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SHORT COMMUNICATION

vsiRNA18 derived from tobacco curly shoot virus can regulate virus infection in *Nicotiana benthamiana*

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Abstract

Virus-derived small interfering RNAs (vsiRNAs) play important roles in regulating host endogenous gene expression to promote virus infection and induce RNA silencing to suppress virus infection. However, to date, how vsiRNAs affect geminivirus infection in host plants has been less studied. In this study, we found that tobacco curly shoot virus (TbCSV)-derived vsiRNA18 (TvsiRNA18) can regulate TbCSV infection in *Nicotiana benthamiana* plants. The virus-mediated small RNA expression system and stable transformation technique were used to clarify the molecular role of TvsiRNA18 in TbCSV infection. The results indicate that TvsiRNA18 can aggravate disease symptoms in these plants and enhance viral DNA accumulation. *ATP-dependent RNA helicase* (*ATP-dRH*) was proven to be a target of TvsiRNA18, and down-regulation of *ATP-dRH* in plants was shown to induce virus-like leaf curling symptoms and increase TbCSV infection. These results suggest that TvsiRNA18 is an important regulator of TbCSV infection by suppressing *ATP-dRH* expression. This is the first report to demonstrate that TbCSV-derived vsiRNA can target host endogenous genes to affect symptom development, which helps to reveal the molecular mechanism of symptom occurrence after the virus infects the host.

KEYWORDS

ATP-dependent RNA helicase, RNA silencing, tobacco curly shoot virus, virus-derived small interfering RNAs

Plant viruses are obligate intracellular parasites and can cause severe diseases in plants, leading to significant economic losses. Over the course of time, plants have evolved various defence strategies, including hormone-mediated, secondary metabolite-mediated, protein degradation-mediated, and immune receptor-mediated defences, to resist virus infections (Calil & Fontes, 2017). For example, it was shown recently that inhibition of *OsAGO2* expression in rice activates the expression of defence-related genes to enhance reactive oxygen species (ROS)-mediated resistance to rice black-streaked dwarf virus infection (Wang et al., 2021). Induction of endogenous jasmonic acid production in rice can enhance rice resistance to rice ragged stunt virus infection (Zhang et al., 2016). In addition, plants use RNA interference (RNAi)-mediated defence to resist viral infections. During virus infection, viral double-stranded RNAs are cleaved to form virus-derived small interfering RNAs (vsiRNAs) 21 to 24 nucleotides (nt) in length by plant Dicer-like enzymes (Szittya & Burgyán, 2013).

Rui Wu and Gentu Wu contributed equally to the paper.

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These vsiRNAs can bind to Argonaute protein (AGO) in the RNA-induced gene silencing complex (RISC) (Ma & Zhang, 2018) and mediate RISC to cleave viral genome transcripts in a sequence-specific manner (Jin et al., 2021). It was revealed that tomato chlorotic mottle virus (ToCMoV) BA-Se1 isolate-derived vsiRNAs can delay ToCMoV symptom development and suppress ToCMoV DNA accumulation in cells by targeting viral genome transcripts (Ribeiro et al., 2007). vsiRNAs derived from the noncoding intergenic region (IR) or coding regions in the pepper golden mosaic virus (PepGMV) genome have also been shown to reduce the severity of PepGMV-induced symptoms and viral DNA accumulation (Rodríguez-Negrete et al., 2009).

Some studies have shown that vsiRNAs can also cleave specific host mRNAs to induce disease-like symptoms in cucumber mosaic virus Y-satellite-infected tobacco plants (Shimura et al., 2011) or in rice stripe virus-infected *Nicotiana benthamiana* plants (Shi et al., 2016). It is well known that vsiRNAs can affect virus infection by interfering with the expression of host endogenous genes. For example, vsiRNAs produced by the genome of cotton leaf curl Multan virus (CLCuMuV) affect viral infection in cotton plants by regulating the expression of host genes (Wang et al., 2016). vsiRNA derived from the IR of tomato yellow leaf curl virus (TYLCV) can induce symptoms such as stunted and curled leaves by targeting *SlLNR1* in tomato, which is beneficial to the infection of TYLCV to tomato (Yang et al., 2019). The vsiRNA-20 produced by Chinese wheat mosaic virus (CWMV) RNA1 can create a more favourable cell environment for CWMV replication by regulating the accumulation of *TaVP* mRNA in wheat (Yang et al., 2020). vsiRNA1 derived from wheat yellow mosaic virus (WYMV) RNA1 can promote the accumulation of ROS by regulating the expression of wheat thioredoxin-like gene (*TaAAED1*), and then enhance the resistance of wheat to WYMV infection (Liu et al., 2021). So far, there are few reports on the function of vsiRNAs derived from geminiviruses.

