Genome-wide comprehensive analysis of miRNAs and their target genes expressed in resistant and susceptible *Capsicum annuum* landrace during *Phytophthora capsici* infection

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Abstract

MiRNAs regulate plants responses to fungal infection and immunity by modulating the gene expression. Despite extensive works on miRNA's role during plant-fungus interaction, work in *Capsicum annuum*-*Phytophthora capsici* pathosystem is limited. Therefore, in the current study, genome-wide known and novel miRNAs were identified in two contrasting chilli pepper landraces, *i.e.* GojamMecha_9086 (resistant) and Dabat_80045 (susceptible) during *P. capsici* infection. The small RNA deep sequencing resulted in 79 known miRNAs corresponding to 24 miRNAs families and 477 novel miRNAs along with 22,895 potential targets, including 30 defence-related genes against *P. capsici* infection. The expression analysis of ~29 known & 157 novel miRNAs in resistant and 30 known and 176 novel miRNAs in susceptible landrace revealed differential accumulation pattern. RT-qPCR of a set of 8 defence related miRNAs representing 4 novel (Pz-novel-miR428-1, Pz-novel-miR160-1, Pz-novel-miR1028-1, Pz-novel-miR204-1) and 4 known (Pz-known-miR803-1, Pz-known-miR2059-1, Pz-known-miR2560-1, Pz-known-miR1872-1) revealed differential accumulation pattern in both resistant and susceptible landrace. Additionally, validation of 8 target genes of corresponding miRNAs using RA-PCR, which as good as 5' RLM-RACE, revealed an inverse relation with their corresponding miRNAs suggesting their key role during disease response. This study provides comprehensive genome-wide information about the repertoire of miRNAs and their target genes expressed in resistant and susceptible chilli pepper landrace, which can serve as a valuable resource for better understanding the post-transcriptional regulatory mechanism during *C. annuum* - *P. capsici* pathosystem.

Key words: *C. annuum*, *P. capsici*, miRNA, defence response, transcriptome, NGS

Introduction

Plants exposed to stress use multiple gene regulatory mechanisms, including post-transcriptional regulation of gene expression, to restore and re-establish cellular homeostasis. During the last two decades, microRNAs (miRNAs) have emerged as one of the ubiquitous and critical regulatory molecules during the entire life cycle of nearly all eukaryotes (Bartel, 2004). The use of miRNAs for trait improvement has been successfully demonstrated wherein overexpression of *OsmiR397* enhanced rice yield by increasing grain size and promoting panicle branching (Zhao *et al.*, 2013). The latest release of miRBase(v22) reported to contains 38,589 hairpin precursors and 48,860 mature microRNAs sequences from 271 organisms showing a continuous increase in the miRNA pool (Griffiths *et al.*, 2019). With the advancement in high throughput technology and rapidly growing bioinformatics, several workers have identified miRNAs, which play a critical role during disease pathogenesis upon fungal invasion in plants by interacting with several classes of resistance genes (Yin *et al.*, 2012; Chen *et al.*, 2012). The data generated by the high throughput sequencing platforms have uncovered the potential role of miRNAs in plants during fungal invasion (Gupta *et al.*, 2014). It has been observed that the host miRNAs, along with their targets either by up or down-regulation, participate in disease modulation in plants upon fungal infection (Zhao *et al.*, 2012; Gupta *et al.*, 2012). For instance, miR1138 was found to be highly accumulated in wheat infected with *P. graminis f.sp. tritici* (62G29-1) (Gupta *et al.*, 2012). Din *et al.* (2016) had profiled and characterized conserved miRNAs and their targets in chilli (*Capsicum annuum* L.) using ESTs. Yang and co-workers (Yang *et al.*, 2021) investigated the heterosis among three cross combinations of *C. annuum* varieties by integrating mRNA and miRNA profiling. Zhang *et al.* (2020) identified miRNAs and their targets involved during anther development in CMS-line N816S and its maintainer line Ning5m pepper (*C. annuum*). Shin and co-workers (Shin *et al.*, 2013) computationally predicted miRNA target in hot pepper (*C. annuum*). The miRNA targets of pepper and their regulatory networks were explored through techniques of MiRTrans (Trans-omics data) (Zhang *et al.*, 2017). Liu and co-workers (Liu *et al.*, 2017) identified and validated miRNAs involved in fruit development and fruit ripening in hot pepper. However, despite these reports, there is no work on identifying and characterizing miRNAs and their targets during *C. annuum* L – *P. capsici* pathosystem.

Therefore, understanding miRNA expression and regulation during *C. annuum* - *P. capsici* pathosystem will help in a better understanding of the molecular and biochemical pathways associated with defence response in chilli pepper.

Pepper (*Capsicum annuum* L.) is one of the most economically important vegetable crops in the world (Catalan *et al.*, 2020) owing to its diverse usages, including spice, either fresh or in powder form (El-Ghoraba *et al.*, 2013), drug, condiment, ointment (Chamikara *et al.*, 2016). On the other hand, its productivity is highly affected by both abiotic and biotic factors. Among biotic factors, the fungal pathogens, especially *P. capsici* imposes severe threats to its production as it can cause losses up to 100% (Babadoost, 2004). The *P. capsici* is a soil-born pathogen triggering diseases like root and crown rot, leaf late blight stems blight, and fruits (Mao *et al.*, 1998). Understanding the molecular mechanism and developing resistant genotypes is a sustainable approach to tackle it as control by chemical and cultural practices remains a challenge (Grumet *et al.*, 2009). Identification and characterization of novel and known miRNAs in chilli pepper in response to *P. capsici* shall widen our current understanding of these miRNAs' role in modulating the molecular and biochemical pathways with disease resistance.

Four small RNA libraries were prepared from control and infected leaves of *P. capsici* resistant and susceptible chilli pepper landraces to fill this gap. The two contrasting chilli pepper landraces, *i.e.* GojamMecha_9086 (resistant) and Dabat_80045 (susceptible), were selected based on our earlier report (Rabuma *et al.*, 2020). The libraries represented small RNAs expressed during control and infection of both resistant and susceptible landraces. We utilized genome-wide sequencing of small RNAs to identify known and novel expressed miRNAs in resistant and susceptible landraces. Target genes of these miRNAs were also detected from our previous transcriptome data (under review) of the same samples. Besides, expression analysis of 8 key miRNAs, along with cleavage/inhibition pattern of their eight corresponding target genes associated with defence response against fungal invasion and immunity, were also validated using RT-qPCR and RA-PCR, respectively.

Materials and Methods

Plant material, fungal strain, and sample preparation

Based on disease score data published earlier (Rabuma *et al.*, 2020), two contrasting landraces of chilli pepper (*C. annuum*), *i.e.* GojamMecha_9086 (resistant) and Dabat_80045

(susceptible), were selected for small RNA sequencing and identification of miRNA to unravel the defence mechanism operating during *C. annuum* – *P. capsici* pathosystem. The details of germination, growth conditions, fungal strain, sporulation and inoculation are the same as described earlier (Andrés Ares *et al.*, 2005; Rabuma *et al.*, 2020). After five days post-inoculation (dpi), samples were harvested from three biological replications of control and infected leaves (4th and 5th leaves) of GojamMecha_9086 and Dabat_80045 representing resistant control (RC), resistant infected (RI), susceptible control (SC) and susceptible infected (SI). The leaf samples were washed and frozen in liquid nitrogen and kept at -80 °C in a deep freeze refrigerator. The total RNA was isolated using ZR Plant RNA Miniprep™ Kit by Zymo Research as per manufacturer instruction. Total RNA was isolated separately from control and infected leaf tissues. The quality and quantity of isolated RNA were checked on 1% denaturing agarose gel and Nanodrop spectrophotometry (Fluorometer, DS-11 FXT, Denovix), respectively. Small RNA enriched samples were sequenced using the Illumina NextSeq500 sequencing platform.

Small RNA library preparation and Illumina sequencing

The small RNA sequencing libraries were prepared from quality checked (QC) passed total RNA using Illumina TruSeq small RNA library preparation kit (Cat. No-RS-200-0024) according to the manufacturer instruction. High-quality small RNA reads were obtained from raw reads by filtering out poor quality reads and removing adapters contamination using Cutadapt (v 1.16) by considering parameters, *i.e.* any reads with < 10% quality threshold (QV) < 20 Phred score were removed, reads with adapters were discarded, a quality with a minimum overlap between the adapter and read was 5bp, reads with ambiguous base pair(N) and reads length below 15bp were trimmed off. After removing the adapter sequences, the quality filtering analysis was performed to trim the low-quality terminal bases using the Trimmomatic (v. 0.38) in a single end mode with a minimum read length of 15bp and base quality cut-off of 20. The quality box plot graph was generated to visualize the quality score of each base with respect to its base position. Quality filtering was performed using the fastq quality filter program, and a quality cut-off (q) of 20 and a minimum percentage (P) value of 100 was assigned for pre-processing. A quality-filtered reads were formatted into a non-redundant FASTA format for the unification of reads. Occurrences of each unique sequence reads were counted as sequence tags. The number of reads for each unique tag reflects the relative expression level of that miRNA. The bioinformatics workflow for Small RNA

sequencing and data analysis is shown in supplementary Fig. S1. The sequence data have been submitted to NCBI. The BioProject ID is....

Identification of known and novel miRNAs

The clean read tags from each library were blasted against the customized Rfam database (<http://www.sanger.ac.uk/software/Rfam>), and any small RNA read tag mapped to the Rfam database were filtered out and excluded from further analysis by considering the parameters, *i.e.* BlastN variant (blastn-short), word size (7bp), low complexity filtering (off), identity (100%), query coverage (100%), mismatch and gaps (not allowed). The reads tags that were not mapped to Rfam were further mapped to known repeat sequence database (Repbase (v 22)) using NCBI blast and were excluded from further analysis if not satisfying the stringent criteria as mentioned above. Read length filtration analysis was performed and reads with length >34nt excluded while <34 nt. (nucleotides) were considered clean putative miRNA reads. These putative miRNA reads were subsequently used for the identification of known and novel miRNA. The putative miRNA reads were mapped to the *Capsicum annuum* (Pepper Zunla v1.0) Genome (GCA_000710875.1) obtained from the NCBI Genome database. Precursor sequences were identified using the tool miRCat from UEA small RNA workbench (v3.2). The precursor stability was analyzed by estimating the MFE (minimum free energy), and only miRNAs observed on stable precursor sequences were reported (Tiwari *et al.*, 2020). To identify known miRNAs and assign miR family, all the unique sequences were BLASTED against the known plant miRNA database (<http://www.mirbase.org>, release v21). Based on the assigned miRbase ID, it was segregated as the known and novel miRNAs.

Differential analysis and target prediction

The sample wise tag counts were used for expression profiling of known and novel miRNA, and miRNAs with at least five reads in any of the four samples were considered for the differential analysis. TPM normalization algorithm was done, and the TPM values were then logged (base 2) transformed to get fold expression analysis. Finally, fold-change values were calculated by subtracting respective fold expression values. Fold change value of above zero was considered as up-regulated, and below zero was considered as down-regulated. For

predicting a potential target, conserved and non-conserved miRNAs of *C. annuum* were used query sequences for blast searches against our *C. annuum* transcriptome sequences (deposited in NCBI database under accession number RC: SAMN16251280; RI: SAMN16251798; SC: SAMN16251797; SI: SAMN16251799 on SRA at the link <https://www.ncbi.nlm.nih.gov/biosample/16251797> using the methods in Wan *et al.*, (2012).

Validation of microRNAs sequencing by qRT-PCR

We utilized the same RNA used for small RNA-seq for qPCR-based validation of selected miRNAs along with their corresponding target genes. The cDNA was synthesized using Mir-XTM miRNA First-Strand synthesis kits according to the manufacturer's protocol. For validation of differentially expressed genes, eight miRNAs representing four novel and four known miRNAs and their eight corresponding target genes associated with modulating the disease pathogenesis during *P. capsici* infection were selected. The list of miRNA specific forward primers is given in Supplementary Table S1. RTQ-UNIr was utilized as a universal reverse primer. To further measure and validate the corresponding target genes' expression levels, regional amplification quantitative RT-PCR (RA-PCR) assay was performed. The RA-PCR was developed to monitor the miRNA-directed cleavage of mRNAs (Oh *et al.*, 2008). MicroRNA mediated cleavage of the mRNA transcripts' target site leads to a decrease in RT-PCR accumulation of any fragment present upstream of the target site (Navarro *et al.*, 2006). The reverse transcription of the miRNA-cleaved mRNA will not generate a cDNA beyond the cleaved site. Therefore, the cDNA segment present upstream of the cleaved site can be expected to be less abundant than a segment present downstream of the cleaved site. RA-PCR has added benefits of including control reaction in the same experiment while other technique such as 5' rapid amplification of cDNA ends (RACE) 5' RACE requires separate reaction and could be skewed by the presence of false positives. For each of the eight target genes, three sets of primer (RA-5' region, RA-middle region and RA-3' region) were designed using Express 3.0.1 primer software (Fig 5; Supplementary Table S2). Actin-7 (XM_016695142.1) was used as an endogenous control gene for data normalization (Gupta *et al.*, 2017).

RT-qPCR reaction was performed in three technical replication from each of the three biological replication using SYBR Green JumpStart Taq ReadyMix 2x on Applied BiosystemsTM StepOneTM Real-Time PCR System. RT-qPCR reaction was set in a 20µl reaction volume containing 10µl SYBR Green JumpStart Taq ReadyMix (2x), 2µl forward primer (10 µM), 2µl RTQ-UNI reverse (10 µM), 4µl cDNA and 2µl of nuclease-free water

of molecular biology grade with the programme of 94 °C and denatured for 2 min, then primary reaction (denaturation at 94°C for 15 seconds, annealing at 55-60°C for 60 seconds, and template extension at 72°C for 15 seconds) for 40 cycles. The *actin-7-like* gene (Sequence ID: XM_016695142.1) and U6 snRNA (snRNA) (GenBank: Z17301.1) was used to normalize target and miRNA gene expression, respectively. The relative expression level was calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). The standard error in expression levels of the three biological replications in the figures was denoted by error bars, and the results were expressed as mean value $\pm$ SD (Hao *et al.*, 2016).

