

Sequence, Secondary Structure, and Phylogenetic Conservation of MicroRNAs in *Arabidopsis thaliana*

Muhammad Waqar Mazhar¹, Nik Yusnoraini Yusof²,
Tayyaba Shaheen¹, Saira Saif¹, Ahmad Raza³
and Fatima Mazhar⁴

¹Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Faisalabad, Pakistan. ²Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Kubang Kerian, Malaysia. ³Department of Biological Sciences, Nuclear Institute for Agriculture & Biology, Faisalabad, Pakistan. ⁴Department of Microbiology, Muhammad Nawaz Sharif University of Agriculture, Multan, Multan, Pakistan.

Bioinformatics and Biology Insights
Volume 16: 1–15
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11779322221142116


ABSTRACT: MicroRNAs are small non-coding RNA molecules that are produced in a cell endogenously. They are made up of 18 to 26 nucleotides in strength. Due to their evolutionary conserved nature, most of the miRNAs provide a logical basis for the prediction of novel miRNAs and their clusters in plants such as sunflowers related to the Asteraceae family. In addition, they participate in different biological processes of plants, including cell signaling and metabolism, development, growth, and tolerance to (biotic and abiotic) stresses. In this study profiling, conservation and characterization of novel miRNA possessing conserved nature in various plants and their targets annotation in sunflower (Asteraceae) were obtained by using various computational tools and software. As a result, we looked at 152 microRNAs in *Arabidopsis thaliana* that had already been predicted. Drought tolerance stress is mediated by these 152 non-coding RNAs. Following that, we used local alignment to predict novel microRNAs that were specific to *Helianthus annuus*. We used BLAST to do a local alignment, and we chose sequences with an identity of 80% to 100%. MIR156a, MIR164a, MIR165a, MIR170, MIR172a, MIR172b, MIR319a, MIR393a, MIR394a, MIR399a, MIR156h, and MIR414 are the new anticipated miRNAs. We used MFold to predict the secondary structure of new microRNAs. We used conservation analysis and phylogenetic analysis against a variety of organisms, including *Gossypium hirsutum*, *H. annuus*, *A. thaliana*, *Triticum aestivum*, *Saccharum officinarum*, *Zea mays*, *Brassica napus*, *Solanum tuberosum*, *Solanum lycopersicum*, and *Oryza sativa*, to determine the evolutionary history of these novel non-coding RNAs. Clustal W was used to analyze the evolutionary history of discovered miRNAs.

KEYWORDS: *Arabidopsis thaliana*, miRNAs, *Solanum lycopersicum*, *Oryza sativa*, phylogenetic analysis

RECEIVED: May 19, 2022. **ACCEPTED:** November 9, 2022.

TYPE: Original Research Article

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research study is self-funded.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Muhammad Waqar Mazhar, Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Faisalabad, 38000, Pakistan. Email: waqarmazhar63@gmail.com

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-flowering 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 including 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, 166g, 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, 394a, 394b, 397a, 397b, 398a, 398b, 398c, 399a, 399b, 399c, 399d, 399e, 399f, 400, 401, 402, 403, 404, 408, 159g, 156h, 158b, 159c, 319c, 164c, 172e, 417, 418, 414, 415, 416, 419, 420, 426, 427a, 427b, 427c, 827, 830, 833a, 835, 836, 837, 839, 841a, 842b, 844, 845a, 846, 848, 850, 851, 847, 855, 854a, 854b, 854c, 856, 857, 858, 859, 860, 861, 865, 845b, 870, 1886, 1888a, 2933a, 2933b, 2936, 3434, 774b, 4221, 854e, 5021, 5024, 5025, 5026, 5029, 5641, 5642a, 858b, 833b, 156i, 156j, 5652, 5653, 5654, 5655, 5656, 5658, 5188, 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 BLASTn 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_2575503, XR_002579567, XR_002582121, XR_002587840, XR_002549854, 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. (Continued)

	<i>H. ANNUUS</i> MIRNA	ACCESSION NO BY BLASTN	NOVEL MIRNA SEQUENCES
			ATATGTATGTATATATATTGATGTATGTA 3p ATCCATCATCTATGGTCTTTGTTGATCAATGA GTTTTAGATTAATTATATAAAGTGTTAAAGAG AGTATC TTGATCATTGGATCTGGTGTGCTCGGACCAGG CTTCATTC ACCESSION XR_002582121 5p GAAGAGAAGAAGAAGCTATATCTTTTGAGGG GAATGTTGTTGGCTCGAGGTCAATAGAAACC AAAGATCTATATCTCTTTTCTCTAACTGTAA TAAAGATGTACATATATATGTATGTATATGAT GTTATAGTCAAC 3p GGTTATTCATTTAGAGTGTTGAATGAGAGAAA CTTGATCATTGGATGATCTGCTGACTCGGAC CAGGCTTCATCCCCCAATTGTTGCTTCA ACCESSION XR_002570335 5p CAATCTTCTCCCTCTCTCTAAGATCACACCTA AAAGATGAACACATTGAAAGCCTTTTATCTT TTGAGGGGATTGTTGTCTGGCTCGAGGCCACT AGAAAGCCTAGATCTTCTCTCTATATACA TATCTATATCA 3p GGGATTGTTGTCTGGCTCGAGGCCACTAGAAA GCCTAGATCTTCTCTCTATATACATATCTA TATCACAGTCAACGCAACCATTGATTACTGAG AGAGATTCTTGATCTGGTGTGCTCGGACCAGG CTTCATTCCCC
4	MIR170	ACCESSION XR_004890935	GATGTTGGTTCGGTTCAATAAGAACTCAATGT TCAAATGATGCATTGAACGTTGCTTTTTGATT GAGCCGTGCCAATATCACGTGCATGTTGCTTC TAAATTTCCACAAGTCTTTTGAAACTTCTGTG AAAAGGACCTA
5	MIR172 a	ACCESSION XR_002592285 ACCESSION	AGAATCTTGATGATGCTGCATCGGAAATCAAT TGACCACTTTAAAAATCAACGCATCAAATACG ATTTTGTGGATCTCATACAAACAAAGGTAAT CTTT TATAGATTGGCTGGAATCTAAGCACAAGGGT

(Continued)

Table 1. (Continued)