Tobacco curly shoot virus is a member of the genus *Begomovirus* in the *Geminiviridae* family and is transmitted in the field by *Bemisia tabaci* (Xie et al., 2002). Tobacco curly shoot virus (TbCSV) infection in tomato and tobacco plants often causes severe leaf curling symptoms and yield reductions (Li et al., 2005; Xie et al., 2002). In the field, some TbCSV isolates are accompanied by beta-satellite DNA, which is named tobacco curly shoot betasatellite (TbCSB) (Li et al., 2005). The TbCSV genome is a circular single-stranded DNA with an IR and six overlapping open reading frames (ORFs) transcribed from opposite directions. The sense-strand viral DNA encodes the V1 and V2 proteins, while the complementary strand viral DNA encodes four proteins: C1, C2, C3, and C4 (Xie et al., 2002). In our recent reports, it was shown that the TbCSV-encoded C3 and V2 proteins are disease symptom determinants (Li et al., 2021; Sun et al., 2020). In TbCSV-infected host plants, the expression of many endogenous genes and microRNAs (miRNAs) altered, and a large number of vsiRNAs were produced (Du et al., 2019; Du et al., 2020; Li et al., 2018; Wu et al., 2019). Compared with previous studies, the function of these vsiRNAs in regulating disease symptoms in TbCSV infected plants has not been reported.

In our previous report, large amounts of vsiRNAs were found in TbCSV-infected *N. benthamiana* plants (Wu et al., 2019). Because tomato is an important host plant of TbCSV in fields, we investigated whether TvsiRNA18, derived from the TbCSV *V1* gene, was also produced in TbCSV-infected tomato plants. Tomato cv. Micro-Tom seeds were planted in pots inside a growth chamber maintained at 24–26°C with a 16 h light/8 h dark photoperiod. At 20 days post-inoculation (dpi) with TbCSV, small RNAs were extracted from the leaves of the TbCSV-infected or mock-inoculated plants using RNAiso reagent (Takara) followed by reverse transcription using TranScript miRNA First-Strand cDNA Synthesis SuperMix (Transgen Biotech). PCR was then carried out using TransTaq high fidelity DNA polymerase (Transgen Biotech). The resulting products were extracted, purified, and then cloned into the pGEM-T-Easy vector prior to sequencing. Similar leaf curling symptoms were discovered in tomato plants and *N. benthamiana* plants inoculated with TbCSV (Figure S1a,b). Analysis of TvsiRNA18 (100 bp) expression in the TbCSV-infected and mock-inoculated tomato plants through reverse transcription (RT)-PCR showed that TvsiRNA18 accumulated in the TbCSV-infected tomato plants but not in the mock-inoculated plants (Figure S1c,d).

To explore whether TvsiRNA18 can play a role in virus infection, an expression vector overexpressing TvsiRNA18 (pCVA-TvsiRNA18) was constructed. TvsiRNA18 was inserted into the pGEM-T-Easy vector (Promega), propagated in *Escherichia coli* DH5a cells, sequenced, and then cloned between the *Kpn*I and *Xba*I cloning sites in the pCVA vector (pCVA and pCVB are cabbage leaf curl virus DNA-A- and DNA-B-based expression vectors) (Tang et al., 2010). The construction of this vector mainly follows the pCVA-mediated small RNA expression system devised by Professor Yule Liu at Tsinghua University (Tang et al., 2010), and our previous research proved that this system can stably express a single small RNA (Wu et al., 2021). Plasmids pCVA and pCVB (pCVA+pCVB) or pCVA-TvsiRNA18 and pCVB (pCVA-TvsiRNA18+pCVB) were co-inoculated into *N. benthamiana* plants through agro-infiltration. Compared to the pCVA+pCVB-inoculated *N. benthamiana* plants (control), the pCVA-TvsiRNA18+pCVB-inoculated *N. benthamiana* plants showed the symptom of leaf curl (Figure 1a). The results showed that TvsiRNA18 was involved in the formation of leaf curling symptoms after virus infection (Figure 1a). At the same time, semiquantitative RT-PCR results showed that there was no significant difference in TvsiRNA18 expression level between pCVA-TvsiRNA18-inoculated plants and TbCSV-inoculated plants (data not shown). Our previous studies have confirmed that after TbCSV infection, the expression of miR1919c-5p and miR167b-3p was down-regulated, and silencing of the two miRNAs could induce the phenotype of leaf curling in *N. benthamiana* (Du et al., 2020; Wu et al., 2021). Based on the above results, we speculate that the stronger leaf curling symptoms were observed in plants inoculated with TbCSV alone, in part because the expression of miRNAs changed after virus infection. Then, TbCSV was inoculated into pCVA+pCVB-infected or pCVA-TvsiRNA18+pCVB-infected *N. benthamiana* plants. The results showed that the pCVA+pCVB+TbCSV-inoculated *N. benthamiana*

