Spatio-temporal expression of miRNAs in tomato tissues upon *Cucumber mosaic virus* and *Tomato aspermy virus* infections

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MicroRNAs (miRNAs) play vital roles in regulating plant growth and development. Recent work has shown that miRNA-mediated regulation of cellular mRNA expression is involved in pathogen–host interactions. However, knowledge about the timing and spatial regulation of plant miRNA expression is still limited. Here, we use stem-loop real-time reverse transcription–polymerase chain reaction to quantify the expression changes of seven miRNAs and their target mRNAs in different tomato tissues during the pathogenic processes. Compared with mock-inoculated plants, the expression levels of investigated miRNAs and mRNAs were enhanced by different degrees upon *Cucumber mosaic virus* (CMV)-Fny and *Tomato aspermy virus*-Bj infections, but were almost unchanged in CMV-FnyD²b (a CMV-Fny 2b-deletion mutant)-infected tomato seedlings. In addition, the obvious up-regulation of several miRNAs and target mRNAs in some tomato tissues suggested their special roles in these tissues’ organogenesis and development. Temporal analyses also revealed that the expressions of these miRNAs and mRNAs were highly regulated by different viral infections. Taken together, the observed spatially and temporally changes in miRNAs and target mRNAs expression levels indicate the abilities of different viruses to interfere with miRNA pathway, and are correlated with their respective functions in phenotype determination in different tomato tissues.

Keywords Solanum lycopersicum; *Cucumber mosaic virus*; *Tomato aspermy virus*; tomato miRNA; gene expression

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Introduction

Symptoms development upon systemic virus infection is regulated by complex interactions between the pathogen and the host plant. The discovery of microRNAs (miRNAs) and small interfering RNAs (siRNAs) in recent years has significant impact on our understanding of this developmental process [1,2]. miRNAs are short (21–24 nt), single-stranded RNA molecules derived from transcripts of endogenous plant loci [3]. Mature miRNAs can target plant mRNAs for degradation or translational repression in a sequence-specific manner. siRNAs are another type of small RNAs (21 nt) that are generated from endogenous aberrant double-stranded RNAs or from exogenous agents such as viruses. It is thought that the primary function of siRNAs is to detect and eliminate exogenous virus invaders, also by sequence-specific RNA degradation [4]. Recent data indicated that the miRNA-mediated gene expression regulation and antiviral siRNA activity share many components such as Dicer and RNA-induced silencing complex, and appear to run along similar pathways [5–7]. Therefore, viral infections are likely to affect cellular gene expression by perturbing miRNA pathway and usually result in developmental abnormalities [8,9].

We have previously reported the expression changes of several miRNAs and their corresponding target mRNAs in tomato leaf tissues upon *Cucumber mosaic virus* (CMV) and *Tomato aspermy virus* (TAV) infections, using stem-loop real-time reverse transcription-polymerase chain reaction (RT–PCR) [10]. However, several publications have also reported the developmental anomalies induced by CMV in other plant tissues such as roots, shoot apical meristem (SAM), and floral organs [11,12]. In addition, tissue-specific viral silencing suppressor effects have been observed previously [12,13]. Thus, the spatio-temporal characterization of miRNA and target mRNA expression is essential for a better understanding of the correlation between virus infection, miRNAs expression, and symptoms induction.

Here, the wild-type CMV-Fny, a mutant CMV-FnyΔ2b (CMV-Fny with the 2b coding sequence deleted), and a natural isolate of TAV-Bj were inoculated on tomato seedlings. At different stages of infection, the expression levels of seven miRNAs and five target mRNAs were quantified in tomato leaves, roots, shoot apices, and stem tissues of virus- or mock-
inoculated plants. By analyzing their expression profiles, we found that tomato miRNA pathways were disrupted by different degrees in these tissues upon CMV-Fny and TAV-Bj infections, but not in CMV-FnyΔ2b-infected tomato seedlings. Moreover, the results also revealed that the spatial and temporal expression of these miRNAs and target mRNAs was highly regulated, and might be closely related with their respective roles in coordinating developmental events.

