Identification and conformational analysis of putative microRNAs in *Maruca vitrata* (Lepidoptera: Pyralidae)

C. Shruthi Sureshan, S.K.M. Habeeb *

Department of Bioinformatics, School of Bioengineering, SRM University, Kattankulathur 603203, Tamil Nadu, India

**Abstract**

MicroRNAs (miRNAs) are a class of small RNAs, evolutionarily conserved endogenous non-coding RNAs that regulate their target mRNA expression by either inactivating or degrading mRNA genes; thus playing an important role in the growth and development of an organism. *Maruca vitrata* is an insect pest of leguminous plants like pigeon pea, cowpea and mung bean and is pantropical. In this study, we perform BLAST on all known miRNAs against the transcriptome data of *M. vitrata* and thirteen miRNAs were identified. These miRNAs were characterised and their target genes were identified using TargetScan and were functionally annotated using Flybase. The importance of the structure of pre-miRNA in the Drosha activity led to study the backbone torsion angles of predicted pre-miRNAs (mvi-miR-9751, mvi-miR-649-3p, mvi-miR-4057 and mvi-miR-1271) to identify various nucleotide triplets that contribute to the variation of torsion angle values at various structural motifs of a pre-miRNA.

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1. Introduction

Insect infestation on a crop leads to loss in yield and quality, and then the insect becomes agricultural pest. The legume pod borer, *Maruca vitrata* (lepidopteran) is one of the serious pest of grain legumes in the tropics and sub-tropics (Sharma, 1998); and they are known to affect overall production by causing damage to pigeon pea (Gopali et al., 2001), mung bean (Zahid et al., 2008) and cowpea (Asante et al., 2010), which in turn depends on the structural motifs like terminal loop, internal loops, and bulges present in the pre-miRNA Terminal loop plays a major role in the cleaving action of Drosha and Dicer on the pre-miRNA (Starega-Roslan et al., 2011). As the Dicer cleaves it to generate mature miRNA (pre-miRNA). The pre-miRNA is transported to cytoplasm where RNase III enzyme called Dicer cleaves it to generate mature miRNA (pre-miRNA).

**Keywords:** MicroRNA, Transcriptome, Precursor microRNA, Torsion angle

*Corresponding author.
E-mail address: habeeb_skm@yahoo.co.in (S.K.M. Habeeb).
angle $\chi$ and there is a correlation between the angles — $\alpha \leftrightarrow \gamma$ and $\epsilon \leftrightarrow \zeta$ (Saenger, 1983). The torsion angle studies had been applied to DNA Holliday Junction structure (Eichman et al., 2002), $\alpha/\gamma$ transitions in B-DNA backbone (Djurinovic et al., 2002), conformational classification of RNA (Schneider et al., 2004) and structural modifications in histones (Sanli et al., 2011). Analysis of torsion angles determines the irregularities in a structure. Every backbone torsion angle consists of certain range of values that maintains the integrity of the structure. Deviation from these values distorts the structure and hence its function (Temiz et al., 2012). In this study, we have investigated the fluctuations imposed on torsion angles with the variations observed in the sequence of miRNAs (Svozil et al., 2008).

Existing transcriptome data were used to identify and characterise the putative miRNAs in *M. vitrata*; in order to predict target genes for the predicted miRNA and functionally annotate them using TargetScan and FlyBase respectively; and also to study the sequence dependant variations in backbone torsion angles in predicted miRNAs.

### 2. Materials and method

#### 2.1. Data collection and identification of putative miRNA and their precursor

The transcriptome data of *M. vitrata* was retrieved from Sequence Read Archive, NCBI. The complete collection of miRNAs was downloaded from miRBase (Griffiths-Jones et al., 2006). The transcriptome data was processed to generate contigs and remove redundant sequences, and only the non-homologue sequences were used for further analysis.

![Fig. 1. The secondary structures of putative miRNAs: The secondary structure consists of a stem and loop structures. The highlighted regions represent the mature microRNA in the hairpin structure.](image-url)
The collected miRNA sequences were used as query for homologous
search against the transcriptome data, using standalone BLAST + 2.2.28
programme (Altschul et al., 1997). The hits obtained were considered as
the candidates for finding precursor miRNAs. These sequences were
submitted to Mfold to predict the secondary structures of precursor
miRNAs (Zuker, 2003). The secondary structures were predicted based
on criteria determined by Zhang et al. (2006):

1. The pre-miRNA could be able to fold into a typical hairpin secondary
structure.
2. The mature miRNA should be located in the stem region of the hairpin structure.
3. miRNA has less than seven mismatches with the complementary sequence in the opposite arm.
4. No loops or breaks are allowed in the miRNA or miRNA*duplex.
5. The negative MFE of the miRNA should be greater than $-18$ kcal/mol and the $(A + U)$ content must be in the range of 40–70%.