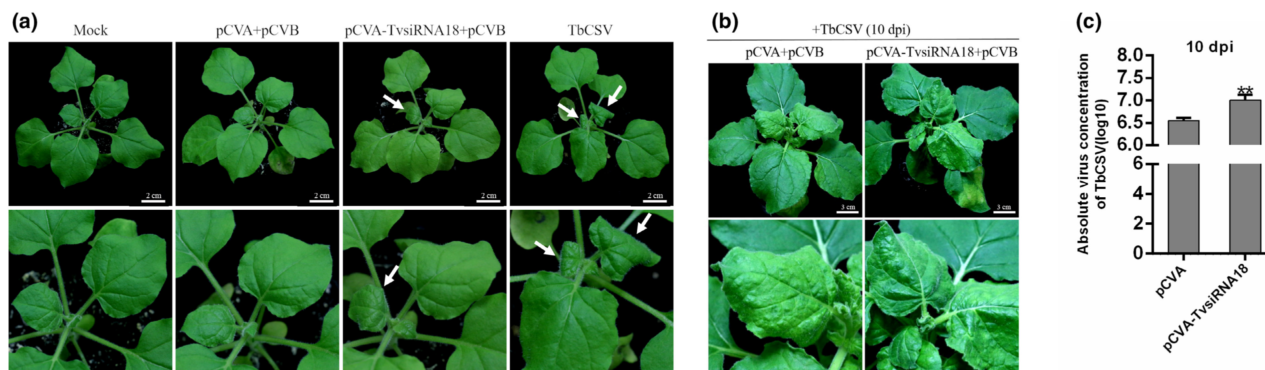

FIGURE 1 Expression of TvsiRNA18 in *Nicotiana benthamiana* plants causes virus-like leaf curling symptoms and enhances the symptoms induced by TbCSV infection. (a) pCVA-mediated TvsiRNA18 expression in *N. benthamiana* plants causes virus-like leaf curling symptoms. The leaves showing curling symptoms are indicated with white arrows. (b) TbCSV was inoculated into pCVA-TvsiRNA18 + pCVB-inoculated or pCVA + pCVB-inoculated plants. The leaf curling symptoms on the pCVA-TvsiRNA18 + pCVB + TbCSV-inoculated plants were much stronger. (c) Quantitative PCR analysis of TbCSV DNA accumulation in the systemic leaves of the assayed plants shown in (b) at 10 days postinoculation (dpi). Statistically significant differences between the two treatments (**$p < 0.01$) were determined using a Student's *t* test.

plants developed moderate leaf curling symptoms, while the pCVA-TvsiRNA18 + pCVB + TbCSV-inoculated *N. benthamiana* plants developed strong leaf curling symptoms (Figure 1b). The accumulation of TbCSV DNA was detected through quantitative PCR (qPCR) using a NovoStart SYBR qPCR Super Mix Plus kit as instructed by the manufacturer (NovoProtein) on a CFX 96 real-time PCR machine (Bio-Rad). qPCR analyses showed that the absolute accumulation level of TbCSV DNA in the pCVA-TvsiRNA18 + pCVB + TbCSV-inoculated plants was significantly higher than that in the pCVA + pCVB + TbCSV-inoculated plants (Figure 1c). These results indicated that the expression of TvsiRNA18 exacerbates symptoms and enhances viral accumulation.

