Indian Journal of Biochemistry & Biophysics
Vol. 62, February 2025, pp. 154-160
DOI: 10.56042/ijbb.v62i2.11834

In silico based profiling and characterization of functional microRNAs and their crucial targets in *Saccharum officinarum*

Abdul Baqi[1,2]*, Samiullah[2], Ghulam Mustafa Khan[2], Asadullah[1], Naqeebullah Khan[2] & Attiq-Ur-Rehman[2]

[1]Colleges, Higher and Technical Education Department, Balochistan, Quetta-87300, Pakistan

[2]Department of Chemistry, University of Balochistan, Quetta-87300, Pakistan

Received 14 June 2024; *revised 25 August 2024*

The functionality of sugarcane (*Saccharum officinarum*) plant cells is influenced by numerous metabolic routes and cell signaling pathways. MicroRNAs (miRNAs) play critical functions in regulating production and providing protection against diverse stressors. This research focuses on identifying and partially characterizing novel miRNAs in sugarcane utilizing computational techniques, along with a preliminary assessment of their potential roles. With the help of *in silico* tools, 20 new non-coding miRNAs associated with 16 miRNA families were predicted. Subsequently, 5, 520 potential protein targets for these miRNAs were obtained using the psRNA Target method. Among these, 17 key targets were associated with managing metabolism, transcription, structural proteins, transport factors and cell signaling pathways. Notably, the miRNA sof-miR5169a was linked to the faciata 1-like protein process. In summary, this study highlights the identification of novel sugarcane miRNAs targeting critical genes, offering insights that may contribute to enhancing sugarcane tolerance under environmental stresses.

Keywords: Abiotic stress, Metabolic proteins, miRNAs, Stem-loop structures, Transportation

MicroRNAs (miRNAs) are small RNA molecules, typically 18 to 26 nucleotides (nt) in length, that originate endogenously within the body. These molecules are a subset of non-coding RNAs and play a crucial role in regulating gene expression, either by directing the cleavage of target mRNAs or by post-transcriptionally inhibiting their translation[1,2].

Mature miRNAs, which are derived from long precursor miRNAs (pre-miRNAs) ranging from 70 to 500 nt, are processed into self-folded hairpin structures by Dicer-like 1 (DCL1) enzymes in plants[3-6]. These mature miRNAs target messenger RNAs (mRNAs) for degradation or inhibit protein translation at the post-transcriptional level[7]. In plants, miRNAs typically exhibit near-perfect or perfect complementarity with their mRNA targets, which facilitates mRNA degradation[8]. Research has shown that miRNAs are vital in various plant developmental processes, including cell development, stress response, nutrient absorption, signal transduction, and assimilation[7-10].

*Correspondence:
E-mail: abdulbaqi.achakzai@gmail.com
Suppl. Data available on respective page of NOPR

In recent years, an increasing number of miRNAs have been identified across animals, plants, and even viruses using both computational and experimental methods. According to miRBase (Release 22), a publicly accessible database, approximately 48, 860 miRNAs from 271 plant and animal species have been identified[11].

After this finding, it was discovered that the genomes of miRNAs from a variety of plant species were completely sequenced[11]. Following this discovery, the genomes of miRNAs from various plant species have been sequenced, leading to a growing body of research aimed at characterizing and annotating miRNAs and their functions. Techniques such as direct cloning, deep sequencing, and other advanced methods have been employed in these efforts. Comparative analyses of miRNAs across different plant species, including mosses and eudicots, have revealed evolutionary conservation of certain miRNAs, indicating their significance[9]. Such conserved miRNAs offer a valuable tool for identifying novel miRNAs across diverse species. Currently, conserved miRNAs from various plant species, including *Sorghum bicolor*[3], maize[4], sugarcane[12], and muskmelon[13], have been profiled using comparative genome-based approaches.

Sugarcane (*Saccharum* spp.), a member of the grass family Poaceae, is one of the most widely cultivated crops globally, contributing over 70% of the world's sugar supply. The plant is known for its high caloric yield per unit growth and serves as the primary source of sugar worldwide. Besides sugar production, sugarcane also provides raw materials for alcohol production and various other products. Traditional methods of sugar production aim to maximize sucrose content[12]. Modern sugarcane cultivars are Saccharum hybrids, with research indicating that 70-80% of the genetic makeup of these hybrids can be traced back to *S. officinarum*[14]. The successful cultivation of sugarcane, like other members of the Poaceae family, relies on genetically robust cultivars, efficient seed production, and the ability to withstand environmental stress. Genetic analysis and testing in diverse environments are crucial for improving plant traits[15-18].

