Genome-Wide Identification of lncRNAs and Analysis of ceRNA Networks During Tomato Resistance to *Phytophthora infestans*

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ABSTRACT

Our previous studies have revealed the function of long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) in tomato in response to *Phytophthora infestans* infection. However, the interaction relationships between lncRNAs and miRNAs during tomato resistance to *P. infestans* infection are unknown. In this study, 9,011 lncRNAs were identified from tomato plants, including 115 upregulated and 81 downregulated lncRNAs. Among these, 148 were found to be differentially expressed and might affect the expression of 771 genes, which are composed of 887 matched lncRNA-miRNA pairs. In total, 88 lncRNAs were identified as endogenous RNAs (ceRNAs) and predicted to decoy 46 miRNAs. Degradome sequencing revealed that 11 miRNAs that were decoyed by 20 lncRNAs could target 30 genes. These lncRNAs, miRNAs, and target genes were predicted to form 10 regulatory modules. Among them, lncRNA42705/lncRNA08711,

lncRNA39896, and lncRNA11265/lncRNA15816 might modulate MYB, HD-Zip, and NAC transcription factors by decoying miR159, miR166b, and miR164a-5p, respectively. Upon *P. infestans* infection, the expression levels of lncRNA42705 and lncRNA08711 displayed a negative correlation with the expression level of miR159 and a positive correlation with the expression levels of MYB genes. Tomato plants in which lncRNA42705 and lncRNA08711 were silenced displayed increased levels of miR159 and decreased levels of MYB, respectively. The result demonstrated that lncRNAs might function as ceRNAs to decoy miRNAs and affect their target genes in tomato plants, increasing resistance to disease.

Keywords: ceRNA, genetics and resistance, interaction network, lncRNA, *Phytophthora*, tomato

Tomato is not only a major crop plant but also an important model plant for studying plant–pathogen interactions (Jiang et al. 2018a; Q. Y. Wang et al. 2019). Tomato late blight is caused by *Phytophthora infestans* (Fry et al. 2019). It occurs worldwide and is regarded as one of the major threats to tomato production, causing major economic losses (Zhang et al. 2013). Therefore, studying the mechanism associated with the resistance of tomato to *P. infestans* infection will help with finding a way to reduce or prevent damage caused by late blight and to develop tomato plants with more effective resistance.

Long noncoding RNAs (lncRNAs) have no protein-coding ability and their lengths are more than 200 nucleotides (nt) (Sun et al. 2018). Based on their genomic location, lncRNAs are classified into several categories, including long noncoding natural antisense transcripts (lncNAT), long intergenic noncoding RNA, generic exonic overlaps, novel isoforms, and others (Cui et al. 2017). Some lncRNAs can compete with endogenous RNAs (ceRNAs) to interfere with the function of microRNAs (miRNAs) (Liu et al. 2017). These ceRNAs contain special regions called endogenous target mimics (eTMs) that can decoy miRNAs (Borah et al. 2018). miRNAs act as key posttranscriptional regulators via inhibiting the expressions of their target genes (Luan et al. 2015). Therefore, acting as a ceRNA is an effective posttranscriptional regulatory mechanism by which lncRNAs interfere with target transcripts (Sen et al. 2014). For example, the *Arabidopsis* ceRNA IPS1 and

Medicago truncatula ceRNA PDIL1 act as decoys for miR399 and suppress its expression (Franco-Zorrilla et al. 2007; T. Wang et al. 2017). Rice lncRNA eTM160 can serve as a decoy for miR160 to regulate rice reproductive development (M. Wang et al. 2017). lncRNAs that act as ceRNAs in plant resistance to pathogen infection have also been reported. For example, during *Paulownia tomentosa* resistance to *Phytoplasma* infection, 23 lncRNAs may act as target mimics and be bound by 33 miRNAs (Fan et al. 2018). The tomato lncRNAs slylnc0195 and slylnc1077 are “decoys” for the miR166 and miR399, respectively, in the tomato–tomato yellow leaf curl virus (TYLCV) interaction (J. Wang et al. 2015).

