**Introduction**

Sunflower (*Helianthus annuus*) belongs to the Asteraceae family. By cloning method, 700 types of miRNA were identified in plants; in 2012, miRNA was identified in *Arabidopsis thaliana*. All the processes of miRNA targets are based on coding and non-coding sequence.\(^1\) Previous studies show that RNA polymerase play important role in the transcription of the miRNA gene.\(^2\)

In recent years, for the identification of miRNA scientists have used high throughput sequencing and computational analysis techniques.\(^3\) Almost all scientists have concluded that microRNAs involved in the regulatory function of flowering and non-flowing plants are conserved.\(^4\) Plants are damaged by 2 types of environmental stresses categorized as biotic and abiotic stress. Damage to living organisms by living organisms like parasites, bacteria, viruses, and fungi is known as abiotic stress as well as damage to a living organism by the source of nonliving factors called abiotic stress.\(^5\) To describe abiotic stress, we should study the function of different organisms that survived in different environments. Stress always affects the plant’s tissues. plants need enough water for their sufficient growth. Up and down movement of water expand plant cells, which causes plant growth. Enough amount of water expands the plant’s cells and transfers the minerals from the soil to the tip of the leaves but stress causes an imbalance in the plant’s routine processes.

According to research every year, we lost 50% of our food production due to abiotic stress. Abiotic stress affects plants’ fruits, crops, metabolism, respiration processes of plants, and at the end plant’s seeds. Seeds are used for next generation; hence, unhealthy seeds affect further production.\(^6\) *H. annuus* typically refer to annual species that tend to spread rapidly and can become aggressive.\(^7\) The plant family improvement depends on the genetically resistant varieties, seed productivity, modern cultivation, and biotic-abiotic stress tolerance. Plant improvement can be assessed by studying its genetic makeup and sowing in a different location.\(^8\) For the human, it acts as an important source of nutrients. Nutritionally, it is the main source of vital nutrients inking carbohydrates, proteins, and...
dietary fibers, and provides almost 20% of the dietary energy supply. According to the miRNA database, *H. annuus* contains a total of 6 precursors and 7 mature microRNA that are compared with *A. thaliana* for the identification of novel microRNA.9 *A. thaliana* contained 205 precursors and 384 mature non-coding RNA. All data regarding miRNA are present in miRBase.

Methodology

Many tools are used as comparative genomics approaches to achieve novel and interesting information about miRNAs in plants and animals. In the initial step, identify sequences and reference sequences, and download them from the microRNA Registry Database. This miRBase Pre-miRNA database was available at https://www.mirbase.org/ freely. Pre-miRNA potential candidates were predicted by subjecting the downloaded mature and precursor miRNAs sequence through the Basic Local Alignment Tool. For this purpose, nucleotide BLAST available freely at Genbank of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) was used. The miRNA* sequences, both mature and precursor, were subjected to BLAST against *H. annuus* expressed sequence tags (ESTs) sequentially using the BLASTn program following a maximum of up to 4 mismatches with miRNAs*.

*EST single-tone selection*

BLASTn program was used and the setting of the parameters was adjusted as expect values, 1000; low complexity, the sequence filter, database, others; organism, *H. annuus*; program selection, somewhat similar sequences; and all other parameters, by default. To identify the coding part of miRNA, we used BLASTx. BLASTx highlights coding regions.10

Prediction of miRNAs secondary structure

MFOLD, a secondary structure prediction tool was used to produce a stem-loop structure for the initially identified potential *H. annuus*. All the initial candidate sequences that failed to develop stable secondary structures were discarded. MFOLD software updated as UNAfold http://www.unafold.org/ and then clicked on MFOLD and then selected application (RNA fold form Version 2.3).11

UNAfold → MFOLD → Application → RNA fold form version

The setting of MFOLD parameters was adjusted as RNA sequence, linear; folding temperature, 37°C, ionic concentration, 1 mol/L of National Center for Biotechnology Information (NCBI) having no divalent ions; percent sub-optimality number, 5; maximum interior loop size 30.

