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ORIGINAL ARTICLE **OPEN ACCESS**

Computational identification of microRNAs and their targets from the expressed sequence tags of Lentil (*Lens culinaris*)

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ABSTRACT
miRNAs are small non-coding RNAs that regulate gene expression either by causing mRNA cleavage or by translational repression in plants and animals, respectively. Although various Experimental approaches are time consuming and expensive techniques to detect miRNAs in plants but comparative genomics complemented with novel bioinformatic tools cover the way for efficient and cost effective identification of miRNAs through homologous sequence search with previously known miRNAs. In this study, using in silico computer-based approaches for identifying miRNAs and their target genes based on a sequence homology search, total 12 novel miRNAs with 73 potential targets belonging to seven miRNA families from 10,190 EST sequences of lentil using in silico approach.. To our knowledge, this is the first report on lcu-miR141, lcu-miR5658, lcu-miR7534, lcu-miR6167, lcu-miR396e, lcu-miR414, lcu-miR10186a, lcu-miR395i, lcu-miR1086b, lcu-miR1522, lcu-miR781a & lcu-miR395e in lentil. 73 potential mRNA targets were also identified by homology searches using psRNATarget server. Most of the lentil miRNA target genes seem to encode transcription factors as well as genes involved in stress response, metabolism, plant growth and development. These findings will contribute to further research in regard to functions and regulatory mechanisms of lentil miRNAs indicating that the EST analysis is an efficient and affordable alternative approach for identifying novel miRNA.
Keyword: *microRNAs, ESTs, GSS, Lentil, M-fold & psRNATarget*

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INTRODUCTION

miRNAs are a class of non-coding small RNAs which play vital roles in plant development processes such as shoot morphogenesis, vegetative to reproductive phase transition, floral differentiation, root initiation, vascular development as well as hormone signalling and homeostasis [1,2]. They also regulate gene expression in response to various environmental stresses, such as pathogen attack, oxidative stress, dehydration and phosphate and sulphate limitation.

In plants, miRNAs initiate from transcripts with strong secondary structures transcribed by RNA polymerase II from coding or intergenic regions that are known as primary miRNA (pri-miRNA) transcripts. These pri-miRNAs are further processed by the RNase III enzyme DICER-LIKE 1 (DCL1) into shorter, stem-loop RNAs known as pre-miRNAs or miRNA precursors. The pre-miRNAs are further cleaved by DCL1 generating a miRNA duplex with a 2-nucleotide 3' overhang. The miRNA duplex is exported from the nucleus into the cytoplasm where the 20-24nt miRNAs are incorporated into an RNA Induced Silencing Complex (RISC) containing one of the Argonaute proteins (Ago) and the miRNA (star sequence) is usually degraded. The miRNAs guide the effector complexes to their target transcripts at their complementary site directing cleavage of the transcripts or inhibiting translation [3]. In higher eukaryotes, such as Drosophila and *Caenorhabditis elegans*, miRNA sequences represent for as much as *ca*. 1% of the total genome size and they are estimated to manage 50% activity of all protein coding genes in mammals [4]. Many plant mature miRNAs have been found to be highly conserved in both structure and sequence across different species. To date 5,24,521 hairpin precursor miRNAs and 30,424 mature miRNAs including thousands of plant miRNAs from more than 200 species deposited in the miRBase Release 21 [5].

There are two main approaches for recognition of miRNAs in plants: Experimental approaches ie direct cloning, high throughput sequencing and Computational approaches ie Genomic survey sequence(GSS) and Express sequence tag(EST) [6,7]. Computational approaches are quicker and more affordable than the experimental approaches and also identify low copy number miRNAs . Computational identification of miRNA is successful not only for those plant species for which full genomic and large EST database is existing but also for those with incomplete genomic information. Different computational miRNA methodologies have been developed based on the conserved characteristics of miRNAs including hairpin stem-loop secondary structures, high level of evolutionary conservation between species and measurements of minimal folding free energy [7,8]. This characteristic has been used as a practical indicator for the identification and prediction of miRNAs by homology searches in other species .

Identification miRNAs using EST analysis has some advantages over other methods. ESTs provide a powerful tool for identification of miRNAs that are conserved among various plant species. There are numerous MiRNA identified based of EST database in mulberry, soybean, finger millet, apple, corn [9].

Lentil (*Lens culinaris*) is a diploid (2n=2X=14) self-pollinating crop with 4 Gbp approximately genome size. It provides affordable supply of dietary proteins (22–35%), minerals, fiber, and carbohydrates to poor people and plays a crucial role in alleviating malnutrition and micronutrient deficiencies. As it exhibits little glycemic index, it is highly recommended by physicians for the people suffering from diabetes, obesity, and cardiovascular diseases [10]. In comparison to major legume crops such as common bean, soybean, chickpea and pigeon pea the pace of development of genomic resources is very slow in lentil. Due to large genome size, narrow genetic base, lack of candidate genes, low density linkage map, and the difficulty in identifying beneficial alleles are the chief limiting factors in genomics improvement in lentil. 10,190 ESTs of Lentil have been deposited in NCBI databases which are useful for the identification of potential miRNAs.

In this regard, there have been no reports on miRNAs in Lentil (*Lens culinaris*), an economically important crop. Therefore, In this study, in silico approach has been used to identify potential miRNAs from the EST-based approach of Lentil. For this, we searched the EST databases to find ESTs matched with the previously known miRNAs from miRBASE database. Then we predicted the secondary structures of the identified ESTs in the first step using RNA MFOLD software. Further, the newly identified miRNAs have been used to find out targets that improve our understanding towards their possible regulatory roles in Lentil.

