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고추, 토마토, 감자 병 저항성과 연관된 microRNA의 진화에 관한 연구

Evolution of MicroRNAs Associated with Disease Resistance in Pepper, Tomato and Potato

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Abstract

Evolution of MicroRNAs Associated with Disease Resistance in Pepper, Tomato and Potato

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MicroRNA (miRNA) is a class of small RNA, which are associated with diverse biological processes in plants, such as growth, development, and disease-resistance. Previously, several plant miRNAs were identified as a trigger of secondary small interfering RNAs (siRNAs) biogenesis, including phased, secondary siRNAs (phasiRNAs), and these small RNAs regulate expression of several gene families. In this study, miRNAs in pepper, tomato and potato were analyzed in an evolutionary aspect. Degradome analysis identified target genes of the miRNAs in three species and some specific miRNAs target numerous genes involved in disease-resistance, encoding nucleotide-binding leucine-rich repeat (NB-LRR) and receptor-like proteins (RLPs). Furthermore, these miRNAs prompt phasiRNA production in pepper, indicating reinforcement of the regulation against disease-resistance genes. miR-n033a-3p, whose target NB-LRRs belonging to specific subgroup CNL-G1 have been duplicated in pepper, targets more CNL-G1 NB-LRRs in pepper than those in potato. These observations suggested that resistance-associated miRNAs might have evolved to regulate numerous genes as the result of

target gene expansion in pepper.

Keywords: degradome, disease-resistance, evolution, microRNA, NB-LRR, pepper, phasiRNA, receptor-like protein

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Introduction

Small RNA-mediated gene silencing is a well-conserved system for gene regulation in eukaryotes (Axtell et al., 2011). Small RNAs are generally 20-24 nucleotides in length and their roles range from plant growth to disease-resistance (Jones-Rhoades et al., 2006; Sunkar et al., 2007; Voinnet, 2008). MicroRNAs (miRNAs), which are classified as small RNAs, regulate various genes in animal and plant. In plant, the length of miRNAs is mostly 21-22 nucleotides and they are generated from stem-loop structured precursors by Dicer-like proteins (DCL). Mature miRNAs are loaded on Argonaute proteins, which are core proteins of the RNA-induced silencing complex (RISC), and repress target mRNA through cleavage if miRNA is near-perfectly paired with targets in sequences (Axtell et al., 2011).

There are particular cases that miRNAs induce the production of secondary small RNAs. It requires that length of the trigger miRNAs is 21 or 22 nucleotides (Chen et al., 2010; Cuperus et al., 2010) and their target mRNAs contain two (Axtell et al., 2006) or one (De felippes et al., 2017) target sites. After cleavage of the transcripts, dsRNAs are generated from the remnants by SUPPRESSOR OF GENE SILENCING 3 (SGS3) and RNA-DEPENDENT RNA POLYMERASE 6 (RDR6). Then, DCL4 and DCL5 process dsRNAs into secondary small interfering RNAs (siRNAs) in a phased pattern (Vazquez et al., 2004; Fei et al., 2013). Given that nature, these siRNAs are known as phased, secondary siRNA (phasiRNA). Most phasiRNAs are 21-nucleotides in length and some phasiRNAs are known to repress target mRNAs in *cis* or *trans*, similar to miRNA (Zhai et al., 2011; Fei et al., 2013). Hundreds of phasiRNA-producing loci (*PHAS* loci) exist in various plants, including Arabidopsis (Axtell et al., 2006), rice (Song et al., 2012) and soybean (Zhai et al., 2011). *PHAS* loci consist of protein-coding genes or *trans*-acting siRNA-generating loci (*TAS*), which are not coding protein (Vazquez et al., 2004;

Allen et al., 2005). miR390-*TAS3*-*ARF* pathway is one of the most well-known example of the *TAS* and it is conserved in land plants (Xia et al., 2017). In the pathway, miR390 induces the phasiRNA production from *TAS3* transcripts, and these phasiRNAs repress auxin response factor (ARF) genes in *trans*.

Diverse studies have reported that miRNAs and phasiRNAs in plants control the expression of various gene families (Pantaleo et al., 2010; Fei et al., 2013; Hwang et al., 2013; Lakhotia et al., 2014; Zhang et al., 2016), including genes associated with disease-resistance. Mechanism of disease-resistance in plants consists of two layers. First, pattern recognition receptors (PRRs) perceive pathogen via pathogen-associated molecular patterns (PAMPs) and trigger resistance responses (PAMP-triggered immunity, PTI). Second, if pathogens overcome PTI by secreting effectors, host plants recognized effectors by resistance proteins (R proteins), and R proteins trigger a subsequent resistance response like rapid and localized cell death (effector-triggered immunity, ETI) (Dodds and Rathjen, 2010). miRNAs belonging to the miR482/2118 superfamily, miR6019, miR6024 and miR6027 are known to regulate genes encoding nucleotide-binding leucine-rich repeat (NB-LRR) proteins, which are a major class of resistance genes (*R*-genes) (Zhai et al., 2011; Li et al., 2012; Shivaprasad et al., 2012; Zhang et al., 2016). Moreover, NB-LRR gene family is also known as *PHAS* gene in numerous plant species including Solanaceae (Zhai et al., 2011; Li et al., 2012; Shivaprasad et al., 2012; Zhang et al., 2016).

Recently, there are several studies that investigated the evolutional history of miRNAs among plant lineages (Abrouk et al., 2012; Zhao et al., 2015). These studies suggested that the divergent evolution of miRNAs are deeply involved in genome duplication and evolution of their target genes. Particularly, NB-LRR associated miRNAs like miR482 superfamily were investigated well regarding evolutionary relationship with target NB-LRRs (De Vries et al., 2015; Gonzalez et al., 2015; Zhang et al., 2016). NB-LRR associated miRNAs are assumed to

originate from tandem duplication of NB-LRRs and evolve with their target NB-LRRs (Zhang et al., 2016). Each plant species and its NB-LRRs evolved respectively, thus, the pool of NB-LRR associated miRNAs is distinguishable in each species (Gonzalez et al., 2015; Zhang et al., 2016).

Solanaceae is one of the most important plant family in agriculture, including pepper (*Capsicum annuum*), tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*). Pepper is widely grown in the world and utilized in food and pharmaceutical industry. Previously, Hwang et al. (2013) identified miRNAs via high-throughput sequencing and validated several target genes of them in pepper (Hwang et al., 2013). Another group reported that pepper miRNAs regulate various transcription factors, which contribute to plant development, through a trans-omics approach (Zhang et al., 2017). Moreover, the other group covered an evolutionary history of the miR482/2118 superfamily and suggested a role of the miR482/2118 family as an evolutionary buffer for *R*-gene diversity (De Vries et al., 2015). However, there is no comprehensive study that reveals the functions and evolutionary history of miRNAs in pepper.

In this study, functions of the miRNAs in pepper were investigated through comparative analyses of miRNA and their target genes using degradome analysis. Surprisingly, miR-n026 and miR-n033, which were specifically annotated in pepper (Hwang et al., 2013), were identified to target *R*-genes as well as several conserved miRNAs like miR482. Furthermore, these miRNAs were predicted to induce phasiRNA biogenesis. miR-n033 family targets numerous NB-LRR genes and most of them belong to CNL-G1 subgroup, which is specifically duplicated in pepper. Based on the estimated divergence times of pepper, tomato and potato, miR-n033 family was presumed to exist in a common ancestor of these species and evolve to regulate more CNL-G1 NB-LRRs, following an expansion of NB-LRR genes in pepper. These results were expected to advance our understanding of the evolutionary relationship between miRNA and target NB-LRRs, and gene

regulation by small RNAs in plant immunity.

Materials and Methods

Information of miRNAs and genes in pepper, tomato and potato

Sequences of precursor and mature miRNAs in pepper (*C. annuum* L.), tomato (*S. lycopersicum* L.) and potato (*S. tuberosum* L.) were extracted from the previous studies and online database (Table S1-3) (Fei et al., 2011; Kozomara and Griffiths-Jones, 2011; Tomato Genome, 2012; Zuo et al., 2012; Hwang et al., 2013; Lakhotia et al., 2014). In pepper, additional miRNAs were identified using almost the same small RNA-Seq data and pipeline from the previous study, excluding a criterion of the expression cut-off (Hwang et al., 2013). Several miRNAs, whose IDs were inconsistent although their sequences are identical between pepper, tomato and potato, were integrated into the same ID. Genome annotation of pepper (v.1.55; http://peppergenome.snu.ac.kr), tomato (ITAG v.2.3; https://solgenomics.net) and potato (JGI v.4.03; https://genome.jgi.doe.gov) was exploited.

Degradome sequencing analysis

Degradome libraries were constructed by Dr. June hyun Park. Degradome Pepper (CM334), tomato (Heinz) and potato (Phureja) were grown under standard conditions (16h light/8h dark; 27℃/19℃). Leaves, roots, stems, green fruits, red fruits and tubers were exploited for the degradome library. Degradome libraries were constructed according to the protocol and sequenced using an Illumina HiSeq2000 (Addo-Quaye et al., 2008). Raw data was preprocessed for a downstream analysis. Adapters were removed from the raw data and reads whose length was outside of the ranges (15-26 nucleotides) were filtered. Filtered reads were analyzed whether they have homology with structural RNAs such as rRNA, tRNA, snRNA and snoRNA using BLASTn with default option against Rfam (http://rfam.xfam.org) (Burge et al., 2013), and non-homologous reads with

structural RNAs were obtained. Likewise, homologous reads with repeats and transposons from the repeat database were also eliminated (Jurka et al., 2005). Because UTR sequences have not been identified in pepper, transcripts were defined to coding sequence (CDS) and ±1 kb flanking sequences. Target genes of the miRNAs were identified using the CleaveLand4 pipeline (Brousse et al., 2014). Targets that *p*-value $\leq$ 0.05 or a score $\leq$ 4 were regarded as significant. Target genes were compared with the NR database through BLASTx, and InterPro domains of the target genes were identified using Blast2go, which were conducted by Dr. Eunyoung Seo.

Northern blot analysis

Northern blot was conducted by Dr. June hyun Park. Total RNA was extracted from leaves of pepper, tomato, potato and *Nicotiana benthamiana* using TRI Reagent (Ambion). Total 20 μg of RNA from each species was respectively separated in a 15% UREA polyacrylamide gel, and transferred to Hybond-NX membrane (GE Healthcare). Transferred RNA was chemically cross-liked via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Pall and Hamilton, 2008). To label with probes, 2 μl of 10 μM oligo, 2 μl of 10X T4 PNK buffer (Takara), 2.5 μl of [γ-^{32}P] ATP, >7000 Ci/mmole (~150 μCi/μl), 12.5 μl of dH_2O, and 1 μl of T4 polynucleotide kinase (Takara) were added to a 20 μl reaction for an hour at 37℃. The labelled probes were purified from unincorporated labels with PERFORMA Spin Columns (Edge Bio) following the manufacturers′ instruction. Probe sequences for northern blot were same with the mature miRNAs in pepper (Table S1). Hybridization and washing were performed as described previously (Sunkar and Zhu, 2004). The membranes were exposed to a phosphorimager, and signals were analyzed using BAS-2500 (Fuji).

PhasiRNA analysis

PhasiRNA prediction pipeline was written in Python language (Fig. S3). Public small RNA-Seq data were exploited in phasiRNA analysis (Fei et al., 2011; Tomato Genome, 2012; Zuo et al., 2012; Hwang et al., 2013; Lakhotia et al., 2014). Adapter sequences were removed from the raw reads, and low-quality reads were filtered. Filtered reads were collapsed using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit). Processed reads were mapped to the genomes of pepper, tomato and potato using Bowtie-1.1.2 with options -v 0 –a (Langmead et al., 2009), and normalized by genome-mapped counts (hit normalize) to prevent the bias overestimating multiply-mapped reads. Then, mapped reads whose abundance is less than three were excluded for efficient analysis. Multiply aligned reads to the genome (> 25) were hard to identify their origin. Thus, they were excluded for downstream analysis. To identify a putative *PHAS* region in the genome, *p*-value was calculated as described (Xia et al., 2013). Predicted *PHAS* regions with *p*-value > 0.001 were excluded and phasing score was calculated in the expanded *PHAS* regions (-500 and +1000 bp) with the modified method (Xia et al., 2013). Only the *PHAS* genes, whose maximum phasing score was 15 or above, were considered *PHAS* genes. *PHAS* genes were characterized by gene annotation data (pepper: v.1.55; tomato: ITAG 2.4; potato: PGSC_DM_v4.03; https://solgenomics.net). *PHAS* genes were examined whether there are miRNA triggers that initiate the phasiRNA production through degradome analysis via CleaveLand4 (Brousse et al., 2014). To predict the target of phasiRNAs, they were analyzed via CleaveLand4 pipeline as the input (Brousse et al., 2014). *PHAS* loci were visualized through the Integrative Genomics Viewer (IGV) (Robinson et al., 2011).

Bioinformatic analyses for miR-n026 and miR-n033

Genome annotation of pepper, tomato and potato were exploited to locate miRNA genes. Syntenic relationships between pepper, tomato and potato genes near

miRNA genes were investigated using MCScanX (BLASTp *e*-value < 1e-10) by Dr. Eunyoung Seo according to Wang et al. (2012). To inspect existence of the miR-n026 and miR-n033 genes in Solanaceous species, homologous regions with them were found by BLASTn with default option in the genome of each species. Evolutionary distances between Solanaceous species were referred from other studies to infer the origin of miRNA genes (Bombarely et al., 2012; Särkinen et al., 2013). Stem-loop structures of the miR-n026 and miR-n033 precursors were predicted by the RNAfold (Mathews et al., 2004). Transposable elements were identified as described (Kim et al., 2014). Subgroups of NB-LRRs in pepper, tomato and potato were classified following the pipeline (Seo et al., 2016). Targeted sequences in NB-LRRs by each miRNA were aligned using Clustal Omega (Sievers et al., 2011), and consensus sequences were identified using WebLogo (http://weblogo.threeplusone.com) (Crooks et al., 2004). Target genes of miR-n026 and miR-n033 were predicted using psRNAtarget (score ≤ 3.5; http://plantgrn.noble.org/psRNAtarget) (Dai and Zhao, 2011). The phylogenetic tree of NB-LRRs was from the study (Seo et al., 2016).

Availability of data

The dataset for this study is available in the Gene Expression Omnibus as below; GSE41654 (pepper small RNA library), GSE18110, GSE32470, GSE76204 (tomato small RNA libraries), GSE52599, GSE32471 (potato small RNA libraries) and GSE102781 (degradome libraries).

Results

Disease-resistance associated miRNAs in Solanaceae were identified via degradome analysis

To investigate target genes of the miRNAs in pepper, tomato and potato, information of miRNAs were exploited from other studies and databases (Fei et al., 2011; Kozomara and Griffiths-Jones, 2011; Tomato Genome, 2012; Zuo et al., 2012; Hwang et al., 2013; Lakhotia et al., 2014). Additional miRNAs in pepper were identified from the previous study, because strict criteria were applied to verify novel miRNAs, which might induce false negative (Hwang et al., 2013). Total 145, 123, and 275 mature miRNAs from a guide and passenger strand and their genes (223, 93, 393) were gathered in pepper, tomato and potato, respectively (Table S1-4). For comprehensive understanding, miRNAs were identified whether they are species-specific with the criteria that sequence of mature and precursor miRNAs was conserved in other species (refer to miRBase) using BLASTn with default option (Table S4). Only 28 miRNA families are conserved among the three species, while 93, 58 and 126 families exist in each species (Fig. S1). It suggests that miRNA genes evolved rapidly after divergence of the lineages.

Parallel Analysis of RNA Ends (PARE) libraries, which are also referred to as degradome libraries, from the pooled tissues (leaves, roots, stems, green fruits, red fruits and tubers) were constructed to identify target genes of miRNAs in pepper, tomato and potato. Degradome analysis verified small RNA-directed target cleavage on a genome-wide scale through sequencing an uncapped 5′ end of RNAs (German et al., 2008; German et al., 2009). Statistics of degradome sequencing were listed in Table S5. Reads with low quality and those which are homologous with structural RNA were filtered for confidence. Target genes of the miRNAs were identified via CleaveLand4 (Brousse et al., 2014).

Hundreds of miRNA-target pairs were identified in pepper, tomato and

potato (436, 352, 169, respectively). There are 99, 72 and 70 miRNAs targeting one or more genes, and 390, 319 and 163 genes cleaved by miRNAs in pepper, tomato and potato, respectively. While there are more degradome reads in potato than those in pepper and tomato, miRNA-target pairs might have been underestimated in potato, because unique reads in potato are lower than those in other species, indicating high redundancy of reads in potato. In results, degradome analysis provided consistent results with the previous study. can-miR-396b and can-miR-396c were identified to target domain-rearranged methyltransferase (DRM) gene as previously validated (Table S6) (Hwang et al., 2013). To investigate target genes of the miRNAs, domains of the target genes were identified and classified (Fig. 1). Interestingly, numerous target genes contain the leucine-rich repeat (LRR) and nucleotide-binding domains shared by APAF-1, R genes and CED-4 (NB-ARC), which are major domains of NB-LRR. Furthermore, there are 115 target genes containing a LRR domain in pepper, which are much more than those in other species (Fig. 1). Majority of the NB-LRRs were targeted by conserved miRNAs including miR482 family, miR6024 and miR6027 (Table S6). miR482 family is conserved in most plant species (Zhang et al., 2016), while miR6024 and miR6027 were only identified in Solanaceae (Li et al., 2012). miR6024 and miR6027 are less conserved than miR482, however, they also targeted numerous NB-LRRs in pepper, tomato and potato. Transcription factors were identified as the second most abundant genes targeted by miRNAs. Transcription factors including Teosinte Branched 1, Cycloidea, and PCF (TCP), ARF and MYB were mostly targeted by conserved miRNAs (Table S6).

Target genes of the species-specific miRNAs were also analyzed in detail (Table 1). In results, more than half of the pepper-specific miRNAs were not identified to trigger target gene cleavage by degradome analysis. It is consistent with the previous studies that most of young miRNAs, generally species-specific, do not have their target genes or functions, because miRNAs have evolved in a

neutral way (Axtell, 2008; Cuperus et al., 2011). However, several pepper-specific miRNAs target numerous genes encoding NB-LRRs, receptor-like proteins (RLPs), F-box proteins and accelerated cell death proteins (Table 1). For example, both can-miR-n002a-c and can-miR-n005a-5p target three F-box proteins (Table S6). Interestingly, these genes are primarily associated with plant defense system (Gou et al., 2009; Fei et al., 2016). Furthermore, can-miR-n026 and can-miR-n033a-3p were identified to cleave numerous RLPs and NB-LRRs (17 and 31, respectively). Generally, only well-conserved miRNAs like miR482 have plenty of target genes (Cuperus et al., 2011), whereas young miRNAs are weakly expressed and have few target genes (Fahlgren et al., 2010; Cuperus et al., 2011; Nozawa et al., 2012; Hwang et al., 2013). However, miR-n026 and miR-n033 were identified to induce cleavage of numerous target genes, although they were only identified in pepper, suggesting their importance in plant defense system. Furthermore, representative examples of cleaved genes associated with disease-resistance by conserved miRNAs (miR482, miR6024 and miR6027) and pepper-specific miRNAs (miR-n026 and miR-n033a) were evident in degradome analysis (Fig. S2). It supported the hypothesis that pepper-specific miRNAs have a significant function to regulate disease-resistance.

PhasiRNAs are also involved in disease-resistance of pepper

Previously, several studies showed that some miRNAs could induce production of secondary small RNAs like phasiRNA to control more target genes efficiently (Zhai et al., 2011; Shivaprasad et al., 2012; Xia et al., 2013). To confirm these findings in this study, putative *PHAS* loci in pepper, tomato and potato were analyzed with the public small RNA libraries (Fei et al., 2011; Tomato Genome, 2012; Zuo et al., 2012; Hwang et al., 2013; Lakhotia et al., 2014) using revised algorithms (Fig. S3) (Chen et al., 2007; Xia et al., 2013). In results, 39, 13, 14 protein-coding *PHAS* loci were identified in pepper, tomato and potato,

respectively (Table 2). There were several gene families including NB-LRR, RLP, nuclear transcription factor Y and transport inhibitor response 1-like (TIR1-like). Most of these protein coding genes were already reported as *PHAS* genes in other plants (Zhai et al., 2011; Xia et al., 2013; Arikit et al., 2014), indicating conservation of the phasiRNA mechanism in diverse plant species. However, *TAS*, which is well-known example of *trans*-acting *PHAS*, was not identified in the result. Interestingly, majority of *PHAS* loci are involved in disease-resistance, especially in pepper. There are 28 NB-LRRs, 8 RLPs and 1 TIR1-like protein genes among *PHAS* of pepper, which are associated with ETI and PTI in plants (Fei et al., 2016).

Triggers of the phasiRNA biogenesis were identified by degradome analysis using CleaveLand4 (Table 2) (Brousse et al., 2014). Length of the identified triggers for phasiRNA biogenesis is generally 22-nucleotides, corresponding with the previous studies (Table S1) (Cuperus et al., 2010; Zhai et al., 2011; Xia et al., 2013). miR482 family is known as a trigger of phasiRNA biogenesis, suggesting that miR482 amplified regulation on NB-LRR through phasiRNA to control disease-resistance extensively (Zhai et al., 2011; Shivaprasad et al., 2012). miR6027 is also known as a trigger like miR482 through cleaving NB-LRR transcripts (Li et al., 2012; Zheng et al., 2015). In this study, miR482 and miR6027 were identified to induce phasiRNA biogenesis by cleaving NB-LRRs (Fig. S4-5). For example, can-miR482c targeted coiled-coil NB-LRR (CNL, CA05g06820), and its cleavage site in CA05g06820 fell into 21-nucleotide phasing window (Fig. S4). In the same context, other miR482 members were also identified as a trigger (Table S7). can-miR6027-3p cleaved another CNL (CA04g14510), and triggered phasiRNA biogenesis (Fig. S5). miR6024 is other miRNA that regulate NB-LRR genes, such as *I2* gene in tomato and *Rx1* gene in potato (Wei et al., 2014). However, there was no evidence that miR6024 is a phasiRNA trigger in pepper and potato, although it was identified as a phasiRNA trigger in tomato and

potato (Li et al., 2012). miR6024 is 22-nucleotides in tomato and potato, but 21-nucleotides in pepper (Table S1). Therefore, can-miR6024 might not induce phasiRNA biogenesis in pepper, considering length of the major phasiRNA triggers (22-nucleotides) (Fei et al., 2013). In the case of stu-miR6024-3p, it might be missed in the result because of the high-redundancy problem in the potato degradome library. miR393 is a conserved miRNA that control *TIR1*-like genes and known as a phasiRNA trigger in Arabidopsis (Si-Ammour et al., 2011). Also, it was reported that miR393 is induced by *Phytophthora sojae* and involved in basal defense response of soybean (Wong et al., 2014). In pepper and potato, miR393 was also identified as a phasiRNA trigger of *TIR1*-like gene (Table 2). Interestingly, pepper-specific miRNAs also triggered phasiRNA biogenesis, and all their target genes are associated with disease-resistance. can-miR-n033a-3p generated phasiRNAs through cleaving four NB-LRRs (Fig. S6). Furthermore, can-miR-n026 induced phasiRNA production by cleaving eight RLPs (Table 2, Fig. 2).