To further investigate the effect of TvsiRNA18 on host plant growth and development, transgenic *N. benthamiana* plants expressing TvsiRNA18 were generated. TvsiRNA18 was cloned into the *Arabidopsis* miR319a precursor in an expression vector to produce pCV-TvsiRNA18. After sequencing, the expression vector was transformed into *N. benthamiana* by the BIOGLE GeneTech Company (Changzhou, China) to produce transgenic *N. benthamiana* plants (referred to as TvsiRNA18-T_0). Genomic DNA and small RNAs were extracted from the TvsiRNA18-T_0 plants and screened through PCR. The results showed that all assayed TvsiRNA18-T_0 transgenic plants contained the expression vector (Figure S2a). Further analysis of small RNA samples through RT-PCR showed that TvsiRNA18-T_0 lines 14, 21, and 22 expressed TvsiRNA18 (Figure S2b). We then collected seeds from plants from the TvsiRNA18-T_0 lines 14, 21, and 22 to produce TvsiRNA18-T_1 lines. The results of the RT-PCR assay confirmed that these three TvsiRNA18-T_1 lines expressed TvsiRNA18 (Figure S2c,d). Analysis of TvsiRNA18-T_1 and wild-type (WT) *N. benthamiana* plants grown inside a greenhouse showed that the growth of the TvsiRNA18-T_1 transgenic plants was delayed compared to that of the WT *N. benthamiana* plants (Figure S2e). TbCSV was then inoculated into TvsiRNA18-T_1 and WT *N. benthamiana* plants. At 14 days postinoculation (dpi), the TbCSV-inoculated TvsiRNA18-T_1 transgenic plants developed stronger leaf curling symptoms than the TbCSV-inoculated WT plants (Figure 2a). qPCR results confirmed that the accumulation of TbCSV DNA in TvsiRNA18-T_1 transgenic plants was 10^{10} readings, while that of TbCSV DNA in WT control plants was only $10^{9.5}$ readings (Figure 2b). Further results showed that the seed germination rate, plant height, and seed-setting rate of the TvsiRNA18-T_1 transgenic plants were significantly reduced compared to those of the WT control plants. In addition, the flowering time of the TvsiRNA18-T_1 transgenic plants was delayed compared to that of the WT control plants (Figure 2c–f). These results confirmed that the expression of TvsiRNA18 in plants can inhibit plant growth and development and repress plant resistance to TbCSV infection.

It is well known that vsiRNAs can interfere with host gene expression (Liu et al., 2021; Shen et al., 2020; Yang et al., 2020). To further elucidate the molecular roles of TvsiRNA18 in plant growth and development and in TbCSV infection, we predicted its target genes in *N. benthamiana* using the psRNATarget website tool (http://plantgrn.noble.org/psRNATarget/) with the maximum expected value parameter set at 2.5. The complementarity score was set to 23 bp, and the target accessibility was set to 20.0. Two potential TvsiRNA18 target genes (Niben101Scf07322g00014.1 and Niben101Scf03009g05001.1) in *N. benthamiana* were found (Table S2), and the relative expression of these target genes was analysed through RT-quantitative real-time PCR (RT-qPCR). The results showed that the expression level of Niben101Scf07322g00014.1 was significantly down-regulated in the TbCSV-infected plants (Figure 3a). This gene encodes an ATP-dependent RNA helicase (ATP-dRH). Consistent with the above results, the expression of *ATP-dRH* was also significantly down-regulated in the pCVA-TvsiRNA18-inoculated *N. benthamiana* plants (Figure 3b), as well as in the leaves of *N. benthamiana* plants transiently expressing TvsiRNA18 (pCV-TvsiRNA18) (Figure 3c). However, there was no change in the expression level of another gene (Niben101Scf03009g05001.1) in the

