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Identification of evolutionary conserved muskmelon non-coding miRNAs and their target proteins using computational approach and its utility in phylogeny analysis

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ABSTRACT

The class of tiny, single-stranded, non-coding RNAs known as microRNAs (miRNAs) consist of 18 to 26 nucleotides. By inhibiting post-transcriptional gene expression, miRNAs govern a variety of biological processes. MiRNAs play a critical role in controlling the growth and development of plants. We studied expressed sequence tags data from different species [*Arabidopsis lyrata* (aly), *Arabidopsis thaliana* (ath), etc.] to identify muskmelon miRNAs and their target genes. By investigating miRNAs in muskmelon, the current study has focused on miRNA verification and expressional analysis. The muskmelon-expressed sequence tags were used in the current investigation to identify 19 novel non-coding miRNAs with minimum folding energy values ranging from -10.10 to -50.40 kcal/mole. The newly predicted muskmelon miRNAs have several target genes using the psRNA-Target tool connected with Gene Ontology enrichment analysis. As a result, a total of roughly 56 targets were predicted in response to biotic and abiotic stress. For instance, the metabolism and enzyme activity of muskmelon is regulated by the miR5043, miR5998, miR11161 and miR11576. Similarly, miR1888, miR2079 and miR5042 typically control plant hormones. Our findings suggest that the Cucurbitaceae family possesses miRNAs that are both conserved and specialized and will help to target genes which will be crucial for Cucurbitaceae plant growth and development.

Keywords: miRNAs, muskmelon, EST, protein targets, molecular functions

INTRODUCTION

MicroRNAs (miRNAs) are small, single-stranded non-coding RNAs lengths range from 18–26 nucleotides and are found in plants, animals and some viruses (Baloch et al., 2018). They are essential for post-transcriptional regulation of gene expression and RNA gene silencing. MicroRNAs carry out important functions via complementary base pairing with Messenger RNA (mRNA) through perfect hybridization (Riolo et al., 2020). MicroRNAs have been described as adversely influencing gene expression by targeting mRNAs for cleavage or translational inhibition (Ghaffar et al., 2023). In plants, miRNAs control tissue development and differentiation, signalling, vegetative or reproductive growth and responses to biotic and abiotic stresses such as salinity, drought and infections (Ghaffar et al., 2024).

One of the economically significant plant families is the Cucurbitaceae family, which includes a variety of plants, such as pumpkin and squash (Cucurbita), cucumber (*Cucumis sativus*), muskmelon (*Cucumis melo*), calabash (*Lagenaria siceraria*), watermelon (*Citrullus lanatus*) and others. The Cucurbitaceae family consists of 975 species and 98 genera and is largely utilized in food and in medicinal use (Ghaffar et al., 2023). This family's members have served as model organisms for

research on the biology of plant vascular systems and sex determination. One of the most widely cultivated fruits in the Cucurbitaceae family is the muskmelon (*Cucumis melo*). The melon's 12 chromosomes are now being used as a model to investigate a variety of biological processes, including color, flavor and texture, throughout fruit development (Ghaffar et al., 2023). In modern studies, *Cucumis melo* has achieved a very significant position in research conducted on plants for the research on biological processes such as fruit development, texture, flavour and colour (Garcia-Mas et al., 2012). Several factors influence the growth of *Cucumis melo* including abiotic and biotic conditions, modern agricultural techniques and high-yield seeds. We can improve the yield and productivity of *Cucumis melo* in different environments by investigating their genetic characteristics through sophisticated methodologies. Muskmelon is considered the best choice among the Cucurbitaceae family due to its short life span, small genomic size, and outstanding fruit development and repining (Garcia-Mas et al., 2012). Among these qualities, it is widely cultivated on over the world with China's No. 1 country producing 49 m tonnes annually almost one-third of the world's production of muskmelon (FAOSTAT, 2022). As previously mentioned *Cucumis melo* is an excellent choice for studying fruit development and repining, sex of determination and phloem physiology, it is widely popular worldwide with an annual production of 26 million tons. Nevertheless, despite the importance of using the melon genome as a model, little research has been done on this genus. Because only 140 miRNAs from various families have been identified in muskmelon and deposited in the miRNA registry database (miRBase) (Ghaffar et al., 2023), there is still room to predict novel non-coding miRNAs. Therefore, identifying novel microRNAs and their targets in a variety of agriculturally important plant species is crucial for basic biology and may have biotechnological applications.

Using expressed sequence tag (EST) analysis for miRNA identification has major advantages for computational methods. ESTs are subsequences which provide quick and efficient information about the miRNA and their prediction. These subsequences produce data sets that may be effectively analyzed to evaluate gene expression (Fizames et al., 2004; Ogihara et al., 2003; Watanabe et al., 2007). To anticipate new miRNAs in muskmelon, many comparative genome-based homolog search techniques were used. For Instance, miRbase was used as a reference sequence tool for the investigation of novel miRNAs. Blastn at the National Centre for Biotechnology Information (NCBI) tool was used to compare the reference sequences to the sequence database and calculate the statistical significance of matches. The Mfold tool created a secondary stem-loop structure with the lowest minimum free energy (MFE) values, which was noted and kept back for additional investigation. The psRNA target tool was used to predict their various protein targets (Ghaffar et al., 2023). Thus, 56 distinct protein targets were identified with the GO analysis tool in muskmelon. Additionally, phylogenetic analysis was done utilizing Clustal Omega (Ghaffar et al., 2024). The results of this study will open the door to further investigation into the physiological and regulatory functions of miRNAs, particularly in muskmelon growth and development.