To investigate whether phasiRNAs also cleave target genes, degradome analysis was performed with the phasiRNAs as input via CleaveLand4 (p-value $\leq$ 0.05 or penalty score $\leq$ 4) (Brousse et al., 2014). A few phasiRNAs from resistance-associated genes acted in *cis* or *trans*. Several phasiRNAs, which were generated from a CNL (CA05g06820) by can-miR482c, regulated their original genes in *cis* or another NB-LRR (CA05g05440) in *trans* (Fig. S4). Likewise, phasiRNAs generated from the RLP (CA08g01140) by can-miR-n026 also acted in *cis* or *trans* (Fig. 2B-C). In the case of can-miR-n033a-3p, phasiRNAs from the NB-LRR (CA06g02150) were identified to only act in *cis* (Fig. S6). These results are consistent with the previous study about a function of phasiRNAs (Zhai et al., 2011), suggesting that phasiRNAs might reinforce regulation of the target genes by miRNAs, especially in disease-resistance.

Evolution of miR-n026 and miR-n033 genes within pepper, tomato and potato

Several miRNAs in pepper were identified to target numerous genes involved in disease-resistance and induce phasiRNA biogenesis. To validate expression of these miRNAs, northern blot was conducted in leaves of pepper, tomato, potato and *N. benthamiana* (Fig. S7). In the result, can-miR-n033a-3p and can-miR-n026 were only expressed in pepper, while can-miR6027 were also expressed in tomato and pototo. It is accordant with the previous classification that miR-n026 and miR-n033 are pepper-specific (Hwang et al., 2013), but miR6027 are conserved in Solanaceae (Li et al., 2012). However, expression of miR6023 was only validated in pepper, although it is known as a conserved miRNA like miR6027 (Li et al., 2012). It might result from difference of miR6023 sequences between pepper and other species, perhaps causing disruption of probe hybridization (Table S1).

To examine whether miR-n026 and miR-n033 are truly pepper-specific, their gene sequences were found in tomato and potato using BLASTn. Interestingly, homologous regions of miR-n026 and miR-n033 were identified on the intergenic regions of chromosome 12 and 10, respectively (Fig. 3). These regions show synteny in tomato and potato. Sequence of miR6027 precursor was conserved among the three species, and miR-n033a and miR-n033b genes were located near miR6027 in pepper and potato (Fig. 3A). Previously, miR-n033a and miR-n033b were not identified in potato. However, miR-n033b and miR6027 seems to share the same precursor structure between pepper and potato (Fig. S8A-C). Furthermore, miR-n033b was identified to express in potato using public small RNA sequencing data (Fig. S8E) (Tomato Genome, 2012; Lakhotia et al., 2014). In the case of miR-n033a, it is also conserved and expressed in pepper and potato, but not in tomato (Fig. S9). Overall, miR-n033 family is not pepper-specific, just not identified in potato previously. To identify whether miR-n033 genes were lost in tomato, miR-n033 genes were examined in other Solanaceous species [eggplant (*Solanum melongena* L.) (Hirakawa et al., 2014), *Petunia axillaris* (Bombarely et al., 2016) and *N. benthamiana* (Bombarely et al., 2012)] via BLASTn. miR-n033a region is

conserved in these species, and miR-n033b region is only conserved in *Capsicum* and *Solanum* species (Fig. S10). Considering that the divergence of *Capsicum* and *Solanum* species was estimated to occur approximately 19 million years ago (mya), and tomato and potato was estimated to diverge from their common ancestor 8 mya (Bombarely et al., 2016), It indicates that miR-n033 genes originated in a common ancestor of *Capsicum* and *Solanum* species and lost in tomato.

Flanking genes of the miR-n026 region were also conserved among pepper, tomato and potato. Interestingly, homologous region of miR-n026 exists in the three species (previously not identified in tomato and potato), but the precursor structure of can-miR-n026 is different from those in tomato and potato (Fig. S11). miR6023 and miR-n026 seem to share the same precursor in tomato and potato, but not in pepper (Fig. 3B). It might result from either pepper-specific translocation of the miR6023 gene from the miR-n026 region or translocation of the miR6023 gene to the miR-n026 region in an ancestor of *Solanum* species. Although precursor structure or miR-n026 is not conserved between pepper and *Solanum* species, expression of the miR-n026 candidates in tomato and potato was confirmed using public small RNA libraries (Mohorianu et al., 2011; Tomato Genome, 2012; Lakhotia et al., 2014; Wu et al., 2016). In summary, miR-n026 and miR-n033 were not pepper-specific and their regions showed dynamic evolution, despite in syntenic regions.

Different target affinity of miR-n033 between pepper and potato

A previous study reported that NB-LRR genes in Solanaceae were classified into 13 subgroups, including 12 CNL and 1 Toll interleukin 1 receptor NB-LRR (TNL) subgroups, based on the phylogenetic relationship of NB-ARC domains (Seo et al., 2016). To compare target specificity of miRNAs regulating NB-LRRs in pepper, the target NB-LRRs were classified into the subgroups. Interestingly, most target NB-LRRs of can-miR-n033 family belong to the CNL-G1 subgroup (Fig. 4A),

which was duplicated after the divergence of pepper from a common ancestor of *Capsicum* and *Solanum* species. Particularly, duplication of the CNL-G1 subgroup was estimated to reach a peak around 10 mya (Seo et al., 2016). Therefore, can-miR-n033 family might have acquired or reinforced their affinity for CNL-G1 NB-LRRs after speciation of pepper from an ancestor. In other words, can-miR-n033 family might have evolved to regulate numerous NB-LRR genes that have recently been duplicated. To investigate how miRNAs targeting NB-LRRs could regulate a number of targets, domains of target NB-LRRs were analyzed (Fig. 4B). Target region of can-miR-n033 family in the NB-LRRs was different with those of other NB-LRR-targeting miRNAs, except can-miR6027. can-miR-n033a-3p and can-miR-n033b targeted the resistance nucleotide binding site (RNBS)-D motif in the ARC2 domain, while can-miR482 family and can-miR6024 targeted the P-loop motif (Fig. 4B). Because RLPs were not classified into subgroups yet, only domains of RLPs regulated by can-miR-n026 and can-miR6023 were analyzed in pepper. Both can-miR-n026 and can-miR6023 were identified to target the region encoding leucine-rich repeat N-terminal domain (LRRNT) (Fig. 4C).

As above, homologous region of the miR-n026 gene exists in tomato and potato, and those of the miR-n033 family exist in potato. Therefore, target affinity of miR-n026 and miR-n033 family was investigated to examine whether there is a difference between pepper, tomato and potato. Because degradome libraries were different in depth, target prediction using psRNAtarget (score $\leq$ 3.5) was adopted to compare target affinity (Dai and Zhao, 2011). Remarkably, can-miR-n033a-3p was predicted to target 37 NB-LRRs (32%) which are more than other miR-n033s (can-miR-n033b-3p: 19, 16%; stu-miR-n033a-3p: 16, 44%; stu-miR-n033b-3p: 12, 33%, respectively; Table 3). It suggests that can-miR-n033a-3p evolved to target more CNL-G1 NB-LRRs, and only a portion of expanded CNL-G1 NB-LRRs was predicted to be targeted by can-miR-n033a-3p. To examine how can-miR-n033a-3p could acquire more target affinity than other miR-n033s, target CNL-G1 NB-LRRs

were analyzed in detail. Target CNL-G1 NB-LRRs of the miR-n033 family in pepper and potato were assigned to the phylogenetic tree from the other study (Seo et al., 2016) and most of them were overlapped with each other (Fig. 5B). It indicates that miR-n033 family might regulate CNL-G1 NB-LRRs before the divergence of *Capsicum* and *Solanum* species. Thus, can-miR-n033a-3p might reinforce its target affinity after the divergence of pepper from a common ancestor of *Capsicum* and *Solanum* species, due to expansion of CNL-G1 NB-LRRs in pepper. This hypothesis is supported by the facts that sequences of mature miR-n033s are different in these species (Fig. 5A) and consensus sequences of the CNL-G1 NB-LRRs targeted by miR-n033a-3p, which correspond with the different sequences in miRNAs, are also different between pepper and potato (Fig. 5C). It suggests that miRNAs and their target CNL-G1 NB-LRRs might evolve together after the divergence of species. In summary, possible scenario is as below: (1) miR-n033 genes existed in a common ancestor of *Capsicum* and *Solanum* species and they regulated CNL-G1 NB-LRRs; (2) after pepper diverging from the ancestor (19 mya) (Bombarely et al., 2016), CNL-G1 subgroup in pepper was highly expanded causing sequence diversification of CNL-G1 NB-LRRs in pepper (Fig. 5B); (3) can-miR-n033a-3p evolved to target more CNL-G1 by mutation (Fig. 5A and Table 3). can-miR-n026 was also predicted to target more RLPs than miR-n026 candidates in tomato and potato (Table S8). Like miR-n033s, sequences of the mature miR-n026s are different (Table S8). Difference in targeting affinity between miR-n026s might result from mutation of the miRNAs and/or expansion of target genes.

Discussion

miRNAs play important roles in the post-transcriptional regulation of gene expression. In this study, miRNAs and their target genes in pepper, tomato and potato were analyzed in a comparative method. Degradome analysis reveals target genes of miRNAs in a genome-wide scale. Results were consistent with the other study, such as can-miR396 was confirmed to target DRM by degradome analysis, which had been identified in pepper and tomato, exclusively (Table S6) (Hwang et al., 2013). Moreover, a number of genes targeted by miRNAs were identified to be associated with the plant disease-resistance. Previous studies showed that several conserved miRNAs are involved in disease-resistance, both PTI and ETI. In Arabidopsis, one of the well-known bacterial flagellin, flg22, induces miR393 which regulates F-box auxin receptors, TIR1, and auxin signaling F-box protein 2 and 3, causing restriction of *Pseudomonas syringae* growth (Navarro et al., 2006). In soybean, it became more susceptible to *Phytophthora sojae* when miR393 was knocked down (Wong et al., 2014). Therefore, suppression of auxin signaling by miR393 results in enhanced PTI. In addition, miR160a positively regulates callose deposition by repressing ARFs, when PAMP is induced (Li et al., 2010). miRNAs regulate ETI via NB-LRRs as well as PTI via control of hormone signaling. miR1507, miR2109 and miR2118 target hundreds of NB-LRRs in *Medicago truncatula* (Zhai et al., 2011; Fei et al., 2015). In Solanaceae, miR482, miR5300, miR6019 and miR6027 were reported to regulate NB-LRRs (Li et al., 2012; Shivaprasad et al., 2012). Moreover, some miRNAs regulating NB-LRRs induce phasiRNA biogenesis to regulate more NB-LRRs. For example, three miRNAs (miR2118, miR2109 and miR1507), whose length is 22-nucleotide, recognize conserved domains of NB-LRRs and trigger the production of phasiRNA in legume (Zhai et al., 2011). In *N. benthamiana*, nta-miR6017 and nta-miR6020 target Toll/interleukin-1 receptor domain in the *N* gene, and overexpression of these miRNAs attenuate *N*-mediated resistance against tobacco mosaic virus (Li et

al., 2012).

Degradome analysis identified that several miRNAs cleaved NB-LRRs and RLPs in pepper. Especially, can-miR-n033 and can-miR-n026 were identified to target dozens of NB-LRRs and RLPs (Table 1), although they might be conserved only in several Solanaceous species. It is a uncommon phenomenon because most miRNAs, whose genes are relatively young and not conserved well, show lower expression than conserved miRNAs or are expressed in specific condition (Rajagopalan et al., 2006). Similarly, most of species-specific miRNAs have a tendency to have few target genes, because they evolved neutrally (Cuperus et al., 2011). Considering that can-miR-n033a and can-miR-n026 induced phasiRNA biogenesis and phasiRNAs regulate NB-LRRs and RLPs in *cis* or *trans* (Fig. S6 and Fig. 2), miRNAs associated with disease-resistance might evolve to regulate a number of genes via phasiRNA, and it might provide long-term benefits for *R* gene evolution. In detail, NB-LRRs generally induce disease-resistance via hypersensitive response (HR) against pathogen infection, causing host cell death (Jones and Dangl, 2006). Previous studies demonstrated that some NB-LRRs could trigger HR in host without pathogen (Michael Weaver et al., 2006; Rairdan et al., 2008; Wang et al., 2016). Thus, if NB-LRRs are not strictly regulated, it could result in serious fitness costs in host plants (Tian et al., 2003). Consequently, miRNAs regulating NB-LRRs might have evolved to control more NB-LRRs by the production of phasiRNAs (Shivaprasad et al., 2012; Fei et al., 2013). It might contribute to relieve the potential problems caused by the rapid evolution of *R* genes like unexpected HR. Hence, miRNAs and phasiRNAs might enable divergent evolution of *R* genes.

It is known that hundreds of NB-LRRs exist in plant genomes. They have evolved during an arms race with pathogens. As above, overactive plant immune systems could be harmful to host because of fitness costs (Tian et al., 2003). For example, a dominant mutant suppressor of npr1-1, constitutive 1 (snc1), which is a

TNL type gene, exhibited constitutive activation of HR without pathogen recognition, and the dwarf phenotype with curly leaves was shown in *Arabidopsis* (Li et al., 2001; Zhang et al., 2003). Therefore, plant should repress NB-LRRs when there is no pathogen, and fine tuning of NB-LRR expression is necessary. In this study, miR-n033 family in pepper was identified to target a number of NB-LRRs via degradome analysis (Table 1). Particularly, there are many CNL-G1 NB-LRRs, whose subgroup was expanded in pepper-specific (Fig. 4-5) (Seo et al., 2016). It suggests the divergent evolution of miRNA and their target NB-LRRs between the different species. However, few CNL-G2 NB-LRRs were found to be targeted by miRNAs, although CNL-G2 was also expanded in pepper (Seo et al., 2016). Therefore, there is a possibility that the other miRNAs or mechanisms, which regulate CNL-G2 NB-LRRs, exist in pepper. It requires further investigation to elucidate it.

miR-n026 was identified to regulate RLPs in pepper. There are few studies about the relationship between miRNA and RLPs. The previous study exhibited that sly-miR6022 and sly-miR6023 regulate *Hcr9*, a Cf-9 homolog in tomato (Li et al., 2012), and the other study showed that sly-miR6022 regulate other RLPs via degradome analysis in tomato fruit (Karlova et al., 2013). Cf-9 genes confer resistance against *Cladosporium fulvum* by recognizing pathogen-derived avirulence factor (Parniske et al., 1997). When Cf-9 recognized the pathogen, *C. fulvum* race 5, necrosis occurred in host. It proposes the importance of Hcr9 in disease-resistance. Meanwhile, an RLP kinase, CaLRR51, was validated to role in the resistance of pepper against *Ralstonia solanacearum*. Particularly, transient overexpression of the *CaLRR51* resulted in HR-like cell death responses, indicating necessity of the strict control of RLPs (Cheng et al., 2017). Degradome analysis revealed that miR-n026 cleaved some *Hcr9* genes in pepper (Table 2), although miR-n026s in tomato and potato were predicted to target few RLPs (Table S8). Hence, miRNAs targeting RLPs in pepper, especially miR-n026, might have

evolved with their target RLPs to regulate more RLPs for avoiding unwanted resistance responses. There might be other mechanisms to compensate for the absence of miR-n026 function in tomato and potato.

Previously, miR-n026 and miR-n033 had been regarded as pepper-specific miRNAs, but their homologs were identified to be also conserved in tomato and potato. There are several possibilities that could explain why miR-n033 and miR-n026 were not annotated in tomato and potato. First, all public small RNA libraries from several studies for each species were merged to examine expression of miR-n026 and miR-n033 (Mohorianu et al., 2011; Tomato Genome, 2012; Lakhotia et al., 2014; Wu et al., 2016). Therefore, expression of miR-n026 and miR-n033 in tomato and potato were too low to be identified as miRNA in the other studies. Second, miR-n033b and miR-n026 shared the same precursor with miR6027 and miR6023, respectively. Thus, miR-n033b and miR-n026 might be excluded, because a precursor of miRNA is generally considered to generate one mature miRNA. Third, length of the miR-n026 and miR-n033 precursors in tomato and potato is quite long (> 200nt). Then, it is hard to identify them with general criteria for miRNA annotation. Because of these possibilities, miR-n026 and miR-n033 in tomato and potato need validation in further study.

In conclusion, several miRNAs in pepper was revealed to target genes encoding disease-resistance associated proteins. miR-n026 and miR-n033 regulate numerous NB-LRRs and RLPs in pepper. This study provides an insight into evolution of miRNAs and target genes involved in disease-resistance, which has promoted differentiation of miRNA function.

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Table 1. Identified target genes of pepper-specific miRNAs via degradome analysis.

miRNA	Target description	# of targets
miR-n002a-c	F-box/kelch-repeat protein	3
miR-n003a-c-3p	ACCELERATED CELL DEATH 6-like	6
miR-n005a-5p	F-box protein CPR30-like	3
miR-n010	receptor-like protein 12-like	2
miR-n016a-b	leucine-rich repeat receptor kinase	2
miR-n022a-c	ATP-citrate synthase beta chain 2	2
	F-box PP2-B10-like	2
miR-n026	receptor-like protein 12-like	17
	acetylajmalan esterase-like	4
miR-n033a	disease resistance protein, late blight resistance homolog[a]	31
miR-n033b	disease resistance protein, late blight resistance homolog[b]	4
miR-n056	disease resistance RPP13	2

[a] and [b] are different from each other

Table 2. List of *PHAS* genes and their trigger miRNAs.

Trigger	Genes producing phasiRNA cluster	Pepper[e]	Tomato	Potato
miR482	disease resistance protein, NB-LRR[a]	20	6	7
miR5300	disease resistance protein, NB-LRR[b]	0	1	0
miR6023	LRR receptor-like protein	0	1	1
miR6024	disease resistance protein, NB-LRR[c]	0	4	0
miR6027	disease resistance protein, NB-LRR[d]	3	1	3
miR8041	disease resistance protein, NB-LRR	0	0	1
miR-n026	LRR receptor-like protein	8	0	0
miR-n033	NB-LRR	4	0	0
miR169	Nuclear transcription factor Y subunit A (NF-YA)	1	0	0
miR171	GRAS family transcription factor	1	0	0
miR319	TCP4	0	0	1
miR393	TRANSPORT INHIBITOR RESPONSE 1-like	1	0	1

[a, b, c] and [d] are different from each other

[e]Number of *PHAS* genes

Table 3. Comparing target prediction results of miR-n033 in pepper and potato.

miRNA	Species	G1[a]	G3	G5	G6	G9	G12	Ng
can-miR-n033a-3p	Pepper	37/116[b]	1/37			6/73	1/17	2
	Potato	7/36	2/17			4/55		
can-miR-n033b-3p	Pepper	19/116	6/37	1/16	1/48	3/73	1/17	
	Potato	12/36	4/17			8/55		1
stu-miR-n033a-3p	Pepper	4/116	1/37			3/73		
	Potato	16/36	1/17			1/55		
stu-miR-n033b-3p	Pepper	3/116	4/37	1/16	2/48	18/73	1/17	
	Potato	12/36	3/17		2/39	9/55		1

[a]G1 to Ng are classes of NLRs. Subgroup G1 to G12 belong to CNL, Ng: NLR

[b]The number of predicted targets (score cutoff = 3.5) / total number of NLRs belonging to each subgroup

Figure 1. Degradome analysis in Solanaceous species.

(A) The numbers of miRNAs targeting at least one gene in pepper (*Capsicum annuum*), tomato (*Solanum lycopersicum*), and potato (*Solanum tuberosum*) were indicated in the parenthesis. (B) The top 10 domains of degradome target genes were identified by InterproScan. X and Y axis indicate the number of genes having certain domains and the description of the domain, respectively. NB-ARC: nucleotide-binding domain shared by APAF-1, Resistance genes, and CED-4; TCP: TEOSINTE BRANCHED1, CYCLOIDEA.

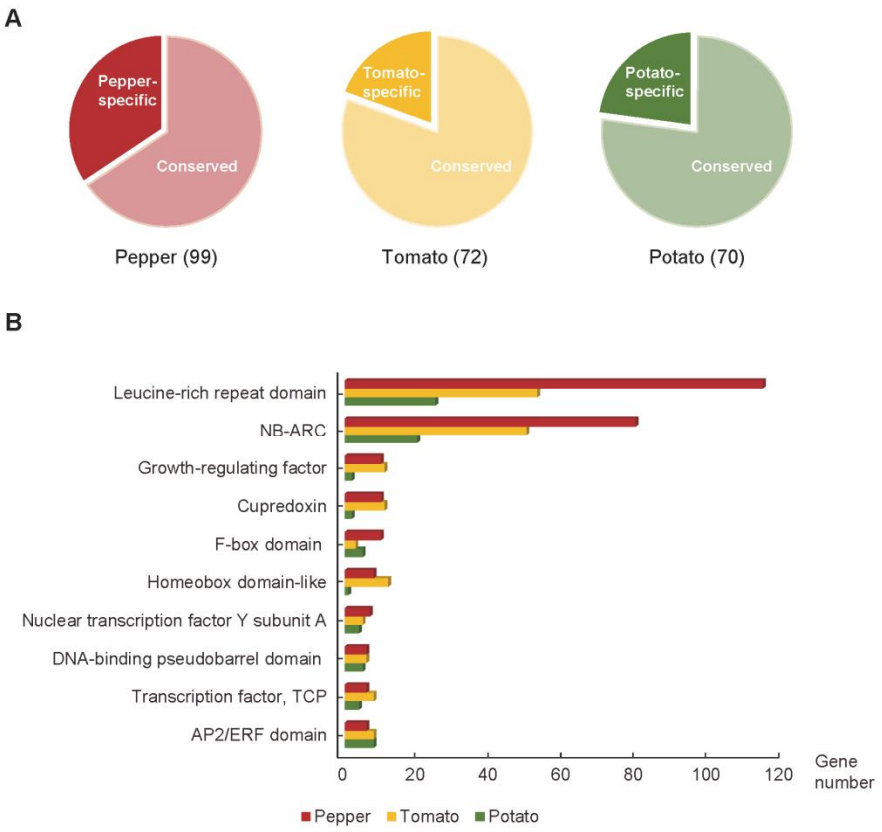

A
Pepper-specific
Conserved
Tomato-specific
Conserved
Potato-specific
Conserved
Pepper (99)
Tomato (72)
Potato (70)
B
Leucine-rich repeat domain
NB-ARC
Growth-regulating factor
Cupredoxin
F-box domain
Homeobox domain-like
Nuclear transcription factor Y subunit A
DNA-binding pseudobarrel domain
Transcription factor, TCP
AP2/ERF domain
0
20
40
60
80
100
120
Gene number
Pepper
Tomato
Potato

Figure 2. can-miR-n026 induces phasiRNA biogenesis by cleaving RLP.

(A) Mapping results of small RNA and degradome reads normalized by mapping count to the genome and phasing score data were viewed using IGV. Red arrow indicates the cleavage site by can-miR-n026. Examples of (B) *cis*-acting phasiRNAs and (C) *trans*-acting phasiRNAs. Red triangles indicate the cleavage sites by small RNAs. In degradome reads panel, pale red color indicates reads mapped to Watson strand and blue color indicates those mapped to Crick strand in the genome.

