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Research Article

IN SILICO PREDICTION OF THE RELATIONSHIP BETWEEN MIRNA AND DIFFERENTIALLY EXPRESSED GENES DURING SUBMERGENCE IN *ORYZA SATIVA*

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ABSTRACT
Plant finely regulates the multifarious pattern of gene expression in response to submergence at the post-transcriptional level. To endure submergence plant genome induces several mRNA, miRNA and regulatory elements. MicroRNAs are short (20–27 nucleotides), non-coding RNA molecules. They play important role in regulation of gene expression. MicroRNAs are well-known as the negative regulator of gene expression via sequence specific recognition of their target mRNA. Here, we have predicted the relationship between the miRNA and mRNAs expressed differentially during submerged condition in *Oryza sativa*. The untranslated regions of the mRNAs are full of transcriptional factors and miRNA target site. Each anaerobically induced mRNA contains a unique combination of cis-acting regulatory elements in their UTRs. We have identified 10 conserved miRNAs families within the genome of *Oryza sativa* induced in response to submergence. The present study uncovers, notable propensity of these miRNAs to interact with the cis-acting regulatory element involved in many biological processes and stress response. These events may modulate the initial signal and produce a new signal and eventually lead to the increased expression of these genes.

***Keywords*:** *Oryza Sativa; MicroRNA; Submergence; Gene Expression; Untranslated Region*

INTRODUCTION
Crop productivity is strictly related to genome stability, an essential requisite for optimal plant growth and development (Macovei *et al.*, 2012). In general there are numerous types of environmental stresses, often crop or location specific, which cause significant crop loses. Environmental stresses can cause severe effects on plant cells sufficient to cause cell death (Umeda and Uchimiya, 1994). Amongst all environmental stresses rice crops especially experience water stress (submergence) in the rainfed lowland of Northeast India. Submergence is considered to be one of the major constraints for crop production in many areas of the world (Kozlowski, 1984). According to FAO (2002) submergence adversely affects 10% of the global land area (Pradhan and Mohanty, 2013). It imposes several often concurrent challenges like starvation of oxygen / carbon-dioxide, hypoxia and anoxia and result in restricted plant growth, development and crop yield. The regulation of gene expression in response to environmental stress is an important factor in plant survival and adaptability. Gene expressional regulation is achieved through a series of complex mechanisms, generally in two distinct steps: firstly at transcriptional level mediated by cis-acting DNA elements such as promoters, enhancers, locus control regions and silencers to produce a mature mRNA (Pesole *et al.*, 2001), secondly at the post-transcriptional control of mRNA nucleo-cytoplasmic transport, translation efficiency, subcellular localization and stability. Recently, miRNAs have been reported to control a variety of biological processes, such as plant development, differentiation, signal transduction or stress responses (Macovei *et al.,* 2012).
MicroRNAs (miRNAs) are a family of small endogenous non-protein coding RNA molecules especially ~20-27 nts, form Watson-Crick base pairs with different target mRNAs and are important post-transcriptional regulators of gene expression regulating various biological activities. Knowing the entire repertoire of these small molecules is the first step to gain a better understanding of their function. The number of miRNAs has expanded rapidly, shortly after the discovery of the first miRNA *i.e.lin-4* & *let-7* RNA in *Caenorhabditis elegans* (Ambros, 2004; Zhang *et al.,* 2007; Sunkar and Jagadeeswaran, 2008). Sequencing data from several species further led to the discovery of many miRNAs, which in turn spurred

