Research article

In-silico based identification and functional analyses of miRNAs and their targets in Cowpea (Vigna unguiculata L.)

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Abstract: Cowpea (Vigna unguiculata L.) is an important leguminous plant and a good diet due to presence of carbohydrate and high protein contents. Currently, only few cowpea microRNAs (miRNAs) are reported. This study is intended to identify and functionally analyze new miRNAs and their targets in cowpea. An in-silico based homology search approach was applied and a total of 46 new miRNAs belonging to 45 families were identified and functionally annotated from the cowpea expressed sequence tags (ESTs). All these potential miRNAs are reported here for the first time in cowpea. The 46 new miRNAs were also observed with stable hairpin structures with minimum free energy, ranging from −10 to −132 kcal mol⁻¹ with an average of −40 kcal mol⁻¹. The length of new cowpea miRNAs are ranged from 18 to 26 nt with an average of 21 nt. The cowpea miRNA-vun-mir4414, is found as pre-miRNA cluster for the first time in cowpea. Furthermore, a set of 138 protein targets were also identified for these newly identified 46 cowpea miRNAs. These targets have significant role in various biological processes, like metabolism, transcription regulation as transcription factor, cell transport, signal transduction, growth & development and structural proteins. These findings are the significant basis to utilize and manage this important leguminous plant-cowpea for better nutritional properties and tolerance for biotic and abiotic stresses.

Keywords: microRNAs; conserved nature; cowpea (Vigna unguiculata L.); homology search

1. Introduction

MicroRNAs (miRNAs) are distinctive regulatory member of the small RNAs that regulate gene silencing at post-transcriptional level. Gene silencing by miRNAs is an important, advance and exciting area of present regulatory RNA research. They are endogenous, non-coding in nature and...
about 18 to 26 nucleotides (nt) in size. They are the negative regulator at post-transcriptional stage of gene regulation [1]. Initially, a self-folded stable hair-pin/stem-loop secondary structure termed as precursor-miRNAs (pre-miRNAs) is generate from long single strand RNA known as primary miRNA (pri-miRNA). Later the pre-miRNAs give rise a small sized (18–26nt) functional RNA known as mature miRNA. This mature miRNA is integrate into argonaute protein and advanced into the RNA induced silencing complex (RISC) [2,3]. The RISC complex having mature miRNA triggers post-transcriptional gene suppression of the messenger RNA (mRNA) either by inhibiting protein encoding or by activating mRNA degradation. This inhibition and degradation capability of the miRNA depends on the scale of complementarity between miRNA and its targeted mRNA [4]. In case of partial pairing between miRNAs and its mRNA target causes its inhibition. While, the complete pairing of miRNAs with it mRNA target causes the mRNAs degradation [1,5]. They participate as gene regulator in almost each and every life activity, such as growth and development, foreign genes suppression, signal transduction, environmental stresses and as a defense against the attacking microbes in various living organisms [1,6-9]. Majority of the miRNAs show conserved behavior among various plant species. Many researchers, based on this conserved nature, have identified a huge number of miRNAs using comparative genomic approaches in a wide range of plant species, including cowpea [10], Brassicanapus [11], Glycinemax [12], cotton species [13,14], Zeamays [15], tobacco [16], switch grass [17], Phaseolus [18], tomato [19], eggplant [20] and chilli [21]. These reports strongly suggest that comparative genomic strategies are valid, highly efficient, convenient, and economical-friendly methods to identify new miRNAs.

Cowpea (Vigna unguiculata L.) is an important leguminous crop of Asia, Africa, Southern Europe and USA [22]. It is a good food due to the presence of carbohydrate and high protein contents. This makes it not only essential diet to the human, but also serve as fodder to livestock. Cowpea is also significant to grow under low soil fertility, heat and drought. It is a key constituent of low-input farming systems for farmers. Cowpea also play vital role in the nitrogen fixation which is necessary for the enhancement of soil productiveness [22,23]. Very little reports and data are available about the miRNAs in this important plant. According to the latest version of miRNA registry database (Version Rfam 21.0, released June, 2014) [24], only few miRNAs are available for cowpea. This situation demands to focus and profile new miRNAs and their targets in cowpea that will act as preliminary data to manage and understand the cowpea at molecular level.

Consequently, a total of 46 new miRNAs belonging to 45 families in cowpea were identified. In this study, one miRNA gene was also found as pre-miRNA cluster (vun-mir4414). Furthermore, these newly identified miRNAs were also validated for their protein targets.

2. Materials and methods

2.1. Identification of raw sequences

A similar methodology [15] with a little modification as described by Barozai MYK, et al. [13] was applied to profile the potential miRNAs from cowpea expressed sequence tags (ESTs). As reference miRNAs, a total of 4739 known plant miRNA sequences, both precursors and matures, were downloaded from the microRNA registry database (Version Rfam 21.0 released June, 2014) [24], and subjected to basic local alignment search tool (BLAST) for alignment against publicly available 187487 ESTs of cowpea from the dbEST (database of EST), release 130101 at http://blast.ncbi.nlm.nih.gov/Blast.cgi, using BLASTn program [25].
2.2. Creation of single tone EST

The repeated ESTs from the same gene were eliminated and a single tone EST per miRNA was produced by using BLASTn program against the cowpea EST database with default parameters [25].

2.3. Elimination of coding sequences

The initial potential miRNA sequences of cowpea, predicted by the mature source miRNAs, were checked for protein coding. The FASTA format of initial potential sequences were subjected against protein database at NCBI using BLASTX with default parameter [26] and the protein coding sequences were removed.

2.4. Creation of hair-pen structures

The initial potential candidate cowpea miRNA sequences, confirming as non-protein coding nature, having 0–4 mismatches with the reference miRNAs and representing single tone gene were subjected to generate hair-pen or secondary structures. Publicly available Zuker folding algorithm http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi, known as MFOLD (version 3.6) [27] was used to predict the secondary structures. The MFOLD parameters were adjusted same as published by various researchers for the identification of miRNAs in various plant and animal species [7,8,28]. For physical scrutinizing, the hair-pen structures either showing the lowest free energy ≤−18 kcal mol\(^{-1}\) or less than or equal to the lowest free energy of the reference miRNAs were preferred. The Ambros et al. [29] threshold values were applied as reference to finalize the potential miRNAs in cowpea. The stem regions of the stem-loop structures were checked and confirmed for the mature sequences with either at least 16 or equal to the reference miRNAs base pairing involved in Watson-Crick or G/U base pairing between the mature miRNA and the opposite strand (miRNA*).

2.5. Convergence and phylogenetic analysis

The convergence and phylogenetic analysis was carried out for the one of conserved cowpea miRNA (vun-mir398). Simply, the vun-mir398, for its conserved behavior in different plant species was checked for convergence and phylogenetic investigation. The vun-mir398 alignment was created with Glycine max (gma), Nicotiana tabacum (nta) and Cucumis melo (cme) by the publicly accessible web logo: a sequence logo generator and ClustalW to produce cladogram tree using neighbor joining clustering method respectively. The results were saved.

