ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Computational Identification of conserved miRNAs and their potential targets in French bean (*Phaseolus vulgaris*)

Nageshbabu R[1]* Jyothi MN[1], Sharadamma N[2], Rai DV[3] and Devaraj VR[4]

[1]Post Graduate Department of Biochemistry, Maharani's Science College for Women, Bangalore-560001 India
[2]Department of Biochemistry, Indian Institute of Science, Bangalore -560012 India
[3]Centre for Bioinformatics, Faculty of Biological Engineering, Shobhit University, Meerut, India
[4]Department of Biochemistry, Central College Campus, Bangalore University, Bangalore -560001 India

ABSTRACT

MicroRNAs (miRNAs) are a novel growing family of endogenous, small, non- coding, single-stranded RNA molecules directly involved in regulating gene expression at the posttranscriptional level. High conservation of miRNAs in plant provides the foundation for identification of new miRNAs in other plant species through homology alignment. Here, previously known plant miRNAs were BLAST against the Expressed Sequence Tag (EST) database of French bean (Phaseolus vulgaris), and according to a series of filtering criteria, a total of 10miRNAs were identified, and 24potential target genes of them were subsequently predicted, most of which seemed to encode transcription factors or enzymes participating in regulation of development, growth and other physiological processes. Overall, our findings lay the foundation for further researches of miRNAs function inFrench bean

Keywords: ESTs, French bean, miRNA.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

MicroRNAs (miRNAs) are a novel growing family of endogenous, small, non- coding, single-stranded RNA molecules encoded in the genomes of plants and animals that repress mRNA translation or mediate mRNA degradation in a sequence-specific manner [1]. The discovery of the first microRNA lin-4 in Caenorhabditiselegans by Ambros laboratory emerged as biology's unusual or unique findings [2]. These tiny bits of RNA play a major role in gene regulation, which involves in negative regulation of gene targets. In recent years, identification and functional studies of miRNA has made great progress in research. The exposition of miRNA in plants is still a continuing process hence, till date a number of plant miRNAshave been discovered and functionally identified. Key roles of miRNA in biological processes are revealed by plant studies, which include regulation of leaf development [3], stem development [4], root development [5], signal transduction, developmental timing, floral differentiation and development, and defense response against every sort of stress. Plant miRNAs are usually evolutionary conserved and are observed in regions of the genome distinct from previously annotated genes. Different approaches used for miRNA identification includes, gene cloning technology and Bioinformatics strategies. Gene cloning is a conventional method to identify the new miRNA accurately, even though it has disadvantages, such as difficulty in finding miRNAs which express at low levels, difficulty in cloning, degradation of RNA during sample separation etc [6].Rapid development in the field of bioinformatics has brought a number of computational programs and other tools to successfully predict the miRNA [7, 8]. This process is purely based on the genomic databases like expressed sequence tags EST and other. Since the miRNAs are more conserved in plant species, it is possible to identify novel miRNAs using computational techniques. Now a days miRNAs are identified using the computational or bioinformatics based approach, since it is very useful in predicting the novel miRNA, which cannot be done by cloning.

In this study, all previously known plant miRNAs from A. thaliana, rice, and other plant species were used to search the P.vulgarishomologs of miRNAs in the publicly available expressed sequence tag (EST), NCBI, (http://www.ncbi.nlm.nih.gov/). A total of 10 potential miRNAs were detected. Using these potential miRNAs sequences, French bean mRNA database was further blasted to find24potential miRNA-targeted genes. Most of the target mRNAs were found to be coding transcription factors which are involved in regulating plant growth, development and metabolism.

MATERIALS AND METHODS

Collection of Reference miRNAs and EST Sequences

All available plant miRNAs and their fold back sequences were obtained from miRBase (http://www.mirbase.org/) on May, 2012. The homolog miRNAs were eliminated and the rest were defined as reference for searching French bean miRNAs. French bean EST sequences (148267 as on May, 2012) and GSS sequences (92239 as on May 2012) were downloaded from

NCBI database (http://www.ncbi. nlm.nih.gov/). All redundant and poor quality sequences were eliminated and created a local EST database.

Potential miRNAs and Their Precursors

The reference sequences were used as a query for homology search against our local French bean EST database at e-value threshold < 0.01 using BLAST + 2.2.22 program. The target sequences with no more than four mismatches were considered for secondary structure prediction using Mfold (online). The precursor sequences were searched at 100 nucleotides upstream or downstream from the location of mature miRNAs with an increment of 10 nucleotides. While selecting a RNA sequence as a candidate miRNA precursor, following criteria were used according to Zhang et al. [1] with minor modifications as: 1) a RNA sequence can fold into an appropriate stem loop hairpin secondary structure, 2) a mature miRNA sequence site in one arm of the hairpin structure, 3) miRNAs had less than seven mismatches with the opposite miRNA sequence in the other arm, 4) predicted secondary structures had higher negative energy MFEs (≤–18 kcal/mol), and iv) 40-70%A+U contents.