MATERIALS AND METHODS

The computational identification and expressional evaluation of new miRNAs in muskmelon were carried out using the "Bioinformatics Algorithms" for homology investigation. The fundamental steps for this study are listed below.

Selection of source miRNAs

MicroRNA Registry Database, viewed on December 19, 2021, at www.miRBase.org (Kozomara et al., 2019), included a collection of 48,860 mature microRNAs from various plant species. The sequences of source microRNAs served as a guide for the prediction of new miRNA sequences in muskmelon. The miRNA sequences within the specified species were subjected to BLASTx analysis with default parameters to weed out repeated sequences and get rid of redundancy or overlaps (Kozomara et al., 2019). A BLASTn search with default parameters was then conducted using the remaining candidate sequences as search query sequences.

Prediction of potential miRNAs

In order to profile new miRNAs in muskmelon, a method developed by Achakzai et al. (2019) was used. The miRNA sequences which matched with muskmelon sequences were used in BLAST to look for homologous sequences in the ESTs and mRNA sequences for species of muskmelon after eliminating the repeated sequences. The ESTs and mRNA sequences with 0–4 nucleotide mismatches to the query miRNA sequences were manually chosen using the BLAST homology search. BLASTX was used to find out the protein-coding sequences and removed them manually.

Using the Zucker folding algorithm and mFold V3.2, the candidate sequences are used to predict the secondary structure of novel non-coding miRNAs (Zuker, 2003). The following criteria, as previously stated by Zhang et al. (2006a) were used to guide the selection of candidate miRNAs and pre-miRNAs. The miRNA should have fewer than six nucleotide mismatches with the complementary miRNA sequence (miRNA*) and the projected mature miRNAs should have no more than four nucleotide mismatches in comparison to the query sequences. Second, the putative miRNA sequence should not be found in the hairpin structure's terminal loop. Instead, it should be found on the same arm of the stem-loop structure as its recognized homologs. The prospective pre-miRNAs also need to have a lower minimal folding free energy -10kcal/mol index than other RNA types and no significant loops or breaks in the miRNA sequence.

Prediction of potential target genes and their functions

By comparing the predicted sequences of the muskmelon miRNAs to the protein-coding nucleotide database from Gene Bank using BLASTn, we were able to identify their putative target genes in this study. The psRNA-Target tool with default parameters was initially used to examine miRNAs produced from melon in order to find possible targets. The methodology employed in this research involved utilizing the Gene Ontology (GO) detection technique to label genes according to their linked biological processes, molecular functions, and cellular components. Bioinformatics tools were used to assign GO terms by matching gene sequences with existing annotations from public databases. This approach offers a thorough understanding of gene functions and relationships, facilitating the recognition of important biological pathways and processes pertinent to our investigation. However, a second approach, as outlined by Barozai (2012) was used for those miRNAs that failed to produce any notable targets. This method includes using the NCBI BLASTn programme to quickly analyse mature miRNA sequences from muskmelon with default parameters (Zhang et al., 2008).

Phylogenetic analysis

Pre-miRNA sequences from 20 plant species were collected from miRBase (version 22, March 2018) (Griffiths-Jones et al., 2007) in order to establish the phylogeny of the predicted miRNAs in muskmelon with default settings. The phylogenic tree was created using Clustal OMEGA 6.0 (Tamura et al., 2013) connected with the iToL alignment tool to align these sequences.

RESULTS

Based on homology search-based computational approaches, 19 conserved novel non-coding miRNAs and 56 target genes in muskmelon were identified by using the publicly accessible database of ESTs (dbEST) at https://blast.ncbi.nlm.nih.gov/ (Altschul et al., 1997). These results demonstrate the presence of a large number of miRNAs in the muskmelon genus and add to the mounting evidence that several miRNAs have undergone significant evolutionary conservation in a variety of plant species.

Characterization of miRNAs in the Muskmelon

A total of 19 novel non-coding miRNAs specific to muskmelon have been successfully predicted, belonging to 16 distinct families. Thirteen families consisted of a single member, except for cme-miR-771, cme-miR-829 and cme-miR-1888, which consisted of two members. The mature sequences of muskmelon miRNAs are found in the 3' or 5' arm of the miRNA stem-loop structure, as shown in Figure 1.