Materials and Methods

Plants and viruses
Seedlings of tomato (Solanum lycopersicum cv. Hezuo903) were grown in a greenhouse at 14 h light/10 h dark (22−28°C). CMV-Fny, the typical strain of CMV subgroup IA, was obtained by in vitro transcription of infectious cDNA clones pFny109, pFny209, and pFny309 (GenBank accession Nos.: D00356, D00355, and D10538, respectively). CMV-FnyΔ2b, a mutant of CMV-Fny in which the 2b open-reading frame was completely deleted, was constructed as previously described [14]. TAV-Bj was isolated from chrysanthemum in Beijing, China, and confirmed by ELISA (Agdia, Elkhart, USA). All of the viruses were maintained on Nicotiana tabacum, and transferred 4−6 days before mechanically inoculating the first true leaves of 15-day-old tomato seedlings. Mock-treated plants were inoculated with phosphate buffer (0.1 M, pH 7.2).

RNA extraction and real-time quantitative RT−PCR
At 7, 14, 21 and 28 days post-inoculation (dpi), 0.2 g fresh tissues were sampled systemically from the upper leaves, roots, stems, and shoot apices of virus- or mock-inoculated plants. Total RNAs were extracted using TRIzol reagent (Invitrogen, Carlsbad, USA), followed by RNase-free DNase treatment (Takara, Dalian, China), and finally resuspended in 30 μl of diethyl pyrocarbonate-treated water.

To quantify the effects of viral infections on expression of miRNAs and target mRNAs by real-time RT−PCR, gene-specific primers were designed using Primer Express version 2.0 (Applied Biosystems, Foster City, USA). For miRNAs, their mature sequences were downloaded from miRNA Registry database (http://miRNA.sanger.ac.uk), and the strategy of stem-loop real-time RT−PCR was adopted with some modifications [15]. Their stem-loop RT primers, forward primers, and reverse primers were designed according to the criteria mentioned in previous work [15,16]. For target mRNAs, the design of conventional forward and reverse primers was based on sequences obtained from clones of ARF8, AGO1-1, and AGO1-2 [10], or from public databases (MYB: AY131230 and AY131231, SCL: DQ087265). All primer pairs were optimized and validated as per our previous work [10], and summarized in Tables 1 and 2.

Then, the total RNAs from 0.2 g tomato tissues were reverse transcribed to cDNAs using stem-loop RT primers for miRNAs, or random primers (6-mer; Takara) for target mRNAs. The cDNA of reference gene (18S rRNA) was synthesized by gene-specific primers in miRNA quantification, and by random primers in mRNA quantification. The reverse transcription procedure was performed as follows: a 1.0 μl aliquot of DNase-treated total RNA was inoculated with 1.0 μl of solution containing 10 μM of each primer, and then the mixture was heated at 80°C for 5 min to denature the RNA and incubated at 60°C for 5 min to anneal the primers. After cooling to room temperature, the remaining reagents (5× buffer, dNTPs, RNase inhibitor, and M-MLV) were added according to the experimental protocol and the pulsed RT reaction proceeded for 30 min at 16°C, followed by 60 cycles at 20°C for 30 s, 42°C for 30 s, and 50°C for 1 s [15]. Finally, the reaction

Table 1 List of primer sequences for tomato mRNAs quantification in this study

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer sequence (5′→3′)</th>
<th>Related mRNA</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYB</td>
<td>TATTTGAGATGCAGAAGACTTG (F)</td>
<td>miR159</td>
<td>AY131230</td>
</tr>
<tr>
<td></td>
<td>ATCTGTTGCTCTGTAATCTTT (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARF8</td>
<td>TGGGAAAGGAGGCTGAAA (F)</td>
<td>miR167</td>
<td>FJ222762</td>
</tr>
<tr>
<td></td>
<td>GCATCCAAGAGTCGATT (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGO1-1</td>
<td>TGGATCAGTAACAGCGAGG (F)</td>
<td>miR168</td>
<td>FJ222763</td>
</tr>
<tr>
<td></td>
<td>TGTAGAGAGGAGAGTTCAACAG (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGO1-2</td>
<td>ATGAACTGAGCCAGACTGACG (F)</td>
<td>miR168</td>
<td>FJ222764</td>
</tr>
<tr>
<td></td>
<td>CCTTACGGGCAACACACCC (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCL</td>
<td>GATGGAGCTATGGTGTTGGATG (F)</td>
<td>miR171</td>
<td>DQ087265</td>
</tr>
<tr>
<td></td>
<td>CACCAGGCTGCTTTTTCG (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18S rRNA</td>
<td>GAGTCATCAGCCTCGCGTTGAC (F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CGGATCATCTCACTCGGTAGGA (R)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*F, forward primer; R, reverse primer.*
mixtures were heated at 85°C for 5 min to inactivate the reverse transcriptase.