<i>H. ANNUUS</i> MIRNA		ACCESSION NO BY BLASTN	NOVEL MIRNA SEQUENCES
		XR_002556375	GTGTTTTTCTGTAGATTTAATAATGAAAATA CTGATCACACACCAGAAAAGAGAAATAGGGC TTTGCTGTATTGTATTTTTTATTGTATTTAGT TTGAAGATTTTG
		ACCESSION XR_004865012	AGAAAGAAAAAGAAAGTGGTTTATGGGCCCC TTGATGGTTTGAGAATCTTGATGATGCTGCAG CGGCAATTGCTGGCTAATTATGATCTTTAAAA CTGGATTATGGTAAGTTGTGACCATGTAGATT TAATCTTAAAATA
6	MIR172 b	ACCESSION XR_004862999	5p TCAAACAACATCAGCAGGTAGCAGCTCCACCT CCTCAGTCACCAGCGCCGACTGCCGCTGCTCC GCCTCT GCCCTCCGGCTGCTCCGCCTTCGACGATTGCT GCCGAAACTTTGTTT 3P ACTAAGTAGCTATTAGCTAATTTTTGTTCTTGT TTAATTTTGAATTCAGGCTGTTTTGAGTTGTGG GATC AAGCTAGAACAAGATTT
7	MIR319 a	ACCESSION XR_004863001	AATTAGCTGCCGACTCATTCAATTAACCACTC AGTAGAAAAGGGTTCACCTTATGCTACTTTGA TTGAGTGAATGATGCGGGAGATAGTTTTCATC CCTTGCTAATCTGTACTTGGACTGAAGGGAGC TCCCTCGTTCTT
8	MIR393 a	ACCESSION XR_002552875	5P TCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTC TCTCTCTCTTACACACACATCTCTCTCCCCGTC TCTATATGTGCGAAGATTACAACGGTAGCTAA AGGACGCATCCAAAGGGATCGCATTGATCCT AAATCCATA 3P CGCATAGTATATGGGTATGATATACCGAGTTG GGATCATGCTATCCTTTTGGATTCTTCTTCGG TTGCTTCTAACTTTATAAATATCACACGCACA AGCCAAGAACAATCAAAGATGGAAGAGAATG TGATGTTTGATT
9	MIR394 a	ACCESSION XR_002554296	CTTTAGGGTTTTTAGGGTTTCTTTCATGGTGGT TTAATAAAGAGTTTCCAGCAGATTTCTTTGGC

(Continued)

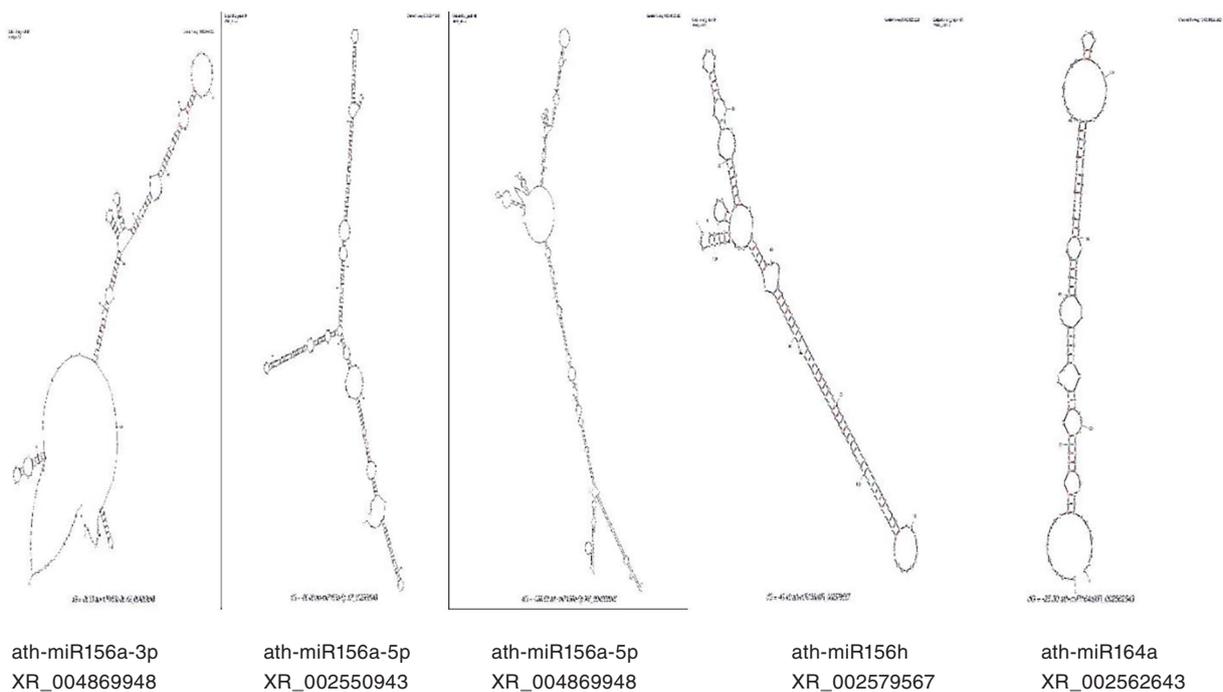
Table 1. (Continued)

