Genome-wide identification, expression profiling, and target gene analysis of microRNAs in the Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), vectors of tospoviruses (Bunyaviridae)

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Abstract

*Thrips tabaci* Lindeman is an important polyphagous insect pest species estimated to cause losses of more than U.S. $1 billion worldwide annually. Chemical insecticides are of limited use in the management of *T. tabaci* due to the thigmokinetic behavior and development of resistance to insecticides. There is an urgent need to find alternative management strategies. Small noncoding RNAs (sncRNAs) especially microRNAs (miRNAs) hold great promise as key regulators of gene expression in a wide range of organisms. MiRNAs are a group of endogenously originated sncRNA known to regulate gene expression in animals, plants, and protozoans. In this study, we explored these RNAs in *T. tabaci* using deep sequencing to provide a basis for future studies of their biological and physiological roles in governing gene expression. Apart from snoRNAs and piRNAs, our study identified nine novel and 130 known miRNAs from *T. tabaci*. Functional classification of the targets for these miRNAs predicted that majority are involved in regulating transcription, translation, signal transduction and genetic information processing. The higher expression of few miRNAs (such as tta-miR-281, tta-miR-184, tta-miR-3533, tta-miR-N1, tta-miR-N7, and tta-miR-N9) in *T. tabaci* pupal and adult stages reflected their possible role in larval and adult development, metamorphosis, parthenogenesis, and reproduction. This is the first exploration of the miRNAome in *T. tabaci*, which not only provides insights into their possible role in insect metamorphosis, growth, and development but also offer an important resource for future pest management strategies.

Keywords

deep-sequencing, miRNAs, sRNAs, Stem-loop RT-PCR, *Thrips tabaci*, tospovirus

1 | INTRODUCTION

Onion thrips, *Thrips tabaci* Lindemann (Figure 1), is an important polyphagous insect pest species (Lewis, 1973) belonging to the family Thripidae. Besides onions, it is known to infest around 300 plant species, including economically important crops such as tobacco, leek, cabbage, pea, melon, lettuce, potato, tomato, carnation (Díaz-Montano, Fuchs, Nault, Fail, & Shelton, 2011; Lewis, 1997; Mandal et al., 2012).
Thrips tabaci is also a vector of two viral pathogens, *Iris yellow spot virus* (IYSV) (Srinivasan et al., 2012) and *Tomato spotted wilt virus* (TSWV) (Pittman, 1927) causing significant disease around the world (German, Ullman, & Moyer, 1992). *Thrips tabaci* is estimated to cause more than U.S. $1 billion in crop losses annually worldwide. To date, chemical insecticides have been widely used for the management of *T. tabaci*, but due to its thigmokinetic behavior and frequent development of insecticide resistance, they have had little use. Therefore, the design of novel insecticides, resistance breeding strategies, an in-depth understanding of genes and gene regulation is necessary for targeting important developmental factors/processes for effective management of this insect. MiRNA analysis is an effective tool to understand gene regulation and expression in both insect and host plant.

MicroRNAs (miRNAs) are a group of small, sequence-specific, endogenously originated noncoding RNA (ncRNA) molecules containing ~18–25 nucleotides (nts), and their main function is to regulate gene expression in animals, plants, and protozoans. MiRNAs controls around 60% of protein-coding gene activities and regulates many cellular processes (Fabian, Sonenberg, & Filipowicz, 2010; Friedman, Farh, Burge, & Bartel, 2009). The function of miRNAs appears to regulate gene expression either by translation repression or by degradation of mRNA through deadenylation (Chekulaeva & Filipowicz, 2009). MiRNA-mediated gene regulation plays a significant role in cellular and developmental processes, for instance in cell division, cell death, disease, hormone secretion, and neural development (Ambros, 2004; Miska et al., 2007; Nohata, Hanazawa, Kinoshita, Okamoto, & Seki, 2012; Singh & Nagaraju, 2008). The first miRNA, Lin-4 gene, was discovered by Lee, Feinbaum, and Ambros (1993) in *Caenorhabditis elegans*. Consequently, several miRNAs have been discovered from wide varieties of organisms including insects (Lagos-Quintana, Rauhut, Lendeckel, & Tuschl, 2001), plants (Bartel, 2004), viruses (Cullen, 2006), and vertebrates (Lim, Glasner, Yekta, Burge, & Bartel, 2003).

Identification of miRNA includes three principle approaches, forward genetics, bioinformatics prediction (Rebijieth et al., 2014; Zhang, Pan, Cannon, Cobb, & Anderson, 2006), and direct cloning and sequencing (Chen et al., 2005; Lagos-Quintana et al., 2001; Lee & Ambros, 2001). High-throughput next-generation sequencing (NGS) emerged as a powerful tool to identify miRNAs from animals and plants (Calla & Geib, 2015; Guillem, Bastian, Maria-Dolors, & Xavier, 2016; Nandety, Sharif, Kamita, Ramasamy, & Falk, 2015; Song et al., 2011; Wang et al., 2012; Wu et al., 2013). It has accelerated the pace of miRNA discovery from various animals and plants (Avesson, Reimegard, Wagner, & Söderbom, 2012; Burnside et al., 2008; Ge et al., 2013; Hu et al., 2012; Kang et al., 2012; Koh et al., 2010; Zhang et al., 2012).

So far, the miRNAome for insects is far behind nematodes, plants, and mammals (Kakumani et al., 2015). MiRNAs are reported from about 25 species of insects belonging to various orders (Stark et al., 2007; Wu et al., 2013). No information is available on *T. tabaci* miRNA content and function. Our study reports the detailed profile of miRNAs from *T. tabaci*. Further analysis identified putative target genes for these miRNAs, which will shed more light on the identification of highly specific miRNAs for thysanopteran pest management in the near future.

## MATERIALS AND METHODS

### 2.1 Insect culture and RNA isolation

*Thrips tabaci* cultures were maintained on *Phaseolus vulgaris* in controlled laboratory conditions at 25°C (DeGraaf & Wood, 2009) with an 8 hr:16 hr light:dark cycle. Total RNA was isolated from whole-body homogenates of sample mix, containing a total of 50 mg of different life stages viz. eggs, larvae, pupae, and adults of *T. tabaci* using TRIzol reagent (Invitrogen, Carlsbad, CA, USA).

### 2.2 Sample preparation and Illumina sequencing

Samples were processed according to Illumina TruSeq™ Small RNA sample preparation guide. Size fractionated small RNA populations (18–28 nts) were extracted, purified, and ligated to 3’ and 5’ adapters using T4 RNA Ligase (Life Technologies, Ambion, USA). Ligated products were reverse transcribed using SuperScript II (Life Technologies, Invitrogen, USA) followed by PCR amplification with 11 cycles and two size selection gels. High-throughput sequencing of the small RNA libraries was performed on Illumina Hiseq2000.

### 2.3 Bioinformatics analysis of small RNA sequencing data

The obtained sequenced dataset was subjected to initial quality check, and the raw reads were taken for adapter trimming and filtering of low-quality data. Thus, obtained sequencing data were queued against Rfam (http://rfam.sanger.ac.uk/) and RepBase (http://
www.girinst.org/repbase/) as references to annotate the ncRNAs viz. rRNAs, tRNAs, snRNAs, snoRNAs, and repeat-associated small RNAs and degraded fragments of expressed genes (exons and introns) in the remaining sequences. Remaining unique sequences were aligned with the miRBase (v21, http://www.mirBase.org/) entries to identify the conserved miRNAs. Novel miRNAs and their star reads were identified using the miRDeep2 (Friedlander, Mackowiak, Li, Chen, & Rajewsky, 2012) and miRCat (http://srna-workbench.cmp.uea.ac.uk/tools/mircat/). Potential secondary hairpin structures for identified novel miRNAs were predicted by employing Mfold (http://mfold.rna.albany.edu/?q_mfold/RNA-folding-form).