Results

Sequencing of small RNA

To identify chilli pepper miRNAs induced by *P. capsici* infection, we analyzed small RNA sequencing data from control and infected chilli pepper leaf tissues of resistant (GojamMecha_9086) and susceptible (Dabat_80045) chilli pepper landrace. Illumina sequencing generated a total of ~70 million high-quality clean reads representing 19, ~7, ~22 and ~20 million from RC, RI, SC, and SI libraries, respectively, after removing low-quality sequences and adapters (Table 1). After filtering the low-quality tags (removing non-coding small RNAs), trimming 3' adaptors and removing contaminants, ~8 million clean reads of 18–24 nucleotide lengths were obtained for downstream analysis of the miRNAs (Table 1). The sequencing data's reliability was ensured by average quality (Q30 score) of 93.45%, ranging from 93.11% to 93.87% across the libraries (Table 1). The miRNAs' length distribution showed the highest abundance of reads of 24 nucleotides followed by 21 nt., 22ntd and 23 nt. in all the four libraries with the maximum in RC and SC compared to RI and SI (Fig. S2).

Identification and expression analysis of known miRNAs during *C. annuum* – *P. capsici* interaction

The clean reads of small RNAs (18–24 nt.) obtained after filtration were mapped against *Capsicum annuum* (Pepper Zunla v1.0) genome (GCA_000710875.1), and known miRNAs were searched against the miRNA database (miRBase 21 released) (Zhang *et al.*, 2020). The small RNA sequence analysis obtained 79 known miRNAs corresponding to 24 miRNAs families in the four-leaf sample libraries of chilli pepper (Table 1). The highest number of known miRNAs (32) was identified in the infected leaf tissue of susceptible landrace

Dabat_80045, SI, whereas the lowest number (30) was identified in resistance control (RC) and resistance infected (RI) leaf (Table 1). Amongst all, thirty commonly expressed known miRNAs were identified in RI and SI leaf, whereas 29 known miRNAs were commonly detected in RC and SC samples (Fig. 4 A; Supplementary Table S3). The differential miRNA expression analysis of known miRNA revealed down-regulation of 4 and 5 miRNAs in RC vs RI and SC vs SI, respectively, whereas up-regulation of 25 miRNAs in RC vs RI and SC vs SI, respectively (Table 3). The most abundant known miRNA families in all the four libraries were Pz-known-miR356-1(mir482), Pz-known-miR1099-1(mir159), Pz-known-miR745-1(mir166), Pz-known-miR2908-1(mir396), and Pz-known-miR3049-1(mir482). Pz-known-miR3686-1(mi395) was abundantly detected only in SI, while Pz-known-miR1186-1(mir156) in only SC leaf. Among all the known miRNA families, Pz-known-miR2908-1(mir396) was most abundant in all the four samples with the highest accumulation in resistant control landrace GojamMecha_9086. Expression analysis of miRNA families further revealed the highest number of miRNA families mir159 (Pz-known-miR1099-1) in RC (36427) sample while lowest in RI (11157) sample. Compared to control conditions, different miRNA families' expression behaviour was greater during *P. capsici* infection. Additionally, the secondary structure of the four most abundant known miRNA families and novel miRNAs were given in Fig. 1A-D and 2A-D, respectively.

Identification, distribution and expression analysis of novel miRNAs during *C. annuum* – *P. capsici* interaction

The miRNA sequencing of RC, RI, SC, SI leaf samples predicted a total of 477 novel miRNA with the highest in SC (~275), followed by RC (~244), SI (~199) and RI (~174) (Table 1). The differential expression analysis of novel miRNA revealed down-regulation of 6 and 32 miRNAs in RC vs RI and SC vs SI, respectively, whereas up-regulation of 151 and 144 miRNAs in RC vs RI and SC vs SI, respectively (Table 3). The most abundant expressed novel miRNA in all the four libraries were Pz-novel-miR3731-1, Pz-novel-miR1981-1, Pz-novel-miR3509-1, Pz-novel-miR2406-1, Pz-novel-miR227-1, and Pz-novel-miR1344-1 (Supplementary Table S4). The Pz-novel-miR3731-1 was the highest abundant in number all four sample with (177934) in SC leaf sample while the lowest in RI (19510) sample (Supplementary Table S4). Most of the known and novel miRNA was found to be common in all the samples, while some of miRNAs were specifically expressed to specific leaf sample. The length of the consensus matured miRNA sequence of the novel miRNAs was in the range

of 19 to 24 nt., of which the majority were 24 nt. (Fig. S2; Table 2). The number of predicted novel miRNAs were more in the control leaf than the infected leaf in the two genotypes (Table 1). Chromosomal distribution analysis of the novel miRNAs showed the involvement of all the chromosomes with the maximum on chromosomes 1 (50) and lowest on chromosome 4 (31), 8(30) and 12 (30) (Fig 3). Additionally, the secondary structure of the novel miRNAs was also predicted, and a representative of the four most abundant novel miRNAs showing mature miRNAs located on either arm of the secondary hairpin structures are shown in Fig. 2A-D). Also, we observed quite a good negative free energy of hairpin structure ranging from -131.29 to -15.3 kcal/mol (Table 2), which is comparable with previous reports in hot pepper (-104.85 kcal/mol), *Arabidopsis thaliana* (-59.5 kcal/mole) and *Oryza sativa* (-71.0 kcal/mol) (Shin *et al.*, 2013). A total of 29 known and novel miRNAs (Supplementary Table S5) were identified as potential players regulating target genes associated with defence response against *P. capsici* infection. Out of that, 18 of these miRNAs were identified as novel miRNAs associated with defence response during *P. capsici* infection.

Comparative expression analysis of miRNAs in control and infected chilli pepper samples

The expression of miRNAs in control and infected leaf were compared for each of the two contrasting chilli pepper landraces. The expression of favoured miRNAs under the control and infected conditions were categorized along with the commonly expressed miRNAs (Fig. 4 and Supplementary Table S3). The result indicated fewer novel and known miRNAs in infected leaf tissue than control (Fig. 4). Further, these miRNAs were analyzed to identify the common miRNAs expressed under infected and control tissue of both the landraces. The result indicated that 87, 17, 98, and 22 novel miRNAs were explicitly expressed in RC, RI, SC, and SI, respectively (Fig.4). Among them, Pz-novel-miR2320-1 (~7.9 fold) and Pz-novel-miR2429-1 (~7.8 fold) were highly explicitly expressed in infected RI and SI library, respectively. Additionally, 177 novel miRNAs were commonly expressed between SC and SI, whereas 157 novel miRNAs were shown commonly expressed between RC and RI libraries. Moreover, 29 known miRNAs were commonly expressed between RC and RI libraries, while 30 known miRNAs were commonly expressed between SC and SI libraries. However, Pz-known-miR2226-1(mir398) (~5.5 fold) and Pz-known-miR504-1(mir1446) (~7.0 fold) were explicitly expressed in RC and RI leaf, respectively, while Pz-

known-miR1186-1 (mir156) (~5.2 fold) and two known miRNAs, i.e. (Pz-known-miR2226-1(mir398) (~8.0 fold) and Pz-known-miR3686-1(mir395) (~7.4 fold)) were expressed explicitly in SC and SI leaf, respectively (Fig 4).

Identification and differential regulation of target genes

The result shows that most of the differentially expressed miRNAs-regulating target genes associated with *P. capsici* infection in SC vs SI leaf samples were novel miRNAs (Supplementary Table S5). To know the corresponding target genes of each of the miRNA during *C. annuum* – *P. Capsici* interaction, extensive target prediction was performed that resulted into identification of a total of 22,895 potential target genes, which was shown to be regulated by 79 known miRNA (corresponding to 24 miRNAs families) and 477 novel miRNAs in all four-leaf samples (Supplementary Table S6). Among these, 82.70 % (18,935) of the targets genes were shown as negatively regulated by miRNA-directed cleavage mechanism, and miRNAs translationally repressed 17.29 % (3,960) target genes. Potential miRNAs targets were predicted from our *C. annuum* transcriptome sequences (deposited in NCBI database under accession number RC: SAMN16251280; RI: SAMN16251798; SC: SAMN16251797; SI: SAMN16251799 on SRA as mentioned above. Hence, target gene annotation analysis revealed that these target genes were involved in different biological functions, including metabolic, cellular, biological processes, molecular activities, development process, resistance to biotic and abiotic stress response and response in the stimulus. Among these target genes, 30 genes were associated with defence response against *P. capsici* infection, which was regulated by 34 miRNAs, i.e. thirteen known and 21 novel miRNAs (Supplementary Table S5). The expression of few essential defence related target genes such as EP3-like gene, PLBRP, PDRP, PGUI, peroxidase 42, PRTA (PTI6), AP2-like ERTF(TOE3) and defensin-like protein genes was regulated by Pz-novel-miR1028-1, Pz-novel-miR428-1, Pz-known-miR2059-1, Pz-known-miR803-1, Pz-known-miR1872-1, Pz-novel-miR160-1, Pz-known-miR2560-1, and Pz-novel-miR204-1, respectively. The pathway for chitin biosynthesis (prominent cell wall component of fungus) was observed to be negatively regulated by Pz-novel-miR1028-1 by cleaving the target NP_001311656.1endochitinase EP3-like precursor gene in the SI leaf sample (Fig. 6B). Compared to RI, the expression of NP_001311656.1endochitinase EP3-like precursor gene was down-regulated in SI, suggesting its vital role in modulating the biosynthesis of chitin (Fig. 6B).

Validation and expression profiling of selected miRNAs and their target genes

To further validate and check the expression pattern of identified miRNAs, four known (Pz-known-miR803-1, Pz-known-miR2059-1, Pz-known-miR2560-1 and Pz-known-miR2560-1) and four novel miRNAs (Pz-novel-miR428-1, Pz-novel-miR1028-1, Pz-novel-miR160-1, and Pz-novel-miR204-1) targeting eight corresponding key genes (*PGUI*, *PDRP*, *AP2-ERTF*, *POD*, *PLBRP*, *EP3*, *PRTA-PT16 like gene*, *DLP*) associated with defence response during *P. capsici* infection were selected. RT-qPCR and RA-PCR were employed for experimental validation of these miRNAs and their targets, respectively, in contrasting chilli pepper landraces. The amplified products were resolved on 2% agarose gel, and amplicons of respective miRNAs and their target mRNAs were detected. The RT-qPCR data was found consistent with the small RNA sequencing data in both resistant and susceptible landraces. The RT-qPCR analysis reveal that, the Pz-novel-miR428-1, Pz-known-miR803-1, Pz-novel-miR1028-1, Pz-novel-miR160-1, Pz-known-miR2059-1, Pz-known-miR2560-1, and Pz-novel-miR204-1 were differentially up-regulated while Pz-known-miR1872-1 was down-regulated in RC vs RI leaf (Fig 6 A-D and 7 A-D). However, all of the eight analyzed miRNAs were differentially regulated in SC vs SI leaf samples (Fig 6 A-D & 7 A-D). Compared to RC vs RI, the expression pattern indicated a varying accumulation of all the studied miRNAs with the highest differential regulation in SC vs SI (Fig. 6 and 7). Amongst all miRNA-target pair, the Pz-known-miR1872-PODs pair showed perfect inverse relation in all four samples (Fig 7D). Fig. 5 depicts the relative positions of the three primer sets of each target genes for RA-PCR. The 5', mid, and 3' regions of each target gene signify upstream, target site and downstream site from the potential cleavage site, respectively. Surprisingly, all the miRNAs' expression at least in one of the four samples was negatively correlated with their target transcripts for the RA-Mid region (Fig. 6 & 7). Further, the RA-Mid region's accumulation pattern of nearly all the transcripts was found to be less than the RA 3' region (Fig. 6 & 7), suggesting that these target genes are cleaved by corresponding miRNAs.

Discussion

Owing to chilli pepper's significant economic importance (*C. annuum* L.), deciphering the crucial role of miRNAs in modulating disease response is essentially

required. As discussed in the introduction section, that despite numerous reports on miRNAs in chilli pepper, the molecular mechanism underlying *C. annuum*-*P. capsici* pathosystem is still a virgin field. Therefore, we performed a three-step experiment, i.e. (1) genome-wide identification of known and novel miRNAs in response to *P. capsici* infection in resistant and susceptible chilli pepper landrace; (2) identification of their corresponding target genes; and (3) validation and expression profiling of vital miRNA-target pairs associated with defence response in two contrasting chilli pepper landraces (resistant: GojamMecha_9086; susceptible: Dabat_80045).