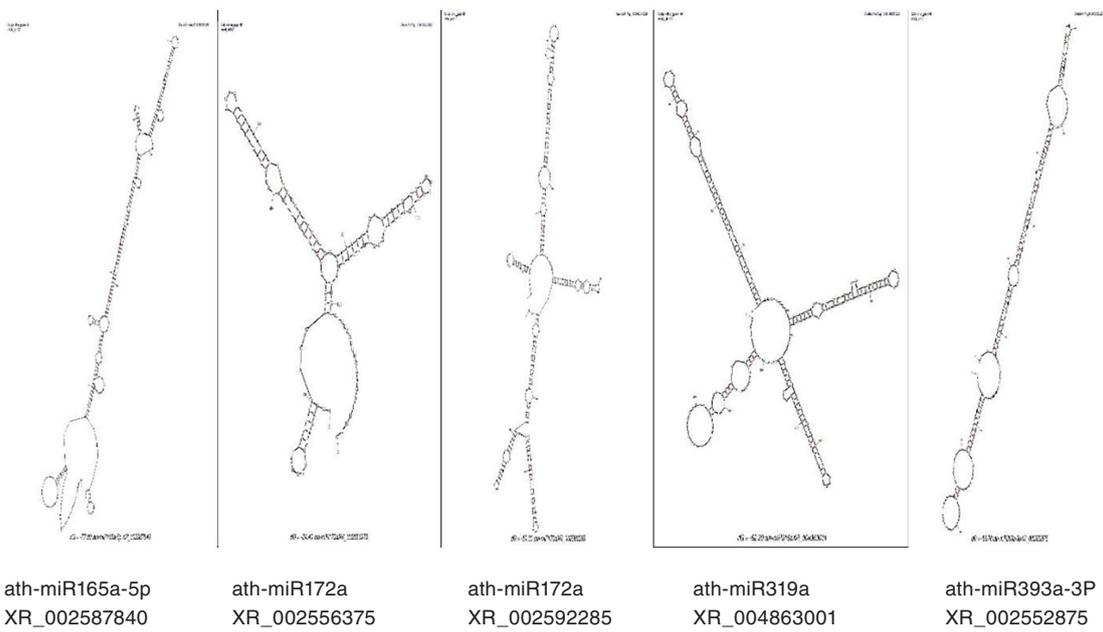
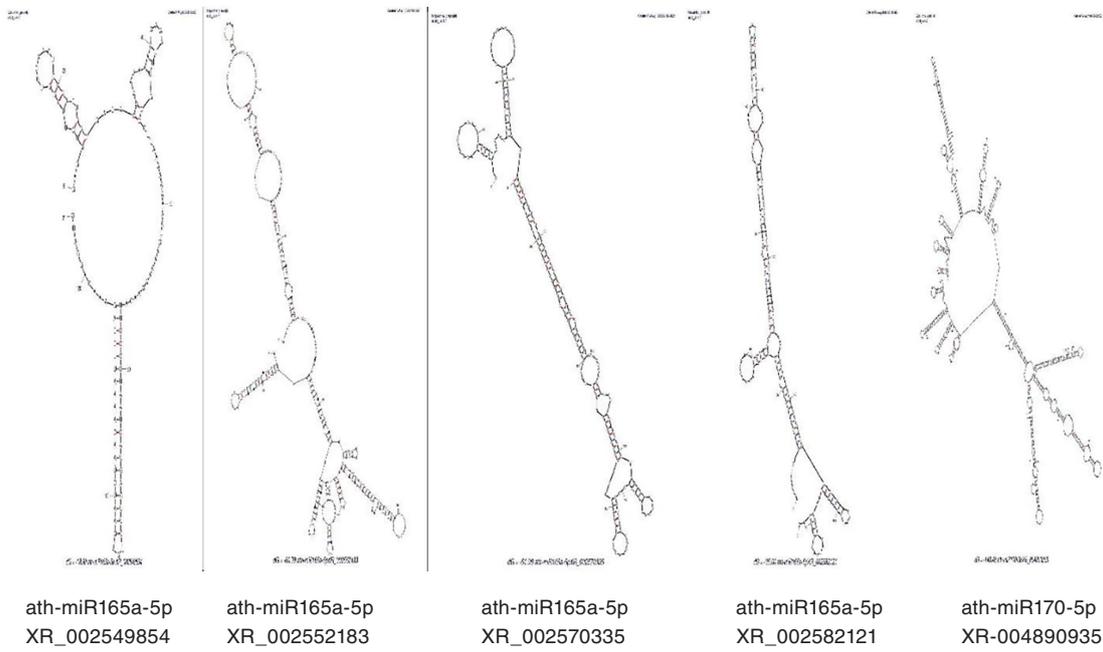
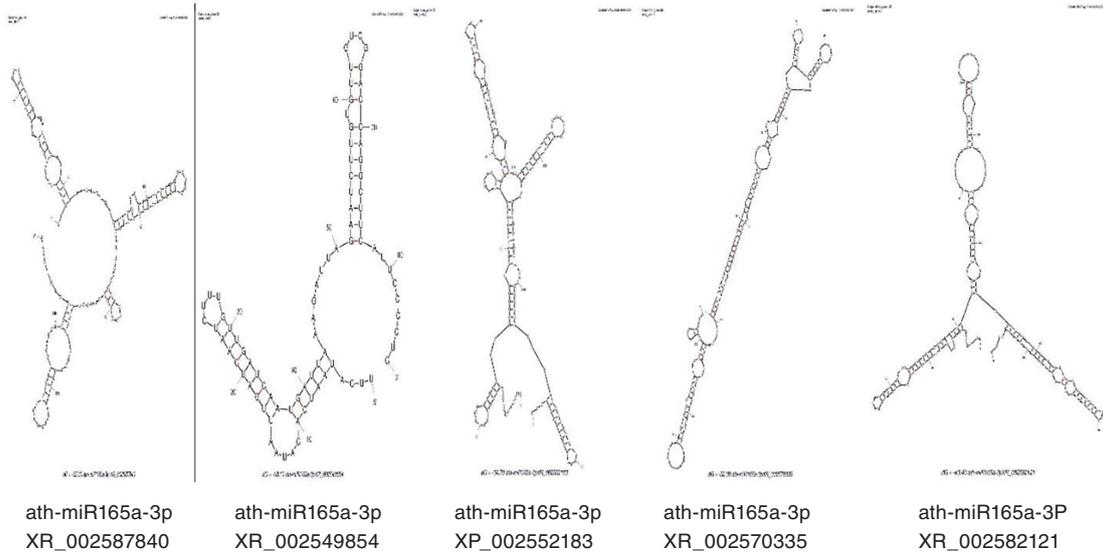
<i>H. ANNUUS</i> MIRNA		ACCESSION NO BY BLASTN	NOVEL MIRNA SEQUENCES
			ATTCTGTCCACCTCCATATTCATTGATCTATGT ATCTCACTGTTGTGTAATGTGTAATTAGGGT TTATTGGTTT
10	MIR399 c	ACCESSION XR_002574508	GGTCGGAAGAAAAGGAGCTGAGACAGCTGGA TGTTTTGAAGCAGGCAATTGTGTTGGGCTGG GTGAAGACAACCTGGCTCTGGGCACGATAACG CAAGGGGTTTTTCCATGCGCCTGCCAATAGAA ATATGCGGTATAGT
11	MIR156h	ACCESSION XR_002579567	ATGTTGATGAAACGGGTTGAAGTGCTCGATG ATGTTGTTGACAGAAGATAGAGAGCACAGAT GACGAAGTTGCAGCTAATATTTGGCATCTTTT TTCTTTGTGCCCTCTATTGTTCTGTATCATCA CATATCTTCTTC
12	MIR414	ACCESSION XR_002575503	ATATTCATTGAGTTATTATTATTTATGAAAAT TTTCATGTTCTTTGAAACTTATCATGTCTTATG AAATCGTTTCAGTCCTCAGTCTTAGGGGCTGT TTGGTTGCCTCTTAATGGCTCCATTAAGAAGC TTGGCCTCTGAATCGGTCAGACATGGGGTGT