The predicted miRNAs were named in accordance with the rules determined in the miRBase (Griffiths-Jones et al., 2006).

2.2. Predicting targets of miRNA

The target genes of the predicted miRNAs were identified using TargetScan (Lewis et al., 2003). TargetScan predict targets of miRNAs by searching for the presence of conserved sites that match the seed region of miRNA. The target genes obtained are then directed to the FlyBase database (Dos Santos et al., 2014), which assist in the functional annotation.

2.3. Predicting three dimensional structure of miRNA and torsion angle analysis

The three dimensional structure of mvi-miR-9751, mvi-miR-6497-3p, mvi-miR-4057 and mvi-miR-1271 was constructed using MC-Sym (Parisien and Major, 2008), which provides a fully automated 3D structure from the user defined secondary structure (in Vienna format) and the sequence of microRNA in study. The PDB files created for the four predicted miRNAs were used to generate the torsion angle data using Curves + (Lavery et al., 2009). Graphs were generated to analyse torsion angle data (.lis file).

3. Results and discussion

In this study, 13 miRNAs were identified in the insect M. vitrata from the transcriptome data.

3.1. Characterisation of miRNA

All the predicted miRNAs have a typical stem-loop structure. The mature miRNAs were located either in 5′ arm (62%) or the 3′ arm (38%) of the stem loop structure. The secondary structures of all the predicted miRNAs are given in Fig. 1.

The length of mature miRNAs varies from 19 to 22 nucleotides and the length of pre-miRNAs varied from 55 to 96 nucleotides. Tables 1 and 2 show the details of precursor miRNAs and predicted mature miRNAs respectively.

Minimum free energy (MFE) calculated for the predicted miRNAs varied from $-51.2$ to $-16$ kcal/mol (Das, 2010). The A + U content for the predicted pre-miRNA varied from 24 to 80% (Asokan et al., 2013).

3.2. Identification of miRNA targets

In animals, the miRNA and microRNA Response Element (MRE) are almost never completely complementary to each other. The “seed” region which constitutes roughly 6–8 nucleotides of the 5′-end generally suffices the functional RISC formation (Brennecke et al., 2005). But recent

### Table 1
Details of predicted precursor miRNAs.

| miRNA         | miRNA sequence | MFE   | E value | A + U content |
|---------------|----------------|-------|---------|---------------|
| mvi-miR-6497-3p* | CGAAGGC CGGAA CGC GGUGCUG CAUUUC | $-38.5$ | 0.001   | 24.44         |
| mvi-miR-6497-3p | AGCGGA AGCGG CGC GCC GCCGCA UGGG | $-51.2$ | 0.001   | 26.08         |
| mvi-miR-4171-5p | GUAUCAU GAUAGU GACGGG GCCAAGG GAAGC | $-16.7$ | 0.003   | 56.75         |
| mvi-miR-466m-3p | UGACUAU AUAAGC CCACGG CAAUGAUA | $-19.4$ | 0.004   | 61.11         |
| mvi-miR-4057  | UGACUCAU CAUAGU GUAUAGU UGUAGU | $-17.9$ | 0.004   | 43.85         |
| mvi-miR-1271  | GACGUCAU CAUAGU GUAUAGU UGUAGU | $-21.7$ | 0.004   | 40            |
| mvi-miR-15b-3p | UGACGUA CGCGG CGCGG CGCGG CGCGG | $-20.8$ | 0.004   | 53.01         |
| mvi-miR-414   | UGACGUA CGCGG CGCGG CGCGG CGCGG | $-25.4$ | 0.004   | 47.91         |
| mvi-miR-33b-3p | GCCAACCU UACCGU GCCGGG GCCGGG GCCGGG | $-17.4$ | 0.004   | 48.61         |
| mvi-miR-6497-5p | UGACGUA CGCGG CGCGG CGCGG CGCGG | $-24.2$ | 2.00E-05 | 46.8          |
| mvi-miR-2966  | GCCGCCG CGCGG CGCGG CGCGG CGCGG | $-26.3$ | 2.00E-04 | 38.09         |
| mvi-miR-9751  | GCCGCCG CGCGG CGCGG CGCGG CGCGG | $-16$   | 4.00E-04 | 80            |
| mvi-miR-4968-3p | UGUUGA CGCGG CGCGG CGCGG CGCGG | $-22.8$ | 8.00E-04 | 56.32         |
studies prove that seed target regions at the 3’-end are conserved and thus demonstrating the predominant regulatory functions of miRNAs through 3’ UTRs (Gu et al., 2007; Friedman et al., 2009). In the current study, we have used 3’ UTR sequence data of Drosophila melanogaster in the TargetScan to confirm our targets (Table 3).