Homology analysis was performed with conserved miRNAs of T. tabaci with the miRNAs of other organisms from the miRBase database (Release 21.0; Griffiths-Jones, Saini, van Dongen, & Enright, 2008). BLASTn embedded in the miRBase database was used to compare the T. tabaci miRNAs with other species, with an E-value of .01 to find out more miRNA homologs. The naming of the miRNAs in this study has been performed according to Griffiths-Jones, Grocock, van Dongen, Bateman, & Enright, 2006. As these miRNAs were predicted from T. tabaci, the prefix for all miRNAs was fixed as “tta.” The rest of the naming convention criteria were in accordance with miRBase (Griffiths-Jones et al., 2006).

### 2.4 Phylogenetic analysis of microRNA family

All the identified miRNAs were classified into different miRNA precursor families (www.rfam.sanger.ac.uk), and primary sequence analyses were performed by employing Bloedit (Hall, 1999) and Weblogo (http://weblogo.berkeley.edu/logo.cgi). Few miRNA families such as miR-8, miR-14, miR-276, and miR-281 were selected for phylogenetic analysis employing RaxML.v.7.0.4 (Stamatakis, 2008).

### 2.5 Target prediction

Targets for identified miRNAs were predicted employing the miRanda program (Enright et al., 2004), against the expressed sequence tags (ESTs) and transcriptome (NCBI Accession: PRJNA203209) database of Frankliniella occidentalis. An alignment score (Smith & Waterman, 1981) greater than or equal to 100 and miRNA:mRNA Minimum Free Energy (MFE, ΔG) less than ~20 kcal/mol were considered as putative targets.

### Table 1
Summary Statistics of Thrips tabaci small RNA data analysis

| Number of trimmed reads | 13,192,454 |
|-------------------------|------------|
| Mapped to mRNA          | 2,378,671  |
| Repbase mapped reads    | 1,396,829  |
| Rfam mapped reads       | 4,181,894  |
| Rfam unmapped reads     | 9,010,560  |
| miRBase mapped reads    | 47,570     |
| Total unmappable for miRNA | 5,187,490 |
| Average length          | 23         |

### Table 2
Small RNAs (Piwi RNAs) with nucleotide lengths larger than 25 nucleotides obtained from Thrips tabaci sequencing data

| smallRNA ID | Sequence (5’→3’) | Length (nt) | Hit in the piRNABank | E-value  |
|-------------|------------------|-------------|----------------------|----------|
| tta_piR1    | ATGGTGGTCTAGTGGTAGAATTCTGGCC | 28         | hsa_piR_018570       | .00065   |
| tta_piR2    | GGGTTCGTTAGTCGGAACCA | 26         | dr_piR_0017650       | .1       |
| tta_piR3    | TTTCCGAGTGGTTACGTCGTCGTTATACGCTTC | 28         | rno_piR_005901       | 1.70E-05 |
| tta_piR4    | CCAAAGCAUGCCAGGCGCACGGCG   | 26         | dr_piR_0052831       | .0047    |
| tta_piR5    | ATGGTGGTCTAGTGGTAGAATTCTGGCC | 28         | hsa_piR_001312       | 1.80E-05 |
| tta_piR6    | CCCTCGGTCTCGGCGTGCTAGC | 27         | No Hit                | NA       |
| tta_piR7    | CCGTGTGGTCTAGTGGTAGAATTCTGGCC | 28         | ona_piR_166322       | .00049   |
| miRNA family | Expression values* (Reads) | Length (nt) | Name of the miRNA | Sequence (5’–3’) | Resource |
|--------------|---------------------------|-------------|--------------------|------------------|----------|
| mir-281      | 468                       | 19          | tta-miR-281a       | AAGAGAGCUAUCGGUCAGC | Aedes aegypti |
| mir-281      | 17583                     | 22          | tta-miR-281b       | AAGAGAGCUAUCGGUCAGCAGU | Bombyx mori |
| mir-281      | 4                         | 22          | tta-miR-281c       | AAGAGAGCUAUCGGUCAGCAGU | Drosophila ananassae |
| mir-281      | 5                         | 21          | tta-miR-281d       | AAGGGAGAUCUGUCGAGCAGU | Lotta gigantea |
| mir-281      | 3                         | 22          | tta-miR-281e       | UGUCAUGAGAUGGUCUCUCUUUU | Branchiostoma belcheri |
| mir-276      | 507                       | 21          | tta-miR-276a       | UAGGAACUUCAUCGGUCAGC | Aedes aegypti |
| mir-276      | 25904                     | 22          | tta-miR-276b       | UAGGAACUUCAUCGGUCAGCUCU | Locusta migratoria |
| mir-276      | 7                         | 22          | tta-miR-276c       | UAGGAACUUCAUCGGUCAGCUCU | Drosophila ananassae |
| mir-306      | 173                       | 21          | tta-miR-306a       | UCAGGUACUGGAGACUCAGUGA | Bombyx mori |
| mir-306      | 3507                      | 22          | tta-miR-306b       | UCAGGUACUGGAGACUCAGAG | Aphiellera |
| mir-306      | 1976                      | 22          | tta-miR-306c       | UCAGGUACUGGAGACUCUCAG | Aedes aegypti |
| bantam       | 61                        | 21          | tta-miR-bantam-a   | UGAGAUCAUUGGAAAGCUAAU | Brugia malayi |
| bantam       | 33                        | 22          | tta-miR-bantam-b   | UGAGAUCAUUGGAAAGCUAGAU | Aedes aegypti |
| bantam       | 1889                      | 23          | tta-miR-bantam-c   | UGAGAUCAUUGGAAAGCUAGAU | Aphiellera |
| bantam       | 5                         | 23          | tta-miR-bantam-d   | UGAGAUCAUUGGAAAGCUAAU | Acryosophon pisum |
| mir-92       | 6                         | 20          | tta-miR-92a        | UAUGACACUGCUGCCCGCUGU | Brugia malayi |
| mir-92       | 8                         | 22          | tta-miR-92b        | UAUGACACUGCUGCCCGCUGU | Bombyx mori |
| mir-92       | 6                         | 22          | tta-miR-92c        | UAUGACACUGCUGCCCGCUGU | Ciona savignyi |
| mir-92       | 76                        | 22          | tta-miR-92d        | UAUGACACUGCUGCCCGCUGU | Oikopleura dioica |
| mir-92       | 4                         | 23          | tta-miR-92e        | UAUGACACUGCUGCCCGCUGU | Tribolium castaneum |
| mir-92       | 1861                      | 22          | tta-miR-92f        | UAUGACACUGCUGCCCGCUGU | Aphiellera |
| mir-92       | 629                       | 22          | tta-miR-92 g       | UAUGACACUGCUGCCCGCUGU | Lytechinus variegatus |
| mir-92       | 46                        | 22          | tta-miR-92 h       | AAUUGCACCGUGGCGCGCCUGA | Aphiellera |
| mir-750      | 4                         | 22          | tta-miR-750a       | CAGAUCUACUGUCCCCAGCUCA | Lotta gigantea |
| mir-750      | 1242                      | 22          | tta-miR-750b       | CAGAUCUACUGUCCCCAGCUCA | Aphiellera |
| mir-750      | 107                       | 23          | tta-miR-750c       | CAGAUCUACUGUCCCCAGCUCA | Capitella teleta |
| mir-10       | 433                       | 21          | tta-miR-10a        | ACCCGUGAUCGCGAAUUGU | Acryosophon pisum |
| mir-10       | 6                         | 21          | tta-miR-10b        | ACCCGUGAUCGCGAAUUGU | Ovis aries |
| mir-10       | 4                         | 22          | tta-miR-10c        | ACCCGUGAUCGCGAAUUGU | Anolis carolinensis |
| mir-10       | 6                         | 22          | tta-miR-10d        | ACCCGUGAUCGCGAAUUGU | Aedes aegypti |
| mir-10       | 9                         | 22          | tta-miR-10e        | ACCCGUGAUCGCGAAUUGU | Gyrodactylus salaris |
| mir-10       | 52                        | 22          | tta-miR-10f        | ACCCGUGAUCGCGAAUUGU | Lotta gigantea |
| mir-10       | 3                         | 23          | tta-miR-10 g       | ACCCGUGAUCGCGAAUUGU | Bos taurus |
| mir-10       | 16                        | 23          | tta-miR-10 h       | ACCCGUGAUCGCGAAUUGU | Anolis carolinensis |
| mir-10       | 3                         | 23          | tta-miR-10i        | ACCCGUGAUCGCGAAUUGU | Schmidtea mediterranea |
| mir-100      | 5                          | 21          | tta-miR-100a       | ACCCGUGAUCGCGAAUUGU | Capra hircus |
| mir-100      | 20                         | 22          | tta-miR-100b       | ACCCGUGAUCGCGAAUUGU | Ateles geoffroyi |
| mir-100      | 57                         | 23          | tta-miR-100c       | ACCCGUGAUCGCGAAUUGU | Branchiostoma floridiae |
| mir-100      | 3                          | 24          | tta-miR-100d       | ACCCGUGAUCGCGAAUUGU | Ascaris suum |
| mir-1000     | 11                         | 18          | tta-miR-1000a      | AUAAUGUCCUGUCACAGC | Tribolium castaneum |

