection of the different viruses (3, 15, 18, 22, 50, 55, 58, 60, 63). Two sets of generic torradovirus primers were designed on the whole-genome sequences of the TI torradoviruses ToTV, ToMarV, ToChSV, and ToChV. These two primer sets proved adequate for the detection of these viruses (66). Primer pair Torrado-1F/Torrado-1R (designed on RNA1 sequences) also appeared useful for detecting some NTI torradoviruses, whereas primer pair Torrado-2F/Torrado-2R (designed on RNA2) was not (22, 67). Both primer pairs proved suitable for the detection of different isolates of CaTV1 (A. Fox, unpublished results). In addition to RT-PCR primer sets, virus-specific detection was also described for ToTV, ToANV, and ToChSV through hybridization with dioxigenin-labeled RNA-riboprobes (23).

Relevance of Torradovirus Infections in the Field

There is still little information on the importance and actual occurrence of torradoviruses in the field. Because of the economic importance of tomato and the uncertain economic impact of

ToTV, the virus was placed on the European and Mediterranean Plant Protection Organization (EPPO) alert list in 2007 (11). A survey in Spain over the period 2001–2008 detected ToTV in several different regions: Alicante, Almería, Barcelona, Murcia, Gran Canaria and Tenerife, and Mallorca. The incidence of ToTV in 451 tested samples varied from 58% in 2001 to 92% in 2005, with an average of 77% over the survey period. ToTV was often (60% of the time) found together with PepMV as well as in combination with other viruses: *Cucumber mosaic virus*, *Parietaria mottle virus*, *Potato virus Y*, *Tomato chlorosis virus*, *Tomato mosaic virus*, *Tomato spotted wilt virus*, and *Tomato yellow leaf curl virus*. These findings led the authors to conclude that torrado symptoms may not be related to ToTV alone (3).

Gómez et al. (28) report on the incidence of ToTV in Murcia (Spain) between 2005 and 2008. ToTV was mostly found in single infections, but double and triple infections of ToTV, PepMV, and/or *Tomato chlorosis virus* occurred. There was no apparent correlation of the severity of torrado symptoms with mixed infections, nor with the presence of PepMV. In single infections, ToTV concentration was highest in the early stages of infection, declining as disease symptoms progressed. In mixed infections, ToTV did seem to lower PepMV titres but apparently in a strain-dependent manner. The authors conclude that mixed infections of ToTV and PepMV may modulate the epidemiology of both viruses by modulating viral fitness.

An infection of ToTV was reported in 2011 from a greenhouse tomato crop in Eastern Sicily, Italy (12). All plants concerned were destroyed, and the current status of ToTV in Italy is unknown. Infections of ToTV in Hungary and Poland were all reported eradicated. Outside of Europe, ToTV has been reported only from Panama (32), Australia (26), and Colombia (61). The occurrence of ToMarV, ToChV, and ToChSV is currently known only from their initial descriptions and seems to be limited to Mexico (ToMarV) and Guatemala (ToChV and ToChSV).

When first noted, ToNDV caused significant losses for the tomato industry in Southern California during the 1980s and was largely responsible for the displacement of commercial tomato production from the region. The virus can cause smaller leaves, leaf necrosis, and severe stunting of tomato plants with little or no fruit production. With fewer tomatoes planted in the region, the virus disappeared and has not been seen in Imperial Valley tomato fields for several years.

A second LNLCV isolate was found in 2013 in lettuce plants with typical LNLCV symptoms subjected for analysis at the Dutch Plant Protection Service (M. Verbeek & J.Th.J. Verhoeven, personal communication). This isolate was collected at a different location in The Netherlands than the original isolate, but no further information on virus distribution is available.

During 2014, limited field surveillance was carried out for CaTV1 in the North of England (A. Fox, personal communication). CaTV1 was detected in 14–25% of plants tested. Unfortunately, owing to the high incidences of several other carrot-infecting viruses in these fields, no conclusions can be drawn as to the effects of CaTV1 on foliar symptoms or yield.