### 3.3. Functional annotation

A total of 141 targets were obtained for 13 miRNAs encoding for metamorphosis, cell signalling, transcription regulation, structural constituents, metabolism, and transmembrane transportation. Thus it proves the multi-level functioning of miRNAs in various molecular and cellular processes.

miRNAs targeted by mvi-miR-466m-3p and mvi-miR-1271 are associated with Hedgehog receptor activity and Ecdysis-triggering hormone receptor activity which are linked to metamorphosis. mvi-miR-9751 was seen to target genes mainly associated with transcription regulation, which is accomplished by sequence specific DNA binding proteins, RNA polymerase II transcription cofactor and histone methyltransferase activity. Further, mvi-miR-9751 also controlled the genes specific to GTPase activity and serotonin activity, which are integral to various signalling pathways. Similarly mvi-miR-4968-3p was found to be associated with transcription regulating proteins as well as signalling molecules (Ras GTPase binding).

mvi-miR-6497-3p* targets miRNAs linked to structural constituents of chorion (the outer shell of the insect egg) along with the protein serine/threonine phosphatase activity. Apart from mvi-miR-6497-3p*, structural constituents of chorion are also targeted by mvi-miR-414 and mvi-miR-35b-3p. mvi-miR-1271 and mvi-miR-4968-3p regulate the genes related to structural constituents of cytoskeleton.

### 3.4. Target multiplicity and cooperativity

Multiplicity is one of the common characteristics of miRNA regulation, such that one 3’ UTR has more than one MREs and thus assisting miRNA in having multiple targets (Ghosh et al., 2007). In our study we identified mvi-miR-9751 to have maximum plausible target miRNAs responsible for transcription regulation and signalling pathways.

Cooperativity is another feature shown by miRNA, where more than one miRNAs regulate a target mRNA, thus establishing an effective silencing (Ghosh et al., 2007). In our study we found that mvi-miR-6497-3p* and mvi-miR-35b-3p participate in the regulation of the gene FBgn0000359 (structure of chorion).

### 3.5. Torsion angle analysis

In order to study the fluctuations observed in torsion angle with respect to the variation in sequences, the structure of mvi-miR-9751 was divided into one loop, three stem sections and one internal loop. Similarly, one loop, five stem sections, one bulge and one internal loop in mvi-miR-649-3p; two stem sections and one internal loop for mvi-miR-4057; one external loop, two stem sections, one bulge and one loop for mvi-miR-1271 were noted down for the analysis. The four torsion angles, $\alpha$, $\gamma$, $\epsilon$ and $\zeta$ have shown deviation from their usual range of values. Similar to DNA sequences, miRNA has relationship between the torsion angles (Saenger, 1983):

1. $\alpha$ and $\gamma$
2. $\epsilon$ and $\zeta$

Figs. 2, 3, 4, 5 shows the relationships and deviations observed in the torsion angles in mvi-miR-9751, mvi-miR-649-3p, mvi-miR-4057 and mvi-miR-1271 respectively. Tables 4 and 5 shows the maximum and minimum values of $\alpha$, $\gamma$, $\epsilon$ and $\zeta$ torsion angles in the stems, loops, internal loops and bulge regions of the two miRNAs.

3.5.1. Deviation of alpha and gamma torsion angles

In general the values of $\alpha$ torsion angles for RNA is specific to the $\gamma$ (30° to 90°) of the Klyne and Prelog cycle. Most of the nucleotides have been found to be in this region, with few exceptions. There were deviations from the $\gamma$ region too. Our study showed deviations from this region, the $\gamma$ region, similarly, due to C3′ exo-puckering respectively. It was noted that most of the nucleotides in our miRNAs had $\gamma$ torsion angles that have occupied a different region other than $\gamma$. The epsilon values were found to be in similar ranges with $\gamma$ regions.

3.5.2. Deviation of epsilon and zeta torsion angles

The usual range of $\gamma$ torsion angles for RNA is $\gamma$ (30° to 90°) of the Klyne and Prelog cycle. All the four microRNA sequences have shown a predominant deviation to $\gamma$, $\epsilon$, $\gamma$, $\epsilon$ and $\zeta$ regions. Overall, both $\alpha$ and $\gamma$ values were found to be in similar ranges with respect to various studies (Schneider et al., 2004).

