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Evaluation of two nontransformative approaches in triggering RNAi against *Tomato Yellow Leaf Curl Virus*

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Abstract

Tomato yellow leaf curl virus-Israel (TYLCV-IL) is a worldwide destructive monopartite begomovirus. Two nontransformative methods, namely the dsRNA (double-stranded RNA) vaccination and the hairpin construct expressing dsRNA were applied to induce RNA interference (RNAi) against TYLCV-IL infection. Analysis of TYLCV-IL-derived small interfering RNAs profile revealed two hot spots triggering post-transcriptional gene silencing, including a part of the coat protein (*CP*) and the replication-associated protein (*Rep*) genes. *CP* and *Rep* derived dsRNAs were produced *in vitro* and applied simultaneously or three days before agroinoculation of TYLCV-IL by rubbing on the leaf surface of *Nicotiana benthamiana*. All tested plants showed symptoms of typical infection at 21 days' post-inoculation (dpi), and viral genomic fragments were amplified in symptomatic plants by PCR as expected. Despite the systemic movement of applied dsRNAs in *N. benthamiana* plants, quantitative PCR showed no statistically significant difference between TYLCV-IL genome accumulation in virus infection alone or at the presence of dsRNA molecules. In contrast, the hairpin RNA construct (hpRep) triggered a high resistance against TYLCV-IL infection in tomato plants by targeting the same *Rep* sequence. HpRep construct suppressed expression of symptoms up to 21 dpi. Moreover, no virus genome was detected in symptomless plants indicating disruption of TYLCV-IL replication. These results indicate the distinct efficiency of two methods of RNAi induction against an important begomovirus species, TYLCV-IL. The role of different factors on the RNAi efficiency against TYLCV-IL has been discussed.

Keywords: TYLCV-IL, dsRNA vaccination, hairpin construct, RNA interference

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Introduction

Tomato yellow leaf curl disease (TYLCD) is one of the most limiting factors in tomato crops worldwide (Czosnek, 2008) caused by different species within the genus *Begomovirus* (family *Geminiviridae*). Begomoviruses are unique members within geminiviruses with one or two genomic single-stranded DNA known as monopartite and bipartite begomoviruses, respectively (Zerbini et al., 2017). The worldwide distribution of the TYLCD in tomatoes is highly dependent on virus transmission (persistent and circulative) by its vector, the whitefly *Bemisia tabaci* (*Hemiptera*: *Aleyrodidae*) (De Barro et al., 2011). Tomato yellow leaf curl virus is a worldwide distributed monopartite begomovirus in tomato and some weeds, ornamental and crop plants (Czosnek, 2008). TYLCV is divided into two phylogenetic strains, TYLCV-Israel (TYLCV-IL) and TYLCV-Mild (TYLCV-Mld), with the former being the type strain (Melita et al., 2021).

Several approaches have been developed to control the TYLCD based on molecular and/or biological characteristics of causing begomoviruses. Some control strategies to restrict vector populations, including physical barriers and/or chemical insecticides (Pereira-Carvalho et al., 2015), are the most popular methods used to control TYLCD in the fields. But, the appearance of insecticides resistant vector populations and eco-friendly concerns must be considered when using these strategies (Rojas et al., 2018). Some other strategies have also been investigated (Shepherd et al., 2009; Pakniat-Jahromy et al., 2010), but are still limited to experimental trials and have not been used in the field (Tabein et al., 2017). Alternatively, several genetic engineering strategies have been evaluated, mainly based on RNA interference (RNAi) (Brunetti et al., 2001).

RNAi is a defense and gene regulatory pathway in almost all eukaryotic cells and acts as an antiviral system in plants (Bisaro, 2006). There are different RNAi pathways which are complexed and occasionally overlapped (Blevins et al., 2006). The post-transcriptional gene silencing pathways involve the cleavage of double-stranded RNA (dsRNA) into small interfering RNAs (siRNAs) by Dicer-like proteins (DCLs) (Blevins et al., 2006). In plants, dsRNA or self-complementary hairpin RNA from engineered inverted repeats constructs were shown to be potent inducers of a gene silencing pathway (Hamilton & Baulcombe, 1999; Waterhouse et al., 1998; Johansen & Carrington, 2001). Furthermore, it has been proven that virus-derived dsRNAs can interfere with infection of positive and negative plant RNA viruses in a sequence-specific manner by directly delivering dsRNAs into plant cells (Petrov et al., 2015; Tenllado & Diaz-Ruiz 2001; Tenllado et al., 2004; Tabein et al., 2020) or by spraying bacterially expressed dsRNAs which is referred to as RNA vaccination (Tenllado et al., 2003; Gan et al., 2010). It has been shown that targeting potyviruses, bromoviruses, tobamoviruses, cucumoviruses, and orthotospovirus genomes by RNA vaccination interfered with viral infection on the host plants (Tenllado & Diaz-Ruiz, 2001; Petrov et al., 2015; Tenllado et al., 2004; Tabein et al., 2020).