Despite its economic importance, only 16 mature miRNAs from sugarcane have been reported in miRBase (Release 22: January 2019). This study aims to contribute to the understanding and profiling of new sugarcane miRNAs. Profiling conserved miRNAs in sugarcane is essential for improving this vital crop. In this work, we have profiled new sugarcane miRNAs and their targets using a comparative genome-based homolog search.

Materials and Methods

Reference miRNA sequences

The miRBase database yielded 6, 164 plant miRNA sequences, including precursor and mature forms[11]. These source miRNAs have been obtained from 16 plant classes (Table 1). These sequences served as reference miRNAs to identify novel conserved miRNAs in sugarcane expressed sequence tags (ESTs).

Retrieving candidate miRNAs

The ESTs of sugarcane were retrieved through a comparative homology-based approach. The Blastn program in the NCBI (National Center for Biotechnology Information) database was employed to analyze sequences from miRBase after selecting the appropriate species. To identify potential conserved miRNAs, reference miRNAs and sugarcane ESTs were analyzed using BLASTn after

Table 1 — Explanation of freshly profiled sugarcane preserved miRNAs.
The sugarcane projected miRNAs are categorized with respect to reference miRNAs

S. officinarum miRNAs	Source miRNAs	[1]PL	[2]MFE	[3]MS (5′→3′)	[4]NM	[5]ML	[6]SE	[7]MSA	[8]GC%	[9]SO	[10]OE
sof-miR160a	zma-miR160a	99	-37. 35	UGCCUGGCU**G**CCUG**C**CUGCCA	3	21	CA242860	5′	71	+	Leaf
sof-miR397a	zma-miR397a	62	-17. 30	UCA**C**UGAGCGCAGCGUUGAUG	1	21	CA183151	3′	57	-	Leaf
sof-miR397b	hvu-miR397a	43	-21. 50	**C**CGUUGAG**C**GC**G**GCGUUGAUG	4	21	CA163560	5′	67	-	Root
sof-miR397c	hvu-miR397a	55	-22. 50	**C**CGUUGAG**C**GC**G**GCGUUGAUG	4	21	CA163560	3′	67	-	Root
sof-miR528a	zma-miR528a	94	-49. 40	UGGAAGGGGCAUGCAGAGGAG	0	21	CA290495	5′	62	+	Seed
sof-miR528b	zma-miR528a	94	-49. 40	UCCUGUGCCUGCCUCUUCCAU	0	21	CA290495	3′	57	+	Seed
sof-miR528c	ata-miR528	48	-28. 40	CCUGUGCCUGCCUCU**G**CC**AUU**	4	21	CA080979	3′	62	-	Leaf
sof-miR1120b	tae-miR1120b	59	-11. 40	**UU**CUU**C**UAUU**U**UGGGACAGAG	4	21	CA087047	5′	38	+	Leaf
sof-miR1127b	tae-miR1127b	88	-19. 94	**A**CA**U**GU**U**UUUCUGGACGGAGG	3	21	CA167120	5′	48	-	Seed
sof-miR1432	zma-miR1432	50	-11. 60	**CU**CAGGAGAGAUGACACCG**AC**	4	21	CA276610	3′	57	-	Seed
sof-miR1435	osa-miR1435	188	-25. 59	U**G**UCUUAAGUCAA**G**CUU**C**U**A**	4	20	CA148668	5′	35	+	Root
sof-miR1846a	osa-miR1846a	105	-38. 45	**AGU**GAGGAGGCCGGGGCCGC**U**	4	21	CA186254	3′	76	+	Seed
sof-miR1846e	osa-miR1846e	76	-35. 90	C**G**ACGAGGAGGCCGG**U**GA**G**C	3	20	CA201353	3′	75	-	Leaf
sof-miR1860	osa-miR1860	147	-75. 40	AGAA**G**ACCAGCUUCCAGAUC**U**	2	21	CA283180	3′	48	+	Seed
sof-miR2925a	osa-miR2925	168	-74. 58	UU**C**GC**C**GCCGCGGGCUUCG	2	19	CA220927	5′	79	+	Leaf
sof-miR2925b	osa-miR2925	89	-53. 50	UGGC**C**GCCGCGGGCUU**CGU**	4	19	CA240884	5′	79	-	Leaf
sof-miR4414a	mtr-miR4414a	53	-14. 10	AGCUG**G**UGAA**U**C**U**UUGGUUCA	3	21	CA094033	5′	43	-	Stem
sof-miR5038a	gma-miR5038a	75	-20. 40	UGAGAAUUUGG**GU**UA**U**UG**G**CCA	4	21	DV732767	3′	43	+	Stem
sof-mir5169a	bdi-mir5169a	138	-22. 90	UUU**U**ACCA**C**GUUU-UAGAAA**A**	4	20	CA167185	5′	25	+	Stem
sof-mir5169b	bdi-mir5169a	141	-33. 48	UUU**U**ACCA**C**GUUU-UAGAAA**A**	4	20	CA167185	5′	25	-	Stem