Prediction of Targets

The prediction of targets for the candidate miRNAs were done with Phaseolus vulgaris DFCI gene index (PVGI) release 3.1. The predicted French bean miRNAs were used as query against French bean DFCI gene index (PVGI) release 3.1 using miRU (http://bioinfo3.noble.org/psRNATarget/) following the criteria as 1) maximum expectation value 3; 2) multiplicity of target sites 2; 3) range of central mismatch for translational inhibition 9-11 nucleotide; 4) maximum mismatches at the complementary site ≤4 without any gaps.

Nomenclature of miRNAs

The predicted miRNAs were named in accordance with miRBase. The mature sequences are designated ‘miR’, and the precursor hairpins are labeled as ‘mir’ with the prefix ‘fb’ for French bean. In the cases where distinct precursor sequences have identical mi RNAs with different mismatch pattern, they were named as fb-mir-1-a and fb-mir-1-b.

RESULTS

Prediction of miRNAs

A total of 148267 ESTs of French Bean were obtained from NCBI EST database. Out of these, 26 sequences had less than five mismatches with previously known plant miRNAs, among which 5 had homologues to more than 3 ESTS and they were ignored. After carefully evaluating the hairpin structures using the criteria mentioned in the method, 10small RNAs were finally identified from French bean ESTs. Details of the predicted miRNAs such as source sequences, location in the source sequences, length of precursor sequences and their minimum

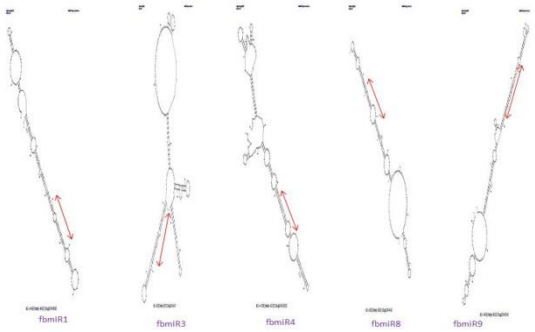
ISSN: 0975-8585

folding free energies and A+U content are tabulated below(Table 1). The newly identified precursor miRNAs have minimum folding free energies (MFE) ranging from -64.80kcal/mol to –33.90 kcal/mol, with an average of about –55.02 kcal/mol and the A+U content were ranges from 34.00 to 65.00% with an average of 53.79%.The length of the precursors ranges from 150-280 with an average of 220nt and mature sequences ranges from 18 to 22 nt. The newly predicted one miRNA (fbmiR8) sequences were perfectly (100%) matched with the corresponding homologue miRNAs, whereas the remaining 9 mature miRNA sequences differ by 1 to 4 nucleotides from their homologues. All the mature miRNAs were found in the stem portion of the hairpin structures (Figure 1) containing less than 6 mismatches in the other arm without break or loop inside the sequences.

Table -1: SP = Start Point, EP = End Point, PL = Pre-miRNA Length, MEF = Minimal Free Energy (in kcal/mol)

New miRNA	Gene ID	SP	EP	Mature miRNA	A+U%	PL	ΔG
fbmiR1	171665565	97	118	UGAAGCUGCCAGCAUGAUCU	45.00%	217	64.80
fbmiR3	171655214	1	19	AAGCUGCCAGCAUGAUCUGA	50.00%	243	53.02
fbmiR4	366411519	98	119	UUGGGCAAAUCUCCUUUGGCA	52.38%	220	41.55
fbmiR8	331946725	99	121	UUGCCGAUUCCACCCAUUCCUAU	52.17%	220	56.00
fbmir9	312044525	113	125	UUAAUUGAGCCGCGUCAAUAUC	59.09%	244	64.50
fbmir10	171671615	122	141	ACGAUGAUGAUGAGGAUGA	57.89%	276	51.76
fbmir11	312052492	86	108	AACAGGGCGGGGAACAGGUGGUG	34.78%	212	62.45
fbmir12	331945455	122	142	AUAUUGGGACGGAGGGAGUAU	52.38%	250	62.05
fbmiR13	331945455	122	142	AUAUUGGGACGGAGGGAGUA	50.00%	204	62.05
fbmiR17	171655214	44	65	ACAGAUCAUGUGGCUGCUUCA	52.38%	110	33.90