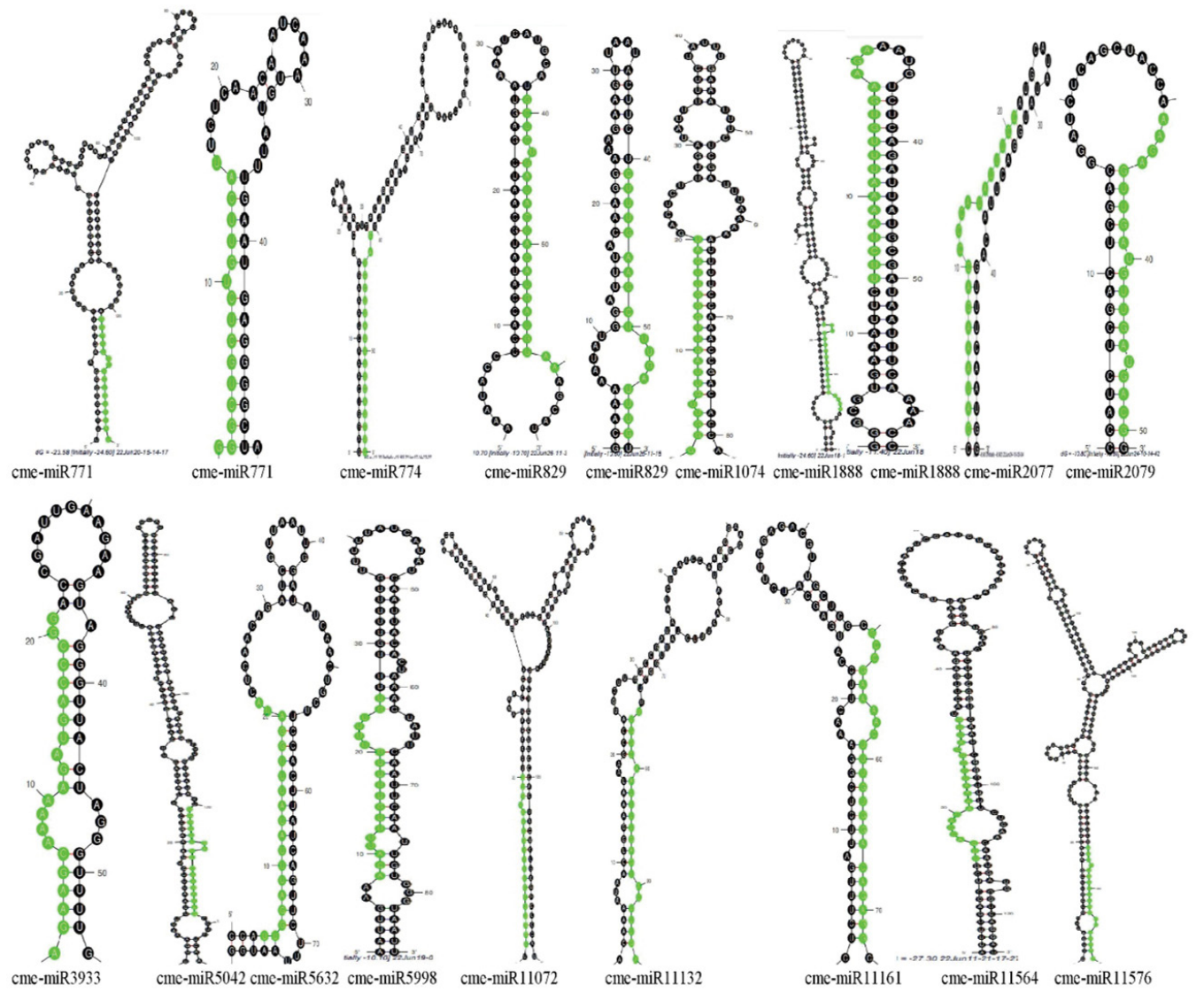

Figure 1. Secondary structures of novel muskmelon miRNA precursors (green color represents regions where mature miRNA are located)

Furthermore 12 out of 19 (63%), miRNAs were found at the 3' arm of the predicted muskmelon species and 7 (37%) on the 5' arm secondary hairpin structure. Typically, muskmelon miRNAs have a length of 17 to 24 nucleotides, but the average length of certain miRNAs was 21 nucleotides (Table 1).

The formation of stem-loop hairpin configurations in miRNAs depends critically on the MFE. A miRNA sequence's secondary structure is more stable when the MFE values are lower. The average MFE values ranged from -10.10 to -50.40 kcal/mole. The measured mismatches were between 0-4, with an average of 2 nucleotides. When compared to their homologs, the miRNAs that had the most mismatches had a maximum of 1 = 05 (26.31%), followed by 0, 3 = 4 (21.05%), and 4, 2 = 3 (15.79%) mismatches. Only 4 among the 19 new miRNAs (21% of all miRNAs) matched its homolog exactly. The identified secondary structures of miRNA precursors are shown in Figure 1.

The mature miRNA and its opposing arm (miRNA*) were shown to have Watson-Crick or G/U base pairs spanning about 17 nucleotides in the secondary structures of muskmelon miRNAs. Except in cases where the miRNA* had fewer base pairs, which are not regarded as miRNAs, stem-loop topologies did not typically imply large internal loops or bulges.

