

PATRÍCIA OLIVEIRA ANDRADE

**INVOLVEMENT OF TRANSLATIONALLY CONTROLLED TUMOR PROTEIN
IN *Tomato yellow spot virus* INFECTION**

Dissertação apresentada à
Universidade Federal de Viçosa, como
parte das exigências do Programa de
Pós-Graduação em Microbiologia
Agrícola, para obtenção do título de
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(Coorientadora)


Poliane Alfenas Zerbini
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Dedico aos meus pais Nélio e Maria Eliza, e a minha filha Sofia.

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ABSTRACT

ANDRADE, Patrícia Oliveira, M.Sc., Universidade Federal de Viçosa, July, 2017. **Involvement of Translationally controlled tumor protein in *Tomato yellow spot virus* infection.** Advisor: Poliane Alfenas Zerbini. Co-advisor: Fernanda Prieto Bruckner.

Viruses are the most abundant and genetically diverse life forms known in our biosphere. To successfully infect hosts, viruses manipulate host cellular components, recruiting host factors necessary for replication, infection, and transmission. In addition, viruses need to supplant various host defense strategies, leading to a complex coevolution mechanism involving virus-host interactions. Different viruses can interact with host cell components similarly or even antagonistic. The presence of the protein translationally controlled tumor protein (TCTP) has been shown to be necessary for the establishment of an efficient potyvirus infection. TCTP is a multifunctional protein found in almost all eukaryotes and is involved in cell growth; ions homeostasis; DNA damage repair and anti-apoptotic activity. Despite numerous studies with TCTP, the involvement of this protein in viral infection is not yet fully understood. Due to its functional diversity, it is possible to imagine that TCTP may be a host factor involved in infections caused by viruses of different groups. Thus, in this work, the effect of TCTP on begomovirus infection was evaluated. *Nicotiana benthamiana* plants silenced for TCTP by VIGS experiments were used to study the effect of TCTP expression on infection by the begomovirus *Tomato yellow spot virus* (ToYSV). TCTP silencing led to higher accumulation of the virus, suggesting that TCTP is a host factor involved in viral infection defense process. Furthermore, TCTP mRNA is highly structured in mammals and is related to the induction of defense response to different viruses. Because it is a highly structured mRNA, it is reasonable to assume that TCTP mRNA may be the target of the plant post transcription gene silencing mechanism, leading to the production of small interfering RNAs (siRNAs) by the cleavage of Dicer proteins and the siRNAs generated might regulate the expression of host endogenous genes involved in virus infection. In silico analysis was performed to evaluate the possible siRNAs generated from the silencing of TCTP mRNA, and the respective targets of this

siRNAs. Genes involved in viral infection, such as those encoding protein kinases, proteins involved in the ubiquitination pathway, transcription and translation factors, and calcium binding proteins were some of the genes identified as possible targets of these predicted siRNAs.

RESUMO

ANDRADE, Patrícia Oliveira, M.Sc., Universidade Federal de Viçosa, julho de 2017. **Envolvimento da proteína Translationally controlled tumor protein na infecção pelo *Tomato yellow spot virus***. Orientadora: Poliane Alfenas Zerbini. Coorientadora: Fernanda Prieto Bruckner.

Os vírus são as formas de vida mais abundantes e geneticamente diversas conhecidas em nossa biosfera. Para infectar hospedeiros com sucesso, os vírus manipulam componentes celulares do hospedeiro, recrutando fatores do hospedeiro necessários para replicação, infecção e transmissão. Além disso, os vírus precisam suplantar diversas estratégias de defesa do hospedeiro levando a um complexo mecanismo de coevolução que envolve diversas interações. Diferentes vírus podem interagir com componentes celulares do hospedeiro de forma semelhante. Foi demonstrado que a presença da proteína translationally controlled tumor protein (TCTP) é necessária para o estabelecimento de uma infecção eficiente por potyvírus. TCTP é uma proteína multifuncional encontrada em quase todos os eucariotos envolvida no crescimento celular, homeostase de íons, reparo de danos no DNA e possui atividade anti-apoptótica. Apesar de inúmeros estudos com TCTP, o envolvimento desta proteína na infecção viral ainda não é totalmente compreendido. Devido a sua diversidade funcional, é possível imaginar que TCTP possa ser um fator do hospedeiro envolvido em infecções causadas por vírus de diferentes grupos. Desta forma, neste trabalho, foi avaliado o efeito de TCTP na infecção por begomovírus. Para isso, plantas de *Nicotiana benthamiana* com TCTP silenciada por VIGS foram utilizadas para estudar o efeito da TCTP na infecção pelo begomovírus *Tomato yellow spot virus* (ToYSV). O silenciamento de TCTP levou a um maior acúmulo de vírus, sugerindo que TCTP é um fator do hospedeiro envolvido no processo de defesa à infecção viral. Além disso, o mRNA de TCTP é altamente estruturado em mamíferos e está relacionado com a indução de resposta a infecções por diferentes vírus. Por ser um mRNA altamente estruturado é razoável supor que o mRNA de TCTP pode ser alvo do processo de silenciamento pós transcricional da planta levando à produção de pequenos RNAs de interferência (siRNAs) através da clivagem por proteínas Dicer e os siRNAs gerados podem regular a

expressão de genes endógenos do hospedeiro. Desta forma, foi realizada uma análise *in silico* para avaliar os possíveis siRNAs gerados a partir do silenciamento do mRNA de TCTP e os possíveis alvos desses siRNAs. Genes que podem estar envolvidos em infecção viral, como aqueles que codificam proteína kinases, proteínas envolvidas na via de ubiquitinação, fatores de transcrição e tradução e proteínas de ligação ao cálcio foram alguns dos genes identificados como possíveis alvos destes siRNAs.

1. Introduction

Viruses are the most abundant and genetically diverse life forms known in our biosphere. All of them are obligate parasites that do not have the molecular machinery necessary to replicate their genomes. So, in order to perpetuate, viruses need to interact efficiently with the host cellular machinery. To do so, they are able to hijack cellular pathways and manipulate cellular components by inducing the expression of host factors required for replication, propagation and movement, or repressing the expression of host factors involved in defense responses against viral infection (Boevink and Oparka, 2005; Wang, 2015; Whitham et al., 2003).

Plant viruses have small genomes, encoding 4-10 proteins, but the infection processes are very complex, involving viral genome replication, cell to cell and systemic movement and transmission to another host. Plant viruses use a number of strategies to modify host cells to establish a successful infection. These modifications include suppression of post-transcriptional gene silencing, interference with regulation of the cell cycle and suppressing innate host defenses, thus creating conditions that favor systemic infections (Whitham et al., 2003; Whitham and Wang, 2004). In contrast, plants can develop mechanisms to protect themselves from viral infections such as post transcriptional gene silencing (Baulcombe, 2004), and general resistance mechanisms such as systemic acquired resistance. These virus-host interactions are typically accompanied by dramatic changes in host gene expression (Whitham et al., 2003).

Because of the essential role of host factors in viral infection, virology researches have been concentrating in their molecular identification. With the improvement of genomic, proteomic and bioinformatics tools, progress in the

identification of host factors required by plant viruses have been made. These studies accelerated the understanding of virus-host interactions and essential biological processes in plants and can be a tool for discovery of new antiviral strategies (Reviewed by Wang, 2015).

Whitham et al. (2003), analyzed changes in gene expression in *Arabidopsis thaliana* plants associated with infection of five positive-strand RNA viruses: The tobamoviruses, *Turnip vein clearing virus* (TVCV) and *Oilseed rape virus* (ORMV), the potexvirus *Potato virus X* (PVX), the cucumovirus *Cucumber mosaic virus* (CMV), and the potyvirus *Turnip mosaic virus* (TuMV). They identified 114 genes with induced expression in response to infections by these viruses. Functional classification of these genes showed that 35 of 114 genes were associated with plant stress or defense responses, demonstrating that plants respond to viruses in a common, coordinated way and that these responses may represent mechanisms that viruses exploit in order to enhance infection.

Alfenas-Zerbini et al. (2009) studied changes in the profile of gene expression during the early stages of infection by the potyvirus *Pepper yellow mosaic virus* (PepYMV) in tomato (*Solanum lycopersicum*). A subtractive library was produced using tomato leaves inoculated with PepYMV 72 hours post-inoculation (hpi). A total of 881 genes differentially expressed were identified and 53 of those genes were confirmed by macroarray analyses or by qRT-PCR. Genes were identified as up- or downregulated and had identity to proteases, transcription factors, heat shock response proteins, proteins involved in signal transduction and in the ubiquitination pathway. The translationally controlled tumor protein (TCTP) was identified as one of the up-regulated genes in this study.