### Table 2
Details of predicted mature miRNAs.

| miRNA          | Contig/singlet | Start position | End position | Strand | miRNA sequence |
|----------------|----------------|----------------|--------------|--------|----------------|
| mvi-miR-6497-3p* | 1768           | 475            | 495          | 3'     | AGGCCGCCAGCCGCCGCCAGC |
| mvi-miR-6497-3p  | 524            | 133            | 153          | 5'     | GAUGCCGCCAGGCUGCUGC |
| mvi-miR-4171-3p  | 5676           | 3              | 22           | 5'     | UACACUGCUUUAGUGUAGC |
| mvi-miR-466m-3p  | 5130           | 450            | 471          | 5'     | UACAUCAACAUCACAUCAGUA |
| mvi-miR-4057    | 4013           | 332            | 311          | 5'     | UUGCCUGCACUCACCAACAGAU |
| mvi-miR-1271    | 1271           | 568            | 547          | 5'     | CUUGGACCCUGCUUAACAGA |
| mvi-miR-15b-3p  | 3546           | 95             | 134          | 3'     | AAGCAUAGUGUGCUUGU |
| mvi-miR-414     | 3442           | 746            | 727          | 5'     | CAUCUCAUCAUCAUCAG |
| mvi-miR-35b-3p  | 4714           | 347            | 327          | 5'     | UCACCGGGAACGUAUAGUU |
| mvi-miR-6497-5p | 367            | 388            | 375          | 5'     | GCCUCUGACGACCGGCGUUGCC |
| mvi-miR-2966    | 570            | 551            | 541          | 5'     | CCCUCCGCGGCUGCCGC |
| mvi-miR-9751    | 290            | 271            | 261          | 5'     | UELUUAACCAUCAUACCCUAAA |
| mvi-miR-4968-3p | 161            | 179            | 173          | 3'     | AGCAAACUGACCGACACAG |

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**Table 1**

miRNAs through three studies prove that seed target regions at the 3’-end are conserved and thus demonstrating the predominant regulatory functions of miRNAs.
| miRNA       | Target gene | Symbol     | Function                                           |
|-------------|-------------|------------|----------------------------------------------------|
| mvi-miR-6497-3p* | FBgn0035746 | CG17742    | Identical protein binding                         |
|             |             | CG1478     | Structural constituent of chorion                 |
|             |             | CG7109     | Serine/threonine phosphatase activity             |
| mvi-miR-6497-3p | FBgn0034100 | CG15709    | Intracellular cyclic nucleotide activated cation channel activity |
| mvi-miR-4171-5p | FBgn0003031 | CG5119     | mRNA 3'-UTR binding                               |
|             |             | CG11280    | Unknown                                           |
| mvi-miR-466m-3p | FBgn0013974 | CG42636    | Guanylate cyclase activity                        |
|             |             | CG2411     | Hedgehog receptor activity                         |
| mvi-miR-466m-3p | FBgn001234   | CG15709    | Intracellular cyclic nucleotide activated cation channel activity |
|             |             | CG18076    | Protein binding                                   |
| mvi-miR-4057 | FBgn0000286 | CG11924    | DNA binding                                       |
|             |             | CG33135    | Voltage-gated cation channel activity             |
| mvi-miR-4057 | FBgn0003892 | CG17941    | Cadherin binding; calcium ion binding             |
|             |             | CG13248    | Voltage-gated cation channel activity             |
| mvi-miR-4057 | FBgn0003520 | CG5753     | mRNA 3'-UTR binding                               |
| mvi-miR-1271 | FBgn0052062 | CG32062    | Transcription factor binding                       |
|             |             | CG11312    | Cytoskeletal adaptor activity                     |
| mvi-miR-1271 | FBgn001235   | CG1478     | Structural constituent of chorion                 |
|             |             | CG5119     | G-protein coupled acetylcholine receptor activity |
| mvi-miR-15b-3p | FBgn0001145 | CG1743     | Glutamate-ammonia ligase activity                 |
|             |             | CG32555    | Rho GTPase activating activity                     |
| mvi-miR-15b-3p | FBgn00266616 | CG17027    | Actin-dependent ATPase activity; protein homodimerization activity; structural constituent of muscle |
|             |             | CG17697    | Wnt-activated receptor activity                   |
| mvi-miR-15b-3p | FBgn0003525 | CG1395     | Protein tyrosine phosphatase activity             |
| mvi-miR-15b-3p | FBgn0003502 | CG1395     | Protein tyrosine kinase activity                  |
| mvi-miR-15b-3p | FBgn0003710 | CG1395     | Sodium channel regulator activity                 |
| mvi-miR-15b-3p | FBgn0004103 | CG5630     | Myosin phosphatase activity                       |
| mvi-miR-414  | FBgn0000359 | CG1478     | Structural constituent of chorion                 |
| mvi-miR-35b-3p | FBgn00021764 | CG5227     | Unknown                                           |
| mvi-miR-35b-3p | FBgn0000360 | CG11213    | Structural constituent of chorion                 |
| mvi-miR-6497-5p | FBgn0046636 | CG1956     | GTPase activity                                   |
| mvi-miR-2966  | FBgn0013995 | CG3254     | Polypeptide N-acetylglalactosaminyltransferase activity |
| mvi-miR-9751  | FBgn0003415 | CG9936     | RNA polymerase II transcription cofactor activity |
| mvi-miR-9751  | FBgn0037351 | CG1475     | Structural constituent of ribosome                |