Recent advances suggest that lncRNAs may act in many vital biological processes, including plant–pathogen interaction. For example, Zhu et al. (2014) reported that the expression of many lncNATs in *Arabidopsis* would change after infection with *Fusarium oxysporum*, and these lncNATs and their sense transcripts can be coinduced upon pathogen infection (Zhu et al. 2014). Another lncRNA, ELENA1, is involved in *Arabidopsis* resistance to pathogens. Plants in which ELENA1 has been knocked out display decreased expression of *PR1* and increased susceptibility to *Pseudomonas syringae* pv. *tomato* DC3000 (Seo et al. 2017). lncRNAs are also involved in tomato resistance to pathogen infection. After infection with potato spindle tuber viroid, the expression levels of many lncRNAs in tomato plants display obvious changes (Zheng et al. 2017). A tomato lncRNA (designated as SILNR1), which is targeted by small interfering RNAs from TYLCV, contributes to disease symptoms (Yang et al. 2019). Another tomato lncRNA16397 can induce the expression of *glutaredoxin* genes to enhance resistance to *Phytophthora infestans* (Cui et al. 2017). Our previous work has shown that lncRNA16397 and lncRNA33732 regulate the accumulation of reactive oxygen species (ROS) to affect tomato resistance to *P. infestans* (Cui et al. 2017, 2019), and we also illustrated the function of miR482b, miR172, and miR1918 in the tomato–*P. infestans* interaction (Jiang et al. 2018b; Luan et al. 2016, 2018). However, the number of lncRNAs associated with tomato

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resistance to *P. infestans* is limited, and the mechanism by which tomato lncRNAs act as ceRNAs to suppress the accumulation of miRNAs to affect gene expression upon *P. infestans* infection is also unknown. To identify the role of lncRNAs in tomato resistance to pathogens infection, we have identified the lncRNAs that were responsive to *P. infestans* infection, predicted the eTM sites of miRNAs in lncRNAs, used degradome sequence to identify the target genes of miRNAs, and constructed interaction networks among lncRNAs, miRNAs, and their target genes. These studies improve our understanding of the function of lncRNAs and the interaction between lncRNAs and miRNAs in the response of tomato to *P. infestans* infection, and will benefit future molecular-based breeding approaches to acquire pathogen-resistant plants.

MATERIALS AND METHODS

lncRNA identification. The transcriptome data of tomato L3708 (a resistant accession to *P. infestans*) infected without (Sp) and with *P. infestans* for 3 days (SpPi) from our previous studies were used to identify the lncRNAs (Cui et al. 2017, 2018b). The clean reads were mapped to the tomato genome iTAGv2.3 (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Slycopersicum) using TopHat. Cufflinks was then used to assemble the mapped reads. The programs CPC and CNCI were used to identify the lncRNAs according to a previous study (Cui et al. 2017). The lncRNAs were classified into four categories based on their genomic locations.

Fragment per kilobase per million reads (FPKM) values represented the expression levels of lncRNAs. Differentially expressed lncRNAs (DELs) between SpPi and Sp samples were identified with $P < 0.05$ and fold change > 1 . Fold change = $\log_2 \text{FPKM}_{\text{SpPi}} / \text{FPKM}_{\text{Sp}}$.

Prediction of lncRNA targets, gene ontology, and Kyoto Encyclopedia of Genes and Genomes pathway analysis. The target genes were searched following the rule that lncRNAs and target genes were separated by < 100 kb. To understand the functional roles of the target genes, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed via Web Gene Ontology Annotation Plot (<http://wego.genomics.org.cn/>) and the KEGG database (<https://www.kegg.jp/>), respectively.

Prediction of ceRNAs. All tomato miRNAs acquired from miRBase (<http://www.mirbase.org/>) and the lncRNAs identified in this study were used to identify potential ceRNAs in tomato.

ceRNAs were predicted using the rules described in a previous study (Jiang et al. 2019). The rules are as follows: (i) bulges are only permitted at the 9th to 12th positions of the 5' end of a miRNA sequence; (ii) the bulge in ceRNAs should be composed of 2 to 4 nt; (iii) G/U pairs are allowed within the region where ceRNA pairs with miRNA and perfect nucleotide pairing is required at the 2nd to 8th positions of the 5' end of the miRNA sequence; and (iv) except for the central bulge, the total mismatches within the ceRNA and miRNA pairing regions should be no more than four, and with no more than two consecutive mismatches. The interaction networks among the ceRNAs, miRNAs, and target genes were constructed using Cytoscape software.