TCTP was first discovered in the 1980s by three different groups who worked independently and had an interest in proteins regulated at the translational level of gene expression. Thomas et al.(1981) referred to the protein as Q23, Yenofsky et al. (1982) as P21 and Böhm et al. (1989) as P23. During this time, the cDNA sequence of this protein was obtained from mouse and human cells and the name “translationally controlled tumour protein” was proposed because it was cloned from a human mammary tumour cell and was under translational control (reviewed by Bommer, 2012; Gachet et al., 1999). Despite its name, new discoveries over the past years have shown that TCTP can also be regulated at the transcriptional level and that it is not exclusively a tumor protein, being found in most mammalian tissues (Bommer et al., 2002). In fact, TCTP is a protein found in almost all eukaryotes and has a high degree of conservation among these phyla, which indicates the importance of this protein for all eukaryotes (Bommer, 2012; Gutiérrez-Galeano et al., 2014).

Numerous studies in different organisms revealed the participation of TCTP on different cellular process. One of the first properties described about TCTP was its calcium binding ability (Haghighat and Ruben, 1992). The protein was found to have microtubule binding properties, associating with microtubules and it was demonstrated that the overexpression of the protein affects cell growth, microtubular network and cell morphology (Gachet et al., 1999; Bommer, 2012). TCTP can bind to the mitotic spindle acting as a general mitotic regulator, playing an important role in the early development of plants and animals. It is also involved in cell growth, ion homeostasis, DNA damage repair and has anti-apoptotic activity (reviewed by Bommer, 2012). Studies on human and *Schistosoma mansoni*

TCTP revealed a chaperone-like activity as TCTP binds and protects proteins against denaturation in thermal shock conditions (Gnanasekar et al., 2009).

The regulation of TCTP occurs under a range of extracellular signals and cellular conditions. TCTP is induced by cell growth signals and stress conditions, oxidative stress, stress induced by heavy metal and toxins, and pro-apoptotic signals (Bommer and Thiele, 2004). TCTP mRNA translation may be regulated by the double-stranded RNA-dependent protein kinase PKR, an eIF2 α protein kinase involved in antiviral defense mechanisms in cells. Humans and mouse TCTP mRNA, which is highly structured with double stranded regions, are able to bind to PKR resulting in the activation of the protein kinase and in consequence inhibiting its own translation (Bommer et al, 2002).

Using TCTP from *Schizosaccharomyces pombe*, Thaw et al. (2001) determined that TCTP is structurally very similar to the human protein MSS4/SDD4, a protein that binds to Rab GTPases (subfamily of Ras GTPases). These findings led the authors to propose that TCTP acts as a guanine nucleotide exchange factor (GEF) that modulates GTPase activity. GTPases regulates innumerable cellular processes, which is an indication to why TCTP is involved in so many different cellular functions (Berkowitz et al., 2008). In *Arabidopsis thaliana*, AtTCTP is able to bind to four GTPases (AtRSBA4a, AtRABA4b, AtRABFF1 and AtRABF2b) and interspecies complementation experiments showed AtTCTP also binding to *Drosophila* Rheb GTPases (Brioude et al., 2010). Knowing that *Drosophila* TCTP controls the target of rapamycin (TOR) activity by activating Rheb GTPases, these experiments suggests that plant TCTP could also be a part of the TOR pathway in plants (Brioude et al., 2010).

Most of the studies conducted with TCTP were done on animals (mouse, human, *Drosophila*) and only a few were conducted on TCTP from plants, using

mostly *A. thaliana* as a model (Gutierrez-Galeano et al., 2014). Characterization of TCTP from *A. thaliana* showed that TCTP from plants and TCTP from non-plants diverged early but still maintained some similar functions (Berkowitz et al., 2008). Specifically for plants, TCTP have been associated to a number of other functions such as root hair development, lateral root formation, auxin homeostasis, gametophyte maturation (Berkowitz et al., 2008) and drought tolerance (Kim et al., 2012). Unlike non-plants organisms that usually have only one gene for TCTP, plants can harbor two or three TCTP genes, indicating a specialization of TCTP functions within plants (Gutierrez-Galeano et al., 2014). For example, *A. thaliana* has two TCTP genes, AtTCTP1 and AtTCTP2. Studies indicated that the two isoforms of the protein perform different functions in the cell as AtTCTP1 is not able to compensate the loss of AtTCTP2. A new function for AtTCTP2 was described as a protein capable of plant regeneration (Toscano-Morales et al., 2015).

There are few studies demonstrating how TCTP influences virus infection. *White spot syndrome virus* (WSSV), a double stranded DNA virus (dsDNA), causes a serious disease in *Litopenaeus vannamei*, the main species of shrimp cultivated in the world. When TCTP was silenced in shrimps, qPCR analysis showed that WSSV load increased significantly. This is an indication of TCTP involvement in WSSV infection (Wu et al., 2013).

Bruckner et al., (2017) analyzed the effect of TCTP silencing on infection of PepYMV in tomato and *N. benthamiana* plants. Based on transient (virus induced gene silencing, VIGS) silencing, *N. benthamiana* leaf discs were collected 72 hours post virus infection to analyze PepYMV accumulation. Results demonstrated that transient TCTP silencing decreases PepYMV accumulation in *N. benthamiana* compared to non-silenced plants during the early stages of infection.

To see how TCTP affects systemic infection, five tomato transgenic lines silenced for TCTP (TCTP5, TCTP6, TCTP11, TCTP22 and TCTP26) were made. From the five transgenic lines, they obtained two strongly silenced plants, TCTP11. All five clonal progenies and wild type tomato plants were sap inoculated with PepYMV and leaves were collected 72 hours post inoculation (hpi) and 14 days post inoculation (dpi). At both time points, results showed PepYMV accumulation in much lower levels in TCTP11 and TCTP26 in comparison to wild type plants and the virus was not able to systemically infect these plants. These findings show the importance of TCTP to the establishment of potyvirus infection in *tomato* and *N. benthamiana*.

To see if TCTP involvement is specific for potyvirus infection or if TCTP affects plant virus infection in a general way, we analyzed the effect of TCTP silencing by VIGS on the infection of the geminivirus *Tomato yellow spot virus* (ToYSV) in *N. benthamiana*.

Geminivirus causes severe economic damage to agricultural production worldwide (Rojas et al., 2005). This group of viruses have circular ssDNA genome ranging from 2.6 to 5.2 kb (reviewed by Inoue-Nagata et al., 2016). In addition, they have a conserved region of nine nucleotides (TAATATTAC), that corresponds to the start site of rolling circle replication (Nawaz-ul-rehman e fauquet, 2009). Replication takes place within the nucleus with the conversion of its ssDNA to dsDNA. The dsDNA is assembled into nucleosomes and is transcribed by host RNA polymerase II, producing replication initiator protein (Rep) which is responsible for initiating rolling-circle replication (Hanley-Bowdoin et al., 2013; Gutierrez et al., 2004).

Based on the genome structure, phylogenetic relationships, vector type and host range, nine genus are recognized: *Begomovirus*, *Mastrevirus*, *Curtovirus*,

Topocuvirus, *Becurtovirus*, *Turncurtovirus*, *Eragrovirus*, *Capulavirus* and *Grablovirus* (Zerbini et al., 2017).

Among the genera, begomoviruses have the highest number of species. Their genomes consists of one or two segments of circular ssDNA, infects dicot plants and are transmitted by the whitefly *Bemisia tabaci* in a persistent circulatory manner (Brown et al., 2015).

The phylogeny of begomovirus is highly structured around its geographical distribution (Rocha et al., 2013). Thus, this genus is divided into two groups: those of the New World (mostly bipartite genomes, with one recently described monopartite genome virus) and those of the Old World (both genome types) (Brown et al., 2015). Begomovirus with bipartite genomes consists of two components with approximately 2.6 kb (DNA-A and DNA-B), and monopartite begomovirus consists of one component (Briddon et al., 2010). DNA-A (and the monopartite genome) encodes the coat protein (CP) that encapsidates the ssDNA. The AV2 protein from DNA-A has been implicated in virus movement and new world begomoviruses lacks this protein (Brown et al., 2015). DNA-A encodes the replication-associated protein (Rep), a transcriptional activator protein (TrAP), a replication enhancer protein (REn) and the AC4 protein. DNA-B, encodes two proteins with movement functions in the host (Hanley-Bowdoin et al., 1999).

Tomato yellow spot virus (ToYSV) is a begomovirus that infects tomato (Calegario et al., 2007). Although it infects tomato, its phylogenetic characteristics are more similar to *Sida mottle virus* (SiMoV), a begomovirus that infects sida, a non-cultivated plant (Andrade et al., 2006). ToYSV induces severe symptoms on its host and can be sap inoculated in several *Nicotiana* species, including *N .benthamiana*. The severity of the symptoms may be due to the tissue tropism of

this virus, since in *N. benthamiana*, the virus is capable of infecting not only the tissues of the phloem, but also the mesophyll cells (Alves-Júnior et al., 2009).