[1]Precursor miRNA length (PL), [2]Minimum free energy (MFE), [3]Mature sequences (MS), [4]Number of mismatches (NM) [denoted in bold and red], [5]Mature sequence length (ML), [6]Source EST (SE), [7]Mature sequence arm (MSA), [8]GC percentage (GC%), [9]Strand orientation (SO), Sense (+), Antisense (-), [10]Organ of expression (OE)

removing repetitive sequences and protein-coding regions[19]. Candidate sugarcane miRNAs exhibiting non-coding characteristics and allowing up to four mismatches with the reference miRNAs were identified, saved in FASTA format, and subsequently analyzed.

Sugarcane miRNAs stem-loop structures

To profile and describe newly conserved miRNAs in sugarcane, preliminary candidate sequences were examined for hairpin structures[8]. MFOLD tool (version 3.6)[20] was used to create hairpin structures of the initially found sugarcane miRNA sequences in order to examine their secondary structures. In the UNAFold software, the sequences finalized from Blastn were placed to acquire probable stem loop structures. Sequences that failed to generate viable secondary structures were excluded. The selection process prioritized candidate miRNA sequences with constant secondary structures, characterized by mature sequences within the stem section, a minimum free energy (MFE) of ≤ -10 Kcal/mol, and approximately 12 nucleotides comprised in Watson-Crick or G/U base pairing with the complementary strand. These selected sequences were preserved and manually examined for further analysis.

Analysis of phylogenetic and conservation relationships

A phylogenetic study of miR-160 was conducted by equating it to other monocotyledonous and dicotyledonous plant precursors connected with *Saccharum officinarum* (sof), *Zea mays* (zma), *Asparagus officinalis* (aof), *Glycine max* (gma), *Eugenia uniflora* (eun) and *Arabidopsis thaliana* (ath) using Clustal Omega. Additionally, a conservation study of various plant precursors, including *Zea mays* (zma), *Eugenia uniflora* (eun) and *Glycine max* (gma) compared to *Saccharum officinarum* (sof), was conducted using the WebLogo tool (version 2.8). The process for logo creation followed previously established methods[4, 21].

Targets assessment

The newly discovered sugarcane miRNAs were evaluated for potential targets using psRNATarget: A Plant Small RNA Target Analysis Server (2017 Update)[22]. The sugarcane database served as the target library, with the analysis performed using updated 2017 parameters, including a mismatch penalty of 1.0 and a maximum expectation cutoff of (5)[23,24].

Results

Sugarcane new potential miRNAs

In this study, 20 new sugarcane miRNAs were profiled through several computational tools. These newly non-coding miRNAs belong to 16 distinct miRNA families (Table 1). Notably, these 20 miRNAs were not formerly reported and are presented here for the first time. These novel miRNAs were derived from source miRNAs such as *A. tauschii* (5%), *B. distachyon* (10%), *G. max* (5%), *H. vulgare* (10%), *M. truncatula* (5%), *O. sativa* (30%), *T. aestivum* (10%), and *Z. mays* (25%).

