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Prediction of cis-regulatory elements for a detailed insight of RuvB family genes from *Oryza sativa*

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ABSTRACT

Cis-regulatory elements (CREs) present in promoter region of a gene, regulates gene expression at transcriptional level. Expression profiling and study of predicted CREs in promoter region which are responsive to various factors, is an approach to predict the role of a gene in stress management of plants and this information can be exploited for the agricultural importance. This study focused on RuvB gene family from rice, which is one of the highly conserved gene family and is scarcely studied in plants. Role of OsRuvB genes to various stress conditions was studied with the help of microarray expression profiling and in silico prediction of CREs present in promoter region of these genes by using various databases- PlantCARE, TENOR and PlantPAN. Study of OsRuvB gene family promoters showed a wide range of CREs involved in hormonal regulation, developmental stages, metabolic processes, temperature response, abiotic and biotic stress tolerance. These CREs were further functionally validated with the real-time analysis for transcript level of these genes under various stress conditions. This study of OsRuvB family CREs gave a detailed insight into the possible functions performed by this conserved family. Further study and research in this family may help to bring us a step forward in stress management study in plants and also provide an important target gene for the agricultural crop improvement.

Key words: *Abiotic, biotic, c-regulatory elements, OsRuvB, promoter, stress*

INTRODUCTION

Genetic material is constant in all cells of a multicellular organism, still there is process of differentiation of cells which allows group of cells to perform specialized functions. This simple concept raises a question of what is behind this process and the simple but powerful answer is 'regulation of gene expression' (Hernandez-Garcia and Finer, 2014). Regulation of gene expression is a very well regulated process which has various degrees involving transcriptional, post transcriptional and post translational levels of regulation. Transcriptional regulation of gene expression involves the upstream DNA sequence of a gene which is called as promoter. Promoter is the DNA sequence where RNA polymerase sits and starts synthesis of mRNA. But binding of RNA pol to promoter and synthesis of mRNA in eukaryotic system is not a simple process. Eukaryotic transcription process involves special proteins called as transcription factors (TF) which bind to specific elements in promoter region and initiate the transcription process. These specific elements are called as cis-regulatory elements (CREs). CREs are highly conserved DNA sequences ranging from 5 to 20 bp and are usually present in the upstream region of transcription start site (TSS) but there are few CREs also which can be present in downstream region (Rombauts, 2003). Specific CREs are conserved for their specific TFs only, for example AUXRR-motif for auxin responsive elements only, ABRE for ABA responsive factors only and so on. Hence, interaction of these CREs with their specific TFs synchronize the expression of genes in response to various spatial, temporal and environmental stimuli (Passricha et al., 2016). Identification of specific CREs in upstream region of genes is a helpful method to elucidate the

mechanism and function performed by various proteins in response to various environmental cues and development and homeostasis (Priest et al. 2009).

Rice genome has been already sequenced and information is available on various databases such as Rice genome annotation project and RAP-DB. Although information available on this crop is ample still there are few families of genes which need to be noted and worked upon, *RuvB* family of genes from *Oryza sativa* is one such family. *RuvB* family is a conserved family which is present across single celled organism (bacteria, yeast) to multicellular organisms (human, *Arabidopsis thaliana*, *Oryza sativa*, Drosophila etc.). Studies in yeast suggest that RuvB proteins are involved in DNA damage repair mechanism and other various cellular functions being the major component of various chromatin remodeling complexes. In *Oryza sativa*, this family is yet untouched although its homologs from different organisms support its involvement in various important cellular mechanisms in response to different stress conditions. In this study, we are trying to look for the role of *OsRuvB* family under various stresses and cis-acting regulatory elements are a source of information which allow us to have that detailed insight. There are 4 members in *OsRuvB* family which are distributed on chromosomes 1, 6 and 7 with locus ID LOC_Os01g62040, LOC_Os06g08770, LOC_Os07g08170 and LOC_Os07g39290 (*OsRuvBL1a*, *OsRuvBL2a*, *OsRuvBL1b* and *OsRuvBL2b*, respectively).

This study is majorly focused on finding the CREs for *OsRuvB* family of genes and to elucidate their role under various stresses. For the identification and prediction of specific CREs Plant CARE and Plant PAN2.0 databases were used. Putative CREs from upstream region of *OsRuvB* family of genes were compared with the expression profiles of these genes on the basis of microarray data from TENOR database and functionally validated with real-time analysis. This comparison and analysis helped to look for the inducible factors for the expression of these genes and hence deduce their probable functions. These genes may be used as future targets for the crop improvement study.

MATERIALS AND METHODS

Microarray expression analysis

For the microarray based expression profiling of *OsRuvB* family genes, TENOR database was used. Gene ID for each gene was submitted in the TENOR database with the selected experimental conditions such as salinity, ABA, drought, jasmonic acid (JA) and cold for shoot and root separately. Microarray expression profiles were depicted in the form of graph with upper-quartile normalized RPK values. Graphs were taken from the TENOR database. Fold Change (FC)>2 was considered as differentially expressed.