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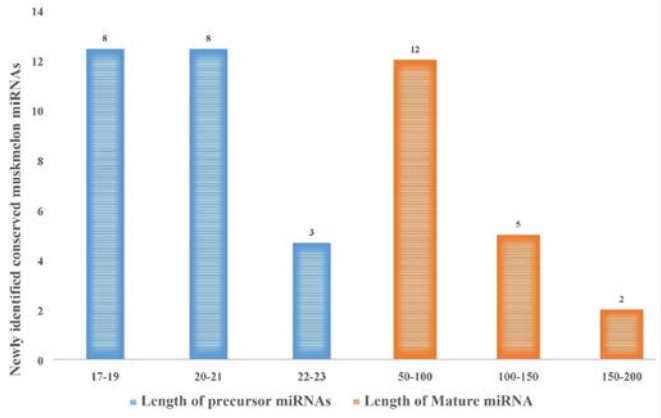
Table 1. Annotation of newly profiled muskmelon conserved miRNAs

Cucumis Melo miRNAs	Source miRNAs	PL	MFE	MS	NM	ML	SE	MSA	GC%	SO	OE
cme-miR771	aly-miR771	137	-23.58	GGGCUGCUC**AGA**UGUUCAU	3	19	JG511394	3'	52.6	-	Fruit
cme-miR771	aly-miR771	50	-12.70	GGGCUGCUC**AGA**UGUUCAU	3	19	JG511394	5'	52.6	-	Fruit
cme-miR774	ath-miR774	98	-12.00	CAUCC**U**UAUUUUCAUCUC	1	18	AM730418	3'	33.3	-	Cotyledon
cme-miR829	ath-miR829	59	-11.30	CUUUGAAGCUUUGAUUUG	0	18	LN681840	3'	33.3	-	Root
cme-miR829	ath-miR829	65	-10.70	GCUCUGAUACCA**C**AUGAUGGAA	1	22	LN713265	3'	45.5	+	Root
cme-miR1074	ppt-miR1074	129	-20.57	GGGUUGUUAGUU**G**GGUUGAU	1	20	LN713261	3'	45.0	-	Fruit
cme-miR1888	ath-miR1888	155	-24.60	UU**UCC**CUAA**A**AUUUGUGAAGA	4	21	AM737930	3'	28.6	-	Fruit
cme-miR1888	ath-miR1888	63	-11.40	UU**UCC**CUAA**A**AUUUGUGAAGA	4	21	AM737930	5'	28.6	-	Fruit
cme-miR2077	ppt-miR2077	50	-10.10	CAUAAAGAC**C**CCAUAUUUCC	2	20	JG536609	5'	40.0	-	Flower
cme-miR2079	ppt-miR2079	51	-10.80	AAGAGUUGAUGUUGAUGACG	0	20	AM741359	3'	40.0	-	Fruit
cme-miR3933	ath-miR3933	54	-10.50	AGAAGCAAAA**A**GA**U**GAC**C**CGG	3	21	AM740788	5'	47.6	-	Fruit
cme-miR5042	gma-miR5042	146	-31.60	GGCUUGAUCCAAGAUAG	0	17	AM737001	3'	47.7	+	Fruit
cme-miR5632	ath-miR5632	77	-12.20	UUGGAUUUAUA**U**UUGGAUA	1	19	AM732940	5'	21.1	-	Cotyledon
cme-miR5998	ath-miR5998	86	-10.60	ACA**U**UUUGU**CA**UUUGUUUUGU	3	21	AM723470	5'	23.3	+	Root
cme-miR11072	lja-miR11072	138	-34.64	UGGU**U**CCUGUGG**G**UGUGUG	2	19	JG553168	3'	57.9	-	Root
cme-miR11132	lja-miR11132	96	-22.40	UU**G**GCAA**U**UUUGAGUACUUUGA	2	22	JG551256	3'	31.8	-	Fruit
cme-miR11161	lja-miR11161	73	-14.40	GCC**G**AAA**A**C**U**U**U**GUGGACAGAGG	4	23	AM738012	3'	52.2	-	Fruit
cme-miR11564	pab-miR11564	125	-27.30	UUGACCACAAAAUCC**G**CAUUG	1	21	DV633699	5'	42.9	-	Fruit
cme-miR11576	pab-miR11576	191	-50.40	UUUGUGGUUUGCAUUUGAA	0	19	AM725094	3'	31.6	-	Leaf

The muskmelon-predicted miRNAs are characterised in terms of source miRNAs, precursor miRNA length (PL), minimum free energy (MFE), mature sequences (MS), number of mismatches (represented in bold (NM), mature sequence length (ML), source EST (SE), mature sequence arm (MSA), GC percentage (GC%), strand orientation (SO) and organ of expression (OE).

The expression patterns for the newly predicted miRNAs in muskmelon are as follows in the fruit, 68.75% (11 out of 19), the roots, 21% (4 out of 19), the cotyledons 10% (2 out of 19), flower and leaf 5% (1 out of 19) were all expressed. These ratios show the relative frequency of miRNA expression in various organs. As noted by numerous studies (Barozai, 2013; Din et al., 2014a; Din et al., 2014b), similar patterns have also been seen in other plant species, including chilli, *Artemisia annua*, eggplant, tomato, and potato. The number and the length of both the precursor and mature miRNAs are shown in (Figure 2).