Sugarcane miRNAs characterization

Several criteria were established to classify and describe the newly profiled sugarcane miRNAs (Table 1). The mature sequences of these newly conserved miRNAs were located within the stem regions of their respective hairpin structures (Fig. S1). In addition, the sugarcane pre-miRNAs ranged in length from 43 to 188 nt, with a mean length of 94 nt. So, the distribution of pre-miRNA lengths showed that 1-50 nt pre-miRNAs accounted for 15% of the

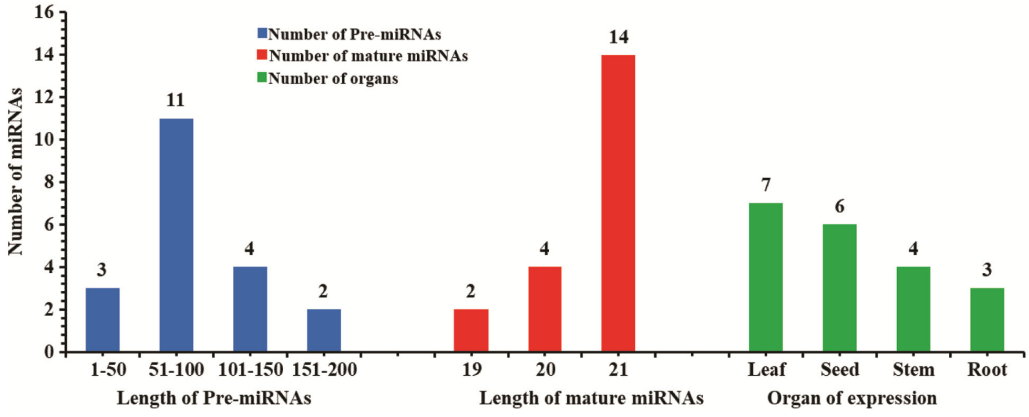

Fig. 1 — The Divisions Recognized in Sugarcane ESTs: Precursor miRNAs length, Mature miRNAs length and organ of expression

total, 51-100 nt made up 55%, 101-150 nt constituted 20%, and 151-200 nt accounted for 10% (Fig. 1).

The minimal free energy (MFE) of freshly discovered sugarcane pre-miRNAs ranged from -75.4 to -11.4 kcal mol^{-1}, with a mean of -33.15 kcal mol^{-1}. Regarding MFE distribution, 10% of pre-miRNAs fell within the -79 to -60 kcal mol^{-1} range, 15% within the -59 to –40 kcal mol^{-1} range, 50% within the –39 to –20 kcal mol^{-1} range, and 25% within the –19 to 0.0 kcal mol^{-1} range. Ranging from 0 to 4, the mismatches between the predicted mature sugarcane miRNAs and their reference sequences averaged 3. Half of the miRNAs exhibited 4 mismatches, 20% had 3 mismatches, 10% had 2 mismatches, 5% had 1 mismatch, and 15% were perfectly matched. Sugarcane miRNAs had mature lengths ranging from 19 to 21 nt, with a mean of 21 nt. Most of the mature sequences (70%) were 21 nt in length (Fig. 1).

Of the mature sequences, 55% were located on the 5' arm of the secondary structures, while 45% were found on the 3' arm. Ranging from 25% to 79%, the GC content, a key parameter, had an average of 55%. The distribution of GC content showed that 20% of sequences had a GC content between 10% and 40%, 35% between 41% and 60%, and 45% between 61% and 80%. The organ of expression for the ESTs of the recently analyzed sugarcane miRNAs were also presented. The majority of miRNAs were expressed in the leaf (7 out of 20), representing 35% of the total, followed by seed (6), contributing 30%, stem (4), accounting for 20%, and root (3), representing 15% (Fig. 1).

Phylogenetic and conservation analysis of sugarcane miRNAs

This study presents an in-depth analysis of sugarcane miRNAs, focusing on their phylogenetic relationships and conservation across various plant species. The phylogenetic tree (Fig. 2) highlights the close relationship between sugarcane and the grass species *Zea mays* (maize), as illustrated by the red-highlighted box. The analysis shows that sof-miRNA160a is closely associated with *Z. mays* (zma) compared to other species such as *A. officinalis* (aof), *G. max* (gma), *E. uniflora* (eun) and *A. thaliana* (ath). Conservation analysis of the pre-miRNA 160a (Fig. 3) further reveals conserved regions within the mature sequences that are shared with other plants, including *Z. mays*, *E. uniflora* and *G. max*, as shown by the red-highlighted areas.