The advancement of NGS technology coupled with bioinformatics analysis has revolutionized the identification of several families of defence-related known and novel miRNAs in plants exposed to various fungal pathogen. Based on the miRNA sequence analysis, 79 known conserved miRNAs corresponding to 24 miRNA families and 477 novel miRNAs were identified in the four chilli pepper libraries upon infection with *P. capsici* (Table 3). Although there is no report on miRNAs in chilli pepper during *P. capsici* infection, however, the current small RNA sequencing analysis obtained a large number of novel miRNAs (477 novel miRNAs) in four libraries as compared to the 35 novel miRNAs identified in ten libraries of *C. annuum*, which are involved in disease resistance-related genes such as verticillium wilt disease resistance proteins, late blight resistance proteins and NBS-LRR type disease resistance proteins (Shin *et al.*, 2013). Tao and co-workers (Tao Li *et al.*, 2015) were identified 71 miRNA families in two genotypes of *C. annuum* (i.e. tolerant hot pepper cultivar ‘R597’ (CaR) and the sensitive cultivar ‘S590’ (CaS) under high temperature and high air humidity stress. Din and co-workers (Din *et al.*, 2016) identified and characterized a total of 88 new miRNAs (from 81 miRNA families) in chilli using expressed sequence tags (ESTs). Furthermore, a total of 59 known miRNAs and 310 novel miRNAs were identified from four libraries involved in fruit development and quality in *C. annuum* (Zou *et al.*, 2017). Also, Robles and co-workers (Robles *et al.*, 2019) identified 104 conserved miRNAs belonging to 37 families and 61 novel in miRNAs in tomato under five biotic and abiotic stress conditions (drought, heat, *P. syringae* infection, *B. cinerea* infection, and herbivore insect attack with *Leptinotarsa decemlineata* larvae). Comparatively, the known conserved miRNAs were highly expressed than the novel miRNAs in the treated sample of resistant and susceptible chilli pepper landrace. As identified, conserved miRNAs vary significantly among families (Cao *et al.*, 2014); we also observed the same trends of

variation of many miRNAs (12) for the can-miR169 family followed by can-miR395 and can-miR172 families. In line with previous reports of Cao *et al.* (2014) and Zhang *et al.* (2018), we also observed more significant variation in the abundance of clean read length distribution of mature miRNAs ranging from 15-29 nt. across all the libraries with 24 nt. being the most abundant (Fig. S2). According to Han *et al.* (2013), most small RNAs were found to be in the range of 20-24nt size, with the 24 nt. class being the most represented of the non-redundant species (75.83 %), followed by the 23 nt. (7.31 %) in *Caragana intermedia* by high-throughput sequencing under salt stress.

As shown in Table 2, compared to known miRNAs, we observed differential expression of a more significant number of novel miRNAs in SC vs SI with nearly 32 novel miRNAs being down-regulated; supporting the report of Shin and co-workers (Shin *et al.*, 2013), reported that some of the novel miRNAs were expressed at a high level in hot Pepper (*C. annuum*). However, Zou and co-workers (Zou *et al.*, 2017) had reported a low expression level of novel miRNAs associated with fruit development and quality in *C. annuum*. This pattern of down-regulation of a large number of novel miRNAs in susceptible, infected chilli pepper landrace might signify miRNA depended on response in modulating disease susceptibility in comparison to resistant landrace by negatively regulating several target genes possibly associated with *P. capsici* infection and intensifying the vulnerability of the treated susceptible genotype to the pathogen infection. Further, differential expression of a nearly equal number of known miRNAs in both the landrace could have a nearly equal contribution of miRNA dependent disease response modulation. Further work on characterizing these novel miRNAs' role using a reverse genetic approach shall impart more understanding of both the landrace's resistance and susceptibility mechanism.

Enrichment analysis of identified known and novel miRNAs (~34) showed their possible involvement in regulating target genes (~30) associated with resistance against *P. capsicum* infection. Compared to SI, conserved miRNA families such as miR482, miR159, and miR166 were the most abundantly expressed and exhibited a high read count 156540.84, 211794.66, and 41876.76, respectively, in RI. This result agrees with previous reports in *C. annuum* and *S. lycopersicum*, where these conserved miRNAs were relatively less expressed than our results (Shin *et al.*, 2013; Robles *et al.*, 2019). This shows that these conserved miRNAs have a more specific role in *C. annuum* in response to *P. capsici* infection. Despite no previous report documented on the role of miR166 in *C. annuum*, there was reported the

role of miR166 in fungal pathogen infection in other plants. For instance, activated *miR166k-166h* expression in rice plant showed enhanced infection resistance by the fungal pathogens *M. oryzae* and *F. fujikuroi*, in which the *ethylene-insensitive 2 (EIN2)* gene was identified as a novel target gene for miR166k (Segundo *et al.*, 2018). Satendra and co-worker (Satendra *et al.*, 2020) identified an induced expression of miR166 under *R. solani* in susceptible and resistant rice cultivars, suggesting these may be basal response regulators against the *R. solani* pathogen. Furthermore, an increase in the accumulation of miR166 and miR159 in cotton plant in response to fungal pathogen *V. dahliae* infection reported (Jin and Guo, 2018). Nevertheless, *Pz-novel-miR1981-1* was the most abundantly expressed with TPM value of 21,280.07 and 15,007.33 in RI and SI samples, respectively, signifying their pathogen and genotypes dependent response to modulate the disease tolerance, which needs further functional investigation to ascertain the regulatory role during *P. capsici* infection.

Target genes identification revealed that most of the identified miRNAs were shown to regulate more target genes associated with resistance against *P. capsici* infection. The Pz-novel-miR3509-1 was observed to have 186 target genes, of which, six target genes (putative disease resistance protein At1g50180 isoform X2, late blight resistance protein R1-A-like, putative late blight resistance protein homolog R1B-14, disease resistance RPP13-like protein 4, ethylene-responsive transcription factor ERF071-like, and putative late blight resistance protein homolog R1A-10) were associated with defence response against *P. capsici* infection. Similarly, Pz-known-miR2059-1(mir172) regulated 141 target genes, including two (putative disease resistance protein (PDRP) RGA1 and leucine-rich repeat receptor-like tyrosine-protein kinase PXC3) genes associated with disease modulation. The RT- qPCR analysis revealed, high expression and differential up-regulation of conserved miRNAs, i.e. Pz-known-miR2059-1(can-mir172), Pz-known-miR2560-1(can-miR172), and Pz-known-miR1872-1(can-miR397) were exhibited inversely regulating their corresponding target putative disease resistance protein (PDRP) RGA1 gene, AP2-like ethylene-responsive transcription factor (AP2-ERTF) TOE3 gene, and peroxidase 42 genes (PODs) expression associated with *P. capsici* infection in SI landrace (Fig. 6). However, comparatively the expression of Pz-known-miR1872-1(can-miR397), Pz-known-miR2059-1(can-mir172), Pz-known-miR2560-1(can-miR172), and Pz-known-miR803-1(can-miR399) were shown to be low in RI landrace, indicating higher expression of their corresponding target genes i.e. peroxidase 42 genes (PODs), putative disease resistance protein (PDRP) RGA1 gene, AP2-like ethylene-responsive transcription factor (AP2-ERTF) TOE3 gene, and

polygalacturonase inhibitor like (PGUI) gene, respectively, which might be one of the reasons of increased resistance to *P. capsici* infection in GojamMecha_9086. Wang *et al.* (2018), up-regulation of several conserved miRNAs including miR159, miR168, miR169, miR172, miR393, miR398, and miR396 in *S. lycopersicum* and *S. habrochaites* during *B. tabaci* infestations suggesting that conserved miRNAs play essential roles in defence. Our results indicated that leaf rust disease-resistance locus receptor-like protein kinase, one of the targets of can-miRNA398, was negatively regulated in *C. annuum* under the *P. capsici* treatment with more significant accumulation in RI compared to SI, signifying its vital role in modulating disease resistance more significantly in resistant chilli pepper. The expression difference in can-miRNA398 - leaf rust disease-resistance locus receptor-like protein kinase target pair might lead to variation in disease resistance response between resistant and susceptible genotype against *P. capsici* infection. The miRNA398 was negatively regulated pathogen-associated molecular patterns (PAMPs) induced callose deposition and disease resistance to bacteria, and the overexpression of miR398b in plants showed enhanced susceptibility to both virulent and non-pathogenic strains of *P. syringae*, indicating its essential role in disease resistance (Zhou *et al.*, 2010).

Among the total predicted target genes, 82.70% (18935) of the targets genes were negatively regulated by miRNA-directed cleavage mechanism, while miRNAs transitionally repressed 17.29%(3960) target genes. The current result shows an inverse correlation between the expression profiles miRNAs and their target genes which is in agreement with the previous report in *S. lycopersicum* during biotic and abiotic stress (Robles *et al.*, 2019). In the current study, from 22,895 identified target genes, 30 targets genes were directly associated with resistance mechanism against *P. capsici* infection, which was regulated by eleven mature known and 21 novel miRNAs. Robles *et al.* (2019) and Zhang *et al.* (2018) reported that the expression of many single target genes was observed to be regulated by different miRNAs of different families during *C. capsici* infection in chilli pepper. For instance, the putative late blight resistance protein homolog R1B-17 gene was regulated by three miRNAs, i.e. Pz-known-miR1099-1(can-miR159), Pz-known-miR1382-1 (can-miR319), Pz-known miR2610-1 (can-miR319) and the polygalacturonase inhibitor-like genes were regulated by four miRNAs, i.e. Pz-known-miR803-1 (can-miR399), Pz-novel-miR1405-1, Pz-novel-miR3245-1, and Pz-novel-miR3502-1. The target squamosa promoter-binding-like protein 6(SBP) is regulated by five miRNAs, i.e. Pz-known-miR1186-1, Pz-

known-miR3461-1, Pz-novel-miR227-1, Pz-novel-miR1107-1, and Pz-novel-miR2181-1. Gong and co-worker have reported the involvement of target SBP the defense response of pepper to *Phytophthora capsici* by regulating defense-related genes(Gong et al., 2020^b). Moreover, our result shows, the Pz-known-miR2059-1(can-miR172) regulated expression of target AP2-like ethylene-responsive transcription factor TOE3 isoform X1 gene, which activates signalling pathway during *P. capsici* infection, and it was in agreement with the report on the role of miR172 in *S. lycopersicum* and *S. habrochaites* under *Bemisia tabaci* infestations (Wang *et al.*, 2018). The RT-qPCR result shows the accumulation of Pz-known-miR1872-1(can-miR397) inversely targeting its corresponding target peroxidase 42 genes in SI than in RI landrace that possibly creates differential disease response between the resistance and susceptible genotype of chilli pepper. The peroxidase enzyme participated in lignin and suberin formation, crosslinking of cell walls components, and synthesis of phytoalexins, leading to the hypersensitive response (HR), a form of programmed host cell death at the infection site associated with limited pathogen development (Almagro *et al.*, 2009). The PR-9 family of peroxidase enzyme have involved in cell wall reinforcement by catalyzing lignification, leading to enhanced resistance against multiple pathogens (Passardi *et al.*, 2004).

Pz-novel-miR1028-1 and Pz-novel-miR177-1 regulate expression of gene encoding endochitinase protein involved in catalyzing the hydrolytic cleavage of the β -1,4 glycoside bond present in biopolymers of N-Acetyl-D-glucosamine, mainly in chitin the wall components of fungus (Ferreira *et al.*, 2007). The CACHi2 chitinase is encoded by a single or two copy genes in the pepper genome (Kim *et al.*, 2000). There was a report on the class IV chitinase, CaChitIV, from pepper plants (*C. annuum*), which interacted with the pepper receptor-like cytoplasmic protein kinase(CaPIK1) and promotes CaPIK1-triggered cell death and defence responses (Kim *et al.*, 2015). NAC transcription factor expression 29-like targeted by Pz-novel-miR34-directed cleavage in chilli pepper has an analogous function with ath-miR164, and the decreased expression leads to increased expression of the NAC transcription factor in *A. thaliana*. The target NAC transcription factors are involved in regulating tissue development in response to biotic and abiotic stress, which was a positive regulator of cell death (Woo *et al.*, 2009; Chuck and O'Connor, 2010). The expression of Pz-known-miR1186-1(~8.0 fold) explicitly in SC leaf negatively was regulated the expression of target squamosa promoter-binding-like protein 6 (SBP) gene. However, expression of this

target SBP in SI leaf enhanced susceptibility to *P.capsici* infection. *CaSBP11* has a negative regulatory effect on defense responses to *Phytophthora capsici* suggests their potentially significant role in plant defense (Gong *et al.*, 2018; Gong *et al.*, 2020^b). Silencing of *CaSBP08* and *CaSBP11* enhanced resistance to *P. capsici* infection in *Nicotiana benthamiana* and pepper, respectively (Gong *et al.*, 2020^a; Gong *et al.*, 2020^b).

Moreover, among eight miRNAs validated, five miRNAs, i.e., Pz-novel-miR428-1, Pz-Novel-miR1028-1, Pz-known-miR1872-1, Pz-known-miR2059 and Pz-known-miR2560-1, negatively regulate the expression of their corresponding target PLBRP, EP3, PODs, PDRP gene and AP2-ERTF, respectively, in SI under *P. capsici* infection. However, the Pz-novel miRNA160-1, Pz-known miR803-1, and Pz-novel-miR204-1 were positively regulated with their corresponding target PRTA PTI6-like gene PGUI, and defensin-like protein gene, respectively, in SI leaf. Similarly, the expression of eight randomly selected miRNAs except, Pz-novel-miR160-1 positively regulate the expression of their corresponding target gene during *P. capsici* infection in the RI leaf sample. Our observation of differential expression of miRNA-target genes in both RI and SI coincides with several reports suggesting an inverse correlation between the miRNA and their corresponding target genes (Robles *et al.*, 2019; Gupta *et al.*, 2017).

In summary, the current small RNA sequencing of chilli pepper exposed to *P. capsici* infection in two contrasting, i.e. resistant and susceptible, chilli pepper landraces identified various conserved and novel miRNAs involved in regulating potential target genes associated with defence response against *P. capsici*. The differential expression behaviour of several conserved and novel miRNAs and their target genes in both RI and SI leaf samples of resistant and susceptible chilli pepper landrace suggests their paramount role in resistance mechanism under *C. annuum* L. - *P. capsici* pathosystem. The variation in the expression level of defence associated miRNAs and their corresponding target genes might be one reason for differential disease response between resistant and susceptible landraces under *C. annuum* L. infection. These results need further functional validation using reverse genetic and/or Crispr/cas9 approaches by selecting few defence associated miRNAs and their corresponding target genes to broaden our current understanding of miRNA based regulation of disease resistance during *C. annuum* L.-*P. capsici* pathosystem.