The mature sequences are shown in blue colour ranging from 17-23 nucleotides, while the precursor sequences are shown in orange and range from 50-200 nt. The majority of mature miRNAs consist of 21 and 19 nt, 5 out of 19 (26.31%) followed by 20 nt 3 out of 19 (15.78%), 12.5% for 18, 22, and 5.26% for 17 and 23 nt.

Figure 2. The calculated length and number of mature and precursor miRNAs

Validation of genus muskmelon miRNAs through BLASTx

The BLASTx tool provided by the National Centre for Biotechnology Information (NCBI) (Altschul et al., 1997) is utilized to determine the coding potential of miRNAs, distinguishing between coding RNAs and noncoding RNAs (Yano et al., 2020). The analysis's findings showed that the muskmelon miRNAs did not exhibit any appreciable similarity to recognized proteins. This prediction indicated that the muskmelon miRNAs are non-coding RNAs since they lack sequence similarity to or alignment with protein-coding genes. This result strengthens the notion that miRNAs regulate gene expression more frequently than they serve as coding templates for protein synthesis.

Phylogenetic analyses

Using muskmelon as the reference species, a phylogenetic and conservation analysis was carried out to evaluate the conservation of a particular 19 miRNA in the plant. Additionally, cladograms and phylogenetic analyses for 19 novel non-coding miRNAs for muskmelon miRNAs were produced. These findings shed light on the kinships and evolutionary links of these new miRNAs within the muskmelon are shown in Figure 3.

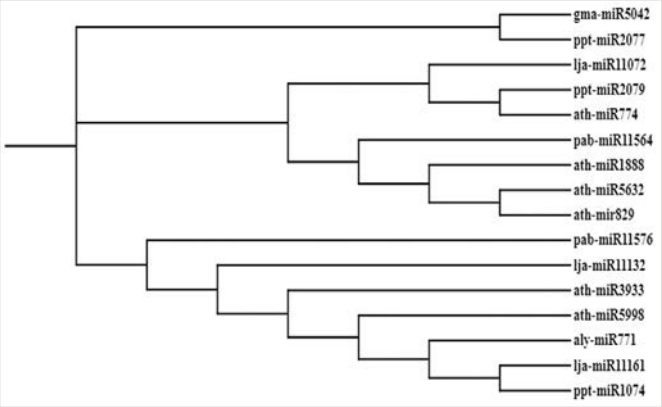


Figure 3. Phylogenetic tree of the newly identified novel non-coding miRNAs in muskmelon (similarities to a number of previously identified miRNAs from different families are visible)

Potential targets of newly identified miRNAs in genus Muskmelon

The identification of conserved miRNAs in plants and animals is made easier through miRNA-related studies, which present a fast and accurate approach. Even though a considerable number of miRNAs have been anticipated in monocot and dicot plants. The primary phytoconstituents found in melon, such as beta-carotene, flavonoids, terpenoids, carbohydrates, volatile compounds, and fatty acids, offer notable medicinal advantages, including antioxidant, analgesic, antiulcer, antidiabetic, anticancer, and hepato-protective properties. Melon is a commercially important plant species that is widely accessible. Due to the importance of melon, a computational technique was employed to forecast new miRNAs by considering ESTs, GSSs, and structural feature standards.

The identification of protein targets for newly predicted novel non-coding miRNAs is one of the

crucial steps in this project. We identified a total of 56 possible targets for 19 miRNAs in muskmelon following the modified version of a well-established technique developed by Zhang et al. (2006b). It's interesting to note that we found multiple miRNA targets shared by a variety of plant species, including rice, Arabidopsis, tomato, poplar, cotton, aubergine, potato, corn and chilli. A comparable category of targets is also present in the muskmelon genome as shown in Figure 4 and Table 2. The specific targets found in the muskmelon genome provide valuable insights into the potential regulatory interactions occurring between the identified miRNAs and their target genes in muskmelon. The present investigation also reveals that miR2079 targets high mobility group protein. In fact, miR3933 regulates the lncRNA-mediated post-transcriptional gene silencing process. Additionally, the miR5632 play an important part in the control of post-transcriptional gene silencing for plant hormones. From the GO study, it is assumed that several miRNAs target the same gene. For instance, the genes miR5042, miR5998, miR11161, and miR11576 control plant metabolism and enzyme activity. Consequently, in the lncRNA-mediated post-transcriptional gene silencing process, miR829, miR2077 and miR5632 took part. Moreover, miR1888, miR2079, and miR5042 are known to control the expression of plant hormone genes. These findings suggest that a number of targets interact to affect plants in a specific way.