Target prediction and functional annotation of sugarcane miRNAs

A critical aspect of this research involved the identification and functional annotation of sugarcane miRNA targets. Using stringent criteria, we predicted the targets of mature sugarcane miRNAs, with results summarized in Table S1 and depicted in (Fig. 4). The

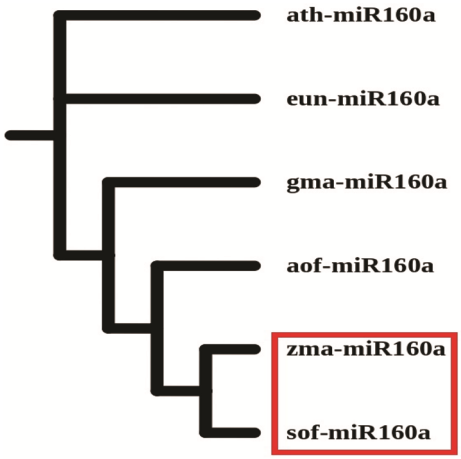

Fig. 2 — Phylogenetic study of sugarcane miRNA. When equated to *Z. mays, A. officinalis, G. max, E. uniflora and A. thaliana*, a tree exposed that *Z. mays* is closely connected to *S. officinarum*. The adjacent plant species were highlighted with the red box

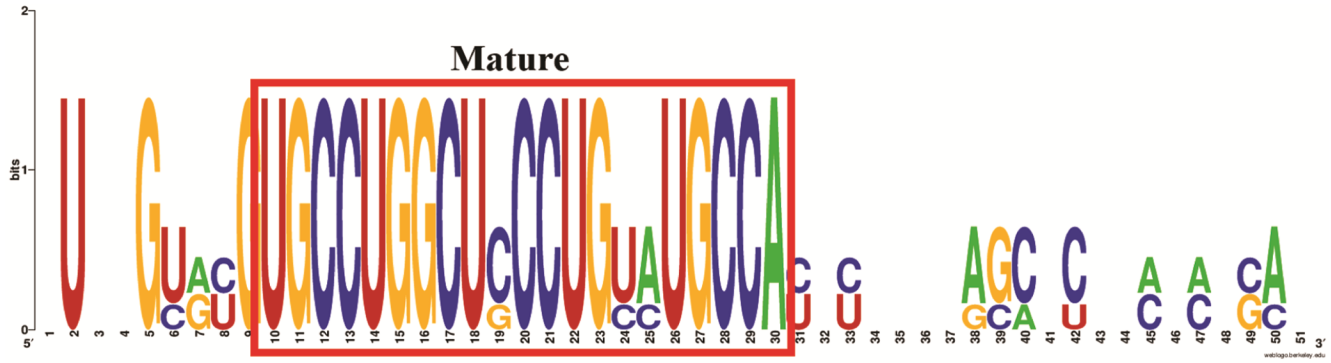

Fig. 3 — Analysis of sugarcane miRNA conservation. The highlighted red boxed area displays mature miRNA sequences along with their conservation status

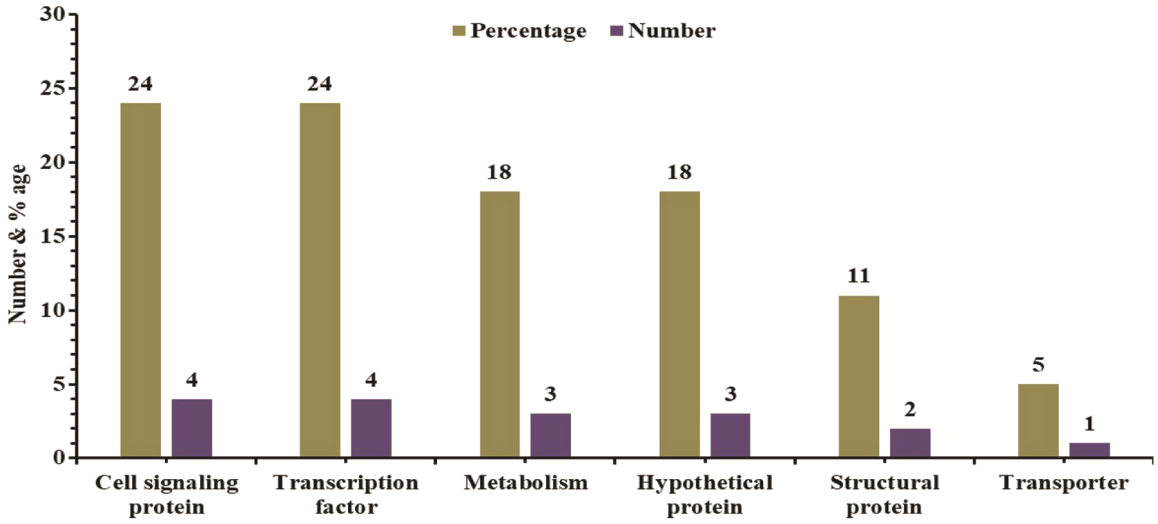

Fig. 4 — The graph displays different proteins targeted by sugarcane miRNAs

analysis identified 5520 potential protein targets, of which 17 were deemed significant and categorized based on their functional roles. These categories include structural proteins, transcriptional regulators, metabolic enzymes, cell signaling proteins, and transporters.