Post-transcriptional gene silencing (PTGS) is a mechanism of RNA-induced gene silencing in plants that regulates gene expression by degrading sequence-specific mRNAs. PTGS is involved in defense against virus infection, and gene regulation. When double stranded RNA (dsRNA) is detected in the cell, whether it is a single-stranded hairpin RNA (hpRNA) or a dsRNA from the replication of viruses or even self-complemented RNAs, it may be a target of Dicer proteins which cleaves it into smaller dsRNAs with approximately 21-24 nucleotides, known as small interfering RNAs (siRNAs) or microRNAs (miRNAs). An endonuclease-containing complex, called RNA induced silencing complex (RISC), incorporates the anti-sense strand of the siRNAs. The RISC complex scans for mRNAs in the cell that have a complementary sequence to the small RNAs incorporated into the complex, promoting their degradation. The silencing signal can move to the other cells causing systemic silencing in the plant (Baulcombe, 2004; Voinnet, 2009; Waterhouse and Helliwell, 2003).

The sequence of tomato TCTP mRNA was obtained in our lab and computer prediction showed a highly secondary structured mRNA. With these result, we hypothesize that the double stranded and hairpins regions of TCTP mRNA may be a target of Dicer proteins and the siRNAs produced by these cleavage might target endogenous mRNAs in which their correspondent proteins may have a role in virus infection.

In this work, we studied if TCTP can be a host factor involved in the infection of the begomovirus ToYSV. We also investigated if TCTP mRNA can be a target of Dicer proteins and if the siRNAs produced have complementary

sequence to endogenous genes in which their correspondent proteins are involved in virus infection.

The results showed an increase in viral accumulation on TCTP silenced *N. benthamiana* plants, suggesting that TCTP is involved in ToYSV infection. This result suggests that TCTP is involved in RNA and DNA virus infection, but in different ways, since transient TCTP silencing in *N. benthamiana* causes a decrease in PepYMV accumulation (Bruckener et al., 2107). TCTP may be involved in general plant processes that support the infection of unrelated plant viruses.

Through in silico analyses, putative siRNAs possibly generated by the cleavage of TCTP mRNA by dicer proteins, had sequence homology to endogenous genes which encodes proteins that are involved in virus infection. Among these proteins we can highlight: protein kinases, proteins involved in ubiquitination pathway and transcriptional and translational factors. This information is a start point of a possible understanding of TCTPs involvement in virus infection.

2. Material and Methods

2.1. Viral isolate

N. benthamiana plants were biobalistically inoculated with ToYSV infectious clone (DNA-A and DNA-B) (Andrade et al., 2006). These plants were used as a source of inoculum for sap inoculation of *N. benthamiana* with 0.2 M potassium phosphate buffer pH 7.2 and 0.1% sodium bisulfate mixed with an abrasive (carborundum). Infected plants were maintained in a greenhouse.

2.2. Virus Induces Gene Silencing (VIGS)

Plants of *N. benthamiana* three weeks after germination were transplanted individually into small plastic cups containing substrate, and transferred to a growth chamber with controlled temperature of 22 ° C and photoperiod of 14h and kept in these conditions for at least five days before agroinfiltration.

Agrobacterium tumefaciens strain C58C1 carrying four viral vectors (*Tobacco rattle virus*, an RNA virus with two components, TRV1 and TRV2) independently, containing cDNA fragments of approximately 400 nucleotides of TCTP (TRV2-TCTP), phytoene desaturase (TRV2-PDS), green fluorescent protein (TRV2-GFP) and with the TRV1 component (Bruckner et al., 2107) were taken from the inventory of the Laboratory of Industrial Microbiology of the Department of Microbiology of Universidade Federal de Viçosa (UFV) and used in the VIGS experiments. The C58C1 strain transformed with each of the viral constructs mentioned above were stratified to petri dishes with LB medium containing the antibiotics kanamycin, gentamycin and ampicillin. After two to three days, an isolated colony present in the petri dish was cultured overnight in a 50 ml tube with 20 to 30 ml of liquid LB medium containing the same antibiotics. The tubes were centrifuged at 4,000 rpm for 10 minutes and resuspended in agroinfiltration buffer

(10 mM MES, 10 mM MgCl₂) and 200 μM acetosyringone), adjusting the optical density (OD₆₀₀) to 1.2. For each volume of agrobacterium suspension transformed with the constructs TRV2-TCTP, TRV2-GFP and TRV2-PDS, an equal volume of an agrobacterium suspension transformed with TRV1 was added. The mixtures were agroinfiltrated in the abaxial face of *N. benthamiana* leaves, using syringes without needles. Eighteen plants were agroinfiltrated with TRV1 + TRV2-TCTP, another eighteen plants with TRV1 + TRV2-GFP and three plants were agroinfiltrated with TRV1 + TRV2-PDS.

With the appearance of the photo-oxidation phenotype of PDS silencing after 14 days, *N. benthamiana* plants were sap inoculated with ToYSV. From the eighteen plants silenced for TCTP (TRV1+TRV2-TCTP) and GFP (TRV1+ TRV2-GFP), nine were inoculated with virus (TRV-TCTP ToY; TRV-GFP ToY) and the other nine were mock inoculated (TRV-TCTP Mock; TRV-GFP Mock). To evaluate the effect of silencing on ToYSV infection, leaves of all plants were collected 96 hours post virus inoculation (hpvi). For each treatment, TRV-TCTP ToY; TRV-GFP ToY; TRV-TCTP Mock and TRV-GFP Mock, three plants were randomly selected to be grouped and their leaves collected to form a single replicate. Thus, for each treatment there were three replicates. All collected material was grinded in liquid nitrogen, placed in 1.5 ml microcentrifuge tube and stored at -80 ° C.

2.3. RNA extraction and cDNA synthesis

Total RNA extraction from the grinded leaves was performed using the Purelink Plant RNA reagent (Thermo Fisher Scientific), according to the manufacturer's instructions. RNA quality and quantity were measured with a NanoDrop (Thermo Fisher Scientific). One microgram of total RNA was treated with DNase I (Promega) using twice as much enzyme as indicated by the manufacturer (2 units of enzyme per microgram of RNA). The treated RNA was used for cDNA synthesis using SuperScript III reverse transcriptase (Invitrogen), according to the manufacturer's instructions, and oligodT as a primer.

2.4. DNA extraction and quantitative PCR

DNA extraction from the grinded leaf tissues were done as described by Doyle and Doyle, (1987) DNA quality and quantity were measured with NanoDrop (Thermo Fisher Scientific) and the DNA amounts of all samples were diluted and standardized to 7 ng/μl. ToYSV viral load were measured by quantitative PCR (qPCR). qPCR reactions were performed in triplicate with CFX96 Real-Time PCR Detection System (Bio-Rad), using Fast SYBR Green Master Mix (Thermo Fisher Scientific) following the manufacturer's instructions. The primers qToY3F and qToY3R for ToYSV DNA-A were used to quantify viral load. Conditions for qPCR amplification were as follows: 20 seconds at 95°C for initial denaturation; 40 cycles of denaturation at 95°C for 3 sec and annealing/extension at 60°C for 30 sec. A standard curve was obtained by regression of Ct values. Viral load was determined by interpolation of the Ct values of each tested sample within the standard curve (Rutledge, 2003).

TCTP expression was quantified by qRT-PCR, using the comparative method of Ct ($\Delta\Delta\text{CT}$), described by Livak and Schmittgen, (2001). The primers qTCTPNb and H2b2 from *N. benthamiana* were used (Bruckner et al. 2017). H2b2 is an oligonucleotide specific for the gene encoding histone 2b and it was used to normalize the reaction.

2.5. Bioinformatics analysis of putative TCTP siRNAs and its protein targets

The secondary structure of tomato TCTP was predicted using RNA Folding Form (version 2.3 energies) software (mFold RNA server) (Zuker, 2003). To map for putative small RNAs from TCTP mRNA, we used the data from a library of miRNAs of tomato infected with the fungus *Alternaria* and healthy tomato plants (<http://www.ebi.ac.uk/ena/data/view/PRJNA305725>). To look for endogenous genes that had complementary sequence to the putative siRNAs of TCTP mRNA, we searched in psRNATarget (Dai and Zhao, 2011). To find the correspondent proteins of these genes, we searched in Sol Genomics Network, in the tomato genome database (Fernandez-Pozo et al., 2015).