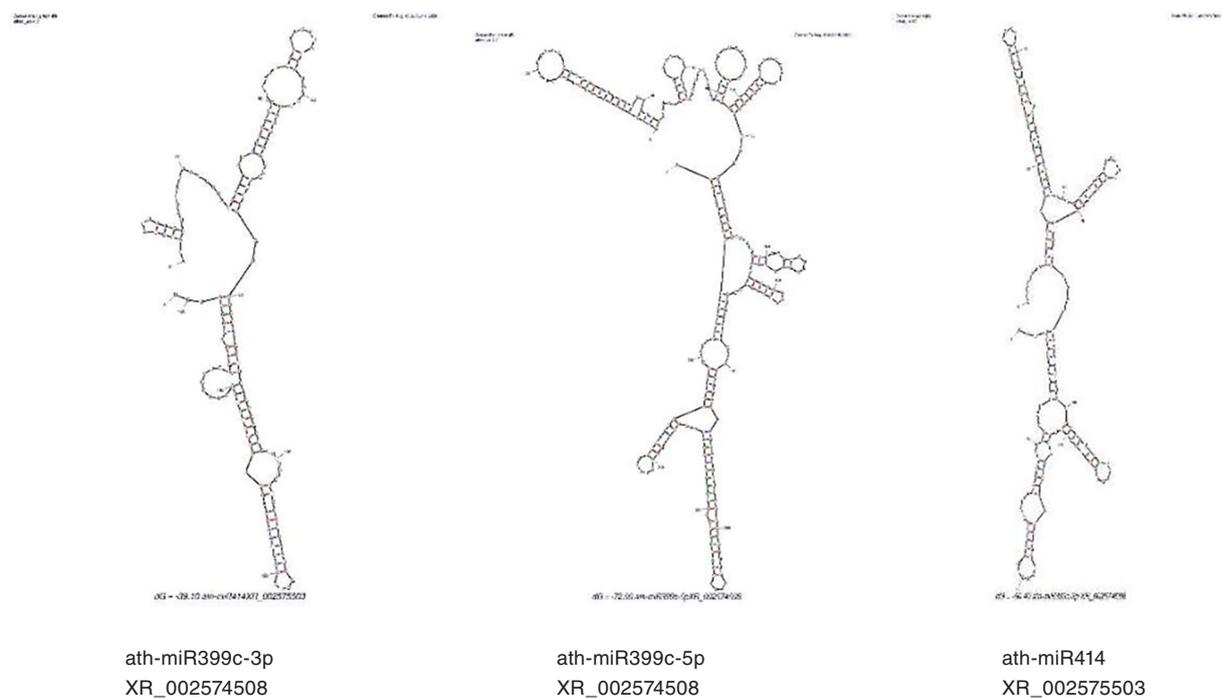
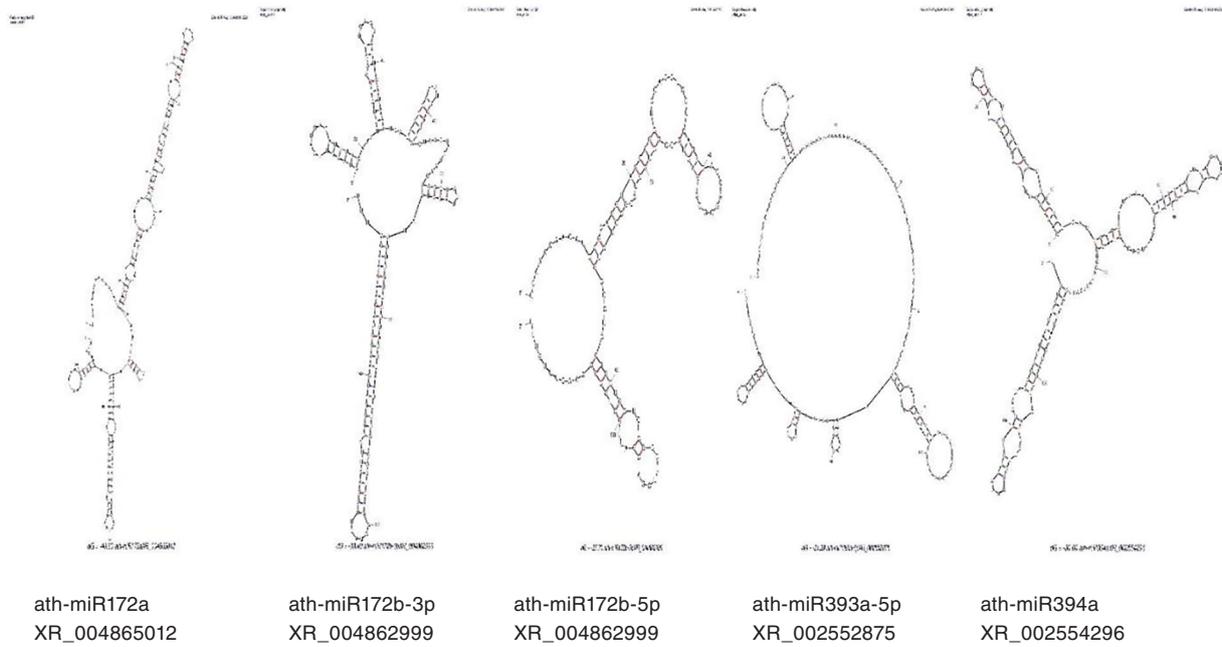
Novel sunflower miRNAs structures predicted from *A. thaliana*

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.







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.

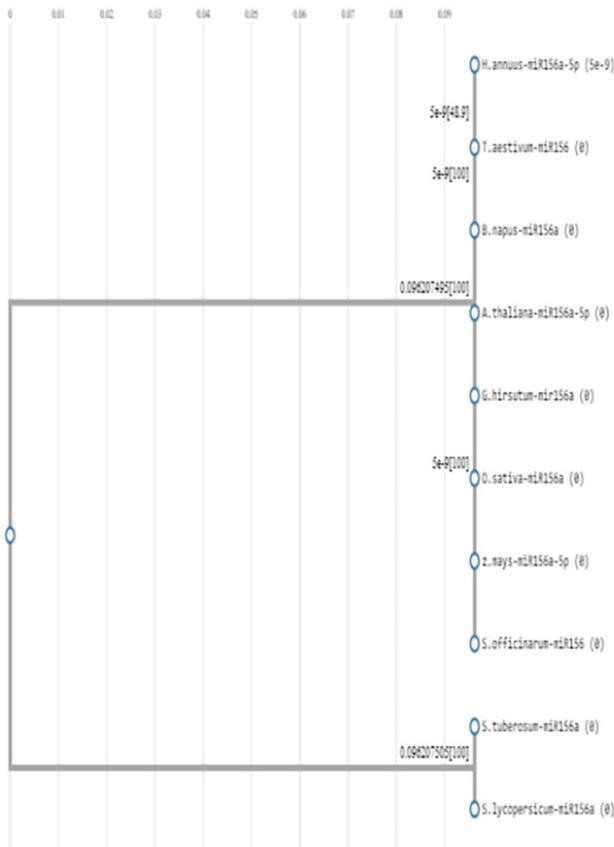


Figure 1. MIR156a.
XR_002550943.