Discussion

The miRNAs identified in this study include homologs from both dicot and monocot species. While some miRNAs are conserved across both groups, others appear to be unique to either dicots or monocots. Comparative genomics, a widely used approach in the discovery of novel miRNAs, led to the identification of 20 new sugarcane miRNAs[24-26]. These newly identified miRNAs were analyzed using empirical formulas (A, B, and D), with principle D proving essential for confirming homologous sequences across multiple plant species[27]. This principle was thus employed to identify and verify new conserved sugarcane miRNAs.

Our analysis demonstrates that the stem section of predicted miRNA hairpin structures contains 11-21 nucleotides involved in G/U or Watson–Crick base pairing between the mature miRNAs and its complementary arms (pre-miRNAs). This structural feature, which lacks significant internal loops or bulges, aligns with findings from similar studies on miRNAs in other plant and animal species[28]. Most identified pre-miRNAs (11 out of 20) were found within a length range of 51–100 nt, with a minimum free energy (MFE) ranging from –75.4 to –11.4 kcal/mol and a mean of –33.15 kcal/mol. These MFE values are consistent with those reported in maize and wheat[4,6].

The study also examined the mismatch rates between predicted mature sugarcane miRNAs and their source sequences, ranging from 0 to 4, with a mean of 3 mismatches. Approximately 15% of the analyzed miRNAs were perfectly matched, a rate comparable to that observed in other plant and animal species[28]. Additionally, the mature miRNA sequences were predominantly 21 nucleotides in length, with 70% of the miRNAs (14 out of 20) falling within this category. This length is consistent with known miRNAs from Coffea species[28].

Notably, 50% of the miRNAs were found on the sense strand, whereas the remaining 50% were located on the antisense strand. Furthermore, 55% of mature miRNAs were located on the 5' arm of secondary structures, with the remaining 45% on the 3' arm. Organ-specific expression of sugarcane miRNAs revealed their involvement in the growth and regulation of various plant tissues, with the highest expression observed in leaves (35%), followed by seeds, stems, and roots. These findings are consistent with organ-specific miRNA expression patterns reported in other plant species, such as cherry and red alga[21,29].

The identified sugarcane miRNAs target a diverse range of proteins, playing crucial roles in various biological processes. It is shown that one single miRNA can target a collection of proteins[3]. According to our analysis 4 out of 17 targeted proteins (24%) were involved in signal transduction, including serine/threonine kinase, nematostella vectensis, mitogen-activated protein kinase, and faciata 1-like protein. Numerous scientists have previously

identified a wide range of cell signaling targets in various plants[28,30].

The second class of proteins includes transcription factors. They are a component of every plant and are essential to the growth of plants[6]. Four targeted proteins (24%) were classified as transcription factors, essential for plant growth and development. These include elongation factor 1-alpha, Os12g0110400 protein, and helix-loop DNA-binding domain-containing protein.

The third class consists metabolic functions in which 3 out of 17 targets (18%) were involved in metabolic processes, such as aspartate aminotransferase, ADP-ribosylation factor, and glycine-rich RNA-binding protein. Metabolism is the main function of these targeted proteins involving in different metabolic functions such as stem formation, in the cell cycle etc. of sugarcane plant[15,31]. The fourth class are hypothetical proteins in which 3 targeted proteins (18%) were classified as hypothetical, with functions yet to be determined. These proteins were identified using methods previously employed in other plant species[6,32].

The fifth class contains the structural protein where two targeted proteins (11%) were involved in structural roles, crucial for the development of roots, shoots, leaves, and flowers. These included histone H2A and probable histone H2A variant 1. Several scientists have stated many structural targeted proteins in numerous plants utilizing this technique[15,33-34]. In the sixth class, one targeted protein (5%), identified as a monosaccharide transport protein, was involved in molecular transportation within the plant. There have been reports of several targeted transport proteins created using the identical method in various plants[28].