Subsequently, the SYBR Green PCR was performed on an Applied Biosystem’s 7300 Sequence Detection System. PCR volumes were set to 10 μl that contained 5 μl of 2× SYBR Green PCR master mix (Takara), 1 μl of the cDNA template (1:10 dilution), and 800 nM each of the corresponding forward and reverse primers (Tables 1 and 2). The cycling profile was 95°C for 10 s, followed by 40 or 50 cycles of 10 s at 95°C, and 30 s at 60°C. All reactions were performed in triplicate, and the controls without template or reverse transcription were included for each gene. Immediately after the final PCR cycle, a melting curve analysis was done to determine the specificity of each reaction. The threshold cycle (CT) values were determined automatically by the instrument, and the fold changes of each gene were calculated as relative quantity (RQ) values using the comparative CT method [10,17].

### Results

**Symptom expression in tomato plants upon CMV-Fny, CMV-FnyΔ2b, and TAV-Bj infections**

In tomato seedlings (cv. Hezuo903), different phenotypes were developed upon CMV-Fny, CMV-FnyΔ2b, and TAV-Bj infections (Fig. 1). CMV-Fny infection severely altered leaf morphogenesis, inducing systemic mosaic, typical reduction of leaflet blades and of whole-plant growth. Furthermore, the CMV-Fny-infected plants exhibited apparently shorter main roots, as well as significantly less lateral roots per centimeter of main root compared with mock-inoculated plants. CMV-FnyΔ2b induced slight mottle at early stages of infection, but then led to a general growth promotion, with increased plant height, leaf area, main root length, and number of lateral root than mock-inoculated plants. TAV-Bj infection produced severe leaf mosaic, stunting, and obvious shortening of internodal distances. Moreover, TAV-Bj also induced significant stunting of main roots and an apparent decrease in the number of lateral roots initiated. When the disease severity was compared, TAV-Bj was the most aggressive and CMV-Fny was less aggressive, whereas CMV-FnyΔ2b had promotion effects on tomato growth in our study.

At 10, 20, and 30 dpi, the accumulation of viral RNAs in leaves of CMV-Fny- and CMV-FnyΔ2b-infected tomato was monitored (Supplementary Table 1), and results demonstrated that the levels of viral RNAs in CMV-FnyΔ2b infection were extremely low throughout the detection time points.
Accumulation levels of miRNAs are differentially altered in virus-infected tomato tissues

To investigate the interference of viral infection with miRNA pathways in different tomato tissues, the expression levels of miR159, miR162, miR164, miR165/166, miR167, miR168, and miR171 in virus- or mock-inoculated plants were quantified at 10, 20, and 30 dpi, using stem-loop real-time RT–PCR (Fig. 2). Some of the quantification results were further confirmed by northern hybridization (Supplementary Fig. S1).

In the upper systemic leaves, CMV-Fny induced the most significant alteration of miRNAs expression at 10 dpi, demonstrated by the ~4–5 folds increases in miR159, miR162, and miR168 levels. However, the higher levels of miR159 and miR162 in CMV-Fny-infected plants were gradually decreased at 20 and 30 dpi, whereas miR168 remained at relatively constant levels. In TAV-Bj-infected plants, the abundance of miR162 and miR168 was most apparently increased at 20 and 30 dpi, respectively, with RQ values of 3.31 and 4.45 [Fig. 2(A)].

In tomato roots, the expression levels of miR165/166 and miR168 in CMV-Fny-infected plants were notably increased to 3.3 folds compared with those of mock infection at 10 dpi, and kept at relatively higher levels throughout the detection time points (RQ between 3.12 and 6.13). In addition, the levels of miR159 and miR164 were also gradually increased at 20 dpi and achieved their maximum levels at 30 dpi. In TAV-Bj infection, the abundance miR165/166 was most significantly altered at 10 dpi (RQ = 2.93), whereas at 20 dpi miR168 was most significantly altered (RQ = 4.6). At 30 dpi, the expression levels of miR159, miR164, and miR168 in TAV-Bj infection were higher than in mock, but obviously lower than those of in CMV-Fny-infected plants [Fig. 2(B)].