MATERIAL AND METHODS

miRNA & EST dataset retrieval:

To find potential miRNA homologs in Lentil, the already known 30,424 plant miRNAs sequences were retrieved (21-24 nt long) from miRNA Registry Database 22. (http://www.mirbase.org/). The redundancy of miRNAs was removed manually, to avoid miRNA duplication and overlapping. After removal of the repeated sequences,10,190 EST were downloaded from GenBank database (http://www.ncbi.nlm.nih.gov).

Identification of potential miRNA homologs and its precursor:

Briefly, the Lentil ESTs sequences were aligned with known mature plant miRNAs using a BLASTn algorithm with an E threshold value of 0.01 and alignment length between 19 and 24 (Fig. 1). Results with no more than 3 nucleotide (<4 nucleotide) substitutions, including insertions, deletions, mutations, and gaps between known miRNAs and homolog sequences, were obtained from the BLASTn search. For each pattern hit, 300 nt of both 5′ and 3′ flanking sequences were extracted from corresponding ESTs as candidate sequences. Since all the miRNA genes are non protein coding, BLASTx online server was used to eliminate the protein coding against NCBI's non redundant (nr) protein database. After removing protein coding sequences, non coding sequences matches with mature miRNA, were further selected.

Secondary structure prediction of miRNA Precursor:

The secondary structures of putative pre-miRNAs were predicted by Mfold (http://mfold.rna.albany.edu). The minimum fold energies (MFEs), minimal free energy indices (MFEIs), and A+U content were employed to distinguish miRNAs from other types of coding and noncoding RNAs. The following criteria were followed to consider an RNA sequence as a miRNA homolog: (i) The sequence should be folded into a suitable stem-loop hairpin secondary structure (ii) miRNA group that is predicted must be exactly or near complementary to the miRNA arrangement in secondary structure (iii) miRNA sequence contain no loops and breaks.

miRNA target predictions:

Target predictions for the miRNAs are based on the principle of nearly perfect complementation between the miRNA and target mRNAs. The small RNA target analysis tool psRNATarget

(http://plantgrn.noble.org/psRNATarget/) was employed to predict the targets of the putative mature miRNAs with default parameters. psRNATarget predicts small RNA targets by reverse complementary matching between small RNA and target transcripts and evaluating the target site accessibility by calculating unpaired energy required to open secondary structure around the small RNA target site. It also predicts the translational inhibition or cleavage degradation by presence/absence of a mismatch in the central complementary region of the small RNA sequence. The potential target genes were also BLASTed against NCBI protein databases for annotation. No more than 4 mismatches between mature miRNAs and their potential target mRNA and no gaps were allowed. The schematic representation followed for identification of potential miRNAs and their targets in Lentil, using ESTs is shown as in Fig:1.

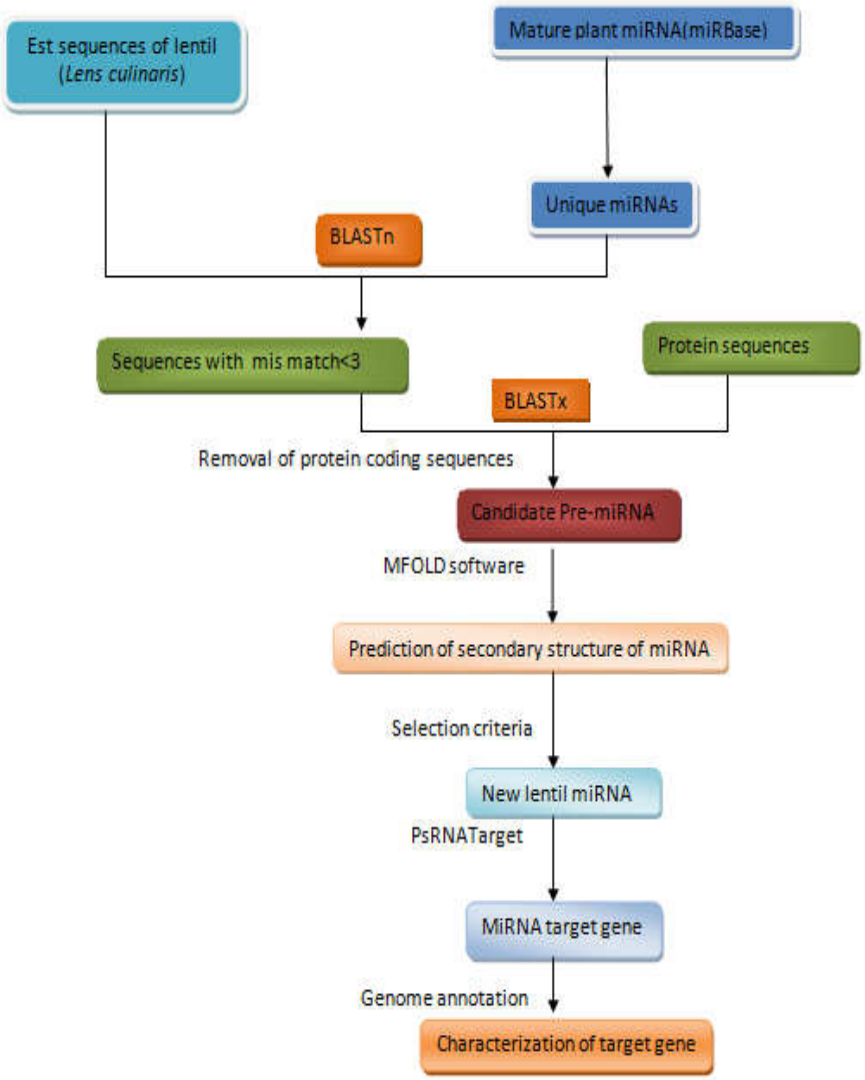


Fig: 1. Workflow of the identification and characterization of potential miRNAs and their target genes in lentil (*Lens culinaris)* using ESTs.