(continued on next page)
Table 3 (continued)

| mRNA       | Target gene | Symbol       | Function                                                                 |
|------------|-------------|--------------|---------------------------------------------------------------------------|
| mvi-miR-9751 |             |              |                                                                           |
| Fh00037834 | CG555       | Histone methyltransferase activity [H4-R3 specific]; protein-arginine omega-N asymmetric methyltransferase activity |
| Fh0000448  | CG2183      | Ligand-activated sequence-specific DNA binding RNA polymerase II transcription factor activity; protein binding |
| Fh00015806 | CG1039      | Ribosomal protein S6 kinase activity                                      |
| Fh0002480  | CG5682      | Unfolded protein binding                                                  |
| Fh00063499 | CG17522     | Glutathione transferase activity                                          |
| Fh00010280 | CG5444      | RNA polymerase II core promoter sequence-specific DNA binding transcription factor activity involved in preinitiation complex assembly |
| Fh00041092 | CG13109     | Ligand-dependent nuclear receptor transcription coactivator; steroid hormone receptor binding |
| Fh00000907 | CG3166      | Protein binding; RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity |
| Fh00000247 | CG13037     | Rab GTPase binding; Rab guanyl-nucleotide exchange factor activity         |
| Fh00002525 | CG6842      | Calcium ion binding; myosin heavy chain binding; myosin V binding         |
| Fh00002823 | CG6384      | Chromatin insulator sequence binding; DNA binding; microtubule binding; POZ domain binding; SNARE binding |
| Fh00003137 | CG33103     | Extracellular matrix structural constituent                               |
| Fh00003410 | CG9949      | Protein binding; protein self-association                                  |
| Fh00003892 | CG2411      | Hedgehog receptor activity; lipoprotein particle receptor activity        |
| Fh00003944 | CG10388     | DNA binding; protein binding; protein domain specific binding; RNA polymerase II distal enhancer sequence-specific DNA binding |
| Fh00004168 | CG16720     | Serotonin receptor activity                                                |
| Fh00004242 | CG3139      | Calcium-dependent phospholipid binding; phosphatidylycerine binding; protein homodimerization activity; SNARE binding |
| Fh00005631 | CG13521     | Heparin binding; protein binding                                          |
| Fh00011217 | CG5425      | Ubiquitin conjugating enzyme activity; ubiquitin protein ligase activity; ubiquitin protein ligase binding |
| Fh00015790 | CG5771      | GTPase activity; protein binding; protein complex binding                 |
| Fh00262872 | CG43227     | Myosin binding                                                            |
| Fh00026391 | CG10961     | Olfactory receptor activity                                                |
| Fh00028675 | CG32975     | Acetylcholine binding                                                      |
| Fh00028996 | CG1922      | RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription |
| Fh00264386 | CG15899     | Low voltage-gated calcium channel activity                                |
| Fh00034691 | CG6562      | Inositol-polyphosphate 5-phosphatase activity                             |
| Fh00036373 | CG10741     | Transcription coactivator binding; transcription factor binding            |
| Fh00037050 | CG14723     | Histamine-gated chloride channel activity                                  |
| Fh00001233 | CG124       | Unfolded protein binding                                                   |
| Fh00002917 | CG1517      | Cation channel activity                                                    |
| Fh00261606 | CG15442     | Structural constituent of ribosome                                         |
| Fh00011224 | CG31000     | miRNA 3′-UTR binding; translation repressor activity, nucleic acid binding |
| Fh00011225 | CG5695      | Actin binding; actin filament binding; calmodulin binding; microtubule binding; myosin light chain binding |
| Fh00013467 | CG18285     | Calmodulin binding                                                        |
| Fh00001122 | CG2204      | GTP binding                                                               |
| Fh00003721 | CG4898      | Actin filament binding                                                     |
| Fh00011656 | CG1429      | RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription |
| Fh00259162 | CG42267     | ATP binding                                                               |
| Fh00042083 | CG3267      | CoA carboxylase activity                                                  |
| Fh00259227 | CG42327     | Protein tyrosine phosphatase activity                                      |
| Fh00002441 | CG5954      | Chromatin insulator sequence binding                                       |
| Fh00002932 | CG19888     | Phosphatidylinositol phosphate binding; protein binding; ubiquitin protein ligase activity |
| Fh00261873 | CG32717     | Protein binding                                                           |
| Fh00004364 | CG8896      | Transmembrane signalling receptor activity                                |
| Fh00003423 | CG1417      | Proline dehydrogenase activity                                            |
| Fh00004656 | CG1956      | GTPase activity; protein binding                                          |
| Fh00001016 | CG8996      | Electron carrier activity; flavin adenine dinucleotide binding             |
| Fh00264855 | CG4260      | Protein transporter activity                                               |
| Fh00020309 | CG14938     | Metal ion binding; nucleic acid binding                                    |
| Fh00027844 | CG7820      | Carbonate dehydratase activity; zinc ion binding                          |
| Fh00031432 | CG9964      | Electron carrier activity                                                  |
| Fh000264815 | CG44007     | 3′,5′-Cyclic-AMP phosphodiesterase activity                               |
| Fh000033095 | CG3409      | Monocarboxylic acid transmembrane transporter activity                    |
| Fh00003317 | CG8635      | Metal ion binding                                                         |
| Fh00033460 | CG1472      | Signal sequence binding; transporter activity; zinc ion binding            |
| Fh00003309 | CG13213     | SAM domain binding                                                        |
| Fh00034967 | CG3186      | Ribosome binding                                                          |
| Fh00035357 | CG1244      | Chromatin binding; nucleosome-dependent ATPase activity; protein binding   |
| Fh000035914 | CG6282     | Oxidoreductase activity, acting on the CH-CH group of donors              |
| Fh00036005 | CG3428      | Contributes to ubiquitin–protein transferase activity                     |
| Fh00036816 | CG3597      | Citrate transmembrane transporter activity; succinate transmembrane transporter activity |
| Fh00050286 | CG30286     | Serine-type endopeptidase activity                                         |
| Fh00025176 | CG42281     | Protein homodimerization activity; sequence-specific DNA binding transcription factor activity |
| Fh00038153 | CG14376     | Ligand-gated ion channel activity                                          |
| Fh00052654 | CG32654     | Ras GTPase binding                                                        |