Isolation of 1Kb upstream sequence of *OsRuvB* family

Rice genome has been completely sequenced and all the genome information is available on Rice Genome annotation Project (http://rice.plantbiology.msu.edu/). 1Kb upstream sequence bulk data was downloaded from the rice genome database and 1Kb upstream sequence for *OsRuvB* family genes was searched in this bulk data with the help of locus ID of these genes.

In silico analysis for identification of CREs

Database used for analysis of 1Kb upstream region of *OsRuvB* family genes was PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). Each sequence was uploaded in search query option and sequence was submitted. Database provided us with all the possible CREs (common and specific) present in the 1Kb upstream region. Presence of specific CREs was confirmed by using other databases also such as PlantPAN (http://plantpan2.itps.ncku.edu.tw/) and TENOR (http://tenor.dna.affrc.go.jp/).

Real-time validation of CREs in selected promoters

For the functional validation of CREs involved in various abiotic and biotic stresses we performed the transcript analysis for genes *OsRuvBL1a* and *OsRuvBL2a* with real-time method. 15 days old rice seedlings were subjected for various stresses such as heat, cold, salinity, drought, abscisic acid (ABA) and salicylic acid (SA). Total RNA was isolated from the treated and non-treated (control) seedlings with TriZOL LS reagent (Invitrogen Life Technologies, USA) as per the manufacturer's instructions. First strand cDNA was synthesized by using oligo-dT primers with iScript Select first strand cDNA synthesis kit (Bio-Rad).

expression analysis of *OsRuvBL1a* and *OsRuvBL2a* in response to various stresses was performed real-time analysis with real-time primers (*OsRuvBL1a* real-time primers forward- 5'-TCAGGAGCTAGGTAGTAAG-3' and reverse- 5'-TTCTGGCGAAAGTTCAG-3' and *OsRuvBL2a* real-time primers forward-5'-CCCTGGTGTTCTGTTTATT-3' and reverse-5'-GTGGTGATCGGTAGTTTG-3'). Actin gene from rice was taken for normalization (OsActin real-time primers -forward-5'-CCTGATGGACAGGTGATCAC-3' and reverse-5'-TCAGCAATACCAGGGAACAT-3'). Fold change in expression of *OsRuvBL1a* in stress conditions as compared to controlled condition was calculated by using $2^{-\Delta\Delta Ct}$ method. Final expression result was derived from three independent biological replicates and three technical replicates.

RESULTS AND DISCUSSION

Expression profile of *OsRuvB* family genes

Microarray gene expression of *OsRuvBL1a* showed altered expression in response to various stress and hormonal treatments as compared to control condition (Fig. 1A i and ii). In shoot tissue, salinity stress imparted less effect on the expression of *OsRuvBL1a* and showed upregulation in response to drought, cold, ABA and jasmonic acid (JA) treatment. In root, only cold treatment led to the upregulation of *OsRuvBL1a* whereas other treatments (salinity, drought, ABA and JA) caused its downregulation (Fig. 1A ii). Another member of *OsRuvBL1* subfamily- *OsRuvBL1b* showed a different expression profile (Fig. 1B). In shoot tissue (Fig. 1B i), it showed substantial downregulation in salinity, drought and cold treatments whereas upregulation in hormonal treatments (ABA and JA). In root tissue, *OsRuvBL1b* showed upregulation in drought, cold and ABA treatments and downregulation in JA treatment (Fig. 1B ii). Expression profile of *RuvB2* gene (*OsRuvBL2a*) in shoot tissue, showed upregulation under ABA and JA treatment and downregulation under drought and cold treatment during initial phase of treatment and then got upregulated during later hours in these treatments (Fig. 1C i). In root tissue also *OsRuvBL2a* gene showed downregulation under drought, cold and JA treatments and remained unaltered during salinity and ABA treatment (Fig. 1C ii). Expression profile of last member of this family