In this study, two nontransformative approaches of RNAi were investigated to protect host plants against TYLCV-IL infection. External application of dsRNAs as well as delivery of hairpin RNA construct of the same viral sequence were examined to evaluate their effects on accumulation of the monopartite begomovirus genome and suppression of symptoms expression. To our knowledge, this is the first report of the external application of dsRNAs against TYLCV-IL.

Materials and Methods

Biological Materials

Construction of an isolate of TYLCV-IL (Genbank Acc. No. DQ144621) in the binary vector pBin19 was used for agroinoculation (Boulton, 2008). The strain LBA4404 of *Agrobacterium tumefaciens* harboring pBin61 was used to produce expression hairpin RNA (hpRNA) construct. *Nicotiana benthamiana* and tomato (cv. Moneymaker) plants were maintained in a growth chamber at 24°C with a light/dark cycle of 16/8 h. Plants with 4-5 fully expanded leaves were used for the bioassay experiments.

***In vitro* dsRNAs Production**

Two-step PCR approach followed by *in vitro* transcription was used to produce virus-derived dsRNA molecules (Voloudakis et al., 2015). In the case of *CP* and *Rep* dsRNAs, total DNA was extracted from TYLCV-IL-infected tomato plants based on Mason et al. (2008). Viral fragments were amplified with specific primers designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). The T7 RNA polymerase promoter sequence was added at the 5′ ends of both the forward and the reverse primers (Table 1). PCR was carried out in a final volume of 50 µl, containing 10X reaction buffer, 1 µl DNA template, 200 µM dNTPs, 0.2 µM of each primer, 1.5 mM $MgCl_2$, and 2.0 units of Platinum *Taq* DNA polymerase (Invitrogen, Thermo Fisher Scientific, Waltham, USA). PCR fragments were loaded on 1% agarose gel and purified by the DNA Clean and Concentrator kit (Zymo Research, Irvine, USA). To synthesize *in vitro* the sense and antisense viral derived ssRNAs, 1 µg of purified DNA template was used in 50 µl transcription reaction containing T7 reaction buffer, 500 µM rNTPs, 5 mM DTT, 50 units of T7 RNA polymerase (Invitrogen, Thermo Fisher Scientific, Waltham, USA), incubated at 37°C for 2 h. After removing the DNA template by TURBO RNase-free DNAse (Ambion, Thermo Fisher Scientific, Waltham, USA), dsRNAs were obtained by mixing the specific sense and antisense ssRNAs, then incubated at 95°C for 3 min, and at 37°C for 30 min. The formation of dsRNAs was confirmed by treatment with Mung Bean Nuclease (New England Biolabs, USA).

Construction of *Rep* RNA Hairpin Construct

The *A. tumefaciens* strain LBA4404 harboring pBin61 vector was used to produce hairpin RNA construct (hpRNA) targeting of *Rep* sequence of TYLCV-IL, designated hpRep. By using of 801 bp length of Phytoene desaturase gene (*PDS*, Genbank Acc. No. NC_015440) sequence of the tomato plant as separating intron, an intron spliced vector containing 796 bp of the central region of *Rep* sequence (overlapping region of C4 and C1 ORFs) was generated in both sense and antisense orientations. The antisense fragment was directionally cloned in *Bam*HI and *Pst*I restriction sites following the cloning of the sense fragment in *Eco*RV and *Xma*I restriction sites at both sides of the PDS intron. LBA4404 cells were transformed with the new recombinant construct, hpRep, under the control of CaMV 35S promoter and used in assays by agroinoculation method.

Plant Bioassays

Bioassays on Tobacco by External Application of dsRNAs

A. tumefaciens culture harboring a tandem-repeat construct of TYLCV-IL in pBin19 were grown at 28°C for 36 to 48 h, before agroinoculation. *N. benthamiana* plants at the four-leaf stage were agroinoculated by injection of 100 µl of the viral culture at different leaf nodes (Boulton, 2008). Ten µg of *in vitro*-produced ds*CP* (500 bp) or ds*Rep* (796 bp) were applied on the upper side of two fully expanded young leaves, previously dusted with carborundum in each plant (10 plants per each treatment), three days before virus agroinoculation or just before virus inoculation. The plants agroinoculated with TYLCV-IL alone and LB4404 harboring empty pBin19 were positive and negative controls, respectively. Treated plants were kept under controlled greenhouse conditions, and symptoms development was monitored until 21 dpi when final observations were recorded. DsRNA derived from *Nsm* of *Tomato spotted wilt virus* (genus *Orthotospovirus*) was used as negative control (non-sequence specific).

Bioassays on Tomato by Injection of hpRep Construct

The recombinant hpRep construct was used in the bioassay on the tomato plants. *A. tumefaciens* cell cultures harboring TYLCV-IL and hpRep construct were grown at 28°C for 36 to 48h. Tomato plants were agroinoculated with 100 µl of the viral culture and hairpin construct when the recombinant hpRep construct inoculated three days before virus agroinoculation or used at the same time. Five tomato Moneymaker plants were used per each treatment. Plants were kept under controlled greenhouse conditions to monitor symptoms expression up to 21 dpi.

