http://doi.org/10.1590/1678-992X-2022-0045

ISSN 1678-992X

SCIENTIA AGRICOLA

Research Article

Tomato fruit blotch virus cytopathology strengthens evolutionary links between plant blunerviruses and insect negeviruses

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Edited by: Jorge Alberto Marques Rezende

Received February 25, 2022

Accepted April 18, 2022

ABSTRACT: Tomato fruit blotch virus (ToFBV) is a blunervirus that causes blotches on mature tomato (*Solanum lycopersicon* L.) fruits in Italy and Australia in 2020, and was newly detected in Brazil. A cytological study on pericarp tissues from the blotched areas of infected fruits collected in Brasília, Brazil, revealed characteristic cell alterations. Small and slender bacilliform particles (ca. 25 nm wide × 100 nm long) were found accumulating in the perinuclear space and the lumen of the endoplasmic reticulum of the epidermis, peri- and mesocarp cells. No viroplasm-like inclusion was observed either in the nuclei or in the cytoplasm. Such cell alterations are reminiscent of those described in cultured mosquito cells infected by negeviruses, an unofficial group of insect viruses. Negeviruses and some other arthropod-borne viruses shared a common ancestor in the RdRp gene with kitavirids, including blunerviruses. Although additional detailed studies are required, we show evidence that ToFBV particles are enveloped and bacilliform, and that such similarity in cytopathology seems to support the evolutionary relationship between plant kitavirids and insect negeviruses.

Keywords: transmission electron microscopy, tomato fruit pericarp, virus-like particles, perinuclear space, kitavirus

Introduction

Tomato (*Solanum lycopersicon* L.) is one of the most cultivated vegetables globally and possibly the plant species in which more viruses and viroids have been registered. In the comprehensive Encyclopedia of Plant Viruses and Viroids (Sastry et al., 2020), 158 viruses and eight viroids are listed as naturally infecting this species. An inventory made in Brazil described 45 virus species officially recognized by the International Committee on Taxonomy of Viruses (ICTV) and eight, still unclassified, infecting cultivated tomatoes (Kitajima, 2020). The blunervirus tomato fruit blotch virus (ToFBV) is a member of the family *Kitaviridae* and genus *Blunervirus* and one of the most recently described viruses infecting tomatoes (Ciuffo et al., 2020). Infected plants present uneven blotchy ripening and dimpling of tomato fruits. Besides Italy and Australia, ToFBV has also been detected in Spain, the Canary Islands, Portugal, Slovenia, and Tunisia[1]. Mechanical transmission assays of ToFBV in tomatoes failed to produce infected plants, and neither virions nor virus-like particles were found in leaf extracts from the infected plants (Ciuffo et al., 2020).

Tomato fruit blotch was recently reported in Brazil infecting organic tomato plants under greenhouse conditions, with a high incidence of fruits bearing chlorotic blotches (Nakasu et al., 2022). No foliar symptom was discerned due to a heavy infestation of the tomato russet mite *Aculops lycopersici* Massee. High throughput sequencing detected two viruses, the crinivirus tomato chlorosis virus (ToCV), and the blunervirus ToFBV isolate MAL. The latter was the only one present in all symptomatic fruits and its genome sequence had > 97 % nucleotide sequence identity with ToFBV isolate Fondi2018 (Genbank accessions MK517477 to MK517480). ToFBV_MAL could be detected only in the pericarp of the fruit lesion (Nakasu et al., 2022).

In an attempt to enrich the knowledge on blunerviruses, an ultrastructural investigation was undertaken on pericarp tissues of blotched areas of ToFBV-infected fruits collected from a commercial field in Brasília, Brazil. Characteristic cytopathic effects have been observed, reminiscent of alterations induced by mosquito-infecting negeviruses (Vasilakis et al., 2013). Furthermore, a phylogenetic analysis conducted with ToFBV and closely related viruses also suggested an evolutionary link between insect-infecting nege-like viruses and plant kitavirids.

Materials and Methods

Tomato fruits from two cultivars (Giacomo and Grazianni) bearing blotched spots (Figure 1) were collected in an organic greenhouse in the surroundings of Brasília, Federal District, Brazil (15°58′32.9″ S, 47°29′44.3″ W, altitude 1,200 m) in Oct 2020, where originally ToFBV_MAL was found (Nakasu et al., 2022). Small fragments of the pericarp (about 1 mm deep, 1 mm wide, and 3 mm long) of the blotched region of tomato fruits (one per fruit, from three fruits) were removed with a sharp razor blade and immediately immersed in a fixative solution (2 % glutaraldehyde, 2.5 % paraformaldehyde in 0.05

[1]A. Tiberini (personal communication, 2021)