Conclusion

This research marks the first identification of 20 novel potential sugarcane miRNAs, distributed across 16 different miRNA families. Advanced bioinformatics approaches were employed to predict and analyze these miRNAs, leading to the identification of 5520 protein targets, with 17 key targets playing roles in processes such as metabolism, cell signaling, transcription, structure, and transportation. Notably, sof-miR397c was found to regulate the monosaccharide transport protein 1, facilitating molecular transport, while sof-miR1860 regulated mitogen-activated protein kinase, which is involved in cell signaling. These findings underscore the diverse and significant roles of sugarcane miRNAs in regulating gene expression, with implications for enhancing sugarcane productivity and resilience.

Acknowledgement

The work for this research has been carried out at the University of Balochistan and the authors are thankful to the faculty members of Department of Chemistry, University of Balochistan.

Conflicts of interest

The authors declare no conflict of interest.

References

1 Singh P, Kumar Gupta S, Ali V, Chhabra R & Verma M, miR-145-5p and miR-203a-5p overcome imatinib resistance in myelogenous leukemic cells through metabolic reprogramming. *Indian J Biochem Biophys,* 60 (2023) 167.

2 Almatroudi A, Non-Coding RNAs in Tuberculosis Epidemiology: Platforms and Approaches for Investigating the Genome's Dark Matter. *Int J Mol Sci,* 23 (2022) 4430.

3 Baqi A, Samiullah, Rehman W, Bibi I, Menaa F, Khan Y & Sattar A, Identification and Validation of Functional miRNAs and Their Main Targets in *Sorghum bicolor*. *Mol Biotechnol,* (2023) 1.

4 Baqi A, Samiullah, Ayub M, Saleem MZ, Khan GM & Asadullah, Genome-wide profiling of novel conserved *Zea mays* microRNAs along with their key biological, molecular and cellular targets and validation using an RT-PCR platform. *Karbala Int J Modern Sci,* 10 (2024a) 4.

5 Yusof K, Rosli R, Abdullah M & Avery-Kiejda KA, The roles of non-coding RNAs in tumor-associated lymph angiogenesis. *Cancers*, 12 (2020) 3290.

6 Achakzai HK, Barozai MYK, Din M, Baloch IA & Achakzai AKK, Identification and annotation of newly conserved microRNAs and their targets in wheat (*Triticum aestivum* L.). *PLoS One,* 13 (2018) e0200033.

7 Jayaswal D, Mainkar P, Kumar K, Agarwal Y, Kalia V & Kansal R, Pyramiding and evaluation of segregating lines containing lectin and protease inhibitor genes for aphid resistance in *Brassica juncea*. *Indian J Biochem Biophys,* 59 (2022) 800.

8 Rani V & Sengar RS, Biogenesis and mechanisms of microRNA-mediated gene regulation. *Biotechnol and Bioengin*, 119 (2022) 685.

9 Hajieghrari B & Farrokhi N, Plant RNA-mediated gene regulatory network. *Genomics,* 114 (2022) 409.

10 Rojas-Pirela M, Andrade-Alviarez D, Medina L, Castillo C, Liempi A, Guerrero-Muñoz J, Ortega Y, Maya JD, Rojas V, Quiñones W & Michels PA, MicroRNAs: master regulators in host–parasitic protist interactions. *Open Biol,* 12 (2022) 210395.

11 Kirchner B, *Functional importance of intra-and extracellular microRNAs and their isoforms in blood and milk* (Doctoral dissertation, Technische Universität München) (2022).

12 Baqi A, Saleem MZ, Ayub M & Saeed S, Identification and annotation of the 21 novel sugar cane (*Saccharum officinarum*) microRNA clusters and their significant