3. Results

3.1. TCTP silencing leads to higher viral accumulation in the early stages of infection by ToYSV.

To see TCTP silencing effect on ToYSV infection, *N. benthamiana* plants had TCTP and PDS expression silenced by VIGS (TRV2-TCTP and TRV2-PDS). Since the GFP gene is not present in plants, the construct TRV-GFP was used as negative control to eliminate the effect of the presence of both *Agrobacterium tumefaciens* and TRV viral vector. TRV2-PDS was used as positive silencing control because of its photo-oxidation phenotype resulting from the silencing of PDS gene in plants. After 15 days, silencing of *N. benthamiana* was well established and plants were sap inoculated with ToYSV.

To evaluate the effect of TCTP silencing on the initial stages of ToYSV infection, relative expression of TCTP mRNA was analyzed by qRT-PCR. Plants agroinfiltrated with TRV-TCTP demonstrated a significant reduction in the expression of TCTP (TRV-TCTP Mock and TRV-TCTP ToY) in comparison to TRV-GFP (TRV-GFP Mock and TRV-GFP ToY), showing that TCTP silencing was successful (Figure 1). Differently from what happens in potyvirus infection (where viral infection increases the expression of TCTP) (Bruckner et al., 2017), TCTP expression was not induced during the firsts steps of the ToYSV infection (Figure 1).

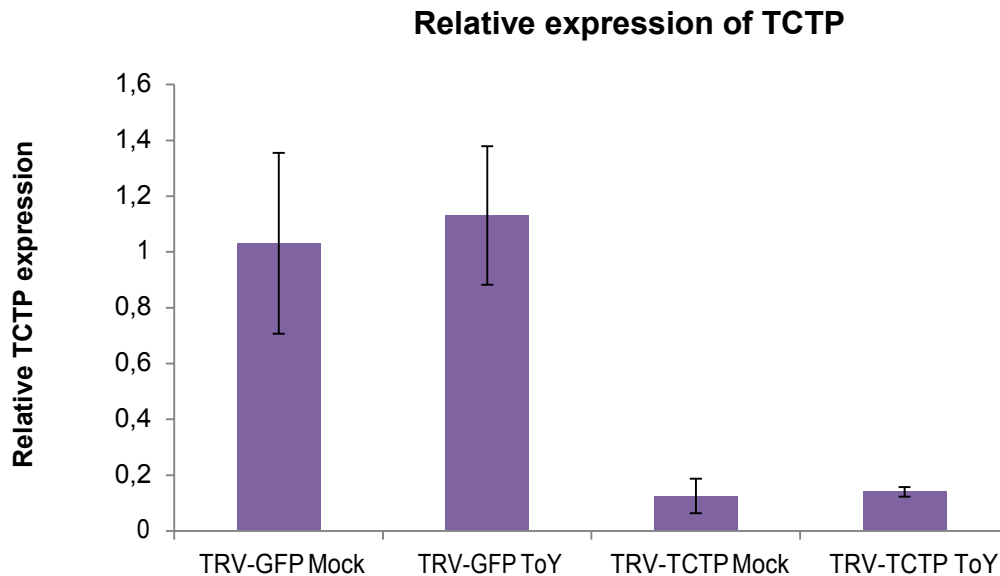


Figure 1: TCTP expression in *N. benthamiana* plants of VIGS assay. TRV-GFP Mock: mean of TCTP expression of three *N. benthamiana* replicates; TRV-GFP ToY: mean of TCTP expression of three *N. benthamiana* replicates inoculated with ToYSV; TRV-TCTP Mock: mean of TCTP expression of three *N. benthamiana* replicates; TRV-TCTP ToY: mean TCTP expression of three *N. benthamiana* replicates inoculated with ToYSV. Error bars correspond to the standard deviation.

The viral load in TCTP silenced and non-silenced plants were determined by absolute quantification. Viral load in TRV-GFP plants inoculated with ToYSV were 175.208,785 copies of viral genome in 14 ng of DNA extraction. In TRV-TCTP plants infected with ToYSV the viral load were 1.379.248, 32 copies of the viral genome (Figure 2). As expected, no copies of viral genome were detected on mock inoculated plants. Bruckner et al. (2017) demonstrated that PepYMV accumulation decreases in plants silenced for TCTP, but in contrast, our results showed that ToYSV accumulated in much higher levels in plants silenced for TCTP (TRV-TCTP) compared to control plants (TRV-GFP).

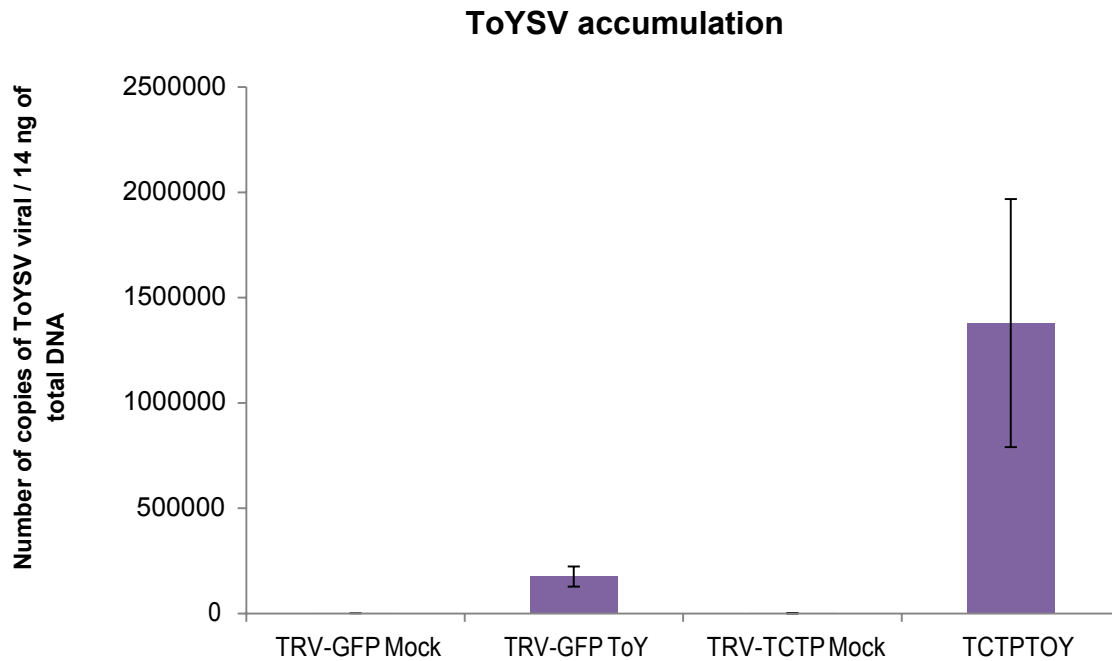


Figure 2: Silencing effect of viral accumulation on ToYSV inoculated leaves 96 hpvi. TRV-GFP mock: mean viral load of three *N. benthamiana* replicates; TRV-GFP ToY, mean viral load of three *N. benthamiana* replicates inoculated with ToYSV; TRV-TCTP Mock, mean viral load of three *N. benthamiana* replicates; TRV-TCTP ToY; mean viral load of three *N. benthamiana* replicates inoculated with ToYSV. Error bars correspond to the standard deviation.

3.2. Analysis of tomato genes that are target of putative siRNAs generated from tomato TCTP mRNA

The secondary structure of tomato TCTP mRNA was predicted in silico (Figure 3) and presented several regions of dsRNA. We hypothesized that these dsRNA regions might be processed by the post-transcriptional gene silencing machinery generating small interfering RNAs (siRNAs). To test this hypothesis, we searched data from a library of miRNA of tomato infected by the fungus *Alternaria* and healthy tomato plants (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75922>) to map putative siRNAs formed from TCTP mRNA. We found two hotspots in the TCTP mRNA structure that might generate siRNAs (Figure 3) and

searched for endogenous genes in the tomato genome that had sequence homology to these siRNAs. We found 34 genes that had sequence homology to the predicted siRNAs generated from TCTP mRNA. Among these genes, it was observed genes encoding protein kinases, calcium binding proteins, transcription and translation factors, RNA binding proteins, genes involved in autophagy and plant defense, among others (Table 1).