M cacodylate buffer, pH 7.2). After 2-3 h of aldehyde fixation, these fragments were washed with 0.05 M cacodylate buffer and post-osmicated in 1 % OsO_4 for 1 h. Fixed tissues were dehydrated in acetone, infiltrated, and embedded in the low viscosity epoxy Spurr's resin (Kitajima and Nome, 1999). Semi-thin sections (ca. 1.5 µm thick) were obtained with a Diatome histological diamond knife, mounted on glass slides, stained with 1 % Azur B and 1 % methylene blue in an aqueous solution, and examined under a photomicroscope. Thin sections from corresponding blocks were made in a Leica EM UC6 ultramicrotome equipped with a Diatome diamond knife. Sections were collected on 300 mesh copper grids, contrasted with 3 % uranyl acetate and Reynold's lead citrate, and examined in a transmission electron microscope equipped with a digital camera. As controls, similar pieces of fruit pericarp from healthy tomato (cv. Santa Clara) were processed and examined.

Fruits from the same batch used for microscopic examinations were submitted to RT-PCR assay using the specific primers Bluner 1F and Bluner 1R (Nakasu et al., 2022). The test was performed in three fruit samples/cv, and positive detection with the amplification of the expected amplicon of 0.5 kbp was obtained, confirming that ToFBV infected these fruits.

For the phylogenetic analysis, amino acid (aa) sequences of the RNA-dependent RNA polymerases (RdRp) of kitaviruses, unclassified kita/nege-like viruses, including those of the groups centivirus, aphiglyvirus, and negevirus, and also sandewaviruses and nelorpiviruses, were retrieved from GenBank (Table 1). RdRp sequences from some viruses of the family *Virgaviridae* were used as an external outgroup. Sequences were aligned using MAFTT (Katoh and Standley, 2013), and informative phylogenetic regions of the multiple sequence alignment were selected using BMGE software (Criscuolo and Gribaldo, 2010) implemented in NGPhylogeny (Lemoine et al., 2019). The substitution models with the lower Bayesian information criterion scores and the Maximum Likelihood trees were obtained using W-IQ-TREE software v. 1.6.12 (Trifinopoulos et al., 2016). One thousand bootstrap replications assessed the reliability of the inferred evolutionary relationships. Sequences of the domains methyltransferase, helicase, and RdRp of blunerviruses were concatenated as previously described (Quito-Avila et al., 2013; Ramos-González et al., 2020). The tree was edited and visualized using Interactive Tree Of Life (iTOL) v 5 (Letunic and Bork, 2019).

Results

At the light microscope level, no significant alteration in tissue organization was apparent. The pericarp of tomato fruits, from either control healthy or the blotched area of ToFBV-infected fruits, revealed the typical organization of epidermis, 5-6 layers of exocarp, and mesocarp formed by larger cells, as described previously (Rančić et al., 2010; Stertz et al., 2005). As expected, pericarp cells were large, with a huge vacuole, embedded in a thin cytoplasm, presenting scarce organelles. The nucleus was similarly large and commonly lobated (Figure 2). Ultrastructural observations, however, revealed significant alterations in the pericarp cells from the blotched areas of ToFBV-infected tomato fruits in all samples (Figures 3-5). Most significantly, under high magnification, the nuclei presented slender, short, enveloped bacilliform particles in the perinuclear space (Figure 3A). These particles were consistently observed in sections forming a single row or grouped in aggregates

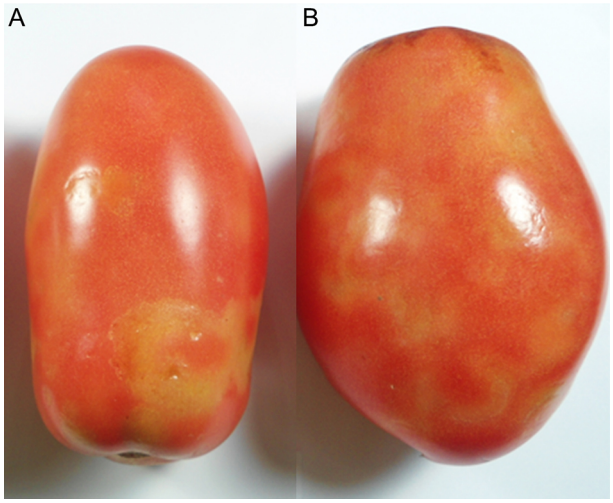

Figure 1 – Blotched tomato fruits from cultivars Giacomo (A) and Grazianni (B), collected from an organic farm in Brasilia, Brazil. Infection by ToFBV (Tomato fruit blotch virus) was confirmed by RT-PCR assay.