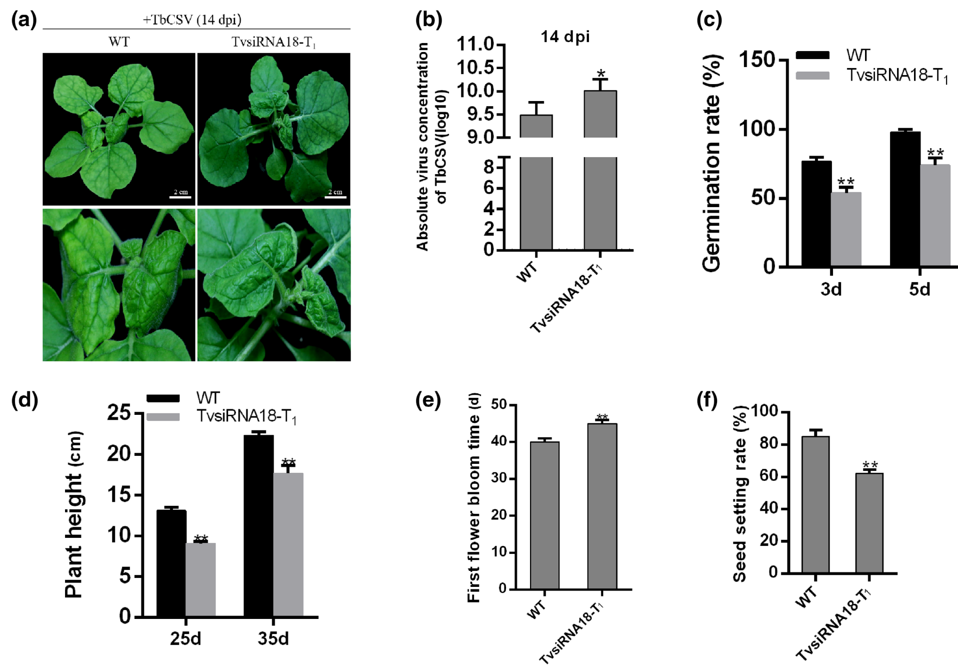

FIGURE 2 TbCSV infection in TvsiRNA18-T_1 transgenic and nontransgenic (wild-type, WT) *Nicotiana benthamiana* plants. (a) TbCSV-induced disease symptoms in the TvsiRNA18-T_1 transgenic and WT *N. benthamiana* plants at 14 days postinoculation (dpi). (b) Assessment of TbCSV DNA accumulation in the systemic leaves of the plants assayed through quantitative PCR. (c) Seed germination rates of the TvsiRNA18-T_1 transgenic and WT *N. benthamiana* plants at 3 and 5 days postgermination. (d) Heights of the TvsiRNA18-T_1 transgenic and WT *N. benthamiana* plants at 25 and 35 days posttransplantation. (e) Flowering times of the TvsiRNA18-T_1 transgenic and WT *N. benthamiana* plants. (f) Seed setting rates of the TvsiRNA18-T_1 transgenic and WT *N. benthamiana* plants. Statistically significant differences between the two treatments (*$p < 0.05$, **$p < 0.01$) were determined using a Student's *t* test.

N. benthamiana plants overexpressing TvsiRNA18 compared with the control plants (data not shown).

To further confirm that *ATP-dRH* is a target gene of TvsiRNA18, a pCV-ATP-dRH::GFP vector was constructed by using primers ATP-dRH-yF and ATP-dRH-yR (Table S1) to amplify the 603 bp *ATP-dRH* sequence (including shear site), double digested with the restriction enzymes *Xba*I and *Kpn*I, and cloned into the pCV-GFP-N1 expression vector (this vector was modified and presented by Professor Fei Yan at Ningbo University; Lu et al., 2011). vsiRNA8 (5′-AGTGT TTTCTAGTCTACCGAA-3′), another vsiRNA derived from TbCSV, served as a control here. The pCV-TvsiRNA8 vector was constructed in our laboratory (authors' unpublished data). The resulting plasmid was transformed into *Agrobacterium tumefaciens* GV3101, propagated, and then infiltrated onto *N. benthamiana* leaves, which were examined under a confocal laser scanning microscope as described previously (Cao et al., 2005; Li et al., 2016). The results showed that the leaves co-infiltrated with pCV-TvsiRNA8 + pCV-GFP, pCV-TvsiRNA18 + pCV-GFP and pCV-TvsiRNA8 + pCV-ATP-dRH::GFP showed strong green fluorescent protein (GFP) fluorescence, while the leaves co-infiltrated with pCV-TvsiRNA18 + pCV-ATP-dRH::GFP showed weak GFP fluorescence (Figure 3d). The expression level of fusion GFP in the infiltrated leaves was analysed through a western blot assay. Total protein was extracted from the infiltrated *N. benthamiana* leaf samples, homogenized in 6× protein loading buffer (TransGen Biotech), and boiled for 10 min, and the protein samples were separated in a Speedy Polyacrylamide gel electrophoresis kit (Cofitt). After transferring protein bands to nitrocellulose membranes, the blots were probed using an anti-GFP antibody (Li et al., 2021). The results showed that the accumulation of ATP-dRH::GFP fusion protein in the leaves co-infiltrated by pCV-TvsiRNA18 + pCV-ATP-dRH::GFP was 0.61 times that the accumulation of GFP protein in the leaves co-infiltrated by pCV-TvsiRNA18 + pCV-GFP. However, there was no significant difference between the accumulation of ATP-dRH::GFP fusion protein in the leaves co-infiltrated by pCV-TvsiRNA8 + pCV-ATP-dRH::GFP and the accumulation of GFP in the leaves co-infiltrated by pCV-TvsiRNA8 + pCV-GFP (Figure 3e), indicating that *ATP-dRH* mRNA is indeed the target of TvsiRNA18.