(Continues)
TABLE 3 (Continued)

| miRNA family | Expression values<sup>a</sup> (Reads) | Length (nt) | Name of the miRNA | Sequence (5′–3′) | Resource |
|--------------|-----------------------------------|-------------|-------------------|------------------|-----------|
| mir-1000     | 192                               | 21          | tta-miR-1000b     | AUAAUGUCCUGUCACAGCAGU | Drosophila melanogaster |
| mir-1000     | 183                               | 22          | tta-miR-1000c     | AUAAUGUCCUGUCACAGCAGUA | Drosophila pseudoobscura |
| mir-8        | 248                               | 22          | tta-miR-8a        | UAAUACUGUACGGUAAAGUGU | Culex quinquefasciatus |
| mir-8        | 15951                             | 23          | tta-miR-8b        | UAAUACUGUACGGUAAAGUGUC | Capitella teleta |
| mir-9        | 5                                 | 22          | tta-miR-8c        | CAUUCUACCUGGCAUCAGAUAAGA | Aedes aegypti |
| mir-9        | 8                                 | 18          | tta-miR-9a        | UCUUUGGAUCCUGACGCGGUA | Aedes aegypti |
| mir-9        | 7                                 | 21          | tta-miR-9b        | UCUCUGGUAUCCUGAUGUAUGA | Tribolium castaneum |
| mir-9        | 6                                 | 21          | tta-miR-9c        | UCUUUGGAUCCUGAUGUAUGA | Capitella teleta |
| mir-9        | 13                                | 23          | tta-miR-9d        | UCUUUGGAUCCUGAUGUAUGA | Capitella teleta |
| mir-9        | 4                                 | 24          | tta-miR-9e        | UCUUUGGAUCCUGAUGUAUGA | Schmidtea mediterranea |
| mir-2        | 21                                | 23          | tta-miR-2a        | UAAUACACCGCCGUUAUGAGC | Apis mellifera |
| mir-2        | 27                                | 23          | tta-miR-2b        | UAAUACACCGCCGUUAUGAGC | Lottia gigantea |
| mir-2        | 27                                | 24          | tta-miR-2c        | UAAUACACCGCCGUUAUGAGC | Aedes aegypti |
| mir-184      | 7145                              | 21          | tta-miR-184a      | UGGACGGAGAACUGAUAAGGG | Anopheles gambiae |
| mir-184      | 142                               | 22          | tta-miR-184b      | UGGACGGAGAACUGAUAAGGGU | Anolis carolinensis |
| mir-184      | 118                               | 22          | tta-miR-184c      | UGGACGGAGAACUGAUAAGGGC | Anopheles gambiae |
| mir-279      | 20                                | 21          | tta-miR-279a      | UGACUAGAUCACACACACAUCC | Acyrthosiphon pism |
| mir-279      | 58                                | 22          | tta-miR-279b      | UGACUAGAUCACACACACAUCC | Lottia gigantea |
| mir-279      | 14                                | 22          | tta-miR-279c      | UGACUAGAUCACACACACAUUUAA | Anopheles gambiae |
| mir-279      | 4                                 | 22          | tta-miR-279d      | UGACUAGAUCACACACACAUUGA | Bombyx mori |
| mir-279      | 105                               | 22          | tta-miR-279e      | UGACUAGAUCACACACACUCUCCA | Apis mellifera |
| mir-279      | 635                               | 22          | tta-miR-279f      | UGACUAGAUCACACACACUCUGU | Bombyx mori |
| mir-279      | 26                                | 24          | tta-miR-279 g     | UGACUAGAUCGAAAACACCGUCC | Apis mellifera |
| mir-279      | 103                               | 25          | tta-miR-279 h     | UGACUAGAUCGAAAACACCGUCC | Apis mellifera |
| mir-279      | 65                                | 21          | tta-miR-279a      | AGGCGCCGCGGAACACUACC | Nasonia vitripennis |
| mir-279      | 5                                 | 22          | tta-miR-279b      | GUAGGCAGCGCGGAACACUACC | Acyrthosiphon pism |
| mir-279      | 168                               | 23          | tta-miR-279c      | GUAGGCAGCGCGGAACACUACC | Acyrthosiphon pism |
| mir-14       | 26                                | 21          | tta-miR-14a       | UCAGUCUUUUCUCUCUCUCUAU | Anopheles gambiae |
| mir-14       | 12427                             | 22          | tta-miR-14b       | UCAGUCUUUUCUCUCUCUCUAU | Acyrthosiphon pism |
| mir-993      | 3                                 | 20          | tta-miR-993a      | UACCCUGAUCAGCGCCGCUUUAGC | Tribolium castaneum |
| mir-993      | 110                               | 23          | tta-miR-993b      | GAAGCGCCGCGGAACACUACC | Acyrthosiphon pism |
| mir-993      | 10                                | 23          | tta-miR-993c      | UACCCUGAUCAGCGCCGCUUUAGC | Manduca sexta |
| mir-993      | 3                                 | 23          | tta-miR-993d      | UACCCUGAUCAGCGCCGCUUUAGC | Drosophila melanogaster |
| mir-1175     | 106                               | 23          | tta-miR-1175a     | AAGUGGAGCAGGGGUCUCUCUAC | Tribolium castaneum |
| mir-1175     | 17                                | 22          | tta-miR-1175b     | AAGUGGAGCAGGGGUCUCUCUAC | Aedes aegypti |
| mir-1175     | 4                                 | 23          | tta-miR-1175c     | UGAGAUACUGCUUGUCUCAUCUACUAC | Apis mellifera |
| mir-1175     | 56                                | 24          | tta-miR-1175d     | UGAGAUACUGCUUGUCUCAUCUACUACUAC | Bombyx mori |
| mir-124      | 106                               | 21          | tta-miR-124a      | UAAGCCACCGCGUGAAUGCCA | Schmidtea mediterranea |
| mir-124      | 84                                | 21          | tta-miR-124b      | UAAGCCACCGCGUGAAUGCCA | Anolis carolinensis |
| mir-263      | 4                                 | 21          | tta-miR-263a      | AAGUGGAGCAGGGGUCUCUCUAC | Bombyx mori |
| mir-263      | 18                                | 23          | tta-miR-263b      | AAGUGGAGCAGGGGUCUCUCUAC | Aedes aegypti |