Virus Detection

Virus Detection by End-point and Quantitative Real-Time PCR

Leaf disks of *N. benthamiana* and tomato cv. Moneymaker plants were collected at 21 dpi. The total DNA of collected samples was extracted according to Mason et al. (2008) and subjected to PCR to detect TYLCV-IL genome. Endpoint PCR was performed using the TY1(+)/TY2(-) primer pair (Table 1). Quantitative real-time PCR (q-PCR) was carried out using an iCycler iQTM Real-Time PCR Detection System with the primers listed in Table 1, employing a previously described program (Tabein et al., 2017). The tobacco gene Niben101Scf02399 encoding the cytochrome c oxidase (NbCOX) was used as a reference gene for relative virus quantification (Tabein et al., 2020). The relative amount of virus was calculated for each sample using the ΔCt method, where ΔCt is $|Ct_{virus} - Ct_{NbCOX}|$. The fold change in the amount of virus between tested dsRNA molecules treatment and virus infection only used as reference was calculated as $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct = \Delta Ct_{dsRNA} - \Delta Ct_{virus}$.

DIG Southern Blot Hybridization

Total DNA extracted from inoculated *N. benthamiana* plants at 21 dpi (approximately 300-500 ng) were loaded onto 1% agarose gel and separated in 0.5 X TBE, containing 0.5 µg/ml ethidium bromide, electrophoresed at 70V for 3 h and blotted onto positively charged nylon membranes (Roche, Basel, Switzerland). Membranes were then hybridized with a digoxygenin labelled CP-specific probe obtained with the TY1(+)/TY2(-) primers (Table 1) (Accotto et al., 2000), following the manufacturer's instructions (Roche, Basel, Switzerland).

DsRNAs Movement in Tobacco

To evaluate systemic movement or stability of dsRNAs at the entry sites (inoculated leaf), 10 µg of ds*Rep* were mixed with 100 µl of sterile water and mechanically inoculated on two carborundum-dusted fully expanded leaves of *N. benthamiana* by rubbing. Treated leaves were washed five times with Triton X-100 (0.05 %) and finally with sterile water. Leaf disks from inoculated leaflets and non-treated new emerged leaflets (systemic) were collected at 1 dpi and 2, 4 and 7 dpi. Total RNA from the leaf disk was extracted by TRIzol reagent (Ambion, Thermo Fisher Scientific, Waltham, USA). *Rep* derived dsRNAs were detected by Northern blot hybridization using synthetized digoxygenin-labelled RNA probe of the sense orientation of *Rep* sequence.

Results

Production of TYLCV-IL Derived DsRNAs

To select the best candidate for triggering RNAi against TYLCV-IL genome, virus derived small RNA (vsRNA) profiles were studied from

Table 1. Details of specific primers used in this study. The T7 binding site is underlined.

Primer	Sequence from 5′ to 3′	Purpose
T7-TYLCV-CP-fw	GAATTAATACGACTCACTATAGGGAGAATGTCGAAGCGACCCGG	dsRNA synthesis
TYLCV-CP-rv	CAAGAAGAACATGACCTGATTAGTGTG	
TYLCV-CP-fw	ATGTCGAAGCGACCCGG	
T7-TYLCV-CP-rv	GAATTAATACGACTCACTATAGGGAGACAAGAAGAACATGACCTGATTAGTGTG	
T7-Rep-TYLCV-Fw	GAATTAATACGACTCACTATAGGGAGATCGTAGAGGGTGACGAAGGT	
Rep-TYLCV-Rv	AACGGTTCTTCGACCTGGTA	
Rep-TYLCV-Fw	TCGTAGAGGGTGACGAAGGT	
T7-Rep-TYLCV-Rv	GAATTAATACGACTCACTATAGGGAGAAACGGTTCTTCGACCTGGTA	
T7_NSm_p105_fw	GAATTAATACGACTCACTATAGGGAGAGGGGTCTTCTAAGTCTGCCAG	
NSm_p105_rv	CCCTTCTTGTATTCTTGGCTGC	
NSm_p105_fw	GGGGTCTTCTAAGTCTGCCAG	
T7_NSm_p105_rv	GAATTAATACGACTCACTATAGGGAGACCCTTCTTGTATTCTTGGCTGC	
TY1(+)	GCCCATGTA(T/C)CG(A/G) AAGCC	Virus detection (End point PCR)
TY-2(-)	GG(A/G)TTAGA(A/G)GCATG(A/C)GTAC	
qRT_NbCOX_fw	CGTCGCATTCCAGATTATCCA	Real-time PCR (qPCR)
qRT_NbCOX_rv	CAACTACGGATATATAAGRRCCRRAACTG	
TY2222(+)	GTCGTTGGCTGTCTGTTGTC	
TY2371(-)	AGGTCAGCACATTTCCATCC	

literature. It was hypothesized that the genomic regions characterized by a high number of mapped vsRNAs could be more subjected to RNAi-mediated degradation (Tabein et al., 2020). Bai et al. (2016) characterized TYLCV-IL derived small RNAs in susceptible and tolerant tomato cultivars. The presented results were checked out to identify the hot spots of vsiRNAs within the TYLCV-IL genome. These data showed TYLCV-IL derived vsiRNAs were mostly 21 nt and 22 nt, and some were 24 nt. It revealed that most TYLCV-IL small RNAs are derived from overlapping regions of coding sequences within the virus genome (Bai et al., 2016). A 500 bp fragment encompasses the overlapping part of V1 and V2 ORFs (referred to as CP), and a 796 bp region including overlapped encoding regions of C1 and C4 proteins (referred to as Rep) with a high number of derived vsiRNAs, were selected as candidate regions to study their interference with TYLCV -IL infection through dsRNA vaccination.