2.6. Prediction of miRNAs targets

Dual schemes were used to predict the potential targets for cowpea miRNAs. In the first scheme, the newly identified cowpea miRNAs were subjected to psRNATarget (http://bioinfo3.noble.org/psRNATarget), with default parameters [30]. The cowpea miRNAs that not produced potential targets through psRNATarget, were subjected to the second scheme as described by Barozai [31]. Briefly, the cowpea mature miRNA sequences were subjected as queries through BLASTn program. The parameters were adjusted as, database: reference mRNA sequences (refseq_mat); organism: Vigna
unguiculata (taxid:4072) and Program Selection: highly similar sequences (megablast). The mRNA sequences showing ≥75% query coverage were selected and further subjected to RNA hybrid—a miRNA target prediction tool [32]. Only targets, confirming stringent seed site located at either positions 2–7 and/or 8–13 from the 5′ end of the miRNAs along with the supplementary site and having minimum free energy (MFE) ≤−20 kcal mol⁻¹ were selected. For more stringency, these targets were subjected to the NTNU microRNA target prediction tool available at http://tare.medisin.ntnu.no/mirna_target/search#results, to confirm the RNA hybrid results. These predicted targets were further analyzed through Gene Ontology (GO) on AmiGO website.

3. Results and discussion

3.1. The new cowpea miRNAs

In order to identify and characterize the potential miRNAs in cowpea, a comparative genomic approach was applied using bioinformatics tools. This is in agreement with the previous reports [8,28,31] that the homology based search by applying comparative genomics is a valid and logical approach to find interesting findings in plants at genomic level. The current study resulted a total of 46 new conserved miRNAs from the analyses of 187487 cowpea ESTs using bioinformatics tools (Table 1). The 46 potential cowpea miRNAs belong to 45 families (vun-miR: 398, 413, 435, 834, 1512, 1514, 1525, 1848, 2095, 2606, 2609, 2622, 2630, 2636, 2657, 2678, 2950, 3434, 4351, 4392, 4408, 4414 (cluster), 4992, 4996, 5012, 5043, 5215, 5216, 5219, 5227, 5241, 5246, 5255, 5261, 5280, 5290, 5298, 5376, 5561, 5758, 5770, 6252, 7696, 8182, 9748). The vun-miR4414 family is observed as cluster pre-miRNA. Available miRNAs literature revealed that all these 46 miRNAs are profiled for the first time in cowpea. In the light of the empirical formula for biogenesis and expression of the miRNAs suggested by Ambros et al. [29], these miRNAs are considered as a valid candidate after justifying the criteria B, C and D. According to Ambros et al. [29] only the criterion D is enough for homologous sequences to validate as potential miRNAs in other species. The present study is in agreement with the other research groups [21,33-36] where similarity based search by applying comparative genomics has produced novel and interesting findings in plants genomics.

![Figure 1. Distribution of the newly identified cowpea pre-miRNAs on the basis of their length.](image-url)
Table 1. The newly identified conserved cowpea miRNAs characterization. Cowpea miRNAs were characterized in terms of precursor miRNA length (PL), minimum free energy (MFE), mature sequence (MS), number of mismatches (NM) (represented in percentage (GC%)), SL = Strand Location and organ of expression (OE).

| miRNAs   | Ref. miRNAs | PL   | MFE   | MS                                                                 | NM | ML  | SE # | MSA | GC% | SL | OE |
|----------|-------------|------|-------|----------------------------------------------------------------------|----|-----|------|-----|-----|----|-----|
| vun-miR398 | mtr-miR398a | 131  | −32.24| TGTGTTTCAGGTCGCCCTTG                                                  | 2  | 21  | FF542932 | 5’  | 61.90 | +  | leaves |
| vun-miR413 | ath-miR413  | 353  | −88.55| TTAGTTTCTTTGTTCTGTT                                                  | 2  | 21  | FG940215 | 5’  | 33.33 | +  | mixed |
| vun-miR435 | osa-miR435  | 347  | −124.38| TTATGAGGCTTTGGAGTGC                                                  | 4  | 20  | FG811172 | 3’  | 40.00 | +  | mixed |
| vun-miR834 | ath-miR834  | 135  | −52.95| TGGTAGCAGTGCGGTTGTTG                                                  | 3  | 21  | FG822669 | 3’  | 66.66 | −  | mixed |
| vun-miR1512| gma-miR1512a| 46   | −10.60| CTTTTAAGAATTCTA-TTA                                                  | 4  | 18  | FG880488 | 3’  | 22.22 | −  | mixed |
| vun-miR1514| gma-miR1514 | 127  | −31.70| TTCATTCTAAATAGGCATC                                                  | 2  | 21  | FF388166 | 5’  | 28.57 | −  | root  |
| vun-miR1525| gma-miR1525 | 78   | −14.10| GGGGTTAAATATGTTTTAGT                                                 | 3  | 21  | FG845219 | 5’  | 28.57 | +  | mixed |
| vun-miR1848| osa-miR1848 | 77   | −32.20| CGCTCGCAGGCCGCGGCGGTCCA                                              | 2  | 22  | FG920123 | 3’  | 86.36 | +  | mixed |
| vun-miR2095| osa-miR2095 | 57   | −17.20| TTTCCATTATGAGCATTTGT                                                | 3  | 20  | FG838629 | 5’  | 30.00 | −  | mixed |
| vun-miR2606| mtr-miR2606a| 69   | −13.00| TTGAAATGCCGTTGCCTCACT                                               | 4  | 21  | FG931806 | 5’  | 42.85 | +  | mixed |
| vun-miR2609| mtr-miR2609a| 70   | −13.00| TTGAAATGCCGTTGCCTCACT                                               | 4  | 21  | FG931806 | 5’  | 42.85 | +  | mixed |
| vun-miR2622| mtr-miR2622 | 210  | −36.85| CTTTGTTGGCAATGTGAGCTTA                                              | 3  | 22  | FG900047 | 3’  | 42.85 | −  | mixed |
| vun-miR2630| mtr-miR2630a| 114  | −24.70| TTGGTTTTGGCTTTGTTTCACT                                             | 3  | 21  | FF391380 | 5’  | 33.33 | +  | root  |
| vun-miR2636| mtr-miR2636 | 191  | −29.40| GGATTTAGTGTTCTGCTAATAT                                              | 4  | 21  | FG814033 | 5’  | 38.09 | −  | mixed |
| vun-miR2657| mtr-miR2657 | 156  | −35.38| TTTATAGTTAGTTAGTTAGTTA                                              | 4  | 22  | FG926034 | 5’  | 18.18 | −  | mixed |
| vun-miR2678| mtr-miR2678 | 136  | −39.32| TAAAATTGGTGGTTGAGTGTC                                                | 3  | 19  | FF389500 | 3’  | 47.36 | −  | root  |
| vun-miR2950| mes-miR2950 | 347  | −83.20| TTCCATCTCTTGGCAGACTGA                                                 | 2  | 21  | FG872933 | 5’  | 42.85 | −  | mixed |
| vun-miR3434| ath-miR3434 | 78   | −17.40| TGAGAGCTACCGGATTCAGA                                                  | 2  | 20  | FF392538 | 3’  | 45.00 | −  | root  |
| vun-miR4351| gma-miR4351 | 148  | −63.30| GTTAGGGTTGCTGTTGGATGTTG                                                | 3  | 22  | FG936300 | 3’  | 50.00 | −  | mixed |
| vun-miR4392| gma-miR4392 | 306  | −80.53| TCTGTTGAAACCCTGGATTTCCGA                                              | 3  | 22  | FG857306 | 5’  | 45.45 | +  | mixed |
| vun-miR4408| gma-miR4408 | 66   | −20.70| CAACACATTGGATGATAGGAGGAGGAGGAGGAGGA                                  | 4  | 24  | FG894682 | 3’  | 37.5  | +  | mixed |