biological, molecular and cellular targets. *Trop Plant Biol,* (2024b) 1.
13 Ghaffar A, Baqi A, Ali I & Shah WA, *In silico* prediction and profiling of novel non-coding miRNAs and their target proteins in muskmelon (*Cucumis melo*). *Pak J Agric Sci,* 60 (2023) 337.
14 Xue A, Li Z, Cai M, Zhang Q, Zhang X, Ming R & Zhang J, Identification and Characterization of microRNAs from *Saccharum officinarum* L by Deep Sequencing. *Trop Plant Biol,* 10 (2017) 134.
15 Achakzai HK, Barozai MYK, Achakzai AKK, Asghar M & Din M, Profiling of 21 novel microRNA clusters and their targets in an important grain: wheat (*Triticum aestivum* L.). *Pak J Bot,* 51 (2019) 133.
16 Fontana DC, de Paula S, Torres AG, de Souza VHM, Pascholati SF, Schmidt D & Neto DD, Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses. *Pathogens,* 10 (2021) 570.
17 Awaad HA, Negm AM & Abu-hashim M, Update, Conclusions, and Recommendations of "Mitigating Environmental Stresses for Agricultural Sustainability in Egypt". In Mitigating Environmental Stresses for Agricultural Sustainability in Egypt. *Springer Cham,* (2021) 561.
18 Rasheed A, Hassan MU, Aamer M, Batool M, Sheng FANG, Ziming WU & Huijie LI, A critical review on the improvement of drought stress tolerance in rice (*Oryza sativa* L.). *Notulae Botani Horti Agrobotan Cluj-Napoca,* 48 (2020).
19 Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ, Basic local alignment search tool. *J Mol Biol,* 215 (1990) 403.
20 ZukerM, Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res,* 31 (2003) 3406.
21 Baloch IA, Barozai MYK & Din M, Bioinformatics prediction and annotation of cherry (*Prunu savium* L.) microRNAs and their targeted proteins. *Turk J Bot,* 42 (2018) 382.
22 Dai X & Zhao PX, psRNATarget: a plant small RNA target analysis server. *Nucleic Acids Res,* 39 (2011) W155.
23 Ganeshpurkar A, Chaturvedi A, Shrivastava A, Dubey N, Jain S, Saxena N & Mujariya R, *In silico* interaction of Berberine with some immunomodulatory targets: A docking analysis. *Indian J Biochem Biophys,* 59 (2022) 848.
24 Baqi A, Samiullah, Saleem MZ, Ayub M & Habibullah, Experimental validation and characterization of Sugarcane Genome-Encoded MicroRNAs and their targets using PCR-based expressional methodology. *J Agric Sci Tech,* (2024c) 1057.
25 Shah SQ, Barozai MYK, Din M, Baloch IA & Wahid HA, 15 RNA secondary structure analysis for abiotic stress resistant and housekeeping genes in *Arabidopsis thaliana* and *Oryza sativa*. *Pure Appl Biol,* 5 (2021) 476.
26 Ghani A, Din M & Barozai MYK, Convergence and divergence studies of plant precursor microRNAs. *Pak J Bot,* 50 (2018) 1085.
27 Ambros V, Bartel B, Bartel DP, Burge CB & Matzke M, A uniform system for microRNA annotation. *RNA,* 9 (2003) 277.
28 Baqi A, Khan GM, Khan N & Ahmed A, Identification and characterization of functional microRNAs and their significant targets in maize plants. *J Plant Biochem Biotech,* 33 (2024) 700.
29 Barozai MYK, Qasim M, Din M & Achakzai AKK, An update on the microRNAs and their targets in unicellular red alga *Porphyridium cruentum*. *Pak J Bot,* 50 (2018) 817.
30 Zamani H, Soleimani M, Kaviani S, Azimi A & Atashi A, DNA methylation is involved in the microRNA-886-3p gene expression and plays a potential role in hematopoietic stem and progenitor cell mobilization through affecting SDF-1. *Indian J Biochem Biophys,* 59 (2022) 103.
31 Demirci MDS & Adan A, Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection. *Peer J,* 8 (2020) e9369.
32 Trivedi GP, Gajera J, Ghanchi IF & Sindhav MG, Simultaneous detection of thirteen exons of dystrophin gene by optimized multiplex PCR assay to screen Duchenne/ Becker muscular dystrophy. *Indian J Biochem Biophys,* 60 (2022) 31.
33 Liu K, Ju Z, Jia Z, Liang G, Ma X & Liu W, Genome-Wide Identification and Characterization of the Oat (*Avena sativa* L.) WRKY Transcription Factor Family. *Genes,* 13 (2022) 1918.
34 Zhang L, Yang B, Zhang C, Chen H, Xu J, Qu C & Li J, Genome-Wide Identification and Post transcriptional Regulation Analyses Elucidate Roles of Key Argonautes and Their miRNA Triggers in Regulating Complex Yield Traits in Rapeseed. *Int J Mol Sci,* 24 (2023) 2543.