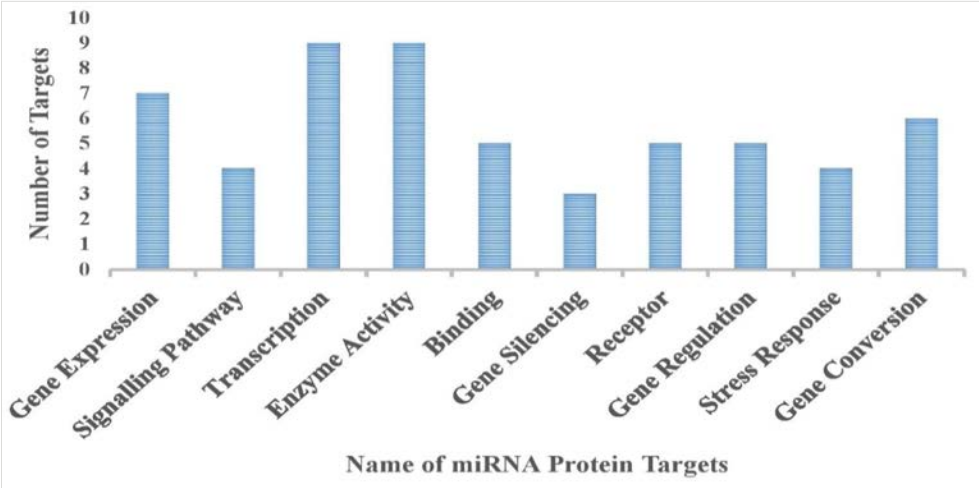


Figure 4. Potential targets of muskmelon miRNAs identified via psRNA Target tool

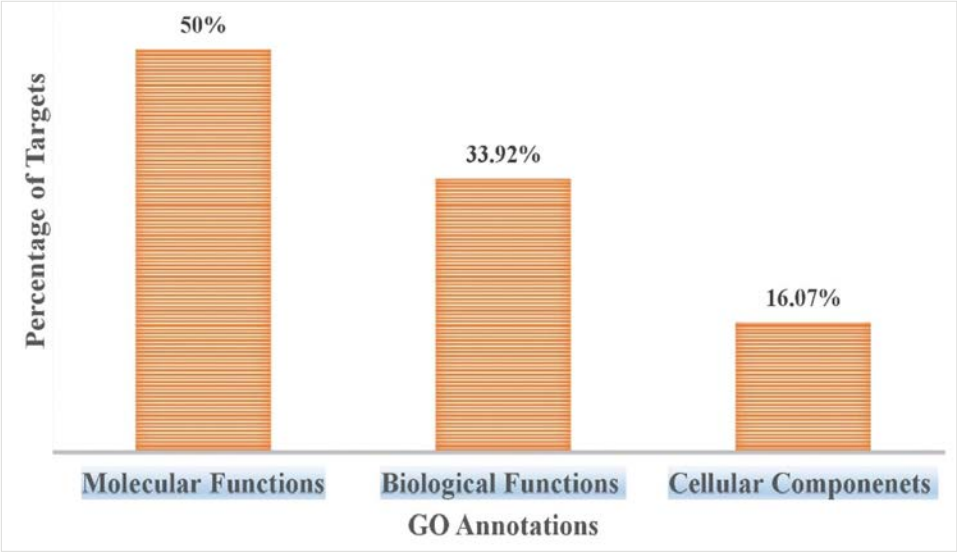


Figure 5. Percentage composition of muskmelon targets assigned based on their mode of action such as molecular functions, biological processes and cellular components (the highest percentage is represented by BP (50%), followed by MF (33.92%) and CC (16.07%)).

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Table 2. The miRNA targets identified for predicted muskmelon miRNAs for coding mRNAs using psRNAs-Target tool and GO-terms

Muskmelon miRNA	Target Acc	GO Annotation	GO	Target Description	Gene No	Alignment
cme-miR771	MELO3C007812	GO:1990715	BP	Gene conversion	1480	miRNA 19 UACUUGUAGACUCGUCGGG 1 ::::::::.:::::::: Target 150 AUGAACAUUUGAGCAGCGA 168
		GO:0035822	MF	mRNA CDS binding		
		GO:0001635	CC	Calcitonin gene-related peptide receptor activity		
cme-miR774	MELO3C007385	GO:0080179	BP	1-methylguanosine metabolic process	345	miRNA 18 CUCUACUUUUAUUCCUAC 1 :::.:::::::::::::: Target 139 GAGGUGAAAAUAAGGAUG 156
		GO:0008662	MF	1-phosphofructokinase activity		
		GO:0005545	MF	1-phosphatidylinositol binding		
		GO:0030121	CC	AP-1 adaptor complex		
		GO:0150056	CC	Amylin receptor complex 1		
cme-miR829	MELO3C033424.	GO:0009292	BP	Horizontal gene transfer	231	miRNA 22 AAGGUAGUACACCAUAGUCUCG 1 :::.:::::::::::::::: Target 202 AUCCGUCAUGUGGUAUCAGAGC 223
		GO:0019080	BP	Viral gene expression		
		GO:0016441	BP	Post-transcriptional gene silencing		
		GO:0004034	MF	Aldose 1-epimerase activity		