(Continues)
| miRNA family | Expression values (Reads) | Length (nt) | Name of the miRNA | Sequence (5′–3′) | Resource |
|--------------|--------------------------|------------|------------------|------------------|-----------|
| mir-263      | 20                       | 24         | tta-miR-263c     | AAUGGCACUGGAAGAAUUCACGGG | Drosophila melanogaster |
| mir-2944     | 18                       | 22         | tta-miR-2944a    | UAUCACAGCAUGAUAAGGACUGA | Aedes aegypti       |
| mir-2944     | 13                       | 23         | tta-miR-2944b    | UAUCACAGCAUGAUAAGGACUGGU | Apis mellifera     |
| mir-13       | 399                      | 22         | tta-miR-13a      | UAUCACAGCAUGAUAAGGACUGA | Tribolium castaneum |
| mir-13       | 17                       | 23         | tta-miR-13b      | UAUCACAGCAUGAUAAGGACUGA | Bombix mori        |
| mir-34       | 15                       | 22         | tta-miR-34a      | UGCGAGUGGUGUGGACUGUGUG | Aedes aegypti      |
| mir-34       | 5                        | 23         | tta-miR-34b      | UGCGAGUGGUGUGGACUGUGUG | Ascaris suum       |
| mir-34       | 3                        | 23         | tta-miR-34c      | UGCGAGUGGUGUGGACUGUGUAG | Lottia gigantea    |
| mir-133      | 15                       | 22         | tta-miR-133a     | UUGGUUCGCCUGUACGACUGU | Schmidtea mediterranea |
| mir-133      | 14                       | 22         | tta-miR-133b     | UUGGUUCGCCUCUACAGCGACUGU | Drosophila persimilis |
| mir-317      | 5                        | 21         | tta-miR-317a     | UGAAACAGCUCCUGUUGAUCUC | Acyrthosiphon pisum |
| mir-317      | 13                       | 24         | tta-miR-317b     | UGAAACAGCUCCUGUUGAUCUC | Lottia gigantea    |
| mir-317      | 13                       | 25         | tta-miR-317c     | UGAAACAGCUCCUGUUGAUCUC | Apis mellifera     |
| mir-317      | 4                        | 25         | tta-miR-317d     | UGAAACAGCUCCUGUUGAUCUC | Capitella teleta   |
| mir-12       | 13                       | 21         | tta-miR-12a      | UGAGAUUAACUGCCAGUGUGU | Tribolium castaneum |
| mir-12       | 13                       | 21         | tta-miR-12b      | UGAGAUUAACUGCCAGUGUGU | Daphnia pulex      |
| mir-252      | 4                        | 22         | tta-miR-252a     | CUAAGACUGUCCGAGGACUG | Drosophila melanogaster |
| mir-252      | 5                        | 23         | tta-miR-252b     | CUAAGACUGUCCGAGGACUG | Saccoglossus kowalevski |
| mir-277      | 11                       | 22         | tta-miR-277a     | UAAAUGCACUAUCUUGUGACGAC | Aedes aegypti    |
| mir-277      | 5                        | 23         | tta-miR-277b     | UAAAUGCACUAUCUUGUGACGAC | Acyrthosiphon pisum |
| mir-31       | 3                        | 21         | tta-miR-31a      | AGGCAAGAUGUCGACAUAGCU | Tribolium castaneum |
| mir-31       | 7                        | 22         | tta-miR-31b      | GGCAAGAUGUCGACAUAGCU | Apis mellifera    |
| mir-3477     | 69                       | 23         | tta-miR-3477a    | UAAAUCUACUGCUACUGAG | Apis mellifera    |
| mir-3477     | 121                      | 22         | tta-miR-3477b    | UAAAUCUACUGCUACUGAG | Apis mellifera    |
| mir-2779     | 5                        | 20         | tta-miR-2779     | AUACAUCGUGUCCACAGA | Bombix mori       |
| mir-929      | 4                        | 22         | tta-miR-929      | AAAUGACUCUACUGAGGACUG | Drosophila melanogaster |
| mir-71       | 172                      | 22         | tta-miR-71       | UCUCACUACCUCUGUCUUCAU | Tribolium castaneum |
| mir-375      | 4                        | 22         | tta-miR-375      | UUUUGUUCGUUGCUCGACUA | Apis mellifera    |
| mir-190      | 3                        | 24         | tta-miR-190      | AGAUAGUUUGAUUUUCUGUGUG | Acyrthosiphon pisum |
| mir-7550     | 3                        | 18         | tta-miR-7550     | AUCCGCUCGCAAGACCA | Ictalurus punctatus |
| mir-482      | 3                        | 22         | tta-miR-482      | GGAUGGGCGUAUGAGGAA | Phaeolus vulgaris  |
| mir-2478     | 3                        | 20         | tta-miR-2478     | GUACCACACUCUGACCA | Bos taurus         |
| mir-316      | 3                        | 21         | tta-miR-316      | UGCUUUUUCUGCUUUGUCUG | Heliconius melpomene |
| mir-3049     | 98                       | 23         | tta-miR-3049     | UCUGGAGUGAGUUGCGGCGGAU | Apis mellifera |
| mir-996      | 57                       | 21         | tta-miR-996      | UGACUAGAUCAUACUGACU | Apis mellifera    |
| mir-275      | 40                       | 23         | tta-miR-275      | UCUGGACUGAAGAUGCCCG | Anopheles gambiae |
| mir-965      | 31                       | 22         | tta-miR-965      | UAAACAGUAUGCUUUCUCUU | Tribolium castaneum |
| mir-67       | 25                       | 24         | tta-miR-67       | UAACACUCUCUGAGAUGAGUGA | Ascaris suum       |
| mir-315      | 21                       | 23         | tta-miR-315      | UUAUGAUGUGUUGCUAAGACCC | Acyrthosiphon pisum |
| mir-305      | 14                       | 23         | tta-miR-305      | UUUGUAUGUAUGAUGUUG | Tetranychus urticae |
| mir-894      | 11                       | 20         | tta-miR-894      | CGUUUCAUGUCGGUUCAC | Physcomitrella patens |