Conservation and phylogenetic analysis

Clustal W was selected for phylogenetic analysis. Clustal W is used for multiple sequence alignment or the alignment of more than one sequence available at https://www.genome.jp/tools-bin/clustalw.

Results

New potential miRNAs in sunflower

In all research, we predicted 152 miRNAs that performed regulatory process against drought-tolerant stress in *A. thaliana*. miRNAs that respond to drought stress are 156a, 156b, 156c, 156d, 156e, 156f, 158a, 159a, 164a, 164b, 165a, 165b, 166a, 166b, 166c, 166d, 166e, 166f, 168a, 168b, 169a, 170, 171a, 172a, 172b, 173, 156b, 319a, 319b, 169b, 169c, 169d, 169e, 169f, 169g, 169h, 169i, 169j, 169k, 169l, 169m, 169n, 171b, 171c, 172c, 172d, 339a, 339b, 169h, 397a, 397b, 397c, 397d, 398a, 398b, 398c, 399a, 399b, 399c, 399d, 399e, 399f, 400, 401, 402, 403, 404, 408, 159g, 156h, 156i, 156k, 159c, 159d, 164c, 172e, 417, 418, 414, 415, 416, 419, 420, 426, 427a, 427b, 427c, 827, 830, 833a, 835, 836, 837, 839, 841a, 842, 843, 844, 845a, 846, 850, 851, 852, 854a, 854b, 854c, 855, 856, 857, 858, 859, 860, 861, 865, 845b, 870, 886, 1888a, 2933a, 2933b, 2936, 3434, 774b, 4221, 854e, 5021, 5024, 5025, 5026, 5029, 5641, 5642a, 858b, 833b, 156i, 156j, 156k, 156l, 156m, 156n, 5652, 5653, 5654, 5655, 5656, 5658, 5688, 1888b, 5666, 5996, 8121, 8165, 8170, 8178, 8180, and 8181. All these miRNA sequences are reported in *A. thaliana*.

Prediction of novel miRNA

In *A. thaliana*, after identifying the 152 microRNA sequences, we performed local alignment of these sequences by BLAST with the parameters of somewhat similarities and *H. annuus* in organisms and selected those BLAST sequences that were 80% to 100% similar. So in local alignment, we got 19 sequences of *A. thaliana* against *H. annuus*. The accession numbers of these 19 sequences are XR_002550943, XR_002552183, XR_002552875, XR_002554296, XR_002556375, XR_002562643, XR_002570335, XR_002574508, XR_002575503, XR_002579567, XR_002582121, XR_002587840, XR_002592285, XR_004862999, XR_004863001, XR_004865012, XR_004869948, and XR_004890935 (see in Table 1). These 19 sequences code for different miRNAs that were novel. In our research, we predicted 12 novel miRNAs in sunflowers against *A. thaliana*. These novel predicted miRNAs are MIR156a, MIR164a, MIR165a, MIR170, MIR172a, MIR172b, MIR319a, MIR393a, MIR394a, MIR399a, MIR156h, and MIR414 (see in Table 2).
Table 1. Novel microRNA in *Helianthus annuus* that is predicted with the reference of *Arabidopsis thaliana* sequences.