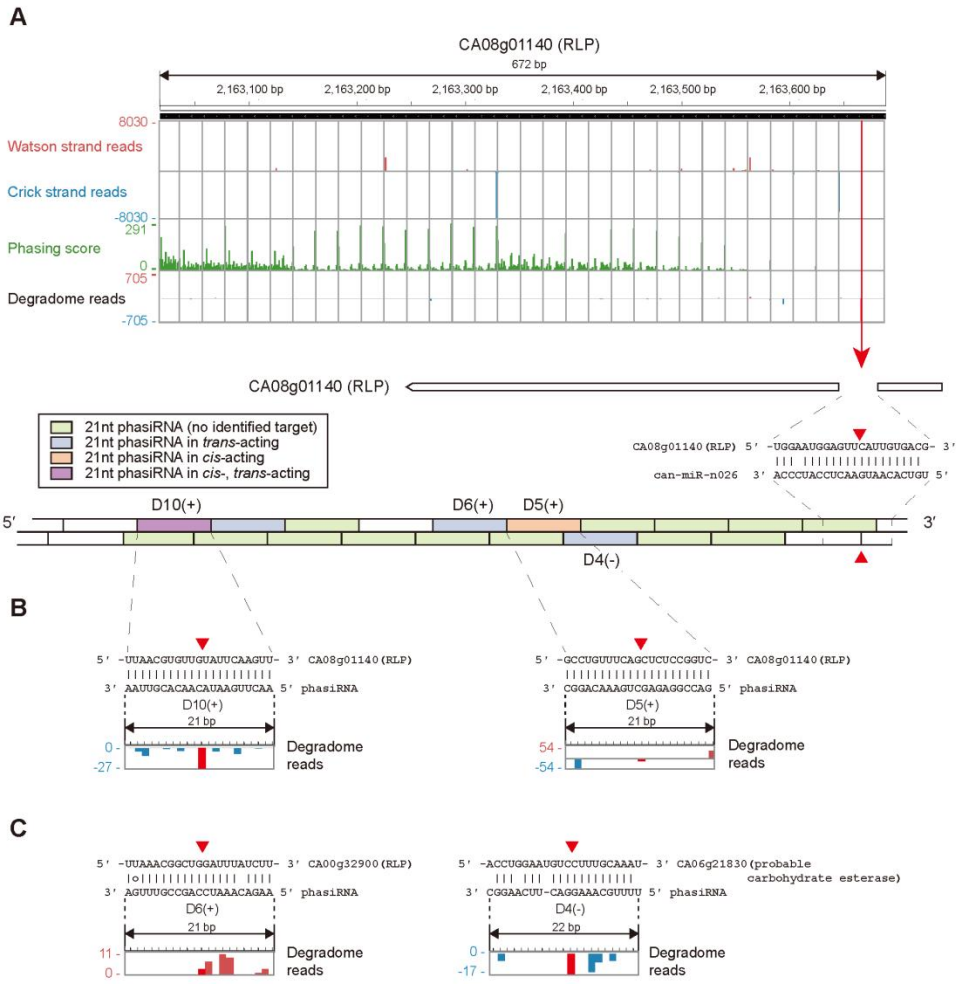

A
CA08g01140 (RLP)
672 bp
2,163,100 bp
2,163,200 bp
2,163,300 bp
2,163,400 bp
2,163,500 bp
2,163,600 bp
8030
Watson strand reads
Crick strand reads
-8030
291
Phasing score
0
705
Degradome reads
-705
CA08g01140 (RLP)
21nt phasiRNA (no identified target)
21nt phasiRNA in trans-acting
21nt phasiRNA in cis-acting
21nt phasiRNA in cis-, trans-acting
CA08g01140 (RLP) 5' -UGGAAUGGAGUUCAUUGUGACG- 3'
can-miR-n026 3' ACCCUACCUCAAGUAACACUGU 5'
5'
D10(+)
D6(+)
D5(+)
D4(-)
3'
B
5' -UUAACGUGUUGUAUUCAAGUU- 3' CA08g01140(RLP)
3' AAUUGCACAACAUAAGUUCAA 5' phasiRNA
D10(+)
21 bp
0
-27
Degradome reads
5' -GCCUGUUUCAGCUCUCCGGUC- 3' CA08g01140(RLP)
3' CGGACAAAGUCGAGAGGCCAG 5' phasiRNA
D5(+)
21 bp
54
-54
Degradome reads
C
5' -UUAAACGGCUGGAUUUAUCUU- 3' CA00g32900(RLP)
3' AGUUUGCCGACCUAAACAGAA 5' phasiRNA
D6(+)
21 bp
11
0
Degradome reads
5' -ACCUGGAAUGUCCUUUGCAAAU- 3' CA06g21830(probable carbohydrate esterase)
3' CGGAACUU-CAGGAAACGUUUU 5' phasiRNA
D4(-)
22 bp
0
-17
Degradome reads

Figure 3. Microsynteny analysis of clusters producing miR-n033 and miR-n026, targeting NLRs and RLPs, respectively.

Red boxes and blue boxes indicate species-specific miRNAs and conserved miRNAs, respectively. Empty boxes with dot line indicate homologous regions of miRNAs that were not annotated. (A) Intergenic regions producing miRNAs targeting NLRs in pepper and the corresponding regions in tomato and potato are depicted. Pink and light green arrows indicate genes encoding receptor protein kinase *TMK1-like* and Coatomer subunit beta-1-like, respectively. Dark blue box indicates LTR/Gypsy element. miRNA genes were depicted as grey boxes. miRNAs targeting NLRs were written in red. (B) Intergenic regions producing miRNAs targeting RLPs in pepper and the corresponding regions in tomato and potato are depicted. Khaki and yellow arrows indicate genes encoding 4Fe-4S ferredoxin and ATP citrate lyase, respectively. miRNA genes were depicted as grey boxes. miRNAs targeting RLPs were written in red.

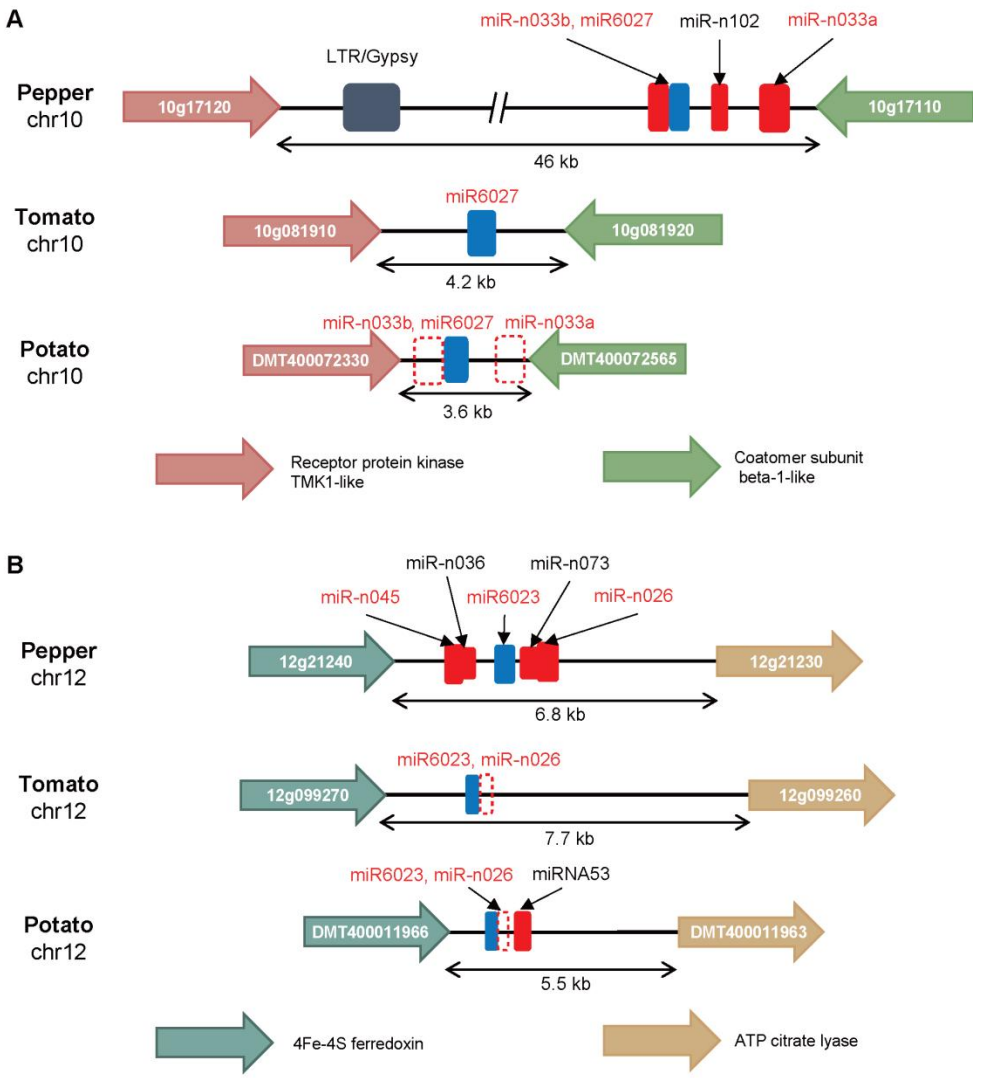

A
Pepper
chr10
LTR/Gypsy
miR-n033b, miR6027
miR-n102
miR-n033a
10g17120
10g17110
46 kb
Tomato
chr10
miR6027
10g081910
10g081920
4.2 kb
Potato
chr10
miR-n033b, miR6027
miR-n033a
DMT400072330
DMT400072565
3.6 kb
Receptor protein kinase
TMK1-like
Coatomer subunit
beta-1-like
B
Pepper
chr12
miR-n036
miR-n073
miR-n045
miR6023
miR-n026
12g21240
12g21230
6.8 kb
Tomato
chr12
miR6023, miR-n026
12g099270
12g099260
7.7 kb
Potato
chr12
miR6023, miR-n026
miRNA53
DMT400011966
DMT400011963
5.5 kb
4Fe-4S ferredoxin
ATP citrate lyase

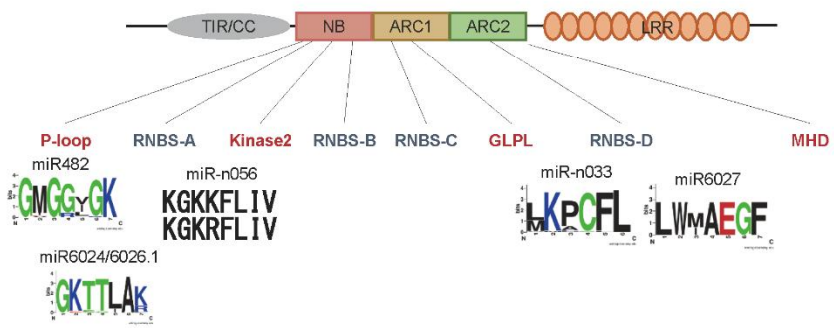
Figure 4. Group-specific NLR targets in pepper.

(A) The number of NLR targets according to their subgroups by miRNAs in pepper were shown as heatmap. NLR subgroups were followed to previous study and subgroup G1 to G12 belong to CNL 50. (B) Targeting regions in NLRs by miRNAs in pepper. Domains and motifs of NLRs were marked. Major and minor motifs were written in red and blue, respectively. Representative amino acid sequences encoded by target regions were shown using WebLogo. Some miRNA targets were unclassified because they are thought to target untranslated regions. (C) Targeting regions in RLPs by miRNAs in pepper. Domains of RLPs were marked. Representative amino acid sequences encoded by target regions were shown using WebLogo.

A

	NLR subgroup												
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	GT
miR482				9	4		2		3	2		3	3
miR6024				6		2			9		3		
miR6026											1		
miR6027		3			2			2					
miR-n019									1				
miR-n033	25								4				
miR-n050									1				
miR-n056				2									

B



C

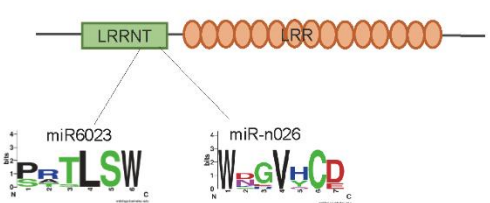
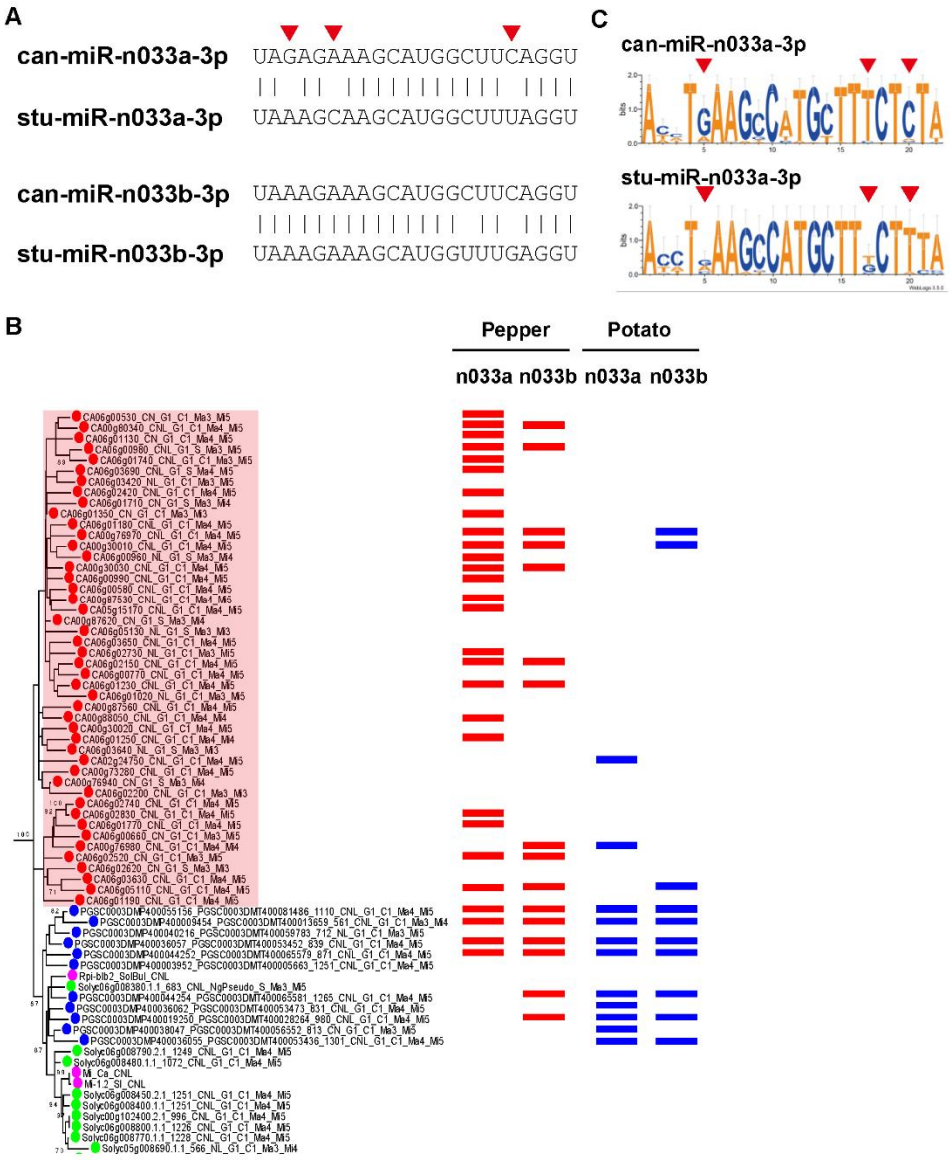


Figure 5. Evolutionary relationship between miR-n033 and CNL-G1.

(A) Alignment of mature miR-n033 sequences. Red triangles indicate different sequences between can-miR-n033a-3p and stu-miR-n033a-3p. (B) Detailed phylogenetic tree of CNL-G1 from our previous study (Refer to Fig. S2 from Seo et al., 2016). Phylogenetic tree of whole CNL-G1 was in Fig. S12. Transparent red box indicates duplicated CNL-G1 in pepper. Red boxes indicate the target CNL-G1 of can-miR-n033. Blue boxes indicate the target CNL-G1 of stu-miR-n033. Note, some CNL-G1 NLRs were excluded in the tree, because their NB-domain did not satisfy the criteria to construct a phylogenetic tree. (C) Consensus sequences of CNL-G1 targeted by miR-n033a. Red triangles indicate the sequences, whose complementary sequences in miRNAs are different.


A
can-miR-n033a-3p
UAGAGAAAGCAUGGCUUCAGGU
stu-miR-n033a-3p
UAAAGCAAGCAUGGCUUUAGGU
can-miR-n033b-3p
UAAAGAAAGCAUGGCUUCAGGU
stu-miR-n033b-3p
UAAAGAAAGCAUGGUUUGAGGU
C
can-miR-n033a-3p
stu-miR-n033a-3p
B
Pepper
Potato
n033a n033b n033a n033b

Supplementary materials

Table S1. List of pepper miRNAs.

ID	Mature sequence	ID	Mature sequence
can-miR1446a-b	UGAACUCUCUCCCUCAAUGGCU	can-miR172f	AGAAUCUUGAUGAUGCUGCAG
can-miR156a-c	UUGACAGAAGAUAGAGAGCAC	can-miR172g	GGAAUCUUGAUGAUGCUGC
can-miR156d-g	UGACAGAAGAGAGUGAGCAC	can-miR172h	AGAAUCUUGAUGCUGCUGCAU
can-miR156h	UUGACAGAAGAGAGUGAGCAU	can-miR172i	GGAAUCUUGAUGAUGCUGCAG
can-miR159a-c	UUUGGAUUGAAGGGAGCUCUA	can-miR319a	CUUGGACUGAAGGGAGCUCCC
can-miR160	UGCCUGGCUCCCUGUAUGCCA	can-miR319b-c	UUGGACUGAAGGGAGCUCCCU
can-miR162a-e	UCGAUAAACCUCUGCAUCCAG	can-miR319d	UCUUGGACUGAAGGGUUCCCU
can-miR162f	UCGAUAAACCUCUGCAUCCGG	can-miR319e	UUGGACUGAAGGGAGCUCCC
can-miR164a-b	UGGAGAAGCAGGGCACGUGCA	can-miR319f	UUGGACUGAAGGGAGCUCCUU
can-miR164c	UGGAGAAGCAGGGCGCGUGCA	can-miR319g	UUGGACUGAAGGGAGCUCCU
can-miR166a-h	UCGGACCAGGCUUCAUUCCCC	can-miR390a-b	AAGCUCAGGAGGGAUAGCGCC
can-miR166i-j	UCUCGGACCAGGCUUCAUUCC	can-miR390c	AAGCUCAGGAGGGAUAGCACC
can-miR166k	UCGAACCAGGCUUCAUUCCCC	can-miR393a-b	UCCAAAGGGAUCGCAUUGAUCC
can-miR166l	UCGGACCAGGCUUCAUUCCUC	can-miR394a	UUGGCAUUCUGUCCACCUCC
can-miR167a-c	UGAAGCUGCCAGCAUGAUCUA	can-miR394b	UUGGCAUUCUGUCUACCUCC
can-miR167d	UGAAGCUGCCAGCAUGAUCUGG	can-miR395a-j	CUGAAGUGUUUGGGGGAACUC
can-miR168a-b	UCGCUUGGUGCAGGUCGGGAC	can-miR396a	UUCCACAGCUUUCUUGAACUG
can-miR168c	UCGCUUGGUGCAGGUCGGGAA	can-miR396b	UUCCACAGCUUUCUUGAACUU
can-miR169a-g	UAGCCAAGGAUGACUUGCCU	can-miR396c	UUCCACAGCUUUCUUGAACUA
can-miR169h	UAUCGGCAGGUCAUCCUUGGC	can-miR397a	UCAUCUACGCUGCACUCAAUC
can-miR171a-e	UGAUUGAGCCGUGCCAAUAUC	can-miR397b	AUUGAGUGCAGCGUUGAUGAC
can-miR171f-g	UGAUUGAGCCGUGUCAAUAUC	can-miR398a-b	UGUGUUCUCAGGUCACCCCUU
can-miR171h	UGAGCCGAACCAAUAUCACUC	can-miR398c	UAUGUUCUCAGGUCACCCCUA
can-miR171i	UUGAGCCGCGCCAAUAUCACU	can-miR398d	UGUGUUCUCAGGUCGCCCCUG
can-miR171j	UUGAGCCGCGCCAAUAUCAUU	can-miR399a-e	UGCCAAAGGAGAAUUGCCCUG
can-miR171k	UUGAGCCGUGCCAAUAUCACGU	can-miR399f-g	UGCCAAAGGAGAGUUGCCCUG
can-miR171l	UUGAGCCGCGCCAAUAUCACG	can-miR399h	CGCCAAAGGAGAGCUGCCCUA
can-miR172a-d	AGAAUCUUGAUGAUGCUGCAU	can-miR403	UUAGAUUCACGCACAAACUCG
can-miR172e	UGAAUCUUGAUGAUGCUGCAU	can-miR408a	UGCACUGCCUCUUCCCUGGCU
can-miR408b	UGCACAGCCUCUUCCCUGGCU	can-miR-n014	CUGAAGUUCGUUACUGUUGUC
can-miR4376	ACGCAGGAGAGAUGAUGCUGGA	can-miR-n015	UUCAGGCCUGAGAAACGAAAAACU
can-miR4414a	AGCUGAUGACUCGUUGAUUCU	can-miR-n016a-b	AUCCAAUACAUCGUCCACAGC
can-miR4414b	AGCUGCUGAAUCAUUGGUUCG	can-miR-n017	CUAAGCAACGCUAUGUCGAGU
can-miR477	CCUCUCCCUCAAGGGCUUCUU	can-miR-n018	AAUUGGACUGGCGCACAUCGGGAG
can-miR482a	UUGCCGAUUCCGUCCAUACCGC	can-miR-n019a-d	UAAUAACUAGUAGUUGAGUGAU
can-miR482b	UUUCCAAUUCCACCCAUUCCUA	can-miR-n020	GGGGAUGUAGCUCAGAUGGUAGA
can-miR482c	UCUUGCCUACACCGCCCAUGCC	can-miR-n021	AUGGAUGAACAAUGCUGAAACAUU

can-miR482d	UCUUACCGAUACCUCCCAUUCC	can-miR-n022a-c	UGAACAAGUAGACACAUGCGUCCU
can-miR482e	CUACCAACUCCACCCAUUCCUG	can-miR-n023	AUGACUUACUUUGACUUGGCACA
can-miR482f	UCUUUCCUACUCCUCCCAUACC	can-miR-n024	UCAACUGCAAACCUGUAAGCCU
can-miR482g	UUUCCUAUUCCACCCAUGCCAA	can-miR-n025	AGGAAGGAACUCCACGUCAUUGCU
can-miR530	UGCAUUUGCACCUGCACCUGU	can-miR-n026	UGUCACAAUGAACUCCAUCCCA
can-miR6022-3p.2	UGGUAUUGUUCCGUUCAGGGA	can-miR-n027	AUGAAAGUUGUCGAUGCCAAC
can-miR6022-5p.2	CCUGAACUGAACAAUACGAUC	can-miR-n028a-b	UCAUCCGAGAUCGUUUCGCUGA
can-miR6023	UUCCAUGAAAGGGUUUUUGGAU	can-miR-n029	UUAGAGUGAGCUCAACAGAGU
can-miR6024-3p	UUUUAGCAAGAGUUGUUUUCC	can-miR-n030	UUCGAGGACCGUCAGUAGCAUA
can-miR6026-3p.1	UUCUUGGCUAGAGUUGUGUUGC	can-miR-n031-5p	UUCCCAGUCCAGGCAUUCCAAC
can-miR6026-3p.2	UUGAACCUUCAGGUGAGUUGC	can-miR-n031-3p	UGGUAUGCUUUGGUUGGGAAAG
can-miR6027-3p	UGAAUCCUUCGGCUAUCCAUAA	can-miR-n032	UGUUCCUGUAGAUAAGCCACU
can-miR6027-5p	AUGGGUAGCGAAGGAUUAACG	can-miR-n033a-3p	UAGAGAAAGCAUGGCUUCAGGU
can-miR827	UUAGAUGAACAUCAACAAACA	can-miR-n033a-5p	CUAAAGCAUGCUUUCUUUAUU
can-miR-n001	UUUCUGUUUUGAUAGUAGGCCU	can-miR-n033b-3p	UAAAGAAAGCAUGGCUUCAGGU
can-miR-n002a-c	UUGCAAACACACCUGAAUCGU	can-miR-n034	ACGGAUGAACUCGCAGAAGGACGA
can-miR-n003a-5p	UGUAGUUGUAGCCAUUCUAUU	can-miR-n035	CUUCGAACUAACUCCUUCUUGACA
can-miR-n003b-5p	UCUAGUUGUAGCCAUUCUAUU	can-miR-n036	AUGUGGGGGCAUGUGACUGAA
can-miR-n003a-c-3p	UAGAGUGGCCACAACUAGAUG	can-miR-n039	UUUGAUGAAGAUCUUACCUUG
can-miR-n004	UAAGAUCGAGCACAAGUUGUU	can-miR-n040	AUCCUUUGUAGCUGACGUGGCACC
can-miR-n005a-5p	UUCGAUACGCACCUGAAUCGCC	can-miR-n041	CACCGAGGACAAGAUCUUAAG
can-miR-n005a-b-3p	GAUUCAGGUUCGUAUGAAAAC	can-miR-n043	GAGACACAUCUGAACUUUAGGAGG
can-miR-n006	CAACAAUCAUCCUUUGGGCUUU	can-miR-n045	CAUCACAUUUAACUCCAUCUCA
can-miR-n008	CGGCAUGAGAGAAAAUAUUGAGAA	can-miR-n046	UUUGAUGCUCUUUGUUUGACA
can-miR-n010	UUAUGAGAUAAGUUCAACACG	can-miR-n048	AGGCACACCUGAUCUUUAGGAGGG
can-miR-n011	AGCAUCGAUUACAAUGACAAAAAG	can-miR-n050	AAGGGUAGCCCGGAACACUGAAGC
can-miR-n012	UUCGGCUCAUCGUUGUUGCAGACG	can-miR-n052	UGUGAUCACGAUUCUCUAGCCU
can-miR-n053	UUUCCAAAAUCUUAUGACAAG	can-miR-n071	UCACGUCAGCUAAAAGGGAUG
can-miR-n055	UUGUCAUAAUGGGUGCUACUG	can-miR-n073	CAGUCAUUUGGCCCGACGUCU
can-miR-n056	ACAAUAAGGAACUUUUUGCCU	can-miR-n081	AGAGGGGUUCACUGAUGCAAGCUG
can-miR-n057	UGAUGAAUCAAAGGAUGUAUCACU	can-miR-n089	UGCACGCAAGAUGGUCCGGACA
can-miR-n061	UCCAGUUGGACAGGUAUAUGCC	can-miR-n092	CGCGGCGACGUGGGCGGUU
can-miR-n063	UACUUUGGGUAUUCAUAUGCU	can-miR-n102	AUGGGUGCAGAAGGAUUGAUG
can-miR-n065	UUGUGAAGUUGGAGUGCAUC	can-miR-n105	AAUAUUUGGACAAGAAGGAGU
can-miR-n067	AGUUCAGGGAUAAUAGGACCC	can-miR-n124	GAGAGUGCACGUAUAGACACA
can-miR-n070	UUUGCCCUUGAUUCUAGCCGA		

Table S2. List of tomato miRNAs.