The two-step PCR approach followed by *in vitro* transcription was used to produce dsRNAs (Volodakis et al., 2015). One µg of DNA template resulted in 50-80 µg dsRNA. The nature of dsRNA molecules was analyzed by Mung Bean Nuclease, which acts as single-stranded nucleic acid endonuclease (Figure 1).

External Application of *Rep* and *CP* Derived dsRNAs Had No Suppression Activity on Symptoms Expression and Genome Replication of TYLCV-IL

Slight yellowing and chlorosis were observed in all the *N. benthamiana* plants which were treated by virus-derived dsRNAs at 10-14 dpi. Typical symptoms, including severe chlorosis and leaf curling, were observed at 21 dpi in these plants (Figure 2A). Southern blot analysis of virus DNAs extracted from tobacco plants treated by dsCP and dsRep was conducted by a specific CP probe. Positive signals, single and double-stranded replicative DNA forms, were detected in all the tested plants that proved infection by the virus even in the presence of the dsRNAs (Figure 2B), indicating virus replication. There was no intensity difference between signals in dsCP, and dsRep derived treatments and infection by TYLCV-IL only (Figure 2B).

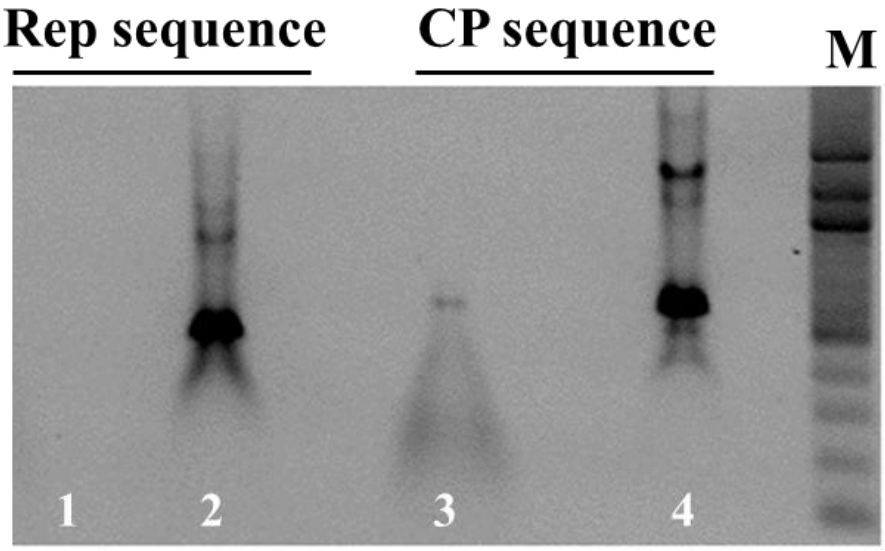

Figure 1. Transcribed single stranded (1 and 3 lanes) and incubated double stranded (2 and 4 lanes) dsRep and dsCP sequences were treated by Mung Bean Nuclease. SsRNAs were completely (Rep derived ssRNA) or incompletely (CP derived ssRNA) degraded, while dsRNAs have not affected by endonuclease activity. M: 1 Kb Plus DNA Ladder (ThermoFisher).