| H. ANNUUS MIRNA | ACCESSION NO BY BLASTN | NOVEL MIRNA SEQUENCES |
|-----------------|----------------------|---------------------|
| MIR156 a        | XR_002550943         | TTTGCTTATATCATATTCTCTTGTTGGCAGAG |
|                 |                      | AAAGAGAAAGTTGGTTGAGAAACTGAGAAGAG AGAGTGACAGCAGCC |
|                 | XR_004869948         | CTCAAACTTTTGCACTCTTTTACTCTGTGTA |
|                 |                      | TTATGGTTCTTGAAGTGGATGATATATGGTTA |
|                 |                      | ACAAGATGGAAGAGAGCTGAGAAGAGAGAAGAGG |
|                 |                      | GAGCATATGCAACCAGTTGATATAGAGTAG |
|                 |                      | CATTATTGGGA |
| MIR164 a        | XR_002562643         | GACATGTAAGCGAGTGGGGTGTGTTTTATAAG |
|                 |                      | ATTACTAAGGTTGGTTGAGCAAGATGAGGAG |
|                 |                      | AAGCAGGGCACCTGCACTACAACCTCAGCAT |
|                 |                      | GCAAACACTTTATCAAATTTCAAAAACACC |
|                 |                      | CTTCTCAGGGTTGA |
| MIR165 a        | XR_002587840         | 5p TCTCTTCCCCCACTTACATACCACACCATTACAC |
|                 |                      | CCATCTTTCTCCTACTAAACTGTAACCCAAA |
|                 |                      | GATGA |
|                 |                      | ACAAGAAGAAAGAAAGCTGAAAGGCTGAAAGCC |
|                 |                      | TTTTCTTGGAGGGGAATGTTGTCTGGCTCGAGG |
|                 |                      | GGCCACT |
|                 | XR_002549854         | 3p AAATGATGTTTTGGAATGTTTGTGATACCTGCG |
|                 |                      | AGAAGTACTGATCGTTGTCTCGGACCGAGG |
|                 |                      | CTTCTTCCCCCAATTTGAGCTCTGCTGTGTC |
|                 |                      | TAAAGAATGTGTGTGTGTGTTGATCATGTTT |
|                 |                      | TCCA |
| MIR165 a        | XR_002550943         | 5p GAGGTCTTGGTTGTTGTTGCACTGACGGG |
|                 |                      | GAATCAAAAGAAATTTGCGCTTAAAAAAATAAT |
|                 |                      | GAAGAGAAGATGGGTGATGAGTACCTGTTT |
|                 |                      | AGAAATCTACAATAACATTCCAGAAGACAGG |
|                 | XR_002549854         | 3p TTCATAATCCATCAGTTGTCAATCTGGTTG |
|                 |                      | ATCAATGTTAAAGATAGAATCTTGTGTTG |
|                 |                      | CGGACAGGCTTCCATCCCCTC |
| MIR165 a        | XR_002552183         | 5p TTCTTTGGAGGGGAATGTTTTTGGGCTGAGG |
|                 |                      | TCATTAGAAAACATTGAGATTTATCTCTCTT |
|                 |                      | TATAT |

(Continued)
| MIRNA | ACCESSION | NOVEL MIRNA SEQUENCES |
|-------|-----------|-----------------------|
| H. Annuus | | ATATGTATGTATATATTTGATGTATGTA |
| | | 3p |
| | | ATCCATCATCTATGGTCTTTTGTGATCAATGA |
| | | GTTTTAGATTTAATTATAAAAGTTAAAGAG |
| | | AGTATC |
| | | TTGATCATTGGATCTGTTGTGCTCGGACCAGG |
| | | CTTCATTC |
| | | 5p |
| | | XR_002582121 |
| | | GAAGAGAAGAAGAAGCTATATCTTTTGGAGGG |
| | | GAATGTTTGGCGTCCAGGCTCAATAAGAAAA |
| | | AAGATCTATATCTCTTCTCTCAACTGGTTAA |
| | | TTGCCATATATATGTATGTATGAT |
| | | GTTATAGTCAAC |
| | | 3p |
| | | GGGATTTTCCTGGTGAGGCTGAGGCCACTAGAAA |
| | | CAGCGTCATGGGATCTGGTGTCGGACCAGG |
| | | CTTCATTCCCC |
| | | 5p |
| | | XR_002570335 |
| | | CAATCTTTCTCCCTCTCTCTAAAGACACCTA |
| | | AAAGATGAAACACATTGAAAGCTTTTTATCTT |
| | | TTGAGGGGATTTGTGTCTGCGTGCGAGCCACT |
| | | AGAAGGCGCATGCTTTCTCTCTCTATATA |
| | | TATCTATATCA |
| | | 3p |
| | | GGGATTTTCCTGGTGAGGCTGAGGCCACTAGAAA |
| | | CAGCGTCATGGGATCTGGTGTCGGACCAGG |
| | | CTTCATTCCCC |