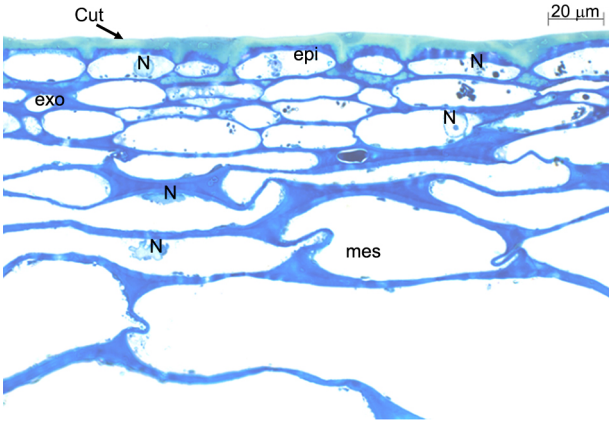

Figure 2 – Light micrograph of a semi-thin, cross-section (ca.1.5 mm thick) from the pericarp of blotched area, resulting from infection by tomato fruit blotch virus (ToFBV), of a tomato fruit, cv. Giacomo. The cuticle (cut)-covered epidermis (epi), a 5-6 cell layer forming the exocarp (exo), and the subjacent mesocarp (mes) and large nuclei (N), some of them lobate, are discernible. No remarkable difference with pericarp from control, uninfected tomato fruit, could be noticed at this level.

Table 1 – List of RNA-dependent RNA polymerase sequences and protein accession numbers from kitaviruses, negeviruses, and other related kita/nege-like viruses used for the phylogenetic reconstruction.

	Genus	Virus	GenBank accession number
Kitaviridae	*Cilevirus*	Citrus leprosis virus C isolate Crd01 (CiLV-C_Crd01)	YP654538
		CiLV-C_SJP01	AKJ79134
		CiLV-C_Asu02	QUM93109
		Citrus leprosis virus C2 isolate Fla (CiLV-C2_Fla)	ATW76030
		CiLV-C2_Hw	AGM16551
		CiLV-C2_Co	YP009508070
		Passion fruit green spot virus isolate Snp1 (PfGSV_Snp1)	QFU28424
		PfGSV_BJL1	QFU28437
		Hibiscus yellow blotch virus (tentative member)	QRG34866
		Ligustrum chlorotic spot virus isolate Crb1 (LigCSV_Crb1) (tentative member)	OK626449
		LigCSV _SPa1 (tentative member)	OK626447
		Ligustrum leprosis virus isolate Cdb1 (tentative member)	OK626451
		Pistachio virus Y (tentative member)	QPL17819
		Solanum violifolium ringspot virus isolate Ctb1 (SvRSV_ Ctb1) (tentative member)	OK626441
		SvRSV _Prb1 (tentative member)	OK626439
	Higrevirus	Hibiscus green spot virus_2	YP004928118
		Pistachio virus X (tentative member)	QPL17815
	Blunervirus	Blueberry necrotic ring blotch virus isolate Georgia (BNRBV_Georgia)	YP_004901700 YP_004901701
		BNRBV_RL	AGI44297 AGI44298
		Tea plant necrotic ring blotch virus (TPNRBV)	YP_009551524 YP_009551530
		Tomato fruit blotch virus isolate Fondi2018 ToFBV Fondi2018	QEL52506 QEL52507
		ToFBV_MAL	QZN83592 QZN83593
Negeviruses[1]	Nelorpivirus[2]	Negev virus	YP009256205
		Brejeira virus	AQM55484
		Castlerea virus	AQZ42313
		Loreto virus	YP009351835
		Ngewotan virus	AQM55317
		Big Cypress virus	YP_009351821
		Culex negev-like virus 2	YP_009388582
		Daeseongdong virus 1	YP_009182191
		Piura virus	AFI24678
		San Bernardo virus	AQM55293
	Sandewavirus[2]	Bustos virus	BAU71147
		Santana virus	AFI24675
		Tanay virus	YP009028558
		Biratnagar virus	AQM55290
		Culex Biggie-like virus	AXQ04820
		Culex negev-like virus 3	YP_009388605
		Dezidougou virus	ARQ15945
		Goutanap virus	AIL49282
		Uxmal virus	AYW01743
		Wallerfield virus	AIS40854

Continue...

Table 1 – Continuation.

Virgaviridae	Tobravirus	Tobacco rattle virus	ACX54058.
		Pea early-browning virus	CAB37343
		Pepper ringspot virus	AAA47081
	Tobamovirus	Tobacco mosaic virus	ABN79257
		Tobacco mild green mosaic virus	AYV75121
		Cucumber green mottle mosaic virus	ANA53152
		Cucumber mottle virus	YP_908760
		Hibiscus latent Fort Pierce virus	ALU84940
		Turnip vein-clearing virus	AEU04724
		Wasabi mottle virus	BAB82440
		Youcai mosaic virus	AAK53541
	Pecluvirus	Peanut clump virus	NP_620047
	Pomovirus	Potato mop-top virus	ARS22098
		Soil-borne virus 2 (tentative member)	ALT22314
	Furovirus	Soil-borne cereal mosaic virus	AAF18330
Kita/nege-like viruses[1]		Tetranychus kitavirus	MN204568
		Beihai anemone virus 1	YP_009333202
		Beihai barnacle virus 2	YP_009333216
		Andrena haemorrhoa nege-like virus	YP_009553581
		Lodeiro virus	YP_009315901
		Muthill virus	AMO03223
		Nephila clavipes virus 4	YP_009552461
		Saiwaicho virus	AWA82269
		Sanxia atyid shrimp virus 1	YP_009336762
		Shayang virga-like virus 1	YP_009333208
		Xingshan nematode virus 1	YP_009333310
		Brandeis virus	AVZ66283
		Culex negev-like virus 1	ASA47301
	Aphiglyvirus[2]	Hubei Wuhan insect virus 9	YP_009337901
		Aphis glycines virus 3	ASH89118
	Centivirus[2]	Wuhan house centipede virus 1	YP_009342435
		Wuhan insect virus 8	YP_009344994
	Frankliniella occidentalis associated negev-like viruses	*Frankliniella occidentalis* associated negev-like virus 1 (Foanegev1)	QNM37802
		Foanegev2	QNM37799
		Foanegev3	QNM37811