To investigate the role of *ATP-dRH* in TbCSV infection in plants, we conducted virus-induced gene silencing (VIGS) assays using a tobacco rattle virus (TRV)-based VIGS vector. For VIGS assays, a 300 bp fragment, representing a partial sequence of *ATP-dRH*, was amplified by RT-PCR using primers ATP-dRH(s)-F and ATP-dRH(s)-R (Table S1) and cloned into the TRV-based VIGS vector to produce TRV-ATP-dRH. The TRV-based vector carrying a fragment from the β-glucuronidase (*GUS*) gene was used as the control. After inoculation of these vectors into *N. benthamiana* plants through agroinfiltration, the TRV-ATP-dRH-inoculated plants developed moderate leaf curling symptoms, but no symptoms were observed in the TRV-GUS-inoculated plants at 10 dpi (Figure 4a). The RT-qPCR results showed that the expression level of *ATP-dRH* in the TRV-ATP-dRH-inoculated plants was significantly down-regulated compared to that in the TRV-GUS-inoculated control plants (Figure 4b). TbCSV

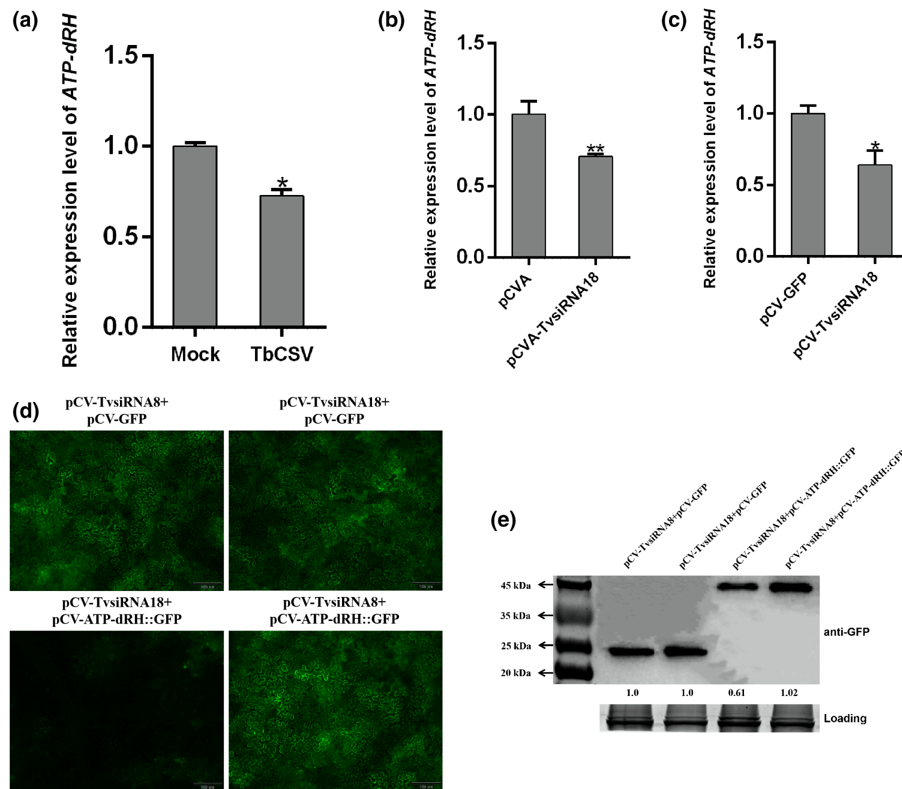

FIGURE 3 TvsiRNA18 targets *Nicotiana benthamiana ATP-dRH* for degradation. (a) The expression level of *ATP-dRH* was down-regulated in TbCSV-infected *N. benthamiana* plants. (b) The expression level of *ATP-dRH* was also down-regulated in the TvsiRNA18-expressing leaves. (c) The expression level of *ATP-dRH* was down-regulated in the leaves transiently expressing TvsiRNA18. Statistically significant differences between the two treatments (*$p < 0.05$, **$p < 0.01$) were determined using a Student's *t* test. (d) Co-infiltrated leaves were harvested at 2 days postinoculation and examined for green fluorescence under a confocal laser scanning microscope. (e) Detection of green fluorescent protein (GFP) and ATP-dRH::GFP fusion protein in the assayed *N. benthamiana* leaves through a western blot assay using a GFP-specific antibody.