Table 1. (Continued)
|   | H. ANNUUS MIRNA | ACCESSION NO BY BLASTN | NOVEL MIRNA SEQUENCES |
|---|----------------|------------------------|-----------------------|
| 6 | MIR172         | XR_004862999           | 5p                    |
|   |                |                        |                       |
|   |                | XR_0025568375          | GGTTTTTTCTGTAGATTATAATGAAATA |
|   |                |                        | CTGATCACACCCAGAAAAGAGAAATAGGGC |
|   |                |                        | TTTGCTGTATTGTTATTTTTTTATTGATTTAGTT |
|   |                |                        | TGAAAAGATTG1           |
|   |                | XR_004865012           | AGAAAGAAAAAGAAAGTGTTTATGGGC |
|   |                |                        | TTGATGTTTGAAGAATCTTGATGCTGCGAG |
|   |                |                        | CGGCAATTTGCGGCTAAATTAGATGCTTTAAAA |
|   |                |                        | CTGGAATTAGGTAAGTTGACCATGAGATT |
|   |                |                        | TAATCTTAAATA            |
| 7 | MIR319         | XR_004863001           | 3p                    |
|   |                |                        |                       |
|   |                | XR_002552875           | AAATTAGGTCCGACTCATTTATTAACCCACTC |
|   |                |                        | AGTAGAAAAGGGTCTACTTTTAAGTATGCTTGA |
|   |                |                        | TTGAGTGAAATGCGGAGATTCTCGGCTTTGATGCTTCC |
|   |                |                        | GCCCCTCGCTGGCTCCCGCTGCTGGACTGATGCTTCC |
|   |                |                        | GCCGAAACTTTT1           |
|   |                |                        | 3P                     |
|   |                | XR_002552875           | ACTAAGTAGCTATTAGCTTTTGTGTTT |
|   |                |                        | TTAATTTTGAAATCCGCTGGTTTTGAGTTG |
|   |                |                        | GATC                    |
|   |                | XR_004863001           | AAGCTAGAAACAAGATTT |
| 8 | MIR393         | XR_002552875           | 5P                    |
|   |                |                        |                       |
|   |                | XR_002552875           | TCCCTCTCTCCTCTCCTCTCTCTCTCTCTCTCTCTCTCTCT |
|   |                |                        | TCTCTCTCTCTACACACACATCTCTCTCCCGTC |
|   |                |                        | CTATATGTGGCAAGATTACAAACGGTAGCTAAGT |
|   |                |                        | AGGACGCATCCAAAGGGGATCGCATGTCCTGATTCTC |
|   |                |                        | AAATCCCATATA            |
|   |                | XR_002554296           | 3P                     |
|   |                |                        |                       |
|   |                | XR_002554296           | CGCATAGTATATGGGAGTATAGTACCCGAGTTG |
|   |                |                        | GGATCATGCTATCTTCTTTGAGATTCTTTCCTGAG |
|   |                |                        | TTGCTTCAACTTTTAAATATCACACGCACA |
|   |                |                        | AGCCAAGAAACAATCAAGAGGAAGAAGATG1 |
|   |                | XR_002554296           | TGATGTTTGGATT                |

(Continued)
Novel sunflower miRNAs structures predicted from *A. thaliana*

In our research, we predicted 12 novel microRNAs in the sunflower that respond against drought stress (MIR156a, MIR164a, MIR165a, MIR170, MIR172a, MIR172b, MIR319a, MIR393a, MIR394a, MIR399a, MIR156a, and MIR414). Here, we explain the miRNAs structures on the base of both 5 and 3 prime by MFold.