[1]Unclassified group of viruses; [2]Proposed name of a tentative genus.

in large expansions of the perinuclear space (Figures 3B-D; 4A and B). A clear continuity of the perinuclear space and adjacent endoplasmic reticulum (ER) could be seen in several cases filled with these bacilliform particles (Figure 4A). These particles were also present in the lumen of the ER, not only in the vicinity of the nucleus (Figure 4B), but also in distant cytoplasmic areas (Figures 4C and D). In rare instances, they were also detected in the ER elements connected to the plasmodesma (Figure 4D). Besides these alterations, no other indication of infection by other viruses was observed in examined tissues.

Measurements of these bacilliform particles in higher magnifications (Figures 5A-C) resulted in a modal value of ca. 25 nm diameter and ca. 100 nm length (Figure 6), being smaller than values reported for cile- and higreviruses (Kitajima et al., 2003; Melzer et al., 2012). These particles are believed to represent ToFBV virions and will be referred to as such from now on. No viroplasm-like inclusion was noticed in the bacilliform particle bearing nuclei, nor the cytoplasm.

Accumulation of virions in the perinuclear space has been previously reported in leaf tissues by membrane-bound plant viruses such as cileviruses, orthotospoviruses, and plant rhabdoviruses, such as dichorhaviruses, cytorhabdoviruses, alphanucleorhabdoviruses, and betanucleorhabdoviruses (Kitajima et al., 1992; 2003; Jackson et al., 2005). Virions maturing at the inner membrane of the nuclear envelope (e.g., alphanucleorhabdoviruses and betanucleorhabdoviruses), budding towards the perinuclear space are observed, although they can migrate to more distant elements of ER (Jackson et al., 2005). Conversely, in the case of viruses maturing in the lumen of the ER, e.g., cilevirus and cytorhabdovirus (Kitajima et al., 2003; Jackson et al., 2005), or via Golgi complex, e.g., orthotospovirus (Kitajima et al., 1992; Kikkert et al., 1999), virion accumulation occurs in the lumen of the ER, but occasionally viral particles are found in the perinuclear space, due to the continuity of the ER and nuclear envelope. In the case of ToFBV, the morphogenesis of these presumed virions is unclear yet.