Continued on next page
| Gene          | Description | Fasta ID 1 | Fasta ID 2 | Score | Match Length | Alignment | Description |
|--------------|-------------|------------|------------|-------|--------------|-----------|-------------|
| vun-mir4414a |             | AGCTGCTGACCTGTTGGTCA |             | 0     | 21           | FF537171  | 5’ 52.38 +  |
| vun-mir4414b |             | ATTCAACGATGCGGGAGCTGC |             | 1     | 21           | FF537171  | 3’ 57.14 +  |
| vun-mir4992  |             | CATCTAAAGATGTTTTTTTTCAG |             | 4     | 22           | FG926352  | 3’ 31.81 -  |
| vun-mir4996  |             | TAGAAGTTACCCATGTTTCCT |             | 2     | 20           | FF388735  | 3’ 40.00 -  |
| vun-mir5012  |             | TTTTGCTGCTCGTGTGTTCC |             | 3     | 21           | FG809429  | 3’ 52.38 +  |
| vun-mir5043  |             | CTTCTCTGTCTCTGACCGACC |             | 3     | 21           | FG810406  | 5’ 57.14 +  |
| vun-mir5215  |             | AGGAGGATGAGCTAGTTGATT |             | 3     | 21           | FG939979  | 5’ 42.85 +  |
| vun-mir5216  |             | TGGGAGTGAAAAACAGTGGAA |             | 2     | 22           | FF399948  | 5’ 40.90 +  |
| vun-mir5219  |             | TCATGGAACTCTAGCTGCAGCAG |         | 1     | 23           | FG850600  | 3’ 52.17 -  |
| vun-mir5227  |             | AGAACAGAAGAAGATTTAGAA |             | 3     | 22           | FG915684  | 5’ 31.81 -  |
| vun-mir5241  |             | TGGGTAATGGAAGAGTGATAT |             | 3     | 21           | FG904590  | 3’ 42.85 +  |
| vun-mir5246  |             | CACCGAGACCTTTGAGATT |             | 4     | 21           | FG856911  | 3’ 47.61 +  |
| vun-mir5255  |             | TGAACAGGATAGAGGACATGAC |         | 4     | 21           | FG910302  | 5’ 47.61 -  |
| vun-mir5261  |             | CGATTGAGATGCGTTGCT |             | 3     | 21           | FG838847  | 5’ 47.61 -  |
| vun-mir5280  |             | TAACTAGAACGCAGGCGAGATCGGG |         | 4     | 26           | FG915361  | 5’ 57.69 -  |
| vun-mir5290  |             | AAAGTAGAGAGAGAAGACACATA |         | 4     | 24           | FG852502  | 5’ 33.33 +  |
| vun-mir5298  |             | TGGATTTCAAGATGAAGATGAAAGA |         | 4     | 25           | FF402284  | 3’ 32.00 -  |
| vun-mir5376  |             | TGAGATTTGGAAGAATATTTGAGA |         | 3     | 23           | FG872123  | 3’ 34.78 +  |
| vun-mir5561  |             | ATCTCTCTCTCTCTTAAATGTATA |         | 3     | 21           | FF390124  | 5’ 33.33 -  |
| vun-mir5758  |             | TAAATTTGAGATCTATGATT |             | 3     | 21           | FG983334  | 3’ 28.57 +  |
| vun-mir5770  |             | TTAGGACTATGTTTGGATA |             | 1     | 21           | FG937135  | 3’ 38.09 -  |
| vun-mir6252  |             | ATGATTTGTTTGAGAGGTTT |             | 4     | 23           | FG841373  | 3’ 43.47 -  |
| vun-mir7696  |             | ACAAGTACTA-AATTCAATA |             | 4     | 20           | FG864277  | 3’ 20.00 -  |
| vun-mir8182  |             | TTGTTTGGCTTGTGAGACT |             | 3     | 22           | FG942892  | 5’ 40.90 -  |
| vun-mir9748  |             | GAAGGAGGTTGAGGAGGAGAG |             | 3     | 22           | FG921211  | 5’ 54.54 +  |
Minimum free energy (MFE Kcal/mol) with numbers of miRNAs occurring

Figure 2. Distribution and classification of newly identified cowpea miRNAs on the basis of their minimum free energies (MFEs).

Figure 3. Distribution of the cowpea miRNAs mismatches (nt) with their reference miRNAs.
3.2. Characterization of cowpea miRNAs

Characterization of newly identified candidate miRNAs is a set crucial step for their validation, as reported earlier [16,17,37]. The pre-miRNA length of the profiled cowpea miRNAs ranges from 46 to 381 nt with an average of 159 nt. The pre-miRNAs were further illustrated on the basis of their length (Figure 1). The minimum folding free energy (MFE) of pre-miRNA is a vital and valid term of characterization. The newly identified potential cowpea pre-miRNAs have shown MFEs in range from $-10$ to $-132$ kcal mol$^{-1}$ with an average of $-40$ kcal mol$^{-1}$ as shown in Figure 2. The numbers of mismatches of mature sequences with their reference sequences were observed in a range of 0–4 with an average of three mismatches as categorized in Figure 3. These values are matched with the previously reported values in different plants [21,37-39]. Mature miRNA sequences lengths were observed from 18 to 26 nt with an average of 21 nt as explained in Figure 4. These findings of mature sequences length are in agreement to prior published data in other plant species [16-18,36]. The 52% cowpea miRNAs sequences were found at 5′ arm, while 48% were at 3′ arm (Figure 5(A),6). The GC content was found from 18 to 86% with an average of 42% as shown in Figure 7. Strand orientation is another important character for the generation of mature miRNAs transcripts. In this study, 24 mature miRNAs were found on minus strand while 22 were observed on plus strand of the transcripts (Figure 8). The same results for plus and minus strand orientation of mature miRNAs are in agreement with the earlier research work [40]. The identified conserved cowpea miRNAs were also characterized on the basis of their organ of expression as presented in Figure 9. These findings are similar with the earlier reports [37] and suggesting organ dependent expression pattern of miRNAs in cowpea. The miRNA organ specific expression would be utilized to manage the organogenesis in cowpea. The secondary self-folded stem-loop structures of the cowpea pre-miRNAs are observed with at least 17 nucleotides engaged in Watson-Crick or G/U base pairing between the mature miRNA and the opposite arms (miRNAs*) in the stem region (Figure 10). Except few where the reference miRNAs have also less base pairing and these precursors do not contain large internal loops or bulges. The mature miRNA sequences are observed in the double stranded stem region of the pre-miRNA secondary structures, as shown in Figure 5(A). Almost similar findings for various plant and animal species were reported by many researchers [16,17,20,37,41,42]. Furthermore, the newly identified
cowpea miRNAs were also confirmed as non-protein coding nature by showing no significant similarity with known proteins. This validation strengthens the expressed nature for computationally identified miRNAs as non-coding RNAs. Similar results were observed in various research papers by many groups [16,43,44].