the development of computational techniques to identify targets. MicroRNAs are evolutionarily conserved throughout the plant kingdom (Zhang *et al.,* 2006). The mode of action of gene expressional regulation mediated by miRNAs differs between plants and animals. MicroRNA triggers translational repression either at the initiation stage (Humphreys *et al.,* 2005; Pillai *et al.,* 2005) or during the elongation phase (Nottrott *et al.*, 2006; Petersen *et al.*, 2006) in animals whereas gene silencing / cleavage seems to be predominant in plants by binding to the complementary sequences on target mRNA (Llave *et al.,* 2002; Jones-Rhoades *et al.,* 2006). In place of suppression of the gene expression recent report suggests that miRNAs also act as inducers of gene expression (Bruno *et al.,* 2011). Presently there are relatively a few indications that miRNAs might be a new class of genes involved in regulation of morphological and metabolic adaptation in cereals during submergence. The identification of putative targets is much more complicated, because miRNA regulates target genes either positively or negatively at a variety of levels, depending on the form of target and miRNA base pairing. Recently, 3′-end of the miRNA has been also shown to target 5'-UTR of targets (Moretti *et al.*, 2010). Much progress has been made in unraveling the complex stress response mechanisms, particularly in the identification of stress responsive protein-coding genes. RNA analysis using submergence-tolerant rice cv FR13A and submergence-intolerant cv IR42 suggested differential transcript levels of genes associated with glycolysis and alcohol fermentation in rice plants under submergence stress (Umeda and Uchimiya, 1994). These results put forward the idea that the anaerobic proteins complicate the genetic engineering approaches for flood tolerance in rice. In addition to submergence responsive genes, recently discovered miRNAs have emerged as the key players in plant stress responses. Zhang *et al.,* (2008), based on microarray analysis identified 39 submergence-responsive plant miRNAs at the 1 % level of significance (Zhang *et al.,* 2008). Here, we present a systematic search for the identification of possible correlations between these protein coding regions and miRNAs expressed differentially during submergence. This will help us understand the dynamic expression pattern of the miRNAs associated with the submergence condition.

MATERIALS AND METHODS

The experimentally validated genes portraying the degree of difference in the expression during anaerobic / submerged condition (Umeda and Uchimiya, 1994; Sachs *et al.,* 1996; Dennis *et al.,* 2000) were retrieved from publicly available nucleotide database NCBI (http://ncbi.nlm.nih.gov/). About 1kb DNA sequence upstream and downstream of the transcription initiation and termination position for these genes was retrieved from Rice Annotation project database (http://rapdb.dna.affrc.go.jp/). In addition to the protein coding sequences, we selected the validated miRNAs showing changed expression profiles from different plant families owing to submergence treatment (Zhang *et al.,* 2008). The mature miRNA sequences were downloaded from miRBase database (http://mirbase.org). Since plant miRNAs are conserved across the species, we used the mature miRNA sequences other than osa-miRNAs as a query sequence to find out the homologous miRNAs present in *Oryza sativa*. The rate of base conservation in the seed region was analyzed for the miRNAs belonging to same family. The miRNAs which showed homology with *Oryza sativa* miRNAs with not more than 2nt mismatch were selected for UTR analysis. The 5'- and 3'-UTR region of the genes targeted by these miRNAs were identified by an in-house perl program. The database PlantCARE was used to find out the cis-acting regulatory element present in the 5'- and 3'- UTR region.

RESULTS AND DISCUSSION

We previously predicted a total of eleven miRNAs and their probable target regions within the genome of *Oryza sativa* based on the genes delivering resistance during submergence i.e. *ABA 8'-hydroxylase 1 (ABA8ox1), submergence tolerance 1 (SUB 1)* and *Oryza sativa cation transport protein (osCTP)* (Paul and Chakraborty, 2013). In view of the fact that the genes and miRNAs involved in submergence were confirmed by the wet lab experiments we speculated that miRNAs might be the key factor for the changed expression profiles of the genes. In order to reduce the ratio of false positive results, here we have used only the experimentally validated submergence responsive genes (table I) and miRNAs to predict the regulatory motifs present in the 5'- and 3'-UTRs of the genes targeted by the miRNAs.