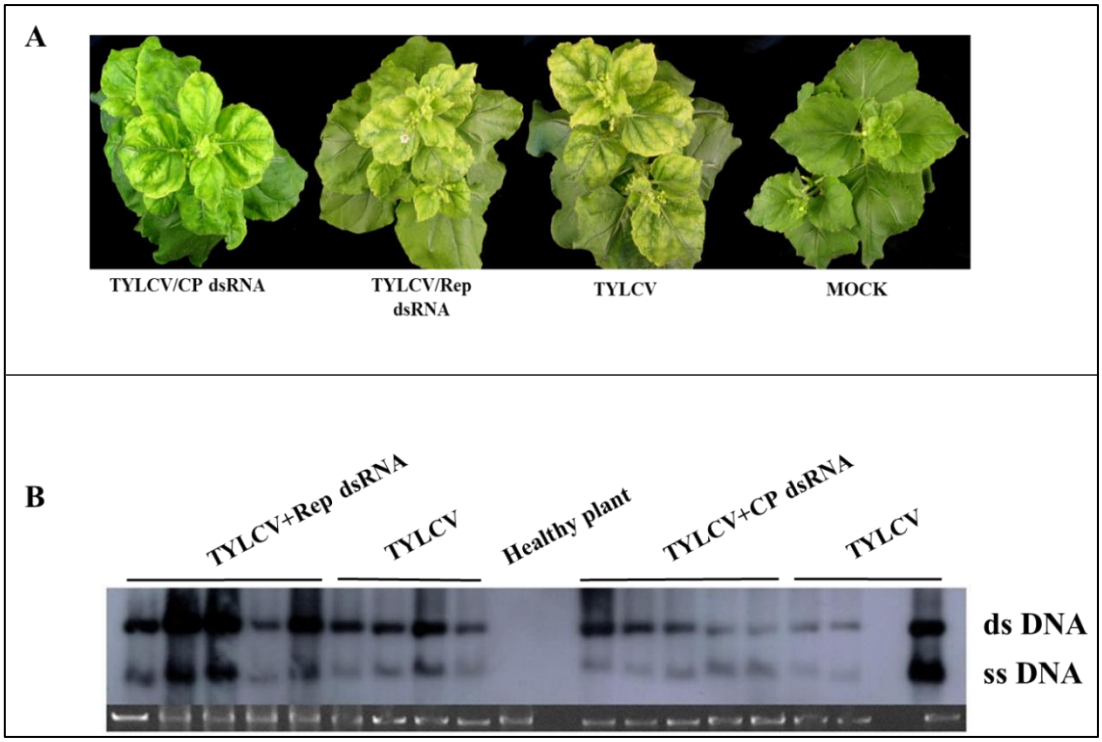

Figure 2. A. Induced systemic symptoms including chlorosis and leaf curling on *N. benthamiana* plants which were treated by TYLCV-IL/dsCP and TYLCV-IL/dsRep in compare to TYLCV-IL infected and healthy (Mock) plants at 21 dpi. B. Southern blot hybridization of total DNA extracted from *N. benthamiana* plants inoculated by TYLCV-IL+dsRep, TYLCV-IL, healthy plant, TYLCV-IL+dsCP and TYLCV-IL at 21 dpi. Loaded DNA of each sample stained with ethidium bromide is shown at the bottom of the blot panel.

Endpoint PCR, as well as qPCR were conducted to have a more precise estimation of the virus genome titer between the different treatments. An expected fragment was amplified in all the tested plants treated by virus alone and virus/dsCP- dsRep, at 21 dpi (Figure 3A). There was no statistically significant differences ($p < 0.05$, ANOVA) in virus titer when the virus was inoculated alone or in the presence of dsRep and dsCP. DsRNA derived from *Nsm* of *Tomato spotted wilt virus* (genus *Orthotospovirus*) was used as negative control (non-sequence specific); as expected, it had no effect on TYLCV-IL titer.

Systemic Movement of DsRNA in *N. benthamiana*

To evaluate systemic movement or stability of dsRNAs at the entry sites, dsRep was applied on the leaves of *N. benthamiana* without virus infection. Sample collections were conducted from upper new emerged leaves at 1, 2, 4, and 7 dpi. Total RNA extracted from these samples was analyzed by Northern blot hybridization. Detected signals revealed the systemic movement of dsRNA molecules after 4 dpi, so that *Rep* derived dsRNA were detectable in the upper new un-treated leaves (Figure 4). Although, there were no positive signals at 1, and 2 dpi for the newly emerged leaves, suggesting a low speed of movement for *Rep* derived dsRNAs from the entry site to the other plant tissues. Also, the detected signal at 7 dpi had lower intensity than 4 dpi, indicating processing of dsRNAs into small interfering RNAs by DCLs.

Efficient Suppression of TYLCV-IL Infection by HpRNA Construct Targeting *Rep*

The silencing hpRep construct was designed (Figure 5A) and used to assess inhibition of virus infection compared to the external application of dsRNAs. The same region of Rep fragment was considered to design hpRep. The results showed that the obtained construct could trigger a high level of resistance against TYLCV-IL infection when used three days before or simultaneously