RESULT AND DISCUSSION

Though miRNAs of different plants have been generally studied in the past several years, no effective study has been performed on Lentil miRNAs, one of the most imperative crops around the World [10]. Despite the fact that there are many computational ways to recognize miRNAs of plants, animals and even microorganisms, EST analysis has a few advantages over others e.g. EST analysis is not confined to model species with published genome to identify conserved miRNAs but it can also be employed to identify miRNAs in other species whose EST information is only available. BLASTn search algorithm can be used for EST analysis. Mostly the bioinformatics techniques that are based on high homology amongst the query and subject sequences are considered as the most effective methodologies. Additionally, the determination of miRNA target genes is vital for understanding the miRNA gene-regulation.

Identification of Potential miRNAs from Lentil:

Due to evolutionary conserved nature of mature miRNAs amongst various plant species, it is easy to predict novel miRNA orthologs or homologs in different plant species [11]. In this study, a comprehensive computational strategy was employed for the identification of potential miRNAs in Lentil (*Lens culinaris*) by searching EST database of Lentil against already known Plant miRNAs.

Finally, after performing Blastn& Blastx, 12 potential Lentil miRNAs belonging to seven miRNA families were identified with their secondary hairpin structure from Lentil EST dataset as depicted in Table: 1 & Suppl-1. In the present study,the length of mature miRNAs ranged from 20 to 24 nucleotides, with 21-nucleotide mature miRNAs being most abundant and the length of predicted miRNA precursors varies from 106 to 166 NT. Majority of known miRNAs in other plants are of same size [1]. The different sizes of the identified miRNAs within the different families suggest that they may perform unique functions in the regulation of miRNA biogenesis or gene expression [1]. The newly-identified miRNAs were named according to miRBase guidelines.

Secondary structure prediction of Pre- miRNA :

Generally, miRNAs are distinguished from other RNAs on the basis of their surrounding sequences ability to adopt the hair-pin structure. Therefore, secondary structures of all the identified miRNAs were predicted (Fig.2). Minimum free energy (MFE) is one of the significant characteristics for the determination of secondary structure of nucleic acids i.e. DNA and RNAThe A+U content of predicted miRNAs ranged from 45.83 to 84.20%. Lower value of MFEs indicates that the secondary structure of the respective sequence is thermodynamically stable. The newly identified miRNAs show MFEIS in range of 0.24 to 1.0 while MFES in range of -17.21to -49.73 kcal/mol. The MFEIs is another useful criterion for distinguishing miRNAs from other types of coding and non-coding RNAs. The miRNA precursors with secondary structures had minimal free energy index (MFEIs) than other different types of RNAs. miRNAs bind more strongly to certain proteins as they have a higher (A+U) content compared to other RNAs.

Prediction of targets for newly identified miRNAs and their putative role:

Compared with their animal counterparts, plant miRNAs recognise a single target site in the coding region to guide mRNA cleavage. miRNA-regulated genes control a wide range of physiological, biological, and metabolic process in plants. It is noted that most of the predicted targets were the genes coding transcription factors, which are mainly involved in plant growth, developmental patterning or cell differentiation. Probably, this is a general characteristic of plant miRNAs that tend to be complementary to their regulatory targets (Table:2). Other targets included proteins involved in metabolism, signal transduction, and stress responses.

In particular, lcu-miR141, lcu-miR395i & lcu-miR395e bound to mRNAs that encode ATP synthesis protein, transcription factor and metabolic enzymes. Similarly, Akula & More [9] & Han et al [12] also reported that cca-miR395a & tae- miR395 encode for ATP synthesis & transcription factor activity also. lcu-miR396e & lcu-miR414 bound to mRNAs that encode Transcription factor, metabolism & growth relegating factor. similarly, Nellikunnumal & Chandrashekar [13] cca-miR396b, cca-miR414c, cca-miR414d & Pan et al. [14] Nnu-miR396 reported to bound with Transcription factor, metabolism and growth relegating factor.

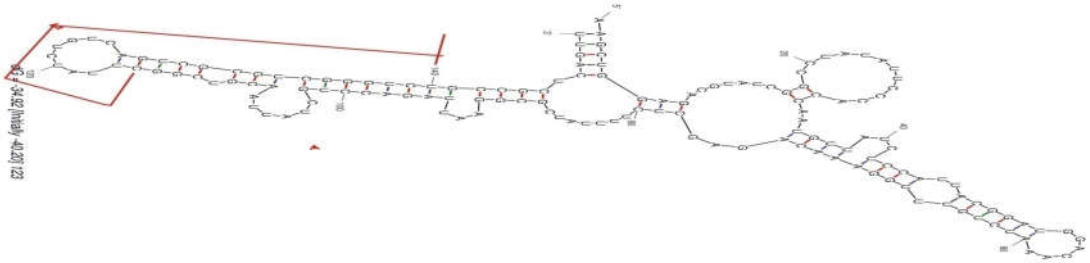
lcu-miR5658 bound to Transcription factor, growth regulating factor. Similarly pvu-miR5658 [15], sin-miR5658 [7] & Vco-miR5658 [13] also reported to bound with transcription factor & growth regulating factor also.

Target multiplicity and co-operativity:

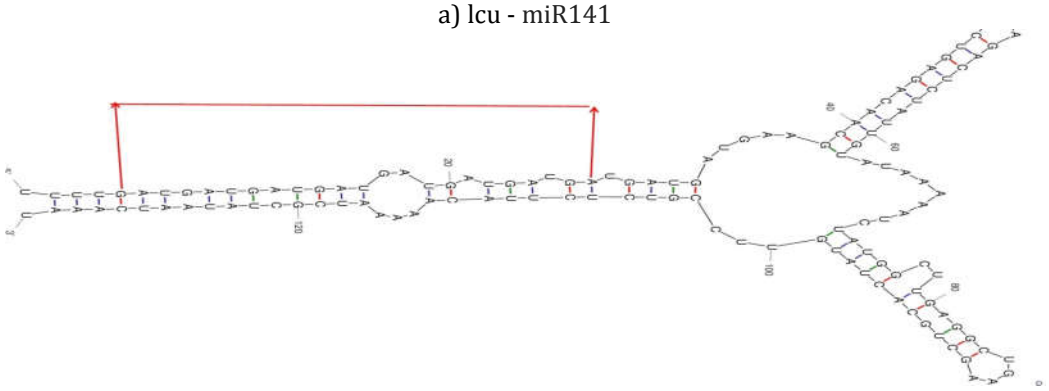
A single miRNA may have multiple target genes and therefore multiple binding sites in the 3'-UTR . In some cases, a miRNA can be complementary to more than one regulatory target. In this case, the targets can be grouped into several gene families, most of our newly-identified miRNAs showed multiple targets ie lcu-miR5658, lcu-miR396e, lcu-miR1522 had a maximum of 10 targets; while lcu-miR414 had 11targets (Table 2). Another important characteristic shown by miRNAs is co-operativity, where more than one miRNA can regulate a single target gene.

In the present study, lcu-miR5658 & lcu-miR396e bound to mRNAs that show co-operativity having same Transcription activator(TC421839), while lcu-miR5658, lcu-miR395i, lcu-miR10186b & lcu-miR781a bound to mRNAs that encode same growth related protein(TC424828) Similarly, lcu-miR5658 &lcu-miR396e bounds to growth related protein(CD411929) while lcu-miR5658 & lcu-miR396e bounds to cellular protein(TC424055) and lcu-miR5658 & lcu-miR396e bounds to cellular protein(TC429333).

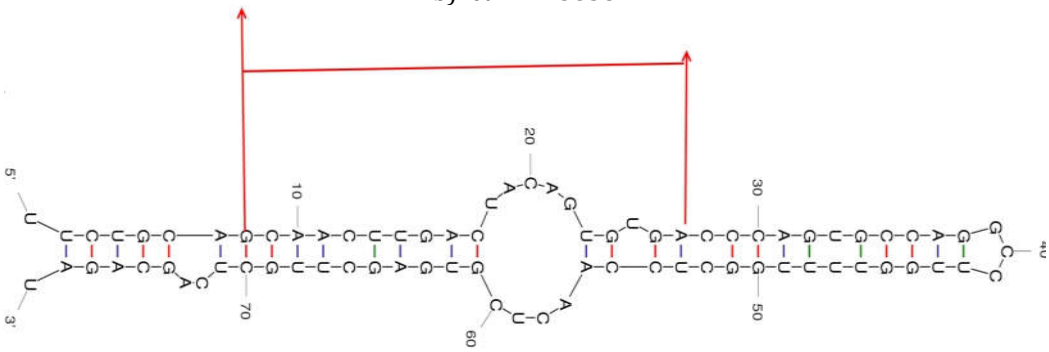
Similarly, lcu-miR414 bound to multiple mRNAs targets that encode multiple cellular protein (TC422551, TC424294) while lcu-miR10186a bound to multiple mRNAs that encode same transcription factor from different sources (TC422658).