Table I: The name and accession number of the submergence responsive genes

Sl No	Gene names	Accession numbers	References
1	*Glucose phosphate isomerase*	*AB107218.1*	(Umeda and Uchimiya, 1994; Dennis *et al.*, 2000)
2	*Phosphofructokinase*	*KC620558.1*	(Umeda and Uchimiya, 1994; Dennis *et al.*, 2000)
3	*Triose phosphate isomerase*	*JQ650258.1*	(Umeda and Uchimiya, 1994)
4	*Glyceraldehyde phosphate dehydrogenase*	*AF357884.1*	(Umeda and Uchimiya, 1994; Sachs *et al.*, 1996)
5	*Phosphoglycerate kinase*	*DQ899741.1*	(Umeda and Uchimiya, 1994)
6	*Enolase*	*U09450.1*	(Umeda and Uchimiya, 1994; Sachs *et al.*, 1996; Dennis *et al.*, 2000)
7	*Pyruvate decarboxylase*	*U07339.1*	(Umeda and Uchimiya, 1994; Sachs *et al.*, 1996; Dennis *et al.*, 2000)
8	*Alcohol dehydrogenase*	*X16296.1*	(Umeda and Uchimiya, 1994; Sachs *et al.*, 1996; Dennis *et al.*, 2000)
9	*Aldolase*	*NM001048392.1*	(Umeda and Uchimiya, 1994; Sachs *et al.*, 1996)
10	*Pyruvate kinase*	*NM001060800.1*	(Umeda and Uchimiya, 1994)
11	*Ribosomal protein YS25*	*D12633.1*	(Umeda and Uchimiya, 1994)
12	*Sucrose synthase*	*NM001063582.1*	(Dennis *et al.*, 2000)
13	*Alpha amylase*	*NM001049545.1*	(Dennis *et al.*, 2000)
14	*Hexokinase*	*NM001048799.1*	(Dennis *et al.*, 2000)
15	*Fructose- 1,6- bisphosphate*	*AK062233.1*	(Dennis *et al.*, 2000)
16	*Lactate dehydrogenase*	*AK105416.1*	(Dennis *et al.*, 2000)
17	*Alanine aminotransferase*	*NM001065251.1*	(Dennis *et al.*, 2000)
18	*Glutamine synthase*	*AK061157.1*	(Dennis *et al.*, 2000)
19	*Nitrite reductase*	*NM001048759.1*	(Dennis *et al.*, 2000)
20	*Nitrate reductase*	*NM001054788.1*	(Dennis *et al.*, 2000)
21	*Formate dehydrogenase*	*NM001064201.1*	(Dennis *et al.*, 2000)
22	*Calcium dependent protein kinase*	*C7830619.1*	(Dennis *et al.*, 2000)

In plants, 7 miRNA families, i.e., miR156/157, miR160, miR159, miR319, miR165/166, miR390 and miR408 are conserved between monocots and dicots. These are also found in primitive land plants such as *Physcometrella* and *Selaginella* (Arazi *et al.,* 2005; Axtell and Bartel, 2005; Axtell *et al.,* 2007). Conserved plant miRNAs play almost similar role across the plant kingdom despite their morphological differences. To study the role of miRNA in submergence, we initially retrieved 39 experimentally validated miRNAs from different plant families. The mature miRNA sequences were used to find out the homologs present in the *Oryza sativa*. Out of the 24 miRNAs other than *Oryza sativa* only 13 miRNAs showed homology to the entire *Oryza sativa* miRNAs present in the miRBase. Our result suggests that these 13 miRNAs play conserved role across the plants species and also implies that miRNAs arose early in eukaryotic evolution, before the divergence of monocots and dicots. The results of the search that had been carried out to find the homologous miRNA are given in the table II.

Table II: Submergence responsive miRNA members

Experimentally validated miRNAs	**Homology to *Oryza sativa* miRNAs *(No. of mismatch)***
ptc-miR159d/f	osa-miR159e *(1)*
ath-miR160a-5p	osa-miR160a/b/d-5p *(0)*
ath-miR166a	osa-miR166d-5p *(1)*
sbi-miR166a	osa-miR166a/b -3p *(1)*
pta-miR159 a/b/c	osa-miR 159 c/d/e/f *(2)*
sof-miR 159e	osa-miR 159 a.1/b *(2)*
ptc-miR166n/p	osa-miR166g- 3p; osa-miR166m *(1)*
ath-miR319c	osa-miR 319a-3p.2/b *(2)*
pta-miR319	osa-miR 319a-3p.2/b *(2)*

MicroRNA seed sequence is the central region for targeting the mRNA. After the seed sequence analysis, out of the 10 newly identified *Oryza sativa* miRNA families homologous to the experimentally validated miRNAs, only osa-miR159, osa-miR160 and osa-miR319 showed the dissimilarity in their seed sequence. Thus a total of 14 miRNAs (including the validated osa-miR528) were used for the mRNA untranslated region (UTR) analysis (table III).