ID	Mature sequence	ID	Mature sequence
sly-miR156_3	CUGGCAGAAGAGAGUGAGCA	sly-miR172_3	GGAAUCUUGAUGAUGCUGCAG
sly-miR156a-c	UUGACAGAAGAUAGAGAGCAC	sly-miR172_5	UGAAUCUUGAUGAUGCUGCAU
sly-miR156d-3p	GCUCACUGCUCUAUCUGUCACC	sly-miR172a-b	AGAAUCUUGAUGAUGCUGCAU
sly-miR156d-5p	UGACAGAAGAGAGUGAGCAC	sly-miR1919a-c-3p	ACGAGAGUCAUCUGUGACAGG
sly-miR156e-3p	GCUUACUCUCUAUCUGUCACC	sly-miR1919c-5p	UGUCGCAGAUGACUUUCGCCC
sly-miR156e-5p	UGAUAGAAGAGAGUGAGCAC	sly-miR319a	CUUGGACUGAAGGGAGCUCC
sly-miR159	UUUGGAUUGAAGGGAGCUCUA	sly-miR319b	UUGGACUGAAGGGAGCUCCCU
sly-miR160a	UGCCUGGCUCCCUGUAUGCCA	sly-miR319c-3p	UUGGACUGAAGGGAGCUCCUU
sly-miR162	UCGAUAAACCUCUGCAUCCAG	sly-miR319c-5p	AGAGCUUCCUUCAGCCCACUC
sly-miR164a-3p	CAUGUGCCUGUUUUCCCCAUC	sly-miR3627_1	UCGCAGGAGAGAUGGCACUUGC
sly-miR164a-b-5p	UGGAGAAGCAGGGCACGUGCA	sly-miR390a-3p	CGCUAUCCAUCCUGAGUUUUA
sly-miR164b-3p	CACGUGUUCUCCUUCUCCAAC	sly-miR390a-5p	AAGCUCAGGAGGGAUAGCACC
sly-miR166a-b	UCGGACCAGGCUUCAUUCCCC	sly-miR390b-3p	CGCUAUCCAUCCUGAGUUUCA
sly-miR166c-3p	UCGGACCAGGCUUCAUUCCUC	sly-miR390b-5p	AAGCUCAGGAGGGAUAGCGCC
sly-miR166c-5p	GGGAUGUUGUCUGGCUCGACA	sly-miR394-3p	AGGUGGGCAUACUGUCAACA
sly-miR167a	UGAAGCUGCCAGCAUGAUCUA	sly-miR394-5p	UUGGCAUUCUGUCCACCUCC
sly-miR167b-3p	AGGUCAUCUAGCAGCUUCAAU	sly-miR395_3	UUGAAGUGUUUGAGGGAACUC
sly-miR167b-5p	UAAAGCUGCCAGCAUGAUCUGG	sly-miR395a-b	CUGAAGUGUUUGGGGGAACUCC
sly-miR168.2	UCCUGCCUUGCAUCAACUGAAU	sly-miR396a-3p	GUUCAAUAAAGCUGUGGGAAG
sly-miR168a-3p	CCUGCCUUGCAUCAACUGAAU	sly-miR396a-5p	UUCCACAGCUUUCUUGAACUG
sly-miR168a-b-5p	UCGCUUGGUGCAGGUCGGGAC	sly-miR396b	UUCCACAGCUUUCUUGAACUU
sly-miR169a	CAGCCAAGGAUGACUUGCCGG	sly-miR397	AUUGAGUGCAGCGUUGAUGA
sly-miR169b	UAGCCAAGGAUGACUUGCCUG	sly-miR399	UGCCAAAGGAGAGUUGCCCUA
sly-miR169c	CAGCCAAGGAUGACUUGCCGA	sly-miR403-3p	CUAGAUUCACGCACAAGCUCG
sly-miR169d	UAGCCAAGGAUGACUUGCCUA	sly-miR403-5p	CGUUUGUGCGUGAAUCUAACA
sly-miR169e-3p	UGGCAAGCAUCUUUGGCGACU	sly-miR4376	ACGCAGGAGAGAUGAUGCUGGA
sly-miR169e-5p	UAGCCAAGGAUGACUUGCCUUU	sly-miR4414_2	UUGCUGCUGAAUCAUUGGCUC
sly-miR171a	UGAUUGAGCCGUGCCAAUAUC	sly-miR477-3p	AGUUCUUGUAGGGUGAGACAAC
sly-miR171b	UUGAGCCGUGCCAAUAUCACG	sly-miR477-5p	UGUCUCUCCCUCAAGGGCUCC
sly-miR171c	UAUUGGUGCGGUUCAAUGAGA	sly-miR482a	UUUCCAAUUCCACCCAUUCCUA
sly-miR171d	UUGAGCCGCGCCAAUAUCAC	sly-miR482b	UCUUGCCUACACCGCCCAUGCC
sly-miR171e	UUGAGCCGCGUCAAUAUCUCU	sly-miR482c	UCUUGCCAAUACCGCCCAUUCC
sly-miR482d-3p	UUUCCUAUUCCACCCAUGCCAA	sly-miR9471b-5p	GAGGUGCUCACUCAGCUAAUA
sly-miR482d-5p	GGAGUGGGUGGGAUGGAAAAA	sly-miR9472-3p	UUCACAAUCUCUGCUGAAAAA

sly-miR482e-3p	UCUUUCCUACUCCUCCCAUACC	sly-miR9472-5p	UUUCAGUAGACGUUGUGAAUA
sly-miR482e-5p	UGUGGGUGGGGUGGAAAGAUU	sly-miR9472-5p.2	UUUUCAGUAGACGUUGUGAAU
sly-miR5300	UCCCCAGUCCAGGCAUUCCAAC	sly-miR9473-3p	AAACGAGUUCAGAUUUACAGC
sly_miR5303.2	AUUUUUGAAGAGUCCGAGCA	sly-miR9473-5p	UGGCUGUAAAUCUAAACUCGU
sly-miR5303	UUUUUGAAGAGUUCGAGCAAC	sly-miR9474-3p	UUUUGUUCGCAGAUACUACAGU
sly-miR5303_5	UUUUUGAAGAGUUUGGGCAAC	sly-miR9474-3p.2	UCUUGGAUUUUAUCGGAGUUA
sly-miR5303_6	UUUUUGAAGGAUCCGAGCAAC	sly-miR9474-5p	UGUAGAAGUCAUGAAUAAAAUG
sly-miR5304	UCAAUGCUACAUACUCAUCCC	sly-miR9475-3p	CUACAAUGUAGAGAUCGUUUU
sly-miR6022	UGGAAGGGAGAAUAUCCAGGA	sly-miR9475-5p	AACGAUCUCUACAUUGUAGGC
sly-miR6023	UUCCAUGAAAGAGUUUUUGGAU	sly-miR9476-3p	AAAAAGAUGCAGGACUAGACC
sly-miR6023-3p.2	UUACUACGGUCAGGAAUCUUAU	sly-miR9476-5p	UCUAGUCCUGCAUCUUUUUUU
sly-miR6024	UUUUAGCAAGAGUUGUUUUACC	sly-miR9477-3p	UUGGGAAAGGGAACAACUGAUAGU
sly-miR6026	UUCUUGGCUAGAGUUGUAUUGC	sly-miR9477-5p	UAUCCGUUGUUCCCUUUUCCUACC
sly-miR6027-3p	UGAAUCCUUCGGCUAUCCAUAA	sly-miR9478-3p	UUCGAUGACAUAUUUGAGCCU
sly-miR6027-5p	AUGGGUAGCACAAGGAUUAAUG	sly-miR9478-5p	GCUUAAAUAUGUAGAUCGAACU
sly-miR1916	AUUUCACUUAGACACCUCAA	sly-miR9479-3p	GAGAAUGGUAGAGGGUCGGACC
sly-miR1917	AUUAAUAAAGAGUGCUAAAGU	sly-miR9479-5p	UCCAGUCCUCUACCCUUCUCCA
sly-miR1918	UGUUGGUGAGAGUUCGAUUCUC	sly-miRn116	UUUGGACGGCAUGAACACUAAUGU
sly-miR5302a	AAACGAGGUUUGUUACUUUGG	sly-miRn32	AGAGGCGGAGUCAGAAUUUUCAAU
sly-miR5302b-3p	UUUUCAACUAUAGCAUUAUUUU	sly-miRn34	AGCUGACGGCGUUAGAUUGAUAUA
sly-miR5302b-5p	UGAAAUGCUAUAGUUGGAAAGU	sly-miRn41	AGGGAUUGUAGGCAGAGAGAUGGC
sly-miR9469-3p	AUUCGGUCUUCUUAUGUGGAC	sly-miRn48	AGUUGACGGCGUUAGAUUGAUACA
sly-miR9469-5p	CCACAUAAGAAGACCGAAUUC	sly-miRn5	AAAGUGAGACGAACAAAUUGAAUC
sly-miR9470-3p	UUUGGCUCAUGGAUUUUAGC	sly-miRn57	AUGGCAGAGACAAUACCUGAAUAU
sly-miR9470-5p	UGAAAUCCAUGAGCCUAAACU	sly-miRn92	UGAGAUGGUAAUAACGGUGAU
sly-miR9471a-3p	UUGGCUGAGUGAGCAUCACGG	sly-miRZ3	AGCUGCUGACCUAUGGAUUCC
sly-miR9471a-5p	CAGGUGCUCACUCAGCUAAUA	sly-miRZ5	UAGGGUGUCGAGUUGAGGAGA
sly-miR9471b-3p	UUGGCUGAGUGAGCAUCACUG		

Table S3. List of potato miRNAs.

ID	Mature sequence	ID	Mature sequence
stu-miR156a-d-5p	UUGACAGAAGAUAGAGAGCAC	stu-miR169f-3p	GCAAGCAUCCUUGGCGACU
stu-miR156d-3p	GCUCUCUAUGCUUCUGUCAUCA	stu-miR171_10	UGUUGGAAUGGCUCAAUCAAA
stu-miR156e,g-k-5p	UGACAGAAGAGAGUGAGCAC	stu-miR171a,c-5p	UAUUGGCCUGGUUCACUCAGA
stu-miR156f-3p	CUCACUUCUCUUUCUGUCAAUC	stu-miR171a,e-3p	UGAUUGAGCCGUGCCAAUAUC
stu-miR156f-5p	CUGACAGAAGAGAGUGAGCA	stu-miR171b-3p	UUGAGCCGCGUCAAUAUCUCU
stu-miR156g-3p	GCUUACUCUCUAUCUGUCACC	stu-miR171b-5p	AGAUAUUGAUGUGGCUCAAUC
stu-miR156h-k-3p	GCUCACUGCUCUAUCUGUCACC	stu-miR171c-3p	UGAUUGAGCCGUGUCAAUAUC
stu-miR159_1	UUUGGAUUGAAGGGAGCUCUA	stu-miR171d-3p	UUGAGCCGUGCCAAUAUCACG
stu-miR160a-3p	GCGUAUGAGGAGCCAAGCAUA	stu-miR171d-5p	AGAUAUUGGUGCGGUUCAAUU
stu-miR160a-b-5p	UGCCUGGCUCCCUGUAUGCCA	stu-miR172_8	GGAAUCUUGAUGAUGCUGCAU
stu-miR162a-b-3p	UCGAUAAACCUCUGCAUCCAG	stu-miR172-6	UGAAUCUUGAUGAUGCUGCAU
stu-miR162a-b-5p	GGAGGCAGCGGUUCAUCGAUC	stu-miR172a-5p	GUAGCAUAAUCAAGAUUCACA
stu-miR164_1-2	UGGAGAAGCAGGGCACGUGCA	stu-miR172a-b,e-3p	AGAAUCUUGAUGAUGCUGCAU
stu-miR164-3p	CAUGUGCUCUAGCUCUCCAGC	stu-miR172b-5p	GCAGCACCAUCAAGAUUCACA
stu-miR164-5p	UGGAGAAGCAGGGCACAUGCU	stu-miR172c-3p	AGAAUCUUGAUGAUGCUGC
stu-miR166a,c-d-3p	UCGGACCAGGCUUCAUUCCCC	stu-miR172c-5p	AGCAUCUUCAAGAUUCACA
stu-miR166a-5p	GGAAUGUUGUCUGGCUCGAGG	stu-miR172d-3p	GGAAUCUUGAUGAUGCUGCAG
stu-miR166b	UCGGACCAGGCUUCAUUCCUC	stu-miR172d-5p	GGAGCAUCAUCAAGAUUCACA
stu-miR166c-5p	GGAAUGUUGUUUGGCUCGAGG	stu-miR172e-5p	GCAACAUCAUCAAGAUUCACA
stu-miR166d-5p	AGAAUGUCGUCUGGUUCGAGA	stu-miR1886a-f	AUGGUAUCGUGAGAUGAAAUCAGC
stu-miR167a-3p	GAUCAUGUGGCAGCCUCACC	stu-miR1886g-3p	UUUCAUAUUGAUUUCAUCUCAU
stu-miR167a-d-5p	UGAAGCUGCCAGCAUGAUCUA	stu-miR1886g-5p	GAGAUGAGAUCAAUGUUUGGACAU
stu-miR167b-3p	GAUCAUGUGGCAGCAUCACC	stu-miR1886h	AUUUUACGUUGAUUUCAUCUCAUG
stu-miR167c-3p	GGUCAUGCUCGGACAGCCUCACU	stu-miR1886i-3p	UUUACGUUGAUUUCAUCUCAUGA
stu-miR167d-3p	GAUCAUGUGGUUGCUUCACC	stu-miR1886i-5p	AUGAGAUGAAAUUAGCGUUUGGAU
stu-miR168_1	UCGCUUGGUGCAGGUCGGGAC	stu-miR1919-3p	ACGAGAGUCAUCUGUGACAGG
stu-miR169_3-5	CAGCCAAGGAUGACUUGCCG	stu-miR1919-5p	UGUCGCAGAUGACUUUCGCCC
stu-miR169a-b-3p	GCAGUCUCCUUGGCUACU	stu-miR2111_2	UAAUCUGCAUCCUGAGGUUUA
stu-miR169a-h-5p	UAGCCAAGGAUGACUUGCCU	stu-miR319-3p	UUGGACUGAAGGGUUCCCUUC
stu-miR169c-3p	GCAGUCUCCUUGGCUACC	stu-miR319-5p	AGGAAACUGUUUAGUCCAACC
stu-miR169d-3p	GCAGGUCAUCUUUAGCUAACU	stu-miR319a-3p	UUGGACUGAAGGGAGCUCCCU
stu-miR169e-3p	GCAAGUUAUCCUGGCUAUC	stu-miR319a-5p	AGAGCUUUCUUCGGUCCACAC
stu-miR319b	UUGGACUGAAGGGAGCUCCU	stu-miR4376-3p	GCAUCAUACUCCUGCAUAUU
stu-miR3627-3p	AAGUGCCUCUGUCUUUCGACA	stu-miR4376-5p	UACGCAGGAGAGAUGAUGCUG
stu-miR3627-5p	UCGCAGGAGAGAUGGCACUUAG	stu-miR4414_1	UGCUGCUGAAUCAUUGGCUC

stu-miR384-3p	AGGGGGCCAAAGUGCCAAAC	stu-miR477a-3p	GAAGCUCUAGCAGGGAGAGCCA
stu-miR384-5p	UUGGCAUUCUGUCCACCUCC	stu-miR477a-5p	CCUCUCCCCUCAAGGGCUUCUC
stu-miR390_1	AAGCUCAGGAGGGAUAGCGCC	stu-miR477b-3p	GAGGUCUUUCGAGUGAGAGUGA
stu-miR390-3p	CGCUAUCCAUCUUGAGUUUUA	stu-miR477b-5p	ACUCUCCCCUCAAAGGCUUCUG
stu-miR390-5p	AAGCUCAGGAGGGAUAGCACC	stu-miR479	UGAGCCGAACCAAUAUCACUC
stu-miR393-3p	AUCAUGCGAUCUCUUCGGAAU	stu-miR482_1	UCUUUCCUACUCCUCCCAUACC
stu-miR393-5p	UCCAAAGGGAUCGCAUUGAUCC	stu-miR482a-3p	UUUCCAAUUCCACCCAUUCCUA
stu-miR395a-j	CUGAAGUGUUUGGGGGAACUC	stu-miR482a-5p	GGAAUUGGUGGAUUGGAAAGC
stu-miR396_1	UUCCACAGCUUUCUUGAACUG	stu-miR482b-3p	UUACCGAUUCCCCCCAUUCCAA
stu-miR396-3p	GUCCAAGAAAGCUGUGGGAAA	stu-miR482b-5p	GGAGUGGGUGGCAUGGUAAGA
stu-miR396-5p	UUCCACAGCUUUCUUGAACUU	stu-miR482c	UUUCCUAUUCCACCCAUGCCAA
stu-miR397_1	UCAUUGAGUGCAGCGUUGAUG	stu-miR482d-3p	UCUUGCCUACACCGCCCAUGCC
stu-miR397-3p	CAUCAACGCUACACUCAAUCA	stu-miR482d-5p	CGUGAGUGGUGGGGUAAGAUA
stu-miR397-5p	AUUGAGUGCAGCGUUGAUGAC	stu-miR482e-3p	UCUUGCCAAUACCGCCCAUUCC
stu-miR398a-3p	UAUGUUCUCAGGUCGCCCCUG	stu-miR482e-5p	AGUGGGUGGUGUGGUAAGAUU
stu-miR398a-5p	GGGUUGAUUUGAGAACAUAUG	stu-miR530_1	UUUUUGAAGAGUCUGGGCAAC
stu-miR398b-3p	UUGUGUUCUCAGGUCACCCCU	stu-miR530_2	UGCAUUUGCACCUGCACCUUA
stu-miR398b-5p	GAGUGUGCCUUAGAACACAGGU	stu-miR5300_1	UCCCCAGUCCAGGCAUUCCAAC
stu-miR399_1-3	UGCCAAAGAAGAUUUGCCCCG	stu-miR5303_3	UUUUUGAAGAGUUCGAGCAAC
stu-miR399a-f-3p	UGCCAAAGGAGAGCUGCCCUG	stu-miR5303a-d	UUUUGGAGAAUCCGACACGCACCC
stu-miR399a-h,k-o-5p	GGGCUACUCUCUAUUGGCAUG	stu-miR5303e	UUUUGGAGAAUCUGACACGGGUGU
stu-miR399g-3p	CGCCAAAGGGGAGCUGCCCUA	stu-miR5303f	AUUUUUGGAGAAUCUGACACGGGU
stu-miR399i-3p	UGCCAAAGGAGAGUUGCCCUA	stu-miR5303g,i	AUAUUUUUGAAGAGUCUGAGCAAC
stu-miR399i-5p	GGGCUACACUCUAUUGGCAUG	stu-miR5303h	AACAUUUUUGAAGAGUCUGAGCAA
stu-miR399j-5p	GGGCUACUCUCUAUUGGCAUA	stu-miR5303j	AAUAUUUUUGAAGAGUCUGAGCAA
stu-miR399j-o-3p	CGCCAAAGGAGAGCUGCCCUG	stu-miR530-3p	AGGUGUUGGUGCUCAUGCAGA
stu-miR403_1	CUAGAUUCACGCACAAACUCG	stu-miR5304-3p	AGAUGAGUAUGGUGCAUUGGA
stu-miR408a-3p	UGCACAGCCUCUUCCCUGGUU	stu-miR5304-5p	CAAUGCAACAUACUCAUCACC
stu-miR408a-5p	ACAGGGACGAGGCAGCGCAUG	stu-miR530-5p	UCUGCAUUUGCACCUGCACCU
stu-miR408b-3p	UGCACUGCCUCUUCCCUGGCU	stu-miR6022	UGGAAGGGAGAAUAUCCAGGA
stu-miR408b-5p	ACGGGGACGAGACAGAGCAUG	stu-miR6023	UUCCAUGAAAGUGUUUUUGGAU
stu-miR4376_1	ACGCAGGAGAGAUGAUGCUGGA	stu-miR6024-3p	UUUUAGCAAGAGUUGUUUUCCC
stu-miR6024-5p	AGAAACAACACUUGCUAAAAGA	stu-miR7992-5p	UUUGACAAUGCACAUCUAGACACU
stu-miR6025	UACCAACAAUUGAGAUAACAUC	stu-miR7993a-d	AUAUUUUAUGUGGUUAACUUAACU
stu-miR6026-3p	UUCUUGGCUAGAGUUGUAUUGC	stu-miR7993b-5p	UUAAGUUAACCACAUAAAAUAUGU
stu-miR6026-5p	AAUACAACUAUUGCCAAGACAA	stu-miR7994a-b-3p	AUAUUAUACUUGGGCAUAAACUCC
stu-miR6027	UGAAUCCUUCGGCUAUCCAUAA	stu-miR7994b-5p	AGUUUAUGCCCAAGUAUAUAAUAUAU