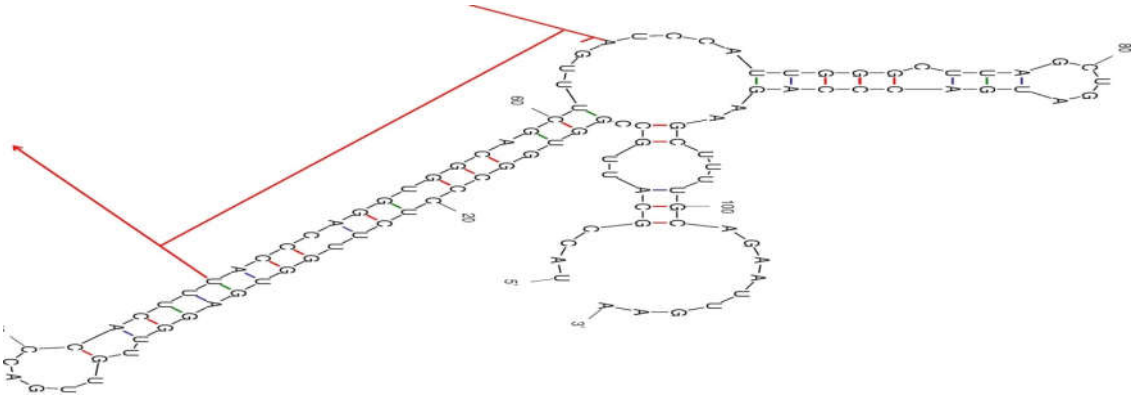
a) lcu - miR141



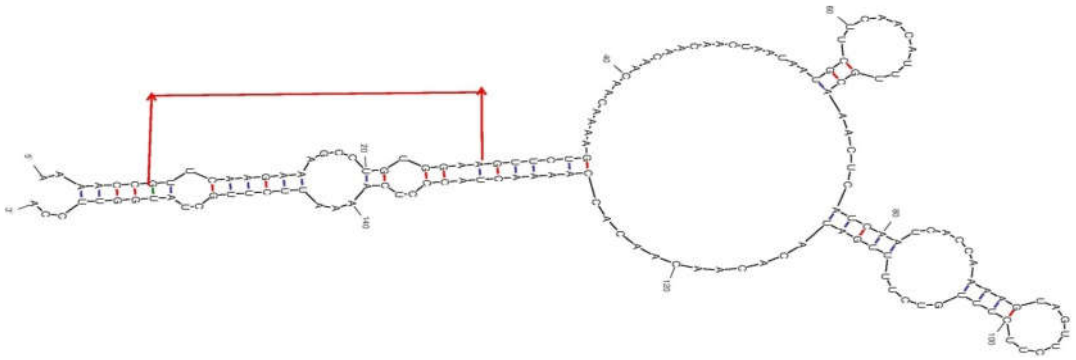
b) lcu-miR 5658



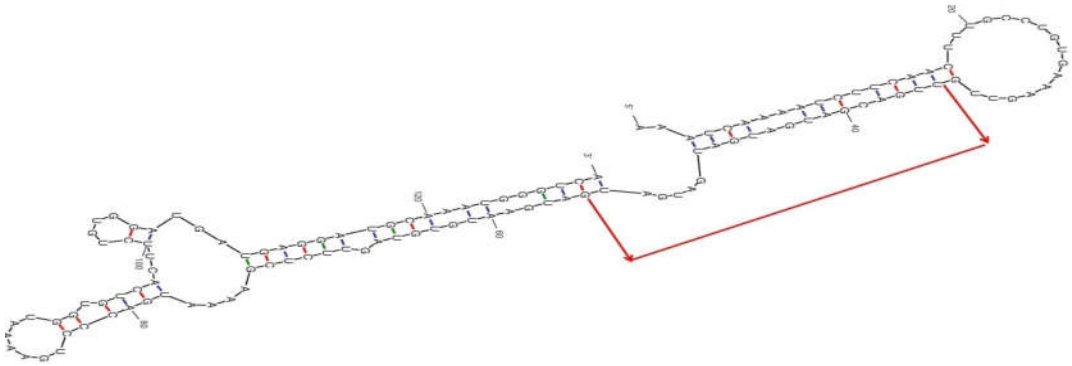
c) lcu-miR7534



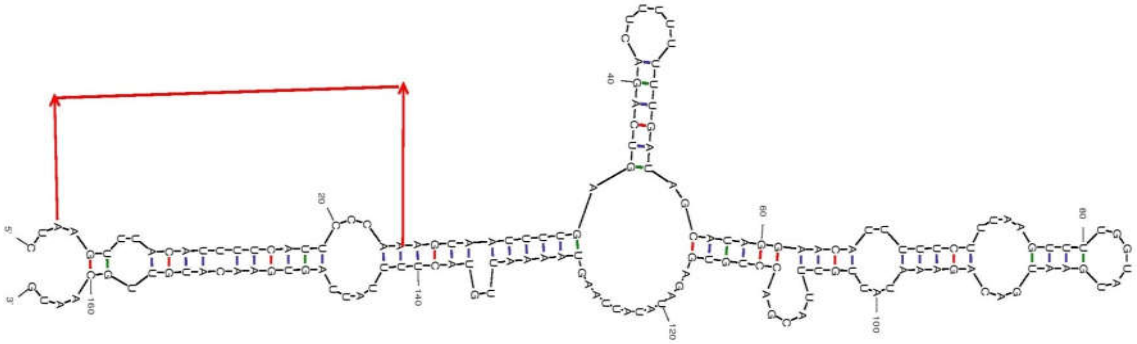
d)lcu-miR6167



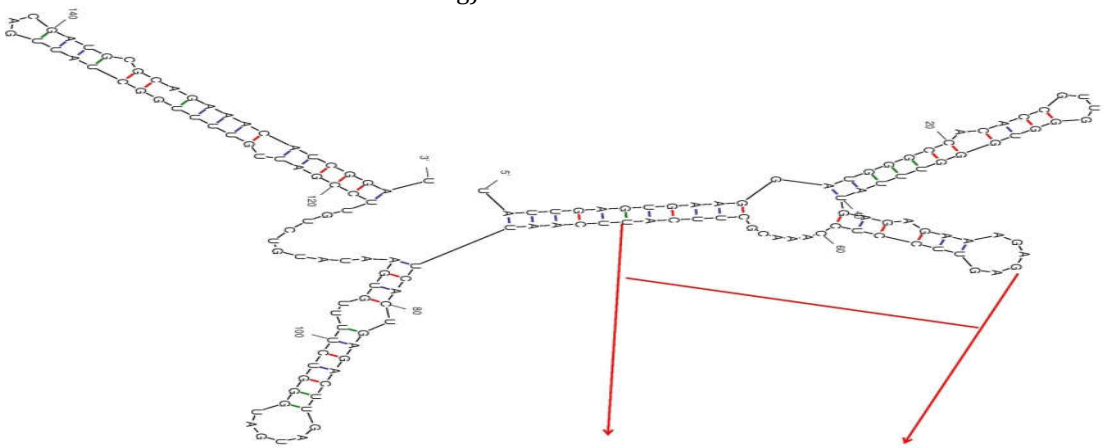
e)lcu-miR396e



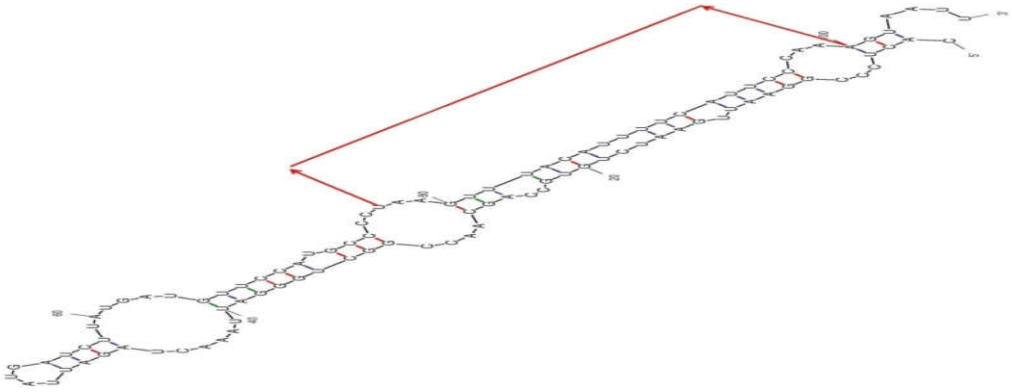
f)lcu-miR414



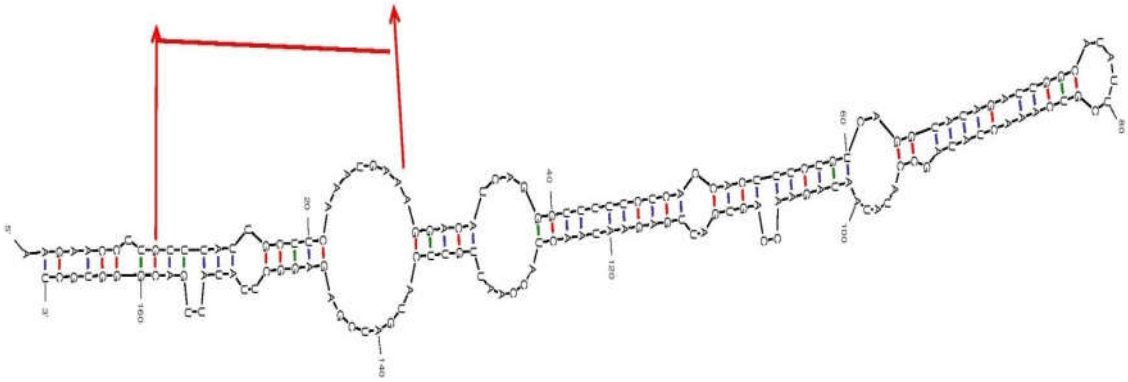
g)lcu-miR10186a



