

# Identification and analysis of miRNA- mRNA Modulation in NAC and Bhlh Genes in Rice

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**Abstract**—Rice (*Oryza sativa*) is an economically important annual food to human beings. It has been used by humans throughout the recorded history for its food, fiber all over the world. So far, plant has been studied extensively at genomics level for the identification of genes and genetics pathways and their regulatory networks. This study is the first step towards the identification of miRNAs and their targets in *Oryza sativa*. MicroRNAs (miRNAs) represent a class of endogenous non-coding small RNAs that play important roles in multiple biological processes by degrading targeted mRNA translation. A total of 3 miRNAs belonging to 2 (NAC & bHLH) independent families were identified in *Oryza sativa* by searching homologs of known plant miRNAs in publically available rice nucleotide sequences. In this expression predicted NAC gene family consist of 2 miRNAs (*ath-miR5021*, *ath-miR5658*) and 10 target sequences. In Bhlh gene family of rice consist of only 1 miRNA (*ath-miR5658*) and 3 target sequences. The potential target genes were assigned to three broad functional categories, i.e., cellular components, biological processes and molecular functions as established for the *Oryza sativa*. These findings of miRNAs will pave the way for understanding the function and processing of *Oryza sativa* small RNAs in future.

**Keywords:** *Oryza sativa*, miRNAs, NAC, Bhlh, Target sequences.

## Introduction

Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize, according to 2012 FAOSTAT data (Das et al. 2016). Since a large portion of maize crops are grown for purposes other than human consumption, rice is the most important grain with regard to human nutrition and caloric intake, providing more than one-fifth of the calories consumed worldwide by humans.

A microRNA is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and other some viruses, that functions in RNA silencing and post-transcriptional regulation of gene expression.

The NAC gene family name was derived from the names of three transcription factors: NAM (no apical meristem, petunia), ATAF1 & Arabidopsis thaliana activating factor and CUC2 (cup-shaped cotyledon, Arabidopsis), which share the same DNA-binding domain (Sour et al. 1996; Aida et al. 1997). NAC genes are found across wide range of plant species and represent one of the largest families of plant-specific transcription factors. Over 50 families of different transcription factors have identified in plants, based on sequence analyses of model species such as rice (Xiong et al., 2005). Together with cis-acting elements, transcription factors are involved in almost all aspects of cellular activity as part of their interrelated roles in regulating gene expression, as reviewed in Xiong et al., 2005. The availability of several complete plant genomic sequences, has led to the identification of 117 NAC genes in Arabidopsis, 151 in rice, 79 in grape, 26 in citrus, 163 in poplar and 152 each in soybean and tobacco (Rtushton et al., 2008; Hu et al., 2010; Nurzzaman et al., 2010, 2012a; Le et al., 2011). In recent years there has been improved knowledge on the molecular mechanisms underlying signaling pathways (Liu et al., 2010) and their involvement in activating defense responses in rice (Valent and Khang, 2010) and of rice innate immune responses, host recognition of pathogens (Skamnioti and Gurr, 2009) and recognition-triggered early signaling events.

The basic/helix-loop-helix (bHLH) transcription factors and their homologs from a large family in plant genomes. They are known to play important roles in specification of tissue types. On other hand, few plant bHLH proteins have been studied functionally. Recent completion of whole genome sequences of model plants Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) allows genome-wide analysis and comparison of the bHLH family in flowering plants. In addition, sequence analysis of potential DNA binding activity, the sequence motifs outside the bHLH domain, and the conservation of intron/exon structural patterns further support the evolutionary relationships among proteins. The genome distribution of rice bHLH genes strongly supports the hypothesis that genome wide and tandem duplication contributed to the expression of bHLH gene family,

consistent with the birth and death theory of gene family evolution. Bioinformatics analysis suggests that rice bHLH proteins can potentially participate in a variety of combinatorial interactions, endowing them with the capacity to regulate a multitude of transcriptional programs.

### Materials & methods:

#### Collection of plant miRNAs:

Plant miRNAs were downloaded from miRNA registry database i.e. miRBase (<http://www.mirbase.org>. Released 20: June, 2013).

First go to (<http://www.mirbase.org>.) then browse ,go expand all . After that sequence download of *Oryza sativa* & mature sequences are converted to Fatch sequences then the species were downloaded.

#### Collection of NAC & bhlh gene family from rice:

Plant transcription factor database (PTFdb), [plantfdb.cbi.pku.edu.cn/](http://plantfdb.cbi.pku.edu.cn/)

It is a portal for the functional and evolutionary study of plant transcription factors. protein sequence of TFdb of the species *Oryza sativa* were downloaded from plant transcription factor database, [plantfdb.cbi.pku.edu.cn/](http://plantfdb.cbi.pku.edu.cn/). From this NAC & bhlh gene family is taken and downloaded the sequences. The sequence were translated with using bioedit or reverse translate (permanent). The gene is edited with copy sequence to clipboard FASTA, and save as OS. Indica.