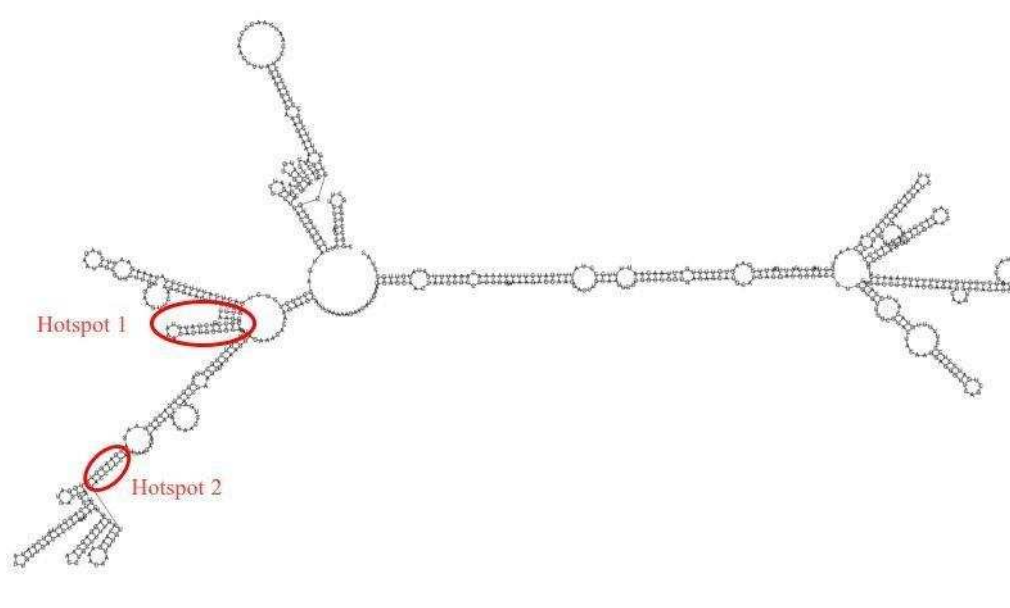


Figure 3: Secondary structure of tomato TCTP mRNA, Secondary structures predicted for TCTP by RNA Folding Form (version 2.3 energies) software (mFold RNA server). The hotspots for putative siRNAs are circled in red.

Table 1: Genes that have sequence homology to the predicted siRNAs located in the two hotspot of tomato TCTP mRNA. Putative siRNAs mapped from a library of miRNAs of tomato infected by *Alternaria* and mock inoculated tomato plants. The access number and the description of proteins corresponds to the information provided by Sol Genomics Network from a tomato genome database

Accession number (Sol Genomics Network)	Protein description	Function
Solyc11g006300.1.1	3-oxo-5-alpha-steroid 4-dehydrogenase family protein	Integral membrane protein
Solyc07g043080.1.1	Agamous-like MADS-box protein AGL80	Probable transcription factor
Solyc00g011880.2.1	AIG2-like protein A	Putative gamma-glutamylcyclotransferase
Solyc01g098430.2.1	Autophagy-related protein	Related to process of autophagy in plants
Solyc04g016400.2.1; Solyc07g065130.2.1	Calcium binding protein	Proteins that participate in calcium cell signaling pathways by binding to Ca ²⁺
Solyc01g097350.2.1	Calcium-binding EF hand family protein	Proteins that participate in calcium cell signaling pathways by binding to Ca ²⁺
Solyc10g008240.2.1	Cc-NBS-LRR	Recognize specific pathogen-derived products and initiate a resistance response
Solyc03g120600.2.1	Cell number regulator 10	Response to oxidative stress
Solyc08g076320.2.1	Cellulose synthase-like protein	beta-glycan synthase that polymerize the backbones of hemicellulose of plant cell wall
Solyc09g013130.2.1	Defective in exine formation	Required for exine pattern formation during pollen development
Solyc12g035870.1.1	DNA-directed RNA polymerase subunit beta	Transcription of DNA into RNA
Solyc10g074700.1.1	ethanolamine-phosphate cytidyltransferase	Biosynthesis of the phospholipid phosphatidylethanolamine
Solyc01g101020.2.1	FACT complex subunit SPT16	Component of the FACT complex, a general chromatin factor that acts to reorganize nucleosomes
Solyc07g053000.1.1	Fasciclin-like arabinogalactan protein	Putative cell adhesion molecules
Solyc03g096270.1.1	gag-pol polyprotein	Transposon Ty1. Integrase protein

Solyc12g011300.1.1	Glutathione S-transferase	Catalyzes the conjugation of electrophilic substrates to glutathione (GSH). Peroxidase and isomerase activities
Solyc08g066810.2.1	Glycoside hydrolase family 5	Hydrolyze the glycosidic bond between two or more carbohydrates
Solyc12g008390.1.1	Golgi candidate	Golgi organization, retrograde transport, vesicle recycling within Golgi
Solyc01g086700.2.1	Phosphoribosylanthranilate transferase	RNA-binding proteins. Mediate gene expression in organelles and nucleus. Facilitates processing, splicing, editing, stability and translation of RNAs
Solyc10g049360.1.1	Cyclin-dependent protein kinase regulator-like protein	Protein kinases. Control cell division and modulate transcription
Solyc10g079170.1.1; Solyc04g015980.2.1; Solyc04g014650.2.1; Solyc08g075620.1.1; Solyc07g005010.2.1; Solyc09g091990.2.1	Receptor-like kinase protein	Protein phosphorylation. Involved in development, growth, hormone perception and response to pathogens
Solyc04g082670.2.1	Ribophorin	Transmembrane glycoprotein located in the membrane of the rough endoplasmic reticulum
Solyc02g078460.2.1	Ring finger protein 141	Involved in the early steps of the plant defense signaling pathway. Involved in protein ubiquitination
Solyc02g078550.2.1	RNA polymerase II C-terminal domain phosphatase-like 1	Multiple roles in RNA processing
Solyc06g065520.2.1	T-complex protein beta subunit	Molecular chaperone. Assists the folding of proteins upon ATP hydrolysis.
Solyc03g058350.2.1	Translation initiation factor	Protein that bind to the small subunit of ribosome during the initiation of translation

We also performed the same analysis for the predicted TCTP siRNAs with the library of miRNAs of tomato infected with *Alternaria* and healthy tomato plants respectively. The results showed a diverse set of genes. Among the genes that had sequence homology to the predicted siRNAs generated from TCTP mRNA, there were genes that encoded protein kinases, proteins involved in ubiquitination pathway, transcriptional and translational factors, calcium binding proteins and pentatricopeptide repeat-containing protein. In the library of infected plants, we found 66 genes that had sequence homology to the predicted siRNAs generated from TCTP mRNA. Among the genes, we can highlight genes that encode proteins involved in ubiquitination pathway (11 genes), protein phosphorylation (11 genes), DNA and RNA binding activities (7 genes) and chaperone activity (2 genes). It was also observed transcription factors (5 genes), genes encoding GTPase, ribosomal proteins, integral membrane, genes involved in autophagy and plant defense, among others (Table 2).

In healthy the library of plants we found 96 genes that had sequence homology to the predicted siRNAs generated from TCTP mRNA. We observed genes that encode proteins involved in ubiquitination pathway (11 genes), phosphorylation and phosphatase activity (9 genes), DNA and RNA binding activities (14 genes), GTPase activity (5 genes) and catalytic activity (3 genes). It was also observed transcription factors (9 genes), calmodulin binding protein, disease resistance protein, chaperones, tRNA synthetase, cyclins, ATP-binding cassette transporter, among others (Table 3).

Table 2: Genes that have sequence homology to the putative siRNAs of tomato TCTP mRNA. Putative siRNAs mapped from a library of miRNAs of tomato infected by *Alternaria*. Protein description and access number corresponds to information provided by Sol Genomics Network from a tomato genome database.

Accession number (Sol Genomics Network)	Protein description	Function
		tRNA synthetase:
Solyc05g056250.2.1	Asparaginyl-tRNA synthetase	Attaches asparagine into its tRNA
Solyc01g081220.1.1; Solyc02g061620.1.1	Phenylalanyl-tRNA synthetase	Binds phenylalanine to its corresponding tRNA
		Chaperone Activity:
Solyc07g064930.2.1	GrpE nucleotide exchange factor	Chaperone. Essential component of the PAM complex
Solyc06g051460.2.1	ATP-dependent chaperone ClpB	Chaperone. Heat shock protein required for acclimation to high temperatures.
		DNA and RNA activity:
Solyc08g067210.2.1	Ribonuclease 3-like protein 2	Cleaves double-stranded RNA (dsRNA)
Solyc01g081560.2.1; Solyc05g006460.1.1	Pentatricopeptide repeat-containing protein	RNA-binding proteins. Mediate gene expression in organelles and nucleus. Facilitates processing, splicing, editing, stability and translation of RNAs
Solyc08g005270.2.1	Poly(ADP-ribose) polymerase	DNA repair, genomic stability, and programmed cell death.
Solyc10g083210.1.1	Auxin response factor 1	DNA-binding protein. Confer specificity to auxin response through selection of target genes as transcription factors
Solyc05g056100.2.1; Solyc11g069460.1.1	Double-stranded RNA binding protein	Regulation of gene expression, host defense, development, translation and RNA editing
		Ubiquitin pathway:
Solyc06g082660.2.1	26S protease regulatory subunit 6B	Involved in ATP-dependent degradation of ubiquitinated proteins
Solyc11g005590.1.	F-box protein SKIP5	Involved in the pathway protein ubiquitination

Table 2. cont.