(Continues)
### TABLE 3 (Continued)

| miRNA family | Expression values (Reads) | Length (nt) | Name of the miRNA | Sequence (5’–3’) | Resource |
|--------------|--------------------------|-------------|--------------------|------------------|----------|
| mir-3533     | 9                        | 20          | tta-miR-3533       | AUGAAGUGUGAGCUAGCAGACAU | Bos taurus |
| mir-307      | 9                        | 20          | tta-miR-307        | UCACAACCUUGUAGUGAG | Daphnia pulex |
| mir-2765     | 664                      | 22          | tta-miR-2765       | UGGUAACUCACCCUGUGGC | Bombyx mori |
| mir-210      | 22                       | 21          | tta-miR-210        | CUUGUGCGUGACACCGCGU | Drosophila melanogaster |
| mir-1        | 650                      | 22          | tta-miR-1          | UGGAAUGUAAGAGUAAGGAG | Drosophila melanogaster |
| mir-87       | 18                       | 21          | tta-miR-87         | GUGAGCAAAGUUCAGGUUG | Ixodes scapularis |
| let-7        | 279                      | 21          | tta-let-7          | TGAGGTAGTGGGTATTGTAT | Drosophila melanogaster |
| mir-3791     | 15                       | 21          | tta-miR-3791       | UCACCCGUGAGAUCAUCA | Apis mellifera |

**Plant-specific miRNA**

| mir-9774     | 6                        | 22          | –                  | CAAGATATGGGTATTGTGTTC | Triticum aestivum |

*Expression value is equivalent to number of miRNA reads from the library.

### TABLE 4 Homology analysis of *Thrips tabaci* miRNA homologs

| tta-miR | Insects | Other Arthropods | Other Invertebrates | Vertebrates | Note |
|---------|---------|------------------|---------------------|-------------|------|
| tta-bantam | √       |        |        |             | Invertebrate specific |
| tta-let-7   | √       | √     |        |             | Highly conserved |
| tta-miR-1   | √       |       |        |             | Insect specific |
| tta-miR-10  | √       | √     |        |             | Highly conserved |
| tta-miR-100 | √       |       |        |             | Highly conserved |
| tta-miR-1000 | √      |       |        |             | Insect specific |
| tta-miR-1175 | √      |       |        |             | Invertebrate specific |
| tta-miR-12  | √       |       |        |             | Insect specific |
| tta-miR-124 | √       | √     |        |             | Highly conserved |
| tta-miR-13  | √       |       |        |             | Insect specific |
| tta-miR-133 | √       | √     |        |             | Highly conserved |
| tta-miR-14  | √       |       |        |             | Insect specific |
| tta-miR-184 | √       | √     |        |             | Highly conserved |
| tta-miR-190 | √       |       |        |             | Highly conserved |
| tta-miR-2   | √       | √     |        |             | Invertebrate specific |
| tta-miR-210 | √       |       |        |             | Highly conserved |
| tta-miR-2478 | –      |       |        |             | Vertebrate specific |
| tta-miR-252 | √       |       |        |             | Invertebrate specific |
| tta-miR-263 | √       | √     |        |             | Invertebrate specific |
| tta-miR-275 | √       | √     |        |             | Arthropod specific |
| tta-miR-276 | √       |       |        |             | Arthropod specific |
| tta-miR-2765 | √      |       |        |             | Insect specific |
| tta-miR-277 | √       |       |        |             | Insect specific |
| tta-miR-2779 | √      |       |        |             | Insect specific |
| tta-miR-279 | √       | √     |        |             | Invertebrate specific |
| tta-miR-2796 | √      |       |        |             | Insect specific |
| tta-miR-281 | √       | √     |        |             | Highly conserved |
| tta-miR-2944 | √      |       |        |             | Insect specific |

*(Continues)*
target genes. The targets were further annotated against NCBI-RefSeq invertebrate protein database and Gene Ontology (GO) terms were assigned (using Blast-2-GO) based on the annotation. The circo plot was generated using Circos (Krzewinski et al., 2009) to visualize the interaction between miRNAs and their targets.

2.6 | Validation of *Thrips tabaci* miRNAs using Stem-loop RT-PCR

We were able to validate six conserved and four novel microRNAs employing Stem-loop RT-PCR primers designed based on previous reports (Chen et al., 2005).

2.7 | Differential expression of *Thrips tabaci* miRNAs using Quantitative Real-Time PCR

Differentially expressed and functionally significant ten miRNAs (six conserved and four novel) were selected for quantitative reverse transcriptase PCR (qRT-PCR). Total RNA was isolated from different life stages viz. larvae, pupae, and adults of *T. tabaci* using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Mir-X-miRNA qRT-PCR SYBR Kit (Clontech Laboratories, Inc., USA) was used for the qRT-PCR reactions. qRT-PCR was performed on Light Cycler 480 (Roche, USA) using 1:20 diluted cDNAs and SYBR Advantage Premix (Clontech Laboratories, Mountain View, USA), according to the manufacturer’s instructions. All the qRT-PCR assays were conducted according to the MIQE guidelines (Bustin et al., 2009). qRT-PCR assays were performed in triplicates for three independent biological replicates, and the relative gene expression data were analyzed using 2^(-ΔΔCt) method (Livak & Schmittgen, 2001). U6 snRNAs was used as an internal control gene for normalization. The values of these three independent experiments were statistically analyzed using one-way ANOVA to calculate the statistical significance.

### RESULTS

3.1 | Illumina sequencing of *Thrips tabaci* small RNAs

The small RNA library prepared for deep sequencing resulted in a total of 13,192,454 raw reads (Table 1). After various mapping (Table 1), the trimmed high-quality small RNA reads were employed to identify
Table 5  Details of Thrips tabaci novel miRNAs and its star strands obtained from this study. Information regarding mature, star and precursor sequences, start and end position, orientation, expression values, MFE value and (A+U) content, etc. have been given.