3.5.3. Nucleotides showing deviation in torsion angles

The torsion angle values under the study have shown to deviate with respect to changes in nucleotide sequence (Svozil et al., 2008). Among all the four nucleotide, G and C have induced the most deviation in torsion angle (Arrigo et al., 2012). The values of torsion angles fluctuated with respect to certain patterns of nucleotide
Fig. 2. Variation in $\alpha$, $\gamma$, $\epsilon$ and $\zeta$ of mvi-miR-9751.

Fig. 3. Variation in $\alpha$, $\gamma$, $\epsilon$ and $\zeta$ of mvi-miR-6497-3p.

Fig. 4. Variation in $\alpha$, $\gamma$, $\epsilon$ and $\zeta$ of mvi-miR-4057.

Fig. 5. Variation in $\alpha$, $\gamma$, $\epsilon$ and $\zeta$ of mvi-miR-1271.
sequence and these patterns were termed as “nucleotide triplets” (Table 6).

Also, when a bulge occurs in the stem region of a miRNA, it leads to variation in torsion angle (Kumar et al., 2012; Popenda et al., 2008).

Hence it is concluded that sequence composition can affect various structural motifs present in a pre-miRNA. This can help in understanding the sequence dependant modulations occurring in the cleaving of pri-miRNA by Drosha to synthesis pre-miRNA (Krol and Krzyzosiak,

| miRNA     | R | POS | ALPHA | GAMMA |
|-----------|---|-----|-------|-------|
|           |   |     | MINIMUM | MAXIMUM | MINIMUM | MAXIMUM |
|           |   |     | BASE | BASE | BASE | BASE | BASE | BASE |
|           |   |     | θ | 3plet | θ | 3plet | θ | 3plet |
| mvr–miR–9751 | S1 | 1–3 | G2 | -131.4 | CGU | U3 | -129.5 | GUU |
|           | S2 | 4–23 | U12 | -145.5 | UUU | G16 | 86.6 | GUU |
|           | S3 | 54–74 | A72 | -178.8 | AAA | A59 | 174.7 | AAA |
|           |   | 26–34 | U26 | -141.4 | GGU | G32 | 138.9 | AGU |
|           |   | 40–48 | U46 | -156.6 | UUA | A48 | -68.6 | AAA |
| IL1       | L  | 49–53 | A52 | -173.7 | AUA | U53 | -39.8 | AUA |
| mvi–miR–6497–3p | S1 | 1–4 | C3 | -122.1 | GCG | A1 | 0 | AGC |
|           | S2 | 5–25 | G22 | -150.5 | GCC | C9 | -11.6 | GCC |
|           | S3 | 69–70 | C69 | -146.6 | UCC | C70 | -97.5 | CCC |
|           | S4 | 33–39 | C35 | -143 | CCA | C37 | -34.2 | AGG |
|           | S5 | 57–63 | G57 | -171.4 | UGC | G60 | 111.7 | GGG |
| IL1       | L  | 64–68 | A66 | -131.1 | GAA | G65 | 77.1 | AGA |
| mvi–miR–4057 | S1 | 1–6 | U6 | -130.8 | GUG | G16 | 0 | CUC |
|           | S2 | 53–57 | C54 | -153.4 | UGC | A57 | 7.7 | UGA |
|           | IL1 | 31–48 | U47 | -150.7 | CUG | C44 | 143.7 | CCC |
| mvi–miR–1271 | S1 | 1–6 | U6 | -130.8 | GUG | G16 | 0 | CUC |
|           | S2 | 53–57 | C54 | -153.4 | UGC | A57 | 7.7 | UGA |
|           | IL1 | 31–48 | U47 | -150.7 | CUG | C44 | 143.7 | CCC |