Fig 1A



Target Prediction

A total of 12 potential targets were identified for the 4 predicted miRNAs based on their perfect or nearly perfect complementarities with their target sequences in Phaseolus vulgaris (Table 2, Figure 1). For all the miRNAs, single binding site was found in the targets without any gaps in the complementary region and expectation value ranges from 0 to 3. These potential miRNA targets were belonged to a number of gene families that involved in different biological

functions such as regulation of cell cycle, metal ion transportation, starch metabolic processes etc. There were 4% of genes encoding transcription factors, 12.5% of genes encoding different enzymes and 4% of genes encoding transporters as well as 80% of genes encoding various proteins of physiological and metabolic processes (Table 2). The fb-miR10 and fbmiR4 did not bind to any target sequences within our filtration criteria. The predicted miRNAs fbmiR12 and fbmiR13 showed 7 targets with Arabidopsis DFCI (ATGI) release 15, and fbmiR11 showed 4 targets with Glycine max DFCI (GMGI) release 16. Hence a total of 24 targets were identified for the predicted miRNAs in French bean.

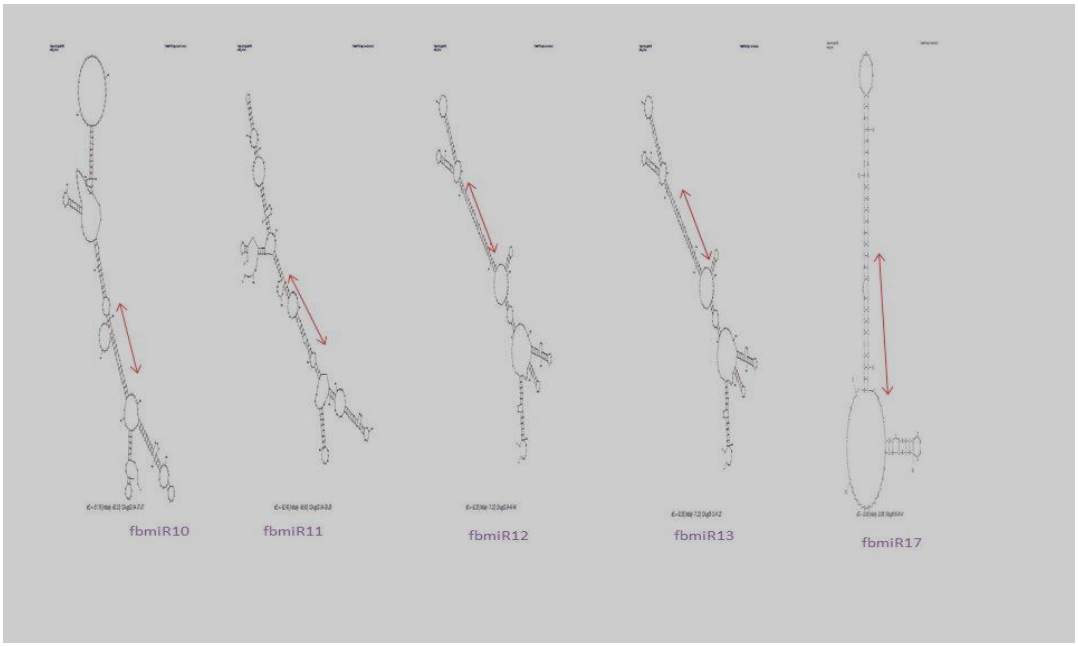
miRNA	Target sites	Gene ID	Target protein	Target involved in	EV	Extent
fbmiR1	1	TC27684	Carotenoid cleavage dioxygenase	Caroteniod cleavage	2.5	Partial
	1	TC20369	Carotenoid cleavage dioxygenase	Caroteniod cleavage	2.5	Partial
	1	TC26796	Chromosome chr9 scaffold	Cell cycle	3.0	Partial
fbmiR3	1	TC27684	CaroTenoid cleavage dioxygenase 1	Caroteniod cleavage	2.5	Partial
	1	TC26796	Chromosome chr9 scaffold	Cell cycle	3.0	Partial
	1	TC20369	Carotenoid cleavage dioxygenase	Carotenoid cleavage	2.5	Partial
fbmiR8	1	CV542270	NADH-ubiquinone oxidoreductase chain 3	Energy metabolism	0.0	Complete
	1	TC26349	Debaryomyceshansenii chromosome E of sTrain CBS767	Cell cycle	3.0	Partial
fbmiR9	1	TC22093	Chromosome chr15	Cell cycle	1.5	Partial
	1	TC20655	scaffold_37 Chromosome chr15 scaffold_37	Cell cycle	1.5	Partial
fbmiR11#	1	TC435652	Chromosome chr14 scaffold_190	Cell cycle	3	Partial
	1	TC443910	Chromosome tndeTermined scaffold_248	Cell cycle	3	Partial
	1	AW832391	Chromosome chr12	Cell cycle	2.5	Partial
	1	TC436634	scaffold_36 Chromosome chr14 scaffold_9	Cell cycle	3.0	Partial
fbmiR12,	1	UC376596	Uncharacterized protein	-	2.5	Paryial
fbmir13##	1	UC387204	Uncharacterized protein AU2g01918.1	-	2.5	Partial
	1	TC362587	Gluthationereductase, chloroplast	Fuel metabolism	3	Complete
	1	TC390712	Glycoprotein	Structural protein	3	Partial
	1	NP1661179	beige/BEACH domain-containing protein	similar to BWF1-like protein [OryzasaUiva]	3	Partial
	1	BP667850	Chromosome chr5 scaffold_2	cell cycle	3	Partial