CsTLV is mostly found in mixed infections in plants displaying root symptoms of frogskin disease. In a recent survey on cassava viruses in Colombia, more than 50% of plants collected in the North Coast region during 2014 were infected with CsTLV ($n = 180$) (W.J. Cuellar, unpublished results). More information is needed about the incidence of this virus in other cassava-growing regions of Colombia and in other regions where the disease has been reported (21).

CONCLUSION

During the past few years, the characterization of torradoviruses demonstrated how an initial description and characterization of a new virus can quickly lead to the identification of a significant number of previously unknown viruses. Genomic sequence information, in particular that obtained

through NGS, allows the identification of unknown plant virus sequences and the development of new means for virus detection and identification.

It is likely that new torradoviruses will be discovered both from solanaceous and nonsolanaceous hosts. We hope future research on torradoviruses will focus not only on the description of new viruses but also on a much better understanding of their biology, including both host ranges and means of (vector) transmission. Currently, the limited knowledge of these topics makes adequate control of these viruses a challenge.

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LITERATURE CITED

1. Adams IP, Skelton A, Macarthur R, Hodges T, Hinds H, et al. 2014. *Carrot yellow leaf virus* is associated with carrot internal necrosis. *PLoS ONE* 9:e109125
2. Alfaro-Fernández A, Bese G, Córdoba-Sellés C, Cebrián MC, Herrera-Vásquez JA, et al. 2009. First report of *Tomato torrado virus* infecting tomato in Hungary. *Plant Dis.* 93:554
3. Alfaro-Fernández A, Cebrián MC, Herrera-Vásquez JA, Córdoba-Sellés MC, Sánchez-Navarro JA, Jordá C. 2010. Molecular variability of Spanish and Hungarian isolates of *Tomato torrado virus*. *Plant Pathol.* 59:785–93
4. Alfaro-Fernández A, Córdoba-Sellés C, Cebrián MC, Herrera-Vásquez JA, Sánchez-Navarro JA, et al. 2008. First report of *Tomato torrado virus* on weed hosts in Spain. *Plant Dis.* 92:831
5. Alfaro-Fernández A, Córdoba-Sellés C, Cebrián MC, Sánchez-Navarro JA, Espino A, et al. 2007. First report of *Tomato torrado virus* in tomato in the Canary Islands, Spain. *Plant Dis.* 91:1060
6. Alfaro-Fernández A, Córdoba-Sellés MDC, Juárez M, Herrera-Vásquez JA, Sánchez-Navarro JA, et al. 2010. Occurrence and geographical distribution of the 'torrado' disease in Spain. *J. Phytopathol.* 158:457–69
7. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–402
8. Amari K, Gonzalez-Ibeas D, Gómez P, Sempere RN, Sanchez-Pina MA, et al. 2008. *Tomato torrado virus* is transmitted by *Bemisia tabaci* and infects pepper and eggplant in addition to tomato. *Plant Dis.* 92:1139
9. Angel SJ, Nolt BL, Pineda LB. 1987. Estudios sobre transmisión por moscas blancas (*Homoptera*: *Aleyrodidae*) de virus asociados con el cuero de sapo en yuca (*Manihot esculenta* Crantz). *Acta Agronómica* 37:27–39 (in Spanish)
10. Angel SJ, Pineda LB, Nolt BL, Velasco AC. 1989. Moscas blancas (*Homoptera*: *Aleyrodidae*) asociadas a transmisión de virus en yuca. *Fitopatologia Colombiana* 13:65–71(in Spanish)
11. Anonymous. 2007. Studies on a new disease of tomato in Spain called 'torrao' or 'cribado'. EPPO Rep. Serv. No. 07-2007, Artic. No. 2007/128
12. Anonymous. 2008. The tomato "marchitez" disease in Mexico is caused by a new virus. EPPO Rep. Serv. No. 04-2008, Artic. No 2008/080
13. Argos P. 1988. A sequence motif in many polymerases. *Nucleic Acids Res.* 16:9909–16
14. Barajas-Ortiz M, León-Sicairos CR, López-Valenzuela JA, Reyes-Moreno C, Valdez-Ortiz A, et al. 2013. Transmission efficiency of Tomato apex necrosis virus by *Bemisia tabaci* (*Hemiptera*: *Aleyrodidae*) biotype B in tomato. *J. Econ. Entomol.* 106:1559–65
15. Batuman O, Kuo YW, Palmieri M, Rojas MR, Gilbertson RL. 2010. Tomato chocolate spot virus, a member of a new torradovirus species that causes a necrosis-associated disease of tomato in Guatemala. *Arch. Virol.* 155:857–69
16. Bazan JF, Fletterick RJ. 1988. Viral cysteine proteases are homologous to the trypsin-like family of serine proteases: structural and functional implications. *Proc. Natl. Acad. Sci. USA* 85:7872–76

