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**Upashna Upadhyay**  
Sam Higginbotham Institute of  
Agriculture, Technology and  
Sciences, Allahabad, U.P., India

**Nepolean Thirunavukkarasu**  
Division of Genetics, Indian  
Agriculture Research Institute,  
New Delhi, India

**Shikha Mittal**  
Division of Genetics, Indian  
Agriculture Research Institute,  
New Delhi, India

**Poonam Singh**  
Sam Higginbotham Institute of  
Agriculture, Technology and  
Sciences, Allahabad, U.P., India

## Insilico analysis of drought tolerant Micro- RNAs in Maize

**Upashna Upadhyay, Nepolean Thirunavukkarasu, Shikha Mittal, Poonam Singh**

### Abstract

Promoters play a very essential role in regulating gene expression at the transcriptional level. Studies of promoters are very crucial and important to improve our basic understanding of gene regulation and will also enhance toolbox of available promoters for further use in agricultural biotechnology. In the present study the promoter sequences have been predicted for seven drought tolerant microRNAs genes and also their contributing cis - acting elements have been predicted, as they provide insights into plant signaling behavior under drought stress and gene regulation. The function of a regulatory RNA molecule to a large extent is determined by its structure. Computational tools and methods for modelling and predicting secondary structure provide us with useful initial models for solving the tertiary structure by NMR. We have predicted the secondary structure of the miRNAs on the basis of minimum free energy. The phylogenetic tree has been generated by using the precursor miRNA sequence has served to clarify significantly the evolutionary relationship of these drought tolerant microRNAs in maize with other crops.

**Keywords:** Promoters, microRNAs, NMR

### Introduction

Research throughout the world have demonstrated that abiotic stress like drought inhibits the germination of seed, seedlings development, chlorophyll biosynthesis and photosynthesis, and that induced oxidative stresses such as reactive oxygen species (ROS) production, it also inhibits plant growth and development (Baohong, 2014; Fernandez, 2014; Mathur *et al.*, 2014; Suzuki *et al.*, 2014) <sup>[1, 3, 6, 11]</sup>. The productivity of over one-third of all fecund or arable land is affected by water scarcity around the world. MicroRNAs are an extensive class of small endogenous RNA molecules that is 20-24 nucleotides in length. Plants contain a high number of species-specific miRNAs and conserved miRNAs such as miR156. The conserved miRNAs may regulate plant morphology and phase change and the species-specific miRNAs may control unique process such as fiber initiation. (Xie *et al.*, 2015) <sup>[12]</sup>. So it suggests that the conserved miRNAs and species-specific miRNA are involved and play important role in plant response to abiotic stress. Therefore to overcome this limitation and to enhance the production of crops, the traits associated with drought tolerance should be improved in cultivated plants (Neumann 2008). MicroRNAs play very important role in drought stress regulation and it is also found in research that they have big role in post-transcriptional gene regulation. So it is necessary to determine their native structure to understand their mechanism of action and predicting secondary structure is a very crucial step for this. The identification of genes and their homologs in several different species indicates that these genes divergence from their common ancestor. This will help us in better understanding of species evolution and can serve to develop plant model system for studying gene function in upcoming research future.

### Materials and Method.

#### A. Drought stress responsive miRNA prediction

All 321 ZeamaysmiRNAssequences (both mature and precursor) were downloaded from miRBase Registry Database (<http://www.mirbase.org/>), Release 21: June 2014. Seven families of microRNAs were selected which have been found to play drought tolerance role in maize (Jayaraman *et al.* 2017) <sup>[4]</sup>.

#### B. Sequence download of seven miRNA genes.

The mature and precursor sequences of miRNA160, miRNA164, miRNA166, miRNA169, miRNA393, miRNA529 AND miRNA2275 from A.thaliana, O.sativa, B. distachyon, S.bicolor, Z.mays were obtained from miRBase.

### Correspondence

**Poonam Singh**  
Sam Higginbotham Institute of  
Agriculture, Technology and  
Sciences, Allahabad, U.P., India

### C. Nomenclature of mature and precursor miRNAs.

The nomenclature of the miRNA used in this study was as per the miR Base Registry database. The sequence was checked for the presence of core promoter elements like TATA and CAAT box using Plant CARE database.