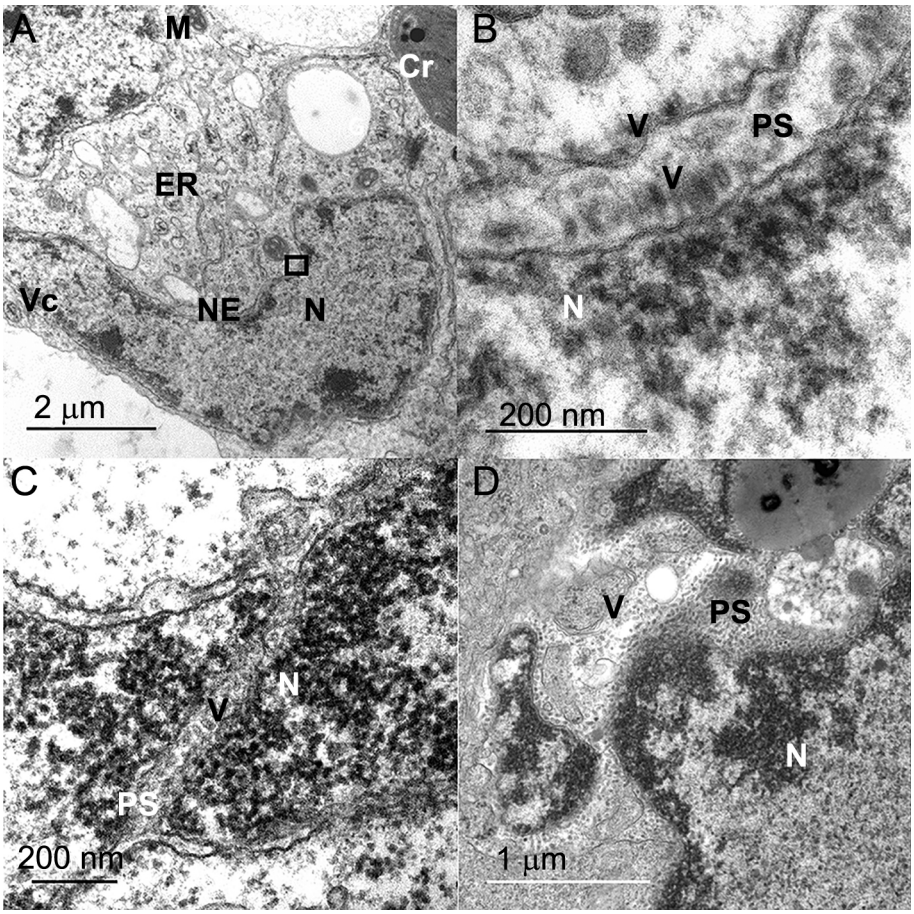

Figure 3 – Transmission electron micrograph of pericarp cells from blotchy lesions on tomato fruits caused by ToFBV (Tomato fruit blotch virus) infection. (A) Low magnification image of part of a mesocarp cell from the pericarp of a blotched region of a cv. Giacomo fruit. A large, lobate nucleus (N) and neighboring cytoplasm, rich in endoplasmic reticulum (ER) elements. (B) Detail of the marked area in A, showing a group of small bacilliform particles (v), side-by-side in the perinuclear space (PS). (C and D) Similar to B, in lesions on fruits of cvs. Grazianni and Giacomo. Note the enlarged perinuclear space filled with bacilliform particles in D. Cr = chloroplast; M = mitochondrion; Vc = vacuole; NE = nuclear envelope.

Possibly they are formed by a budding process at the level of the nuclear envelope, but no clear-cut evidence is available yet.

The phylogenetic analyses using the deduced aa sequences of the RdRp of accepted and tentative species of the family *Kitaviridae*, and several unclassified arthropod-infecting viruses, showed a branch comprising the three genera of the family *Kitaviridae.* In contrast, the sister branch encompassed monopartite viruses of Nelorpivirus and Centivirus (Figure 7). Basal to these, several branches, including viruses of the groups Sandewavirus, Aphiglyvirus, and others found infecting several invertebrates, such as mites, crustaceous, and insects were also observed. This result indicated a close relationship between the kitaviruses and the nege-like viruses. Plant-infecting viruses closer to kitavirids belonging to the family *Virgaviridae* were represented in this tree by some members of the genera *Tobamovirus*, *Tobravirus*, *Pecluvirus*, *Furovirus*, and *Pomovirus,* and served as an outgroup.

Discussion

The genus *Blunervirus* is a member of the recently created family *Kitaviridae* (https://talk.ictvonline.org/taxonomy/), which also incorporates two more genera, *Cilevirus* and *Higrevirus.* Members of this family have divided genomes (*Cilevirus*- two molecules, *Higrevirus*- three, and *Blunervirus*- four) of (+) sense ssRNA. Before ToFBV, two other blunerviruses have been described, the blueberry necrotic ring blotch virus (BNRBV) and the tea plant necrotic ring blotch virus (TPNRBV). The blueberry necrotic ring blotch virus was detected in the southern United States, infecting blueberry plants (interspecific hybrids of *Vaccinium corymbosum* L., Ericaceae) and eriophyid mites of the genus *Calacarus* are suspected to be their vector (Burkle et al., 2012; Martin et al., 2012; Cantu-Iris et al., 2013; Quito-Ávila et al., 2013; Robinson et al., 2016). A metagenomic study based on next-generation sequencing of tea plants (*Camellia sinensis* L.) in China, with virus-like