Declarations

This research did not involve the use of any animal or human data or tissue.

Author's contributions

Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing original draft, Software, Validation, T.R, Om.P.G and V.C., Project administration, Resource, V.C, Writing, Revising and Editing, Supervision, Om.P.G, and V.C.

Declaration of competing interest

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Fig. legends

Fig. 1. Secondary structure of known miRNA identified from small RNA sequencing of *C. annuum* during infection with *P. capsici*: A) Pz-known-miR803-1 (can-mir399), B) Pz-known-miR1872-1 (can-mir397), C) Pz-known-miR2059-1 (can-mir172), D) Pz-known-miR2560-1 (can-mir172) used in the validation of RT-qPCR (the green colour shows the matured miRNA sequence site).

Fig. 2. Secondary structure of novel miRNAs identified from transcriptome sequence of *C. annuum* during infection of *P. capsici* and the matured miRNAs sequence marked with green colour: A) Pz-novel-miR160-1, B) Pz-novel-miR204-1, C) Pz-novel-miR428-1, and D) Pz-novel-miR1028-1

Fig. 3. Novel miRNA identified in *C. annuum* A) Distribution of novel miRNAs in control (dark green colour bars) and in infected (red colour bars) samples of *C. annuum*; B) Distribution of novel miRNAs in different chromosomes of the *C. annuum* genome.

Fig. 4. Venn diagram showing common and exclusively expressed miRNAs in resistance and susceptible chilli pepper. The red colour circle represents the control samples, whereas the blue colour circle represents the *P. capsici* infected leaf samples. 1) known miRNAs in A) Resistance Control Vs. Resistance Infected and B) Susceptible inoculated; 2) Novel miRNAs in A) Resistance Control Vs. Resistance Infected and B) Susceptible Control Vs. Susceptible Infected.

Fig. 5. The diagram of regional amplification of qRT-PCR of target genes: A) The three site of primers used for regional amplification of target gene cleaved by miRNAs (5' side both pair of forward and reverse primers, middle side both forward and reverse primers, middle side both forward and reverse primers, 3' side both pair of forward and reverse primers); B) The miRNA cleavage site (▲) of eight target genes associated to the infection of *P. capsici*, the start and stop codon was illustrated by (▶ and ◀) respectively.

Fig. 6. The RT-qPCR result analysis of the randomly selected DEGs of miRNAs regulating its corresponding identified target gene associated with defence response against *P. capsici* infection: A) Pz-novel-miR428-1 with its target putative late blight resistance protein homolog (PLBRP) RIP-13 gene, B) Pz-novel-miR1028-1 gene with its target endochitinase (EP) EP3-like precursor gene, C) Pz-novel-miR160-1 regulating its target PRTA-PTI6-like gene, and D) Pz-novel miR204-1 gene-regulating its target defensin-like protein(DLP) gene.

Fig. 7. The RT-qPCR result analysis of the randomly selected DEGs of miRNAs regulating its corresponding identified target gene associated with defence response against *P. capsici* infection: A) Pz-known-miR2560-1(Can-miR172) gene-regulating its target AP2-like ethylene-responsive transcription factor (AP2-ERTF) TOE3 gene, B) Pz-known-miR2059-1 gene-regulating its target putative disease resistance protein (PDRP) RGA1 gene, C) Pz-known-miR803-1 with its target polygalacturonase inhibitor like (PGUI) gene, and D) Pz-known-miR1872-1(Can-miR397) with its target peroxidase 42 genes (PODs).

Supplementary Fig.

Fig. S1. The bioinformatics workflow for small RNA sequencing and data analysis

Fig. S2. Length distribution of final cleaned reads (15-29nt.) for the four-leaf sample of chilli pepper

Table legends

Table 1. Summary of small RNA sequencing and analysis in control and *P. capsici* infected libraries of *C. annuum* L.

Table 2. Some identified novel miRNAs and their abundance with minimum free energy across the four-leaf library of *C. annuum* under *P. capsici* infection.

Table 3. The number of differentially expressed miRNA in control and infected leaf sample library combination of RC vs. RI and SC vs. SI.

Supplementary Tables

Table S1. The designed primers for randomly selected differentially expressed miRNA genes for validation of miRNA Seq using RT-qPCR

Table S2. The designed primers for eight miRNAs target genes and exogenous actin-7-like primer randomly selected for validation using RT-qPCR

Table S3. List of commonly expressed known and novel miRNA along with their fold change value in *C. annuum* L. under *P. capsici* infection.

Table S4. List of all the novel miRNA and their fold change value in RC vs RI and SC vs SI sample of *C. annuum* L. under *P. capsici* infection.

Table S5. The differentially expressed genes of known and novel miRNA with their corresponding target genes associated with *P. capsici* infection in resistance and susceptible *C. annuum* L (in sample combination of resistance control and resistance inoculated and susceptible control and susceptible infected).

Table S6. List of the target genes along with their potential corresponding miRNAs

Table 1. Summary of small RNA sequencing and analysis in control and *P. capsici* infected libraries of *C. annuum* L.

Sample name	Total Number of Reads	Clean Reads before Data Filtering	High quality reads	Q30 (%)	Clean Reads (18–24 nt)	% Alignment	Identified Known miRNAs	Identified Novel miRNAs
RC	33,114,392	32,621,939	19,905,381	93.11	2,837,843	66.7	30	244
RI	8,126,377	8,030,871	7,074,044	93.44	1,265,530	67.8	30	174
SC	26,454,028	26,144,603	22,662,602	93.47	2,624,705	68.4	31	275
SI	25,076,472	24,760,107	20,371,470	93.78	1,591,445	65.8	32	199

Table 2. Some identified novel miRNAs and its abundance with minimum free energy across the four-leaf library of *C. annuum* under *P. capsici* infection.

Name	Sequence(5' → 3')	RC	RI	SC	SI	% GC	C.annu. Chrs	Chromosome start	Chromosome end	Strand	Minimum Free Energy(kcal/mol)
Pz-novel-miR1028-1	TAAATAGTCGGTGATAGTTCA	49	20	11	47	31.74	12	11868979	11868999	-	-29.9
Pz-novel-miR428-1	ACAGTAGAAATCTGAGATCT	14	9	27	17	31.33	9	234968668	234968688	+	-75.1
Pz-novel-miR1304-1	AGATTAGTCTTTGGGTTGGGC	0	0	44	6	45.98	1	270341727	270341813	-	-31.6
Pz-novel-miR1405-1	TGCCAAAGGAGAGTTGCCCT	0	0	0	12	42.65	10	17578994	17579013	+	-35.07
Pz-novel-miR177-1	GAGAGACTGTTTCAATAGACCCCT	10	0	11	7	42.26	9	151636093	151636116	+	-26.2
Pz-novel-miR1443-1	TTTCGGTCCACTGGTGAGCTT	44	14	28	21	44.16	6	207649764	207649784	-	-20.6
Pz-novel-miR160-1	TCAAGTCCCGGCAACGGAAC	0	0	10	6	36.05	1	169007162	169007181	-	-24.09
Pz-novel-miR2808-1	ATTGAGTTCAGGAATAATAGGACC	10	25	0	6	30.25	9	7219559	7219582	+	-68.64
Pz-novel-miR3720-1	ATTCGTCTCTTAGGTAGTTAAGTT	34	0	8	7	27.08	4	199525677	199525700	-	-47.36
Pz-novel-miR2082-1	TATCGTGTCAAGCGGGACT	11	7	9	33	64.04	10	4007779	4007797	-	-49.3
Pz-novel-miR204-1	CTTTCTTGAGTAGACATGCCT	6	0	12	5	35.39	6	157425199	157425220	+	-17.3
Pz-novel-miR3247-1	TAATGTTAGGAATAAAGTCAGC	42	0	16	17	36.37	11	95787727	95787748	+	-46.9
Pz-novel-miR2077-1	TTTTGGCATTGATTGTTTGGC	15	5	12	12	39.14	5	177247046	177247067	-	-19
Pz-novel-miR2682-1	TTGAGATGAAACGTGTAGAACT	21	13	43	11	35.36	12	45482703	45482724	-	-52.2
Pz-novel-miR1059-1	AAGTAGGAAACTCAAACCCGAT	5	0	11	5	28.02	1	288547809	288547832	-	-61.96
Pz-novel-miR1088-1	AAGTAGCAACTTGTGTTGAACAAGT	5	0	11	0	35.71	11	152704710	152704733	+	-38.4
Pz-novel-miR1112-1	TGTGTGAAATTTCTGAATGCCT	686	91	522	195	36.11	3	19907297	19907318	+	-19.5
Pz-novel-miR3731-1	TCCGCTGTAGCACTGGC	173306	19510	177934	33049	47.67	1	284452126	284452142	+	-28.5
Pz-novel-miR1163-1	AAGAGACTGTGATGAATTTTCGTC	19	24	18	12	37.36	2	144189274	144189297	+	-75.8
Pz-novel-miR1167-1	AAGTTTGAAATTCTGAATCCGCCT	219	59	97	80	36.61	12	219141945	219141968	-	-59.57
Pz-novel-miR1171-1	AGTTGTTATCATGTGATCAGGAGG	9	0	13	0	37.03	10	10831799	10831822	+	-42.7
Pz-novel-miR1250-1	ATGTAGAATTTAAAGAAGAGTAT	6	0	26	0	32.63	10	128092855	128092877	-	-29.8
Pz-novel-miR1257-1	ACAAGTTTAAATTTCTGAATCCGT	87	36	41	41	40.82	11	205287434	205287457	+	-70.21
Pz-novel-miR1290-1	AGCTGACGTGAAACCTTTGACGGG	15	12	35	7	40	11	10245314	10245337	-	-22.1
Pz-novel-miR1293-1	AGGCTGTTTTCAAGGCTTGAACCT	10	7	5	0	43.75	1	282994374	282994397	+	-17.8
Pz-novel-miR1344-1	AAACTCGAGAAGCTGATTAACGA	259	88	174	139	45.25	6	8095423	8095446	+	-43.3
Pz-novel-miR1425-1	GAGACACATCTGAAGTTAGGAGG	29	19	16	9	43.87	10	1189211	1189234	-	-67.92
Pz-novel-miR1443-1	TTTCGGTCCACTGGTGAGCTT	44	14	28	21	44.15	6	207649764	207649784	-	-20.6
Pz-novel-miR1457-1	TGCACAGCCTCTCCCTGGCT	19	0	13	0	50.58	8	134522186	134522206	-	-43
Pz-novel-miR1578-1	TCGATGGATAGAAATTGAGCT	10	5	29	6	42.96	10	2637472	2637492	+	-71.8
Pz-novel-miR1612-1	AACTGTTGAAGTGAGGTACTCGAG	0	5	10	0	41.67	1	5186596	5186619	-	-15.3
Pz-novel-miR1728-1	ATCTTAAACTATACGCGAAAGGC	17	0	0	0	37.62	5	59051153	59051176	-	-111.5
Pz-novel-miR175-1	GATCTGTAAGAAGCTTGAGCT	12	0	13	0	41.77	6	98725044	98725064	+	-53.1
Pz-novel-miR2669-1	ACCATTTTAGATAGTTGAAGGAC	18	9	0	26	37.78	11	172818461	172818486	+	-101.94
Pz-novel-miR431-1	CCCAGTGAAGTTGGAGTGT	91	10	134	20	40.74	10	754664	754682	+	-131.29

Name	Mature miRNA sequence (5' to 3')	Primer (5' to 3') to be synthesized
RTQ-UNIr	-	CGAATTCTAGAGCTCGAGGCAGG
U6snRNA	-	AACAGTCTGACTTGCCCTTC
Pz-novel-miR428-1	ACAGUAGAAAUCUGAGAUCU	ACAGTAGAAATTCTGAGATCT
Pz-known-miR803-1	UGCCAAAGGAGAGUUGCCUG	TGCCAAAGGAGAGTTGCCCTG
Pz-novel-miR1028-1	UAAAUAGUCGGUGAUAGUUA	TAAATAGTCGGTGATAGTTCA
Pz-known-miR1872-1	AUUGAGUGCAGCGUUGAUGAC	ATTGAGTGCAGCGTTGATGAC
Pz-novel-miR160-1	UCAAGUCCCGCAACGGAAC	TCAAGTCCCGCAACGGAAC
Pz-known-miR2560-1	GAAUCUUGAUGAUGCUGCAU	GAATCTTGATGATGCTGCAT
Pz-novel-miR204-1	CUUUUCUUGAGUAGACAUGCCU	CTTTTCTTGAGTAGACATGCCT
Pz-known-miR2059-1	AGAAUCUUGAUGAUGCUGCA	AGAATCTTGATGATGCTGCA

Table 3. The number of differentially expressed miRNA in control and infected leaf sample library combination of RC vs. RI and SC vs. SI.