### D. Promoter prediction for precursor miRNAs.

Promoters are basically made up of groups or clusters of short sequences that provide them with binding sites for transcription factors that is the cis-regulatory elements. The precursor miRNAs sequences were submitted to get the promoter sequences using online tool ([www.fruitfly.org/sequence\\_tools/promoter](http://www.fruitfly.org/sequence_tools/promoter)). (Reese M.G., 2001).

### E. Transcriptional Regulatory motif (cis-regulatory elements) analysis of potential maize miRNA Gene Promoter.

miRNAs were transcribed by RNA Polymerase II (Cui (2009); Megraw (2006); Meng (2011) and Zhou (2010). cis-regulatory elements on miRNA gene promoter was analyzed by using tools designed for it such as Plant CARE

(<http://bioinformatics.psb.ugent.be/webtools/plantcare/html>) (Lescot., 2002).

The parameters were set as default. The sequence was checked for the presence of core promoter elements like TATA and CAAT box using Plant CARE database.

### F. Prediction of Secondary Structure

The precursor sequences formed the hairpin structure through Vienna RNA package.

### G. Sequence Alignment and Phylogenetic Analysis.

The CLUSTAL W aligned sequences was used for reconstructing the phylogenetic tree using FigTree.v.1.4.3pre tool.

## Results and Discussion

### Identification of Potential Maize miRNA

A good number of microRNAs are present in different plant species from mosses to Flowering plants. These homologous miRNAs families typically have conserved the regulatory functions associated with them (Zeng *et al.* 2006) [13]. Studied in Euphorbiaceous plants about the species and condition specific miRNA expression patterns. In the present study potential miRNAs belonging to seven miRNAs families were identified and selected for further in silico studies.

### Cis-Regulatory Elements in Maize miRNA Promoter Regions

The precursor sequences of the maize miRNA genes were analyzed by PlantCARE to reveal the earlier known cis-

regulatory elements that can regulate the gene expression (Meng *et al.*, 2011) [9]. It has been known that promoter regions of many miRNA genes have been found to contain a TATA box (a core promoter sequence served for initiation of the transcription process) and a CAAT box (a common cis-element in promoter and enhancer region), concluding us with the information that miRNA genes were transcribed by RNA polymerase II similar to other protein coding genes (Lee; 2004, Cui; 2009) [5, 2]. The cis-regulatory element involved in light responsiveness were found more prevalent on miRNA genes, which gives us the information to conclude that light plays an important role in miRNA gene expression.

Among the putative cis-elements shown in Table 1, several are known stress-responsive elements, such as the cis-acting element involved in abscisic acid (ABA) responsiveness (CE3), the ABA-responsive element (motif Iib), the TATA-box as the core element for start of transcription, and the GC motif involved in anoxic-specific induction. Some other regulatory elements, namely the TATC-box and the P-box for gibberellin responsiveness, Skn-1 and GCN4 for endosperm expression, the A-box for  $\alpha$ -amylase promoters, previously reported to be stress-relevant in the analysis of regulatory sequences in the promoters of zinc finger proteins Zat12 and Zat7, and transcription factor MRKY25 (Rizhsky *et al.*, 2004) [10]. Therefore, these elements can tentatively be related to the drought stress response or tolerance process. The G-box for the light stress response, although not clearly confirmed to have a role in drought stress responsiveness, was proven to be involved in the complex biochemical systems to perceive and respond to light of different wavelengths for photosynthetic organisms (Martínez-Hernández *et al.*, 2002) [6].

According to PLACE database almost there are almost 469 cis-regulatory elements present in plants. The core sequence ACGT is considered to be one of the most functionally important element in various promoters that respond different environmental stimuli such as light, anaerobiosis and hormones such as abscisic acid and Auxin (Mehrotra *et al.*, 2015) [8]. The regulation of gene expression is by ABRE and other cis-regulatory elements such as CRE, DRE etc. Table 1 show us some stress responsive elements such as ABRE (cis-regulatory element involved in abscisic acid responsiveness), MBS (a MYB binding site involved in drought –inducibility) and other stress responsive elements such as Skn-1 motif (cis element involved in endosperm expression, but also has been reported possibly involved in abiotic stress), TC- rich repeats (cis element involved in defense and stress responsiveness). Skn-1 motif, ABRE, MBS motifs found in these miRNA genes are in accordance with the finding of Patanum 2012; in Cassava that show us that these cis-regulatory motifs are relevant to drought stress in the miRNA genes. The results of this study present us a complete set of transcription factor binding site that should be further investigated for miRNA regulation in Maize.