3.3. Cluster pre-miRNA gene in cowpea

In animals, a large number of miRNAs have been found in clusters and have been predicted to have similar expression profiles and functions [45]. The miRNA clusters have rarely been detected in plants. They were first reported by Jones-Rhoades and Bartel [46]. In this study, we also identified one pre-miRNA (mir4414) as cluster in cowpea having two mature miRNAs within Figure 5(B). On the basis of current available literature, this miRNA family (miR4414) was found for the first time in cowpea as a cluster.

3.4. Convergence and phylogenetic studies

The newly characterized cowpea miRNA vun-mir398, due to its conserved nature, was investigated for convergence and phylogeny. Simply, the cowpea miRNA vun-mir398 alignment and cladogram tree, using neighbour joining clustering method, were created with Glycine max (gma), Nicotiana tabacum (nta) and Cucumis melo (cme) by the publicly available Web-Logo, a sequence logo generator [47] and ClustalW, a multiple sequence alignment tool [48]. The cowpea miRNA vun-mir398 is observed in convergence with Glycine max (gma), Nicotiana tabacum (nta) and Cucumis melo (cme) as shown in Figure 11(A). The Phylogenetic cladogram tree, as illustrated in Figure 11(B), clearly showed that on the basis of sharing a more recent common ancestor the cowpea miRNA is more closely related to Glycine max (gma) than Nicotiana tabacum (nta) and Cucumis melo (cme). Zeng et al. [49] have also reported conserved nature in Euphorbiaceous plants.

3.5. The potential cowpea miRNAs targeted genes

Profiling the potential cowpea miRNAs targeted genes is a vital step for validation of the computationally identified miRNAs. A total of 138 targeted genes were predicted for the 46 potential cowpea miRNAs. The detail description is mentioned in Table 2. Different cowpea miRNAs targeting same proteins and vice versa were predicted here. This showed that one miRNA target more than one mRNAs and a single mRNA targets by many miRNAs [50]. The profiled targeted genes are categories as, 27% (37 of 138) are engaged in metabolism, 26% (36 of 138) are playing role as transcription factors, 11% (15 of 138) are involved in transport activities, 11% (15 of 138) are shown with stress related, and the rest are engaged in hypothetical protein, signal transduction, growth and development, structural proteins and diseases related. Almost all of these targets were already reported as miRNA targets in other plants [7,16,17].
Figure 5. (A) The newly identified cowpea miRNAs’ secondary structures. Cowpea pre-miRNAs secondary structures were developed through Mfold algorithm. These structures clearly showing the mature miRNAs in stem portion of the stem-loop structures. (B) Cowpea pre-miRNA cluster. Cowpea miRNA (vun-miR4414) was found as a pre-miRNA cluster with two mature miRNAs (miR4414a and miR4414b). The pre-miRNA cluster secondary structure was created by Mfold (version 3.6), showing mature sequences in green within the same pre-miRNA sequence.
Figure 6. Distribution of mature miRNAs location on the either arms of hair-pen structures and numbers (frequency%) of miRNAs occurring.

Figure 7. Percentage distribution of GC content and numbers (frequency%) of miRNAs occurring.

Figure 8. Percentage distribution of strand orientation and numbers (frequency%) of miRNAs occurring.
Figure 9. Percentage distribution of organ expression and numbers (frequency%) of miRNAs occurring.

Figure 10. Percentage distribution of base pairing between the mature miRNA and the opposite arms (miRNAs*) in the stem region and numbers (frequency%) of miRNAs occurring.

Majority (27%) of the newly characterized cowpea miRNAs are observed to regulate the metabolic proteins. Such findings regarding metabolism related genes targeted by miRNAs are similar with the prior publications in plants and animals [28,43,44]. Pectin methylesterase (PME) is an important enzyme that acts on pectin, a major component of plant cell wall. PME catalyzes reactions according to the double-displacement mechanism [51]. In this study, the PME is predicted as a putative target for vun-miR1882. Thus the vun-miR1882 is a valuable resource to regulate cell wall. Another important enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) is a key enzyme in photosynthesis and photorespiration, where it catalyzes the fixation of CO₂ and O₂, respectively. Due to its rate-limiting property in photosynthesis, it is the prime focus of improving the plant productivity [52]. The cowpea miRNA (vun-miR2657) is predicted to target this important enzyme which is the potential resource to modify Rubisco expression and ultimately plant productivity.
| miRNA    | Target Acc. | Target Description                          | Function            | miRNA   | Target | Alignment |
|----------|-------------|---------------------------------------------|---------------------|---------|--------|-----------|
| vun-mir398 | TC8412      | Predicted protein                           | Hypothetical protein | miRNA   | 21     | GUCCCCGGUGAGUCUCUUUGU 1 |
|          |             |                                             |                     | Target  | 24     | CAGGGACAGUCUGUAAACACA 44 |
| vun-mir413 | TC18010     | H/ACA ribonucleoprotein complex             | Transcription factor | miRNA   | 21     | UUCCUCUUUCUUUCUUUGAUU 1 |
|          |             |                                             |                     | Target  | 432    | AAGCAGAACAGAGAAACUA 452 |
| vun-mir413 | FF538223    | Tropinone reductase                         | Metabolism          | miRNA   | 21     | UUCCUCUUUCUUUCUUUGAUU 1 |
|          |             |                                             |                     | Target  | 321    | GAGCAGAAUAUGGGAACUA 341 |
| vun-mir413 | TC16544     | Valyl-tRNA synthetase                       | Metabolism          | miRNA   | 21     | UUCCUCUUUCUUUCUUUGAUU 1 |
|          |             |                                             |                     | Target  | 1013   | AGGCCAGAACAGGGAAGAUAA 1033 |
| vun-mir413 | TC9044      | Uroporphyrinogen decarboxylase              | Metabolism          | miRNA   | 21     | UUCCUCUUUCUUUCUUUGAUU 1 |
|          |             |                                             |                     | Target  | 59     | GAGAAGAAGAGAGAACUGA 79 |
| vun-mir435 | TC9534      | Chromosome chr12 scaffold_238,             | Hypothetical protein | miRNA   | 20     | AGUUGAGGUUUCGCAAGAUU 1 |
|          |             |                                             |                     | Target  | 242    | UCAACUCCAAUGUUUCAUGA 261 |
| vun-mir435 | FF387447    | Chromosome chr9 scaffold_7,                | Hypothetical protein | miRNA   | 20     | AGUUGAGGUUUCGCAAGAUU 1 |
|          |             |                                             |                     | Target  | 386    | UCAAUUUCAGGCCUCUUAA 405 |
| vun-mir435 | TC16349     | Ripening related protein                    | Growth and development | miRNA   | 20     | AGUUGAGGUUUCGCAAGAUU 1 |
|          |             |                                             |                     | Target  | 474    | UCAACUCCAAUGGCCUCUGA 493 |
| vun-mir435 | FG810938    | Protein kinase                              | Signal transduction | miRNA   | 523    | UUAACUCCAAACCUUUUAUGA 542 |
|          |             |                                             |                     | Target  | 532    | UUAACUCCAAACCUUUUAUGA 542 |
| vun-mir834 | TC4272      | SCOF-1                                      | Transcription factor | miRNA   | 21     | GUGUGGUGGUGGAGCAUGGU 1 |
|          |             |                                             |                     | Target  | 474    | UCAACUCCAAUGGCCUCUGA 493 |
| vun-mir834 | TC8566      | Cytochrome P450 monooxygenase CYP83E9       | Metabolism          | miRNA   | 20     | GUGUGGUGGUGGAGCAUGGU 1 |
|          |             |                                             |                     | Target  | 465    | CAGCCACACGACACUGCA 484 |
| vun-mir834 | TC7191      | DnaJ-like protein                           | Stress related      | miRNA   | 21     | GUGUGGUGGUGGAGCAUGGU 1 |
|          |             |                                             |                     | Target  | 173    | CCGCCACCCACUCACCGACCC 193 |