Plant material collection and *P. infestans* inoculation. Tomato L3708 and *P. infestans* were cultured according to a previous study (Jiang et al. 2018b). The five-leaf-stage tomato plants were infected with *P. infestans* spores (10^6 zoospores/ml), as described previously (Cui et al. 2018a). At 0 and 3 days post-infection (dpi), the whole fifth leaf from each tomato plant was removed for RNA isolation.

Degradome library construction and sequencing. Total RNA from the tomato leaves infected with *P. infestans* for 3 days was used to prepare the degradome library by LC Biotech in Hangzhou, China. The process of library construction was as follows: (i) approximately 150 ng of poly(A)+ RNA was used as input RNA and annealing with Biotinylated Random Primers; (ii) Strapavidin capture of RNA fragments through Biotinylated Random Primers; (iii) 5' adaptor ligation to only those RNAs containing 5'-monophosphates; (iv) reverse transcription and PCR; and (v) libraries were sequenced using the 5' adaptor only, resulting in the sequencing of the first 36 nt of the inserts that represented the 5' ends of the original RNAs. Single-end sequencing (36 bp) was then performed on an Illumina HiSeq2500.

Analysis of degradome data. Degradome data were analyzed according to Hou's method (Hou et al. 2017). The CleaveLand tool was used to analyze the degradome data and identify the targets of miRNAs. The degradome sequences were mapped to the transcriptome sequences to generate a degradome density file. Target prediction with alignment score ≤ 4 was performed with the program TargetFinder. Thereafter, the degradome density file and results of the target prediction were compared and significant hits were identified as target plots.

Virus-induced gene silencing constructs, *Agrobacterium* infiltration, and disease-resistance analysis. TRV2 and TRV1 plasmids used for virus-induced gene silencing (VIGS) were

Fig. 1. Characteristic analysis of long noncoding RNAs (lncRNAs) from samples of tomato infected without (Sp) and with *Phytophthora infestans* for 3 days (SpPi). **A**, Number of identified lncRNAs and differentially expressed lncRNAs. **B**, Composition of different types of lncRNAs in SpPi sample; x = antisense of mRNA, u = intergenic region, o = generic exonic overlaps, and j = novel isoforms.

provided by Prof. Liu from Tsinghua University of China. The ligation independent cloning method was used to clone the VIGS sequence into the TRV2 vector. The freeze-thaw method was used to transform the TRV-VIGS constructs into *Agrobacterium tumefaciens* strain GV3101.

A. tumefaciens carrying TRV1 and suspensions containing TRV2-derived constructs or TRV2 empty vector were mixed prior to infiltration into tomato leaves (Jiang et al. 2018b). Based on our previous study (Jiang et al. 2019), plants were maintained

for 21 days, and the fifth leaves were sampled for subsequent experiments.

Each detached leaf was inoculated with 20 µl of a *P. infestans* zoospore suspension, while whole-plants were sprayed with the same zoospore suspension. Leaf lesion diameter and disease index (DI) were calculated based on the method of Jiang et al. (2018b).

Reverse-transcription quantitative PCR analysis. miRNAs, lncRNAs, and gene expression were quantified by reverse-transcription

Fig. 2. Selected differentially expressed long noncoding RNAs (DELs) validated by reverse-transcription quantitative PCR (RT-qPCR). **A**, Relative expression levels of 25 DELs between RNA-sequencing (RNA-Seq) and RT-qPCR from samples of tomato infected without (Sp) and with *Phytophthora infestans* for 3 days (SpPi). **B**, Consistency of the expression levels of the 25 selected DELs between RNA-Seq data and RT-qPCR data.

quantitative PCR (RT-qPCR) using TransScript Green miRNA Two-Step qRT-PCR SuperMix (Transgen Biotech) and SYBR Premix Ex Taq II kit (TaKaRa). The tomato *actin* gene was used as a reference gene, with all primer sequences listed in Supplementary Table S1. All reactions were carried out using three biological replicates. The $2^{-\Delta\Delta CT}$ method was used to determine and normalize the levels of transcripts in each case.