Table III: Submergence Responsive *Oryza Sativa* Mirnas And Their Mature Sequences

Sl. No	**Micro RNAs**	**Accession numbers (miRBase)**	**Mature sequences**
1	osa-miR159a.1	*MIMAT0001022*	UUUGGAUUGAAGGGAGCUCUG
2	osa-miR159e	*MIMAT0001026*	AUUGGAUUGAAGGGAGCUCCU
3	osa-miR160b-5p	*MIMAT0000629*	UGCCUGGCUCCCUGUAUGCCA
4	osa-miR166d-5p	*MIMAT0022858*	GGAAUGUUGUCUGGCUCGAGG
5	osa-miR166m	*MIMAT0001087*	UCGGACCAGGCUUCAUUCCCU
6	osa-miR319a-3p.2-3p	*MIMAT0001028*	UUGGACUGAAGGGUGCUCCC
7	osa-miR167d-5p	*MIMAT0001039*	UGAAGCUGCCAGCAUGAUCUG
8	osa-miR168a-5p	*MIMAT0001045*	UCGCUUGGUGCAGAUCGGGAC
9	osa-miR168b	*MIMAT0001046*	AGGCUUGGUGCAGCUCGGGAA
10	osa-miR171b	*MIMAT0001063*	UGAUUGAGCCGUGCCAAUAUC
11	osa-miR171h	*MIMAT0001077*	GUGAGCCGAACCAAUAUCACU
12	osa-miR396d	*MIMAT0013835*	UCCACAGGCUUUCUUGAACGG
13	osa-miR399a	*MIMAT0000984*	UGCCAAAGGAGAAUUGCCCUG
14	osa-miR528-5p	*MIMAT0002884*	UGGAAGGGGCAUGCAGAGGAG

In plant, approximately 7% of the coding sequence codes for the transcription factors (TFs) (Udvardi *et al.,* 2007). TFs play a major role in gene expression regulation by binding to specific regions of the mRNA and confer submergence tolerance. In multicellular organisms, TFs and miRNAs are the major families of gene regulators. The transcriptionally regulated miRNAs in response to submergence stress target mRNA for cleavage / translational repression at the post-transcriptional level. According to Zhang *et al.,* (2009) miRNA target mRNA encodes TFs, mostly involved in root growth and morphogenesis (Zhang *et al.*, 2009). This results in the promotion of adventitious roots. MicroRNA shows dynamic expression pattern; a single miRNA may have multiple targets and a single mRNA may be targeted by multiple miRNAs. To identify the dynamic expression pattern played by these miRNAs we have analyzed their role in the UTRs of all the transcriptionally regulated genes. The mRNA UTRs is involved in several post-transcriptional regulatory pathways that control mRNA localization, stability and translation efficiency. After analyzing the UTRs of the genes, it was evident that the transcriptionally regulated genes maintain above 50% base conservation. This result implies that the genes induced during anaerobisis possess similar sequence in their promoter region and also suggests the involvement of the common TFs. More specifically, since the AU rich elements (AREs) and miRNA targeted region are more than 70% conserved in doing so, it put forward an idea that AREs are involved in the regulation of expression of these genes with clinical and developmental consequences. These evidences reveal that preferred synthesis of genes induced during anaerobisis involves transcriptional as well as significant post-transcriptional regulation of gene expression. The graphical representation for the miRNA osa-miR159a.1 and its targets (UTR) are given in the figure 1.