| MicroRNA Family | Name of the Novel miRNA | miRNA sequence | miRNA* sequence | Hairpin sequence | Start | End | Orientation | miRNA Reads | miRNA* Reads | MFE |
|-----------------|-------------------------|----------------|-----------------|-----------------|-------|-----|-------------|--------------|--------------|-----|
| Novel miRNA-1   | tta-miR-N1              | AGGUAAUACACU   | NO              | NO              | 3630  | 3650| - [Negative strand] | 28           | Nil          | -32.9 |
|                 |                         | UGCGAGGCCA     |                 |                 |       |     |             |              |              |      |
| Novel miRNA-2   | tta-miR-N2              | UUCGUUGUGCGG   | NO              | NO              | 1439  | 1460| + [Positive strand] | 6            | Nil          | -40  |
|                 |                         | AAAAUGGAU      |                 |                 |       |     |             |              |              |      |
| Novel miRNA-3   | tta-miR-N3              | AUCAGCGAGUUC   | NO              | NO              | 11956 | 11976| + [Positive strand] | 15           | Nil          | -39.6 |
|                 |                         | UGCGACUAC      |                 |                 |       |     |             |              |              |      |
| Novel miRNA-4   | tta-miR-N4              | UGACUACAGUC    | NO              | NO              | 8647  | 8667| + [Positive strand] | 3414         | Nil          | -37.2 |
|                 |                         | UCACUCGUCU     |                 |                 |       |     |             |              |              |      |
| Novel miRNA-5   | tta-miR-N5              | UGGUAACUAC     | NO              | NO              | 13477 | 13497| - [Negative strand] | 305          | Nil          | -35.1 |
|                 |                         | UGCGGGGCCCA    |                 |                 |       |     |             |              |              |      |
| Novel miRNA-6   | tta-miR-N6              | UUGGUUCGCU     | NO              | NO              | 1925  | 1964| + [Positive strand] | 528          | 14           | -37.9 |
|                 |                         | CGCGUCGGUGUG   |                 |                 |       |     |             |              |              |      |
| Novel miRNA-7   | tta-miR-N7              | UCAGGUACCAG    | NO              | NO              | 12973 | 12993| - [Negative strand] | 1978         | 1            | -36  |
|                 |                         | AAGUAGGCGC     |                 |                 |       |     |             |              |              |      |
| Novel miRNA-8   | tta-miR-N8              | UCUCUUGGGAU    | NO              | NO              | 14289 | 14310| + [Positive strand] | 22           | 12           | -29.3 |
|                 |                         | UUGCGGAUGUGG   |                 |                 |       |     |             |              |              |      |
| Novel miRNA-9   | tta-miR-N9              | CGGCUCGGUGUG   | NO              | NO              | 274   | 294 | + [Positive strand] | 12           | 3            | -34.8 |
both known and novel miRNAs. Size distributions of the trimmed high-quality reads were varied from 18 to 26 nts with a peak at the 23 nts (Figure 2). A small portion of our library consisted of read length of around 26–28 nts, which could be putative piwi-interacting RNAs (pi-RNAs) from T. tabaci as the homology search against the piRNABank database revealed that some of these were similar to previously reported piRNAs (Table 2).

3.2 | Identification of known miRNAs from *Thrips tabaci*

Our analyses on the trimmed high-quality reads resulted in a total of 130 conserved miRNAs representing 55 different miRNA families (Table 3). Among the known miRNAs, miR-276, miR-281, miR-8, and miR-14 are highly expressed with an expression value of 26,418, 18,063, 16,204, and 12,453, respectively (Table 3). Analysis of the 55 miRNA families revealed that most of them were present in arthropod species (Table 4), with many homologous miRNAs from *Aedes aegypti*, *Apis mellifera*, *Bombyx mori*, *Acyrthosiphon pisum*, and *Tribolium castaneum* (Figure S1).

3.3 | Identification of novel miRNAs from *Thrips tabaci*

Miranalyzer pipeline identified a total of nine novel miRNAs from *T. tabaci* for the first time (Table 5), with their predicted precursor secondary structures (Figure 3). The complete details of the mature miRNAs and their corresponding pre-miRNAs have been given in Table 5. The length of the novel miRNAs ranged from 21 to 22 nucleotides with a preference of Uracil (66.7%) followed by Adenine (22.2%) at the 5′ end. The length of the pre-miRNAs was in the range of 63–76 nucleotides with an average Minimum Free Energy (MFE) of ~35.97 kcal/mol, indicating pre-miRNAs are readily folded into their secondary structures. Among these nine miRNAs, three were located in the 5′ arm while the other six arose from 3′ arm (Table 5, Figure 3). tta-miR-N4 (3414 copies) and tta-miR-N7 (1978 copies) were having the highest abundance compared to the remaining novel miRNAs (Table 5).

3.4 | The presence of miRNA star strands

It is very difficult to identify the star strand (miRNA*) sequences from the library, as it will be degraded soon after being exported to the cytosol. However, our results revealed that ten *T. tabaci* miRNA* families (mir-14, mir-184, mir-8, mir-276, mir-210, mir-1, mir-3477, mir-71, mir-13, and let-7) were identified within the known miRNA category (Table 6). The expression values (number of reads) of all miRNA* s were lower than that of their corresponding miRNAs (Table 6). Among the miRNA* family, mir-8 and mir-276 families were having the highest abundance with 308 and 258 copies, respectively. Our results

![FIGURE 3](image-url) Stem-loop structures of nine novel *Thrips tabaci* miRNAs indicating mature miRNA sequence (green color) and miRNA star strand sequences (red color)
**TABLE 6** Details of *Thrips tabaci* miRNA*s* obtained from this study. Information regarding mature, star and precursor sequences, start and end position, orientation, expression values, MFE value and (A+U) content, etc. have been given.

| MicroRNA Family | Name of the miRNA* | miRNA | miRNA* | Hairpin sequence | Start | End | miRNA* Abundance | Minimum Free Energy(MFE) | Hairpin G/C% |
|-----------------|---------------------|-------|--------|------------------|-------|-----|------------------|---------------------------|-------------|
| mir-14          | tta-tta-miR*-14     | UCAGUCUUUUCUC UUCUCUCAU | GGGGAGAGAUAGGGCUUU GGUUGGCU(5) | GGGGAGAGAUAGGGCUUU GGUCAGAUUUUAGAUCAGU CUAGCUUUUUUCUCUCUCUCC | 345   | 366 | 5                | -26.7                     | 45.614033   |
| mir-184         | tta-miR*-184       | UGGACCGGAGAAUCUG AUAAAGGG | CCUCUGUAUUCUCG UGUCCGCGU(21) | CGCCUCUCUGUCAUUCUGGU UCUCUGGUUGCAUCA CUAACUGGAGGGAACUCU GAUAAGGGGCG | 592   | 612 | 21               | -33.5                     | 54.411762   |
| mir-8           | tta-miR*-8         | UAAUACUGUCAGG UAAAGAUGUC | CAUCUUACC GGCAUAGA(308) | UCCUGUUCACAUUCAUACG CGGAGAUAAUACUGAG UCAGUUAAAGAUGUCUCAG | 3,662 | 3,684 | 308              | -27.7                     | 43.421055   |
| mir-276         | tta-miR*-276       | UAGGAACUCAUA CCGUCGCU | UAGCGGAGUAAAGAUCAGU ACAGGCUGGCAAGA(7) | UCCAGUACUGGAGGUAUG AGUUCGUGCGCUUCUGUGCU AGUCAGAAGAAACUCUAA UACCGUGUCUCUGG | 4,052 | 4,073 | 258              | -33.9                     | 49.275364   |
| mir-210         | tta-miR*-210       | CUUUGUGCGUGU GACAGCGGCU | AGCUGCGUGGACACU GCACACUGGCAAGA(196) | AGCUGCGGACUGCAGACAA GAUAAGACCUUUGGAAAC UCUGUUCGUGCGGACAGCGGCU | 10,847 | 10,867 | 8                | -28.09                    | 50          |
| mir-1           | tta-miR*-1         | UGGAAGUAAUAG AAGUAUGGAG | CCAUACUCUUCUUGGCU CCCUAAUACUCUACUCUACUCUCCUAU(4) | GUUCCUCAUCUUCCUGGCUUC CCCUUUACUUCUUGGAGAUAUGUAACUUGAAACCUAAU GGAUGUAAAGAAGAUAGGAGC | 581   | 602 | 11               | -24.86                    | 38.46154    |
| mir-3477        | tta-miR*-3477      | UAAUACUGUUGU GGUACUGUGA | UAGGCGGGUGCCCG UAGGUGUG(1) | GUUACUACUGUUGUACUGUUGUAGACGUUCCGGGUUC CGCUGGAGGUUGU GC | 5,735 | 5,756 | 1                | -31.3                     | 49.152542   |
| mir-71          | tta-miR*-71        | UCUCUACUGCU UGUCUUCUAUG | UGAAAGACAUUGGGUAG UGAGAU(19) UGAAAGACAUUGGGUAG UGAGAU(20) | GGUGUAGCUUGAAGACAUUG GUGAUGCUUGAAGACAUUGUUCGUGAUGACUCAUCUCAUCUGCUUUCUCAUGUGUC | 1,176 | 1,197 | 39               | -47.1                     | 46.575344   |
| mir-13          | tta-miR*-13        | UAAUCACAGCCAC UUUGAUGGAG | GCCUACUAUACUCGCGCU GUGAGACUCACACUGCGAUGGCAGC(17) | GAGGGCGGAGCCGAUCAUAC GGCUGAGACACACACUGCGAUGGCAGC UCUUCAUGUGGCUCU | 1,427 | 1,449 | 81               | -41                       | 51.282055   |
| let-7           | tta-miR*-let-7     | UGAGGUGUAGU GGUUACUGA | CUGUACAAUUGGCUA ACUUCUC(2) CUGUACAUCUUCUACUCUACUUCUCUUC(4) | GCGGGGUGUAGUAGUAGUGUG GGUUAAGUAAAGAACACAAACUCUUUGGAGUACUGAUCUGGUACUG | 1,826 | 1,846 | 6                | -31.9                     | 45.56962    |
also indicated the presence of miRNA* sequences in four of our novel miRNAs such as tta-miR-N6, tta-miR-N7, tta-miR-N8, and tta-miR-N9, although the abundance was low (Table 5). The complete characteristic features of these miRNA* sequences and their corresponding pre-miRNA’s have been given in Tables 5 and 6.