Differential combination (Control_vs_ treated)	Type of miRNA	Number of down- regulated miRNA	Number of up- regulated miRNA
RC_vs_RI	Known	4	25
	Novel	6	151
SC_vs_SI	Known	5	25
	Novel	32	144

Table S1. The designed primers for randomly selected differentially expressed miRNA genes for validation of miRNA Seq using RT-qPCR

Table S2. The designed primers for eight miRNAs target genes and exogenous actin-7-like primer randomly selected for validation using RT-qPCR

Sr. No .	miRNA	miR binding site on target (bp)	Target	Target total length (bp)	Primer Name	Primer region (bp)	F Primer (5' to 3')	R Primer (5' to 3')	Amplicon size (bp)	Tm (°C) (Primer Blast)
1.	>Pz-novel-miR428-1	1317	>XM_016720598.1	3193	RA-5'	1156-1295	CATCGAACCCCTTCCCTGCTT	AGAGGTTGCCAATCCCATGT	140	59
					RA-Mid	1278-1424	ATGGGATTGGCAACCTCTGT	TCCCACTTGTTGTTGCATTGG	147	59
					RA-3'	1606-1755	ACCAAAGTTGATGCCACAAGC	CCTTTCGTGTTTGTCGTGCC	150	59
2.	>Pz-known-miR803-1	982	>XM_016706793.1	1251	RA-5'	628-775	AGCCTTACTGGACAAGTGCC	CCAATGCGTTCTCGACAGA	148	59
					RA-Mid	936-1047	AACGTTGCAGACCTTCGACA	CAGCACTCTGAAACCGTGGA	112	60
					RA-3'	1028-1158	TCCACGGTTTCAGAGTGCTG	GTTTCTCTGCTCAACTGGACC	131	59
3.	>Pz-novel-miR1028-1	689	>NM_001324727.1	955	RA-5'	578-682	ACTTCAACTACGGACCTGCTG	ACCACAATGCTGTCTTGAAGGA	105	60
					RA-Mid	662-802	CCTTCAAGACAGCATTGTGGT	CCTTCTAGCAACAGTTTGAGGA	141	59
					RA-3'	772-916	GGTGGTAATCCTCAAACCTGTTGC	AGTCTGTGAGCGCACTTAGTGT	145	59
4.	>Pz-known-miR1872-1	1050	>XM_016704636.1	1227	RA-5'	889-1032	ACCACCAACTAGCAACTGACA	TGATCTCACCTTTGTCCCAG	144	59
					RA-Mid	1000-1099	ACAACCCTCTTACTGGGACA	ACGAGTACAAGCACACAGCA	100	58
					RA-3'	1127-1229	TATGTGAGGGAGGTAGCGGA	GTCACCACCACTTTCATGCC	103	59
5.	>Pz-novel-miR160-1	1009	>XM_016722112.1	1596	RA-5'	622-771	GGCCGGAATACTGTACGGAG	CCGTTGTCGAACACCTCTGA	150	59
					RA-Mid	998-1139	ACGCTGTTACGTTGTCACT	TTCTCCGGCATCCCAAACCTC	142	59
					RA-3'	1102-1201	TCATTCTTTCGGTTGCCGGA	ACTACCTAGCCTCAAGGGCA	100	59
6.	>Pz-known-miR2560-1	1919	>XM_016705550.1	2340	RA-5'	1606-1739	ACCTTGCAACTATTGAGCCAGA	TGGGATTGATCACCAGGTCTC	134	59
					RA-Mid	1882-2028	TGGAAATCCAATTTTCTCCTCA	AGTGAATAGTATGGTCAAGATGGT	147	58
					RA-3'	2126-2275	AGGCTAGCTCTTGGTTGGAA	GCCTCCAGTTAGTATTGGAGTACA	150	59
7.	>Pz-novel-miR204-1	210	>XM_016697277.1	602	RA-5'	80-153	TCAAAGAGATTATGGCTCTTTCCA	TGAATTTTGGCTTTCTGTTTTGC	120	58
					RA-Mid	130-283	TGGGGTGGAAGCTCAACAAA	TGGCTTAGTGCATAGACACCT	154	58
					RA-3'	443-527	AGTTTATAATGTTGTGTCTTGGCT	TCCAAAAAGGATTTTGATTGTGAC	85	58
8.	>Pz-known-miR2059-1	2285	>XM_016718449.1	2484	RA-5'	1094-1242	TGGCTGTGAAAACCTTGGA	TGATGGCATCTGTTTCATAGCTCA	149	59
					RA-Mid	2178-2301	TGATTTGATGGACGGAGAGGG	CTTTGATGTTGCAGGAGCGG	124	59
					RA-3'	2331-2478	GGTAGAGCTTCCAGAGTGCC	TCCTCCACTTAGCAATGGACAG	148	59
Internal control genes										

Sr. No	Gene	F Primer (5' to 3')	R Primer (5' to 3')	Amplicon size (bp)	Tm (°C)
3.	actin-7-like	TCCTCGCATCACTTAGCACC	GCCCATCTCAAAGACTTGCC	138	58

Table S3. List of commonly expressed known and novel miRNA along with their fold change value in *C. annuum* L. under *P. capsici* infection.

Sr. No.	miRNA-Acc	miR_Family	Mature Sequence	Fold Change	Remark
Known miRNAs					
RC vs RI					
1	Pz-known-miR1872-1	mir397	ATTGAGTGCAGCGTTGATGAC	-0.4	Down Regulated
2	Pz-known-miR2300-1	mir482	TTTCCAATTCCACCCATTCTTA	-0.7	Down Regulated
3	Pz-known-miR3230-1	mir172	AGAATCTTGATGATGCTGCAT	-0.1	Down Regulated
4	Pz-known-miR825-1	mir408	TGCACTGCCTCTTCCCTGGCT	-0.5	Down Regulated
5	Pz-known-miR1099-1	mir159	TTTGGATTGAAGGGAGCTCTA	0.8	Up Regulated
6	Pz-known-miR1382-1	mir319	TTGGACTGAAGGGAGCTCCC	0.9	Up Regulated
7	Pz-known-miR1393-1	mir171	TGATTGAGCCGTGCCAATATC	0.9	Up Regulated
8	Pz-known-miR1398-1	mir169	TAGCCAAGGATGACTTGCCT	1.6	Up Regulated
9	Pz-known-miR1703-1	mir162	TCGATAAACCTCTGCATCCGG	2.0	Up Regulated
10	Pz-known-miR2059-1	mir172	AGAATCTTGATGATGCTGCA	0.9	Up Regulated
11	Pz-known-miR2547-1	mir4376	ACGCAGGAGAGATGATGCTGGA	1.1	Up Regulated
12	Pz-known-miR2560-1	mir172	GAATCTTGATGATGCTGCAT	0.6	Up Regulated
13	Pz-known-miR2610-1	mir319	TTGGACTGAAGGGAGCTCCT	1.1	Up Regulated
14	Pz-known-miR2633-1	mir162	TCGATAAACCTCTGCATCCAG	1.7	Up Regulated
15	Pz-known-miR2782-1	mir160	TGCCTGGCTCCCTGTATGCCA	1.3	Up Regulated
16	Pz-known-miR2908-1	mir396	TTCCACAGCTTTCTTGAACCTG	0.3	Up Regulated
17	Pz-known-miR2921-1	mir319	TTGGACTGAAGGGAGCTCCTT	1.2	Up Regulated
18	Pz-known-miR2947-1	mir398	TGTGTTCTCAGGTCGCCCTG	1.3	Up Regulated
19	Pz-known-miR3049-1	mir482	TCTTGCCTACACGCCCATGCC	1.4	Up Regulated
20	Pz-known-miR3121-1	mir394mir384	TTGGCATTCTGTCCACCTCC	0.2	Up Regulated
21	Pz-known-miR3168-1	mir390	AAGCTCAGGAGGGATAGCGCC	2.8	Up Regulated
22	Pz-known-miR3389-1	mir167	TGAAGCTGCCAGCATGATCTA	1.3	Up Regulated
23	Pz-known-miR3461-1	mir156mir157	TTGACAGAAGATAGAGAGCAC	2.7	Up Regulated
24	Pz-known-miR356-1	mir482	TCTTTCCTACTCTCCCATACC	0.8	Up Regulated
25	Pz-known-miR400-1	mir168	TCGCTTGGTGCAGGTCGGGAC	1.6	Up Regulated
26	Pz-known-miR588-1	mir403	TTAGATTACGCACAAACTCG	1.0	Up Regulated
27	Pz-known-miR745-1	mir166	TCGGACCAGGCTTCATTCCCC	1.1	Up Regulated
28	Pz-known-miR803-1	mir399	TGCCAAAGGAGAGTTGCCCTG	2.5	Up Regulated
29	Pz-known-miR98-1	mir396	TTCCACAGCTTTCTTGAACCTT	1.0	Up Regulated
SC vs SI					
1	Pz-known-miR1382-1	mir319	TTGGACTGAAGGGAGCTCCC	-2.2	Down Regulated

2	Pz-known-miR3049-1	mir482	TCTTGCCTACACCGCCCATGCC	-0.6	Down Regulated
3	Pz-known-miR504-1	mir1446	TGAACCTCTCTCCCTCAATGGCT	-0.3	Down Regulated
4	Pz-known-miR2610-1	mir319	TTGGACTGAAGGGAGCTCCT	-0.2	Down Regulated
5	Pz-known-miR2633-1	mir162	TCGATAAACCTCTGCATCCAG	-0.1	Down Regulated
6	Pz-known-miR3168-1	mir390	AAGCTCAGGAGGGATAGCGCC	0.2	Up Regulated
16	Pz-known-miR825-1	mir408	TGCACTGCCTCTTCCCTGGCT	1.2	Up Regulated
17	Pz-known-miR1398-1	mir169	TAGCCAAGGATGACTTGCCT	1.3	Up Regulated
18	Pz-known-miR588-1	mir403	TTAGATTACGCACAAACTCG	1.4	Up Regulated
19	Pz-known-miR1393-1	mir171	TGATTGAGCCGTGCCAATATC	1.5	Up Regulated
20	Pz-known-miR1099-1	mir159	TTTGGATTGAAGGGAGCTCTA	1.6	Up Regulated
21	Pz-known-miR3230-1	mir172	AGAATCTTGATGATGCTGCAT	1.6	Up Regulated
22	Pz-known-miR1872-1	mir397	ATTGAGTGCAGCGTTGATGAC	1.6	Up Regulated
23	Pz-known-miR98-1	mir396	TTCCACAGCTTTCTTGAACCT	1.8	Up Regulated
24	Pz-known-miR2059-1	mir172	AGAATCTTGATGATGCTGCA	1.8	Up Regulated
25	Pz-known-miR3461-1	mir156mir157	TTGACAGAAGATAGAGAGCAC	1.9	Up Regulated
26	Pz-known-miR2547-1	mir4376	ACGCAGGAGAGATGATGCTGGA	2.3	Up Regulated
27	Pz-known-miR2560-1	mir172	GAATCTTGATGATGCTGCAT	2.3	Up Regulated
28	Pz-known-miR3389-1	mir167	TGAAGCTGCCAGCATGATCTA	2.5	Up Regulated
29	Pz-known-miR2300-1	mir482	TTTCCAATTCCACCCATTCTTA	3.3	Up Regulated
30	Pz-known-miR2908-1	mir396	TTCCACAGCTTTCTTGAACCTG	4.5	Up Regulated
Novel miRNAs					
RC vs RI					
1.	Pz-novel-miR1112-1		TGTGTGAAATTTCTGAATGCCT	-0.4	Down Regulated
2.	Pz-novel-miR2041-1		AAGCCAATTTGCAGAGCAAGGGT	-0.5	Down Regulated
3.	Pz-novel-miR3731-1		TCCGCTGTAGCACTGGC	-0.6	Down Regulated
4.	Pz-novel-miR410-1		TTTCCCTATTCTTTGAT	-2.6	Down Regulated
5.	Pz-novel-miR431-1		CCCAGTGAAGTTGGAGTGT	-0.7	Down Regulated
6.	Pz-novel-miR661-1		AGACTCCGAATTCTGGATCCGCCT	-0.9	Down Regulated
7.	Pz-novel-miR1021-1		CAGTTGTGCAATAATTACGCCT	3.0	Up Regulated
8.	Pz-novel-miR1028-1		TAAATAGTCCGGTGATAGTTCA	1.2	Up Regulated
9.	Pz-novel-miR1239-1		AGTACAGGGGTAATAGAACTCTCC	3.2	Up Regulated
10.	Pz-novel-miR1257-1		ACAAGTTTAAAATTCTGAATCCGT	1.2	Up Regulated
11.	Pz-novel-miR1269-1		AAACTTGTATAAAATTGAACAACCT	3.1	Up Regulated
12.	Pz-novel-miR1290-1		AGCTGACGTGAAACCTTTGACGGG	2.2	Up Regulated
13.	Pz-novel-miR1293-1		AGGCTGTTTCAAGGCTTGAACCT	2.0	Up Regulated
14.	Pz-novel-miR1327-1		AATAGGAATCGTTACTGTCAGTAA	3.7	Up Regulated
15.	Pz-novel-miR1328-1		TTCCCGCTGGTGCAC	2.0	Up Regulated
16.	Pz-novel-miR1353-1		AATGGCTAAGTAGAAGTATCCACT	1.9	Up Regulated
17.	Pz-novel-miR1364-1		ATTCATTTGGATGGGTCTGGCT	0.7	Up Regulated
18.	Pz-novel-miR1397-1		AAAAGGACTGACTTGGAGAGAGGT	1.1	Up Regulated
19.	Pz-novel-miR1404-1		GAGACTTGAACCTTGTGTTGAAGCT	2.5	Up Regulated
20.	Pz-novel-miR1425-1		GAGACACATCTGAACCTTAGGAGG	1.9	Up Regulated
21.	Pz-novel-miR143-1		AAACGTGACAAGTAAAAAGTGAACA	2.8	Up Regulated
22.	Pz-novel-miR1443-1		TTTCGGTCCACTGGTGAGCTT	0.9	Up Regulated
23.	Pz-novel-miR1481-1		AATTGTTGGGACAACCTTCTATAGT	2.4	Up Regulated