stu-miR6149-3p	UGAUUCAGGUUUGUAUGCAAAC	stu-miR7995	UUACACGUAGACAAGUUGACCAUU
stu-miR6149-5p	UUGCAACACACCUGAAUCGUC	stu-miR7996a-c	AUGUGGUACAUAUGAAAUUUGAAA
stu-miR7122-3p	ACAGCGUUUCUCUGUAUAACC	stu-miR7997a-b	AUGCUGCUCGGACUCUUCAAA
stu-miR7122-5p	UUAUACAGAGAAACCGCUGUCG	stu-miR7997c	AUAUUGCUCGGACUCUUCAAAAAU
stu-miR827-3p	UUAGAUGAACAUCAACAAACA	stu-miR7998	ACGGACCGUAGAUCAAUCCACAGU
stu-miR827-5p	UUUGUUGAUGGUCAUCUAUUC	stu-miR7999-3p	ACGACCCGUAGAACUGCCCACGAC
stu-miR7979	AGGUACAUGAACUCUAACGAGGCA	stu-miR7999-5p	CUGGGUCACUUCUACGGGUCCUUC
stu-miR7980a	AUGAGAUGAAGUCAAUGUUUGGAC	stu-miR8000	ACACCGAAGAACUGACACCGAAGA
stu-miR7980b-3p	GAGAUGGAAUCAGUGUUUGGACAU	stu-miR8001a	UCCUGGGGAUUAGUAUGAAAAUUC
stu-miR7980b-5p	GUCCAAACACUGAUUCCAUCUCAU	stu-miR8001b-3p	GGAUUUUCAUACUAAUUCCUAGAA
stu-miR7981-3p	AUAGGACUUUAGUUUAGUUAAGGU	stu-miR8001b-5p	AUGGGGAUUAGUAUGAAAAUUUGC
stu-miR7981-5p	GUUAAUUAAACUAUGGUCCUAUUA	stu-miR8002-3p	AUUCCAUUAUUAUCAAGAAAAAAG
stu-miR7982a-b	AAGUUGGAUGAUAAUAAUAUAUAU	stu-miR8002-5p	UUUUUCGUGAUAAUAAUGGAAUCA
stu-miR7983-3p	ACUAAUGCCGGUAAAGACUUUAAC	stu-miR8003	AUUUCGGUAUACAAAUGGGAUGAC
stu-miR7983-5p	UAAAGUCUUUAGCGACAUUGGUUC	stu-miR8004	AGGGGUUGUGUAUGUGUUUGGCCU
stu-miR7984a-b	AUACCGAACUUUGGAAAUGACCUU	stu-miR8005a-c	UUUAGAGUUUAAGGUUUAGAGUUU
stu-miR7984b-3p	GGUCUUUCAUAAAAUUUGGUAUCG	stu-miR8005b-5p	ACUCUAAAUUUUAAAUUCUAAAUC
stu-miR7984c-3p	CCUUCAUAAAGUUUGGUAUCGUAA	stu-miR8006-3p	UGCCCUGCCGUCCAAAAAAUAGA
stu-miR7984c-5p	ACGAUACCAAACUUUAUGAAGGAC	stu-miR8006-5p	UAGUUUUUGGACGACAGGGGCACC
stu-miR7984d-3p	CAAUUUCGGUCAAAGUUCGGAUAU	stu-miR8007a-3p	CGAAAAAUGAAAAGUGCCACAUAA
stu-miR7984d-5p	AUCCGAACUUUGACCGAAAUUGCU	stu-miR8007a-5p	AUGUGGCACUUUUCGGAUUUUGAG
stu-miR7985	CGGGCUUGCCUAGAACGGGUUACC	stu-miR8007b-3p	CGAAAAAUGAAAAGUACCACAUAA
stu-miR7986	AGUUUAAAACGUUACUGUCGGUAA	stu-miR8007b-5p	AUGUGACACUUUUUGAAUUUCGAG
stu-miR7987	ACACUAGUUGAUCUUAUUGAUGAC	stu-miR8008a	AUUUCCAGAAAAGCGACGGACAGU
stu-miR7988	AACGGAAAAGGGCCAAAAAUACCC	stu-miR8008b	AAACCCAGAAAAGCGACGGACAGU
stu-miR7989	ACAAAUAAGUCCAUUACCUGAACC	stu-miR8010	AUAGGACCCUAGUUAAAUUUAGGU
stu-miR7990a	UUCAAAUGAUCGUAACUUUGGCCU	stu-miR8011a-3p	AAUAAAAAGAAGCCUCACACAACU
stu-miR7990b	GAAUUUUCAAAUGAUCGUAACUUU	stu-miR8011a-5p	UUGUGUGAGGUUUCUUUUUGUUUC
stu-miR7991a-c	AGGAGGUCGGAAUUUUUAAUGAAU	stu-miR8011b-3p	UUCGUGAGACAAAAAGAAGCCU
stu-miR7992-3p	UGUCUAGAUGUGCAUUUCAAAGU	stu-miR8011b-5p	ACUCAUUUUUGUCUCACAAAAA
stu-miR8012	AUGACUUUAAGUCGCGUCUGGCCC	stu-miR8036-3p	UAUGUCUUUCCGAUGCCUCCCA
stu-miR8013	AGAAGAAAAUCGCUCCGUCAGAAG	stu-miR8036-5p	GGAGGAAUCGAAAGAUAUAAG
stu-miR8014-3p	AUGAAUACAAUGUUUGGAUAAAUU	stu-miR8037	AUAAUUUGGAGGAAUAGGAACC
stu-miR8014-5p	AUUGUUUCAUAUUGUAUUGUAUUU	stu-miR8038a-b-3p	GUUCAACUUGCUCACUUGGAG
stu-miR8015-3p	GUUUCAUUUUCAAGGUCCAAUAGC	stu-miR8038a-b-5p	CCUUGUGAGUAAGUUGAAUCUC
stu-miR8015-5p	UAUUGGAUAUUGAAAAUGAAACUU	stu-miR8039	UUUCCUAUCUGAACUAUCACC
stu-miR8016	AUUUUUGAAUGGAAGGCCCAUGUG	stu-miR8040-3p	CUUAUAAUUGUAAUUAUGAUC

stu-miR8017	AUCCAAGUGAAGUGUAUCGUCUCA	stu-miR8040-5p	UCAUAAUUACAAUUAUAAGCC
stu-miR8018	ACGAACCGUAGAUCCCAUCCGUGG	stu-miR8041a-b-3p	AUGAUGUAUAGCAAAGAGCCU
stu-miR8019-3p	AAAAGAAUGACCUGGUUUGACUUG	stu-miR8041a-b-5p	GUGCUUUGCUAUUUUCAUUG
stu-miR8019-5p	AGGGAAGCAGGUCAUUCUUUAUG	stu-miR8042	AUUAGACUGAAGUGCUGAUCU
stu-miR8020	AAUUUCAUUGAGUAUGUUGUUGUU	stu-miR8043	UGAUAUAAUUGGACUUUGGCC
stu-miR8021	AUUCAAGGCUCAAACUCGAGACCU	stu-miR8044-3p	UCUCCAGCGAUAUUUGAAACU
stu-miR8022	UUUAAAAUGAGAAUUUUGGACUAUU	stu-miR8044-5p	UUUCAAAUAUGGUUGGAGAUG
stu-miR8023	UUUGGCACAAUUUCAUUGGCAACC	stu-miR8045	AUUGAUAGUUGAGGUGUGUUU
stu-miR8024a-5p	UUGAAGAAUUUAAAGACUUCAACU	stu-miR8046-3p	CGCUGAAAUUUCGAUCAUAAU
stu-miR8024a-b-3p	UUGGAGGAUUUGAAGAUUUCAACU	stu-miR8046-5p	UAUGAUCGAAGUUUCAAUGAC
stu-miR8025-3p	UUUAAUUGCAUGCCAAGUGUGUGG	stu-miR8047	CCAUUUUUUCGAAAUUAGACC
stu-miR8025-5p	ACAUACUCGACAUGCAAUUAAAUU	stu-miR8048-3p	AGAUGGACAUGCUAAUGAACA
stu-miR8026	AUGUAGAGAAUAUGUGGUAACCCU	stu-miR8048-5p	CUCAUUAGCAUCUCCAUCUUG
stu-miR8027	AUCUCGAGAUAAGUUAUUCUGGAC	stu-miR8049-3p	CAUGUCUACAUGAGCCUGAUA
stu-miR8028-3p	GUUCAUAAUUAUAGUAUAAGGAUG	stu-miR8049-5p	CAAGGCUCAUGCAGACAUGCA
stu-miR8028-5p	UCCUUAUGCUACAAUUGUGAACAA	stu-miR8050-3p	UGACUUGAGAUUCCUACUUGG
stu-miR8029	AGCCAUUUUUCUUUGUUUUGGAGC	stu-miR8050-5p	AAGUAGGAAUCAAGGUCAAU
stu-miR8030-3p	UUAAAACCAAAUCAACCCAAAU	stu-miR8051-3p	UAUUUCUUCUACCAUACUAUU
stu-miR8030-5p	UUGGGUUGGUUUGGUCUCGGGUU	stu-miR8051-5p	UAGUAUGGUAGAAAGAUUCA
stu-miR8031	UUAGACACCUCAACUAAGACUUG	stu-miRn146	GGGUGUUGGUCGAUUAAGACA
stu-miR8032a,e-3p	AGUGUGAGUCGGUGUGAUUAGG	stu-miRNA134	UAAAGAGGACGGGAUGACAGA
stu-miR8032a-g-5p	UGGUCGGCAUGACUCCCGAGGU	stu-miRNA152	UAUCUGAGUAGCAUAGGGAAUU
stu-miR8032b,f,g-3p	AGUGUGAGUCGGUGCGAUUAGG	stu-miRNA42	UAGGGCAGUUCUCCGUUUGGCA
stu-miR8032d-3p	AGUGUGAGUUGGUGCGAUUAGG	stu-miRNA5	UAUAUGCUCUAGAUUUUGGAC
stu-miR8033-3p	UCAAUUCUGCAGCUUUAGGAGU	stu-miRNA53	UUCCAUGAGACUGUUUUUGGGU
stu-miR8033-5p	UUCCAAAGCUGCAGAAAUGAGU	stu-miRNA72	UUGGUUGAGUGAGCAUCUAAG
stu-miR8034	UAUGACAAACACUGCAAAAACU	stu-miRNA231	UGUCUAGACGUGCAUACUCAA
stu-miR8035	UCCAUCUUCAAUAUCACUUUCU	stu-miRNA232	UGUACAGUUCAUCUUCGGCGCC
stu-miRNA261	UUCGAUACGCACCUGAAUCGA		

Table S4. Number of the miRNAs and miRNA genes used in this study.

	Pepper	Tomato	Potato
Number of miRNAs	145	123	275
Conserved	80	81	145
species-specific	65	42	130
Number of miRNA genes	223	93	393
conserved	143	63	283
species-specific	80	30	110

Table S5. Number of the miRNAs and miRNA genes used in this study.

	Pepper	Tomato	Potato
Total reads	251,689,162	226,553,118	371,124,547
No adaptor & low quality	61,432,588	19,447,550	84,937,163
Rfam & repeat filtered	38,441,544	103,199,875	16,233,245
Final reads	151,815,030	103,905,693	269,954,139
Unique reads	17,172,509	25,382,981	4,817,402
Aligned to CDS	83,954,715	64,038,011	199,670,938
Not aligned	67,860,315	39,867,682	70,283,201
Aligned %	55.30	61.63	73.96

Table S6. Results of degradome analysis in pepper.

miRNA	Score[a]	*p*-value	Description
can-miR1446a-b	3	0.0421	GRAS family transcription factor
can-miR1446a-b	4	0.0698	neurofilament medium polypeptide-like isoform X2
can-miR156h	4	0.4661	Aldehyde dehydrogenase
can-miR156a-c	2	0.0012	LIGULELESS1 protein, putative
can-miR156d-g	1	0.0018	LIGULELESS1 protein, putative
can-miR156d-g	1	0.0920	LIGULELESS1 protein, putative
can-miR156h	1.5	0.1137	LIGULELESS1 protein, putative
can-miR156a-c	4	0.2913	non-specific lipid-transfer protein 3-like
can-miR156h	1	0.0421	squamosa promoter-binding protein 1-like
can-miR156a-c	1	0.0003	squamosa promoter-binding protein 1-like isoform X2
can-miR156d-g	4	0.8804	squamosa promoter-binding protein 1-like isoform X2
can-miR156d-g	1	0.0015	squamosa promoter-binding-like protein 16-like isoform 1
can-miR156h	2.5	0.1348	squamosa promoter-binding-like protein 16-like isoform 1
can-miR156a-c	2	0.0006	Promoter-binding protein SPL9
can-miR156d-g	1	0.0698	Promoter-binding protein SPL9
can-miR156d-g	1	0.0471	SPL domain class transcription factor
can-miR156h	1.5	0.0009	SPL domain class transcription factor
can-miR159a-c	3.5	0.0068	Actin cytoskeleton-regulatory complex protein pan1, putative isoform 2
can-miR159a-c	8	0.0280	AG-motif binding protein-5
can-miR159a-c	3.5	0.0074	Detected protein of unknown function
can-miR159a-c	2.5	0.0920	Detected protein of unknown function
can-miR159a-c	2.5	0.0003	GAMyb-like1
can-miR159a-c	4	0.0009	GAMYB-like2
can-miR159a-c	5	0.0083	NHL domain-containing protein isoform 2
can-miR159a-c	5	0.0116	uncharacterized N-acetyltransferase ycf52-like
can-miR160	1.5	0.0003	Auxin response factor 17
can-miR160	1	0.0006	Auxin response factor 10
can-miR160	0.5	0.0009	Auxin response factor, putative
can-miR160	1.5	0.0015	Auxin response factor, putative
can-miR160	1	0.0012	Auxin response factor 10
can-miR162f	2.5	0.0003	Dicer-like 1 protein (Fragment)
can-miR164a-b	4	0.0031	NAC domain-containing protein 21/22, putative
can-miR164a-b	2	0.0012	Salicylic acid-induced protein 19
can-miR164a-b	4	0.0025	NAC domain-containing protein 21/22, putative
can-miR164a-b	4	0.3365	NAC domain class transcription factor
can-miR164a-b	4	0.1952	UDP-glucuronate decarboxylase 1
can-miR164c	3	0.0009	protein CUP-SHAPED COTYLEDON 2-like
can-miR164c	4	0.7366	Detected protein of unknown function
can-miR164c	5	0.0022	Beta-fructofuranosidase (Fragment)
can-miR166a-h	3	0.0015	Class III HD-Zip protein 8
can-miR166a-h	3	0.0012	Class III HD-Zip protein 3
can-miR166a-h	2.5	0.0006	DNA binding protein, putative
can-miR166a-h	3	0.0698	DNA binding protein, putative

can-miR166i-j	2	0.0238	DNA binding protein, putative
can-miR166i-j	2	0.0471	Class III HD-Zip protein 5
can-miR166i-j	2.5	0.0920	Class III HD-Zip protein 8
can-miR166i-j	2.5	0.0698	Class III HD-Zip protein 3
can-miR166i-j	2.5	0.1137	DNA binding protein, putative
can-miR166l	2.5	0.0003	Class III HD-Zip protein 5
can-miR167a-c	2.5	0.0920	Multidrug resistance pump, putative
can-miR168c	4	0.0009	eukaryotic translation initiation factor 2c, putative
can-miR168c	4.5	0.0421	Serine/threonine-protein kinase PBS1, putative
can-miR169a-g	3	0.0012	Transcription factor CCAAT
can-miR169a-g	2	0.0009	Nuclear transcription factor Y subunit A3
can-miR169a-g	3	0.0061	YA3
can-miR169a-g	2.5	0.0022	Nuclear transcription factor Y subunit A-3, putative
can-miR169a-g	3	0.0015	nuclear transcription factor Y subunit A-10-like
can-miR169a-g	3	0.2913	YA4
can-miR169a-g	4	0.7533	YA4
can-miR169a-g	4	0.8733	Dentin sialophosphoprotein, putative
can-miR171f-g	1.5	0.0471	GRAS family transcription factor
can-miR171f-g	1.5	0.0238	GRAS family transcription factor
can-miR171f-g	1.5	0.0009	BAC19.14
can-miR171h	0	0.0238	GRAS family transcription factor
can-miR171i	1	0.0006	GRAS family transcription factor
can-miR171i	3	0.0009	GRAS family transcription factor
can-miR171k	1.5	0.0238	BAC19.14
can-miR171l	2	0.0003	GRAS family transcription factor
can-miR172a-d	4	0.8550	Dopamine beta-monooxygenase, putative
can-miR172a-d	4	1.0000	Cysteinyl-tRNA synthetase, putative
can-miR172e	2.5	0.0006	APETALA2B
can-miR172e	4	1.0000	Multidrug resistance protein ABC transporter family
can-miR172e	3	0.9102	CD2 antigen cytoplasmic tail-binding protein
can-miR172e	3.5	1.0000	Pentatricopeptide repeat-containing protein
can-miR172f	4	1.0000	Ubiquitin-protein ligase, putative
can-miR172f	3.5	0.9997	Serine/threonine-protein kinase
can-miR172g	1	0.0003	AP2 transcription factor SlAP2b
can-miR172g	1	0.0006	Transcription factor APETALA2
can-miR172g	4	1.0000	Plant synaptotagmin
can-miR172g	3	0.3524	Nucleic acid binding protein, putative
can-miR172g	2.5	0.9989	Xyloglucan galactosyltransferase KATAMARI1, putative
can-miR172h	4	0.3203	UDP-glucosyltransferase, putative
can-miR172h	4.5	0.0421	Detected protein of unknown function
can-miR172i	1.5	0.0006	AP2 transcription factor SlAP2d
can-miR172i	1.5	0.0003	AP2 transcription factor SlAP2d
can-miR172i	1.5	0.0698	AP2 transcription factor SlAP2c
can-miR319a	3.5	0.1936	TCP transcription factor
can-miR319a	3.5	0.1582	TCP transcription factor
can-miR319a	7.5	0.0434	Ef-hand calcium binding protein, putative

can-miR319b-c	2.5	0.0005	TCP transcription factor 1
can-miR319b-c	2.5	0.0015	TCP transcription factor
can-miR319b-c	2.5	0.0012	TCP transcription factor
can-miR319b-c	4	0.0825	GAMyb-like1
can-miR319d	2	0.0421	PfkB-like carbohydrate kinase family protein
can-miR319d	4	0.1211	TCP transcription factor
can-miR319d	4	0.0825	TCP transcription factor
can-miR319e	2	0.0018	Transcription factor, putative
can-miR319f	3.5	0.0009	Lanceolate
can-miR319f	3.5	0.0012	Lanceolate
can-miR319g	4	0.2144	GAMYB-like2
can-miR319g	4	0.4526	NHL domain-containing protein isoform 2
can-miR390c	3.5	0.2144	Protein phosphatase, putative
can-miR393a-b	2	0.0015	Transport inhibitor response 1 (Fragment)
can-miR393a-b	2	0.0018	TIR1-like protein
can-miR393a-b	3	0.0037	Transport inhibitor response 1 (Fragment)
can-miR393a-b	2	0.0028	Detected protein of confused Function
can-miR393a-b	2	0.0012	TIR1-like protein
can-miR393a-b	2	0.0471	Transport inhibitor response 1 (Fragment)
can-miR393a-b	2	0.0025	Transport inhibitor response 1
can-miR393a-b	2	0.0238	protein TRANSPORT INHIBITOR RESPONSE 1-like
can-miR393a-b	3	0.0049	Detected protein of confused Function
can-miR393a-b	2	0.0698	Transport inhibitor response 1
can-miR393a-b	2	0.0890	Detected protein of unknown function
can-miR393a-b	2	0.1554	Transport inhibitor response 1
can-miR393a-b	7	0.0068	Transport inhibitor response protein
can-miR394a	7	0.0471	Eukaryotic translation initiation factor 3 subunit, putative
can-miR394b	1	0.0003	F-box family protein
can-miR395a-j	3	0.0012	Sulfate transporter, putative
can-miR395a-j	2	0.0003	Sulfate/bicarbonate/oxalate exchanger and transporter sat-1
can-miR395a-j	2.5	0.0471	Sulfate/bicarbonate/oxalate exchanger and transporter sat-1
can-miR395a-j	4	0.7004	ATP-dependent RNA helicase, putative
can-miR395a-j	3.5	0.0698	Sulfate adenylyltransferase
can-miR396a	3	0.0034	Detected protein of unknown function
can-miR396a	3	0.0037	growth-regulating factor 4-like
can-miR396a	3	0.0040	growth-regulating factor 4-like
can-miR396a	3	0.0031	growth-regulating factor 3-like
can-miR396a	3.5	0.9568	60S ribosomal protein L35a-3-like
can-miR396a	5.5	0.0238	AT4g27650
can-miR396b	2.5	0.0006	DNA (cytosine-5)-methyltransferase DRM1-like
can-miR396b	4	0.0455	putative late blight resistance protein homolog R1A-10-like
can-miR396b	4	0.9021	Germin-like protein subfamily 1 member 20
can-miR396b	3.5	0.4912	prohibitin-3, mitochondrial-like
can-miR396b	4	0.0920	putative B3 domain-containing protein At3g49610-like
can-miR396c	4	0.0028	growth-regulating factor 8-like
can-miR396c	4	0.0031	growth-regulating factor 1-like

can-miR396c	4	0.0037	UPA17
can-miR396c	3	0.0003	DNA (cytosine-5)-methyltransferase DRM1-like
can-miR396c	4	0.0022	growth-regulating factor 3-like
can-miR396c	3	0.0009	Detected protein of confused Function
can-miR396c	4	0.2913	growth-regulating factor 6-like isoform X1
can-miR396c	4	0.8804	Terpene synthase
can-miR396c	5	0.0119	Growth-regulating factor
can-miR396c	8	0.0077	Metallo-hydrolase/oxidoreductase superfamily protein isoform 1
can-miR397a	4	0.5392	At5g65760
can-miR397b	1	0.0238	Laccase-like multicopper oxidase
can-miR397b	2	0.0471	Diphenol oxidase
can-miR397b	3.5	0.1952	laccase-11-like
can-miR397b	3.5	0.1756	laccase-11-like
can-miR397b	3.5	0.2514	laccase-2-like
can-miR397b	1.5	0.0698	Laccase 110a
can-miR397b	4	0.5950	Laccase 110b
can-miR398d	6	0.0144	Glycerol-3-phosphate transporter, putative
can-miR398d	4.5	0.0471	Os12g0570700
can-miR399a-e	2	0.0471	PHO2
can-miR399a-e	1.5	0.0238	PHO2
can-miR399a-e	3	0.0698	PHO2
can-miR399f-g	4	0.5034	Detected protein of confused Function
can-miR403	1	0.0003	AGO2A
can-miR408a	3	0.0009	basic blue protein-like
can-miR408a	3.5	0.0006	plastocyanin-like domain-containing family protein
can-miR408a	4	0.0025	Unknown protein
can-miR408b	4.5	0.0025	psbP-like protein 2, chloroplastic-like
can-miR4376	3	0.0238	Auto-inhibited Ca2+-transporting ATPase 10
can-miR4414a	4	0.1211	Unknown protein
can-miR4414b	4	0.9912	Protein phosphatase 2C
can-miR4414b	7	0.0421	Transcription factor Pur-alpha
can-miR4414b	11	0.0074	Unknown protein
can-miR482a	4	0.0698	NL27
can-miR482b	3	0.0009	TIR-NBS-LRR RCT1-like resistance protein
can-miR482b	3	0.0006	Nematode resistance-like protein
can-miR482b	2.5	0.9966	Detected protein of unknown function
can-miR482b	7.5	0.0077	Serine/threonine-protein kinase
can-miR482c	3	0.3524	CC-NBS-LRR type resistance protein (Fragment)
can-miR482c	3	0.3829	Disease resistance protein I2C-5, putative
can-miR482c	3	0.3365	CC-NBS-LRR protein
can-miR482c	3	0.4977	CC-NBS-LRR type resistance protein (Fragment)
can-miR482c	5.5	0.0455	disease resistance protein RPP13-like
can-miR482c	5.5	0.0464	R2 late blight resistance protein
can-miR482c	5.5	0.0461	SNKR2GH2 protein
can-miR482c	4.5	0.0034	SNKR2GH2 protein
can-miR482c	4.5	0.0040	SNKR2GH2 protein