was then inoculated into ATP-dRH-silenced and nonsilenced (TRV-GUS) *N. benthamiana* plants. The results showed that the TRV-ATP-dRH + TbCSV-inoculated plants developed stronger leaf curling symptoms than the TRV-GUS + TbCSV-inoculated plants at 14 dpi (Figure 4c). RT-qPCR analysis showed that the expression level of *ATP-dRH* in the TRV-ATP-dRH + TbCSV-inoculated plants was significantly reduced compared to that in the TRV-GUS + TbCSV-inoculated control plants (Figure 4d). In contrast, the accumulation level of TbCSV DNA in the TRV-ATP-dRH + TbCSV-inoculated *N. benthamiana* plants significantly increased compared to that in the TRV-GUS + TbCSV-inoculated plants (Figure 4e). Consequently, we concluded that TvsiRNA18 suppresses *ATP-dRH* expression in plants to promote TbCSV infection.

Because pathogen-derived siRNAs can move in plants and silence host gene expression, many reports have termed siRNAs "siRNA effectors" to describe their functions (Ming et al., 2015; Weiberg et al., 2015). These siRNAs include those found in *Botrytis cinerea*-infected tomato and *Arabidopsis* plants (Weiberg et al., 2013), in nematodes and mammalian cells (Buck et al., 2014), and in *E. coli* and *Caenorhabditis elegans* (Liu et al., 2012). In this study, we also found that TvsiRNA18 can regulate host gene expression. To further investigate how TvsiRNA18 affects TbCSV infection in plants, we constructed a pCV-TvsiRNA18 expression vector and used it to stably transform *N. benthamiana*. Analyses showed that the growth and development of these TvsiRNA18 transgenic plants were clearly retarded, but the infection of TbCSV in these plants was enhanced. To date, vsiRNA-induced host susceptibilities have been reported in DNA and RNA virus-infected plants. For example, a large number of vsiRNAs were produced after CLCuMuV infected cotton, which affected the virus infection of cotton plants by targeting host transcripts (Wang et al., 2016). The expression of TYLCV-derived vsiRNA can inhibit the expression of *SlLNR1* in tomato and then enhance the symptoms and accumulation of virus-infected tomato plants (Yang et al., 2019). Recently, it was reported that vsiRNA-20 from CWMV genomic RNA can affect the expression of *TaVP* in wheat to boost CWMV infection (Yang et al., 2020). By silencing the wheat chlorophyll synthase gene, barley yellow dwarf virus-derived vsiRNA8856 causes leaves to turn yellow and aggravates symptoms after virus infection (Shen et al., 2020). In this study, we found that the expression of the *ATP-dRH* gene in *N. benthamiana* was significantly suppressed by TbCSV infection or TvsiRNA18 expression, resulting in stronger leaf curling symptoms. Thus, this evidence suggested that TvsiRNA18 enhances TbCSV infection in host plants by targeting *ATP-dRH* expression.