Continued. Table 2

Muskmelon miRNA	Target Acc	GO Annotation	GO	Target Description	Gene No	Alignment
cme-miR1074	MELO3C023400	GO:0070058	BP	tRNA gene clustering	912	miRNA 20 UAGUUGGGUUGAUUGUUGGG 1 : :::.::::: :::::::: Target 609 AACAAUCCAACCAACAACCC 628
		GO:1901522	BP	Positive regulation of transcription from RNA polymerase II promoter involved in cellular response to chemical stimulus		
		GO:0003700	MF	DNA-binding transcription factor activity		
cme-miR1888	MELO3C011448	GO:1903799	BP	Negative regulation of miRNA processing		miRNA 21 AGAAGUGUUUAAAAUCCCUUU 1 :::::.::: :::::::::: Target 1397 UCUUCGUAAAAUUUAGGGAAA 1417
		GO:0010468	BP	Regulation of gene expression		
		GO:0001635	MF	Calcitonin gene-related peptide receptor activity		
cme-miR2077	MELO3C005084	GO:1900370	BP	Positive regulation of post-transcriptional gene silencing by RNA	1770	miRNA 20 CCUUUAUACCCCAGAAAUAC 1 : :::::::::.:::.:: Target 1475 UCAUAAAUGGGGUUUUUGUG 1494
		GO:0023019	BP	Signal transduction involved in regulation of gene expression		
		GO:0070721	CC	ISGF3 complex		
cme-miR2079	MELO3C009353	GO:0010467	BP	Gene expression	1107	miRNA 20 GCAGUAGUUGUAGUUGAGAA 1 :::::::::::::::::::: Target 93 CGUCAUCAACAUCAACUCUU 112
		GO:1903705	BP	Positive regulation of siRNA processing		
		GO:0045944	BP	Positive regulation of transcription by RNA polymerase II		

Continued. Table 2

Muskmelon miRNA	Target Acc	GO Annotation	GO	Target Description	Gene No	Alignment
		GO:1903231	MF	mRNA base-pairing translational repressor activity		
cme-miR3933	MELO3C014561	GO:0045003	BP	Double-strand break repair via synthesis-dependent strand annealing	1673	miRNA 21 GGCCCAGUAGAAAAACGAAGA 1 ..:::::. :::::::::::::: Target 335 UUGGGUU-UCUUUUUGCUUCU 354
		GO:0000512	BP	lncRNA-mediated post-transcriptional gene silencing		
		GO:0140869	MF	miRNA inhibitor activity via base-pairing		
cme-miR5042	MELO3C007772	GO:0019080	BP	Viral gene expression	1255	miRNA 17 GAUAGAACCUAGUUCGG 1 :::::.::::::::: .: Target 165 CUAUUUUGGAUCAAAUC 181
		GO:0140053	BP	Mitochondrial gene expression		
		GO:0004522	MF	Ribonuclease A activity		
		GO:1990406	CC	CGRP receptor complex		
cme-miR5632	MELO3C015657	GO:0016441	BP	Post-transcriptional gene silencing	1170	miRNA 19 AUAGGUUUAUAUUUAGGUU 1 : ::.::::::::.:::::: Target 187 UUUCUAAAUAUAGAUCCAA 205
		GO:1990715	MF	mRNA CDS binding		
		GO:0005606	CC	Laminin-1 complex		
		GO:0045323	CC	Interleukin-1 receptor complex		

Continued. Table 2

Muskmelon miRNA	Target Acc	GO Annotation	GO	Target Description	Gene No	Alignment
cme-miR5998	MELO3C012292	GO:0040029	BP	Epigenetic regulation of gene expression	2251	miRNA 21 UGUUUUGUUUACUGUUUUACA 1 .: ::..::::::..:::::: Target 2115 UUAUAAUGAAUGAUGAAAUGU 2135
		GO:0008889	MF	Glycerophosphodiester phosphodiesterase activity		
		GO:0018740	MF	2'-hydroxybiphenyl-2-sulfinate desulfinase activity		
cme-miR11072	MELO3C011763.	GO:0032601	BP	Connective tissue growth factor production	1962	miRNA 19 GUGUGUGGGUGUCCUUGGU 1 ::: ::.:::::::::::: Target 816 AACAAACUCACAGGAACCA 834
		GO:0070576	MF	Vitamin D 24-hydroxylase activity		
		GO:0003700	MF	DNA-binding transcription factor activity		
cme-miR11132	MELO3C002017	GO:0031047	BP	Regulatory ncRNA-mediated gene silencing	2160	miRNA 22 AGUUUCAUGAGUUUUAACGGUU 1 .:::::::::.::.:::.::. Target 1844 AUAAAGUACUCGAAGUUGUCAG 1865
		GO:0008157	MF	Protein phosphatase 1 binding		
		GO:0101002	CC	Ficolin-1-rich granule		
		GO:0000164	CC	Protein phosphatase type 1 complex		
cme-miR11161	MELO3C020121	GO:0010608	BP	Post-transcriptional regulation of gene expression	1104	miRNA 23 GGAGACAGGUGUUUCAAAAGCCG 1 :::::::::::::::::::::::: Target 467 CCUCUGUCCACAAAGUUUUCGGC 489