*Continued on next page*
| mir & protein ID | Description/Function | Transcription factor | miRNA sequence | Target sequence |
|-----------------|----------------------|----------------------|-----------------|-----------------|
| FG876294 vun-mir834 | Zinc finger-like protein | Transcription factor | 21 GGUGGUGGCGGUGACGAUGGU 1 | 138 CCACCACCGCCACCGCCACCA 158 |
| TC4023 vun-mir834 | GroEL-like chaperone, ATPase | Stress related | 21 GGUGGUGGCGGUGACGAUGGU 1 | 78 CCUCGCCGCCGCCACUGUUGCCA 98 |
| TC7031 vun-mir834 | Oxophytodienoate reductase | Metabolism | 21 GGUGGUGGCGGUGACGAUGGU 1 | 19 UCAUCAGCCACUGCUUCCA 39 |
| TC15421 vun-mir834 | MYB | Transcription factor | 20 GGUGGUGGCGGUGACGAUGGU 1 | 138 CCACCACCGCCACCGCCACCA 158 |
| GH622195 vun-mir834 | Ribosomal protein | Structural protein | 21 GGUGGUGGCGGUGACGAUGGU 1 | 78 CCUCGCCGCCGCCACUGUUGCCA 98 |
| TC7768 vun-mir834 | Calcium-binding EF-hand | Transcription factor | 21 GGUGGUGGCGGUGACGAUGGU 1 | 110 CCACCACCGCCACUGUUGCCA 130 |
| XM_013230906 vun-mir1512 | Biomphalaria glabrata dual oxidase | Metabolism | 490 UUACUCUGUCUAGUACUACCA | 687 GAUGCCUAUUUUAGAAAUGAA 707 |
| XM_006957329 vun-mir1512 | Nucleoside triphosphate hydrolase protein | Transcription factor | 3' A 5' | 95 UGCUACUGCUAUCUGUACCA 105 |
| KC463855 vun-mir1512 | NB-LRR receptor (RSG3-301) | Transcription factor | 3' C CCC GG U 3' | 195 AAUGAAAUUCUGAAUUCAG 205 |
| EF076031 vun-mir1512 | Phosphatidic acid phosphatase alpha (PAPa) | Metabolism | 3' A AAGGGG G A 5' | 295 UGGUAAAGUCUGAAUUGCUAG 305 |
| AF413209 vun-mir1512 | Dolichos biflorus chloroplast ribulose-1,5-bisphosphate carboxylase | Metabolism | 3' A A 5' | 395 UGCUCACUCACUGAAUUCAG 405 |
| FF388166 vun-mir1514 | NAC domain-containing protein 78 | Transcription factor | 21 CUACGCAUAAUACUCUACUU 1 | 110 CCACCACCGCCACUGUUGCCA 130 |

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| miRNA   | Accession No. | Enzyme/Protein Name                          | Function | miRNA      | Target    |
|---------|---------------|---------------------------------------------|----------|------------|-----------|
| vun-mir1514 | FF540114      | Phosphate transporter family protein transporter | miRNA 20 | UACGGAUA AAUCUUUACUU 1 |
| vun-mir1514 | TC15423       | NAM-like protein                            | miRNA 20 | UACGGAUA AAUCUUUACUU 1 |
| vun-mir1514 | TC869         | ATP-binding cassette sub-family f member 2   | miRNA 21 | CAUCGGAUA AAUCUUUACUU 1 |
| vun-mir1514 | FG830151      | Starch branching enzyme                     | miRNA 20 | UACGGAUA AAUCUUUACUU 1 |
| vun-mir1514 | TC5197        | Cytochrome c biogenesis protein-like        | miRNA 20 | UACGGAUA AAUCUUUACUU 1 |
| vun-mir1525 | TC17248       | Salt-tolerance protein                      | miRNA 21 | UGAAUUGUA AAUGGAAUUGGG 1 |
| vun-mir1525 | FG915097      | UDP-N-acetylmuramoylalanine-D-glutamate ligase | miRNA 21 | UGAAUUGUA AAUGGAAUUGGG 1 |
| vun-mir1525 | TC14268       | Non-specific lipid-transfer protein          | miRNA 20 | GUAUUGUA AAUGGAAUUGGG 1 |
| vun-mir1848 | EG424245      | Radical SAM domain protein                  | miRNA 20 | GUAUUGUA AAUGGAAUUGGG 1 |
| vun-mir2095 | FF402667      | Resistance protein MG55                     | miRNA 21 | UGAAUUGUA AAUGGAAUUGGG 1 |
| vun-mir2095 | TC2784        | Vacuolar protein sorting-associated protein  | miRNA 20 | GUAUUGUA AAUGGAAUUGGG 1 |
| vun-mir2066 | TC406838      | SNF1 related protein kinase                 | miRNA 20 | CACUCUGGUGUA AAUGGAAUUGGG 1 |
| vun-mir2066 | TC401737      | ATP binding protein                          | miRNA 20 | CACUCUGGUGUA AAUGGAAUUGGG 1 |