#### Standalone BLAST of miRNAs against rice gene family:

Using Bioedit, alignment of all plant miRNAs against the local nucleotide sequence databaf “MYB” & “MYB related” gene family of *oryza sativa* was carried out using Standalone BLAST 2.2.10 (oct-19-2004) program at an e-value threshold  $\leq 0.001$  (Altschul et al., 1997). Values were find from obtained database. From the found hits with maximum 3 nt mismatches and without gap were considered for extracting the precursor sequences (pre-miRNA). From miRBase precursor sequence and mature sequence of selected miRNAs were collected. The fold-back secondary structures of pre-miRNAs were predicted using Mfold (Zuker, 2003). The potential target genes of the identified miRNAs were predicted using the plant miRNA target finder program ([www.http://plantgrn.noble.org/psRNATarget](http://www.http://plantgrn.noble.org/psRNATarget)). The predicted miRNA/target pairs were obtained and the sequences of the target accession were downloaded.

## Results & Discussion

Table 1

Sl no.	MIRN As	Id no.	Length	Target	Start	End	Strand	Match	Score	e-Value
1	ath-miR5021	MIMAT0020525	20	BGIOSGA027881-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA027881-PA	202	218	++	17/17	34	9expo005
				BGIOSGA029774-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA029774-PA	243	258	++	16/16	32	3expo0042
2	ath-miR5658	MIMAT0022431	21	BGIOSGA034998-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA034998-PA	660	642	+	19/19	38	6expo006
				BGIOSGA032905-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA032905-PA	131	133	+	19/19	38	6expo006
				BGIOSGA032901-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA032901-PA	117	119	+	19/19	38	6expo006
				BGIOSGA015018-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA015018-PA	660	642	+	19/19	38	6expo006
				BGIOSGA009257-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA009257-PA	771	753	+	19/19	38	6expo006
				BGIOSGA030048-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA030048-PA	117	119	+	18/18	36	2expo005
				BGIOSGA027877-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA027877-PA	821	838	+	18/18	36	2expo005
				BGIOSGA021116-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA021116-PA	953	936	+	18/18	36	2expo005
				BGIOSGA005950-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA005950-PA	951	933	+	18/18	36	2expo005
				BGIOSGA014332-PA  <i>Oryza sativa</i> _subsp._indica NAC BGIOSGA014332-PA	841	825	+	17/17	34	9expo005

Table 2

MiRNAs	Id no.	Length	Target	Start	End	Strand	Match	Score	e-VALUE
ath-miR5658	MIMAT0022431	21	BGIOSGA000206-PA  <i>Oryza sativa</i> _subsp._indica bHLH BGIOSGA000206-PA	153	133	+/-	21/21	42	7expo004
			BGIOSGA030958-PA  <i>Oryza sativa</i> _subsp._indica bHLH BGIOSGA030958-PA	159	141	+/_	19/19	38	6expo006

		BGIOSGA029759-PA  <i>Oryza sativa</i> subsp. <i>indica</i>  bHLH BGIOSGA029759-PA	156	138	+/-	19/19	38	6expo006
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**Discussion:** The NAC gene family name was derived from the names of three transcription factors: NAM (no apical meristem, petunia), ATAF1 & Arabidopsis thaliana activating factor and CUC2 (cup-shaped cotyledon, Arabidopsis), which shared the same DNA-binding domain (Sour et al. 1996; Aida et al. 1997). NAC genes are found across wide range of plant species and represent one of the largest families of plant-specific transcription factors. Over 50 families of different transcription factors have identified in plants, based on sequence analyses of model species such as rice (Xiong et al., 2005). Together with cis-acting elements, transcription factors are involved in almost all aspects of cellular activity as part of their interrelated roles in regulating gene expression, as reviewed in Xiong et al., 2005.

The basic/helix-loop-helix (bHLH) transcription factors and their homologous from a large family in plant genomes. They are known to play important roles in specification of tissue types. On other hand, few plant bHLH proteins have been studied functionally.

**Conclusion:** In this study, genome-wide computational prediction of miRNAs in rice, and dozens of target mimics were identified. A total of 3 miRNAs belonging to 2 (NAC & bHLH) independent families were identified in *Oryza sativa* by searching homologs of known plant miRNAs in publically available rice nucleotide sequences. In this expression predicted NAC gene family consist of 2 miRNAs (ath-miR5021, ath-miR5658) and 10 target sequences. In Bhlh gene family of rice consist of only 1 miRNA (ath-miR5658) and 3 target sequences. MiR5021 play an important role in gene regulation in plants. Gene regulation at the transcriptional and transcriptional level leads to diversity in phenotypes and function in organisms to contain a binding site for the microRNA (ath-miR5021). Ath-miR5658 play an important role in salt stress and sensory transduction.

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