Ubiquitin pathway:		
Solyc08g077340.2.1	F-box/LRR-repeat protein	Involved in the pathway protein ubiquitination
Solyc04g063380.2.1	F-box/LRR-repeat protein 2	Involved in the pathway protein ubiquitination
Solyc02g072080.1.1	U-box domain-containing protein 17	Involved in the pathway protein ubiquitination
Solyc12g042000.1.1	G2/M phase-specific E3 ubiquitin-protein ligase	Involved in the pathway protein ubiquitination. Essential in early embryonic development to prevent apoptotic death
Solyc06g073930.2.1	Ubiquitin carboxyl-terminal hydrolase	Involved in the processing of poly-ubiquitin precursors as well as of ubiquitinated proteins
Solyc01g111650.2.1	Skp1	Involved in ubiquitination and subsequent proteasomal degradation of target proteins
Solyc01g010820.2.1	Cullin C	Involved in ubiquitination and subsequent proteasomal degradation of target proteins.
Solyc03g118670.2.1	ERAD-associated E3 ubiquitin-protein ligase component HRD3A	Involved in ubiquitin-dependent degradation of misfolded endoplasmic reticulum proteins.
Solyc07g042570.2.1	Eukaryotic translation initiation factor 3 subunit 11	ERAD substrate-recruiting factor that recognizes misfolded proteins for ubiquitin ligase complex. Targets the misfolded LRR receptor kinase
Phosphorylation Activity		
Solyc12g099980.1.1; Solyc02g070910.1.1; Solyc10g079170.1.1; Solyc09g091990.2.1; Solyc09g098290.2.1; Solyc02g081490.2.1; Solyc02g085430.2.1; Solyc06g008270.2. Solyc12g100020.1.1	Receptor kinase protein	Protein phosphorylation. Involved in development, growth, hormone perception and the response to pathogens
Solyc05g015610.2.1	Histidine kinase cytokinin receptor	Protein phosphorylation. Involved in cytokinin signaling
Solyc04g079700.2.1	WD-40 repeat family protein; myosin heavy chain kinase	Protein phosphorylation. Signal transduction, transcription regulation, apoptosis

Table 2. cont.

Transcription factors:		
Solyc06g083980.1.1; Solyc05g011810.2.1; Solyc02g063430.2.1	BHLH transcription factor	Controls the transcription from DNA to mRNA
Solyc07g037910.1.1	Homeobox-leucine zipper protein ROC7	Transcription factor. Controls the transcription from DNA to mRNA
Solyc07g066330.2.1	NAC domain protein	Transcription factor. Controls the transcription from DNA to mRNA
Solyc00g012540.1.1	Zinc finger transcription factor 3 SIC3H3	Transcription factor. Controls the transcription from DNA to mRNA
Solyc05g015510.2.1	Squamosa promoter-binding-like protein 11	Transcription factor. Leaf development, vegetative phase change, Gibberelic acid signaling and toxin response
Proteins with different functions		
Solyc09g092140.2.1	Methyltransferase	Transferase
Solyc03g058350.2.1	Translation initiation factor 5B	Translational GTPase that catalyzes the joining of the 40S and 60S subunits to form the 80S initiation complex
Solyc05g053570.2.1	Pleiotropic drug resistance protein; ATP-binding cassette transporter	Transmembrane transport using ATP. Involved in pathogen resistance
Solyc03g013160.2.1	Amino acid transporter family protein	Transport of amino acids across membranes
Solyc10g084210.1.1	Syntaxin-71	Vesicle trafficking protein that functions in the secretory pathway
Solyc05g051230.2.1	Adenylyltransferase and sulfurtransferase MOCS3	Biosynthesis of the molybdenum cofactor.
Solyc04g049330.2.1	V-type proton ATPase subunit G 1	Catalytic subunit of the peripheral V1 complex of vacuolar ATPase
Solyc03g083870.2.1	Pectinesterase	Cell wall associated protein. Modifications to the wall
Solyc02g070120.1; Solyc02g070130.1.1	FAD-binding domain-containing protein	Electron carrier activity

Proteins with different functions

Solyc11g044880.1.1	Kinesin like protein	Motor protein that functions in the NACK-PQR MAP kinase signaling pathway, which is essential for somatic cell cytokinesis
Solyc02g094180.2.1	Peroxidase 1	Oxidizing enzymes that oxidize organic substrates, having hydrogen peroxide as the electron acceptor molecule.
Solyc01g094680.2.1	Signal peptide peptidase	Cleaves parts of other proteins
Solyc06g082120.2.	Ran GTPase binding protein	GTPase
Solyc01g091460.2.1	ARF guanine-nucleotide exchange factor 2	Guanine nucleotide exchange factor
Solyc02g087150.2.1	Heavy metal transport/detoxification protein	Heavy metal transport
Solyc01g105320.2.1	Metacaspase	Induce programmed cell death
Solyc03g097600.2.1	MtN3-like protein	Integral membrane protein
Solyc05g056430.1.1	Auxin responsive SAR protein	Effectors of Hormonal and Environmental Signals in Plant Growth
Solyc05g005680.2.1	Xyloglucan endotransglucosylase/hydrolase	Cleaves and religates an essential constituent of the primary cell wall. Participates in cell wall construction of growing tissues.
Solyc08g080470.2.1	Calmodulin binding protein	Proteins which bind calmodulin
Solyc01g102840.2.	Tir-nbs, resistance protein	Recognize specific pathogen-derived products and initiate a resistance response
Solyc05g055970.2.1	Accelerated cell death 6	Regulates the salicylic acid (SA) signaling pathway leading to cell death and modulating cell fate
Solyc09g013130.2.1	Defective in exine formation	Required for exine pattern formation during pollen development
Solyc08g014550.2.1	Ribosomal L9-like protein	Ribosomal protein

Table 3: Genes that have sequence homology to putative siRNAs of tomato TCTP mRNA. Putative siRNAs mapped from a library of miRNAs of mock tomato plants. Protein description and access number corresponds to information provided by Sol Genomics Network from a tomato genome database.

Accession number (Sol Genomics Network)	Protein description	Function
Ubiquitin Pathway		
Solyc04g071320.2.1	Ubiquitin carboxyl-terminal hydrolase	Involved both in the processing of ubiquitin precursors and of ubiquitinated proteins.
Solyc02g038770.1.1	RING finger protein	Involved in protein ubiquitination. Involved in the early steps of the plant defense signaling pathway.
Solyc03g082630.2.1	26S protease regulatory subunit 7	Involved in the ATP-dependent degradation of ubiquitinated proteins
Solyc06g007320.2.1	Ubiquitin-activating enzyme E1	Involved in the first step of ubiquitination reaction
Solyc08g077340.2.1	F-box/LRR-repeat protein	Involved in the pathway protein ubiquitination
Solyc08g013800.2.1	F-box family protein	Involved in the pathway protein ubiquitination
Solyc01g057190.1.1	F-box family protein	Involved in the pathway protein ubiquitination
Solyc01g095810.2.1	Ring H2 finger protein	Involved in the pathway protein ubiquitination, involved in the early steps of the plant defense signaling pathway
Solyc07g005530.2.1	Ubiquitin carboxyl-terminal hydrolase	Involved in the processing of poly-ubiquitin precursors as well as of ubiquitinated proteins
Solyc09g005150.1.1	Ubiquitin-protein ligase E3	Recognizes a protein substrate and catalyzes the transfer of ubiquitin from E2 ubiquitin-conjugating enzyme to the protein substrate.
Solyc06g084440.2.1	Nuclear protein localization 4	Binds to ubiquitinated proteins and is necessary for the export of misfolded proteins from the ER to the cytoplasm
Phosphorylation and Phosphatase Activity		
Solyc06g065690.2.1	Serine/threonine-protein phosphatase 2A regulatory subunit delta 1	Protein phosphatase that regulates multiple aspects of cell growth and metabolism
Solyc05g056010.2.1	Serine/threonine-protein phosphatase	Phosphoprotein phosphatase that acts upon phosphorylated serine/threonine residues

Table 3: Cont.