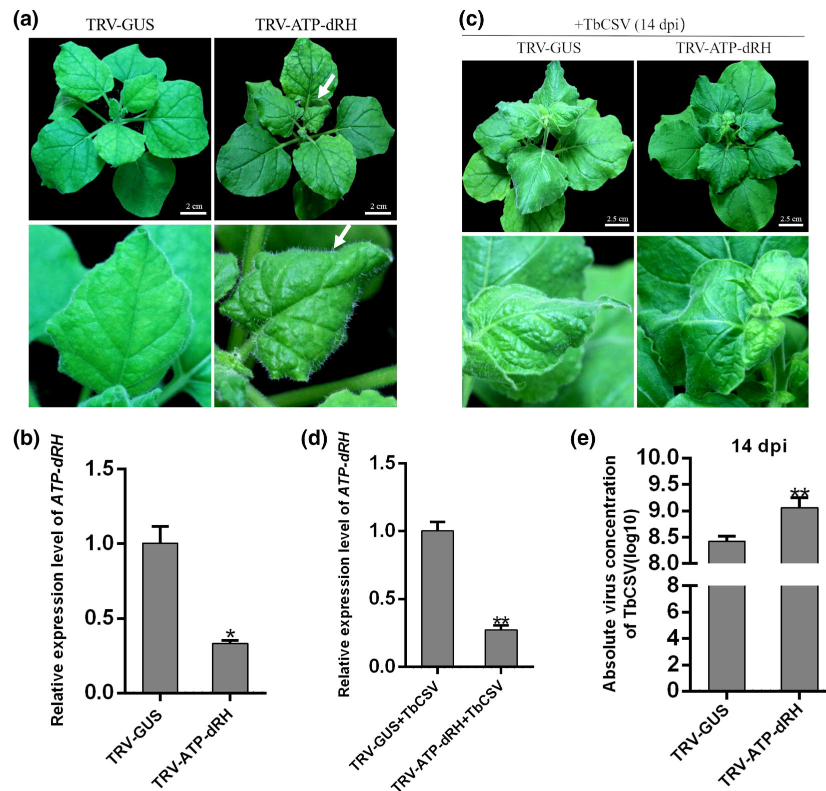

FIGURE 4 Effect of *ATP-dRH* on TbCSV infection in *Nicotiana benthamiana* plants. (a) A tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) vector (TRV-ATP-dRH) was used to silence *ATP-dRH* expression in *N. benthamiana* plants. Plants inoculated with TRV-GUS were used as controls. The plants were photographed at 10 days postinoculation (dpi). The leaves showing curling symptoms are indicated with white arrows. (b) The expression level of *ATP-dRH* in the TRV-ATP-dRH-inoculated or TRV-GUS-inoculated plants was determined through reverse transcription-quantitative PCR (RT-qPCR) at 10 dpi. (c) Symptoms after TbCSV was inoculated onto TRV-ATP-dRH-inoculated or TRV-GUS-inoculated plants. (d) The expression level of *ATP-dRH* in the TRV-ATP-dRH + TbCSV-inoculated or TRV-GUS + TbCSV-inoculated plants was determined through RT-qPCR at 14 dpi. (e) The accumulation level of TbCSV DNA in the systemic leaves of the TRV-ATP-dRH + TbCSV-inoculated or TRV-GUS + TbCSV-inoculated plants was determined through qPCR at 14 dpi. Statistically significant differences between the two treatments (*$p < 0.05$, **$p < 0.01$) were determined using a Student's *t* test.

DEAD-box RNA helicases are a large protein family in eukaryotes and most prokaryotes (Cruz et al., 1999; Rocak & Linder, 2004). Genes related to DEAD-box RNA helicases have been found in many plants, and some studies have reported their biological functions in plants. For example, many DEAD-box RNA helicases have been shown to regulate key steps in plant embryo development, plastid function, and floral meristem formation (Jacobsen, 1999; Kobayashi et al., 2007; Wang et al., 2000). DEAD-box RNA helicases have also been shown to regulate plant defence against environmental stresses (Vashisht & Tuteja, 2006) by targeting various signalling pathways. For example, *Arabidopsis* LOS4 has been shown to play important roles in the host response to cold and heat stresses (Gong et al., 2002, 2005), and STRS1 and STRS2 function as negative regulators of many abiotic stress signalling pathways (Kant et al., 2007). To date, how DEAD-box RNA helicases regulate plant responses to virus infections is largely unknown.

TbCSV infection causes severe damage to many important crops (Qing et al., 2010; Xie et al., 2002). Because our current knowledge on host resistance to TbCSV infection and disease symptom development is limited, current control of TbCSV is less effective. It was found that there was a 14-nucleotide deletion in the complementary region between the *SlLNR1* gene and TYLCV-derived vsiRNA in a disease-resistant tomato variety that greatly weakened the sensitivity of the tomato to TYLCV infection (Yang et al., 2019). Our study revealed that TvsiRNA18 can inhibit *ATP-dRH* expression in plants to promote viral DNA accumulation. Therefore, in the future, it might be possible to use gene editing technology to modify the target genes of vsiRNAs, which can help the plant escape the attack by vsiRNAs, and thus plant resistance to viral infection may be improved.

AUTHOR CONTRIBUTIONS

R.W. and G.T.W. carried out the main experiments, data processing and analysis, and developed the research programme. Y.J.H. participated in vector construction and symptom observation. H.L.Z. and J.X.T. collected the samples and extracted nucleic acid. M.J.L. provided advice for the research plan. L.Q. conceived the study and revised the manuscript. All the authors contributed to this article and approved the submitted version.

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further enquiries can be directed to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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