3.5 | Identification of plant miRNA family in *Thrips tabaci* sRNA library

Interestingly, this study has identified mir-9774 (Expression value 6), a plant microRNA family in our *T. tabaci* sRNA library (Table 3).

3.6 | Phylogenetic analysis of *Thrips tabaci* miRNAs

Phylogenetic analyses revealed that most of the known miRNAs are highly conserved (Table 4, Figure 4a1–d1 and Figure 4a3–d3) among various species within the Kingdom and the phylogenetic trees for miR-8, miR-14, miR-276, and miR-281 revealed that *T. tabaci* miRNAs grouped with the closely related species of insects (Figure 4a2–d2). Figure 4 also revealed that *T. tabaci* miRNAs are well conserved, particularly in the seed region compared to the homologous miRNAs from other species.

3.7 | Identification of targets for *Thrips tabaci* miRNAs

Targets were predicted for known and novel miRNAs of *T. tabaci* employing miRanda with a scale of 0–7 to indicate the stringency of miRNA-target pairing with the smaller numbers representing higher stringency. ESTs and transcriptome of *F. occidentalis* were used as a reference for target searches with a cut-off score 140.

3.7.1 | Targets for known miRNAs from *Thrips tabaci*

One hundred and thirty known miRNAs were searched for targets against ESTs and transcriptome sequences of *F. occidentalis*. A total of 218 and 1,025 targets were obtained from ESTs and transcriptome, respectively (Tables S1 and S2). The Blast-2-GO enrichment analysis was performed employing gene ontology (GO) terms for genes targeted by these miRNAs (Figure 5a,b). For those targets in the ESTs, three motifs were over-represented in GO–BP (biological process) category viz. “metabolic process,” “transport,” and “catabolic process.” The GO–MF (molecular function) category was over-represented by the motif “oxidoreductase activity” and “catalytic activity” (Figure 5a). On the other hand, GO terms enrichment analysis of miRNA targets in the transcriptome yielded motifs for “transport,” “signal transduction,” and “metabolic process” in GO–BP category; while, GO–MF category was over-represented with motifs for “ATP binding,” “transferase activity,” and “binding” (Figure 5b). Complete details of the Blast-2-GO analysis were provided in Tables S3 and S4.

3.7.2 | Targets for novel miRNAs from *Thrips tabaci*

Novel miRNAs were searched for their targets in the *F. occidentalis* transcriptome. A total of 65 miRNA-target pairs were obtained (Table S5), and further Blast-2-GO analysis indicated the over-representation of “Transport” and “ATP binding” as GO–BP and GO–MF category, respectively (Figure 6 and Table S6).

3.7.3 | Synteny analysis using Circos

The synteny analysis of the *T. tabaci* miRNAs and their targets were performed by employing circos (Krzywinski et al., 2009). In brief, the Blast analysis was performed using *T. tabaci* miRNA sequences (known and novel) against *F. occidentalis* scaffolds (Approx. largest 200). The positions of miRNAs were identified and their targets are represented in the Circos plot (Figure 7).

3.8 | Validation of *Thrips tabaci* microRNAs

This study revealed 130 known and nine novel miRNAs from *T. tabaci*. However, further validation of these miRNAs was performed by (1) stem-loop endpoint reverse transcriptase PCR (RT–PCR) and (2) real-time quantitative reverse transcriptase PCR (RT-qPCR). Using stem-loop endpoint RT-PCR, we have validated six conserved viz. tta-miR-281, tta-miR-276, tta-miR-10, tta-miR-100, tta-miR-184, and tta-miR-3533 and four novel miRNAs viz. tta-miR-N1, tta-miR-N4, tta-miR-N7, tta-miR-N9 from *T. tabaci* using the primer sets as described (Table S7). All of these miRNAs were amplified with an approximate product size of 75 bp (Figure 8a).

Our study also quantified the expression level of the above-mentioned ten miRNAs from *T. tabaci* larva, pupa, and adult using RT-qPCR (Table S8, Figure 8b). Results suggested that the miRNA expression was higher in pupal and adult stages compared to larval stages in six microRNAs such as tta-miR-281, tta-miR-184, tta-miR-3533, tta-miR-N1, tta-miR-N7, and tta-miR-N9 (Figure 8b).
FIGURE 4 Continued
4 | DISCUSSION

The onion thrips, *Thrips tabaci*, is an important pest species and a tospovirus vector causing significant negative impacts on yield and quality of various economically important crops (German et al., 1992). Although microRNAs are key gene regulators and are involved in many biological processes, including growth and development, no previous study has been conducted on the identification and validation of miRNAs in *T. tabaci*. MicroRNAs are known from more than 25 insect species, (Stark et al., 2007). Several miRNAs have been reported from various orders of insects such as Diptera, Hymenoptera, Coleoptera, Orthoptera, Lepidoptera, Hemiptera, Homoptera (Wu et al., 2013), and Thysanoptera (Rebijith, Asokan, Hande, & Krishna Kumar, 2016). This study reports the complete miRNA profile from onion thrips, *Thrips tabaci*. A small RNA library was prepared from the pooled samples of different developmental stages of *T. tabaci* and the high-throughput Illumina deep-sequencing technology (Avesson et al., 2012; Burnside et al., 2008; Ge et al., 2013; Koh et al., 2010) was used to identify miRNAs from the prepared library.