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| vun-mir2606 | NP305366 | Hypothetical protein | Hypothetical protein | miRNA | 21 | UCAUCUUGGUUUGCUAGAGG 1 |
|-------------|----------|----------------------|----------------------|-------|----|------------------------|
|             |          |                      |                      | Target| 420 | ACUGAGAUAUGCUAGCUAGCAG 440 |
| vun-mir2609 | NP038997 | Jasmonate induced protein | Stress related | miRNA | 21 | UCAUCUUGGUUUGCUAGAGG 1 |
|             |          |                      |                      | Target| 220 | ACUGAGAUAUGCUAGCUAGCAG 240 |
| vun-mir2609 | NP568563 | SEC14-like protein | Transcription factor | miRNA | 21 | UCAUCUUGGUUUGCUAGAGG 1 |
|             |          |                      |                      | Target| 417 | ACUGAGAUAUGCUAGCUAGCAG 437 |
| vun-mir2609 | TC406838 | SNF1 related protein kinase-like protein | Signal transduction | miRNA | 20 | UCAUCUUGGUUUGCUAGAGG 1 |
|             |          |                      |                      | Target| 1051 | ACUGAGAUAUGCUAGCUAGCAG 1070 |
| vun-mir2622 | TC9003   | Alpha-expansin 2 | Metabolism | miRNA | 22 | AUUCUGUGUUUACUGUGUUC 1 |
|             |          |                      |                      | Target| 64 | UAACUCUCAAAAGCAGAGCA 85 |
| vun-mir2630 | TC15462  | Auxin influx transport protein | Transporter | miRNA | 20 | UUUUGUGUUUACUGUGUUC 1 |
|             |          |                      |                      | Target| 293 | UAACUCUCAAAAGCAGAGCA 321 |
| vun-mir2630 | FF390661 | Serine/arginine repetitive matrix 1 | Transcription factor | miRNA | 20 | UUUUGUGUUUACUGUGUUC 1 |
|             |          |                      |                      | Target| 349 | UAACUCUCAAAAGCAGAGCA 368 |
| vun-mir2630 | FG865319 | Monosaccharid transport protein | Transporter | miRNA | 20 | UUUUGUGUUUACUGUGUUC 1 |
|             |          |                      |                      | Target| 109 | UAACUCUCAAAAGCAGAGCA 128 |
| vun-mir2630 | TC4441   | Ras-related protein RAB8-1 | Transcription factor | miRNA | 20 | UUUUGUGUUUACUGUGUUC 1 |
|             |          |                      |                      | Target| 75 | AAACUCAAA-AACAAAGCAGA 93 |
| vun-mir2630 | TC1550   | Homeodomain leucine zipper protein HDZ3 | Transcription factor | miRNA | 21 | AUUUUGGGUGUUUACUGUGU 1 |
|             |          |                      |                      | Target| 1253 | UAACUCUCAAAAGCAGAGCA 1273 |
| vun-mir2630 | FC457466 | Pseudouridylate synthase | Metabolism | miRNA | 21 | AUUUUGGGUGUUUACUGUGU 1 |
|             |          |                      |                      | Target| 504 | AAACUCAAAAGCAGAGCA 524 |
| vun-mir2630 | TC6720   | Ubiquitin carrier protein | Transporter | miRNA | 20 | UUUUGUGUUUACUGUGUUC 1 |
|             |          |                      |                      | Target| 685 | AAACUCAAAAGCAGAGCA 704 |
| vun-mir2636 | TC7750   | NADH-ubiquinone oxidoreductase chain 2 | Metabolism | miRNA | 21 | AUUUUGGGUGUUUACUGUGU 1 |
|             |          |                      |                      | Target| 225 | AUUUUGGGUGUUUACUGUGU 245 |

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| vun-mir2636 | FF537611 | Na+/H+ antiporter | Metabolism | miRNA | 20 | AUAAGUCGUGUGAUGUGAGG 1 |
| vun-mir2636 | TC1711 | Beta-1,3-glucanase-like protein | Metabolism | miRNA | 19 | UAGUCGUGGUAUGUGAGG 1 |
| vun-mir2657 | TC7897 | Proteinase inhibitor 20 | Metabolism | miRNA | 20 | UGGUGUGUGUGUGAUGUGU 1 |
| vun-mir2657 | FG852576 | Heat shock protein 70 cognate | Stress related | miRNA | 22 | GUGUGUUGUGUGUGUGUGU 1 |
| vun-mir2678 | EF472252 | Bound starch synthase | Metabolism | miRNA | 19 | UGUAGUCGUGUGAUGUGAGG 1 |
| vun-mir2678 | D88122 | CPRD46 protein | Stress related | miRNA | 3' | UG GA AAU 5' |
| vun-mir2678 | AY466858 | Peroxisomal ascorbate peroxidase | Metabolism | miRNA | 3' | UG G GA 3' |
| vun-mir2678 | AB028025 | YLD mRNA for regulatory protein | Metabolism | miRNA | 3' | UG G GA 3' |
| vun-mir2950 | TC11773 | F-box/Kelch-repeat protein | Transcription factor | miRNA | 21 | AAGUCGACGUGUGUUCUC 1 |
| vun-mir2950 | TC2831 | Ethylene responsive protein | Stress related | miRNA | 20 | AGAGUACGACGUGUUGAA 1 |
| vun-mir3434 | TC7167 | Protein transport protein Sec24-like At3g07100 | Transporter | miRNA | 20 | AGAGUACGACGUGUUGAA 1 |