R—region; S—stem; IL—internal loop; B—bulge; EL—external loop; L—loop; POS—base position; θ—torsion angle value; 3plet—triplets. The coloured cells represent the torsion angle values that have deviated from the Klyne and Prelog cycle. Violet colour represents the highest deviation value and the orange colour represents the lowest deviation value.
2004; Starega-Roslan et al., 2011). Studies have shown that the size, location and the distribution of terminal loops and internal loops can affect the cleavage by Dicer. Therefore a shift in the cleavage sites of the enzymes Drosha and Dicer can result in the formation of isomiRs (isoforms of mature miRNAs) (Fernandez-Valverde et al., 2010; Neilsen et al., 2012).

| mRNA   | R   | POS | Epsilon | Zeta |
|--------|-----|-----|----------|------|
|        |     |     | MINIMUM  | MAXIMUM  |
|        | BASE | φ   | 3plet | BASE | φ | 3plet |
|        | BASE | φ | 3plet | BASE | φ | 3plet |
| mvi-miR | -9751 | 1–3 | G2 | -164.8 | CGU | U3 | -142.7 | GUU | U3 | -48 | GUU | C1 | -38 | CGU |
| S1     | 75–77 | G77 | -142.7 | CGU | A75 | -126.1 | AAC | G77 | -51 | CGU | C76 | -43.2 | ACG |
| S2     | 4–23 | U18 | -159.6 | UUU | A7 | 0 | UAG | U17 | -107.2 | GUU | A7 | 0 | UAG |
| S3     | 54–74 | A71 | -161.2 | AAA | C68 | 55 | CCG | C68 | -128.7 | CCG | A70 | 118.8 | GAA |
| S3     | 26–34 | U34 | -172.3 | UUA | U27 | -131.3 | UUA | A29 | -77.1 | AAA | U34 | 130.7 | UUA |
| S4     | 40–48 | U42 | -153.3 | AUU | C68 | 81.6 | AAA | A40 | -75.2 | AAA | A48 | 126.1 | AAA |
| IL1    | 24–25 | G25 | -146.2 | GGU | U2 | 0 | GGU | U2 | -131.3 | UUA | A29 | -77.1 | AAA |
| L      | 49–53 | A5 | -169.3 | AAA | A52 | 0 | AAU | A51 | -42.3 | AAA | A50 | 128.2 | AAA |
| L      | 35–39 | A37 | -171.7 | AAA | A35 | 0 | UAA | A37 | -98.8 | AAA | A36 | 87.1 | AAA |
| mvi-miR | -6497-3p | 1–4 | A1 | -146.1 | AGC | G2 | -140.9 | AGC | C3 | -65.1 | GCG | G4 | -37.6 | GGA |
| S1     | 91–92 | C91 | -128.7 | CGG | G92 | 0 | CCG | C91 | -59.5 | CCG | G92 | 0 | CCG |
| S2     | 5–25 | G17 | -168.9 | UGC | G22 | -102.9 | GGC | G8 | -115.8 | UGC | G22 | -20.5 | GGC |
| S3     | 71–90 | C78 | -167.2 | GCG | G72 | -105.9 | GGA | U88 | -101.3 | GUU | U80 | -14.1 | GUU |
| S4     | 26–27 | G26 | -149.7 | UGG | G27 | -146.1 | GGU | G27 | -79 | GCU | G26 | -73.7 | UGC |
| S5     | 69–70 | C70 | -155.6 | CGG | C69 | -124.2 | UGC | C69 | -36.3 | UCC | C70 | -23 | CCG |
| S4     | 33–39 | C39 | -160.8 | UGG | G35 | -116 | CCG | G35 | -98 | GGU | G38 | -35.4 | CCG |
| S4     | 57–63 | G59 | -161.1 | CGG | C63 | -116 | GGU | G60 | -98 | GGU | G58 | -35.4 | CCG |
| S4     | 41–45 | U45 | -168.5 | CUC | U43 | -124.8 | GUC | C41 | -98.5 | UGC | G44 | -36.4 | UCU |
| S4     | 50–54 | A52 | -153.5 | GAC | G50 | 160 | GGG | G51 | -44.4 | GGA | G54 | 0 | GGU |
| IL1    | 28–32 | G30 | -161.6 | CGG | C32 | -127.1 | ACG | G30 | -94.3 | GGA | G29 | -50.3 | UGC |
| B      | 40    | U40 | -153.9 | CUC | U40 | -68.7 | CUC | U40 | -68.7 | CUC | U40 | -68.7 | CUC |
| L      | 55–56 | U55 | 0 | GUU | U56 | 179.6 | UUG | U56 | -47 | UUG | U55 | 0 | GUU |
| L      | 46–49 | U47 | -159.9 | CUC | C48 | -130.5 | UGC | C46 | -87.7 | UCU | G49 | -55.3 | CAG |
| mvi-miR | -4057 | 1–6 | GA | -164.9 | CGG | G5 | -138.8 | GGU | G5 | -60.2 | GGU | G4 | -8.4 | CGG |
| S1     | 53–57 | G56 | -161 | UGA | A57 | 0 | UGA | G56 | -85.7 | UGA | A57 | 0 | UGA |
| S2     | 31–48 | C39 | -163.1 | GCC | G43 | 174.3 | GCC | G46 | -81 | CCG | G48 | 159.3 | UGC |
| IL1    | 1    | G7 | -141.3 | UGC | G7 | -68.1 | UGC | G7 | -68.1 | UGC | G7 | -68.1 | UGC |
| B      | 49–52 | A50 | -162.6 | GAG | G52 | -122.8 | GGA | G50 | -120.5 | GAG | G52 | 122.4 | GGA |
| L      | 2–4 | C2 | -138.9 | GCC | A4 | 0 | CAC | C3 | -66 | CCA | A4 | 0 | CAC |
| S2     | 51–53 | G52 | -147.3 | CUG | U55 | 0 | GGU | G52 | -60.7 | UGC | G53 | -50.9 | GGG |
| S2     | 6–26 | A20 | -170.4 | CAG | G13 | -48.8 | UGC | G13 | -151.8 | UGC | C26 | 84.5 | CCA |
| S2     | 32–50 | C44 | -169.4 | ACA | C41 | 0 | CCG | G35 | -107.7 | CCG | G41 | 0 | CCG |
| EL     | 1    | G1 | -140 | GCC | G13 | -50.9 | GCC | G13 | -50.9 | GCC | G13 | -50.9 | GCC |
| B      | 5    | C5 | -81.9 | ACG | G5 | -48.9 | ACG | C5 | -48.9 | ACG | G5 | -48.9 | ACG |
| L      | 27–31 | G29 | -159.3 | AGG | A27 | -124.8 | AAA | G29 | -85.4 | AGG | G30 | -32.3 | GGA |