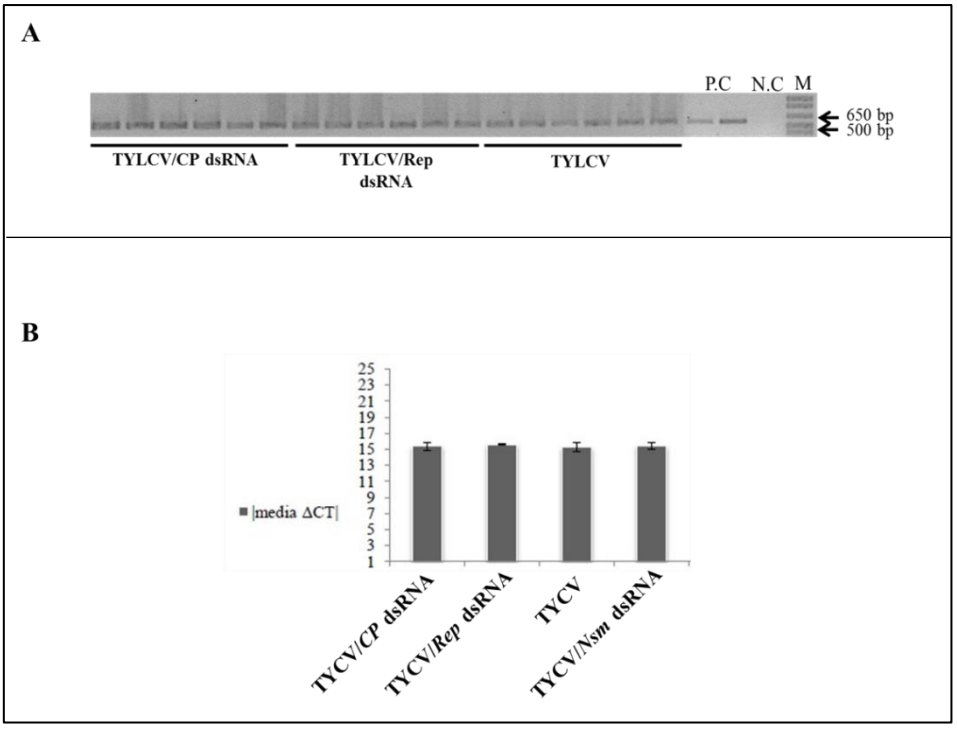

Figure 3. (A) Electrophoresis pattern of amplified genomic fragments of TYLCV-IL in different treatments of external application dsRNAs in agarose gel. P.C.: Extracted cloning plasmid containing full length sequence of TYLCV-IL was used as positive control. N.C.: Extracted DNA of a healthy plant was used as template in negative control. M: 1 Kb Plus DNA Ladder (ThermoFisher). (B) Quantification of TYLCV-IL genome titer by quantitative real-time PCR. Vertical axis shows ΔCt values. There was no statistically significant difference between applied treatments.

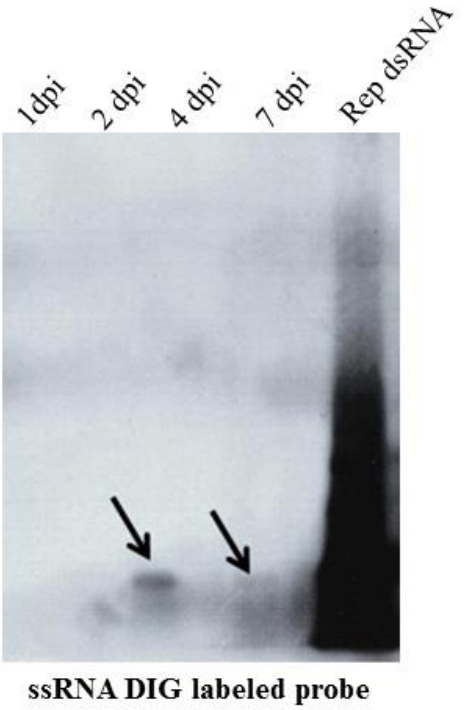

Figure 4. Detection of *Rep* derived dsRNA by northern blot hybridization using ssRNA DIG labeled probe at 1, 2, 4 and 7 dpi. Positive signal was detected at 4 dpi with more intensity in compare to 7 dpi (indicated by arrows). *In vitro* synthetized dsRNA was used as positive control (Rep dsRNA).