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| vun-mir4351 | TC5899 | Expressed protein               | Hypothetical protein               | miRNA          | Target miRNA          | Target miRNA          |
|-------------|--------|---------------------------------|-----------------------------------|----------------|-----------------------|-----------------------|
|             | FF391835 | NADH-ubiquinone oxidoreductase chain 2 | Metabolism                      | 22 GGUUGAGGUUCACUGGGGAUUG 1 | 27 CCACUCCCACUGAACCUCUAC | 48                  |
| vun-mir4392 | TC14606 | AKIN beta1                      | Signal transduction              | 20 UUGAGGUUCACUGGGGAUUG 1 |                      |                       |
| vun-mir4392 | TC9038  | SNF1-related protein kinase regulatory beta subunit 1 | Signal transduction              | 22 AGGCUCUGAGAGAAGGUUCU 1 | 22 AGCCUUCUGAGAAGGUUCU 1 |                       |
| vun-mir4408 | TC2049  | Monoxygenase                    | Metabolism                       | 791 UGCUAAAUGGUUGAACAAC 1 | 979 UGCUAAAUCACUGCUUAGA 1000 |                       |
| vun-mir4992 | FG809835 | TFIIE                           | Transcription factor             | 369 UGCAUAUUUCACAAAAGGUUGUG 392 |                      |                       |
| vun-mir4992 | TC11468 | Uncharacterized protein At2g03890.2 | Hypothetical protein             | 22 GACUUUUUUCUGUGAAGAUCAC 1 |                      |                       |
| vun-mir4992 | TC414   | Zinc finger protein 7           | Transcription factor             | 247 CUGAAAAAAAACCAUCAUAGAUG 268 |                      |                       |
| vun-mir4992 | TC2268  | Zinc finger protein 4           | Transcription factor             | 836 UGCAUUAAUCACAUUUUGAUG 857 |                      |                       |
| vun-mir5012 | TC1335  | Ribosomal protein L30          | Structural protein                | 739 UGCAUUAAUCACAUUUUGAUG 760 |                      |                       |
| vun-mir5012 | TC59    | Acireductone dioxygenase        | Metabolism                       | 22 GACUUUUUUCUGUGAAGAUCAC 1 |                      |                       |
| vun-mir5012 | TC12731 | Mn-specific cation diffusion facilitator transporter | Transporter                      | 857 UGCAUUAAUCACAUUUUGAUG 878 |                      |                       |
| vun-mir5043 | FF401363 | Ran-specific GTPase-activating protein | Transcription factor              | 21 CCGCUUGUGUGCUGCGUUUU 1 |                      |                       |
| vun-mir5215 | FG909052 | Ferredoxin I precursor         | Metabolism                       | 20 UUGUUAGUCAUGGGAAGAGA 40 |                      |                       |

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| vun-mir5215 | GH620837 | L-lactate dehydrogenase | Metabolism | miRNA | 20 | UAGUGAAGAGGUAGAGGA | 1 |
|vun-mir5215 | TC8326 | 50S ribosomal protein L21 | Structural protein | miRNA | 21 | UUAGUGUCAUGGAGAGGA | 1 |
|vun-mir5215 | FG849457 | Vancomycin resistance protein | Stress related | miRNA | 20 | UAGUGUGAGGAGGA | 1 |
|vun-mir5215 | TC6816 | General substrate transporter | Transporter | miRNA | 21 | UUAGUGUGAGGAGGA | 1 |
|vun-mir5216 | FG851044 | Metal ion binding | Transcription factor | miRNA | 22 | AAGGUGACAAAAAGUGAGGU | 1 |
|vun-mir5216 | FG841236 | T5I8.13 | Transcription factor | miRNA | 22 | AAGGUGACAAAAAGUGAGGU | 1 |
|vun-mir5216 | FG931306 | Predicted protein | Hypothetical protein | miRNA | 21 | AAGGUGACAAAAAGUGAGGU | 1 |
|vun-mir5219 | TC16320 | Tumor-related protein | Growth and development | miRNA | 20 | UUUAGUGACAAAAAGUGAGGU | 1 |
|vun-mir5227 | TC9947 | TINY-like protein | Transcription factor | miRNA | 22 | AAGGUGACAAAAAGUGAGGU | 1 |
|vun-mir5227 | FG842691 | HMG1/2-like protein | Transcription factor | miRNA | 20 | GACGUCGACGCUG | 1 |
|vun-mir5227 | FG886406 | Probable intracellular septation protein | Growth & development | miRNA | 20 | GACGUCGACGCUG | 1 |
|vun-mir5227 | TC17852 | Glutathione S-transferase PM24 | Metabolism | miRNA | 20 | GACGUCGACGCUG | 1 |
|vun-mir5227 | TC10272 | DNA-directed RNA polymerase subunit | Transcription factor | miRNA | 20 | GACGUCGACGCUG | 1 |
|vun-mir5241 | TC10790 | VDAC-like porin | Transporter | miRNA | 20 | GACGUCGACGCUG | 1 |
| vun-mir5241 | TC18525 | Peptidyl-prolyl cis-trans isomerase | Metabolism | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5241 | FG863193 | Probable plastid-lipid-associated protein 13 | Stress related | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5241 | TC7362 | Serine/threonine protein kinase | Signal transduction | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5241 | TC16629 | Multidrug resistance protein | Disease related | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5241 | TC2781 | Non-specific lipid-transfer protein | Transporter | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5241 | TC212 | Chaperone GrpE type 2 | Stress related | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5245 | TC8912 | Pyruvate kinase | Signal transduction | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5245 | TC18327 | Cysteine protease | Metabolism | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5261 | FG838847 | Chromosome undetermined scaffold_221 | Hypothetical protein | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5261 | FF398912 | TIR | Stress related | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5290 | TC3168 | Hydroxyproline-rich glycoprotein | Disease related | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5290 | FG844083 | PAS sensor protein | Signal transduction | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5290 | FG871448 | Eco57I restriction endonuclease | Metabolism | miRNA: 20 AAGUGAAGAAAGGAUGGGU
| vun-mir5290 | TC11392 | Ribonuclease III | Transcription factor | miRNA: 20 AAGUGAAGAAAGGAUGGGU