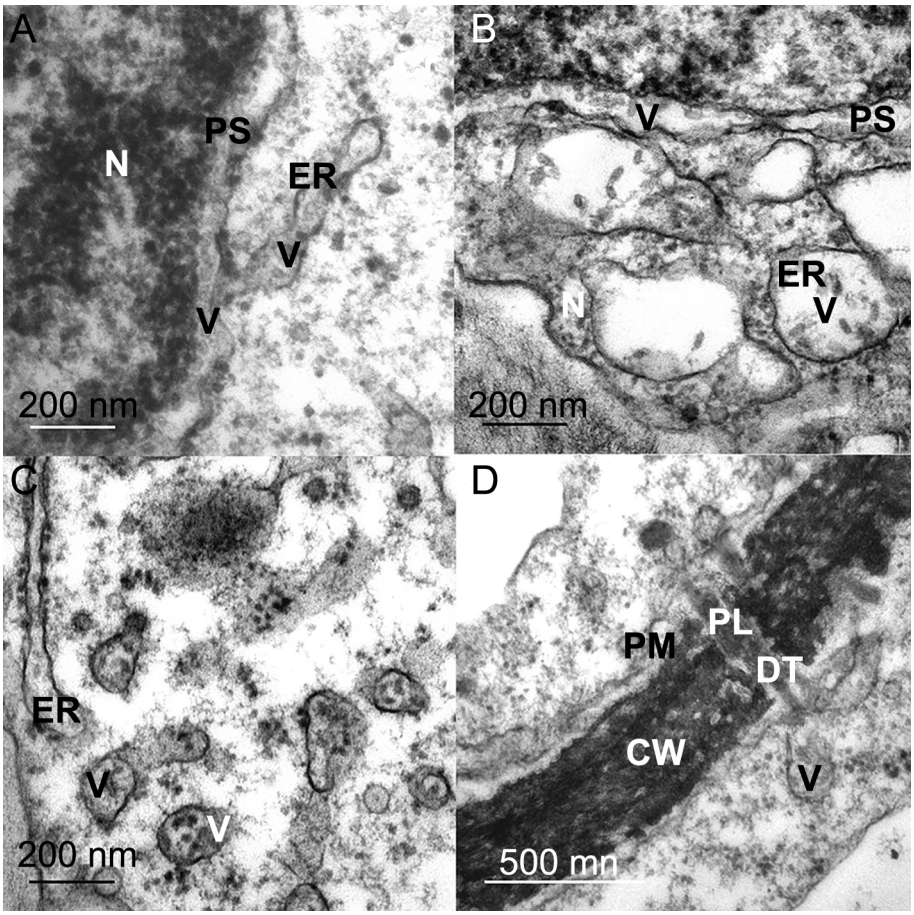

Figure 4 – (A-C) Bacilliform virus-like particles (v) in the lumen of the endoplasmic reticulum (ER), in blotchy areas of fruits from cvs. Giacomo (A and C) and Grazianni (B). In D, elements of ER are continuous, as desmotubule (DT), to the plasmodesma (PL), in a fruit pericarp cell from cv. Grazianni. CW = cell wall; N = nucleus; PS = perinuclear space; PM = plasma membrane.

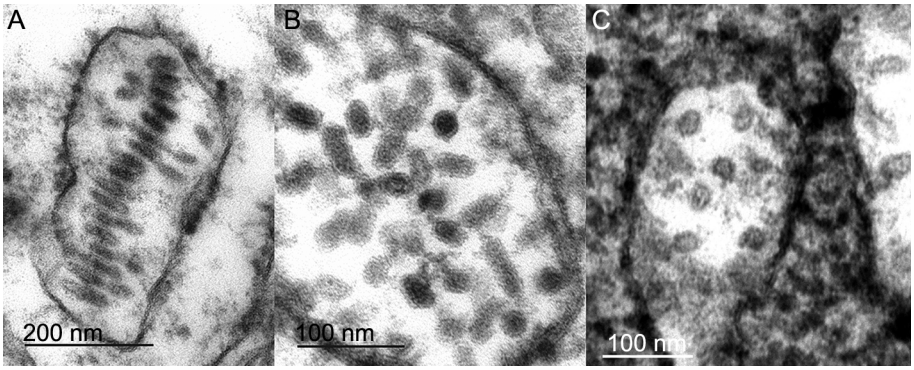

Figure 5 – (A-C) High magnification images of the short, bacilliform particles, which are the presumed virions of ToBFV (Tomato fruit blotch virus), from pericarp cells of blotchy areas of tomato fruits infected by ToFBV, respectively cvs. Giacomo (A and C), Grazianni (B).

discoloration symptoms, revealed a mixed infection by a presumed ilarvirus, tea line pattern virus (TLPV), and the blunervirus TPNRBV (Hao et al., 2018).

Cileviruses are the most studied among kitavirids, with detailed virion morphology and cytopathology information. Virions are bacilliform and enveloped (50-60 nm × ca. 120 nm), accumulating within membrane-bounded cavities of the ER and, occasionally, in the perinuclear space. A characteristic electron dense, vacuolated and polymorphic viroplasm is present in the infected cells (Kitajima et al., 2003; Freitas-Astúa et al., 2018; Ramos-González et al., 2020). Virions