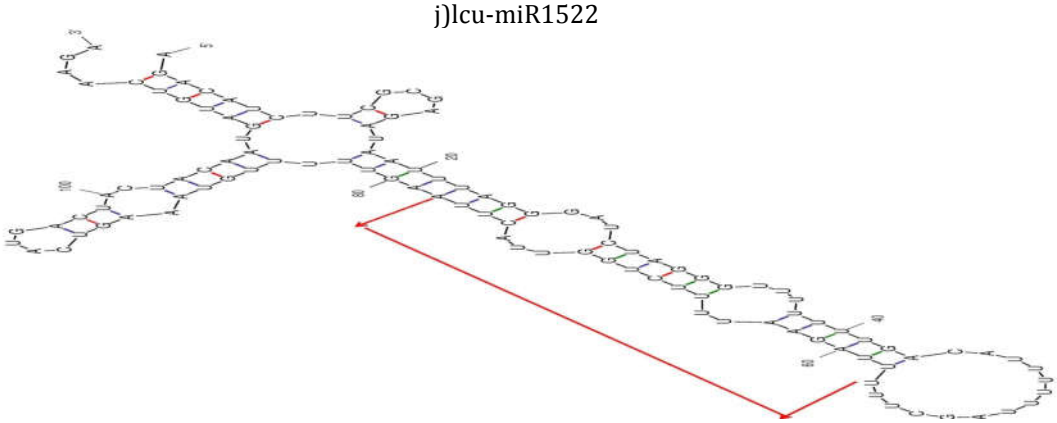
h)lcu- miR395i



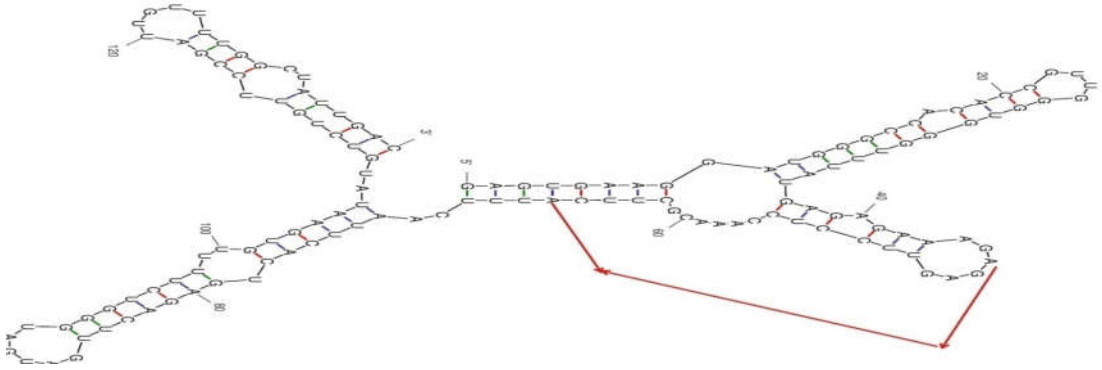
i)lcu-miR10186b



j)lcu-miR1522



k)lcu-mir781a



l)lcu-miR395e

Fig 2: Predicted secondary structures of identified precursor miRNAs in Lentil using MFOLD program.

* mature miRNAs sequences are marked with red line.

Table: 1. Conserved miRNAs identified in Lentil by EST analysis and their characteristic features