**Table 1:** Type of cis-regulatory elements relevant to drought stress in maize miRNA

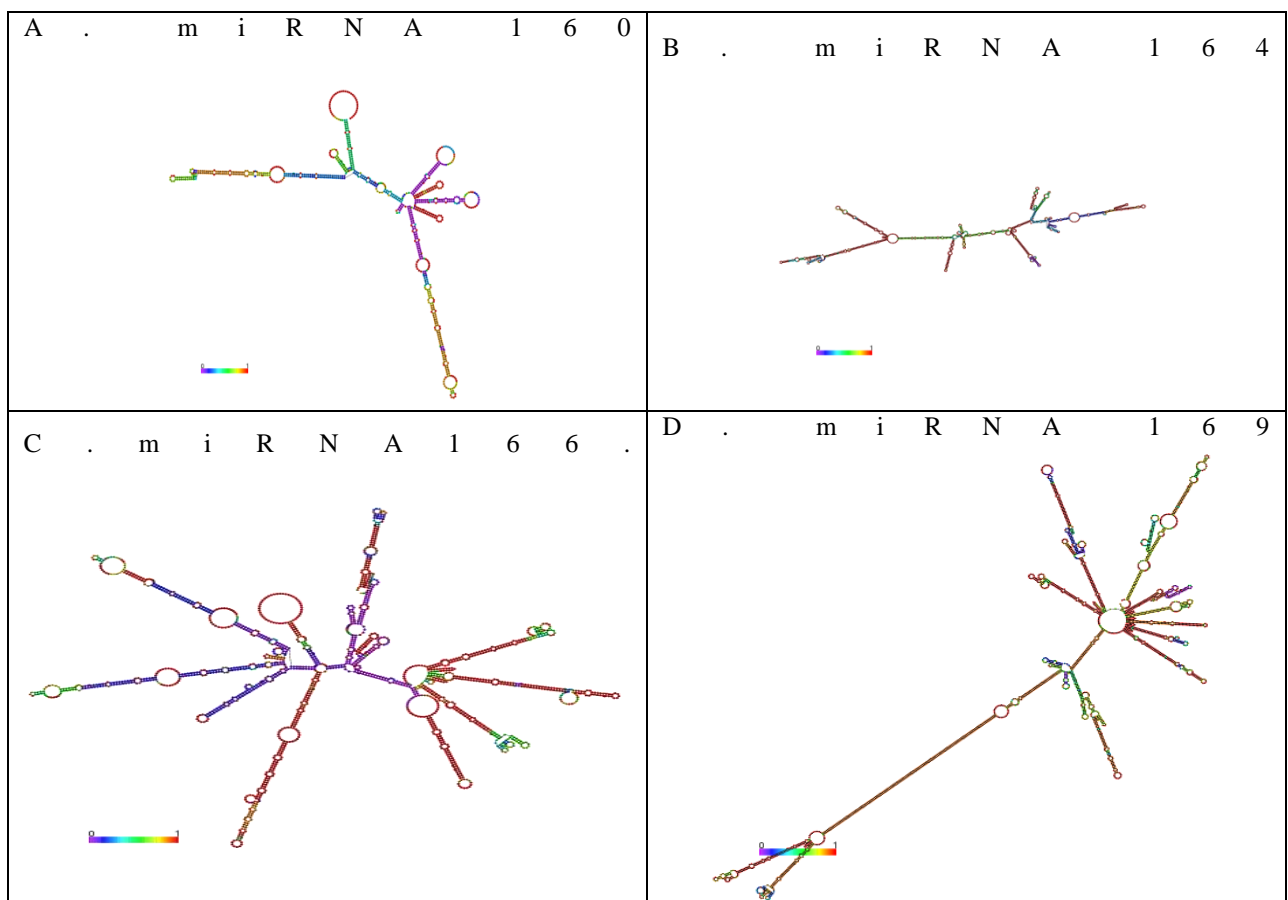
Cis-regulatory element type	E l e m e n t f u n c t i o n	miRNAs with cis-regulatory element
C A A T - b o x	common cis-acting element in promoter and enhancer regions	160b, 160g, 166b, 169d, 169e, 169h, 169l, 169o, 169r, 393a, 393b, 2275b, 2275c, 2275d
O 2 - s i t e	cis-acting regulatory element involved in zein metabolism regulation	1 6 0 b , 1 6 0 g
S p l	light responsive element	1 6 0 b , 1 6 0 g 1 6 9 j , 1 6 9 n , 1 6 0 q
T A T A - b o x	core promoter element around -30 of transcription start	160c, 166k, 169f, 169p, 529, 2275c, 2275d
c i r c a d i a n	cis-acting regulatory element involved in circadian control	1 6 0 c , 1 6 0 g , 3 9 3 b , 3 9 3 c
M B S	M Y B B i n d i n g S i t e	1 6 0 d , 2 2 7 5 b
S k n - 1 _ m o t i f	cis-acting regulatory element required for endosperm expression	160d, 169c, 169e, 169g, 169i, 169p
G A T A - m o t i f	part of a light responsive element	1 6 0 f
I - b o x	part of a light responsive element	1 6 0 f