can-miR482c	4.5	0.0037	SNKR2GH2 protein
can-miR482c	5	0.0247	CC-NBS-LRR type resistance protein (Fragment)
can-miR482c	5	0.0225	Disease resistance protein
can-miR482d	2.5	0.0012	CC-NBS-LRR type resistance-like protein
can-miR482d	2.5	0.0009	Protein YigZ (Fragment)
can-miR482d	3	0.0046	Disease resistance protein RGH9
can-miR482d	2.5	0.0015	Disease resistance protein RGH3
can-miR482d	2.5	0.0006	Detected protein of unknown function
can-miR482d	3	0.6705	Detected protein of unknown function
can-miR482d	4	0.1952	CC-NBS-LRR type resistance protein (Fragment)
can-miR482d	4	0.6457	Disease resistance protein RGH3
can-miR482d	5	0.0113	putative late blight resistance protein homolog R1A-10-like
can-miR482d	5	0.0116	putative late blight resistance protein homolog R1A-10-like
can-miR482d	5.5	0.0268	putative late blight resistance protein homolog R1B-16-like
can-miR482e	3.5	0.9636	DEAD-box ATP-dependent RNA helicase
can-miR482e	6	0.0162	Transcription factor, putative
can-miR482f	1	0.0009	Detected protein of unknown function
can-miR482f	3	0.0289	Late blight resistance protein Rpi-sto1
can-miR482f	2	0.0003	NBS-LRR type disease resistance protein (Fragment)
can-miR482f	2	0.0471	Nbs-lrr resistance protein
can-miR482f	2.5	0.0012	CC-NBS-LRR type resistance-like protein
can-miR482f	2.5	0.0015	I2 (Fragment)
can-miR482f	4	0.0071	Nematode resistance-like protein
can-miR482f	4	0.9961	Protein binding protein, putative
can-miR482f	4	0.7149	Detected protein of unknown function
can-miR482f	4.5	0.0028	Blight resistance protein
can-miR530	1	0.0003	Detected protein of unknown function
can-miR530	3.5	0.3037	Eukaryotic translation initiation factor 3 subunit, putative
can-miR530	3.5	0.7760	Lanceolate
can-miR530	4.5	0.0095	Aprataxin, putative
can-miR6022-3p.2	4	0.8267	Cf-4
can-miR6022-3p.2	4	0.9997	Hcr9-OR2C
can-miR6022-3p.2	4	0.9996	Hcr9-OR2A
can-miR6022-3p.2	2.5	0.6284	Verticillium wilt disease resistance protein
can-miR6022-3p.2	1.5	0.0890	receptor-like protein 12-like
can-miR6022-3p.2	3	0.6591	Hcr2-0A
can-miR6022-3p.2	1.5	0.2601	Hcr2-0A
can-miR6022-3p.2	1.5	0.1348	receptor-like protein 12-like
can-miR6022-3p.2	2.5	0.3037	receptor-like protein 12-like
can-miR6023	2	0.0238	Hcr9-Avr4-per1
can-miR6023	1.5	0.0037	Hcr9-OR2A
can-miR6023	4	0.4788	Cf-4
can-miR6023	2	0.2144	Hcr9-OR2C
can-miR6023	4	0.7411	receptor-like protein 12-like
can-miR6023	2	0.3365	NL0E
can-miR6023	2	0.4526	receptor-like protein 12-like

can-miR6023	2.5	0.3829	Hcr9-Avr9-hir4
can-miR6023	2	0.3037	Hcr9-OR2A
can-miR6023	4	0.1952	Hcr9-0
can-miR6023	2.5	0.3678	Hcr9-Avr4-per1
can-miR6023	4	0.2917	NL0D
can-miR6023	2	0.2332	NL0E
can-miR6024-3p	2.5	0.0012	Detected protein of unknown function
can-miR6024-3p	2.5	0.0061	Detected protein of unknown function
can-miR6024-3p	2.5	0.0330	Detected protein of unknown function
can-miR6024-3p	3	0.6441	putative late blight resistance protein homolog R1B-12-like
can-miR6024-3p	3.5	0.9998	Disease resistance protein R3a-like protein
can-miR6024-3p	4	0.8683	Tm-2^2 ToMV resistance protein
can-miR6024-3p	3	0.1952	Tm-2 protein
can-miR6024-3p	4	0.1554	Reistance protein F
can-miR6024-3p	3	0.2144	Tm-2
can-miR6024-3p	3.5	0.6282	Reistance protein F
can-miR6024-3p	3	0.9545	putative late blight resistance protein homolog R1B-16-like
can-miR6024-3p	4	1.0000	Detected protein of unknown function
can-miR6024-3p	3	0.0790	putative late blight resistance protein homolog R1A-10-like
can-miR6024-3p	3	0.9988	putative late blight resistance protein homolog R1A-10-like
can-miR6024-3p	3	0.9705	putative late blight resistance protein homolog R1B-16-like
can-miR6024-3p	3	0.1623	Detected protein of unknown function
can-miR6024-3p	3.5	0.4661	Detected protein of unknown function
can-miR6024-3p	3.5	0.2225	Detected protein of unknown function
can-miR6024-3p	3.5	0.9831	CC-NBS-LRR type resistance protein (Fragment)
can-miR6024-3p	4	0.9999	putative disease resistance RPP13-like protein 1-like isoform X2
can-miR6024-3p	3.5	0.7988	CC-NBS-LRR type resistance protein (Fragment)
can-miR6024-3p	3.5	0.9857	CC-NBS-LRR type resistance protein (Fragment)
can-miR6024-3p	3.5	0.9864	CC-NBS-LRR type resistance protein (Fragment)
can-miR6024-3p	7	0.0009	RecA
can-miR6026-3p.1	1.5	0.0003	Tm-2
can-miR6026-3p.1	6	0.0455	ATP-binding cassette transporter, putative
can-miR6027-3p	2.5	0.0238	HJTR2GH1 protein
can-miR6027-3p	2.5	0.0471	disease resistance protein RPP13-like
can-miR6027-3p	3.5	0.0366	Disease resistance protein BS2
can-miR6027-3p	3	0.0698	Disease resistance protein RGH2
can-miR6027-3p	4	0.4661	disease resistance protein RPP13-like
can-miR6027-3p	4	0.4788	R2 late blight resistance protein
can-miR6027-3p	4	0.4530	SNKR2GH2 protein
can-miR6027-3p	3.5	0.1137	NBS-coding resistance gene analog (Fragment)
can-miR6027-3p	4	0.0684	NRC1
can-miR6027-3p	4	0.9843	NRC1
can-miR827	6	0.0256	Detected protein of unknown function
can-miR-n002a-c	0	0.0003	F-box/kelch-repeat protein At3g23880-like, partial
can-miR-n002a-c	0	0.0006	F-box protein CPR30-like
can-miR-n002a-c	1	0.0009	F-box/kelch-repeat protein At3g23880-like, partial

can-miR-n003a-5p	3.5	0.3498	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 1-like
can-miR-n003a-c-3p	2	0.0421	ankyrin repeat-containing protein At3g12360-like
can-miR-n003a-c-3p	2.5	0.0471	ankyrin repeat-containing protein At5g02620-like isoform 1
can-miR-n003a-c-3p	4	0.5392	Detected protein of unknown function
can-miR-n003a-c-3p	3	0.2601	Detected protein of unknown function
can-miR-n003a-c-3p	3	0.2276	Ankyrin repeat-containing protein, putative
can-miR-n003a-c-3p	4	0.9487	Detected protein of unknown function
can-miR-n003a-c-3p	4	0.5152	Ankyrin repeat-containing protein, putative
can-miR-n004	3.5	0.7592	Ran-binding protein
can-miR-n005a-5p	1	0.0003	Ubiquitin-protein ligase, putative
can-miR-n005a-5p	1	0.0006	F-box family protein
can-miR-n005a-5p	1	0.0009	Ubiquitin-protein ligase, putative
can-miR-n005a-5p	3.5	0.8619	Acyl ACP-thioesterase
can-miR-n010	4	0.1701	Hcr9-Avr4-per1
can-miR-n010	4	0.2440	Hcr9-OR2C
can-miR-n016a-b	2	0.0238	Hcr2-0A
can-miR-n016a-b	3	0.4285	Hcr2-p2
can-miR-n016a-b	4	0.2867	Hcr2-2A
can-miR-n019a-d	2.5	0.3772	protein TAP1 precursor-like
can-miR-n019a-d	4	0.9873	U1 small nuclear ribonucleoprotein A-like isoform X1
can-miR-n019a-d	3	0.7584	putative late blight resistance protein homolog R1A-4-like
can-miR-n022a-c	2	0.3240	Detected protein of unknown function
can-miR-n022a-c	1.5	0.5268	Detected protein of unknown function
can-miR-n022a-c	2	0.4011	Peroxidase
can-miR-n022a-c	3	0.6063	Pentatricopeptide repeat-containing protein, putative
can-miR-n022a-c	2	0.7584	At1g28280
can-miR-n022a-c	3.5	0.8241	Detected protein of unknown function
can-miR-n022a-c	4	0.9940	pleiotropic drug resistance protein 1-like
can-miR-n022a-c	4	0.6763	F-box protein PP2-B10-like
can-miR-n022a-c	4	0.9690	mitochondrial substrate carrier family protein ucpB-like
can-miR-n022a-c	1.5	0.3113	Zinc finger protein, putative
can-miR-n022a-c	1.5	0.7004	ATP-citrate lyase B-1
can-miR-n022a-c	3.5	0.6733	F-box protein PP2-B10-like
can-miR-n022a-c	2.5	0.4544	protein MNN4-like
can-miR-n022a-c	2	0.5556	myb-like protein X-like
can-miR-n022a-c	2.5	0.1224	Detected protein of unknown function
can-miR-n022a-c	2	0.3726	Phospholipase D
can-miR-n022a-c	2	0.2917	Phospholipase C, putative
can-miR-n023	0.5	0.4285	Cytochrome P450
can-miR-n023	2.5	0.2716	Crossover junction endonuclease MUS81
can-miR-n023	3	0.2648	uncharacterized LOC101221004
can-miR-n023	3.5	0.7411	Dihydrolipoamide S-acetyltransferase, putative
can-miR-n024	9	0.0421	Scythe/bat3, putative
can-miR-n025	4	0.3829	At1g01920
can-miR-n026	1.5	0.0012	Detected protein of confused Function
can-miR-n026	4	0.0092	Peru 2

can-miR-n026	2.5	0.0375	Detected protein of confused Function
can-miR-n026	2	0.0006	Hcr9-9D
can-miR-n026	2	0.0031	Hcr9-OR2A
can-miR-n026	1	0.0003	Hcr9-OR2C
can-miR-n026	1.5	0.0058	receptor-like protein 12-like
can-miR-n026	1.5	0.0055	receptor-like protein 12-like
can-miR-n026	1.5	0.0052	Hcr9-OR2A
can-miR-n026	3.5	0.8288	Hcr9-Avr4-per1
can-miR-n026	3.5	0.8212	Hcr9-OR2C
can-miR-n026	2.5	0.4788	Hcr9-Avr4-per1
can-miR-n026	3	0.9694	GDSL-motif lipase/hydrolase family protein (Fragment)
can-miR-n026	2	0.4119	Hcr9-Avr4-par1
can-miR-n026	3.5	0.1756	Hcr9-Avr4-per1
can-miR-n026	4	0.6624	GDSL lipase-like chlorogenate-dependent caffeoyltransferase (Precursor)
can-miR-n026	2	0.4285	GDSL lipase-like chlorogenate-dependent caffeoyltransferase (Precursor)
can-miR-n026	3	0.5950	GDSL lipase-like chlorogenate-dependent caffeoyltransferase (Precursor)
can-miR-n026	2.5	0.8914	NL0E
can-miR-n026	2.5	0.8940	Hcr9-Avr4-par1
can-miR-n026	7	0.0238	Hcr9-OR2A
can-miR-n031-3p	3.5	0.4757	Serine/threonine-protein phosphatase
can-miR-n031-5p	4	0.9297	glycerate dehydrogenase, putative
can-miR-n032	4	0.0421	ATP binding protein, putative
can-miR-n033a-3p	1	0.0058	Root-knot nematode resistance protein
can-miR-n033a-3p	2	0.0119	Late blight resistance protein Rpi-blb2
can-miR-n033a-3p	1	0.0185	Detected protein of confused Function
can-miR-n033a-3p	2	0.0018	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	3	0.0247	Root-knot nematode resistance protein
can-miR-n033a-3p	3	0.0037	Root-knot nematode resistance protein
can-miR-n033a-3p	4	0.9846	Detected protein of unknown function
can-miR-n033a-3p	4	0.8025	Detected protein of unknown function
can-miR-n033a-3p	0.5	0.1211	Late blight resistance protein Rpi-blb2
can-miR-n033a-3p	1	0.3365	Root-knot nematode resistance protein
can-miR-n033a-3p	4	0.0615	NBS-coding resistance gene analog (Fragment)
can-miR-n033a-3p	4	0.0525	Detected protein of unknown function
can-miR-n033a-3p	4	0.0534	putative late blight resistance protein homolog R1B-12-like
can-miR-n033a-3p	4	0.9857	putative late blight resistance protein homolog R1B-12-like
can-miR-n033a-3p	2	0.1305	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	3.5	0.9818	Root-knot nematode resistance protein
can-miR-n033a-3p	4	0.6860	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	4	0.6457	Late blight resistance protein Rpi-blb2
can-miR-n033a-3p	1	0.3976	NBS-LRR root-knot nematode resistance protein
can-miR-n033a-3p	3	0.8651	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	4	0.5805	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	4	0.8887	Disease resistance gene homolog Mi-copy2
can-miR-n033a-3p	4	0.6624	Disease resistance gene homolog Mi-copy2
can-miR-n033a-3p	3	0.5598	Root-knot nematode resistance protein

can-miR-n033a-3p	3	0.5491	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	2	0.2144	Root-knot nematode resistance protein
can-miR-n033a-3p	3	0.4544	Late blight resistance protein Rpi-blb2
can-miR-n033a-3p	3	0.8015	Root-knot nematode resistance protein
can-miR-n033a-3p	2	0.3772	Root-knot nematode resistance protein
can-miR-n033a-3p	1	0.1545	NBS-LRR resistance protein-like protein
can-miR-n033a-3p	4.5	0.0440	putative late blight resistance protein homolog R1C-3-like
can-miR-n033b-3p	4	0.5598	Disease resistance gene homolog Mi-copy2
can-miR-n033b-3p	4	0.3037	Root-knot nematode resistance protein
can-miR-n033b-3p	7	0.0171	putative late blight resistance protein homolog R1C-3-like
can-miR-n033b-3p	7	0.0144	Detected protein of unknown function
can-miR-n036	3.5	0.7130	TBCC domain-containing protein 1-like isoform X1
can-miR-n039	4	0.9996	Nuclear RNA binding protein (Fragment)
can-miR-n040	3	0.0068	Glutathione S-transferase/peroxidase
can-miR-n040	7	0.0152	DNA ligase 1-like
can-miR-n041	5	0.0093	Non-symbiotic hemoglobin, putative
can-miR-n041	5	0.0276	DNA polymerase alpha subunit B
can-miR-n043	3	0.0080	ycf49-like protein-like isoform 1
can-miR-n043	4	0.9021	Detected protein of unknown function
can-miR-n043	2	0.2276	Detected protein of unknown function
can-miR-n043	3	0.3365	AT1G30620 protein
can-miR-n043	2.5	0.5586	TMV resistance protein N-like
can-miR-n048	3	0.0455	Detected protein of unknown function
can-miR-n048	4	0.1386	Detected protein of unknown function
can-miR-n048	2.5	0.0805	disease resistance protein At4g27190-like
can-miR-n048	4	0.5772	Anthranilate N-benzoyltransferase protein, putative
can-miR-n048	3	0.2276	Serine esterase family protein
can-miR-n048	3.5	0.4757	Clathrin assembly protein, putative
can-miR-n048	4	0.2332	AT1G30620 protein
can-miR-n048	3	0.0698	zeatin O-glucosyltransferase-like
can-miR-n050	3.5	0.9620	Importin subunit alpha
can-miR-n050	4	0.9987	uncharacterized LOC101224558, partial
can-miR-n050	4	0.7832	50S ribosomal protein L12-2, chloroplastic-like isoform 1
can-miR-n050	3.5	0.7533	Detected protein of unknown function
can-miR-n053	3.5	0.9999	AP2 transcription factor SlAP2d
can-miR-n056	1	0.0421	CC-NBS-LRR protein
can-miR-n056	3	0.9666	Disease resistance protein R3a-like protein
can-miR-n061	2	0.0276	MYB-related transcription factor (Fragment)
can-miR-n061	2	0.2913	MYB-related transcription factor (Fragment)
can-miR-n061	2	0.1554	MYB-related transcription factor (Fragment)
can-miR-n063	2.5	0.0238	Suppression of tumorigenicity, putative
can-miR-n063	3	0.1211	secoisolariciresinol dehydrogenase-like
can-miR-n063	4	0.9336	NADH dehydrogenase
can-miR-n063	4	0.7706	Putative taxane 13-alpha-hydroxylase cytochrome P450
can-miR-n063	4	0.9731	Unknown protein
can-miR-n063	3.5	1.0000	COBRA-like protein

can-miR-n063	3.5	0.8733	Serine/arginine rich splicing factor, putative
can-miR-n065	2	0.1386	Serine protease
can-miR-n065	3	0.9998	Calmodulin, putative
can-miR-n065	2.5	0.2225	NB-LRR type disease resistance protein
can-miR-n065	3	1.0000	MATE efflux family protein 6-like
can-miR-n065	4	1.0000	Detected protein of unknown function
can-miR-n065	4	0.9390	RING/U-box superfamily protein, putative
can-miR-n065	4	0.9999	Trans-2-enoyl-CoA reductase, mitochondrial, putative, expressed
can-miR-n067	4	0.9960	UDP-glucose:glucosyltransferase
can-miR-n067	3.5	0.4067	Synaptic vesicle 2-related protein
can-miR-n067	3	0.5300	Detected protein of unknown function
can-miR-n067	2	0.0632	casparian strip membrane protein 1-like
can-miR-n067	3.5	0.5472	Detected protein of unknown function
can-miR-n067	4	0.9991	Phosphatidylinositol-4-phosphate 5-kinase, putative
can-miR-n067	4	0.7783	DNA (Cytosine-5)-methyltransferase 3A
can-miR-n070	3	0.0238	Heavy metal cation transport atpase, putative
can-miR-n071	1	0.1545	Leucine-rich repeat-containing protein, putative
can-miR-n071	4	0.9464	Detected protein of unknown function
can-miR-n071	2.5	0.9983	thioredoxin X, chloroplastic-like
can-miR-n071	0.5	0.1211	Clathrin assembly protein, putative
can-miR-n071	3	0.4337	aluminum-activated malate transporter 9
can-miR-n071	4	0.5598	aluminum-activated malate transporter 9
can-miR-n071	4	0.9938	protein ECERIFERUM 3-like
can-miR-n102	4	0.6346	probable uridine nucleosidase 2-like
can-miR-n105	4	0.7783	Atypical receptor-like kinase 1

[a]Penalty score

Table S7. Result of phasiRNA analysis in pepper.

miRNA trigger	Maximum phasing score	Annotation[a]
can-miR169a-g	39.63	Nuclear transcription factor Y subunit A-3, putative
can-miR171i	19.72	BAC19.14
can-miR393a-b	129.39	TIR1-like
can-miR482b	86.87	TIR-NBS-LRR RCT1-like resistance protein
can-miR482b	44.04	NB-LRR
can-miR482c	399.89	NB-LRR
can-miR482c	395.86	NB-LRR
can-miR482c	317.92	NB-LRR
can-miR482c	274.71	NB-LRR
can-miR482c	179.32	NB-LRR
can-miR482c	83.74	NB-LRR
can-miR482c	73.10	NB-LRR
can-miR482c	52.58	autoimmune
can-miR482d	243.61	NB-LRR
can-miR482d	97.76	NB-LRR
can-miR482d	80.17	NB-LRR
can-miR482d	72.26	NB-LRR
can-miR482d	64.01	NB-LRR
can-miR482d	58.20	NB-LRR
can-miR482d	53.53	NB-LRR
can-miR482e	144.06	autoimmune
can-miR482f	602.24	NB-LRR
can-miR482f	89.56	NB-LRR
can-miR482f	51.16	NB-LRR
can-miR6027-3p	195.90	NB-LRR
can-miR6027-3p	136.12	NB-LRR
can-miR6027-3p	35.99	NB-LRR
can-miR-n026	255.30	Hcr9-OR2A
can-miR-n026	90.18	receptor-like protein 12-like
can-miR-n026	62.15	Hcr9-Avr4-par1
can-miR-n026	54.90	NL0E
can-miR-n026	41.14	receptor-like protein 12-like
can-miR-n026	31.86	Hcr9-OR2C
can-miR-n026	22.58	Hcr9-OR2A
can-miR-n026	16.07	Hcr9-9D
can-miR-n033a	80.91	NB-LRR
can-miR-n033a	75.74	NB-LRR
can-miR-n033a	50.98	NB-LRR
can-miR-n033a	21.99	NB-LRR

[a]Red: NB-LRR, blue: RLP

Table S8. Comparing target prediction results of miR-n026.

miRNA	Species	RLP[a]
can-miR-n026	Pepper	17
	Tomato	16
	Potato	5
sly-miR-n026	Pepper	2
	Tomato	2
	Potato	1
stu-miR-n026	Pepper	0
	Tomato	0
	Potato	1

[a]Number of the predicted target RLPs (score ≤ 3.5)

Figure S1. miRNA classification in pepper, tomato and potato.

A Venn diagram showed conserved miRNA families in Solanaceae. Numbers in the parenthesis indicate miRNAs which are conserved in other plant species.