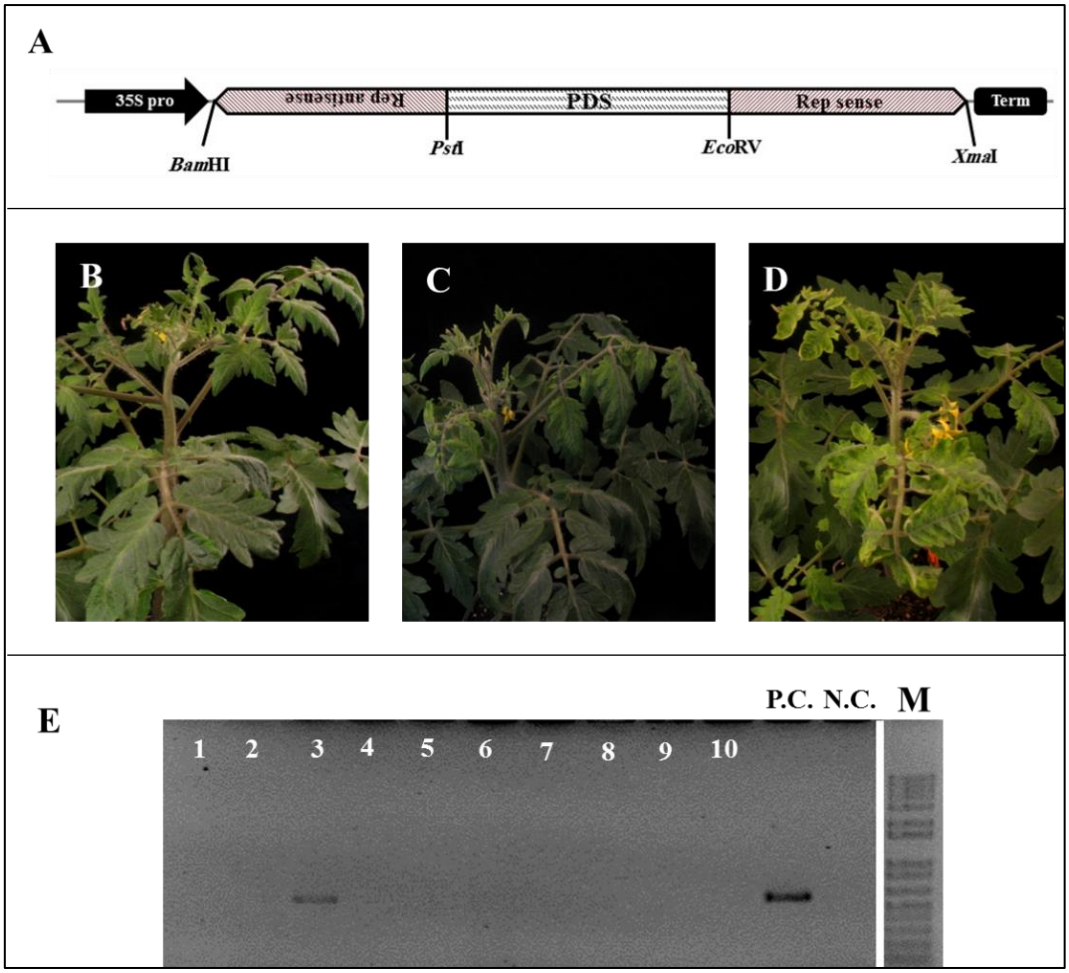

Figure 5. (A) Schematic representation of pBin61+Rep798 hpRNA construct with PDS sequence as intron. The expression cassette is controlled by CaMV 35S promoter. Sens and antisense orientation of *Rep* sequence have created right and left arms of the construct. Each used mono site digestion enzyme have been shown at the ends of both orientations of *Rep* sequence. (B) Treated tomato moneymaker plant by hpRNA construct in 3 days before inoculation of TYLCV was remained symptomless up to 21 dpi. (C) Symptomless tomato plants which were inoculated simultaneously by hpRNA construct and TYLCV-IL infectious clone. (D) Expressed typical symptoms in a positive control tomato plant which was inoculated with TYLCV infectious clone at 21 dpi. (E) Detection of viral DNA fragment in tomato plants inoculated with TYLCV infectious clone+hpRNA construct at 3 days before virus inoculation (lanes 6, 10) and simultaneously inoculation of *A. tumefaciens* harboring infectious clone and hpRNA construct (Lanes 1, 5). N.C.: Extracted DNA from healthy tomato plant was used as negative control; P.C.: Extracted DNA from TYLCV-IL infected tomato plant was used as positive control in PCR; M: 1 Kb Plus DNA Ladder (ThermoFisher).

infiltrated with TYLCV-IL. All five tested tomato cv. Moneymaker plants, which received hpRep construct at three days before virus inoculation, remained symptomless until three weeks post inoculation. There was no detection of virus genomic fragment up to this time point (Figure 5B, E). However, when hpRep was co-agroinfiltrated with TYLCV-IL, no symptoms were observed at 21 dpi, but a faint band was observed in one of the five treated plants following PCR detection TYLCV-IL using a TYLCV specific primer pair (Figure 5C, E). Electrophoresis of PCR results on the agarose gel showed a relatively low concentration of virus genome in RNAi treatment (Figure 5E), which could be related to the lack of symptoms in this positive PCR sample at 21 dpi. Positive control plants inoculated with virus alone showed typical TYLCD symptoms at 21 dpi, including leaf curling and yellowing, specifically in upper new emerged leaves (Figure 5D).

Discussion

RNAi plays a vital role against virus infections (Swevers et al., 2018). Over the past

two decades, RNAi has become a powerful tool for studying gene functions in various organisms by delivering gene-specific dsRNA and is now also recognized as a new plant pathogens control tool (Wang et al., 2017), including plant infecting viruses. Several studies have reported that expression of chimeric hairpin dsRNA constructs that target coding sequences of different begomoviruses genes can interfere with infection by stable transformation or transient expression (Ammara et al., 2015; Fuentes et al., 2016; Khatoon et al., 2016). But, there are some concerns regarding the use of plant transformation based RNAi method on human and environment health (Das & Sherif, 2020). Generally, transformative hpRNA constructs showed high protection of plants against geminiviruses. But, high rate of geiminiviruses evolution (Jeske et al., 2001), and related health concerns resulted into the need of more reliable and sustainable approaches for control (Das & Sherif, 2020).

Application of dsRNA, siRNA, and hpRNAs on the surface of plant tissues which referred to as RNA vaccination is a newly developed approach to control plant virus infections. While exogenously application of dsRNAs has been successfully used against many positive and negative plant RNA viruses (Mittter et al., 2017; Petrov et al., 2015; Tabein et al., 2020; Tenllado & Diaz-Ruiz, 2001; Tenllado et al., 2004), efficiency of this approach in protecting plants against various infectious viruses, especially geminiviruses, needs to be further elucidated (Namgial et al., 2019; Melita et al., 2021).