24.	Pz-novel-miR149-1		GTTATACTGTTATCTGTTCTGAGC	2.5	Up Regulated
25.	Pz-novel-miR1523-1		GTAAGACTGTGTACAATAGATCCT	1.9	Up Regulated
26.	Pz-novel-miR1576-1		AGATAACTAATCTCGGAATGAGTT	1.5	Up Regulated
27.	Pz-novel-miR1578-1		TCGATGGATAGAAATTGAGCT	1.5	Up Regulated
28.	Pz-novel-miR1605-1		ACAAACTTAAAATTCTGGATCCGT	1.3	Up Regulated
29.	Pz-novel-miR1632-1		CATGTAGAAAACCTCTGTTTGGAGT	2.5	Up Regulated
30.	Pz-novel-miR165-1		AAGGACATGTAGGCTCAGCCTATT	1.7	Up Regulated
31.	Pz-novel-miR168-1		AACCGAACCTGAATCAAACCGACA	2.2	Up Regulated
32.	Pz-novel-miR1725-1		TCGGGTTTCTAAAGTAGACACC	1.8	Up Regulated
33.	Pz-novel-miR174-1		AGAACCTTGTATCTATTGAACACT	3.1	Up Regulated
34.	Pz-novel-miR1790-1		ATGCGATGTGATTCTGAAATTAGT	2.8	Up Regulated
35.	Pz-novel-miR1846-1		AACAGGTAAGTAAGTCTGTCC	3.2	Up Regulated
36.	Pz-novel-miR185-1		AGGCTAGTGAACCTTTGTACGCTAT	2.4	Up Regulated
37.	Pz-novel-miR210-1		AAGAGACATAAATTTGGGCT	3.0	Up Regulated
38.	Pz-novel-miR213-1		AACGATCTTGGGATAGAAGAAGGT	2.9	Up Regulated
39.	Pz-novel-miR2808-1		ATTGAGTTCAGGAATAATAGGACC	3.8	Up Regulated
40.	Pz-novel-miR2813-1		ATCCGGGATTTATGTAAGTTACTC	1.9	Up Regulated
41.	Pz-novel-miR285-1		AGTAGGAATCGTACTGACAGTAA	3.1	Up Regulated
42.	Pz-novel-miR2989-1		TTTCCTACTTGAACATACACC	2.5	Up Regulated
43.	Pz-novel-miR329-1		AGTGACTGTTATGATGGTGACGGT	1.4	Up Regulated
44.	Pz-novel-miR3301-1		GGTGAAAGAGACATAAATTTGGGCT	3.5	Up Regulated
45.	Pz-novel-miR3321-1		TTCTGAGATATTAACCTGTAGT	2.3	Up Regulated
46.	Pz-novel-miR428-1		ACAGTAGAAATTCTGAGATCT	1.9	Up Regulated
47.	Pz-novel-miR433-1		AAGGGTCTGTAGATGACTTAAGCC	1.4	Up Regulated
48.	Pz-novel-miR954-1		AGTGACTGTTTCAGATAATTGTGTC	3.3	Up Regulated
49.	Pz-novel-miR96-1		AGGAAGACTGTCTGATTGTAAAGA	3.5	Up Regulated
50.	Pz-novel-miR974-1		AACTTGTCCTCCAGTTGTAGAACAAC	1.4	Up Regulated
SC vs SI					
1.	Pz-novel-miR1328-1		TTCCCGGCTGGTGCAC	-3.1	Down Regulated
2.	Pz-novel-miR3301-1		GGTGAAAGAGACATAAATTTGGGCT	-1.1	Down Regulated
3.	Pz-novel-miR1304-1		AGATTAGTCTTTTGGGTTGGGC	-1.0	Down Regulated
4.	Pz-novel-miR3407-1		GAGTGTATAAGAACAATGCTGAAT	-1.0	Down Regulated
5.	Pz-novel-miR328-1		AGAGAGATTGTTTTCGATAGAACT	-1.0	Down Regulated
6.	Pz-novel-miR431-1		CCCAGTGAAGTTGGAGTGT	-0.9	Down Regulated
7.	Pz-novel-miR1053-1		ATTGGTCCAGAGGATAATAGAACC	-0.8	Down Regulated
8.	Pz-novel-miR214-1		AAATAGAATTTCTGACTGTATGGT	-0.7	Down Regulated
9.	Pz-novel-miR2869-1		AGACTAGTTTTTTGGGTTGGGC	0.0	No Change
10.	Pz-novel-miR204-1		CTTTTCTTGAGTAGACATGCCT	0.6	Up Regulated
11.	Pz-novel-miR42-1		TGCTAAGATTGAGTTCCGGGT	0.6	Up Regulated
12.	Pz-novel-miR2252-1		AGACTCTAAATCTGAATCCGCCT	0.9	Up Regulated
13.	Pz-novel-miR3546-1		AGAGAATCGTTCAAAGCACCGAAA	0.9	Up Regulated
14.	Pz-novel-miR2214-1		AGACTCCAAATCTGAATCCGCCT	1.0	Up Regulated
15.	Pz-novel-miR3321-1		TTCTGAGATATTAACCTGTAGT	1.0	Up Regulated
16.	Pz-novel-miR138-1		TCTGAAAAGTTTGTGGTGTGATT	1.0	Up Regulated
17.	Pz-novel-miR2638-1		TTGGCATTCTGTCTACCTCC	1.0	Up Regulated

18.	Pz-novel-miR3002-1		AACCAGAGGTCTGTAGTTAGAGCC	1.0	Up Regulated
19.	Pz-novel-miR1425-1		GAGACACATCTGAACCTTTAGGAGG	1.0	Up Regulated
20.	Pz-novel-miR2813-1		ATCCGGGATTTATGTAAGTTACTC	1.0	Up Regulated
21.	Pz-novel-miR2705-1		AGGATAACTAATTCTGAAATGAGT	1.0	Up Regulated
22.	Pz-novel-miR3381-1		ACAGAAAAATCTGAGCCCT	1.0	Up Regulated
23.	Pz-novel-miR3509-1		TTTTAGCAAGAGTTGTTTTCC	1.0	Up Regulated
24.	Pz-novel-miR2176-1		TCTTGCCGATTCCGTCCATACC	1.1	Up Regulated
25.	Pz-novel-miR348-1		AGAATTTATATTGTGACGGACGAT	1.1	Up Regulated
26.	Pz-novel-miR143-1		AAACGTGACAAGTAAAAGTGAACA	1.1	Up Regulated
27.	Pz-novel-miR160-1		TCAAGTCCCGGCAACGGAAC	1.1	Up Regulated
28.	Pz-novel-miR3523-1		CGGTACAGTAGATTTCTGAGC	1.1	Up Regulated
29.	Pz-novel-miR428-1		ACAGTAGAAATTCTGAGATCT	1.2	Up Regulated
30.	Pz-novel-miR177-1		GAGAGACTGTTTCAATAGACCCCT	1.2	Up Regulated
31.	Pz-novel-miR1523-1		GTAAGACTGTGTACAATAGATCCT	1.2	Up Regulated
32.	Pz-novel-miR778-1		AATGAATTCTGAACGTGTTAAAGT	1.2	Up Regulated
33.	Pz-novel-miR954-1		AGTGACTGTTTCAGATAATTGTGTC	1.3	Up Regulated
34.	Pz-novel-miR3290-1		AGAGAGACTGTTTTCGATAGACT	1.3	Up Regulated
35.	Pz-novel-miR2964-1		ACCCTTTGACTGTTGACCTTGCCCT	1.7	Up Regulated
36.	Pz-novel-miR822-1		AGGATTTGATATGAATTTCTCCGT	1.7	Up Regulated
37.	Pz-novel-miR3513-1		ATTTAGGATTTAGGTAGAGTAGCC	1.7	Up Regulated
38.	Pz-novel-miR227-1		TCTTACCGATAACCTCCCATTC	1.8	Up Regulated
39.	Pz-novel-miR3570-1		GTTATGTATATTAATAGAGAGAGT	1.8	Up Regulated
40.	Pz-novel-miR1806-1		GAGAATGCGTCGGTAACAATAAGG	2.0	Up Regulated
41.	Pz-novel-miR540-1		TGACAAGGTTCAAAGAAAGGGTGG	2.0	Up Regulated
42.	Pz-novel-miR2618-1		ACTGAAACCTACAGCTGAACGCCT	2.1	Up Regulated
43.	Pz-novel-miR1853-1		TTGAGTTACCATCACTTGCCCT	2.9	Up Regulated
44.	Pz-novel-miR974-1		AACTTGTCGCCAGTTGTAGAACAAC	2.9	Up Regulated
45.	Pz-novel-miR1349-1		AAGGAGCAATAATCATTTTCTTGT	3.2	Up Regulated
46.	Pz-novel-miR644-1		CAGATCAGTAGAGAAGAAACATCT	3.3	Up Regulated
47.	Pz-novel-miR2082-1		TATCGTGTCAAGCGGGACT	3.7	Up Regulated
48.	Pz-novel-miR1028-1		TAAATAGTCGGTGATAGTTCA	3.9	Up Regulated
49.	Pz-novel-miR3127-1		AGGACAAGAATATCACTCAACTCT	4.4	Up Regulated
50.	Pz-novel-miR2841-1		TTTGTCTATCTCATCCCTCATACC	5.1	Up Regulated

Table S4. List of all the novel miRNA along with their fold change value in RC vs RI and SC vs SI sample of *C. annuum* L. under *P. capsici* infection.

Sr. No.	miRNA-Acc	Mature Sequence	Abundance level		Fold Change	Remark
Novel miRNAs			RC	RI		
RC vs RI						
1.	Pz-novel-miR3731-1	TCCGCTGTAGCACTGGC	173306	19510	-0.6	Down Regulated
2.	Pz-novel-miR1981-1	TTGCAAACACACCTGAATCGT	1424	1121	2.2	Up Regulated
3.	Pz-novel-miR1112-1	TGTGTGAAATTTCTGAATGCCT	686	91	-0.4	Down Regulated

4.	Pz-novel-miR410-1	TTTCCTATTCTTTGAT	559	16	-2.6	Down Regulated
5.	Pz-novel-miR3509-1	TTTAGCAAGAGTTGTTTTCC	418	244	1.7	Up Regulated
6.	Pz-novel-miR227-1	TCTTACCGATAACCTCCCATTC	391	83	0.3	Up Regulated
7.	Pz-novel-miR2406-1	TTCAGGCCTGAGAAAACGAAAACT	328	149	1.4	Up Regulated
8.	Pz-novel-miR1344-1	AAACTCGAGAACTCTGATTAAACGA	259	88	1.0	Up Regulated
9.	Pz-novel-miR2638-1	TTGGCATTCTGTCTACCTCC	234	71	0.8	Up Regulated
10.	Pz-novel-miR1167-1	AAGTTTGAAATTCTGAATCCGCCT	219	59	0.6	Up Regulated
11.	Pz-novel-miR2176-1	TCTTGCCGATTCCGTCCATACC	190	38	0.2	Up Regulated
12.	Pz-novel-miR2905-1	GAGGGAAATTTCTGAATGCCT	187	53	0.7	Up Regulated
13.	Pz-novel-miR661-1	AGACTCCGAATTCTGGATCCGCCT	128	12	-0.9	Down Regulated
14.	Pz-novel-miR479-1	CTTCTAGAAGCTGTAGGACTTC	105	42	1.2	Up Regulated
15.	Pz-novel-miR1933-1	GCGTAAGAACTCAACGGGA	101	45	1.3	Up Regulated
16.	Pz-novel-miR3429-1	ACAAGTTTGAAATTCTGAATCCGT	100	36	1.0	Up Regulated
17.	Pz-novel-miR2041-1	AAGCCAATTTGCAGAGCAAGGGT	99	12	-0.5	Down Regulated
18.	Pz-novel-miR1975-1	AAGTTTGAAATTCTGGATCCGCCT	96	36	1.1	Up Regulated
19.	Pz-novel-miR3708-1	TTGAGCCGTGCCAATATCACGT	93	44	1.4	Up Regulated
20.	Pz-novel-miR431-1	CCCAGTGAAGTTGGAGTGT	91	10	-0.7	Down Regulated
21.	Pz-novel-miR545-1	TTGAGCCGCGCCAATATCACG	88	27	0.8	Up Regulated
22.	Pz-novel-miR1257-1	ACAAGTTTAAAAATTCTGAATCCGT	87	36	1.2	Up Regulated
23.	Pz-novel-miR2407-1	ACATGGGTAAAGTAAATGAAACT	84	62	2.1	Up Regulated
24.	Pz-novel-miR3301-1	GGTGAAAGAGACATAAATTTGGGCT	83	169	3.5	Up Regulated
25.	Pz-novel-miR210-1	AAGAGACATAAATTTGGGCT	65	88	3.0	Up Regulated
26.	Pz-novel-miR2743-1	TGTAGCTCTGGGTTTATGGTT	58	12	0.2	Up Regulated
27.	Pz-novel-miR3523-1	CGGTACAGTAGATTCTGAGC	52	19	1.1	Up Regulated
28.	Pz-novel-miR3570-1	GTTATGTATATTAATAGAGAGAGT	51	15	0.7	Up Regulated
29.	Pz-novel-miR1142-1	TACTTGTTAGAATTGTTGGCCC	50	19	1.1	Up Regulated
30.	Pz-novel-miR1028-1	TAAATAGTCGGTGATAGTTCA	49	20	1.2	Up Regulated
31.	Pz-novel-miR2252-1	AGACTCTAAATTCTGAATCCGCCT	48	12	0.5	Up Regulated
32.	Pz-novel-miR390-1	AACCGAACCGAATTTGTAGAAATC	47	32	2.0	Up Regulated
33.	Pz-novel-miR214-1	AAATAGAATTTCTGACTGTATGGT	44	50	2.7	Up Regulated
34.	Pz-novel-miR2452-1	AAGGGACATAGTGCTGAACCACTA	44	53	2.8	Up Regulated
35.	Pz-novel-miR1443-1	TTTCGGTCCACTGGTGAGCTT	44	14	0.9	Up Regulated
36.	Pz-novel-miR1829-1	AGACTTCGAATTCTGGATCCGCCT	41	13	0.9	Up Regulated
37.	Pz-novel-miR2182-1	CTTTTGAAAGAAGGATTGCCT	40	26	1.9	Up Regulated
38.	Pz-novel-miR1725-1	TCGGGTTTCTAAAGTAGACACC	40	24	1.8	Up Regulated
39.	Pz-novel-miR407-1	TTGGACACGTAGACACATGCG	38	7	0.1	Up Regulated
40.	Pz-novel-miR778-1	AATGAATTCTGAACGTGTTAAAGT	37	22	1.8	Up Regulated
41.	Pz-novel-miR3722-1	ACCGAGGACATGAACCTAGATAGG	36	10	0.7	Up Regulated
42.	Pz-novel-miR3064-1	TACTTTGGGTATTCATATGCT	36	11	0.8	Up Regulated
43.	Pz-novel-miR421-1	GGGATAGTAGTTCAAT	35	8	0.4	Up Regulated
44.	Pz-novel-miR1239-1	AGTACAGGGGTAATAGAACTCTCC	34	54	3.2	Up Regulated
45.	Pz-novel-miR2480-1	AGGTATCCTGTAGAATTAATTGAG	29	18	1.8	Up Regulated
46.	Pz-novel-miR1425-1	GAGACACATCTGAACCTTAGGAGG	29	19	1.9	Up Regulated
47.	Pz-novel-miR450-1	TGATAGCCAAGGATGACTGGT	28	8	0.7	Up Regulated
48.	Pz-novel-miR1853-1	TTGAGTTACCATCACTTGCCCT	28	7	0.5	Up Regulated