In shoot apices, the abundance of most miRNAs (except for miR167 and miR171) in CMV-Fny infection was only slightly up-regulated (RQ between 1.22 and 1.93) at 10 dpi, but in TAV-Bj-infected plants they were increased to 1.85–2.50 folds higher than in mock infection. At 20 and 30 dpi, the expression levels of miR162 were significantly increased in both infections (RQ between 2.04 and 4.82) [Fig. 2(C)].

In stem tissues, the abundance of most miRNAs was almost unchanged at 10 dpi except for miR162, miR167, and miR168 in CMV-Fny infection (~RQ = 1.7), and miR159 and miR162 in TAV-Bj infection (~RQ = 1.5). However, the expression levels of miR165/166, miR167, and miR168 in CMV-Fny infection and miR162 in TAV-Bj infection were notably increased at 20 dpi (RQ between 2.53 and 3.49). At 30 dpi, the abundance of miR168 in CMV-Fny infection, as well as miR162 and miR168 in TAV-Bj infection were up-regulated by ~2.5 folds, whereas the levels of other miRNAs were almost unchanged (RQ < 1.5) [Fig. 2(D)].

Generally, we found that the expression levels of tested miRNAs were altered with evident strain-specific differences. Compared with mock-inoculated plants, CMV-Fny and TAV-Bj induced higher accumulation levels of most tested miRNAs, whereas only slightly alterations were observed in CMV-FnyΔ2b-infected plants. Furthermore, the miRNAs abundance in leaves and roots was more severely altered than in shoot apices and stem tissues. Besides these, we found that miR159 was particularly up-regulated in leaf and root tissues of CMV-Fny- or TAV-Bj-infected plants. miR167 was specially over-accumulated in stems of virus-infected plants, whereas miR164 was over-accumulated in all of the tested tissues except for stem. miR162, miR165/166, and miR168 showed continuous up-regulation in all tissues of virus-infected plants, but miR171 levels were almost unchanged throughout the detection time points compared with mock infection.

The levels of miRNA-regulated transcripts are affected differentially by the three viruses

Next, the transcript levels of AGO1, ARF8, MYB, and SCL, as targets of miR168, miR167, miR159, and miR171, were...
also quantified using the same RNA preparations, respectively.

In tomato leaves, both CMV-Fny and TAV-Bj induced clear increases in MYB, ARF8, AGO1-1, and AGO1-2 expression levels at 10 dpi (Fig. 3). At 20 dpi, the abundance of AGO1-2 was most significantly increased in the two infections, with RQ values of 2.65 and 3.69, respectively. At 30 dpi, ARF8 levels in CMV-Fny- and TAV-Bj-infected plants were increased by 2.47 and 1.98 folds, whereas no apparent alterations were observed for other mRNAs (RQ < 1.5) [Fig. 3(A)].

In tomato roots, the abundance of MYB was remarkably increased in both CMV-Fny- and TAV-Bj-infected plants at 10 dpi (RQ between 1.90 and 2.51), whereas the over-accumulation of AGO1-1 and AGO1-2 was more remarkable at 20 and 30 dpi (RQ between 0.85 and 3.06) [Fig. 3(B)].

In shoot apices, the abundance of AGO1-2 was most apparently increased in CMV-Fny- and TAV-Bj-infected plants at 10 dpi (RQ between 1.64 and 2.54), whereas at 20 dpi it was AGO1-1. At 30 dpi, the expression levels of tested mRNAs were almost unchanged in CMV-Fny infection, but the abundance of ARF8, AGO1-1, and AGO1-2 in CMV-FnyΔ2b-infected plants was notably increased to 2.86, 1.67, and 2.13 folds of those in mock infection, and ARF8, AGO1-2 levels in TAV-Bj infection were approximately double those of mock-inoculated plants [Fig. 3(C)].

In stem tissues, the alteration of mRNAs levels was less apparent than in other tissues. At 10 dpi, the most notable

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**Figure 2** Quantitative analyses of seven miRNAs in different tomato tissues upon CMV-Fny, CMV-FnyΔ2b, and TAV-Bj infections. At 10, 20, 30 dpi, relative quantities (RQs) of tested miRNAs in leave (A), root (B), shoot apex (C), and stem (D) tissues of virus- or mock-inoculated tomato seedlings were determined by quantitative stem-loop real-time RT–PCR. According to the comparative method (RQ = 2^(-ΔΔCt)), the expression level of each miRNA was first normalized to 18S rRNA (reference gene), and then made relative to the amount of corresponding miRNAs in mock-inoculated sample, representing the calibrator. Columns represent mean fold change from three biological replicates and vertical bars indicate standard errors.
change was the MYB level in CMV-Fny infection (RQ = 1.77). At 20 dpi, the abundance of AGO1-2 was notably increased in both CMV-Fny and TAV-Bj infections (∼RQ = 2.1). At 30 dpi, the expression levels of ARF8 in CMV-FnyΔ2b and TAV-Bj infections were increased to 2.50 and 2.04 folds of that in mock-inoculated plants, whereas no apparent alteration was observed in CMV-Fny-infected plants [Fig. 3(D)].