Statistical analysis. All data represented the means $\pm$ standard deviations of three independent experiments. Differences among the groups were indicated at a $P = 0.05$ level of significant difference.

RESULTS

Identification of lncRNAs and DEL analysis. After assembly of two RNA-sequencing (RNA-Seq) datasets and mapping and analysis of the length, coding potentials, and coverage of reads, 9,331 lncRNAs were obtained from the SpPi and Sp samples (Fig. 1A). Of these, only 9,011 lncRNAs were expressed in the SpPi sample. According to the locations of lncRNAs in the tomato genome, 6,635, 2,271, 72, and 33 lncRNAs were found to be antisense of mRNA, intergenic region, generic exonic overlaps, and novel isoforms, respectively (Fig. 1B). In total, 196 DELs were identified, including 115 upregulated and 81 downregulated lncRNAs in the SpPi sample (Fig. 1A; Supplementary Table S2).

To validate the DELs, 25 lncRNAs were selected to confirm the differential expression by RT-qPCR. Fold changes from RT-qPCR were compared with those from the RNA-Seq expression analysis results. The RNA-Seq results were confirmed by the RT-qPCR results (Fig. 2A). In addition, the levels of lncRNAs from RT-qPCR and RNA-Seq results exhibited similar trends ($R^2 = 0.8079$) (Fig. 2B), confirming the reliability and accuracy of the RNA-Seq analysis.

Prediction of lncRNA targets. To examine the DEL function, we predicted the potential targets of DELs. In total, 887 sequences matched to the lncRNA-mRNA pairs of 148 DELs and 771 genes (Supplementary Table S3).

To understand the function of the DELs, we analyzed the GO terms (level 2) of the target genes of the DELs. In total, 296, 129, and 381 genes were assigned with one or more GO terms for molecular

function, cellular component, and biological process, respectively (Supplementary Fig. S1). For molecular function, major categories were found for binding (GO:0005488) and catalytic activity (GO:0003824). For cellular component, genes involved in cell (GO:0005623), cell part (GO:0044464), and membrane (GO:0016020) were highly represented. For biological process, metabolic process (GO:0008152) was the most represented GO term, followed by cellular process (GO:0009987).

Among these GO terms, response to stimulus (GO:0050896) included 23 genes that were affected by 20 DELs (Supplementary Table S4). Among these genes, an abscisic acid receptor gene (Soly12g055990.1) affected by lncRNA45893 was assigned to response to biotic stimulus (level 3, GO:0009607), which was directly associated with plant response to pathogen infection (Fig. 3A). Upon *P. infestans* infection, the transcript levels of Soly12g055990.1 and lncRNA45893 were downregulated, as revealed by RNA-Seq and RT-qPCR (Fig. 3B and C).

The KEGG pathway analysis showed that 79 genes were assigned to 115 KEGG pathways (Supplementary Table S5). Plant-pathogen interaction (k04626) was directly involved in plant resistance to pathogens. Three genes were found in this pathway, including Soly03g123800.1, Soly05g050350.1, and Soly06g066370.2, which were affected by lncRNA13670, lncRNA1114, and lncRNA24371, respectively (Fig. 3A). After infection with *P. infestans*, their expression levels also increased according to the RT-qPCR and RNA-Seq results (Fig. 3B and C).

lncRNAs as ceRNAs decoy miRNAs. lncRNAs can decoy miRNAs via their eTM sites. The eTMs usually contain bulges or mismatches in the middle of the miRNA binding sites during binding between lncRNAs and miRNAs. Therefore, identification of the lncRNAs that might act as the eTMs of miRNAs was performed based on a program used in our previous study (Jiang et al. 2019). In total, 46 miRNAs could be decoyed by 88 lncRNAs, forming 39 miRNA-lncRNA duplexes (Supplementary Table S6; Supplementary Fig. S2). Among these duplexes, 15 lncRNAs were found to suppress the expression of miR482d-5p, and the regulatory module mediated by lncRNA34658 was the most complex, including 3 miRNAs and 8 lncRNAs.