A B R E	cis-acting element involved in the abscisic acid responsiveness	164a, 164b, 164d, 164f, 164g, 166i, 166k, 393c
G - B o x	cis-acting regulatory element involved in light responsiveness	164a, 164b, 164d, 164f, 164g, 166i, 166k, 393c, 529
M N F l	light responsive element	164b, 164d, 164g, 393c
m o t i f I i b	abscisic acid responsive element	1 6 4
A - b o x	cis-acting regulatory element	166a, 166i, 169o
C C G T C C - b o x	cis-acting regulatory element related to meristem specific activation	166a, 166i, 169o
G C N 4 _ m o t i f	cis-regulatory element involved in endosperm expression	166f, 169m
R Y - e l e m e n t	cis-acting regulatory element involved in seed-specific regulation	166g, 169g, 169r
T G A - e l e m e n t	auxin-responsive element	166k, 169l
C G T C A - m o t i f	cis-acting regulatory element involved in the MeJA-responsiveness	169a, 169b
T G A C G - m o t i f	cis-acting regulatory element involved in the MeJA-responsiveness	169a, 169b
a s - 2 - b o x	AtGATGinvolved in shoot-specific expression and light responsiveness	1 6 9
S k n - 1 _ m o t i f	cis-acting regulatory element required for endosperm expression	160d, 169c, 169e, 169g, 169i, 169p
T C - r i c h r e p e a t s	cis-acting element involved in defense and stress responsiveness	1 6 9
G A R E - m o t i f	gibberellin-responsive element	1 6 9
C C A A T - b o x	M Y B H v l b i n d i n g s i t e	2 2 7 5