Taken together, it was found that the abundance of investigated mRNAs varied with the same tendency of their corresponding miRNAs, with higher values in CMV-Fny and TAV-Bj infections, but no or very limited changes in CMV-FnyΔ2b infection. Moreover, the abundance of MYB was particularly up-regulated in leaves and roots of virus-infected plants, AGO1-1 and AGO1-2 were over-accumulated in all of the four tissues, whereas SCL levels were unchanged throughout the detection time points. All these observations match the spatial alterations of their corresponding miRNAs. However, the ARF8 levels were notably increased in leaves of CMV-Fny- and TAV-Bj-infected plants, which was not consistent with the expression changes of miR167. These results indicated that although with some exceptions, there is a substantial correlation between the accumulation levels of target mRNAs and their corresponding miRNA species, consistent with the hypothesis by which different viral infections would
differentially perturb miRNA-guided regulation of gene expression.

Discussion

Although miRNAs were discovered many years ago [18], our knowledge of their functions is still limited. In recent years, hundreds of miRNAs have been predicted to be present in different plants, regulating the expression of multiple mRNAs; thus, one of our tasks is to understand their biological roles in various development and morphogenesis processes. Reliable and thorough analysis of the spatio-temporal expression of miRNAs and target mRNAs is a prerequisite for understanding the function of individual miRNAs in pathogen–host interaction. However, difficulties accompanying these studies consist in the transient, cell-specific expression of many miRNAs and their induction at low levels caused only by particular endogenous cues or environmental stimuli [19,20]. Here, we quantified the expression alterations of seven miRNAs and five target mRNAs in different tomato tissues upon viral infections, using the modified stem-loop real-time RT–PCR methods. Based on the expression characters of these miRNAs and mRNAs, several interesting findings were drawn from our results.

First, the spatial accumulation of tomato miRNAs and target miRNAs were highly regulated, and correlated with their roles in coordinating developmental events. For example, in CMV-Fny- and TAV-Bj-infected plants, it was found that miR164 and miR165/166 levels were up-regulated in several organs (leaves, roots, and shoot apices), indicating the involvement of miR164 and miR165/166 in symptoms determination of these tissues. These speculations were further supported by previous reports, which showed that miR164 was expressed on the flanks of tomato SAM and throughout young leaf primordial, and target of miR164, NAC1, was involved in lateral root emergence and regulated lamina outgrowth on a grander scale than that of serrations [21–23]. miR165/166 was reported to target homeodomain-leucine zipper (HD-ZIP) gene, and reduction in HD-ZIP gene expression resulted in prominent phenotypes including alterations of organ polarity, inhibition of vascular development and aberrant differentiation of the interfascicular fibers [24,25]. These data are in agreement with the observed leaf- and root-altered morphology caused by CMV-Fny and TAV-Bj infections.

However, the up-regulation of miR159 in leaf and root tissues, and miR167 in stems of CMV-Fny- and TAV-Bj-infected plants need to be further investigated, as miR159 was reported to accumulate mainly in the inflorescence and floral tissues of Arabidopsis, tobacco, and barley, while miR167 controlled the patterns of ARF6 and ARF8 expressions and was essential for the fertility of both ovules and anthers in Arabidopsis [26,27]. It would be very interesting to further investigate whether they have direct roles in these tissues’ morphogenesis in tomato.

The second interesting finding is the observed temporal regulation of miRNAs and target mRNAs expression, suggesting that timing of miRNA accumulation in developing tissues is also an important factor for miRNA-mediated gene regulation. For example, miR164 levels were unaltered in roots of CMV-Fny- and TAV-Bj-infected plants at 10 dpi, but increased gradually at 20 and 30 dpi. Moreover, we found in tomato leaves, CMV-Fny induced the highest accumulation levels of most miRNAs at 10 and 20 dpi, whereas in TAV-Bj infection these were observed at 30 dpi. In roots, the levels of most miRNAs were higher in CMV-Fny-infected plants than those of in TAV-Bj infection throughout the detection time points. In shoot apices, TAV-Bj induced higher miRNAs expression levels than CMV-Fny at 10 and 20 dpi, but comparable levels were found in both infections at 30 dpi. These data suggested that different models are applied by the two viruses to interference with the tomato miRNA pathway.