Analysis of degradome data and identification of targets silenced by miRNAs. Degradome sequencing was performed to

Fig. 3. Genes affected by differentially expressed long noncoding RNAs (DELs) in response to stimulus (GO:0050896) and plant-pathogen interaction (k04626). **A**, Cytoscape result showing predicted interaction networks. Circles and squares represent genes and DELs, respectively. Expression levels of these genes and long noncoding RNAs by **B**, RNA-sequencing and **C**, reverse-transcription quantitative PCR; dpi = days postinfection. The $\log_2$ fragment per kilobase per million reads values were used to create the heatmap.

generate 18,759,023 raw reads from the SpPi sample. Of these raw reads, approximately 99.12% were mapped to the tomato genome and 5,325,281 mapped reads were mapped only once in a genome, which are called unique mapped reads. After the reads were mapped to the transcriptome sequences, it was found that 14,319,481 reads were mapped to 24,239 transcripts, with 3,810,948 unique transcript mapped reads (Table 1). After the degradome density file was generated using CleaveLand, it was found that 11 miRNAs, which were decoyed by 20 lncRNAs, could target 30 genes (Table 2). Cytoscape results showed that these lncRNAs, miRNAs, and target genes formed 10 regulatory modules (Fig. 4). Among them, the regulatory module of miR160a contained the most abundant elements, including one miRNA, four genes, and four lncRNAs, and the regulatory module of miR6026 contained the least abundant elements, including only one miRNA, one lncRNA, and one gene.

lncRNA-miRNA transcription factor networks. Three of these modules were found to associate with the expression of transcription factors (TF). lncRNA42705/lncRNA08711, lncRNA39896, and lncRNA11265/lncRNA15816 could modulate MYB, HD-Zip, and NAC TFs by decoying miR159, miR166b, and miR164a-5p, respectively (Fig. 4). A 2- or 3-nt bulge on eTMs located between the 9th and 12th positions at the 5' end of the miRNA sequence was required by eTMs for the decoying of miR159 in lncRNA42705

and lncRNA08711 (Fig. 5A). The predicted binding sites of miR159 among these two lncRNAs were well conserved at the second to eighth positions of the 5' end of the miRNA sequence (Fig. 5B).

The results of degradome sequencing showed that miR159 could target three members of the tomato MYB TFs family, including Solyc01g090530.1, Solyc06g073640.1, and Solyc01g009070.2 (Fig. 4). The regions from 956 to 976, 85 to 105, and 1,043 to 1,063 nt in Solyc01g009070.2, Solyc01g090530.1, and Solyc06g073640.2, respectively, could well be binding sites for miR159. The cleavage sites were located at 967, 96, and 1,054 nt, respectively (Fig. 5C).

The correlation between the expression of lncRNA42705 and lncRNA08711, miR159, and MYB genes was investigated by RT-qPCR after infection with *P. infestans*. Both lncRNA42705 and lncRNA08711 exhibited a dramatic increase in expression upon *P. infestans* infection whereas the expression of miR159 was down-regulated, suggesting that the expression levels of lncRNA42705 and lncRNA08711 are negatively correlated with the expression level of miR159 (Fig. 5D). In addition, the three target genes of miR159 were also upregulated. These results indicated that lncRNA42705 and lncRNA08711 could modulate MYB TFs by decoying miR159.

Silencing of lncRNA42705 and lncRNA08711 affects tomato resistance to *P. infestans*. To identify the roles of lncRNA42705 and lncRNA08711, we used VIGS to silence these two lncRNAs in tomato plants. The expressions of both lncRNA42705 and lncRNA08711 were reduced to approximately 40% (Fig. 6A and B). Tomato plants in which lncRNA08711 (TRV2::08711) was silenced showed no significant change in lncRNA42705 expression. The expression of miR159 was dramatically increased, whereas that of three MYB genes was down-regulated (Fig. 6A). Likewise, tomato plants in which lncRNA42705 had been silenced (TRV2::42705) exhibited no change in the accumulation of lncRNA08711. The expression of miR159 was upregulated, whereas that of three MYB genes was

TABLE 1. Summary of degradome data of the tomato plants infected with *Phytophthora infestans*