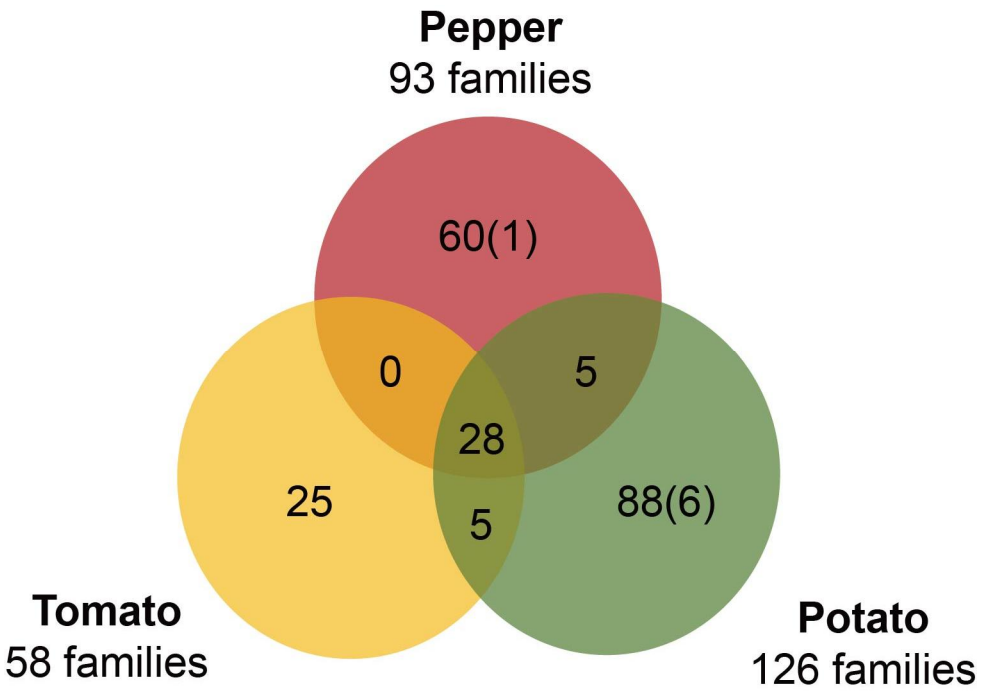
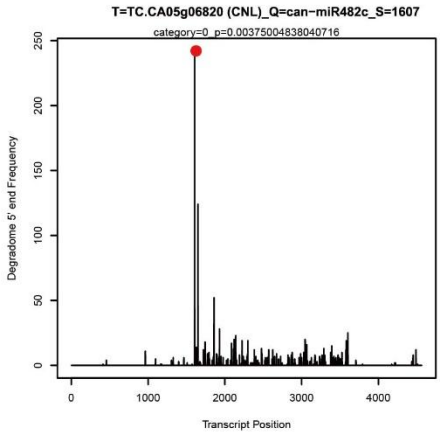
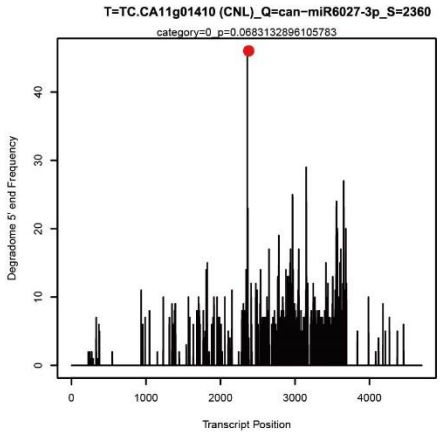
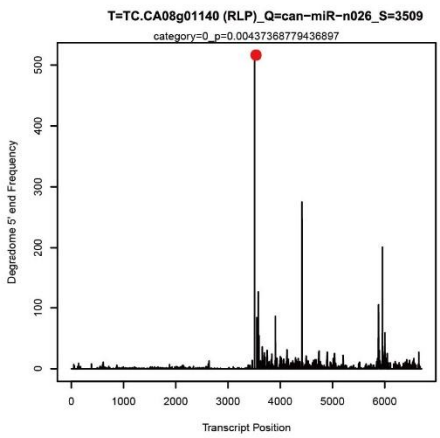

Pepper
93 families
60(1)
0
5
28
25
5
88(6)
Tomato
58 families
Potato
126 families

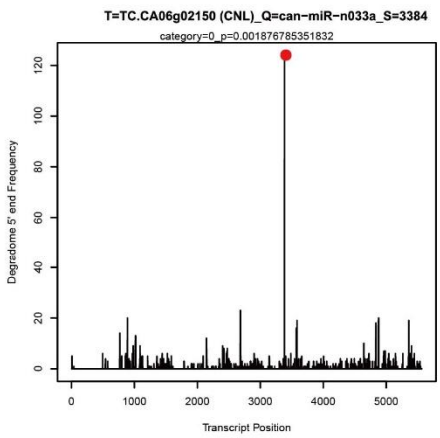
Figure S2. Representative examples of miRNAs cleaving disease-resistance genes.

Plot shows results of the degradome analysis in pepper. x-axis and y-axis indicate 5' end position of the mapped degradome reads and abundance of the mapped degradome reads, respectively. Red dot indicates cleavage site in the transcript by miRNA.


T=TC.CA05g06820 (CNL)_Q=can-miR482c_S=1607
category=0 p=0.00375004838040716
Degradome 5' end Frequency
Transcript Position


T=TC.CA11g01410 (CNL)_Q=can-miR6027-3p_S=2360
category=0 p=0.0683132896105783
Degradome 5' end Frequency
Transcript Position


T=TC.CA08g01140 (RLP)_Q=can-miR-n026_S=3509
category=0 p=0.00437368779436897
Degradome 5' end Frequency
Transcript Position


T=TC.CA06g02150 (CNL)_Q=can-miR-n033a_S=3384
category=0 p=0.001876785351832
Degradome 5' end Frequency
Transcript Position

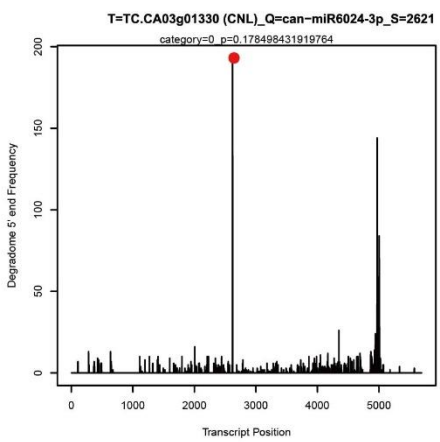
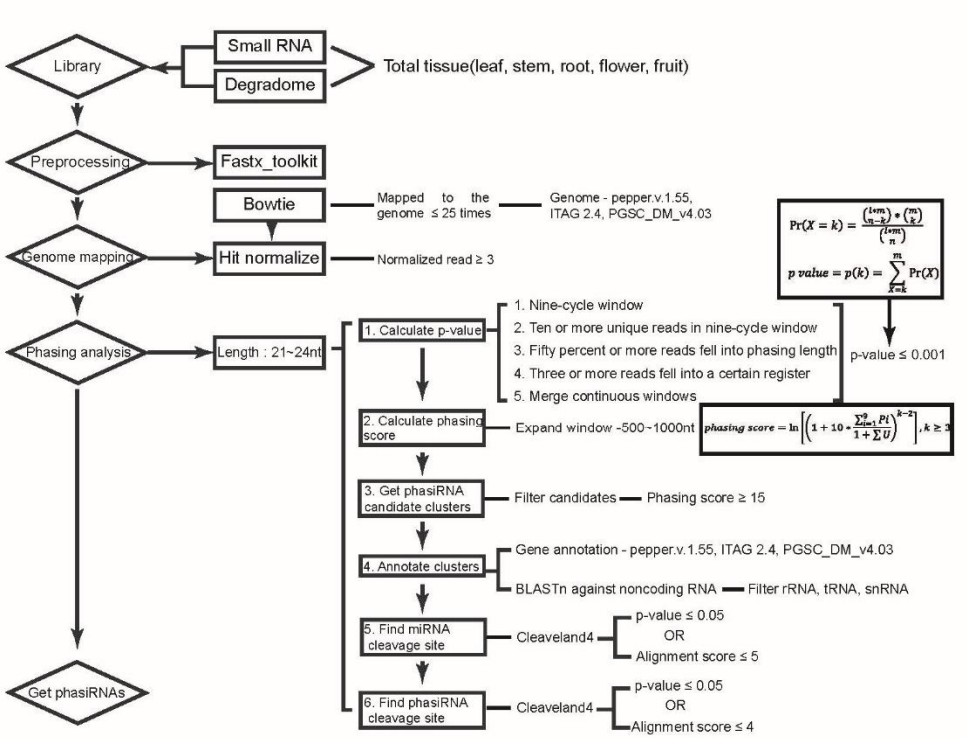

T=TC.CA03g01330 (CNL)_Q=can-miR6024-3p_S=2621
category=0 p=0.178498431919764
Degradome 5' end Frequency
Transcript Position

Figure S3. Pipeline for phasiRNA analysis.

Pipeline for phasiRNA analysis in this study is modified algorithm from the study of Xia et al. (2013).



※ In the formula for calculating p value, l is the length for phasing analysis; m is number of the phases within a window; k is number of the unique small RNAs mapped to the phased positions; n is number of the unique small RNAs within a window. Small RNAs of the "l" length are only used for calculation.

※ In the formula for calculating phasing score, P is total abundance of the reads mapped to the phased positions within a nine-cycle window, whose length is "l"; U is total abundance of the reads which are mapped to out of the phase and whose length is "l"; k is equal.

Figure S4. can-miR482c induces phasiRNA biogenesis by cleaving CNL.

(A) Mapping results of small RNA and degradome reads normalized by mapping count to the genome and phasing score data were viewed using IGV. Red arrow indicates the cleavage site by can-miR482c. Examples of (B) *cis*-acting phasiRNAs and (C) *trans*-acting phasiRNAs. Red triangles indicate the cleavage sites by small RNAs. In degradome reads panel, pale red color indicates reads mapped to Watson strand and blue color indicates those mapped to Crick strand in the genome.

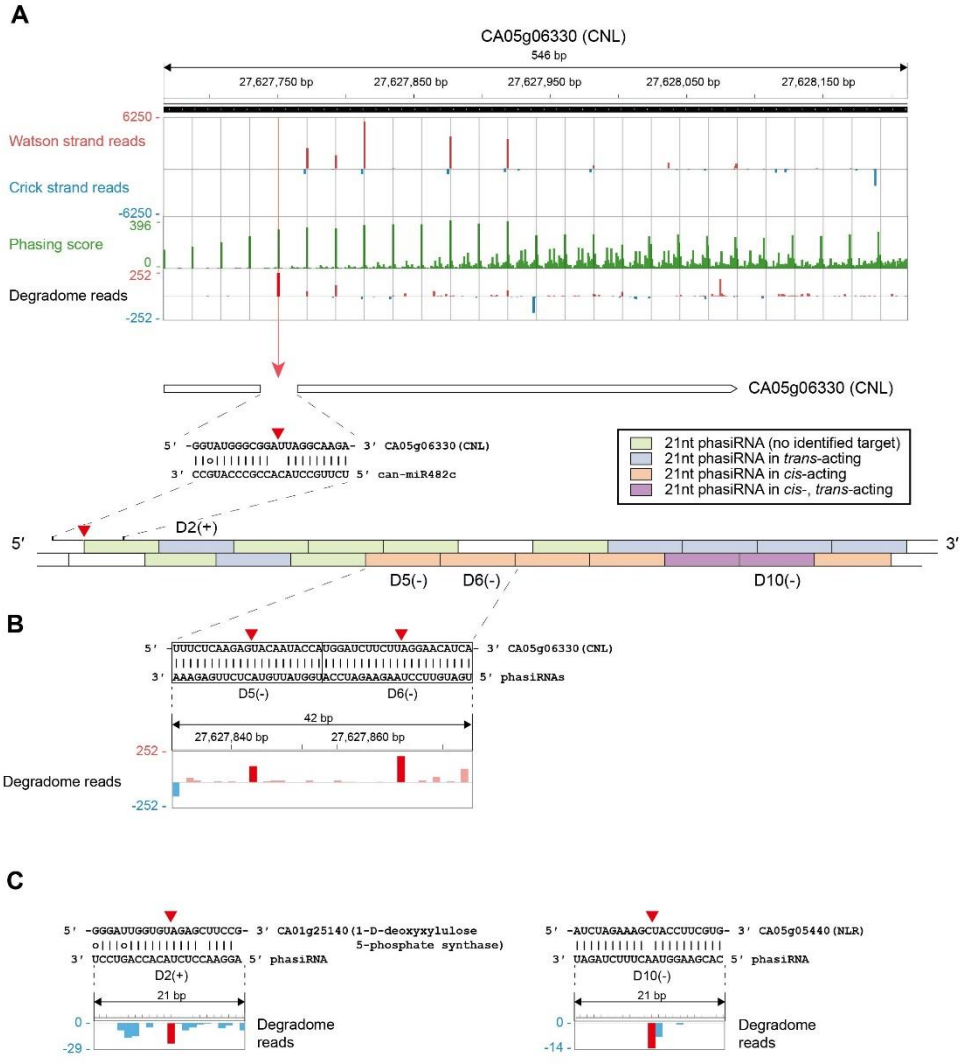

A
CA05g06330 (CNL)
546 bp
27,627,750 bp
27,627,850 bp
27,627,950 bp
27,628,050 bp
27,628,150 bp
6250
Watson strand reads
Crick strand reads
-6250
396
Phasing score
0
252
Degradome reads
-252
CA05g06330 (CNL)
5′ -GGUAUGGGCGGAUUAGGCAAGA- 3′ CA05g06330(CNL)
3′ CCGUACCCGCCACAUCCGUUCU 5′ can-miR482c
21nt phasiRNA (no identified target)
21nt phasiRNA in trans-acting
21nt phasiRNA in cis-acting
21nt phasiRNA in cis-, trans-acting
5′
D2(+)
3′
D5(-)
D6(-)
D10(-)
B
5′ -UUUCUCAAGAGUACAAUACCAUGGAUCUUCUUAGGAACAUCA- 3′ CA05g06330(CNL)
3′ AAAGAGUUCUCAUGUUAUGGUACCUAGAAGAAUCCUUGUAGU 5′ phasiRNAs
D5(-)
D6(-)
42 bp
27,627,840 bp
27,627,860 bp
252
Degradome reads
-252
C
5′ -GGGAUUGGUGUAGAGCUUCCG- 3′ CA01g25140(1-D-deoxyxylulose
5-phosphate synthase)
3′ UCCUGACCACAUCUCCAAGGA 5′ phasiRNA
D2(+)
21 bp
0
-29
Degradome reads
5′ -AUCUAGAAAGCUACCUUCGUG- 3′ CA05g05440(NLR)
3′ UAGAUCUUUCAAUGGAAGCAC 5′ phasiRNA
D10(-)
21 bp
0
-14
Degradome reads

Figure S5. can-miR6027 induces phasiRNA biogenesis by cleaving CNL.

(A) Mapping results of small RNA and degradome reads normalized by mapping count to the genome and phasing score data were viewed using IGV. Red arrow indicates the cleavage site by can-miR6027. Examples of (B) *cis*-acting phasiRNAs and (C) *trans*-acting phasiRNAs. Red triangles indicate the cleavage sites by small RNAs. In degradome reads panel, pale red color indicates reads mapped to Watson strand and blue color indicates those mapped to Crick strand in the genome.

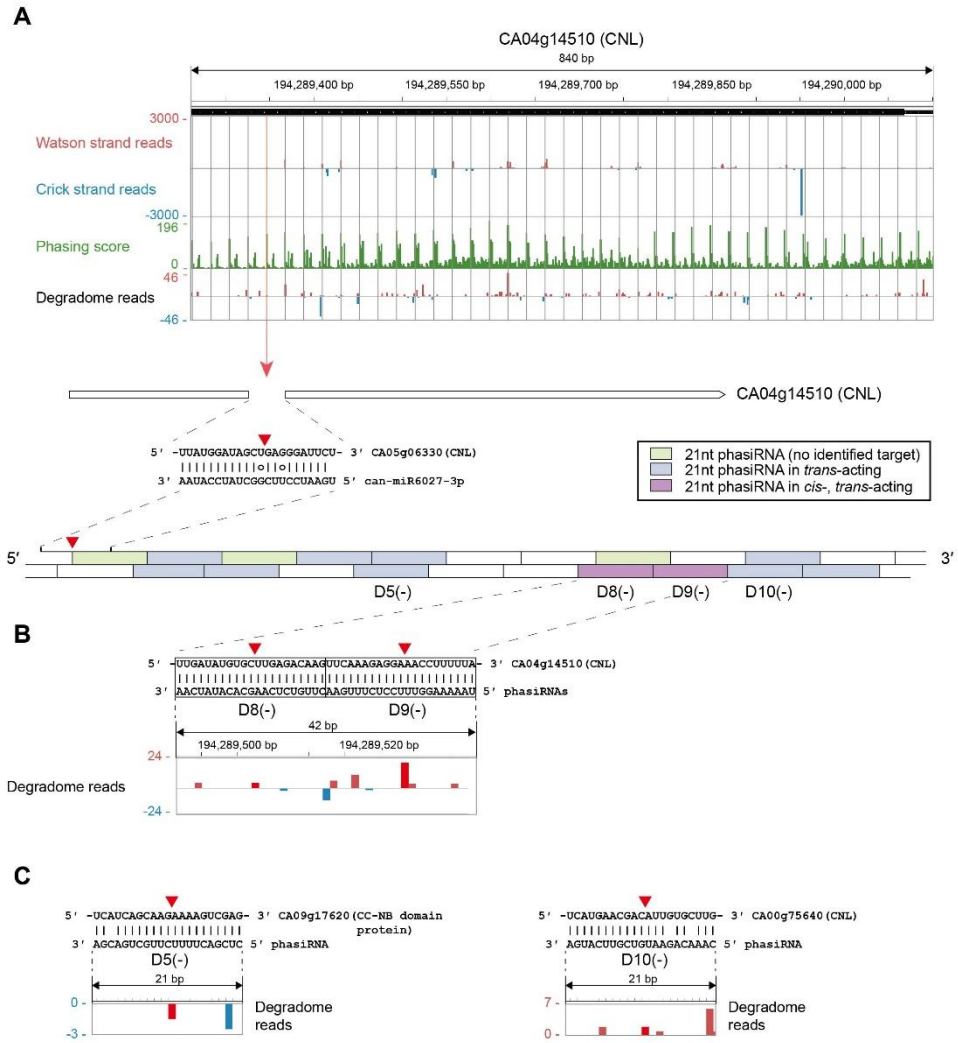

A
CA04g14510 (CNL)
840 bp
194,289,400 bp
194,289,550 bp
194,289,700 bp
194,289,850 bp
194,290,000 bp
3000
Watson strand reads
Crick strand reads
-3000
196
Phasing score
0
46
Degradome reads
-46
CA04g14510 (CNL)
5′ -UUAUGGAUAGCUGAGGGAUUCU- 3′ CA05g06330(CNL)
3′ AAUACCUAUCGGCUUCCUAAGU 5′ can-miR6027-3p
21nt phasiRNA (no identified target)
21nt phasiRNA in trans-acting
21nt phasiRNA in cis-, trans-acting
5′
3′
D5(-)
D8(-)
D9(-)
D10(-)
B
5′ -UUGAUAUGUGCUUGAGACAAGUUCAAAGAGGAAACCUUUUUA- 3′ CA04g14510(CNL)
3′ AACUAUACACGAACUCUGUUCAAGUUUCUCCUUUGGAAAAAU 5′ phasiRNAs
D8(-)
D9(-)
42 bp
194,289,500 bp
194,289,520 bp
24
Degradome reads
-24
C
5′ -UCAUCAGCAAGAAAAGUCGAG- 3′ CA09g17620(CC-NB domain protein)
3′ AGCAGUCGUUCUUUUCAGCUC 5′ phasiRNA
D5(-)
21 bp
0
-3
Degradome reads
5′ -UCAUGAACGACAUUGUGCUUG- 3′ CA00g75640(CNL)
3′ AGUACUUGCUGUAAGACAAAC 5′ phasiRNA
D10(-)
21 bp
7
0
Degradome reads

Figure S6. can-miR-n033a induces phasiRNA biogenesis by cleaving NLR.

(A) Mapping results of small RNA and degradome reads normalized by mapping count to the genome and phasing score data were viewed using IGV. Red arrow indicates the cleavage site by can-miR-n033a. (B) An example of *cis*-acting phasiRNA. Red triangles indicate the cleavage sites by small RNAs. In degradome reads panel, pale red color indicates reads mapped to Watson strand and blue color indicates those mapped to Crick strand in the genome.

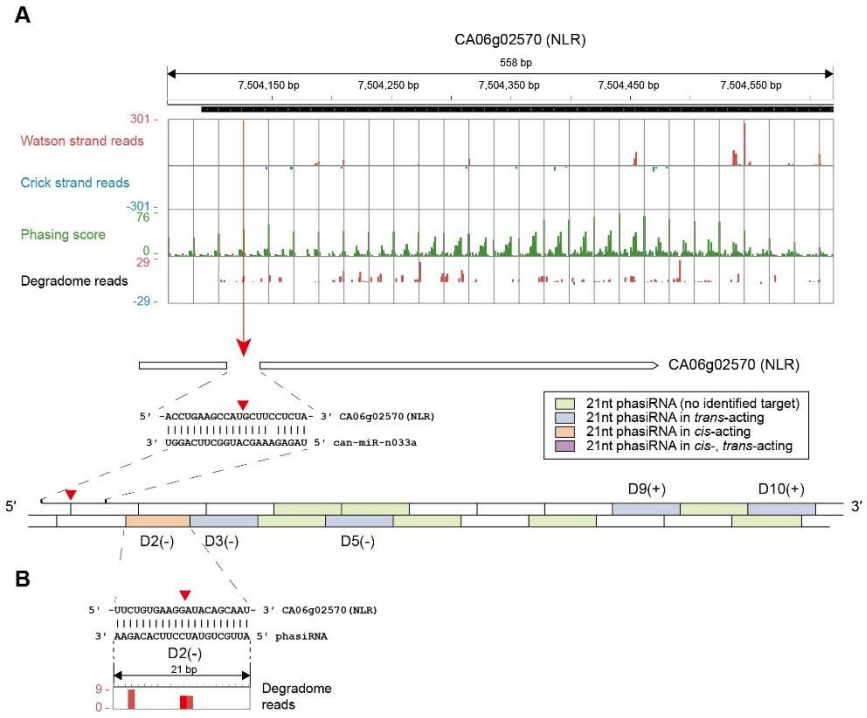

A
CA06g02570 (NLR)
558 bp
7,504,150 bp
7,504,250 bp
7,504,350 bp
7,504,450 bp
7,504,550 bp
301 -
Watson strand reads
Crick strand reads
-301 -
76
Phasing score
0 -
29 -
Degradome reads
-29 -
CA06g02570 (NLR)
5′ -ACCUGAAGCCAUGCUUCCUCUA- 3′ CA06g02570(NLR)
3′ UGGACUUCGGUACGAAAGAGAU 5′ can-miR-n033a
21nt phasiRNA (no identified target)
21nt phasiRNA in trans-acting
21nt phasiRNA in cis-acting
21nt phasiRNA in cis-, trans-acting
5′
D9(+)
D10(+)
3′
D2(-)
D3(-)
D5(-)
B
5′ -UUCUGUGAAGGAUACAGCAAU- 3′ CA06g02570(NLR)
3′ AAGACACUUCCUAUGUCGUUA 5′ phasiRNA
D2(-)
21 bp
9 -
0 -
Degradome reads

Figure S7. Validation of the disease-resistance associated miRNAs via northern blot.

Total RNA was extracted from leaf of pepper, tomato, potato and *Nicotiana benthamiana*. tRNAs are shown as a loading control.

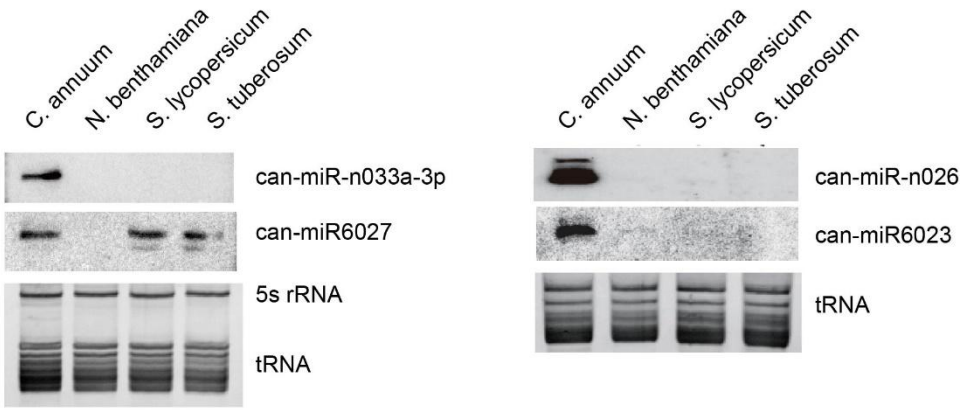

C. annuum
N. benthamiana
S. lycopersicum
S. tuberosum
can-miR-n033a-3p
can-miR6027
5s rRNA
tRNA
C. annuum
N. benthamiana
S. lycopersicum
S. tuberosum
can-miR-n026
can-miR6023
tRNA

Figure S8. Hairpin structures of the miR-n033b genes and their expression.