Sr. No	miRNA with accession ID	Lentil EST ID (gene source)	Predicted miRNA 5'-3'	miRNA Length	Pre-miRNA length	Strands	(A+U)%	MFES kcal/mol	MFEIS
lcu-miR141	rgl-miR5141 (MIMAT0020652)	GT620850.1	AGACCCGACGCGACTGACAGATAA	24	151	+/-	45.83	-34.92	0.45
lcu-miR5658	ath-miR5658 (MIMAT0022431)	GT625020.1	TCCACAGGCTTTCTTGAACGG	21	136	+/+	47.00	-23.25	0.32
lcu-miR7534	lja-miR7534 (MIMAT0029359)	GT621094.1	GCAACTTGACTACAGTTTGAC	21	77	+/+	56.84	-24.00	0.73
lcu-miR6167	hbr-miR6167 (MIMAT0024785)	GT626379.1	TACCCAGGTGGAAGCTTTGA	20	110	+/+	50.00	-26.33	0.47
lcu-miR396e	fve-miR396e (MIMAT0044551)	GT623488.1	TTCCACAGGCTTTCTTGAAC	20	159	+/-	55.00	-17.21	0.24
lcu-miR414	ath-miR414 (MIMAT0001322)	GT627921.1	TCATCTTCATCATCATCGTCA	21	129	+/-	61.00	-22.55	0.45
lcu-miR10186a	gma-miR10186a (MIMAT0040951)	GT624031.1	TTGGGAATTAAAATGTAAACTT	22	164	+/-	76.00	-25.79	0.65
lcu-miR395i	gma-miR395i (MIMAT0024921)	GT625332.1	ATGAAGTGTTTGGGGGAACTC	21	159	+/-	52.37	-49.73	0.65
lcu-miR10186b	gma-miR10186b (MIMAT0041642)	GT624031.1	TTTGGGAATTAAAATGTAAACTT	23	106	+/-	78.26	-24.10	1.0
lcu-miR1522	gma-miR1522 (Mimat0007383)	GT626612.1	TTTATTGCTTAAAATGAAA	19	166	+/+	84.20	-41.70	1.72
lcu-miR781a	ath-mir781a (MIMAT0003940)	GT622528.1	TTAGAGTTTTCTGGATACTTA	21	114	+/+	71.41	-22.10	0.67
lcu-miR395e	Lus-miR365e (MIMAT0027208)	GT625332.1	TGAAGTGTTTGGAGGAACTC	20	136	+/-	55.00	-37.43	0.61

Table: 2. Major potential target genes for newly identified miRNAs in Lentil

miRNAs	Targeted proteins acc.	Target function	GO annotation (biological process)	Inhibition
lcu-miR141	TC420920	ATP synthase subunit alpha, Ripening-related expansin,	ATP synthesis	Cleavage
lcu-miR5658	TC421839	NDX1 homeobox protein		Cleavage
	TC424828	SDL5A	Transcription factor	Cleavage
	CD411929	Auxin down-regulated protein	Cellular processes	Cleavage
	TC461554	60S ribosomal protein L27a-3	Growth factors	Cleavage
	TC424055	60S ribosomal protein L27a-3		Cleavage
	TC429333	60S ribosomal protein L27a-3		Cleavage
	TC441225	MYB transcription factor MYB187		Cleavage
	TC432435	SDL12A	Transcription factor	Cleavage
	TC425647	ER lumen protein retaining receptor	Cellular processes	Cleavage
	TC424278	ER lumen protein retaining receptor		Cleavage
lcu-miR7534	TC464192	SNF-1-like serine/threonine protein kinase	Cellular processes	Cleavage

lcu-miR6167	TC422480 TC445641 TC474736	Lipoxygenase-10 NAC domain protein NAC1 Lipoxygenase	metabolic processes	Cleavage Cleavage Translation
lcu-miR396e	TC421839 TC463570 TC428481 TC461554 TC424055 TC429333 TC425811 TC421784 CD411929 TC420869	NDX1 homeobox Histone H2A ABC transporter-like protein 60S ribosomal protein L27a-3 60S ribosomal protein L27a-3 60S ribosomal protein L27a-3 Histone H2A Phosphatidylinositol transfer-like protein III Auxin down-regulated protein Ribonucleoside-diphosphate reductase	Transcription factor Protein phosphorylation Cellular processes Protein phosphorylation Cellular processes Growth factors Protein phosphorylation	Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage
lcu-miR414	TC468432 TC422551 TC433089 BE801284 TC437607 TC422551 TC433089 TC424294 TC429001 TC468432 TC424294	Nascent polypeptide-associated complex NAC; UBA-like; Nascent polypeptide-associated complex NAC; UBA-like Nascent polypeptide-associated complex NAC; UBA-like; Malate dehydrogenase Nascent polypeptide-associated complex NAC; UBA-like Glycinin Nascent polypeptide-associated complex NAC; UBA-like; Malonyl-CoA:isoflavone 7-O-glucoside-6''-O-malonyltransferase FACT complex subunit SPT16 Nascent polypeptide-associated complex NAC; UBA-like Nascent polypeptide-associated complex NAC; UBA-like	Cellular processes oxidative metabolism Growth related Cellular processes	Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage
lcu-miR10186a	TC422658 TC424301 TC425870 TC441436 TC422658	BZIP transcription factor bZIP105 Histone-fold/TFIID-TAF/NF-Y Cyclin Resistance-like protein KNBS2 BZIP transcription factor bZIP105	Transcription factor oxidative metabolism, stress related Transcription factor	Cleavage Cleavage Cleavage Cleavage Translation
lcu-miR395i	TC432804 TC452601 TC433245 TC454368 TC420828	ATP sulfurylase Auxin down-regulated protein MYB transcription factor MYB64 Homogentisate phytyltransferase VTE2-2 NAC5 protein	ATP synthesis Growth related Transcription factor Cellular process	Cleavage Cleavage Cleavage Cleavage Cleavage
lcu-miR10186b	TC432804 TC433245 TC454368 TC420828	ATP sulfurylase MYB transcription factor MYB64 Homogentisate phytyltransferase VTE2-2 NAC5 protein	ATP synthesis Transcription factor Protein phosphorylation Cellular process	Cleavage Cleavage Cleavage Cleavage
lcu-miR1522	TC449581 TC434072 TC430116 TC477053 TC432799 TC483461 TC424261 TC448647 TC457919 BQ627603 BI701050	intracellular transporter Chloroplast lipocalin Proline dehydrogenase Auxin down-regulated protein Cytochrome P450 monooxygenase CYP51G1 Alpha-tubulin 1-aminocyclopropane-1-carboxylic acid oxidase Autophagy-related protein 8C precursor 60S ribosomal protein L38 Arabinogalactan protein Arabinogalactan protein	Transporter protein Protein phosphorylation Protein phosphorylation Growth factor oxidative metabolism ATP synthesis Metabolic process	Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage Cleavage
lcu-miR781a	TC443421 TC437161 TC438046	Serine hydroxymethyltransferase Clathrin heavy chain Photosystem II type I chlorophyll a/b-binding	Metabolic process photo synthesis	Cleavage Cleavage Cleavage