Sample	Number
Raw reads	18,759,023
Mapped reads	18,593,529
Unique raw reads	5,369,063
Unique mapped reads	5,325,281
Transcript mapped reads	14,319,481
Unique transcript mapped reads	3,810,948

TABLE 2. Target genes identified by degradome sequencing

microRNA	Target gene	Annotation	Score	Range
miR159	Solyc01g090530.1.1	MYB transcriptional regulator	3.5	85–105
	Solyc01g009070.2.1	MYB domain protein	2.5	956–976
	Solyc06g073640.2.1	MYB domain protein	3	1,043–1,063
	Solyc12g014120.1.1	...	3.5	461–481
miR160a	Solyc09g082890.1.1	ATPase E1-E2 type family protein	4	159–179
	Solyc11g069500.1.1	Auxin response factor	1	1,302–1,322
	Solyc11g013470.1.1	Auxin response factor	1.5	543–563
	Solyc06g075150.2.1	Auxin response factor	1	1,491–1,511
miR164a-5p	Solyc09g007810.2.1	Auxin response factor	0.5	1,597–1,617
	Solyc06g069710.2.1	NAC domain containing protein	4	623–643
	Solyc07g062840.2.1	NAC transcriptional regulator	2	713–733
	Solyc07g066330.2.1	NAC domain containing protein 1	2	751–771
miR166b	Solyc03g115850.2.1	NAC domain containing protein	4	692–712
	Solyc08g066500.2.1	Homeobox-leucine zipper protein	3	566–586
	Solyc11g069470.1.1	Homeobox-leucine zipper protein	3	560–580
	Solyc02g069830.2.1	Homeobox-leucine zipper protein	3	618–638
miR168a-5p	Solyc03g120910.2.1	Homeobox-leucine zipper protein	2.5	849–869
	Solyc02g024070.2.1	Homeobox-leucine zipper protein	3	808–828
	Solyc12g044410.1.1	Homeobox-leucine zipper protein	2.5	548–568
	Solyc06g072300.2.1	Stabilizer of iron transporter SufD	4	377–397
miR394-5p	Solyc05g015520.2.1	Galactose oxidase	1	1,248–1,267
	Solyc04g051600.2.1	tRNAHis guanylyltransferase	4	554–573
	Solyc09g082860.2.1	ATP sulfurylase 1	3.5	477–497
	Solyc09g082860.2.1	ATP sulfurylase 1	3.5	477–497
miR395a	Solyc11g007360.1.1	F-box protein	1	705–725
	Solyc05g007570.2.1	Protein of unknown function	0.5	2,515–2,535
	Solyc01g005870.1.1	Receptor like protein 32	1	1,186–1,206
	Solyc01g006550.2.1	Receptor like protein 32	1	1,196–1,216
miR395b	Solyc01g009690.1.1	Receptor like protein 32	1	1,207–1,227
	Solyc06g048960.2.1	Dicer-like 2	3	54–75
miR5303				
miR6022				
miR6026				

downregulated (Fig. 6B). These results suggested that lncRNA42705 and lncRNA08711 could silence miR159 to affect the expression of *MYB* genes.

The disease phenotypes for the control (TRV2::00), TRV2::08711, and TRV2::42705 tomato plants were examined upon *P. infestans* infection. The detached leaves of TRV2::08711 and TRV2::42705 tomato plants displayed more severe disease symptoms than those of TRV2::00 plants (Fig. 6C). The leaves of TRV2::08711 and TRV2::42705 tomato plants showed larger lesion diameters (Fig. 6D). Additionally, after whole-plant infection with *P. infestans*, DIs of TRV2::08711 and TRV2::42705 tomato plants were higher than the DIs of TRV2::00 tomato plants (Fig. 6E). The results suggest that the silencing of lncRNA42705 and lncRNA08711 lead to a decreased resistance of tomato.