### Table 1. (Continued)

| H. ANNUUS MIRNA | ACCESSION NO BY BLASTN | NOVEL MIRNA SEQUENCES |
|-----------------|------------------------|-----------------------|
| MIR399 | ACCESSION | XR_002574508 | ATCTGTCCACCTCCATAATTCCATTGATCTATGT |
|           |           |           | ATCTCAGTTGTGTAATGTGATAATGGA |
|           |           |           | TTATGGTTT |
| MIR156h | ACCESSION | XR_002579567 | ATGTTGATGAACGGGTGAGTTGCTCTCATG |
|           |           |           | ATGTTGATGACAAGAGTAGAGAGACACAGAT |
|           |           |           | GACGAAGTTGAGCTAATATTGGGCTCTTTT |
|           |           |           | TTCTTTTGTCCTCTCTATTGTTCGTTCATTCATCA |
|           |           |           | CATATCTTCTTC |
| MIR414 | ACCESSION | XR_002575503 | ATATTCATTTGAGTTATTATTATTATTATGAAAT |
|           |           |           | TTTCATTTGGCTCTCTATTATGCTTATG |
|           |           |           | AAATCGTTTCTGCTCCAGTCTCTTTAGGGGCTGT |
|           |           |           | TTGGTTGAGGCTCTTTATAGGAGGCTCTG |
|           |           |           | TGGCCCTGCTGACTGCGAGACTGAGGGGCTGT |

![miRNA structures](image-url)
Ancestral conservation of H. annuus. In addition, novel sunflower precursor miRNAs were selected for conservation studies. For this process, all novel miRNAs selected to evaluate their conservation in other plants. For conservation studies, we selected 10 different types of plants like *Gossypium hirsutum, H. annuus, A. thaliana, Triticum aestivum, Saccharum officinarum, Zea mays, Brassica napus, Solanum tuberosum, Solanum lycopersicum*, and *Oryza sativa*. Using the same process, many researchers have determined precursor conservation and phylogenetic analysis in different plants.
Table 2. Conservation analysis of *Helianthus annuus* with other organisms such as *Gossypium hirsutum*, *H. annuus*, *Arabidopsis thaliana*, *Triticum aestivum*, *Saccharum officinarum*, *Zea mays*, *Brassica napus*, *Solanum tuberosum*, *Solanum lycopersicum*, and *Oryza sativa*.

|                | *G. hirsutum* | *H. annuus* | *A. thaliana* | *T. aestivum* | *S. officinarum* | *Z. mays* | *B. napus* | *S. tuberosum* | *S. lycopersicum* | *O. sativa* |
|----------------|---------------|-------------|---------------|---------------|------------------|-----------|-----------|---------------|-------------------|------------|
| MIR156a        | present       | present     | present       | present       | present          | present   | present   | present       | present           | present    |
| MIR164a        | present       | Non         | present       | Non           | present          | present   | present   | present       | present           | present    |
| MIR165a        | Non           | non         | present       | non           | non              | Non       | non       | non           | non               | non        |
| MIR170         | non           | non         | present       | non           | non              | Non       | non       | non           | non               | non        |
| MIR172a        | present       | non         | present       | non           | non              | present   | present   | present       | present           | present    |
| MIR172b        | present       | non         | present       | non           | non              | present   | present   | present       | present           | present    |
| MIR319a        | non           | non         | present       | present       | non              | present   | non       | present       | present           | present    |
| MIR339a        | present       | non         | present       | Non           | non              | present   | present   | present       | present           | present    |
| MIR339b        | present       | non         | present       | non           | non              | present   | present   | present       | present           | present    |
| MIR339c        | present       | non         | present       | present       | non              | present   | present   | present       | present           | present    |
| MIR339d        | present       | non         | present       | present       | non              | present   | present   | present       | present           | present    |
| MIR339e        | present       | non         | present       | present       | non              | present   | present   | present       | present           | present    |
| MIR339f        | present       | non         | present       | present       | non              | present   | present   | present       | present           | present    |
| MIR414         | non           | non         | present       | non           | non              | non       | non       | non           | non               | present    |
Phylogenetic analysis of non-coding microRNAs

The study of the evolutionary history of a species or a group of organisms or a particular characteristic of an organism. Here we have done phylogenetic analysis by clustal W.