Third, the five target genes here studied are involved in developmental control and response to hormones (MYB, ARF8, and SCL transcription factors), or miRNA biogenesis (AGO1-1 and AGO1-2). Consistent with the up-regulation of miR168, paralleled increasing in AGO1 expression levels were observed in all of the tested tissues of CMV-Fny- and TAV-Bj-infected plants, indicating the disruption of normal miRNA-dependent gene expression regulation. As the similar tendency of miR159, the abundance of MYB was up-regulated in leaves and roots of CMV-Fny- and TAV-Bj-infected plants. For miR171/SCL pair, no alteration of both their expression was observed throughout the detecting time points, confirming that they were not involved in differentiation and development of tomato leaves, roots, shoot apices, and stems. However, we found a notable increase in ARF8 in the leaves of CMV-Fny- and TAV-Bj-infected tomato, whereas miR167 levels were only slightly altered in these samples. Thus, it is suspected that the expression levels of ARF8 are affected by non-miRNA mechanisms in this case, or these are probably resulted from tissue-specific expression of individual miRNA, which has been demonstrated in inflorescence tissue of P1/Hc-Pro transgenic Arabidopsis [13,20].

Finally, it was found that tomato plants harboring CMV-Fny and TAV-Bj infections had decreased numbers of lateral roots per centimeter of the main root, and their main roots were stunted relative to mock-inoculated plants in our study. In contrast, CMV-FnyΔ2b infection caused apparently increased numbers of lateral roots per centimeter of main root, when compared with mock controls. However, these observations are obviously different from
the previously reported root phenotypes in CMV-Fny-infected or CMV-Fny 2b-transgenic Arabidopsis [12]. The differences may be caused by different plant species, and indicate fundamental similarities and also remarkable differences in interaction mechanisms between viral pathogens versus model plants such as A. thaliana, and versus crop plants like tomato.

Using the same method, Cillo et al. [28] also quantified the increased miRNAs/miRNAs levels in leaves of CMV-Fny-infected tomato, and CMV sequence implicated in the perturbation of miRNA metabolism was mapped to 3′ region of RNA 2. As CMV-Fny, CMV-Ls (a mild strain) and CMV-Fny(Ls2b) (a recombinant strain) were used in their study, the influence of other viral factors on miRNAs pathway could not be excluded. Our study proved and extended their investigation by comparing miRNAs/miRNAs expression alterations upon CMV-Fny and CMV-FnyΔ2b infections, and another species of Cucumovirus (TAV-Bj) was included as well; thus, the role of 2b protein in perturbing tomato miRNA pathway could be analyzed directly. Besides these, more samples were collected from different tissues of virus-infected tomato during the pathogenic processes, and the results demonstrated that spatio-temporal alterations of miRNAs/miRNAs expression were also implicated in virus–host plants interaction.

Recently, Lewsey et al. [29] reported the disruption of Arabidopsis salicylic acid and jasmonic acid (JA)-mediated defensive signaling pathways by CMV 2b protein. Our results also showed the expression levels of miR164, miR159/MYB, and miR167/ARF8 that were believed to involve in phytohormone response pathways were noticeably altered by CMV-Fny and TAV-Bj infections in tested tissues. However, they suggested that the 2b protein may not be the only CMV-encoded factor that inhibits JA responses in Arabidopsis [29]. But, according to our results, the effects of other viral factors on miRNAs or miRNAs expression were not apparent, which probably occurred because of the limited transcripts studied in our experiments.

In conclusion, this study quantified the expressions of seven miRNAs and five target miRNAs in different tomato tissues after CMV-Fny, CMV-FnyΔ2b, and TAV-Bj infections. Based on our observations, it was found that the spatial and temporal expression of tomato miRNAs and miRNAs was highly organized, and was implicated in different symptoms determination. It is expected that this study will provide a new insight to investigate the interactions between hosts and pathogens.

**Supplementary Data**

Supplementary data is available at ABBSc online.

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**References**