DISCUSSION

Research on the roles of lncRNAs in plants tends to lag behind the research on the roles of lncRNAs in mammals. Experimental proofs display that a few plant lncRNAs have been found to be involved in

the regulation of fruit ripening (Li et al. 2018; Yu et al. 2019), affecting development and growth (Meng et al. 2018; Y. Wang et al. 2018) and response to nutritional deficiency (T. Wang et al. 2017), drought and salt stresses (Qin et al. 2017), and pathogen infection (Zhu et al. 2014). As for plant-pathogen interaction, a number of fungi-responsive lncRNAs have been identified from *Triticum aestivum* after infection with stripe rust and powdery mildew (Zhang et al. 2016). In *Brassica napus*, 3,181 lncRNA candidates were identified 24 and 48 h after infection with *Sclerotinia sclerotiorum* (Jain et al. 2017). Numerous lncRNAs have also been identified in tomato, cotton, rice, and *Arabidopsis* following TYLCV, *Verticillium dahliae*, *Magnaporthe oryzae*, and *F. oxysporum* infection, respectively (Joshi et al. 2016; J. Wang et al. 2018; Zhang et al. 2018; Zhu et al. 2014). In general, the functions of lncRNAs have been less extensively studied compared with the number of lncRNAs that have been identified. The silencing of the tomato lncRNA slylnc0957 was found to enhance the resistance of the plant to TYLCV (J. Wang et al. 2018). Cotton plants in which GhIncNAT-ANX2 and GhIncNAT-RLP7 have been silenced display the enhanced resistance to *V. dahliae* and *Botrytis*

Fig. 4. Predicted interaction network among long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and target genes by Cytoscape. Circles, squares, and triangles represent miRNAs, lncRNAs, and target genes, respectively. Boxes represent the networks contained transcription factors.

cinerea (Zhang et al. 2018). In this study, the data obtained from transcriptome analysis suggested that lncRNAs in tomato might play an important role in the response to *P. infestans* infection. For example, 196 lncRNAs were responsive in *P. infestans* infection (Fig. 1A). Among them, 148 could affect the expression of 771 genes, which comprised 887 matched lncRNA-mRNA pairs (Supplementary Table S3). These results suggest that these lncRNAs are responsive to *P. infestans* infection and important components of the antifungal networks in tomato.

miRNAs are also involved in the resistance of plants to pathogen infection. For example, tomato miR394 is responsive to *B. cinerea* infection, and its overexpression in *Arabidopsis* can decrease the resistance to pathogens (Tian et al. 2018). The miR156/SPL network affects *Arabidopsis* immune response by regulating ROS accumulation and activating the salicylic acid signaling pathway (Yin et al. 2019). Tomato plants where miR482b has been silenced also show enhanced resistance to *P. infestans* (Jiang et al. 2018b). To show the biological function of miRNAs in general, it is necessary to identify their target genes. Degradome sequencing is an effective method to identify target genes of miRNAs. For example, using degradome sequencing, we identified four

members of the nucleotide bind site leucine-rich repeat family as the target genes of miR482b in tomato plants (Jiang et al. 2018a). Degradome sequencing has been used to identify the target genes of miR172. It was found that miR172 acts as a positive regulator through cleaving its target genes AP2/ERF (Soly11g072600.1) upon *P. infestans* infection (Luan et al. 2018). In this study, the target genes of miRNAs were identified in the tomato leaves infected with *P. infestans* using degradome sequencing (Table 2). It was found that miR166b could target six members of the HD-Zip family, whereas miR164a-5p could target four members of NAC family, while miR159 could target three members of the MYB family (Figs. 4 and 5C). In addition, the expression of miR159 was negatively correlated with the expression of the *MYB* genes upon *P. infestans* infection (Fig. 5D). Likewise, the accumulation of miR159 is also negatively correlated with its target gene *MYB33-like* during tomato resistance to tomato leaf curl virus (ToLCV) infection (Naqvi et al. 2010). An NAC TF as the target of ta-miR164 acts by affecting wheat resistance to stripe rust (Feng et al. 2014). In addition, miR166-mediated silencing of HD-Zip TF is involved in tomato resistance to TYLCV infection (J. Wang et al. 2015).