We used the *F. occidentalis* genome sequence as a reference for *T. tabaci*, as the complete genome *T. tabaci* is still not available in the database. The higher percentage of mapping (91%) was possible only because both these insects belong to the same family, Thripidae. Employing this approach, our study revealed 130 conserved and nine novel miRNAs from *T. tabaci*. The size distributions of the high-quality reads were varied from 18 to 28 nts in our library and the peak was at the 25 nt, which was on par with previous studies (Ge et al., 2013; Liang, Feng, Zhou, & Gao, 2013; Sattar et al., 2012). Our study indicated the unique read distributes of 26–28 nts with a relative lower abundance, which is common in many small RNA libraries (Chang et al., 2016; Jagadeeswaran et al., 2010; Surridge et al., 2011; Zhang et al., 2013), indicating the presence of piRNAs. Piwi RNAs

![Gene Ontology (GO) classification of the putative target genes for the conserved *T. tabaci* miRNAs against ESTs (a) and transcriptome (b) sequences of *F. occidentalis*. GO terms was assigned to each target gene based on the annotation and were summarized into three main GO categories viz. (1) biological process (BP), (2) molecular function (MF), and (3) cellular component (CC). Only top ten subcategories are presented here.](image-url)
(piRNAs) are the class of small RNAs mediating chromatin modifications (Ross, Weiner, & Lin, 2014) which are derived mainly from retrotransposons and other repetitive elements with high sequence diversity (Ross et al., 2014; Siomi, Sato, Pezic & Aravin, 2011; Zhang et al., 2013). Thus, our results indicated that T. tabaci genome not only harbors miRNAs but also other small RNAs such as piRNAs that might be involved in the transgenerational epigenetic inheritance (Weick & Miska, 2014).

MiRNAs are evolutionarily conserved regulators of gene expression (Rebijith et al., 2014; Zhang et al., 2009), and few can even act as markers in defining the evolutionary relationship in a wide range of insect species (Kakumani et al., 2015). Our homology and phylogeny analysis revealed that insect miRNAs are well-conserved, despite considerable diversity in the genome (Figure 4a–d). MiRNA’s are not easily detectable as it degrades soon after being exported to the cytosol (Wu et al., 2013). However, our results indicated the presence of several miRNA’s (Tables 5 and 6) that matched to the same precursor sequences with their mismatched complementary mature miRNAs.

We identified the presence of a plant-specific miRNA family, mir-9774 in the T. tabaci sRNA library, and the same has been recently reported from Triticum aestivum L. and Brachypodium distachyon (L.) Beav (Wei et al., 2009). Previous miRNA studies on cotton/melon aphid, A. gossypii also reported six plant miRNA family (Sattar et al., 2012). They also showed that such microRNAs were transformed into the aphid tissues (especially in gut contents) during the phloem sap ingestion. However, none of those six have been identified in our sRNA library.

Our results showed that the highest expression is for tta-miR-276 with an expression value of 26,418. Very recent studies showed that miR-276 expressed in the ovaries of female locusts mediates progeny egg-hatching synchrony by upregulating its target brahma (brm), a transcription coactivator gene (He et al., 2016). Thus, it is plausible that miR-276 enhances brm expression to promote developmental synchrony and provide insight into the regulation of developmental homeostasis in T. tabaci. The second highest expression is for miR-281 with an expression value of 18,063 and might be involved in the development and metamorphosis of T. tabaci as recent studies showed that miR-281 regulates the expression of ecdysone receptor (EcR) isoform B, in Bombyx mori (Jiang et al., 2013). Another interesting microRNA obtained in the current study was miR-8, and it can target the Wingless signalling pathway to regulate secretion of yolk protein precursors by the female mosquito fat body and accumulation into the developing ovaries (Lucas et al., 2015, http://www.smartscitech.com/index.php/RD/article/view/815). Therefore, it is quite possible that miR-8 may play a key role in the reproductive processes of T. tabaci. An insect-specific miR-14 was identified in T. tabaci with an expression value of 12,453 and studies on lepidopteran insects showed the antiapoptotic role of miR-14 (Kumarswamy & Chandna, 2010). The rest of the species-specific miRNAs identified in T. tabaci might play important role in insect-specific features, such as metamorphosis, parthenogenesis, and biogenesis of pheromones (Zhang et al., 2007). Whereas, the other invertebrate- and vertebrate-specific miRNAs (Table 3) identified from T. tabaci required special attention, as their non-existence in other species of insects could be due to the absence of complete genomic information for most of those insects (Ge et al., 2013).

The expression profile of miRNA varies spatiotemporally among different developmental stages (Li, Cassidy, Reinke, Fischboeck, & Carthew, 2009; Xu, Zhou, Wang,Auersperg, & Peng, 2006), and the developmental expression profiles (larval, pupal and adult stage) of ten microRNAs were studied by RT- qPCR (Figure 8b). The higher expression of tta-miR-281, tta-miR-184, tta-miR-3533, tta-miR-N1, tta-miR-N7, and tta-miR-N9 in T. tabaci pupal and adult stages reflected their possible role in parthenogenesis, adult development, and sexual reproduction. The high levels of miR-276 in the larval stage...
indicated their possible involvement in insect-specific features such as metamorphosis.

miRNAs regulate the gene expression through targeting transcripts that can bring about mRNA cleavage, mRNA decay or translational repression of target miRNAs by binding to 3’ UTRs, 5’ UTRs, and even to coding regions (Lytle, Yario, & Steitz, 2007). Thus, it is important to identify the gene targets and thereby we can understand the biological role of a particular miRNA. As miRNA targets have been identified using the (1) expressed sequence tags (ESTs) and (2) transcriptomic sequences of *F. occidentalis*. The GO annotations for the predicted targets were classified as potential biological process, cellular component, and molecular function. The putative targeted genes included signal transduction pathways, transcription factors, reproduction, embryo development, insect molting, immune response, and even metabolism. Overall, the results from our study indicated that these conserved and novel miRNAs identified from *T. tabaci* might play crucial regulatory role in the regulation of thrips growth and development.
In summary, the result from our study add to the pool of miRNA databases and is the first report of small RNAs from *Thrips tabaci*, a nonmodel insect lacking genome information. One hundred and thirty conserved and nine novel miRNAs were identified with high confidence and sufficient evidence is the major contribution of our study. Sequence analyses revealed that most of the *T. tabaci* miRNAs are highly conserved in various species, making miRNAs, a hallmark of evolutionarily conserved regulators of gene expression. To harmonize the data and to provide more useful biological insights, we have also carried out in silico analysis of identifying potential targets for these miRNAs. Our results indicated that the list of putative mRNA targets was very extensive and most of the putative target genes for *T. tabaci* miRNAs were associated with several KEGG pathways such as metabolic process, transport, translation, signal pathways, and oxidative phosphorylation. However, further experiments are required for the validation of these targets. Expression levels of *T. tabaci* miRNAs were validated by RT-qPCR, and the results indicated few of these miRNAs have been predicted in the adult development process, which can be further utilized in gene functional studies through RNAi-based approach or in developing miRNA mimics both for feeding and *in planta* expression (Agrawal, Sachdev, Rodrigues, Sowjanya Sree, & Bhatnagar, 2013; Jayachandran, Hussain, & Asgari, 2013; Nandety et al., 2015) as novel pest management strategies based on gene silencing and insect transgenesis.

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**DATA AVAILABILITY**

All relevant data are within the paper and its Supporting Information files. The small RNA Sequence data has been submitted to NCBI under the BioSample project ‘PRJNA350618’; BioSample Accession: ‘SAMN05943039’.

**CONFLICT OF INTEREST**

The authors have declared that no competing interests exist.

**AUTHORS’ CONTRIBUTIONS**

Conceptualization: KBR HRH, Experiments: KBR HRH, Reagents/materials: KBR RA SG, Writing—original draft: KBR, Writing—review and editing: KBR HRH RA SG NKK.
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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

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