17. Boratyn G, Schaffer A, Agarwala R, Altschul S, Lipman D, Madden T. 2012. Domain enhanced lookup time accelerated BLAST. *Biol. Dir.* 7:12
18. Budziszewska M, Obrępalska-Stęplowska A, Wieczorek P, Pospieszny H. 2008. The nucleotide sequence of a Polish isolate of *Tomato torrado virus*. *Virus Genes* 37:400–6
19. Budziszewska M, Wieczorek P, Zhang Y, Frishman D, Obrępalska-Stęplowska A. 2014. Genetic variability within the Polish tomato torrado virus Kra isolate caused by deletions in the 3′-untranslated region of genomic RNA1. *Virus Res.* 185:47–52
20. Calvert LA, Cuervo M, Lozano I. 2012. Cassava viral disease in South America. In *Cassava in the Third Millennium: Modern Production, Processing, Use and Marketing Systems*, ed. B Ospina, H Ceballos, pp. 309–18. Cali, Colombia: CIAT
21. Calvert LA, Cuervo M, Lozano I, Villareal N, Arroyave J. 2008. Identification of three strains of a virus associated with cassava plants affected by frogskin disease. *J. Phytopathol.* 156:647–53
22. Carvajal-Yepes M, Olaya C, Lozano I, Cuervo M, Castaño M, Cuellar WJ. 2014. Unraveling complex viral infections in cassava (*Manihot esculenta* Crantz) from Colombia. *Virus Res.* 186:76–86
23. Da Silva DM Jr, Ferriol I, Falk B. 2014. Specific detection of three *Torradovirus* species with dioxigenin-labeled probes. *BMC Proc.* 8(Suppl. 4):P125
24. Davino S, Bivona L, Iacono G, Davino M. 2010. First report of *Tomato torrado virus* infecting tomato in Italy. *Plant Dis.* 94:1172
25. Gamarra HA, Fuentes S, Morales FJ, Glover R, Malumphy C, Barker I. 2010. *Bemisia afer* sensu lato, a vector of *Sweet potato chlorotic stunt virus*. *Plant Dis.* 94:510–14
26. Gambley CF, Thomas JE, Persley DM, Hall BH. 2010. First report of *Tomato torrado virus* on tomato from Australia. *Plant Dis.* 94:486–86
27. Gómez P, Sempere R, Aranda MA. 2012. *Pepino mosaic virus* and *Tomato torrado virus*. Two emerging viruses affecting tomato crops in the Mediterranean basin. In *Advances in Virus Research*, 84:505–32. San Diego: Elsevier Acad.
28. Gómez P, Sempere RN, Amari K, Gómez-Aix C, Aranda MA. 2010. Epidemics of *Tomato torrado virus*, *Pepino mosaic virus* and *Tomato chlorosis virus* in tomato crops: Do mixed infections contribute to torrado disease epidemiology? *Ann. Appl. Biol.* 156:401–10
29. Gorbalenya AE, Donchenko AP, Blinov VM, Koonin EV. 1989. Cysteine proteases of positive strand RNA viruses and chymotrypsin-like serine proteases: a distinct protein superfamily with a common structural fold. *FEBS Lett.* 243:103–14
30. Gorbalenya AE, Koonin EV, Wolf YI. 1990. A new superfamily of putative NTP-binding domains encoded by genomes of small DNA and RNA viruses. *FEBS Lett.* 262:145–48
31. Hanssen IM, Lapidot M. 2012. Major tomato viruses in the Mediterranean basin. *Adv. Virus Res.* 84:31–66
32. Herrera-Vásquez JA, Alfaro-Fernández A, Córdoba-Sellés MC, Cebrián MC, Font MI, Jordá C. 2009. First report of *Tomato torrado virus* infecting tomato in single and mixed infections with *Cucumber mosaic virus* in Panama. *Plant Dis.* 93:198
33. Idriss M, Abdallah N, Aref N, Haridy G, Madkour M. 1997. Biotypes of the castor bean whitefly *Trialeurodes ricini* (Misra) (Hom., *Aleyrodidae*) in Egypt: biochemical characterization and efficiency of geminivirus transmission. *J. Appl. Entomol.* 121:501–9
34. Jones DR. 2003. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* 109:195–219
35. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. 2012. *Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier Acad.
36. Knudsen B, Hein J. 2003. Pfold: RNA secondary structure prediction using stochastic context-free grammars. *Nucleic Acids Res.* 31:3423–28
37. Koonin EV. 1991. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *J. Gen. Virol.* 72:2197–206
38. Larsen RC, Duffus JE, Liu HY. 1984. Tomato necrotic dwarf—a new type of whitefly transmitted virus. *Phytopathology* 74:795
39. Le Gall O, Christian P, Fauquet CM, King AMQ, Knowles NJ, et al. 2008. *Picornavirales*, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T = 3 virion architecture. *Arch. Virol.* 153:715–27