R—region; S—stem; IL—internal loop; B—bulge; EL—external loop; L—loop; POS—base position; φ—torsion angle value; 3plet—triplets. The coloured cells represent the torsion angle values that have deviated from the Klyne and Prelog cycle. Violet colour represents the highest deviation value and the orange colour represents the lowest deviation value.
The outcome of this study can be implemented to investigate the effect of sequence variation in miRNAs and the resulting conformational changes observed during the binding of miRNAs to the RISC.

4. Conclusion

In the current study we identified thirteen putative miRNAs from *M. vitrata*. These miRNAs regulate miRNAs related to metamorphosis, cell signalling, transcription regulation, structural constituents, metabolism, and transmembrane transportation. miRNAs identified in the pest *M. vitrata* can be the initial step for an effective pest management programme.

Backbone torsion angles of precursor structures of mvi-miR-9751, mvi-miR-6497-3p, mvi-miR-4057 and mvi-miR-1271 show that secondary structure programme.

The outcome of this study can be implemented to investigate the effect of sequence variation in miRNAs and the resulting conformational changes observed during the binding of miRNAs to the RISC.

| Triplet(s) | Region | Alpha | Gamma | Epsilon | Zeta |
|-----------|--------|-------|--------|----------|------|
| CCG      | Stem   | –97.5 | 93.5   | –155.6  | –23  |
| GGU      | Stem   | 86.6  | –142.5 | –157.2  | –17.5|
| GGU      | Stem   | –131.4| 119.0  | –122.8  | –91.8|
| GCG      | Stem   | 133.3 | –91.4  | 160.0   | –2.1 |
| CCA      | Stem   | –127.3| 112.7  | –129.5  | 84.5 |
| UCC      | Internal loop | 166.3 | 159.2  | –141.3  | –68.1|
| GUA      | Internal loop | –49.1 | 52.1   | –161.6  | –94.3|
| AUA      | Loop   | 33.0  | –166.4 | –171.7  | 98.8 |
| AGG      | Loop   | 127.8 | –153.0 | –159.3  | –85.4|
| AGG      | Bulge  | 82.0  | 7.4    | –81.9   | –48.9|

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