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_002562643” 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*.

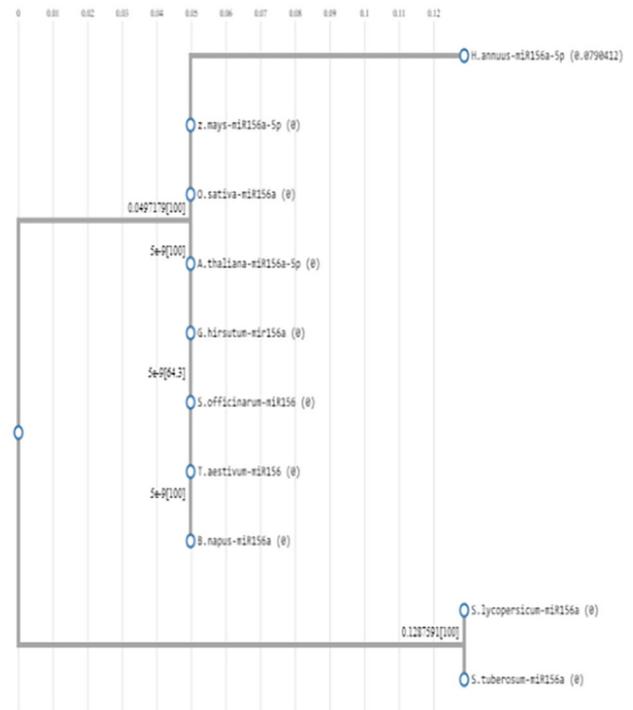


Figure 2. MIR 156.
XR_004869948.

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*, *S. tuberosum* and shows a distance relationship with *G. hirsutum*.

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_002556375” shows close relation with *A. thaliana*, *Z. mays*, *S. lycopersicum*, *O. sativa*, *B. napus* and with “XR_004865012” shows close relationship 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_004863001” 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*

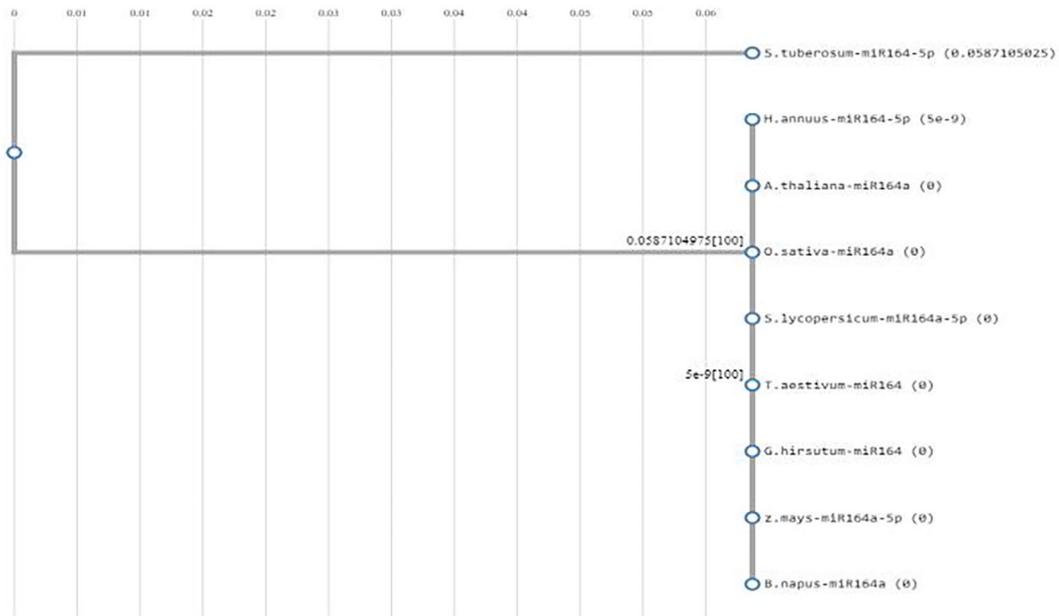


Figure 3. MIR164a. XR_002562643.

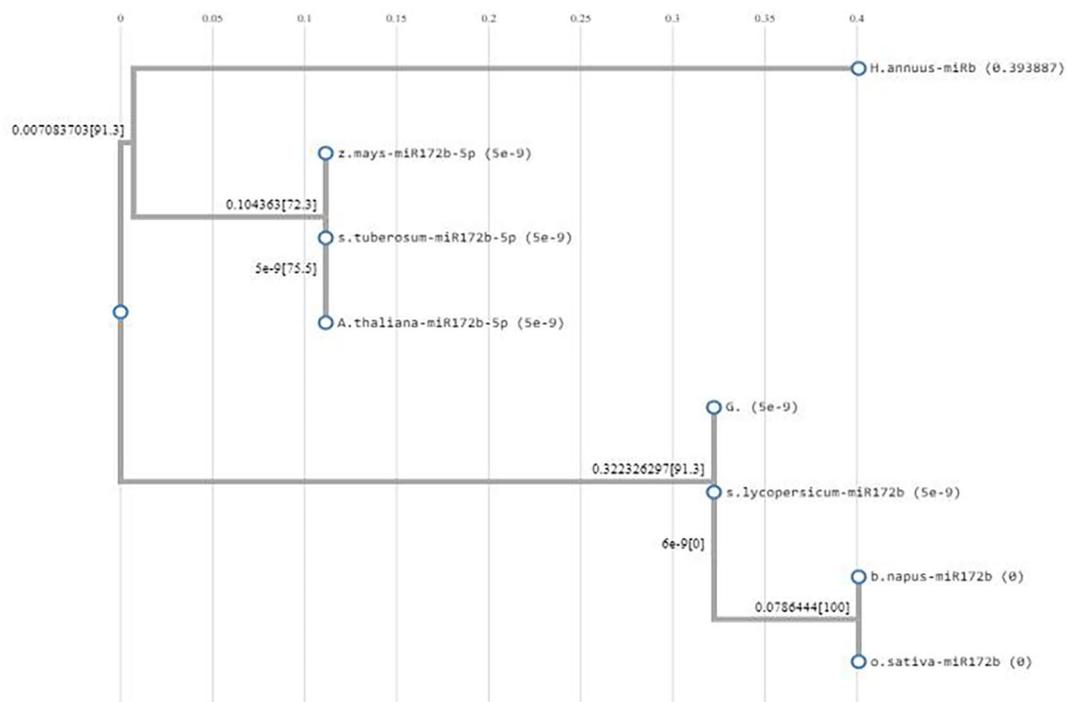


Figure 4. MIR172b. XR_004862999.