	BU765684	protein precursor;	Metabolic process	Cleavage
	TC420828	ADR12 protein		Cleavage
	TC420799	NAC5 protein		Cleavage
		Seed calcium dependent protein kinase		
	TC432804	ATP sulfurylase		Cleavage
	TC454368	Homogentisate phytyltransferase VTE2-2	ATP synthesis	Cleavage
lcu-miR395e	TC455127	Acetyl-CoA carboxylase	Metabolic process	Cleavage
	TC429679	MYB transcription factor MYB135		Translation
	TC446787	MYB transcription factor MYB135	Transcription factor	Translation
	TC421106	MYB transcription factor MYB136		Translation

CONCLUSION

In this study, comparative-genome based prediction and characterization of miRNAs provide an alternative to RNAi mediated technology. According to our knowledge based on a literature survey, for the first time we have identified total 12 novel miRNAs with 73 potential targets belonging to seven miRNA families from 10,190 EST sequences of lentil using in silico approach. The potential roles of these miRNAs in lentil include regulation of transcription and signal transduction pathways. Thus, they may regulate stress responses, auxin responses, protein phosphorylations, leaf and flower development, ATP synthesis and enzymes involved in metabolisms. The findings from this study are believed to contribute to further researches on the function and regulatory mechanisms of miRNAs in lentil. It also indicates that the EST analysis is an efficient and affordable alternative approach for identifying novel miRNA.

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Suppl. 1: Precursor sequences of conserved miRNAs identified in Lentil

Sr. No	miRNA with accession ID	miRNA Sequences
1	rgl-miR5141 (MIMAT0020652)	AAGCTGGAGACGTATCGGCCTATATTCCCACCAATGTTATCTCCATTACGGATGGACAAA TCTGTTTGGAAACAGAGCTCTTTTATCGCGGAATTAGACCTGCTATTAACGTCGGCTTAT CTGTCAGTCGCGTCGGGTCTGCCGCTCAGTT
2	ath-miR5658 (MIMAT0022431)	TTTTGATGATGATGATGATGATGATGATGATGATGAAGCAACAGAGTCTCAGACTCTATT GTATAAAAATCTATGGCTTGAGGCTGAAGCTGCACTATGTTCCGTCTCTTACAAAAATCG CTATAATCAAATGAAG
3	lja-miR7534 (MIMAT0029359)	GTTCTGCAGCAACTTGACTACAGTGTGACCCAGTGCCAGGCCTTGGTTTTGGCTCCAACTC GTGAGCTTGCTCAGCA
4	hbr-miR6167 (MIMAT0024785)	TACCGCATTGCCGGTGGCCCTCTTGGTGAGGTTGTTGACCCACTTTACCCAGGTGGCAGCT TTGATCCATTGGGCTTAGCTGATGACCCAGAAGCTTTTGCAGAATTGAA
5	fve-miR396e (MIMAT0044551)	AAAACCGTTCAAGAAAGCCTGTGGAAGTTCTGAAAACAACAACAACAACTAATAATGCTT CAACATTTGCAAACTCATCAATCACCAAAAGTAGTTCTTCTTTGTCTTTTGATACACAAC AACACCAAAACTACCCTCAAAATTCTTGCTATGGTTCCA
6	ath-miR414 (MIMAT0001322)	AAATCAAAATCTTCAACTTTGCCTGTGAAAGTTGTTGACGATGATGATGATGATGATGAA TGTGTAGTTCTCGAAAATGACCCTGAAAATGGTGTCACTTCTGTGGATGATGAGGATGCA AATGGGTCA
7	gma-miR10186a (MIMAT0040951)	CTAAGTTTACATTTTCATTCCCAAAGTAATTTTGAGTCAGACTTTTTTTTGATAGCATAG GAACATTTTTCTTTAAGTTTTGGTATGAATGACAGAAATATTGTTTACGACCTGTGAGAT ATATTAAGTAAAATTGTACTTTTATTAGTGAACATGTTGCAATG
8	gma-miR395i (MIMAT0024921)	TATTGAGTGAAGGATGGGCCACACCGTTGGGTGGGTTTATGAGAGAAAGAGAGTTCCTCC AAACGCTTCATTCAATTCACTGAGACTTGATGATGGGTCTTTTGTGAATATGTCTGTTCC GATTGTTTTGGCTATTGACGATGCGCAGAAACATCGGAT
9	gma-miR10186b (MIMAT0041642)	CACTCCCGGAATTGAATCTGTGCCAGCAACCGGCTGGGATTAAACTAGATTATGATCTTA TGATGTTCCATGCCCCTAAGTTTACATTTTCATTCCCAAAGTAATT
10	Gma-miR1522 (Mimat0007383)	AAGAACCTTGTTTATTGCTTCAAATGAAAGGACATCAGGGTTTTTCTCACCACTTTCTGT CAGGTATAGATTGGCATATTCGTCAAACTATAGCCATATAATAGAACCAGTGATTGAGAA TAACTACCAATTGTTCATGATCGAGAGGCTTATATTGACGGGTGCT
11	ath-mir781a (MIMAT0003940)	AGACATCTTCGCGAGATAATTTAGGGATCTAGGGTTTTTTTGACATTTTTTAGCTTTTTA GAATTTTCTGGTTACTTAAGTTTTTGTAAAGTCATGACTACTACAATGATGTCA
12	Lus-miR365e (MIMAT0027208)	GAGTGAAGGATGGGCCACACCGTTGGGTGGGTTTATGAGAGAAAGAGAGTTCCTCCAAAC GCTTCATTTCAATTCACTGAGACTTGATGATGGGTCTTTTGTGAATATGTCTGTTCCGAT TGTTTTGGCTATTGAC

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