49.	Pz-novel-miR1353-1	AATGGCTAAGTAGAAGTATCCACT	27	18	1.9	Up Regulated
50.	Pz-novel-miR1182-1	AGACTCCGAATTCTGAATCCGCCT	27	9	0.9	Up Regulated
SC vs SI						
1.	Pz-novel-miR3731-1	TCCGCTGTAGCACTGGC	177934	33049	-0.6	Down Regulated
2.	Pz-novel-miR1981-1	TTGCAAAACACACCTGAATCGT	3655	1204	0.2	Up Regulated
3.	Pz-novel-miR3509-1	TTTAGCAAGAGTTGTTTTCC	525	301	1.0	Up Regulated
4.	Pz-novel-miR1112-1	TGTGTGAAATTTCTGAATGCCT	522	195	0.4	Up Regulated
5.	Pz-novel-miR410-1	TTTCCCTATTCTTTGAT	458	124	-0.1	Down Regulated
6.	Pz-novel-miR3301-1	GGTGAAAGAGACATAAECTTTGGGCT	442	59	-1.1	Down Regulated
7.	Pz-novel-miR2905-1	GAGGGAAATTTCTGAATGCCT	337	117	0.3	Up Regulated
8.	Pz-novel-miR2406-1	TTCAGGCCTGAGAAACGAAAACT	261	228	1.6	Up Regulated
9.	Pz-novel-miR2638-1	TTGGCATTCTGTCTACCTCC	210	115	1.0	Up Regulated
10.	Pz-novel-miR210-1	AAGAGACATAAECTTTGGGCT	189	41	-0.4	Down Regulated
11.	Pz-novel-miR1328-1	TTCCCGGCTGGTGCAC	181	6	-3.1	Down Regulated
12.	Pz-novel-miR227-1	TCTTACCGATACCTCCATTCC	175	169	1.8	Up Regulated
13.	Pz-novel-miR1344-1	AAACTCGAGAACTCTGATTAACGA	174	139	1.5	Up Regulated
14.	Pz-novel-miR2041-1	AAGCCAAATTGCAGAGCAAGGGT	168	32	-0.6	Down Regulated
15.	Pz-novel-miR1933-1	GCGTAAGAACTCAACGGGA	153	124	1.5	Up Regulated
16.	Pz-novel-miR2176-1	TCTTGCCGATTCCGTCCATACC	140	82	1.1	Up Regulated
17.	Pz-novel-miR431-1	CCCAGTGAAAGTTGGAGTGT	134	20	-0.9	Down Regulated
18.	Pz-novel-miR1975-1	AAGTTTGAAATTTCTGGATCCGCCT	116	78	1.3	Up Regulated
19.	Pz-novel-miR661-1	AGACTCCGAATTCTGGATCCGCCT	114	55	1.3	Up Regulated
20.	Pz-novel-miR2407-1	ACATGGGTAAAGTAAATGAAACT	109	40	0.4	Up Regulated
21.	Pz-novel-miR1167-1	AAGTTTGAAATTTCTGAATCCGCCT	97	80	1.5	Up Regulated
22.	Pz-novel-miR545-1	TTGAGCCGCGCCAATATCACG	86	64	1.4	Up Regulated
23.	Pz-novel-miR1142-1	TACTTGTTAGAATTGTTGGCCC	85	17	-0.5	Down Regulated
24.	Pz-novel-miR214-1	AAATAGAATTTCTGACTGTATGGT	79	14	-0.7	Down Regulated
25.	Pz-novel-miR1725-1	TCGGGTTTCTAAAGTAGACACC	75	36	0.8	Up Regulated
26.	Pz-novel-miR3429-1	ACAAGTTTGAAATTTCTGAATCCGT	67	45	1.3	Up Regulated
27.	Pz-novel-miR1239-1	AGTACAGGGGTAATAGAACTCTCC	67	30	0.7	Up Regulated
28.	Pz-novel-miR3708-1	TTGAGCCGTGCCAATATCACGT	65	43	1.2	Up Regulated
29.	Pz-novel-miR328-1	AGAGAGATTGTTTTTCGATAGAACT	63	9	-1.0	Down Regulated
30.	Pz-novel-miR390-1	AACCGAACCGAATTTGTAGAAATC	62	28	0.7	Up Regulated
31.	Pz-novel-miR1829-1	AGACTTCGAATTTCTGGATCCGCCT	56	23	0.5	Up Regulated
32.	Pz-novel-miR2452-1	AAGGGACATAGTGCTGAACCACTA	52	39	1.4	Up Regulated
33.	Pz-novel-miR407-1	TTGGACACGTAGACACATGCG	51	15	0.1	Up Regulated
34.	Pz-novel-miR231-1	AACCGAAAAATCGAACTGTTAAGGA	51	9	-0.7	Down Regulated
35.	Pz-novel-miR2252-1	AGACTCTAAATTTCTGAATCCGCCT	47	25	0.9	Up Regulated
36.	Pz-novel-miR3523-1	CGGTACAGTAGATTCTGAGC	45	27	1.1	Up Regulated
37.	Pz-novel-miR1353-1	AATGGCTAAGTAGAAGTATCCACT	45	19	0.6	Up Regulated
38.	Pz-novel-miR479-1	CTTCTAGAAGCTGTAGGACTTC	44	57	2.2	Up Regulated
39.	Pz-novel-miR2682-1	TTGAGATGAAACGTGTAGAACT	43	11	-0.1	Down Regulated
40.	Pz-novel-miR3064-1	TACTTTGGGTATTCATATGCT	42	16	0.4	Up Regulated
41.	Pz-novel-miR3503-1	AAACCGAAAATGAACCGTTAAGG	42	7	-0.8	Down Regulated

42.	Pz-novel-miR1257-1	ACAAGTTTAAAAATTCTGAATCCGT	41	41	1.8	Up Regulated
43.	Pz-novel-miR2253-1	AAACTCGAAAACTCTGATTAAGGA	40	27	1.3	Up Regulated
44.	Pz-novel-miR285-1	AGTAGGAATCGTTACTGACAGTAA	39	7	-0.7	Down Regulated
45.	Pz-novel-miR1831-1	AGGTAAGACTGAATACAGTGCACC	39	12	0.1	Up Regulated
46.	Pz-novel-miR2043-1	TTTGACACTTTTGTGCTGAGTTGGC	37	12	0.2	Up Regulated
47.	Pz-novel-miR870-1	AACCTGAGACCTCTGATTAAGGAA	36	9	-0.2	Down Regulated
48.	Pz-novel-miR2283-1	ACTCATAGGATATCATGTAGAACA	35	8	-0.3	Down Regulated
49.	Pz-novel-miR1290-1	AGCTGACGTGAAACCTTTGACGGG	35	7	-0.5	Down Regulated
50.	Pz-novel-miR3407-1	GAGTGTATAAGAACAATGCTGAAT	35	5	-1.0	Down Regulated

Table S5. The differentially expressed genes of known and novel miRNA with their corresponding target genes associated with *P. capsici* infection in resistance and susceptible *C. annuum* L (in sample combination of resistance control and resistance inoculated and susceptible control and susceptible infected).

No.	Name of miRNA	miRNA ID	FC Analysis	Exp of miRNA In RC vs RI	FC Analysis	Exp of miRNA In SC vs SI	Target CDS transcript	Target genes
1	Pz-known-miR2610-1	Can-miR319	1.06	Up Regulated	-0.24	Down regulated	CDS_14512_Unigene_36255_Transcript_54747	XP_016542959.1 putative late blight resistance protein homolog R1B-17
2	Pz-novel-miR1028-1		1.22	Up regulated	3.92	Up regulated	CDS_8413_Unigene_24374_Transcript_37395	XP_015160125.1 putative late blight resistance protein homolog R1B-14
3	Pz-novel-miR428-1		1.88	Up regulated	1.16	Up regulated	CDS_9005_Unigene_25430_Transcript_38979	XP_016576084.1 putative late blight resistance protein homolog R1B-13
4	Pz-novel-miR1304-1		Nil	NiL	-1.05	Down regulated	CDS_7053_Unigene_21909_Transcript_33661	XP_020546966.1 putative late blight resistance protein homolog R1B-16
5	Pz-known-miR803-1	Can-miR399	2.52	Up regulated	0.50	Up regulated	CDS_13788_Unigene_34777_Transcript_52630	XP_016562279.1 polygalacturonase inhibitor-like
6	Pz-novel-miR1405-1		Nil	NiL	Nil	NiL	CDS_13788_Unigene_34777_Transcript_52630	XP_016562279.1 polygalacturonase inhibitor-like
7	Pz-novel-miR1028-1		1.22	Up regulated	3.92	Up regulated	CDS_18560_Unigene_4481_Transcript_6845	NP_001311656.1 endochitinase EP3-like precursor
8	Pz-novel-miR177-1		Nil	NiL	1.17	Up regulated	CDS_16858_Unigene_41152_Transcript_61771	XP_016580739.1 endochitinase 4
9	Pz-novel-miR1443-1		0.86	Up regulated	1.41	Up regulated	CDS_19421_Unigene_46658_Transcript_69807	XP_016556000.1 probable endo-1,3(4)-beta-glucanase ARB_01444
10	Pz-known-miR1872-1	Can-miR397	-0.44	Down regulated	1.61	Up regulated	CDS_4939_Unigene_18363_Transcript_28254	XP_016560122.1 peroxidase 42
11	Pz-novel-miR160-1		Nil	NiL	1.08	Up regulated	CDS_20661_Unigene_49476_Transcript_73985	XP_016577598.1 pathogenesis-related genes transcriptional activator PTI6
12	Pz-novel-miR2808-1		3.83	Up regulated	Nil	NiL	CDS_30957_Unigene_8586_Transcript_13357	XP_016537600.1 putative L-ascorbate peroxidase 6
13	Pz-novel-miR3720-1		Nil	NiL	1.63	Up regulated	CDS_2925_Unigene_1491_Transcript_2236	XP_016575755.1 L-ascorbate peroxidase 1, cytosolic
14	Pz-known-miR2059-1	Can-miR172	0.86	Up regulated	1.82	Up regulated	CDS_1373_Unigene_1226_Transcript_1798	XP_016548531.1 ethylene-responsive transcription factor RAP2-7
15	Pz-known-miR2560-1	Can-miR172	0.58	Up regulated	2.34	Up regulated	CDS_20368_Unigene_48746_Transcript_72877	XP_016561036.1 AP2-like ethylene-responsive transcription factor TOE3
16	Pz-novel-miR2082-1		1.86	Up regulated	3.70	Up regulated	CDS_21759_Unigene_52006_Transcript_77824	XP_016547331.1 vegetative cell wall protein gp1 isoform X2
17	Pz-novel-miR204-1	Nil	Nil		0.56	Up regulated	CDS_27383_Unigene_66986_Transcript_102384	XP_016579284.1 defensin-like protein
18	Pz-known-miR1393-1	Can-miR171	0.86	Up regulated	1.55	Up regulated	CDS_9792_Unigene_26823_Transcript_41105	PHU19092.1 GDSL esterase/lipase
19	Pz-novel-miR3247-1		Nil	NiL	1.91	Up regulated	CDS_17963_Unigene_43538_Transcript_65143	PHT87102.1 Hypersensitive-induced response protein 1