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.¹² 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

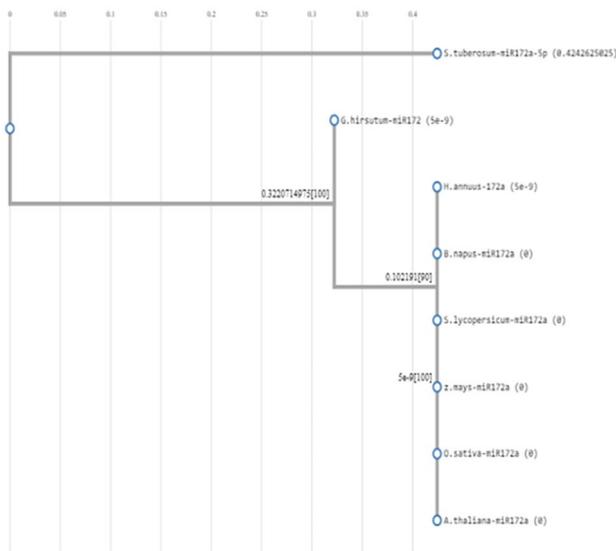


Figure 5. MIR 172a (XR_002592285).

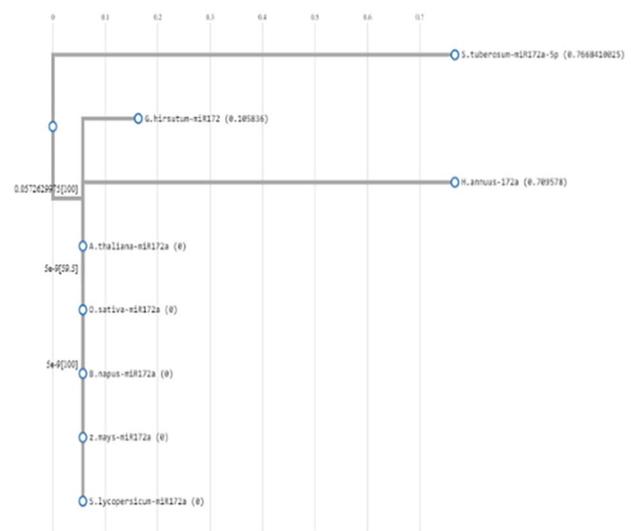


Figure 6. MIR172a (002556375).

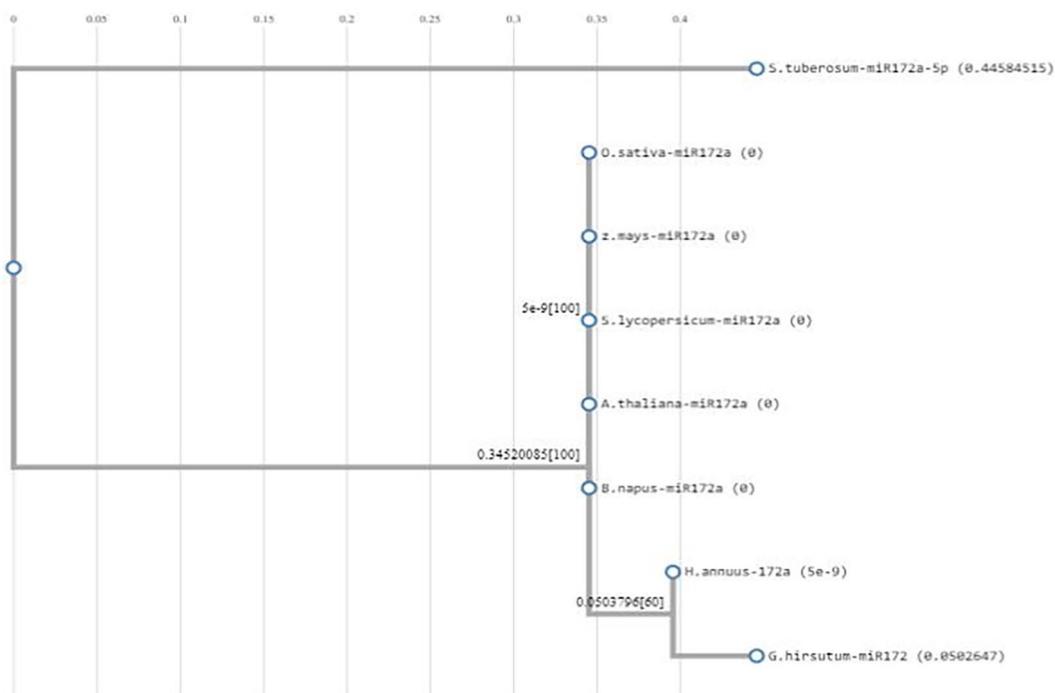


Figure 7. MIR172a (004865012).

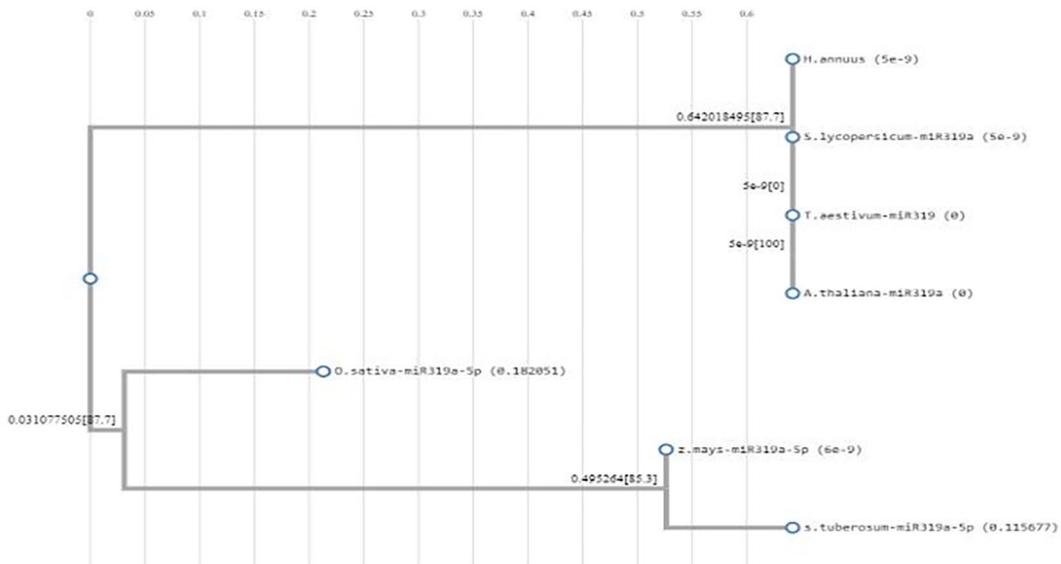


Figure 8. MIR319(XR_004863001).