Phylogenetic analysis of MIR156a

The phylogenetic analysis of mir156a is described in Figures 1 and 2. According to the MIR156a phylogenetic analysis, H. annuus with the Accession number “XR_002550943” and “XR_4869948” shows close relation with T. aestivum, B. napus, A. thaliana, G. hirsutum, O. sativa, Z. mays, and S. officinarum.

Phylogenetic analysis of MIR164a

The phylogenetic analysis of mir164a is described in Figure 3. According to the phylogenetic analysis of MIR164a, H. annuus with the Accession number “XR_002556375” shows close relation with T. aestivum, B. napus, A. thaliana, O. sativa, Z. mays, S. lycopersicum, and G. hirsutum and shows a distance relationship with S. tuberosum.

Phylogenetic analysis of MIR172b

The phylogenetic analysis of mir172b is described in Figure 4. According to the phylogenetic analysis of MIR172b, H. annuus with the Accession number “XR_004862999” shows close relation with A. thaliana, Z. mays, S. lycopersicum, and S. tuberosum.

Phylogenetic analysis of MIR172a

Phylogenetic analysis of mir172a is described in Figures 5 to 7. According to the phylogenetic analysis of MIR172a, H. annuus with the Accession number “XR_002592285” and “XR_004865012” shows close relation with G. hirsutum.

Phylogenetic analysis of MIR319

The phylogenetic analysis of mir319 is described in Figure 8. According to the phylogenetic analysis of MIR319, H. annuus with the Accession number “XR_004865012” shows close relation with A. thaliana, S. lycopersicum, and T. aestivum.

Phylogenetic analysis of MIR393a

The phylogenetic analysis of mir393 is described in Figure 9. According to the phylogenetic analysis of MIR393a, H. annuus
with the Accession number “XR_002552875” shows close relation with A. thaliana, G. hirsutum, O. sativa, S. tuberosum, and Z. mays.

**Phylogenetic analysis of MIR394**

The phylogenetic analysis of mir393 is described in Figure 10. According to the phylogenetic analysis of MIR94, H. annuus with the Accession number “XR_002554296” shows a distance relation with A. thaliana, G. hirsutum, O. sativa, B. napus, S. lycopersicum, and Z. mays.

**Phylogenetic analysis of MIR399**

The phylogenetic analysis of mir399 is described in Figure 11. According to the phylogenetic analysis of MIR94, H. annuus with the Accession number “XR_002574508” shows a distance
relation with *A. thaliana*, *G. hirsutum*, *O. sativa*, *B. napus*, *S. lycopersicum*, and *Z. mays*.

**Discussion**

Sunflower is the fourth biggest oil-seed crop in the world. The seeds of sunflowers are used in food as well as their dried stalk is used as fuel. It has previously been used as an ornamental plant and was also used in ancient ceremonies. Moreover, different parts of sunflowers are used in body painting, decorations, and making dyes for the textile industry. Its oil is used in the manufacturing of margarine and salad dressings, and cooking. With roasted seeds, a coffee type could be made. In industry, it is used in cosmetics and paints. Due to its lack of anti-nutritional factors and high nutritional values, it is a potential source of protein for human consumption. Due to its metabolic, physiological, and morphological adaptation strategies, the sunflower is one of the most important oil-seed crops and is resistant to various abiotic stresses. This crop is of special interest for its adaptation to limited water availability, high temperatures, high salinity, and heavy-metal concentrations in soil. The dried stems...
which are used for fuel contain potassium and phosphorous which can be composed and returned to the soil as fertilizer.  