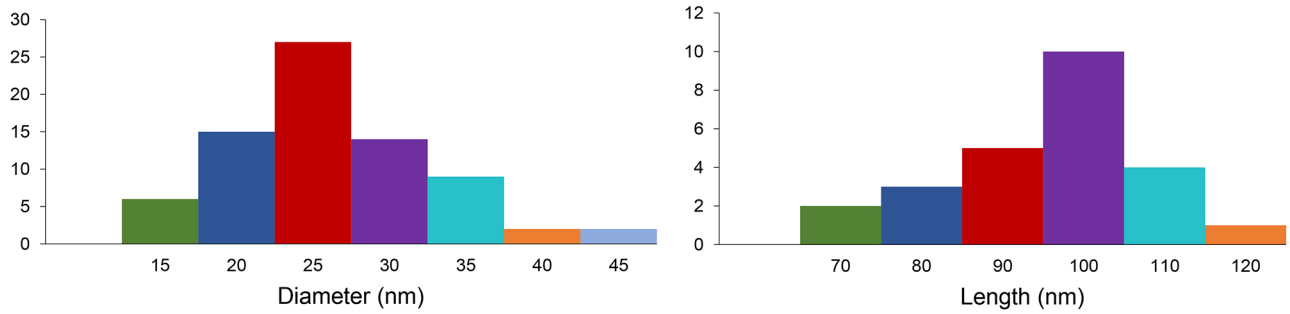

Figure 6 – Histogram of measurements of the size of short, slender, bacilliform particles present in the perinuclear space and the lumen of the endoplasmic reticulum (ER), in pericarp cells from blotchy lesions of tomato fruits, infected by ToFBV (Tomato fruit blotch virus). Particles in tissues from the blotched lesions from two tomato cultivars. Modal diameter is ca. 25 nm, and modal length is ca. 100 nm.

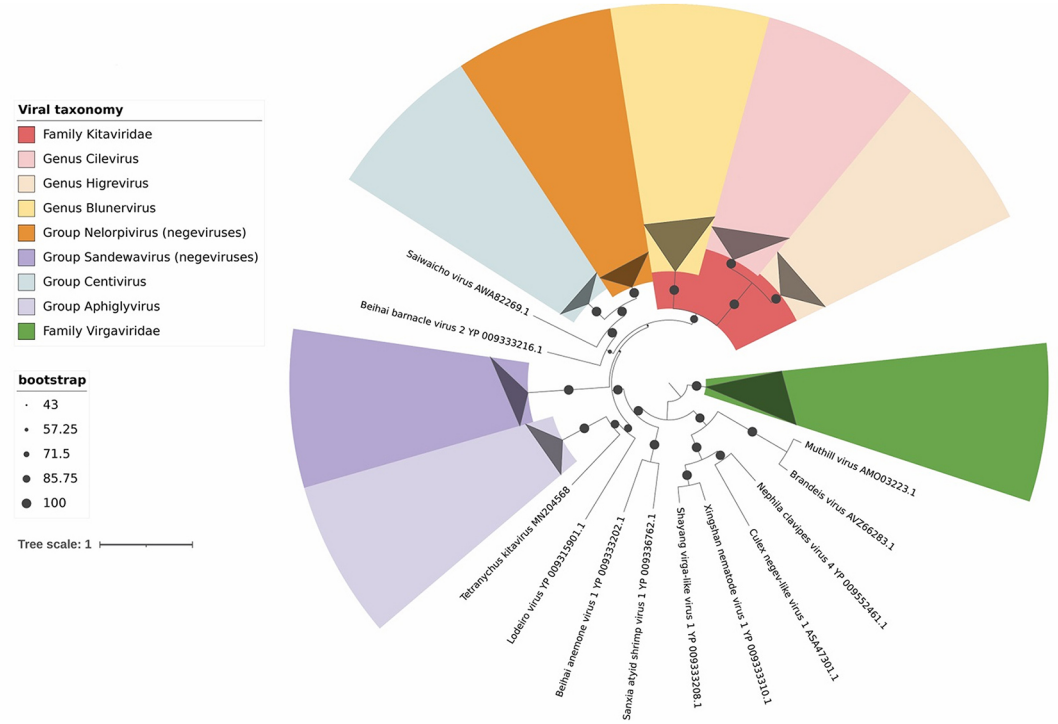

Figure 7 – ML tree inferred using the aa sequences of RdRp from kitavirids, negeviruses, and kita/nege-like viruses. Phylogenetic informative regions of the multiple sequence alignment included 546 residues that were selected using BMGE software and its evolutionary history was inferred based on the model LG + F + I + G4. The scale bar specifies the average number of amino acid substitutions per site. Branch colors indicate bootstrap values according to the graphic legend. Sequences of some members of the family *Virgaviridae* were used as an outgroup.

have also been observed in the viruliferous mite vector (*Brevipalpus yothersi* Baker), between adjacent cells of the caecal epithelium, anterior prosomal gland, and nearby cells, but not within cells (Alberti and Kitajima, 2014). Particles similar to cileviruses, but not viroplasm, were described in leaf cells of the higrevirus hibiscus green spot virus 2 (HGSV 2) (Melzer et al., 2012). TPNRBV is the only blunervirus for which some information regarding the morphology of virions is available. They are non-enveloped, isometric, with ca. 85 nm in diameter, and scattered in the cytoplasm (Hao et al., 2018). This description, coupled with the reported systemic infection by TPNRBV, contrasts sharply with descriptions of other known kitavirids (Melzer et al., 2012; Freitas-Astúa et al., 2018). Additional analyses are needed to clarify this incongruency. Overall, cytopathic effects observed in the pericarp of the blotched area of tomato fruits infected by ToFBV are in line with those reported for kitavirids. Presumed virions are enveloped and accumulate in membrane-bounded cavities (lumen of ER and/or perinuclear space), although significant differences in size are likely. A previous study on tomato

samples, collected in the same greenhouse in 2019 and showing the chlorotic blotch symptoms, demonstrated that the plants were infected with ToFBV and tomato chlorosis virus (ToCV), a crinivirus, using a metagenomic approach (Nakasu et al., 2022). Criniviruses have filamentous and flexuous particles and are restricted to the phloem cells. Such particles were not observed in the phloem of the vascular region of the fruits or other tissue types. As no other known tomato viruses have negev-like virus particles, our results present the first evidence that ToFBV particles are most likely enveloped bacilliform particles.