Phosphorylation and Phosphatase Activity		
Solyc05g052520.2.1	Protein phosphatase 2C	Protein phosphatase that regulates multiple aspects of cell growth and metabolism
Solyc10g017620.2.1	fructokinase	Catalyzes the phosphorylation of fructose to produce fructose-1-phosphate.
Solyc01g109080.2.1	Protein kinase Phosphatidylinositol 3- and 4-kinase	Cell growth, proliferation, differentiation, motility, survival and intracellular trafficking
Transcription factor		
Solyc01g009170.2.1	Ethylene insensitive 3 class transcription factor	Transcription factor acting as a positive regulator in the ethylene response pathway
Solyc00g050430.2.1; Solyc08g083170.1.1	BHLH transcription factor	Controls the transcription from DNA to mRNA
Solyc08g080580.2.1	Myb transcription factor	Transcription factor as key factor in controlling development, metabolism and responses to biotic and abiotic stresses.
Solyc10g009360.2.1	Dof zinc finger protein 2	Transcription factor .Regulates a photoperiodic flowering response
Solyc03g044300.2.1	APETALA2a AP2a	Transcription factor. Plays a role in flower development
Solyc10g019260.1.1	R2R3MYB transcription factor 1	Transcription factor. Involved in plant development, metabolism, and responses to biotic and abiotic stresses
Solyc04g014380.2.1	Kinase interacting protein 1	Transcription factor. Involved in chromatin biding, DNA biding
Solyc06g034340.1.1	NAC domain protein	Transcription factor. Involved in developmental processes and plant hormonal control and defense
DNA and RNA properties		
Solyc05g055010.2.1	RNA-binding protein	Are able to bind to the double or single stranded RNA and participation forming ribonucleoprotein complex
Solyc11g069460.1.1	DsRNA-binding protein 2	Regulation of gene expression, host defense, development, translation and RNA editing
Solyc12g009590.1.1	Methyl-CpG DNA binding	Binds to DNA that contains one or more symmetrically methylatedCpGs

Table 3: Cont.

DNA and RNA properties		
Solyc08g005270.2.1	Poly(ADP-ribose) polymerase,	DNA repair, genomic stability, and programmed cell death.
Solyc03g098620.2.1	DNA-binding protein RHL1	Component of the DNA topoisomerase VI complex involved in chromatin organization and progression
Solyc05g012730.1.1	DNA topoisomerase	Unwind DNA
Solyc03g063340.2.1	DNA polymerase subunit B	Component of the polymerase delta complexes, plays a role in high fidelity genome replication
Solyc02g062780.2.1	Chromodomain-helicase-DNA-binding protein 6	Chromatin-remodeling protein
Solyc01g099460.1.1	ATP-dependent DNA helicase RecQ type	Genome maintenance. Unwinding of DNA
Solyc06g076340.2.1	mRNA binding protein Pumilio 2	Posttranscriptional/translational repressor
Solyc02g093880.2.1	DNA-binding bromodomain-containing protein	A protein that binds to single or double stranded DNA.
Solyc01g095110.2.1; Solyc06g065510.2.1	Pentatricopeptide repeat-containing protein	RNA-binding proteins. Facilitates processing, splicing, editing, stability and translation of RNAs
Solyc04g078990.2.1	Alkylated DNA repair protein alkB	Repair alkylation damage to DNA and RNA
Catalytic Activity		
Solyc05g008910.2.1	Glutamine amidotransferase	Catalyze the removal of the ammonia group from a glutamine molecule and its subsequent transfer to a specific substrate
Solyc04g049330.2.1	V-type proton ATPase subunit G 1	Catalytic subunit of the peripheral V1 complex of vacuolar ATPase
Solyc02g085660.1.1	UDP-glucosyltransferase	Catalyzes the addition of the glycosyl group from a UTP-sugar to a small hydrophobic molecule

Table 3: Cont.

		GTPase Activity
Solyc01g111240.2.1	Translocase of chloroplast 90	GTPase involved in protein precursor import into chloroplasts
Solyc09g011480.2.1	Rop guanine nucleotide exchange factor 1	Nucleotide exchange factor. Acts as an activator of Rop (Rho of plants) GTPases
Solyc10g086030.1.1	Interactor of constitutive active ROPs	Acts as a scaffold, mediating interaction of ROPs (GTGases) with different proteins
Solyc01g105810.2.1	Rab-GDP dissociation inhibitor	GTPase protein. Involved in regulation of intracellular vesicle trafficking
Solyc10g006540.2.1	Formin 3	Rho GTPases. Involved in the polymerization of actin
		Proteins with different functions
Solyc07g064930.2.1	GrpE nucleotide exchange factor	Chaperone. Essential component of the PAM complex
Solyc01g087920.1.1	Serine carboxypeptidase	Cleaves a peptide bond at the carboxy-terminal (C-terminal) end of a protein or peptide.
Solyc05g005680.2.1	Xyloglucan endotransglucosylase/hydrolase 2	Cleaves and religates xyloglucan polymers, an essential constituent of the primary cell wall,
Solyc07g066030.2.1	Proteasome activator subunit 4-like	Component of the proteasome that recognizes acetylated histones and promotes degradation of histones during DNA damage response
Solyc04g072130.1.1	Initiation factor eIF-4 gamma	Component of the protein complex eIF4F, which is involved in the recognition of the mRNA cap
Solyc07g049480.2.1	Cleavage and polyadenylation specificity factor subunit 3	Component of theCPSF complex that play a key role in pre-mRNA 3'-end formation
Solyc04g008610.2.1	Histone acetyltransferase	Enzymes that acetylate conserved lysine amino acids on histone
Solyc05g015610.2.1	Histidine kinase cytokinin receptor	Histidine kinase functions as a cytokinin receptor
Solyc01g105320.2.1	Metacaspase	Induce programmed cell death
Solyc08g005940.1.1	Trypsin proteinase inhibitor	Inhibitor that reduces the biological activity of trypsin enzyme, an enzyme involved in the breakdown of many different proteins

Table 3: Cont.

Proteins with different functions		
Solyc03g083340.1.1	Response regulator 8	Mediates cell's response to changes in environment as part of a two-component regulatory system. Coupled to specific histidine kinases
Solyc09g097890.2.1	Cytochrome b561/ferric reductase transmembrane	Membrane protein. Function as electron transporters, shuttling electrons across membranes from ascorbate to an acceptor molecule
Solyc04g076610.1.1	Receptor expression-enhancing protein 4	Microtubule-binding protein required to ensure proper cell division
Solyc11g010920.1.1	Kinesin	Motor protein that functions in the NACK-PQR MAP kinase signaling pathway, which is essential for somatic cell cytokinesis
Solyc06g068740.2.1	Reductase SDR family member 7B	NAD- or NADP-dependent oxidoreductases.
Solyc03g007660.2.1	ETC complex I subunit	NADH dehydrogenase.
Solyc12g042260.1.1	Low affinity nitrate transporter	Nitrate transport
Solyc07g052550.1.1	Peroxidase	Oxidizing enzymes that oxidize organic substrates, having hydrogen peroxide as the electron acceptor molecule.
Solyc03g118640.2.1	D-tagatose-1,6-bisphosphate aldolase subunit gatY	Oxidoreductase
Solyc05g005250.2.1	Pantothenate kinase	Participates in Coenzyme A (CoA) biosynthetic pathway
Solyc09g009700.2.1	GDSL esterase/lipase	Participates on lipid catabolic process. Has hydrolase activity. Acts on ester bonds
Solyc11g010550.1.1	GPI ethanolamine phosphate transferase	Plays a role in glycolipid biosynthesis.
Solyc08g068240.1.1	Xenotropic and polytropic retrovirus receptor	Plays a role in phosphate homeostasis. It has virus receptor activity
Solyc09g074950.2.1	Katanin p60 ATPase-containing subunit	Promote rapid reorganization of cellular microtubule arrays
Solyc03g098730.1.1	Kunitz trypsin inhibitor	Protease inhibitor. Trypsin Inhibitors are usually specific for either trypsin or chymotrypsin.
Solyc01g094680.2.1	Signal Peptide Peptidase	Protein that cleaves parts of other proteins.