40. Le Gall O, Sanfaçon H, Ikegami M, Iwanami T, Jones T, et al. 2007. *Cheravirus* and *Sadwavirus*: two unassigned genera of plant positive-sense single-stranded RNA viruses formerly considered atypical members of the genus *Nepovirus* (family *Comoviridae*). *Arch. Virol.* 152:1767–74
41. Legg JP, Raya MD. 1998. Survey of cassava virus diseases in Tanzania. *Int. J. Pest Manage.* 44:17–23
42. Maruthi MN, Hillocks RJ, Mtunda K, Raya MD, Muhanna M, et al. 2005. Transmission of *Cassava brown streak virus* by *Bemisia tabaci* (Gennadius). *J. Phytopathol.* 153:307–12
43. Melcher U. 2000. The '30K' superfamily of viral movement proteins. *J. Gen. Virol.* 81:257–66
44. Mushegian AR. 1994. The putative movement domain encoded by nepovirus RNA-2 is conserved in all sequenced nepoviruses. *Arch. Virol.* 135:437–41
45. Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S. 2011. Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol.* 49:219–48
46. Di Tommaso P, Moretti S, Xenarios I, Orobitg M, Montanyola A, et al. 2011. T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. *Nucleic Acids Res.* 39:W13–W17
47. Pospieszny H. 2005. Preliminary study on the spherical virus transmitted by greenhouse whitefly (*Trialeurodes vaporariorum*). *Proc. Joint Conf. Work. Group Legume and Veg. Viruses, 2nd, Ft. Lauderdale, FL*, April 10–14, p. 30, Gainesville: Univ. Fla.
48. Pospieszny H, Borodynko N. 2014. Seed transmission of *Tomato torrado virus* in pepper (*Capsicum annuum*). *Proc. Int. Cong. Mol. Plant-Microbe Interact, XVI*, Rhodes, Greece, July 6–10, p. P168
49. Pospieszny H, Borodynko N, Hasiów-Jaroszewska B, Rymelska N. 2013. *Seed transmission of Tomato torrado virus in tomato*. Presented at Int. Symp. Plant Virus Epidemiol., 12th, Arusha, Tanzania
50. Pospieszny H, Borodynko N, Obrępalska-Stęplowska A, Hasiów B. 2007. The first report of *Tomato torrado virus* in Poland. *Plant Dis.* 91:1364
51. Pospieszny H, Budziszewska M, Hasiów-Jaroszewska B, Obrępalska-Stęplowska A, Borodynko N. 2010. Biological and molecular characterization of Polish isolates of *Tomato torrado virus*. *J. Phytopathol.* 158:56–62
52. Poudel B, Wintermantel WM, Cortez AA, Ho T, Khadgi A, Tzanetakis IE. 2013. Epidemiology of *Blackberry yellow vein associated virus*. *Plant Dis.* 97:1352–57
53. Sanfaçon H, Iwanami T, Karasev AV, van der Vlugt RAA, Wellink J, et al. 2012. *Secoviridae*. In *Ninth Report of the International Committee on Taxonomy of Viruses*, ed. AMQ King, MJ Adams, EB Carstens, EJ Lefkowitz, pp. 881–99. San Diego: Elsevier Academic
54. Sanfaçon H, Wellink J, Le Gall O, Karasev A, van der Vlugt RAA, Wetzel T. 2009. *Secoviridae*: a proposed family of plant viruses within the order *Picornavirales* that combines the families *Sequiviridae* and *Comoviridae*, the unassigned genera *Cheravirus* and *Sadwavirus*, and the proposed genus Torradovirus. *Arch. Virol.* 154:899–907
55. Seo J-K, Kang M, Kwak H-R, Kim M-K, Kim C-S, et al. 2014. Complete genome sequence of motherwort yellow mottle virus, a novel putative member of the genus *Torradovirus*. *Arch. Virol.* 160:587–90
56. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25:4876–82
57. Thompson JR, Kamath N, Perry KL. 2014. An evolutionary analysis of the *Secoviridae* family of viruses. *PLoS ONE* 9:e106305
58. Turina M, Ricker MD, Lenzi R, Masenga V, Ciuffo M. 2007. A severe disease of tomato in the Culiacan area (Sinaloa, Mexico) is caused by a new picorna-like viral species. *Plant Dis.* 91:932–41
59. Tzanetakis I, Wintermantel W, Poudel B, Zhou J. 2011. *Diodia vein chlorosis virus* is a group-1 crinivirus. *Arch. Virol.* 156:2033–37
60. Van den Heuvel JFJM, Maris PC, Verbeek M, Dullemans AM, van der Vlugt RAA. 2010. Plant virus desginated Tomato torrado virus. *Patent No. 2006213170/WO2006085749 A1*
61. Verbeek M, Dullemans AM. 2012. First report of *Tomato torrado virus* infecting tomato in Colombia. *Plant Dis.* 96:592
62. Verbeek M, Dullemans AM, Raaij HG, Verhoeven JTJ, van der Vlugt RAA. 2014. Lettuce necrotic leaf curl virus, a new plant virus infecting lettuce and a proposed member of the genus *Torradovirus*. *Arch. Virol.* 159:801–5