20	Pz-novel-miR3523-1		1.06	Up regulated	1.08	Up regulated	CDS_28493_Unigene_70107_Transcript_107512	XP_016577453.1 glycolipid transfer protein 1
21	Pz-known-miR1872-1	Can-miR397	-0.44	Down regulated	1.61	Up regulated	CDS_24735_Unigene_59496_Transcript_89837	XP_016576412.1 callose synthase 9
22	Pz-novel-miR1364-1		0.67	Up regulated	0.24	Up regulated	CDS_4748_Unigene_18055_Transcript_27786	NP_001311841.1 lignin-forming anionic peroxidase-like precursor
23	Pz-known-miR3121-1	Can-miR394	0.16	Up regulated	0.84	Up regulated	CDS_5863_Unigene_1989_Transcript_3011	XP_016539926.1 glycine-rich protein 2-like
24	Pz-known-miR2059-1	Can-miR172	0.86	Up regulated	1.83	Up regulated	CDS_20518_Unigene_49112_Transcript_73432	XP_016573935.1 putative disease resistance protein RGA1
25	Pz-novel-miR2077-1		0.92	Up regulated	1.83	Up regulated	CDS_29171_Unigene_7209_Transcript_11179	XP_012853252.1 putative disease resistance protein At1g50180
26	Pz-novel-miR2682-1		1.82	Up regulated	-0.14	Down regulated	CDS_13171_Unigene_33510_Transcript_50817	XP_016582136.1 stellacyanin
27	Pz-known-miR2947-1	Can-miR398	1.27	Up regulated	0.29	Up regulated	CDS_7657_Unigene_23021_Transcript_35345	CAL91170.1 phenylalanine ammonia-lyase
28	Pz-novel-miR1304-1		Nil	Nil	-1.04	Down regulated	CDS_20315_Unigene_4863_Transcript_7480	XP_016573455.1 pectin acetyltransferase 8-like
29	Pz-known-miR803-1	Can-miR399	2.51	Up regulated	0.50	Up regulated	CDS_12274_Unigene_31731_Transcript_48232	XP_016569239.1 probable pectin methyltransferase QUA2
30	Pz-known-miR1186-1	mir156	Nil	Nil		Only Expressed in SC	CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6
31	Pz-novel-miR227-1		0.3	Up regulated	1.8	Up-regulated	CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6
32	Pz-known-miR3461-1	mir156mir157	2.7	Up regulated	1.9	Up-regulated	CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6
33	Pz-novel-miR1107-1			Only Expressed in RI	-0.2	Down-regulated	CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6
34	Pz-novel-miR2181-1		1.6	Up regulated	0.2	Up-regulated	CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6

Table S6. List of some of the target genes along with their potential corresponding miRNAs

CDS_Target_Acc	Description	Target_Aligned_Fragment	miRNA_Acc	miRNA_Aligned_Fragment	Inhibition
CDS_14512_Unigene_36255_Transcript_54747	XP_016542959.1 putative late blight resistance protein homolog R1B-17	UGGAGGUGCCUUCACUCCAGA	Pz-known-miR1099-1	UUUGGAUUGAAGGGAGCUCUA	Cleavage
CDS_8413_Unigene_24374_Transcript_37395	XP_015160125.1 putative late blight resistance protein homolog R1B-14	GGAACUAAUACCGACAAUUUG	Pz-novel-miR1028-1	UAAAUAGUCGGUGAUAGUUCA	Cleavage
CDS_9005_Unigene_25430_Transcript_38979	XP_016576084.1 putative late blight resistance protein homolog R1B-13	UGGUCGUGGGAUUUUACUUU	Pz-novel-miR428-1	ACAGUAGAAAUCUGAGAUCU	Cleavage

CDS_7053_Unigene_21909_Transcript_33661	XP_020546966.1 putative late blight resistance protein homolog R1B-16	AUCUAGCUCAGAAGAUGAAUCA	Pz-novel-miR1304-1	AGAUUAGUCUUUUGGGUUGGGC	Cleavage
CDS_14512_Unigene_36255_Transcript_54747	XP_016542959.1 putative late blight resistance protein homolog R1B-17	UGGAGGUGCCUUCACUCCAG	Pz-known-miR2610-1	UUGGACUGAAGGGAGCUCCU	Cleavage
CDS_13788_Unigene_34777_Transcript_52630	XP_016562279.1 polygalacturonase inhibitor-like	UUUGCGGCUCUCCUUUGCCG	Pz-novel-miR1405-1	UGCCAAAGGAGAGUUGCCCU	Cleavage
CDS_13788_Unigene_34777_Transcript_52630	XP_016562279.1 polygalacturonase inhibitor-like	UUUGCGGCUCUCCUUUGCCG	Pz-novel-miR1405-1	UGCCAAAGGAGAGUUGCCCU	Cleavage
CDS_18560_Unigene_4481_Transcript_6845	NP_001311656.1 endochitinase EP3-like precursor	ACAAUUGUCAUUCACUAAUUA	Pz-novel-miR1028-1	UAAAUAGUCGGUGAUAGUUA	Cleavage
CDS_16858_Unigene_41152_Transcript_61771	XP_016580739.1 endochitinase 4	GAGGUCUUUUGGAAACGGACUCUU	Pz-novel-miR177-1	GAGAGACUGUUUCAAUAGACCCU	Cleavage
CDS_19421_Unigene_46658_Transcript_69807	XP_016556000.1 probable endo-1,3(4)-beta-glucanase ARB_01444	CUUGUUUCUGUUGGCUAACUCUU	Pz-novel-miR517-1	AAGAGUUGAGAGAAUAGAGAGAGU	Translation
CDS_19421_Unigene_46658_Transcript_69807	XP_016556000.1 probable endo-1,3(4)-beta-glucanase ARB_01444	GCACUCACUAGUGUACCCAAA	Pz-novel-miR1443-1	UUUCGGUCCACUGGUGAGCUU	Cleavage
CDS_4940_Unigene_18364_Transcript_28255	XP_016560122.1 peroxidase 42	GGUGAGAUCAGAAAGCAGUGUAAU	Pz-novel-miR149-1	GUUAUACUGUUAUCUGUUCUGAGC	Cleavage
CDS_2925_Unigene_1491_Transcript_2236	XP_016575755.1 L-ascorbate peroxidase 1, cytosolic	GCUCUAACCACUUAAGGGACGUGU	Pz-novel-miR3720-1	AUUCGUCUCUUAGGUAGUUAAGUU	Cleavage
CDS_20661_Unigene_49476_Transcript_73985	XP_016577598.1 pathogenesis-related genes transcriptional activator PTI6-like	UUUCGGUUGCCGGAGUUUGG	Pz-novel-miR160-1	UCAAGUCCCGCAACGGAAC	Cleavage
CDS_30957_Unigene_8586_Transcript_13357	XP_016537600.1 putative L-ascorbate peroxidase 6	GGCUCUAUUCUUUUUGAACUAGAC	Pz-novel-miR2808-1	AUUGAGUUCAGGAAUAAUAGGACC	Cleavage
CDS_1373_Unigene_1226_Transcript_1798	XP_016548531.1 ethylene-responsive transcription factor RAP2-7-like isoform X1	CCGGGAAGAGAAUAGAUGUAGGUA	Pz-novel-miR359-1	AACUGACAUUUUAGCUCUUAACU	Cleavage
CDS_21759_Unigene_52006_Transcript_77824	XP_016547331.1 vegetative cell wall protein gp1 isoform X2	AGUAGCGUUUGGUGUGAUA	Pz-novel-miR2082-1	UAUCGUGUCAAGCGGGACU	Cleavage
CDS_27383_Unigene_66986_Transcript_102384	XP_016579284.1 defensin-like protein	AGCCAUGUGUAUUUGAGAAUAA	Pz-novel-miR204-1	CUUUUCUUGAGUAGACAUGCCU	Cleavage
CDS_9792_Unigene_26823_Transcript_41105	PHU19092.1 GDSL esterase/lipase	ACAAUUGGAAUGGUUCAACA	Pz-known-miR1393-1	UGAUUGAGCCGUGCCAAUUAUC	Cleavage
CDS_17963_Unigene_43538_Transcript_65143	PHT87102.1 Hypersensitive-induced response protein 1	GGGAAUGCCUCACUGGAACUCUAU	Pz-novel-miR2497-1	AUAGAAUUCUGAUGAGGCUUGCUU	Cleavage
CDS_28493_Unigene_70107_Transcript_107512	XP_016577453.1 glycolipid transfer protein 1	GUUUAGAAAUUACUGGAGCA	Pz-novel-miR3523-1	CGGUACAGUAGAUUUCUGAGC	Cleavage
CDS_4749_Unigene_18056_Transcript_27787	NP_001311841.1 lignin-forming anionic peroxidase-like precursor	GGUCACACCCAAUCAACUGGAU	Pz-novel-miR1364-1	AUUCAUUUGGAUGGGUCUGGCU	Translation

CDS_5863_Unigene_1989_Transcript_3011	XP_016539926.1 glycine-rich protein 2-like	GAAAGUGGACAGAGGGCUAA	Pz-known-miR3121-1	UUGGCAUUCUGUCCACCUC	Cleavage
CDS_20518_Unigene_49112_Transcript_73432	XP_016573935.1 putative disease resistance protein RGA1	AGUGGUAACAUCAAGUUUCU	Pz-known-miR2059-1	AGAAUCUUGAUGAUGCUGCA	Cleavage
CDS_29171_Unigene_7209_Transcript_11179	XP_012853252.1 putative disease resistance protein Atlg50180	GCUAAAGAAGUUAUUGUUGAAA	Pz-novel-miR2077-1	UUUUGGCAUUGAUUGUUUUGGC	Cleavage
CDS_13171_Unigene_33510_Transcript_50817	XP_016582136.1 stellacyanin	ACCUUUGUACAUUUUUUUUCAG	Pz-novel-miR2682-1	UUGAGAUGAAACGUGUAGAACU	Cleavage
CDS_7657_Unigene_23021_Transcript_35345	CAL91170.1phenylalanine ammonia-lyase 3	GUGGAGUUAUCUGAGGAUGCA	Pz-known-miR2947-1	UGUGUUCUCAGGUCGCCCCUG	Cleavage
CDS_20315_Unigene_4863_Transcript_7480	XP_016573455.1 pectin acetylerase 8-like	AUCCAUCCAGAAGAUUGAUUG	Pz-novel-miR1304-1	AGAUUAGUCUUUUGGGUUGGGC	Cleavage
CDS_12274_Unigene_31731_Transcript_48232	XP_016569239.1 probable pectin methyltransferase QUA2	UAGGACAGCUCUCUCUUGGUG	Pz-known-miR803-1	UGCCAAAGGAGAGUUGCCCUG	Cleavage
CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6	AUGCUCUCUCUCUUCUGUCA	Pz-known-miR1186-1	UGACAGAAGAGAGUGAGCAC	Cleavage
CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6	CAAAUGAGAGGUGUUGGCAACA	Pz-novel-miR227-1	UCUUACCGAUACCUCCAUUCC	Cleavage
CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6	AUGCUCUCUCUCUUCUGUCAU	Pz-known-miR3461-1	UUGACAGAAGAUAGAGAGCAC	Cleavage
CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6	UUCACUACUGCUGAGUCCAUG	Pz-novel-miR1107-1	UAACGGAUUCAGCAGGGCUGAC	Cleavage
CDS_17079_Unigene_4161_Transcript_6368	XP_016545418.1 squamosa promoter-binding-like protein 6	GGCAUAAAGUAUGUGAGGUUCACU	Pz-novel-miR2181-1	AGUGAACUUUGUACGCUAUGUGGU	Cleavage

Figure 1. Secondary structure of known miRNA identified from small RNA sequencing of *C. annuum* during infection with *P. capsici*: A) Pz-known-miR803-1 (can-mir399), B) Pz-known-miR1872-1 (can-mir397), C) Pz-known-miR2059-1 (can-mir172), D) Pz-known-miR2560-1 (can-mir172) used in the validation of RT-qPCR (the green colour shows the matured miRNA sequence site).

Figure 2. Secondary structure of novel miRNAs identified from transcriptome sequence of *C. annuum* during infection of *P. capsici* and the matured miRNAs sequence marked with green colour: A) Pz-novel-miR160-1, B) Pz-novel-miR204-1, C) Pz-novel-miR428-1, and D) Pz-novel-miR1028-1

Figure 3. Novel miRNA identified in *C. annuum* A) Distribution of novel miRNAs in control (dark green colour bars) and in infected (red colour bars) samples of *C. annuum*; B) Distribution of novel miRNAs in different chromosomes of the *C. annuum* genome.

Figure 4. Venn diagram showing common and exclusively expressed miRNAs in resistance and susceptible chilli pepper. The red colour circle represents the control samples, whereas the blue colour circle represents the *P. capsici* infected leaf samples. 1) known miRNAs in A) Resistance Control Vs. Resistance Infected and B) Susceptible inoculated; 2) Novel miRNAs in A) Resistance Control Vs. Resistance Infected and B) Susceptible Control Vs. Susceptible Infected.

Figure 5. The diagram of regional amplification of qRT-PCR of target genes: A) The three site of primers used for regional amplification of target gene cleaved by miRNAs (5' side both pair of forward and reverse primers, middle side both forward and reverse primers, middle side both forward and reverse primers, 3' side both pair of forward and reverse primers); B) The miRNA cleavage site (▲) of eight target genes associated to the infection of *P. capsici*, the start and stop codon was illustrated by (► and ◄) respectively.

Figure 6. The RT-qPCR result analysis of the randomly selected DEGs of miRNAs regulating its corresponding identified target gene associated with defence response against *P. capsici* infection: A) Pz-novel-miR428-1 with its target putative late blight resistance protein homolog (PLBRP) RIP-13 gene, B) Pz-novel-miR1028-1 gene with its target endochitinase (EP) EP3-like precursor gene, C) Pz-novel-miR160-1 regulating its target PRTA-PTI6-like gene, and D) Pz-novel miR204-1 gene-regulating its target defensin-like protein(DLP) gene.

Figure 7. The RT-qPCR result analysis of the randomly selected DEGs of miRNAs regulating its corresponding identified target gene associated with defence response against *P. capsici* infection: A) Pz-known-miR2560-1(Can-miR172) gene-regulating its target AP2-like ethylene-responsive transcription factor (AP2-ERTF) TOE3 gene, B) Pz-known-miR2059-1 gene-regulating its target putative disease resistance protein (PDRP) RGA1 gene, C) Pz-known-miR803-1 with its target polygalacturonase inhibitor like (PGUI) gene, and D) Pz-known-miR1872-1(Can-miR397) with its target peroxidase 42 genes (PODs).

Figure S1. The bioinformatics workflow for small RNA sequencing and data analysis

Figure S2. Length distribution of final cleaned reads (15-29 nt.) for the four-leaf sample of chilli pepper