Phylogenetical studies comparing RdRp sequences of kitavirids indicate they share an evolutionary relationship with several arthropod-infecting viruses, especially mosquito viruses of the negevirus group (Vasilakis et al., 2013; Kallies et al., 2014; Nunes et al., 2017; Quito-Ávila et al., 2020; Ramos-González et al., 2020; Morozov et al., 2020; Chiapello et al., 2021). These negeviruses are still not accepted as formal taxons by ICTV, but they are overall grouped in the clades Nelorpivirus and the Sandewavirus (Nunes et al., 2017). The genome of kitaviruses, negeviruses, and other kita-like viruses also has another orthologous ORF (ORF3 in the RNA3 of ToFBV) encoding a protein showing the motif SP24 (pfam16504) (Kuchibhatla et al., 2014). The motif occupies the central region of the protein, contains several transmembrane domains, and is possibly involved in the virion structure (Solovyev and Morozov, 2017). The analysis of purified virions of the negevirus Castlerea virus revealed that this is probably a membrane protein (O'Brien et al., 2017). On the other hand, a phylogenetic reconstruction using the RNA-dependent RNA polymerase amino acid sequences shows that kitaviruses, nelorpiviruses, and centiviruses are the closest descendant from a common ancestor, also shared with sandewaviruses, aphiglyviruses, and other unclassified arthropods-infecting viruses, all potentially forming the nege-like virus group.

The present report of the cytopathic effects caused by ToFBV seems to add another element supporting the link between negeviruses and kitavirids. Indeed, several ultrastructural studies on negevirus-infected cultured mosquito cells (C6/C36 from *Aedes albopictus* Skuse) report converging results. Infection of the negeviruses, such as Negev, Piura, Loreto, Wallerfield, Ochlerotatus caspius negevirus, Santana, BC2-5, Tanay, results in large expansions of the perinuclear space containing tubular particles ca. 25 nm wide, or of variable lengths (Vasilakis et al., 2013; Auguste et al., 2014; Carapeta et al., 2015; Popov et al., 2019; Zhao et al., 2019). Additionally, spheroidal particles have been found in membrane-bounded cavities in the cytoplasm. Although a few negeviruses have been purified (Nabeshima et al., 2014; O'Brien et al., 2017; Zhao et al., 2019; Colmant et al., 2020), the most detailed morphological description was made by Colmant et al. (2020). Using cryo-electron microscopy imaging, these authors described negevirus virions as "prolate spheroid-like particles, with most particles presenting a plate-like projection suspended from one end of the virion" and having a stratified layer at the surface, indicative of particles enveloped. However, the intracellular localization of such particles is not clear yet. The structures in the perinuclear space are tubular and thinner than the purified particles. On the other hand, some spheroidal particles observed in cytoplasmic vacuoles resemble purified virions. Additional studies using immunogold labeling with specific antibodies against viral structural protein may provide a clear answer. Compared to the present case of ToFBV, gross cell modifications are similar to those reported for negeviruses. However, there are still unclear points. In the case of ToFBV, particles in the perinuclear space and ER seem to represent virions, but the nature of tubular structures observed in the perinuclear cavities of C6/36 cultured mosquito cells infected by several negeviruses is unclear yet. These structures differ from the ellipsoidal particles seen in purified preparations.

Cytopathic effects may be considered a feature of viral biology and directly related to viral genomic traits. Indeed, cell alterations and intracellular localization of virions are similar within a virus genus or even across the family. Thus, similarities in cytopathology between kitavirids and negeviruses seem to favor the proposed phylogenetic relationship, a common ancestry based on hallmark genes, that is, those encoding the RdRP and the SP24-containing protein, and some aspects of their genomic organization.

Conclusion

Ultrastructural studies on pericarp tissues from the blotched area of tomato fruits, associated with infection by the blunervirus tomato fruit blotch virus (ToFBV, family *Kitaviridae*) revealed significant cell alterations, represented by the accumulation of short, slender, bacilliform particles in the perinuclear space and the lumen of the ER. Such alterations are similar to those reported for several negeviruses. Since genetically closer viruses cause similar cell changes in infected cells, the symmetry in cytopathology between kitavirids and negeviruses seems to stress their phylogenetical connection.

Acknowledgments

This research had financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2019/25078-9).

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