The presented results in this study showed, unlike hpRep construct, *in vitro* synthesized dsRNAs derived from *Rep* and *CP* sequences of TYLCV-IL were inefficient to interfere with infection of the cognate begomovirus species. Symptoms were expressed in treated plants by dsRNAs such as control plants that were agroinoculated with TYLCV-IL alone. Viral genomic fragments were detected in all the treated plants, and qPCR showed no difference in TYLCV-IL genome titer between treatments. Recently, Melita et al. (2021) showed that the topical application of C4 and V2 derived dsRNAs from a mild strain of *Tomato yellow leaf curl virus* (TYLCV-Mld) could reduce disease severity and incidence in tomato plants. Therefore, several factors appear to be involved in efficiency of exogenous applied dsRNAs against begomoviruses infection.

Exogenously applied dsRNAs is a dose dose-dependent mechanism (Dubrovina & Kiselev, 2019; Tenllado & Díaz-Ruíz, 2001). External application of dsRNAs introduces a certain amount of dsRNAs that might not be enough to protect plants against a high rate of replication in geminiviruses. In contrast, the hairpin construct provides a higher dsRNA concentration level by the continuous expression of dsRNA (Gou et al., 2007). Moreover, the induction of resistance by the external application of dsRNAs largely depends on selecting target sequences (Tabein et al., 2020). Recently, it has been shown that dsRNA-mediated vaccination targeting C4 and V2 sequences from TYLCV-Mild can repress the disease incidence and severity of the cognate virus in tomato plants (Melita et al., 2021). Also, targeting non-coding conserved sequences within intergenic regions of begomoviruses by a hpRNA construct represented resistance against a broad spectrum of species (Abhary et al., 2006). These results suggest the need for more studies to investigate the efficiency of RNA vaccination by targeting different sequences of begomoviruses. It must be considered that using different suppressors, including C4 and V2, and nuclear replication in infected host cells (Brown et al., 2012) could protect geminivirus genomes in confronting against RNAi pathway (Hanley-Bowdoin et al., 2013).

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ارزیابی دو رویکرد غیرتراریخت‌سازی در راه‌اندازی تداخل آرانِ‌ای علیه ویروس پیچیدگی برگ زرد گوجه‌فرنگی

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چکیده

سویه شدید ویروس پیچیدگی برگ زرد گوجه‌فرنگی (Tomato yellow leaf curl viru, TYLCV-IL) یک بگوموویروس تک‌بخشی مخرب با گسترش جهانی است. دو روش غیرتراریخت‌سازی، واکسیناسیون آران‌ای دولا و کاربرد سازه سنجاق‌سری بیان‌کننده آران‌ای دولا، به منظور القای تداخل آران‌ای بر علیه ویروس پیچیدگی برگ زرد گوجه‌فرنگی مورد استفاده قرار گرفت. بررسی پروفایل آرانِ‌ای‌های کوچک مداخله‌گر مشتق شده از ژنوم ویروس، دو نقطه داغ شامل قسمت‌هایی از توالی ژن‌های کدکننده پروتئین پوششی و پروتئین همراه با همانندسازی را در راه‌اندازی خاموشی ژن پس از ترانویسی مشخص نمود. آرانِ‌ای‌های دولای مشتق شده از این توالی‌ها در شرایط درون‌شیشه‌ای تولید شدند و همزمان و یا سه روز قبل از مایه‌زنی همسانه عفونت‌زای ویروس، بر روی سطح برگ گیاهان توتون مورد استفاده قرارگرفتند. تمام گیاهان علایم مشخص آلودگی را در زمان ۲۱ روز پس از مایه‌زنی نشان دادند و قطعات ژنومی ویروس در گیاهان دارای علایم توسط واکنش زنجیره‌ای پلی‌مراز تکثیر شد. علی‌رغم حرکت سیستمیک آران‌ای‌های دولایِ مورد استفاده در گیاهان توتون، واکنش زنجیره‌ای پلی‌مراز در زمان حقیقی، هیچ تفاوت آماری معناداری در سطح تجمع ژنوم ویروس در بین تیمارهای مختلف نشان نداد. در رویکرد دوم، کاربرد سازه سنجاق‌سری بیان‌کننده آران‌ای دولا، بروز علایم آلودگی را تا زمان ۲۱ روز پس از مایه‌زنی سرکوب کرد. همچنین ژنوم ویروس در گیاهان فاقد علایم ردیابی نشد که نشان‌دهنده قطع همانندسازی ژنوم ویروس است. این نتایج، کارآیی متمایز دو روش القای تداخل آرانِ‌ای را علیه یک گونه مهم بگوموویروس نشان می‌دهد. نقش عوامل مختلف تأثیرگذار بر کارآیی تداخل آرانِ‌ای علیه ویروس پیچیدگی برگ زرد گوجه‌فرنگی مورد بحث قرار گرفته است.

کلمات کلیدی: ویروس پیچیدگی برگ زرد گوجه‌فرنگی، واکسیناسیون آران‌ای، سازه سنجاق‌سری، تداخل آرانِ‌ای

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