ISSN: 0975-8585

	1	EL202278	Undetermined protein	-	3	Partial
fbmiR17	1 1	TC28539 TC27529	BZIP Tanscription factor bZIP50 HmsH protein	Transcription factor -	3 3	Partial Partial

EV= expectation Value, # target with glycine max DFCI release 16, ## targets with Arabidopsis DFCI release 15

FIG 1B



DISCUSSION

In plant kingdom most of the mature miRNAs are evolutionarily conserved from species to species. This information enables us to predict new miRNA homologs or orthologs by in-silico method [13].Therefore, we used all previously known plant mature miRNAs from miRBase to search for homologs of miRNAs and their target genes in French bean in the publicly available EST and GSS database of P.vulgaris. Finally, 10 potential French bean miRNAs were identified. In the present study, the length of predicted miRNA precursors varies from 110 to 276. 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 [13]. All the mature sequences of French bean miRNAs are in the stem portion of the hairpin structures, as shown in Figure 3. According to the estimation approximately 10,000 ESTs in plants contain one miRNA [13] that means 148267 ESTs in French bean should contain 14miRNAs. But in this study, 10miRNAs were detected. The current results confirm that the approach of EST analysis is a relatively efficient way to identify miRNAs. To understand the biological function of miRNAs in plant development, it is necessary to identify their targets. In miRNA target prediction, the

screening criterion was set according to the description in Methodology. Finally, 24 potential targets for French bean miRNAs were identified Table 2.It is identified that, most of the predicted miRNA targets were coding genes for transcription factors mainly involved in the synthesis of enzymes participating in regulation of development, growth and other physiological processes. The general characteristic of the miRNA sequence is, it is complementary to their target gene, and in some case single miRNA can be complementary to more than one target gene. fbmiR17 found to target the transcription factor, whereas the mi RNAs fb miR12 and fbmiR13 had more than one targets which are involved in free radical metabolism (glutathione reductase) and structural protein (plastid glycoprotein). Many of the miRNAs were targeting chromosomal scaffold proteins and involved in cell cycle regulation.

CONCLUSION

This paper, with a bioinformatics approach, 10 mature miRNAs along with 24 target genes were identified in P.vulgaris. In-silicostudies stand as initial point for understanding miRNAs role in gene regulation. Thus, identification of miRNAs and their target genes help in understanding function and regulatory mechanisms in French bean.

REFERENCES

[1] Zhang B, Pan X and Anderson AT. FEBS Letters 2006; 580(15): 3753-3762.
[2] Zhou X, Ruan J, Wang G and Zhang W. PLoS Computational Biol 2007; 3(3): e37.
[3] Singh J and Nagaraju J. Insect Mol Biol 2008; 17(4): 427-436.
[4] Zhang B, Pan X, Cobb GP and Anderson TA. Develop Biol 2006; 289(1): 3-16.
[5] Mallory CA and Vaucheret H. Nature Gen 2006; 38: S31-S36.
[6] Sunkar R, Chinnusamy V, Zhu J and Zhu KJ. Trends Plant Sci 2007; 12(7):301-309.
[7] Zhang B, Pan X, Cannon CH, Cobb GP and Anderson AT. Plant J 2006; 46(2): 243-259.
[8] Eckardt N. The Plant Cell 2005; 17: 1335-1338.
[9] Guo SH, Xie Q, Fei FJ and Chua HN. The Plant Cell 2005; 17: 1376-1386.
[10] Jones-Rhoades MW and Bartel PD. Mol Cell 2004; 14: 787-799.
[11] Sunkar R and Zhu JK. The Plant Cell 2004; 16(8): 2001-2019.
[12] Lu Y, Gan Q, Chi X and Qin S. Plant Cell Reports 2008; 27(10): 1571-1579.
[13] Alba R, Fei Z, Payton P, Liu Y, Moore LS, Debbie P, Cohn J and D'Ascenzo M. The Plant J 2004; 39(5): 697-714.