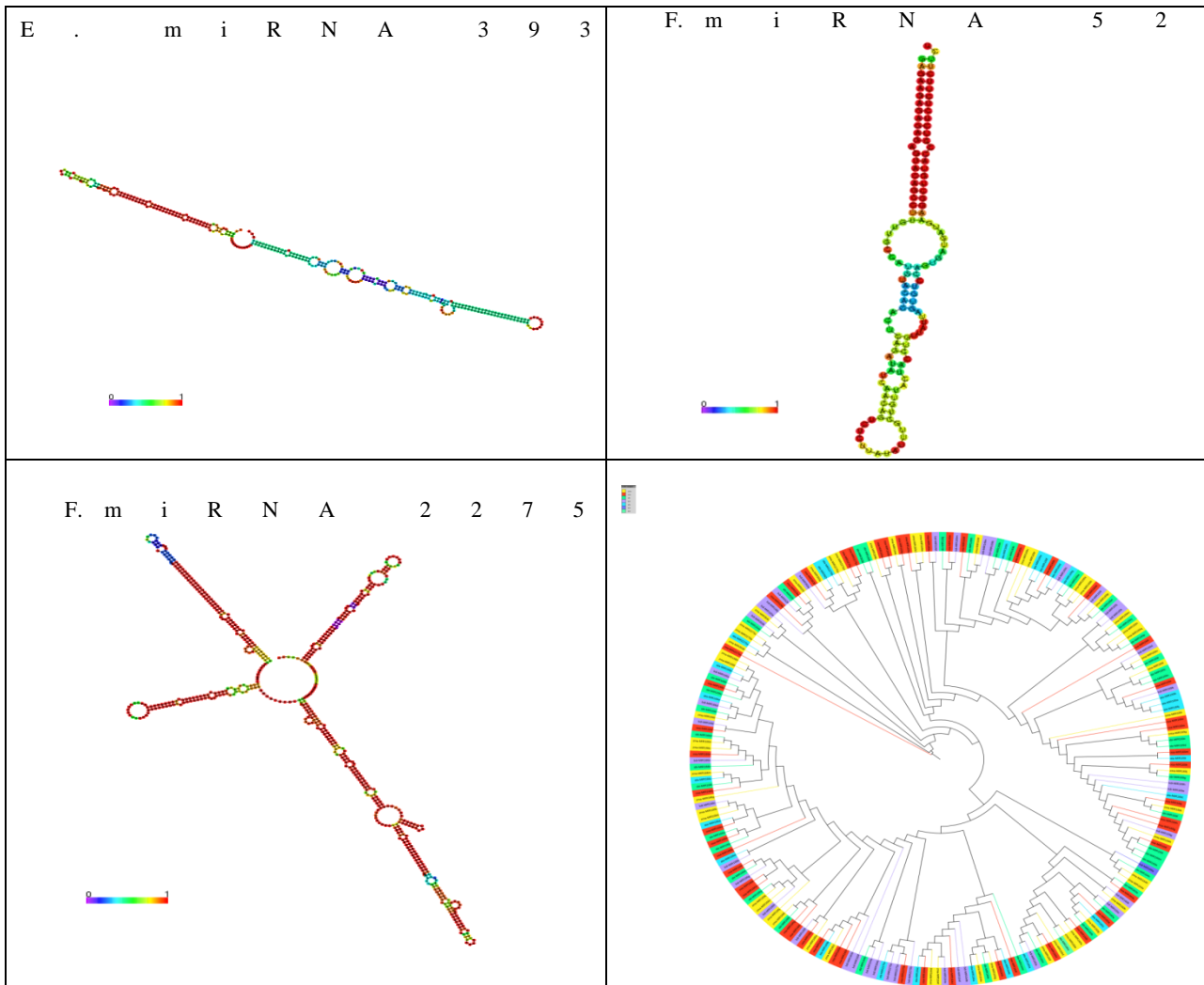
### Secondary Structure Prediction and Phylogenetic Analysis.

The newly found miRNA genes are tolerant to drought stress which is potentially very interesting. RNA has potentially very important functions which also includes regulation of transcription and translation. Structure of the miRNA play very important role in regulating all these activities by molecular interaction or conformational changes. Therefore function of a miRNA molecule is determined by its secondary structure. Figure 1 shows us the predicted secondary structure of these miRNA genes. The tendency for RNA folding to be primarily driven by secondary structure features is a tremendous advantage for structural and functional studies of

RNAs (Ding; 2010). The phylogenetic analyses suggest us that different miRNAs might evolve at different rates not only within the same plant species, but also in different plant species. To gather the information about the evolutionary relation of miRNAs in plant species a combined phylogenetic tree was constructed with the published precursor sequences of the miRNAs in five plant species. By deeply observing the phylogenetic tree it can be clearly stated that all these microRNA genes have a common ancestral relationship and they all play a major role in regulating the gene expression to combat the harsh effect of drought and make the plant tolerant to drought stress which can be analyzed in figure 2.



**Fig 1:** Predicted Secondary Structure of the miRNAs.



**Fig 2:** The combined Phylogenetic tree of plant miRNA

### Summary and Conclusion

A brief study in looking insight to these newly found miRNAs help us to better understand the mechanism behind how they regulate the gene expression and the way they alter the mechanism to withstand the drought effect. None of the predicted miRNAs showed identity with the previously reported miRNAs in maize and these are addition into maize miRNA data set. In addition, these microRNAs appear to be related to the development, growth, metabolism and other physiological processes under stress response. Identification of new miRNAs and their target genes will provide the future path leading to the understanding of the core regulatory interactions during abiotic stress in maize. Researcher can further verify these predicted miRNA experimentally by high throughput sequencing of small RNA libraries.

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