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| vun-mir5290  | TC12655  | Calcium dependent protein kinase | Signal transduction | mIRNA  | GUUAAAUCUCUACUAGGUUGAAU  |
|-------------|----------|---------------------------------|---------------------|--------|--------------------------|
| vun-mir5290 | TC4908   | ACC oxidase                      | Growth & development|        |                          |
| vun-mir5290 | FG874464 | RNA-binding protein              | Transcription factor|        |                          |
| vun-mir5298 | TC16082  | Translation initiation factor IF | Transcription factor|        |                          |
| vun-mir5298 | TC11481  | Non-specific lipid-transfer protein | Transporter       |        |                          |
| vun-mir5298 | TC16211  | (Iso) Flavonoid glycosyltransferase | Metabolism        |        |                          |
| vun-mir5376 | TC18575  | Zgc:158399 protein              | Hypothetical protein|        |                          |
| vun-mir5376 | TC16446  | Predicted protein                | Hypothetical protein|        |                          |
| vun-mir5376 | FC457472 | Chromosome chr1 scaffold_135    | Hypothetical protein|        |                          |
| vun-mir5561 | TC1062   | H\(^+\)/Ca\(^{2+}\) exchanger 2 | Transporter        |        |                          |
| vun-mir5561 | TC8162   | GTPase                           | Metabolism         |        |                          |
| vun-mir5561 | TC11798  | Cold shock domain                | Stress related     |        |                          |
| vun-mir5758 | TC975    | Chromosome chr11 scaffold_13    | Hypothetical protein|        |                          |
| vun-mir5758 | TC5742   | Pyrophosphate-dependent phosphofructo-1-kinase | Signal transduction|        |                          |
| miRNA ID | Gene ID | Description | Functional Category | miRNA Sequence | Target Sequence | Gene Products |
|----------|---------|-------------|---------------------|----------------|----------------|---------------|
| vun-mir5758 | TC16939 | Chromosome undetermined scaffold_310 | Hypothetical protein | GUAGGUUUGAUGGUUGUA | 509 AAAUCAUUGAUCCAGCUA |  |
| vun-mir5770 | TC1925 | | Amine oxidase | Metabolism | AGUAGGUUUGUAGCAUGAU |  |
| vun-mir5770 | TC5168 | | Copper amine oxidase | Metabolism | AGUAGGUUUGUAGCAUGAU |  |
| vun-mir5770 | TC18480 | | Ribonuclease H | Transcription factor | GUAGGUUUGAUGGUUGUA |  |
| vun-mir5770 | TC1738 | | Allyl alcohol dehydrogenase | Metabolism | GUAGGUUUGAUGGUUGUA |  |
| vun-mir6252 | FG841373 | | Nucleoporin-like protein | Transcription factor | GUAGGUUUGAUGGUUGUA |  |
| vun-mir6252 | FG857360 | | Membrane protein | Transporters | GUAGGUUUGAUGGUUGUA |  |
| vun-mir6252 | TC15301 | | Homeobox domain, ZF-HD class | Transcription factor | GUAGGUUUGAUGGUUGUA |  |
| vun-mir7696 | FG864277 | | BZIP transcription | Transcription factor | GUAGGUUUGAUGGUUGUA |  |
| vun-mir7696 | FF383199 | | Olfactory receptor | Signal transduction | GUAGGUUUGAUGGUUGUA |  |
| vun-mir8182 | TC3507 | | Pectin methylesterase | Metabolism | GUAGGUUUGAUGGUUGUA |  |
| vun-mir9748 | TC16306 | | Lectin-like protein kinase | Signal transduction | GUAGGUUUGAUGGUUGUA |  |
| vun-mir9748 | TC1064 | | Zinc finger, RING-type: Thioredoxin-related | Transcription factor | GUAGGUUUGAUGGUUGUA |  |
| vun-mir9748 | TC9843 | | Beta-xylosidase/alpha-L-arabinosidase | Metabolism | GUAGGUUUGAUGGUUGUA |  |

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| miRNA   | Function                          | Target Sequence       | miRNA Sequence       |
|---------|-----------------------------------|-----------------------|----------------------|
| vun-mir9748 TC15743 | Heat shock protein | TC15743 | Stress related | GAGGAGGGAGUUGUGAAGGAAG |
| vun-mir9748 TC15591 | Transcription factor AHAP2 | TC15591 | Transcription factor | GAGGAGGGAGUUGUGAAGGAAG |
| vun-mir9748 TC298 | Glutathione reductase | TC298 | Metabolism | GAGGAGGGAGUUGUGAAGGAAG |
| vun-mir9748 TC1040 | Glycine-rich protein 2b | TC1040 | Transcription factor | GAGGAGGGAGUUGUGAAGGAAG |

Figure 11. (A) Cowpea miRNA’s conservation studies. Alignment of *V. unguiculata* (vun) miRNA (vun-mir398) with *G. max* (gma), *N. tabacum* (nta) and *C. melo* (cme) was generated using Web logo: a sequence logo generator, showing conserved nature mature miRNA sequences. The mature sequences highlighted in a rectangle red box. (B) Cowpea miRNA’s phylogenetic analysis. *V. unguiculata* (vun) miRNA (vun-mir398) with *G. max* (gma), *N. tabacum* (nta) and *C. melo* (cme) was done with the help of ClustalW and cladogram tree was generated using neighbor joining clustering method. The phylogenetic tree showed that the *V. unguiculata* (vun) is more closed to *G. max* (gma) than *N. tabacum* (nta) and *C. melo* (cme). The closed plant species highlighted in a rectangle red box.
The transcription factor myeloblastosis (MYB) is an important regulator of many developmental and physiological processes in plants. Ballester et al. [53], suggested that the MYB also plays a significant role in regulating the flavonoid pathway in plants. The newly identified cowpea miRNA family vun-834 is found to target the MYB transcription factors. Thus this miRNA is an important resource to fine tune the MYB regulation for the desirable traits in cowpea fruit. The transcription factor, zinc finger is believed to be involved in many biotic and abiotic stresses as responding gene to manage the plant under these stresses [54]. The same family of transcription factor is also reported to play a crucial role in plant development [55]. The newly identified cowpea miRNA families vun-miR834 and 4992 are found to target this zinc finger transcription factor family. These miRNAs are important resources to regulate the zinc finger family proteins for the betterment of cowpea under various biotic and abiotic stresses and fruit development.

Similarly 12% targeted genes by cowpea miRNAs are engaged in transport activities. ATP-binding cassette transporters comprise a highly conserved family of ATP-binding proteins that are involved in transporting of various molecules across plasma membrane. Here vun-miR1514 is identified to target ATP-binding cassette transporters. Such findings are in agreement with the other workers in the miRNA field [37,43].

Biotic and abiotic stresses like salinity, drought, temperature extremities, heavy metals, pathogen attacks, and pollution cause huge yield reductions in plants [56]. Naturally plants have various systems to protect themselves from these stresses that occur at various levels, i.e., at whole plant, tissue, cellular, sub-cellular, genetic and molecular levels [56-60]. Many studies suggest that plant miRNAs are involved in these stresses [9,17,61]. In this study identified miRNAs such as vun-miR1525, 2657 and 9748 also targeted heat shock proteins that expressed in response of heat stress. This suggests the role of these miRNAs during the heat stressed condition of plants. Similar findings were reported in switch grass [17].

Some miRNAs of cowpea were observed to target the protein functioning in the process of cell signal transduction. Almost similar findings were observed by many researchers in various organisms [42,43]. Protein kinases are key regulators of cell function and play crucial role in protein phosphorylation and dephosphorylation that are major signaling pathways induced by osmotic stress in higher plants. Similarly, SNF1 (sucrose non-fermenting-1) is an osmotic-stress-activated protein kinase in Arabidopsis thaliana that can significantly impact drought tolerance of Arabidopsis thaliana plants [62]. These two important proteins were targeted by cowpea miRNAs families, like vun-miR435, 2606, 2609 and 4392 respectively. Serine/threonine protein kinase (STPKs) is another protein kinase that is targeted by miRNA family (miR5241), act as sensors of environmental signals and regulate different developmental changes and also host pathogen interactions [63].

In this study, newly profiled cowpea miRNAs were also observed to target hypothetical proteins, growth and development, structural proteins and disease related proteins. Such findings were also published earlier [19,21,37].

4. Conclusion

The current study is resulted 46 new miRNAs and their 138 targeted genes in an important commercial plant cowpea. All these miRNAs are profiled for the first time in cowpea. These findings will serve as resources to fine tune cowpea plant at micro-molecular level. This will help us to enhance the production ability of cowpea against biotic and abiotic stress tolerance. Furthermore these miRNAs and their targets are also powerful functional genomic resources in the Kingdom plantae.
Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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