63. Verbeek M, Dullemans AM, van den Heuvel JFJM, Maris PC, van der Vlugt RAA. 2007. Identification and characterisation of *Tomato torrado virus*, a new plant picorna-like virus from tomato. *Arch. Virol.* 152:881–90
64. Verbeek M, Dullemans AM, van den Heuvel JFJM, Maris PC, van der Vlugt RAA. 2008. *Tomato marchitez virus*, a new plant picorna-like virus from tomato related to *Tomato torrado virus*. *Arch. Virol.* 153:127–34
65. Verbeek M, Dullemans AM, van den Heuvel JFJM, Maris PC, van der Vlugt RAA. 2010. Tomato chocolàte virus: a new plant virus infecting tomato and a proposed member of the genus *Torradovirus*. *Arch. Virol.* 155:751–55
66. Verbeek M, Tang J, Ward LI. 2012. Two generic PCR primer sets for the detection of members of the genus *Torradovirus*. *J. Virol. Methods* 185:184–88
67. Verbeek M, van Bekkum PJ, Dullemans AM, van der Vlugt RAA. 2014. Torradoviruses are transmitted in a semi-persistent and stylet-borne manner by three whitefly vectors. *Virus Res.* 186:55–60
68. Verdin E, Gognalons P, Wipf-Scheibel C, Bornard I, Ridray G, et al. 2009. First report of *Tomato torrado virus* in tomato crops in France. *Plant Dis.* 93:1352
69. Wintermantel WM, Hladky LL. 2013. Genome characterization of Tomato necrotic dwarf virus, a torradovirus from southern California. *Phytopathology* 103:160
70. Wintermantel WM, Wisler GC. 2006. Vector specificity, host range, and genetic diversity of *Tomato chlorosis virus*. *Plant Dis.* 90:814–19