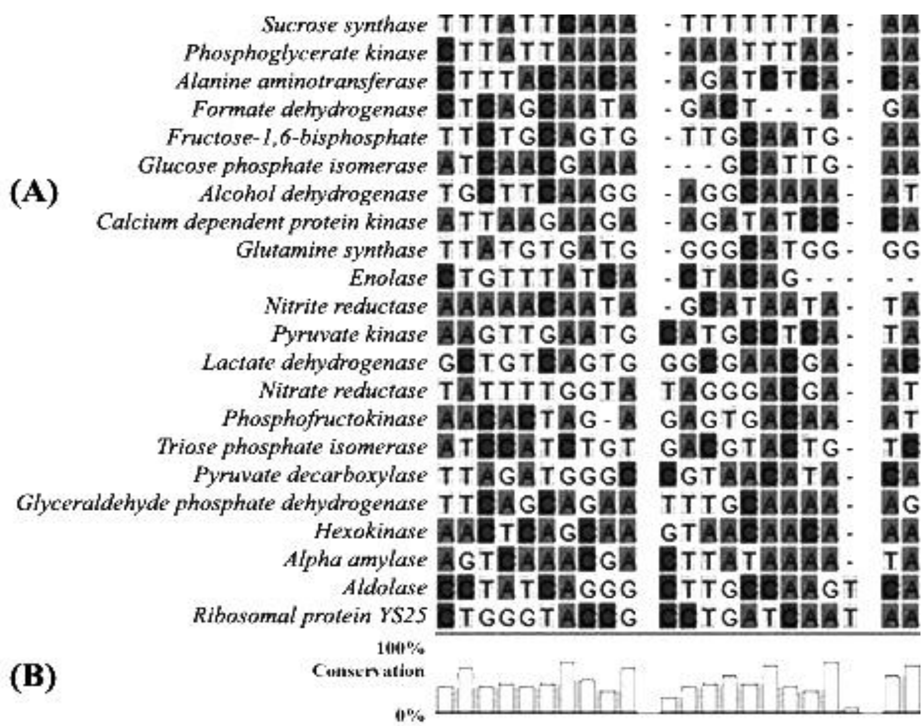

Figure 1: (A) osa-miR159a.1 target region (301-322nt) at the 3'-UTR of *sucrose synthase;* (B) percentage of base conservation

Taken altogether the results, we summarized that the metabolically related genes maintain a defined genetic pattern despite the morphological differences of the plants.

To analyze the propensity of miRNAs to interact with all the cis regulatory motifs playing role in gene expression pattern with regard to submergence, we have analyzed the presence of motifs flanking to the miRNA target site within 1-kb downstream and upstream of the genes. A number of functionally significant cis-acting regulatory elements that are associated with plant stress response were identified upstream and downstream of the genes coding sequence in rice (table IV).

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Table IV: List of cis-regulatory elements presents adjacent to transcriptionally regulated *Oryza sativa* miRNAs

Genes	miRNA	5'-UTR *(Distance from the initiator codon)*	3'-UTR *(Distance from the terminator codon)*
Glucose phosphate isomerase	osa-miR168a	CGTCA *(263)*	
	osa-miR168b		MBS *(683)*
Phosphofructokinase	osa-miR160b		GT1 *(830)*
	osa-miR528	G box *(102)*, sp1 *(129)*, motif IIb *(45)*	
Triose phosphate isomerase	osa-miR160b		Box 4 *(784)*, CCAAT *(794)*
	osa-miR528	ABRE *(54)*, G box *(54)*, LTR *(100)*, Sp1 *(8)*	
Phosphoglycerate kinase	osa-miR159a.1		GT1 *(455)*
	osa-miR168b		Box 4 *(412)*, GT1 *(455)*
	osa-miR396d	A box *(315)*, CCGTCC box *(315)*, MNF 1 *(216)*	
Enolase	osa-miR167d	AAAC *(904)*, ATCT *(876)*, TCCC *(944)*	
	osa-miR168a		GCN 4 (*490*), sp1 (*598*)
	osa-miR171b	Box 4 (*117*)	I box *(543*)
Pyruvate decarboxylase	osa-miR528		Box III *(551*)
Alcohol dehydrogenase	osa-miR160b	5'-UTR py-rich stretch (*464*), ABRE (*396*), G box (*399*)	
	osa-miR168a	Box 4 (*570*), Box 1 *(525*)	
	osa-miR171b		Box I (*457*), GA (*388*), LTR (*417*)
Aldolase	osa-miR167d		A box (*659*), CCGTCC *(659*), sp1 (*642*), motif IIb (*534*)
Pyruvate kinase	osa-miR168b	AE box (*254*), GC (*183)*, sp1 (*170)*	
Ribosomal protein YS25	osa-miR160m	ABRE (*93*),sp1 (*60*), motif IIb (*103*)	
Sucrose synthase	osa-miR159a.1		TGG (*324*)
	osa-miR160m	ATGCAAAT (*197*), Gap box (*194*), TGACG (*167*)	
	osa-miR171b		GAG *(614*)
	osa-miR528	W box (*626)*	
Lactate dehydrogenase	osa-miR159e	GAG (*899)*	
	osa-miR168a	CGTCA *(954*), GAG (*899*)	
	osa-miR171b	Box 4 (*117*), G box (*151*)	
	osa-miR171h		Skn1 (*14*), TCT (*118*)
	osa-miR396d		Skn1 (*495*)
Alanine	osa-miR168a		GAG (551), TCT