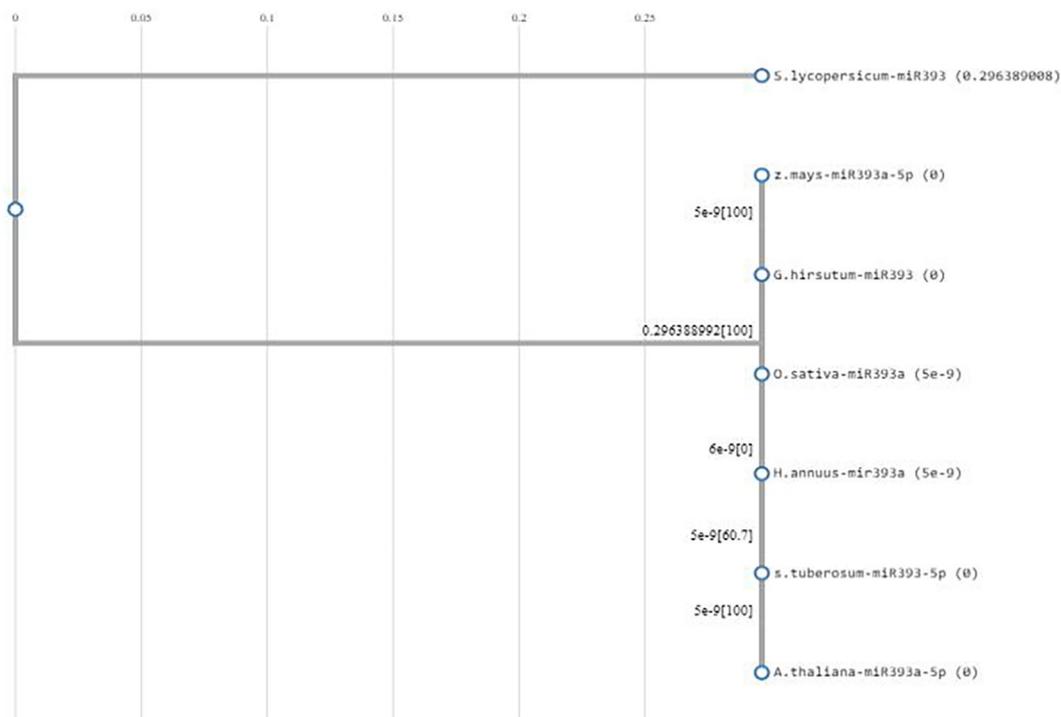


Figure 9. MIR393a(XR_002552875).

which are used for fuel contain potassium and phosphorous which can be composed and returned to the soil as fertilizer.¹³

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.^{14,15} 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.¹⁶

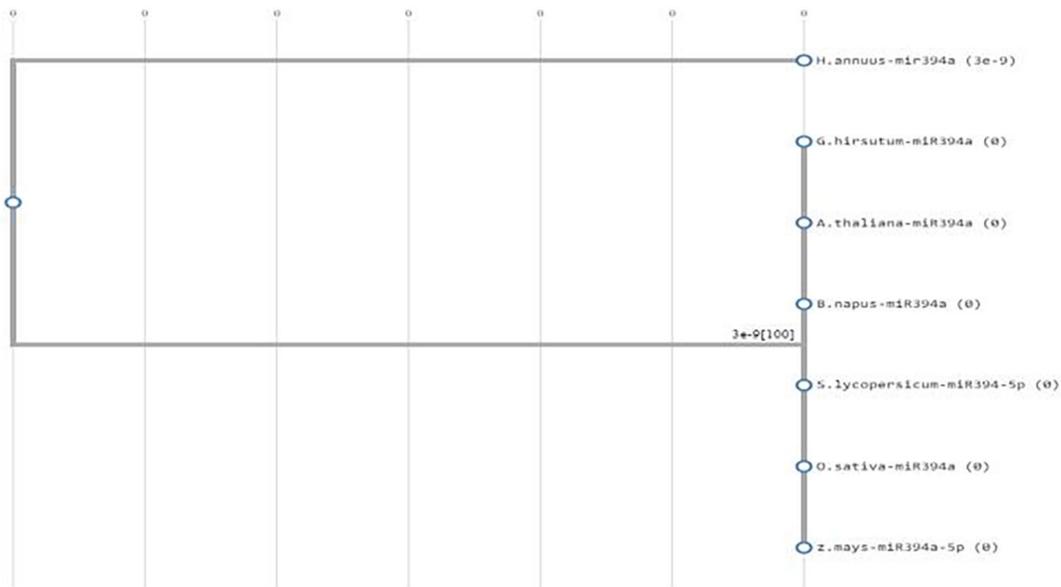


Figure 10. XR_002554296.