Table 3: Cont

		Proteins with different functions
Solyc08g014550.2.1	60s ribosomal protein L-9	Protein that is a component of 60s ribosome
Solyc10g079790.1.1	Calmodulin-binding protein	Proteins which bind calmodulin
Solyc11g043060.1.1	Disease resistance protein	Recognition of specific pathogen effectors
Solyc03g119730.2.1	Histone deacetylase	Remove acetyl groups from lysine amino acid on a histone, allowing the histones to wrap the DNA more tightly.
Solyc06g072100.1.1	ABC transporter G family member 11	Required for pollen coat development. Controls cutin transport to the extracellular matrix
Solyc07g053070.1.1	Purple acid phosphatase	Role in Plant Tolerance to Phosphate Limitation
Solyc04g016370.2.1	DNA ATP-dependent helicase	Separates two annealed nucleic acid strands using energy derived from ATP hydrolysis
Solyc03g121480.2.1	Thioredoxin family protein	Serves as a general protein disulfide oxidoreductase. It interacts with a broad range of proteins
Solyc05g014480.2.1	Glyceraldehyde-3-phosphate dehydrogenase	Serves to break down glucose for energy and carbon molecules.
Solyc01g086890.2.1	Splicing factor 4-like protein	Spliceosome assembly
Solyc08g081240.2.1	Hydroxyproline-rich glycoprotein family protein	Structural proteins in plant cell walls
Solyc11g067000.1.1; Solyc05g053570.2.1; Solyc05g053610.2.1	ATP-binding cassette transporter	Transmembrane transport using ATP. Involved in pathogen resistance
Solyc10g084210.1.1	Syntaxin-71	Vesicle trafficking protein that functions in the secretory pathway
Solyc04g077900.1.1	D5-type cyclin	Activates cyclin-dependent kinase (Cdk) enzymes.
Solyc08g077450.2.1	CSC1-like protein	Acts as an osmosensitive calcium-permeable cation channel
Solyc05g056250.2.1	Asparaginyl-tRNA synthetase	Attaches asparagine into its tRNA

4. Discussion

TCTP silencing leads to higher viral accumulation in the early stages of infection by ToYSV

TCTP is a ubiquitous protein in eukaryotes involved in different numbers of cellular processes. Alfenas-Zerbini et al. (2009) demonstrated up-regulation of TCTP in tomato infection by PepYMV and Bruckner et al. (2017) showed a decrease on PepYMV accumulation in both transient TCTP silenced *N. benthamiana* and stable silencing in tomato. In this study, we demonstrated that TCTP is also involved in ToYSV infection, but differently from Bruckner et al. (2017) results, TCTP transient silencing led to higher ToYSV accumulation in *N. benthamiana* infected plants and TCTP expression was not induced by ToYSV infection.

With similar results, a study on the effect of TCTP silencing in shrimps infected by WSSV, a dsDNA virus, revealed more severe symptoms in shrimps and an increase in virus accumulation occurred. They also demonstrated that mRNA levels of replication protein VP28 of WSSV increased in TCTP silencing shrimps. These results suggest an important role of TCTP in host immune response (Wu et al., 2013)

Unlike potyviruses that have ssRNA genome, ToYSV is an ssDNA virus. The difference in their genome has different implications on infection establishment. Geminivirus needs to reprogram cell cycle to use the replication machinery off infected cells, because normally, infected cells have exited the cell cycle. Plants, later in their development, transit to an endocycle where replication and cell expansion occur but not cell division. Many geminiviruses induce plants to

re-enter endocycle and redirect host factors to their own replication processes and overall infection (reviewed by Hanley-Bowdoin et al., 2013). Transcriptome analysis of *Arabidopsis* during geminivirus infection showed that geminiviruses activates genes expressed during the S phase (where replication occurs)/G2 phase of cell cycle and inhibits genes expressed during M (phase where mitosis occurs) /G1 phase (Ascencio-Ibáñez et al., 2008). Studies have demonstrated the capacity of TCTP to control cell cycle progression both in animals and plants (Brioude et al., 2010; Gachet et al., 1999; Bommer et al., 2015). In mutant TCTP *Nicotiana tabacum* lineages, regulation of cell cycle was altered. When TCTP was silenced, cells had a tendency to stay in G1/S phase exhibiting an extended G1 phase and in consequence M/G2 phase was delayed, that is, cells were able to replicate their genome but the mitosis phase and in consequence cell division are delayed (Brioude et al., 2010). The involvement of TCTP in cell cycle might be an explanation to why ToYSV accumulation increases in silenced TCTP plants, since geminivirus controls the endocycle where replication and cell expansion occur but not cell division. Since TCTP mRNA is not induced in ToYSV infection, maybe TCTP is not directly involved in ToYSV infection and its silencing could indirectly help ToYSV establishment in infected cells because of its function on cell cycle regulation.

Since TCTP has multiple functions and participates in central cellular processes, a number of possibilities in which it could be affecting virus infection are possible. In *Drosophila melanogaster*, for example, it was demonstrated that TCTP functions as a GEF by activating Ras GTPases dRheb, a GTPase that regulates the TOR kinase pathway (Hsu et al., 2007). Interspecies complementation experiment showed that *A. thaliana* TCTP is able to bind to four

different Rab GTPases (AtRABA4a, AtRABA4b; AtRABF1 and AtRABF2) and is also capable of binding to *D. melanogaster* dRheb (Brioudes et al., 2010). Thus, TCTP has also been suggested to be a GTPase modulator of the TOR pathway in plants. TOR protein kinase signaling pathway is a central regulator of intra and extracellular signals, controlling cell metabolism and regulating mRNA translation and cell cycle (reviewed by Ouibrahim et al., 2015). In mammals, virus infection modulates mTOR signaling pathway to make host cells suitable for viral replication (Le Sage et al., 2016). With this evidence, we speculate that since TCTP is a multifunction protein, one of the ways in which plant TCTP may participate in viral infection is by acting through the TOR pathway.

More studies have to be done to better understand the exact role of TCTP on virus infection. Since TCTP silencing interferes with the infection process of RNA and DNA viruses in different ways, we speculate that TCTP may be involved in different plant processes that affect the infection of unrelated plant viruses.

Analysis of tomato genes that are target of putative siRNAs generated from tomato TCTP mRNA

While studying the importance of TCTP for potyvirus replication, Bruckner (2016) observed in western blot analysis that TCTP mRNA up-regulation induced by potyvirus infection does not result in protein increase, and thus hypothesized that TCTP mRNA rather than the protein could be involved on virus infection. However, the expression of a non-translatable form of TCTP mRNA had no effect on virus accumulation (Bruckner, 2016).

Since TCTP mRNA forms secondary structure with dsRNA regions, we speculated that it might be a target of Dicers proteins in PTGS and the siRNAs

produced might target the expression of protein involved in virus replication an overall infection.

We didn't find any transcriptome analysis of tomato small RNAs infected with virus to use as a basis for mapping putative siRNAs of TCTP mRNAs, so instead we searched for small RNA analysis with other plant pathogens, since it triggers similar pathogen responses in cells. To test our hypothesis, we searched data from a library of tomato miRNAs infected with the fungus *Alternaria* and miRNAs from mock tomato plants to map putative siRNAs from TCTP mRNA. The software psRNATarget (Dai and Zhao, 2011) was used to find genes that are complementary to the predicted TCTP siRNAs.

When analyzing the genes from Table 2, almost all of them might affect virus infection, especially genes encoding proteins involved in ubiquitination pathway, protein kinases and transcription factors. Genes from Table 3 encodes proteins that are more diverse in terms of function, but still, protein kinases, ubiquitin proteins and transcriptional factors are the most predominant genes. Those proteins that might participates in virus infection are as follows: protein kinases, proteins involved in ubiquitin pathway, transcription and translation factors, proteins involved in hormone pathway, calcium and calmodulin binding proteins, ribonucleases dsRNA binding proteins, cyclins, pathogen resistance protein, proteins involved in DNA repair and stability and ribosomal proteins

Most of the endogenous small RNAs detected in plants are noncoding RNAs of 20-24 nt. The first eukaryotic small RNA was a miRNAs that was isolated in *Caenorhabditis elegans*, and since then innumerous miRNAs were detected both in plants and in animals (reviewed by Yu and Kumar, 2003). One of the first's publications of PTGS in plants demonstrated that the progeny of the transgenic

Nicotiana tabacum SR1 plants containing the *Nicotiana plumbaginifolia* beta-1,3-glucanase *gn1* gene revealed an unexpected phenomenon of gene suppression of the GN1 protein (de Carvalho et al., 1992) In a further study, it was demonstrated that specific endogenous beta-1,3-glucanase genes are cosuppressed with *gn1* in tobacco plants (de Carvalhol et al., 1995). Sanders et al. (2002) demonstrated that in the presence of the *gn1* transgene in tobacco, an endogenous glucanase gene is activated for production of siRNAs, leading to a more active silencing than would occur in a passive involvement. It was the first evidence of endogenous genes actively involved in the silencing process in plants.

These studies demonstrate the potential for siRNAs generated from endogenous genes to affect complex signaling pathways that may be involved in viral infection process.

5. Conclusions

- TCTP is a protein involved in RNA and DNA virus infection, but with different outcomes. While TCTP transient silencing in *N. benthamiana* causes a decrease in PepYMV accumulation, ToySV accumulates in TCTP silenced *N. benthamiana* plants.
- Putative siRNAs generated from TCTP mRNAs have sequence homology to genes encoding proteins with diverse types of function that can be involved in virus infection.
- Further studies need to be done to confirm the generation of these putative siRNAs from TCTP mRNA, its targets and their relationship to viruses infection.

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