aminotransferase			(559)
Glutamine synthase	osa-miR159a.1		ABRE *(189)*, MBS *(191)*, TATC *(230)*
	osa-miR159e		G box *(391)*
	osa-miR167d	Box 4 *(146)*, HSE *(127)*, MBS *(136)*, TCA *(173)*	
Nitrite reductase	osa-miR167d	Pc-CMA2c *(102)*	
	osa-miR171h	5'-UTR py-rich stretch *(773)*, GA *(827)*	
	osa-miR528	Pc-CMA2c *(102)*, sp1 *(8)*	
Nitrate reductase	osa-miR396d		Skn1 *(702)*
	osa-miR528	Skn1 *(394)*, TCT *(389)*	
Formate dehydrogenase	osa-miR399a	ABRE *(416)*, G box *(414)*	
Calcium dependent protein kinase	osa-miR319a-3p.2		P box *(685)*, TGACG *(696)*
	osa-miR167d	A box (*92*), CCGTCC *(92)*, G box *(207)*, GC *(117)*, GCN 4 *(145)*, sp1 (*152-163*)	
	osa-miR168b		TGACG ()*696*, P box *(685)*
	osa-miR171h	AE box (*309*), ARE (*687*), AT rich element (*649*)	

Osa-miR168a targets the 3'-UTR region of the gene *enolase* and 5'-UTR region of *glucose phosphate isomerase, alcohol dehydrogenase* and *lactate dehydrogenase*. Osa-miR168a targets directly and downstream of the CGTCA motif for *glucose phosphate isomerase,* and *lactate dehydrogenase*, respectively such that it possibly acts as an obstacle for the motif signal. Osa-miR168a targets a region downstream to GCN4 motif and acts as a negative regulator of endosperm expression, whereas upstream to sp1 motif for the 3'-UTR of the gene *enolase* as a positive regulator of gene expression. In the 5'-UTR of the gene, *alcohol dehydrogenase* and *nitrate reductase,* osa-miR160b and osa-miR171h bind to a region upstream of the pyrimidine rich stretch, which is a cis-acting element involved in conferring high transcriptional level. As a consequence our result suggests that osa-miR160b and osa-miR171h may act as enhancers for gene expression and contribute to the tissue specific control of protein translation. The complete findings of our miRNA: UTR interaction analyses are given in the table number IV. All submergence-responsive miRNAs targeted more than one gene involved in carbohydrate concentration / and alcohol fermentation. Hence, gene expression in submergence regulates through a complex network. These results, taken together with those of previous studies, indicate that there is an integrated co-regulatory network between the gene and miRNAs expressed during submergence.