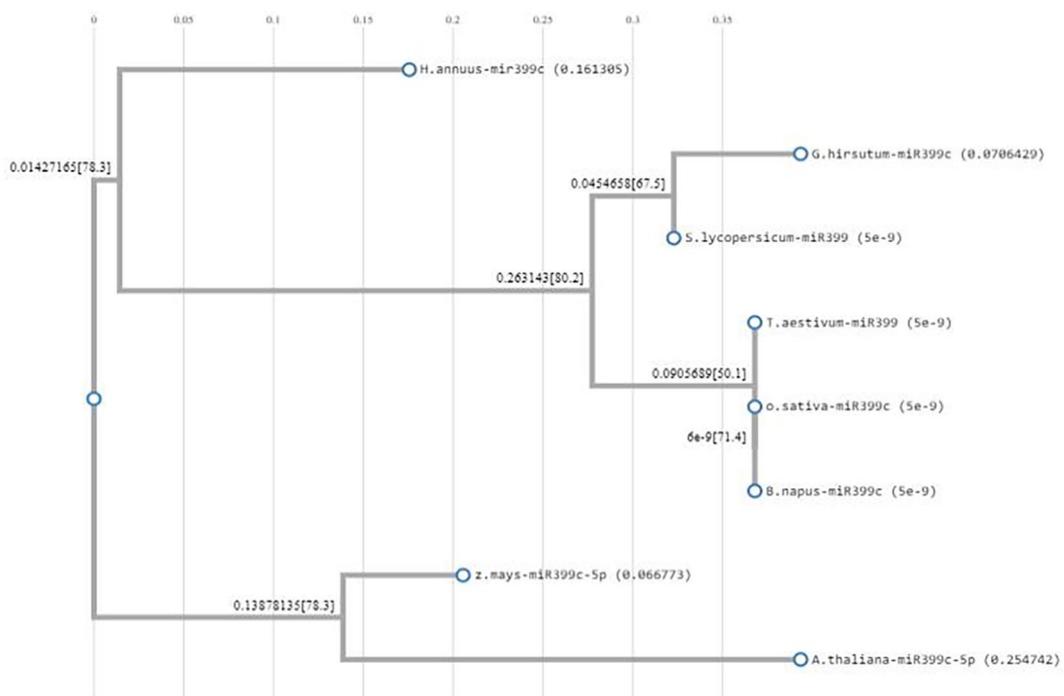


Figure 11. MIR399(XR_002574508).

By regulating gene expression, miRNA plays a vital role to regulate the developmental processes of organisms.¹⁷ The negative regulation of miRNAs in gene expression in both plants and animals has been demonstrated.¹⁸ MicroRNAs have been revealed to modulate diverse developmental processes, including polarity, identity, and organ separation, and to regulate their function and biogenesis.¹⁸ 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, mir319a, 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. officin*;¹⁹ mir-165a and

mir-170 are present in only *A. thaliana*; mir-172a and mir172-b are present in all except *H. annuus*, *T. aestivum*, and *S. officin*;²⁰ mir-319a is present in all except *G. hirsutum*, *H. annuus*, *S. officin*, and *Z. mays*; mir-393a is present in all except *H. annuus*, *T. aestivum*, and *S. officin*; mir-394a is present in all except *H. annuus*, *T. aestivum*, *S. officin*, and *B. napus*; mir-399c is present in all except *H. annuus* and *T. aestivum*; mir-156h is present in all except *G. hirsutum*, *H. annuus*, *B. napus*, and *S. lycopersicum*; 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, mir394a, 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.

REFERENCES

1. Ma X, Tang Z, Qin J, Meng Y. The use of high-throughput sequencing methods for plant microRNA research. *RNA Biol.* 2015;12:709-719.
2. Hajheidari M, Koncz C, Eick D. Emerging roles for RNA polymerase II CTD in Arabidopsis. *Trends Plant Sci.* 2013;18:633-643.
3. Mao W, Li Z, Xia X, Li Y, Yu J. A combined approach of high-throughput sequencing and degradome analysis reveals tissue specific expression of microRNAs and their targets in cucumber. *PLoS ONE.* 2012;7:e33040.
4. Stobrawa K. Poplars (*Populus* spp.): ecological role, applications and scientific perspectives in the 21st century. *Balt For.* 2014;20:204-213.
5. Ahmad M, Ali Q, Hafeez M, Malik A. Improvement for biotic and abiotic stress tolerance in crop plants. *Biol Clin Sci Res J.* 2021;2021:50.
6. Sabagh A, Hossain A, Islam MS, et al. Consequences and mitigation strategies of heat stress for sustainability of soybean (*Glycine max* L. Merr.) production under the changing climate. In: Hossain, A, ed. *Plant Stress Physiology*. IntechOpen; 2020. <https://www.intechopen.com/chapters/71856>
7. Nguyen DTC, Nguyen TT, Le HT, et al. The sunflower plant family for bioenergy, environmental remediation, nanotechnology, medicine, food and agriculture: a review. *Environ Chem Lett.* 2021;19:3701-3726.
8. Bresghehlo F, Coelho ASG. Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *J Agric Food Chem.* 2013;61:8277-8286.
9. Jeyaraj A, Zhang X, Hou Y, et al. Genome-wide identification of conserved and novel microRNAs in one bud and two tender leaves of tea plant (*Camellia sinensis*) by small RNA sequencing, microarray-based hybridization and genome survey scaffold sequences. *BMC Plant Biol.* 2017;17:1-16.
10. Barozai MY, Baloch IA, Din M. Identification of microRNAs and their targets in *Helianthus*. *Mol Biol Rep.* 2012;39:2523-2532.
11. Gupta H, Tiwari T, Patel M, Mehta A, Ghosh A. An approach to identify the novel miRNA encoded from *H. Annuus* EST sequences. *Genom Data.* 2015;6:139-144.
12. Naz S, Fatima Z, Iqbal P, et al. Agronomic crops: types and uses. In: Hasanuzzaman, M, ed. *Agronomic Crops*. Springer; 2019:1-18.
13. Fernández-Luqueño F, López-Valdez F, Miranda-Arámbula M, Rosas-Morales M, Pariona N, Espinoza-Zapata R. An introduction to the sunflower crop. In: Arribas, JA, ed. *Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases*. Nova Science Publishers; 2014:1-18.
14. Hajjehgari B, Farrokhi N. Plant RNA-mediated gene regulatory network. *Genomics.* 2022;114:409-442.
15. Blair CD, Olson KE. The role of RNA interference (RNAi) in arbovirus-vector interactions. *Viruses.* 2015;7:820-843.
16. Takezawa D, Komatsu K, Sakata Y. ABA in bryophytes: how a universal growth regulator in life became a plant hormone? *J Plant Res.* 2011;124:437-453.
17. Zhang B, Wang Q, Pan X. MicroRNAs and their regulatory roles in animals and plants. *J Cell Physiol.* 2007;210:279-289.
18. Dugas DV, Bartel B. MicroRNA regulation of gene expression in plants. *Curr Opin Plant Biol.* 2004;7:512-520.
19. Wojciechowska N. Mechanizmy procesu starzenia liści i korzeni chłonnnych topoli kalifornijskiej (*Populus trichocarpa* Torr. Gray). Published 2020. <https://repozytorium.amu.edu.pl/handle/10593/25657>
20. Baloch IA, Barozai MYK, Baloch AH, Din M. Bioinformatic prediction and annotation of apple microRNAs and their targets. *Pak J Bot.* 2019;51:909-922.