Continued. Table 2

Muskmelon miRNA	Target Acc	GO Annotation	GO	Target Description	Gene No	Alignment
		GO:0023019	BP	Signal transduction involved in regulation of gene expression		
		GO:0009616	BP	RNAi-mediated antiviral immune response		
cme-miR11564	MELO3C005059	GO:0080185	BP	Effector-mediated induction of plant hypersensitive response by symbiont	633	miRNA 21 GUUACGCCUAAAACACCAGUU 1 :: ::.::::::::::.:. Target 394 AUUUGGGGGUUUUGUGGUUAG 414
		GO:0098790	BP	ncRNA transcription associated with protein coding gene TSS/TES		
cme-miR11576	MELO3C017116	GO:0080188	BP	Gene silencing by RNA-directed DNA methylation	1207	miRNA 19 AAGUUUACGUUUGGUGUUU 1 :::::::::::::::::: Target 97 UUCAAAUGCAAACCACAAA 115
		GO:1990407	MF	Calcitonin gene-related peptide binding		
		GO:0008988	MF	rRNA (adenine-N6-)-methyltransferase activity		

Where, (MF) Molecular Function, (BP) Biological Process, (CC) Cellular Component, and (GO) Gene Ontology

DISCUSSION

Several miRNAs exhibit evolutionary conservation across various plant species, as reported by Chavez-Montes et al. (2014). In this study, we focused on identifying miRNAs and their target genes within the muskmelon species by thoroughly analysing wide-ranging EST data from distinct species, we effectively predicted 19 miRNAs and 56 protein target genes in muskmelon. The miRNAs predicted in the muskmelon are members of at least sixteen different plant miRNA families are shown in Table 1.

The relevance and broad existence of these sixteen miRNA families in plants is highlighted by the remarkable degree of conservation in the Plantae kingdom. These results suggested that the highly conserved miRNAs have important and preserved roles in a variety of aspects of plant development, including the production of flowers, fruits, leaves and roots. These miRNAs play essential functions that are constant across all plant species. This discovery supports earlier studies that showed how miRNAs play a role in controlling these processes such as developmental process, stress responses, nutrient homeostasis, and metabolism. It emphasizes the adaptability of miRNAs by demonstrating how a single miRNA can target several genes in various plant species, possibly influencing a variety of elements of plant life (Ghaffar et al., 2023).

It is well known that miRNAs can target many locations on a single transcript (Forman et al., 2010). By taking into account the variety of target sites, one can evaluate the recognition ability of a miRNA towards its mRNA target. Based on this study we predicted multiple target sites observing the differences between evaluated miRNA and the target transcript of more than three consecutive nucleotide positions. The research revealed that the majority of the target transcripts had multiple miRNA binding sites. Multiple binding sites for the detected miRNAs imply a better degree of precision for the miRNA-target duplex, according to the analyzed techniques (Song et al., 2012).

Additional target genes identified by psRNA-Target using the melon ESTs database showed that the genes were involved in several processes, positive regulation of siRNA processing, positive regulation of transcription by RNA polymerase II, and mRNA base-pairing translational repressor activity. The fact that numerous distinct miRNAs were involved in targeting the same route was one intriguing finding of the GO analysis.

Due to their fast divergence and the absence of well-defined activities for many miRNA families, it is difficult to comprehend the evolutionary dynamics of plant miRNAs. However, several theories have been put out to explain the trends in miRNA divergence and conservation. The miRNA genes frequently go through birth-and-death evolution, with new miRNA genes continuously arising and other miRNA genes gradually losing their function or disappearing. The pre-miRNA sequence alone is not always sufficient for inferring phylogenetic relationships, hence it is significant to note that the variance in phylogenetic patterns could be due to the chosen sequences.

The study of miRNAs has been extremely beneficial in predicting new and conserved non-coding miRNAs in plants and mammals. This study focuses on muskmelon since it is recognized to have a wide range of phytochemicals with therapeutic properties. Beta-carotene, flavonoids, terpenoids, carbohydrates, volatile compounds, and fatty acids are only a few of the major phytochemicals that are plentiful in muskmelons. Furthermore, these phytochemicals have several beneficial effects, including hepatoprotective, anti-tumour, anti-diabetic, anti-ulcer, and antioxidant properties. Whereas model plants of both monocots and dicots have been discovered to contain a number of microRNAs. Muskmelon is an important plant variety for future research because of its commercial viability and significance (Li et al., 2019).

CONCLUSION

The ESTs-biased computational method that takes advantage of the structural characteristics of the muskmelon was used to predict new miRNAs in the muskmelon. Nineteen non-coding miRNAs were predicted in muskmelon using bioinformatics techniques. We also found 56 targets for 19 of these miRNA families. Computational validation was performed on each of the predicted miRNAs and their targets. The study's findings have improved our understanding of the molecular mechanisms underlying the muskmelon's regulatory system. The experimental validation of the anticipated candidate miRNAs is a vital next step in developing a better understanding of microRNA-mediated post-transcriptional regulation and gene silencing in muskmelon. This study will provide a better understanding to researchers to apprise of how melon miRNAs function and how they regulate their gene expression. Consequently, this research work will not only improve the yield of muskmelon but will also manage the environment to enhance productivity.

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