From our analysis it was evident that all the transcriptionally regulated miRNAs bind to precise regions which are either downstream or upstream to the motif sequences. These regions play significant roles in gene expression, stability and many other key stress responsive factors like abscisic acid, salicyclic acid, gibberellin, temperature and so forth. All the miRNAs except osa-miR319a-3p.2 bind to a region adjacent to light responsive motifs (LRMs). A total of forty-seven miRNA targets were predicted, close to the light responsive motif. In majority of the cases (64.5%) it was observed that miRNA targets the 5'-UTRs. The abscisic acid, gibberallin, MeJA, heat, temperature and drought associated cis-acting elements were also present at varying frequencies in close proximity to the miRNA target sites. Out of all the light responsive motifs 63.8%, 17.02% and 19.14% motifs are present before, within and after the miRNA target site, respectively. Light responsive cis-acting regulatory element GT1 acts to decrease the transcription rate by binding to box II and III in dark-adapted transgenic tobacco (Kuhlemeier *et al*., 1987). The action of GT-1 motif may be confined by osa-miR160b, osa-miR159a.1, osa-miR171b and osa-miR528 in dark-specific repression, which results in the normal transcription of the genes *phosphofructokinase, triose phosphate isomerase, phosphoglycerate kinase, enolase* and *pyruvate decarboxylase* in submergence. G-box binding factors are the large family of TFs has been linked to a diverse group of activities in plants including stress responses (Menkens and Cashmore, 1994). The core and the downstream sequence of the G-box were found as a target by osa-miR159e, osa-miR399a and osa-miR160b, osa-miR167d, osa-miR171b, osa-miR528, respectively.

The gap-box found in the promoter region of *Sucrose synthase* was also found as a target site by osa-miR160m. Similarly all other LRMs present in the UTRs of the genes were targeted by the miRNAs expressed during submergence. Although light responsive elements and their binding TFs have been discovered, none of the elements has been identified solely to confer light responsiveness to minimal heterologous promoters (López-Ochoa *et al.*, 2007; Ibraheem *et al.*, 2010).

A set of experimental observations suggest that the light responsive cis-acting elements present in the promoter regions function as silencers in the absence of light (Kuhlemeier *et al.*, 1987; Kuhlemeier *et al.*, 1989; Stockhaus *et al.*, 1989). In support of this, we speculated from our analysis that the transcriptionally regulated miRNAs bind to these 65.95% light responsive cis-acting elements present in the promoter region and hold back their action in submerged condition. Plant shows resistance against several environmental stress conditions through the regulation of phytohormone signal transduction (Feys and Parker, 2000), and eventually several miRNAs (miR159, miR160 and miR167) were induced by phytohormones (Zhang *et al.*, 2005; Liu *et al.*, 2009).

Hence, the occurrence of hormone-related cis-elements (GRE, MBS, CGTCA motif, and ABRE) in the promoter region of the genes which are probable target of miRNAs implies that the pathway mediated by phytohormones is also crucial in the regulation of the miRNA-mediated gene expression. Osa-miR159a.1 was found to target the MBS motif, a MYB binding site involved in the down-regulation of *glutamine synthase*.

In that way it makes the MBS inaccessible to MYB-binding protein, resulting in normal/increased level of transcript in stress condition. Furthermore, the miRNAs were found to target the abscisic acid (ABA) responsive motifs (ABRE & motif IIb) by complementary binding to the motif core sequence. Pertaining to the previous research on ABA (Bartholomew *et al.*, 1991; Chang and Walling, 1991; Staneloni *et al.*, 2008), our result revealed that the miRNAs block the action of ABA in down-regulation of gene expression during submergence. It possibly results in increased number of anaerobic gene transcripts required for the survival and tolerance of plants during the submergence stress.

Conclusion

Submergence tolerance is associated with crops grown in high-rainfed areas of the world. During submerged condition, rice expresses a number of transcription factors and miRNAs at high transcriptional level. It was observed that amongst all the differentially expressed miRNAs, nearly 55% are conserved. The study also presents the nucleotide base conservation at UTRs, which in turn indicates the presence of common transcriptional factors. Unlike the non-conserved miRNAs which likely target diverse genes that function in a broad range of biological processes, these conserved miRNAs have the tendency to interact with several transcription factors in the UTRs of the genes involved in carbohydrate concentration and alcohol fermentation. The present *in-silico* analysis detecting the presence of cis-acting motifs targeted by miRNAs in the UTRs, gives an indication about the nature of submergence stress signal that might induce the expression of these genes. Furthermore, the plant light responsive elements are composite, which interact to regulate gene expression at both transcriptional and post-transcriptional level. As a result of the presence of a large number of cis-regulatory elements, it is still mysterious in genomics whether these light responsive motifs are involved in negative or positive regulation of gene expression.

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