Hairpin structures of (A) can-miR-n033b, (B) stu-miR-n033b and (C) sly-miR6027, respectively. Color indicates the possibility of base-pairing. (D-F) Small RNA mapping results on the homologous region of miR-n033b in pepper, potato and tomato, respectively.

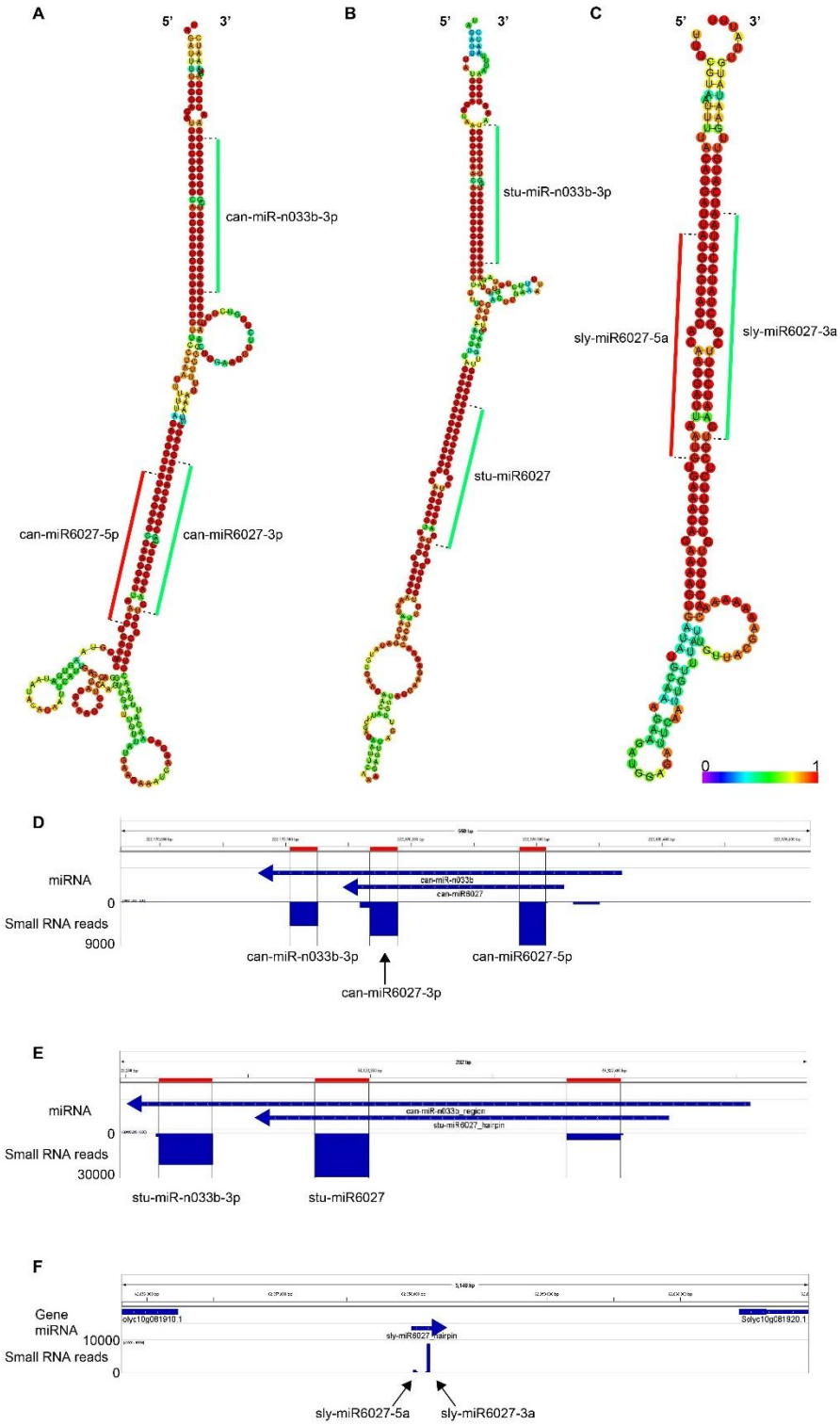

A
5'
3'
can-miR-n033b-3p
can-miR6027-5p
can-miR6027-3p
B
5'
3'
stu-miR-n033b-3p
stu-miR6027
C
5'
3'
sly-miR6027-5a
sly-miR6027-3a
0
1
D
miRNA
0
Small RNA reads
9000
can-miR-n033b
can-miR6027
can-miR-n033b-3p
can-miR6027-5p
can-miR6027-3p
E
miRNA
0
Small RNA reads
30000
can-miR-n033b_region
stu-miR6027_hairpin
stu-miR-n033b-3p
stu-miR6027
F
Gene
miRNA
10000
Small RNA reads
0
Solyc10g081910.1
Solyc10g081920.1
sly-miR6027_hairpin
sly-miR6027-5a
sly-miR6027-3a

Figure S9. Hairpin structures of the miR-n033a genes and their expression.

Hairpin structures of (A) can-miR-n033a, (B) stu-miR-n033a, respectively. miR-n033a does not exist in tomato. Color indicates the possibility of base-pairing. (C-E) Small RNA mapping results on the homologous region of miR-n033a in pepper, potato and tomato, respectively.

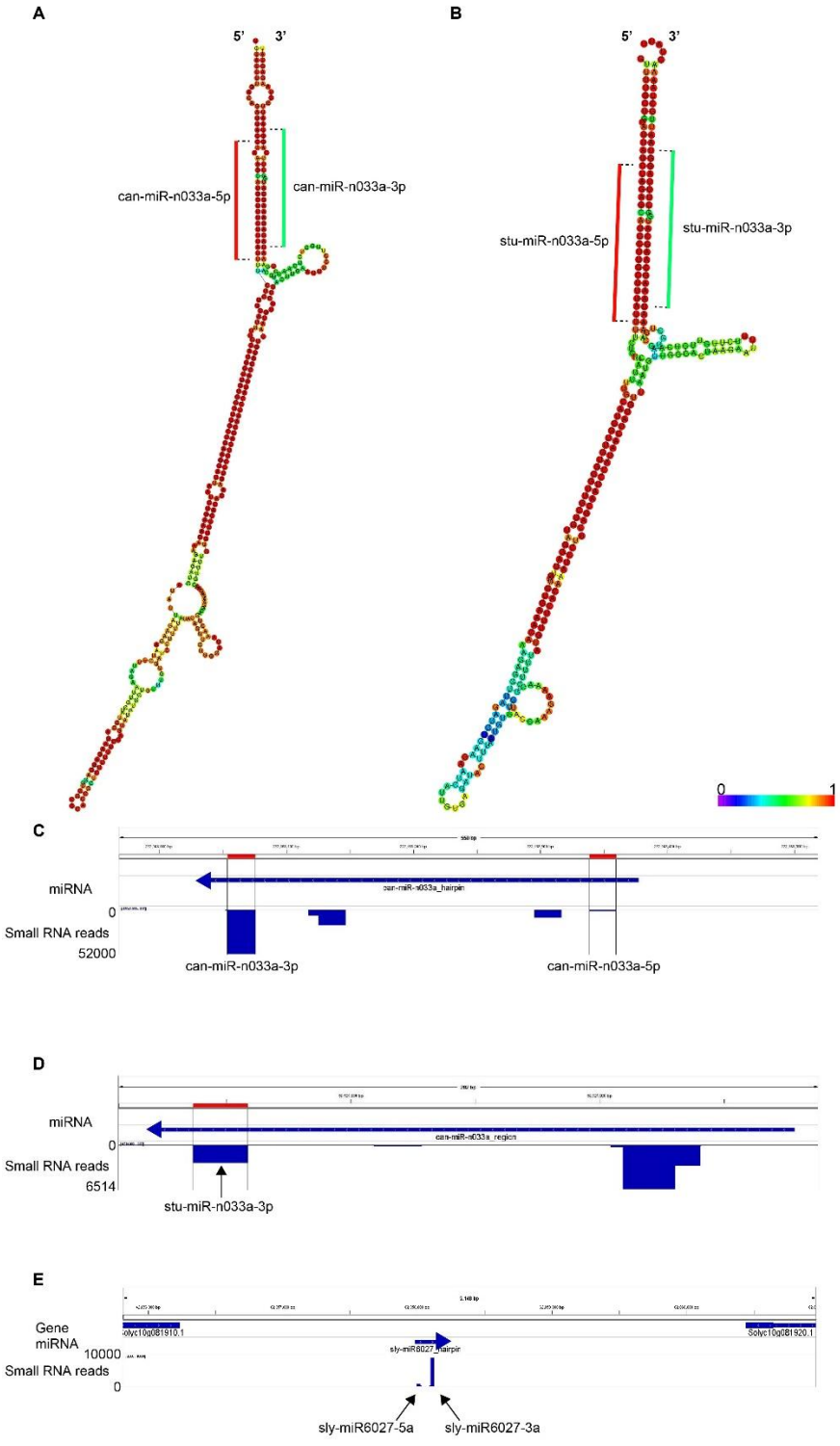

A
5'
3'
can-miR-n033a-5p
can-miR-n033a-3p
B
5'
3'
stu-miR-n033a-5p
stu-miR-n033a-3p
0
1
C
miRNA
can-miR-n033a_hairpin
0
Small RNA reads
52000
can-miR-n033a-3p
can-miR-n033a-5p
D
miRNA
can-miR-n033a_region
0
Small RNA reads
6514
stu-miR-n033a-3p
E
Gene
miRNA
Solyc10g081910.1
Solyc10g081920.1
sly-miR6027_hairpin
10000
Small RNA reads
0
sly-miR6027-5a
sly-miR6027-3a

Figure S10. Conservation of miR-n033 and miR-n026 in Solanaceous species.

(A) Evolutionary distances between Solanaceous species. (B) Conservation status of miR-n026 and miR-n033 in Solanaceous species. Homologous region of miRNA was identified using BLASTN (default option) and hairpin structure was predicted using RNAfold. Red color indicates that both sequences of miRNA-homologous region including mature miRNA and hairpin structure are conserved. Orange color indicates that sequences of miRNA-homologous region including mature miRNA is conserved, but hairpin structure is not because of insertion.

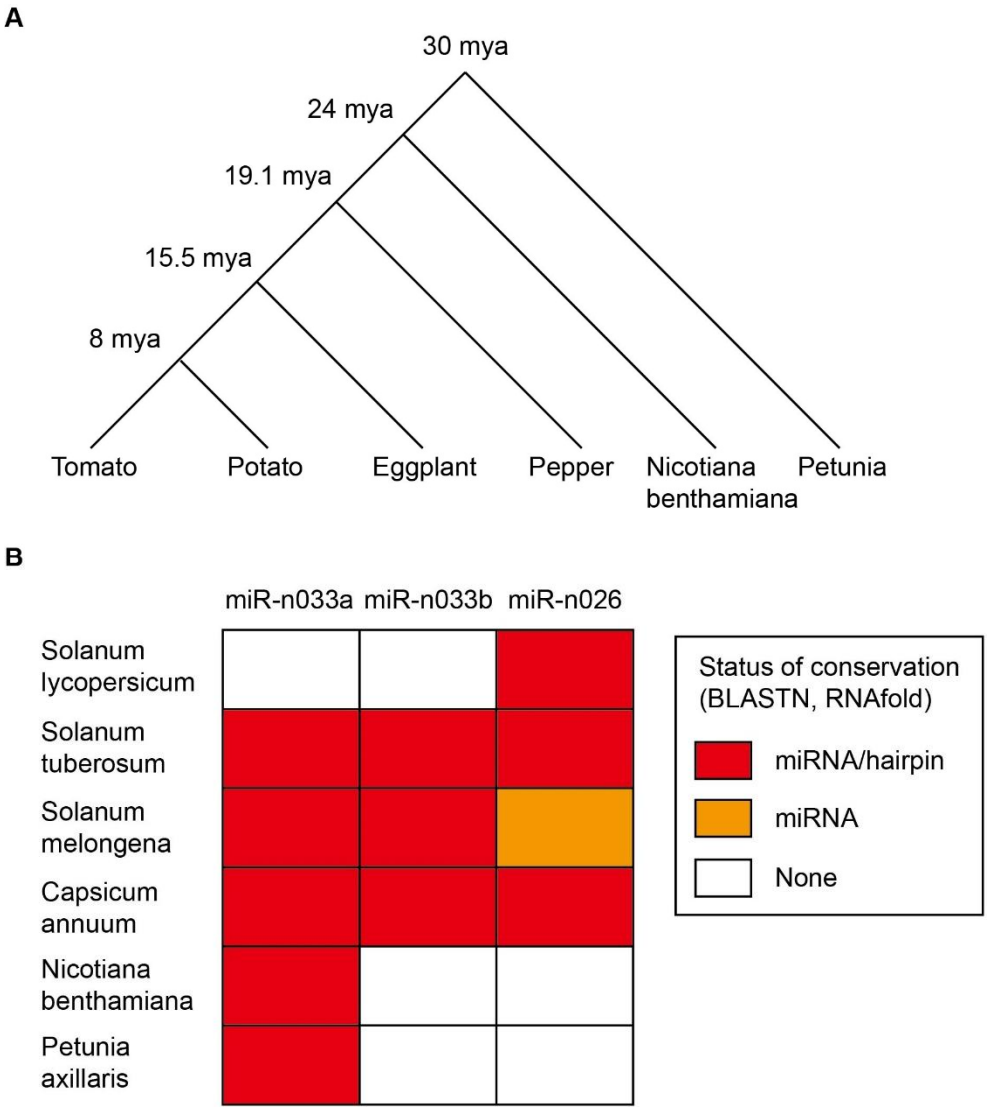

A
30 mya
24 mya
19.1 mya
15.5 mya
8 mya
Tomato
Potato
Eggplant
Pepper
Nicotiana benthamiana
Petunia
B
miR-n033a
miR-n033b
miR-n026
Solanum lycopersicum
Solanum tuberosum
Solanum melongena
Capsicum annuum
Nicotiana benthamiana
Petunia axillaris
Status of conservation (BLASTN, RNAfold)
miRNA/hairpin
miRNA
None

Figure S11. Hairpin structures of the miR-n026 genes and their expression.

Hairpin structures of (A) can-miR-n026, (B) stu-miR-n026 and (C) sly-miR-n026, respectively. Color indicates the possibility of base-pairing. (D-F) Small RNA mapping results on the homologous region of miR-n026 in pepper, potato and tomato, respectively.

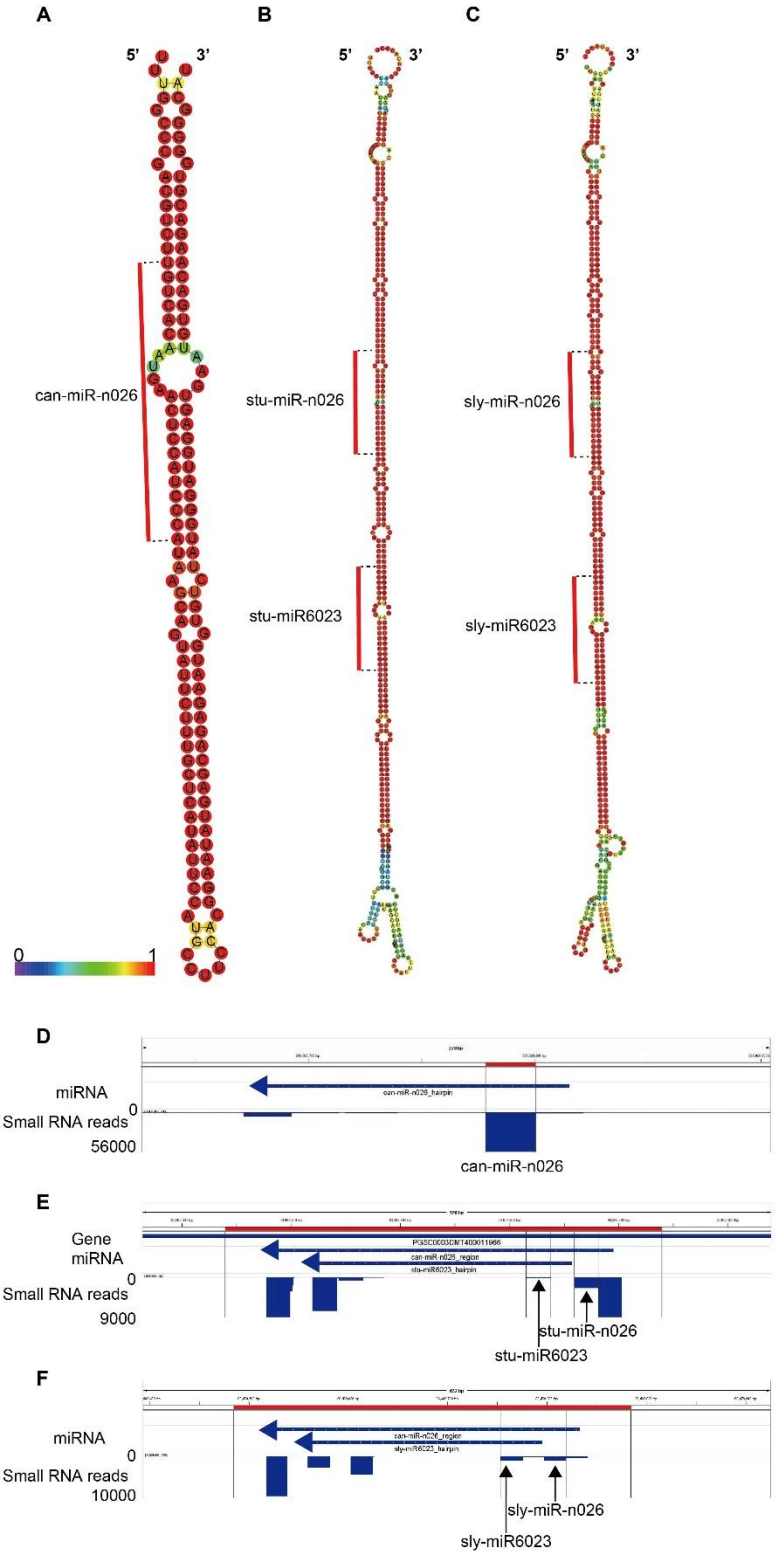
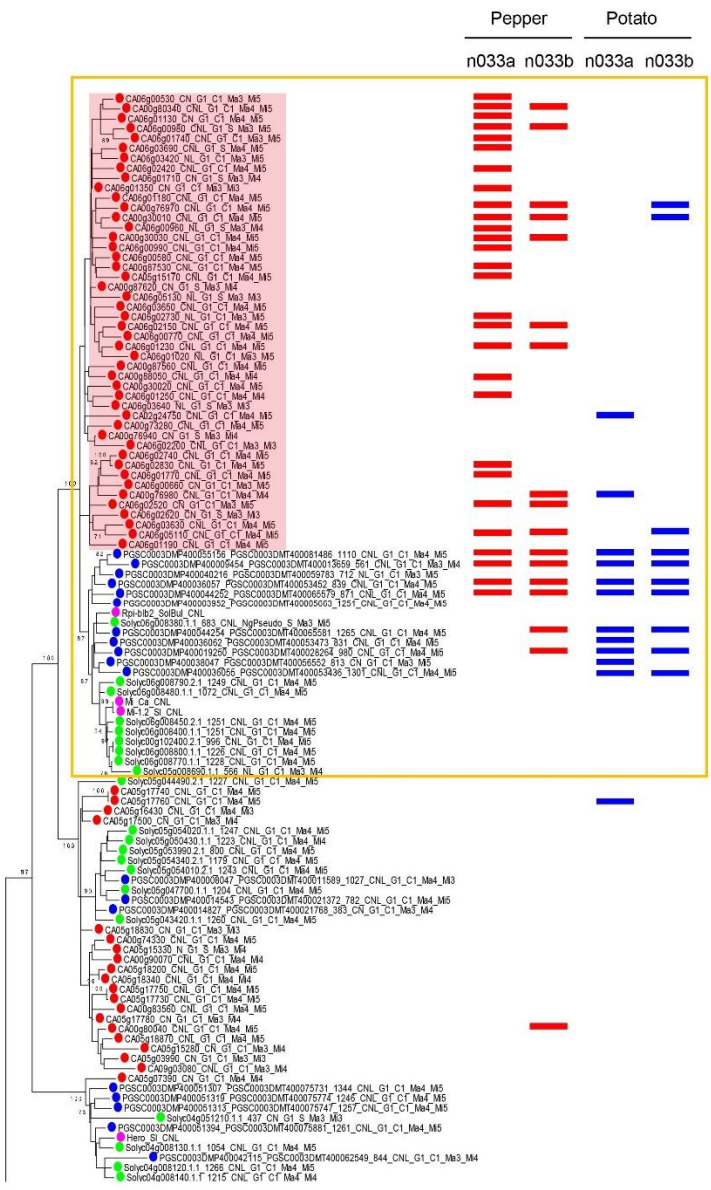

A
B
C
5'
3'
can-miR-n026
stu-miR-n026
stu-miR6023
sly-miR-n026
sly-miR6023
0
1
D
miRNA
Small RNA reads
0
56000
can-miR-n026
E
Gene
miRNA
Small RNA reads
0
9000
stu-miR-n026
stu-miR6023
F
miRNA
Small RNA reads
0
10000
sly-miR-n026
sly-miR6023

Figure S12. Phylogenetic relationships of whole CNL-G1 in pepper, tomato and potato.

The part in yellow boundary corresponds with Fig. 5b. Red boxes indicate the predicted target CNL-G1 of can-miR-n033. Blue boxes indicate the predicted target CNL-G1 of stu-miR-n033. Note, some CNL-G1 NLRs were excluded in the tree, because their NB-domain did not satisfy the criteria to construct a phylogenetic tree.


Pepper
Potato
n033a
n033b
n033a
n033b

Abstract in Korean

마이크로RNA는 small RNA의 일종으로, 식물에서 생장, 발달, 병 저항성과 같은 다양한 생물학적 과정에 연관되어 있는 것으로 알려져 있다. 이전 연구결과에 따르면, 몇몇 식물 마이크로RNA는 phased, secondary siRNA (phasiRNA)와 같은 이차 small interfering RNA의 생성을 유도하며, 생성된 small RNA들은 몇몇 유전자 군의 발현을 조절한다는 사실이 알려져 있다. 본 연구에서는 고추, 토마토, 감자 마이크로RNA를 진화적인 측면에서 분석하였다. Degradome 분석을 통해 세 종에서의 마이크로RNA 표적 유전자를 발굴하였고, 일부 마이크로RNA들이 병 저항성과 연관된 수많은 유전자들, 가령 nucleotide-binding leucine-rich repeat (NB-LRR)과 receptor-like protein (RLP)와 같은 유전자들을 조절한다는 사실을 확인할 수 있었다. 더욱이 해당 마이크로RNA들이 고추에서 phasiRNA 생성을 유도하여 병 저항성 유전자들에 대한 조절을 강화한다는 사실을 밝혔다. 특히, miR-n033a-3p는 감자에 비해 고추에서 더 많은 NB-LRR 유전자들을 조절할 것으로 예측되었는데, 해당 마이크로RNA의 표적 NB-LRR 유전자들은 주로 고추 특이적으로 팽창된 CNL-G1 분류에 속한다는 사실을 확인할 수 있었다. 이러한 관찰 결과들을 종합하여, 병 저항성에 연관된 마이크로RNA들이 고추에서 표적 유전자 팽창으로 인해 많은 유전자를 조절하도록 진화하였음을 짐작할 수 있었다.

주요어: 고추, 마이크로RNA, 병 저항성, 진화, degradome, NB-LRR, phasiRNA, receptor-like protein

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