Fig. 5. Long noncoding RNA (lncRNA)42705 and lncRNA08711 function to modulate *MYB* genes by decoying miR159. **A**, Predicated base-pairing interactions between miR159 and endogenous target mimic (eTM) sites of two lncRNAs. Shaded letters represent bulges. **B**, Conservation analysis of eTM sites of two lncRNAs decoyed miR159. Shaded letters represent conserved sequence. **C**, Cleavage sites between miR159 and its target genes Soly1g009070.2, Soly1g090530.1, and Soly1g06g073640.2. Shaded lines and letters represent cleavage sites. **D**, Expression levels of lncRNA08711, lncRNA42705, miR159, and its target genes between samples of tomato infected without (Sp) and with *Phytophthora infestans* for 3 days (SpPi).

In plants, miRNA expression level is affected by various molecular mechanisms. For example, during plant-*Phytophthora* sp. interaction, *P. sojae* effectors PsPSR1 and PsPSR2 suppress host miRNA accumulation to affect plant resistance (Qiao et al. 2013). In addition, various TFs activate miRNA expression through binding *cis*-acting elements in the promoter of the premiRNA. *Arabidopsis* AtMYB2 and tomato RIN TFs can promote the expression of miR399f and miR172, respectively (Baek et al. 2013; Gao et al. 2015). ceRNAs can also affect the expression levels of miRNA via eTM sites decoying miRNA. Recently, it has been experimentally studied that some ceRNAs can serve as a decoy for miR399, miR160, miR166, and miR482b in plants, including *Arabidopsis*, rice, *Medicago truncatula*, and tomato (Franco-Zorrilla et al. 2007; Jiang et al. 2019; J. Wang et al. 2015; M. Wang et al. 2017; T. Wang et al. 2017). In this study, from tomato leaves infected with *P. infestans*, we identified 88 lncRNAs as the decoys for 46 miRNAs (Supplementary Table S6). Among them, lncRNA42705/lncRNA08711, lncRNA39896, and lncRNA11265/lncRNA15816 contained eTMs of miR159, miR166b, and miR164a-5p (Fig. 4). The expression levels of both lncRNA42705 and lncRNA08711 were negatively correlated with the expression level of miR159 and positively correlated with the expression levels of *MYB* genes after infection with *P. infestans* (Fig. 5D). Silencing of lncRNA42705 and lncRNA08711 in tomato plants led to an increase in miR159

accumulation (Fig. 6A and 6B). These results suggest that lncRNA42705 and lncRNA08711 functioned as eTMs that may decoy miR159.

In our previous study on phylogenetic analysis of the tomato *MYB* gene family, we found that the target genes of miR159, Solyc06g073640.1, and Solyc01g009070.2 are clustered into subgroup 18 and exhibit a closer relationship with AtMYB33 and AtMYB65 (Cui et al. 2018a). Tomato MYB33 is responsive to ToLCV infection (Naqvi et al. 2010). In *Arabidopsis*, AtMYB33 is involved in plant responses to the root-knot nematode *Meloidogyne incognita* and it acts in galls at early stages of gall development (Medina et al. 2017). *Arabidopsis* MYB33 and MYB65 are associated with disease symptoms of cucumber mosaic virus (Du et al. 2014). These findings suggest that Solyc06g073640.1 and Solyc01g009070.2, similar to homologs MYB33 and MYB65, may affect tomato resistance to pathogens. In this study, the expression of Solyc06g073640.1 and Solyc01g009070.2 were increased upon *P. infestans* infection (Fig. 5D). The tomato plants with silenced lncRNA42705 and lncRNA08711 displayed a decrease in Solyc06g073640.1 and Solyc01g009070.2 accumulation and an increase in tomato susceptibility (Fig. 6). The results suggest that lncRNA42705 and lncRNA08711 act in plant resistance to the pathogen by affecting MYB expression.

Fig. 6. Silencing of long noncoding RNA (lncRNA)42705 and lncRNA08711 affects tomato resistance. Expression levels of lncRNA42705, lncRNA08711, miR159, and *MYB* genes in the **A**, TRV2::08711 and **B**, TRV2::42705 tomato plants. **C**, Phenotypes and **D**, diameter of the lesion in the detached leaves from TRV2::00, TRV2::08711, and TRV2::42705 tomato plants at 5 days postinfection (dpi). Scale bars = 1 cm. **E**, Disease index of TRV2::00, TRV2::08711, and TRV2::42705 tomato plants at 5 dpi.

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