MiRNAs arise from primary longer RNA transcripts that include a self-complementary fold-back, from which the mature miRNAs are excised. They are short RNA molecules containing 19-24 nucleotides in size. They are familiar as regulators of gene expression by binding to open reading frames (ORF) or untranslated regions (UTR) of specific mRNAs, targeting them for directing or cleavage translation inhibition at the mRNA level. It has been demonstrated that around 60% of protein-coding genes are targets of miRNAs and are modulated by these small RNAs.

miRNAs are derived from hairpin pre-miRNA from which both miRNA and the imperfectly complementary miRNA* strands are released. Their sequences are not conserved between plants and animals, and even not have been seen in fungi. Many miRNAs within the kingdom have an ancient origin, some being completely conserved among sunflower, Arabidopsis, rice, and even liverworts, mosses, and hornworts.
By regulating gene expression, miRNA plays a vital role to regulate the developmental processes of organisms. The negative regulation of miRNAs in gene expression in both plants and animals has been demonstrated. MicroRNAs have been revealed to modulate diverse developmental processes, including polarity, identity, and organ separation, and to regulate their function and biogenesis. In our study, we used the miRBase database to find miRNAs from *A. thaliana* that were tolerant against drought stress that was 152 in strength and then performed local alignment of these miRNAs against sunflower and found 12 novel miRNAs (MIR156a, MIR164a, mir165a, mir170, mir172a, mir172b, mir193a, mir393a, mir394a, mir399c, mir156h, and mir414). The secondary structure of these 12 novel miRNAs, including forward and reverse strands, was forecasted by MFOLD software by using default parameters. Later, conservation and phylogenetic analysis were done by selecting 10 different organism type such as *G. hirsutum, H. annuus, A. thaliana, T. aestivum, S. officinarum, Z. mays, B. napus, S. tuberosum, S. lycopersicum*, and *O. sativa*. In our study, all novel miRNAs are present in only *A. thaliana*. 

In our study, mir-156a is present in all species; mir-164 is present in all except *H. annuus* and *S. officinarum*; mir-156b and
mir-170 are present in only A. thaliana, mir-172a and mir172-b are present in all except H. annus, T. aestivum, and S. officinarum;10– 13 miR-319a is present in all except G. bursutum, H. annus, S. officinarum, and Z. mays; mir-393a is present in all except H. annus, T. aestivum, and S. officinarum; mir-394a is present in all except H. annus, T. aestivum, S. officinarum, and B. napus; mir-399c is present in all except H. annus and T. aestivum; mir-156h is present in all except B. napus, and H. annus; mir-399c is present in all except H. annus, T. aestivum; mir-394a is present in all except Z. mays, H. annus, B. napus, and S. officinarum; and mir-414 is absent in all except A. thaliana, and O. sativa.

**Conclusions**

Twelve novel miRNAs (MIR156a, MIR164a, mir165a, mir170, mir172a, mir172b, mir319a, mir393a, mir399c, mir156h, and mir414) were identified against drought stress in A. thaliana. We targeted these miRNAs to cope with the drought tolerance in sunflowers. In our study, all novel miRNAs are present in only A. thaliana. Moreover, different parts of sunflowers are used in body painting, decorations, and making dyes for the textile industry. Its oil is used in the manufacturing of margarine and salad dressings, and cooking. With roasted seeds, a coffee type could be made. In industry, it is used in cosmetics and paints. The improvement method also increases the production of sunflowers and benefits economically.

**Author Contributions**

All authors have contributed to this study.

**Availability of Data and Materials**

The data associated with a paper are available on demand through email contact of co-author waqarmazhar63@gmail.com.

**Ethical Approval**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Consent for Publication**

This study is based on research.

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