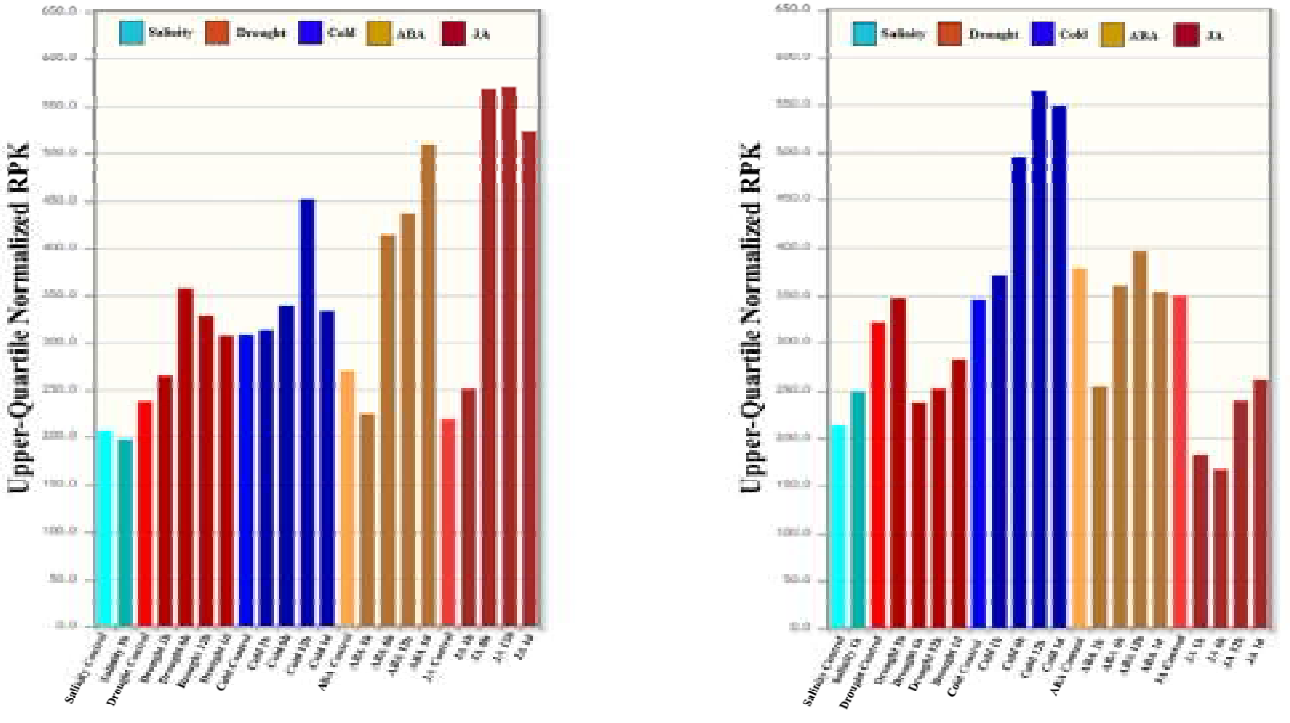

Fig. 1.(A) Microarray expression profile from TENOR database. Expression profile of *OsRuvBL1a* gene in presence of salinity (1h), drought, cold, ABA and JA (1h, 6h, 12h and 1day) in (i) shoot tissue and (ii) root tissue.

Table 1. List of cis-regulatory elements present in 5' upstream region of *OsRuvB* family genes using PlantCARE database

Responsive Factors	CREs	Sequence	Present in	PlantCARE ID	Function
Hormonal responsive	CGTCA	CGTCA	OsRuvBL1a OsRuvBL1b OsRuvBL2a OsRuvBL2b	U83904	involved in MeJA responsiveness
	TGACG	TGACG	OsRuvBL1a OsRuvBL1b OsRuvBL2a	U83904	cis-acting regulatory element involved in the MeJA-responsiveness
	ABRE	CACGTG	OsRuvBL1b OsRuvBL2a OsRuvBL2b	D13044	cis-acting element involved in the abscisic acid responsiveness
	motif IIb	CCGCCGCGCT	OsRuvBL1a OsRuvBL1b	Synthetic	abscisic acid responsive element
	TGA	AACGAC	OsRuvBL1b OsRuvBL2a	X98521	auxin-responsive element
	TCA-element	GAGAAGAATA, CCATCTTTTT	OsRuvBL1a	X98521	cis-acting element involved in salicylic acid responsiveness
	AuxRR-core	GGTCCAT	OsRuvBL2b	D85911	cis-acting regulatory element involved in auxin responsiveness
	G-box	GACATGTTGGT	OsRuvBL1a OsRuvBL1b OsRuvBL2a OsRuvBL2b	X03710	light responsive element
	circadian	CAANNNNATC	OsRuvBL1a OsRuvBL2a OsRuvBL2b	M14445	cis-acting regulatory element involved in circadian control
	as-2-box	GATAATGATC	OsRuvBL1a OsRuvBL2b	L02124	involved in shoot-specific expression and light responsiveness
	GT1-motif	GGTTAA	OsRuvBL1b OsRuvBL2a	X98080	light responsive element
Light Responsive	Sp1	GGGCGG	OsRuvBL1b OsRuvBL2a	Synthetic	light responsive element
	CATT	GCATTC	OsRuvBL1b OsRuvBL2b	D13044	part of a light responsive element
	Box 4	ATTAAT	OsRuvBL2a OsRuvBL2b	X15473	part of a conserved DNA module involved in light responsiveness
	AT1	ATTAATTTTACA	OsRuvBL1a	Z13987	part of light responsive module
	Box1	TTTCAAA	OsRuvBL1a	M21356	Light-responsive element
	GATA	AAGGATAAGG	OsRuvBL1b	Z13987	part of a light responsive element
	chs-Unit 1 ml	ACCTAACCCGG	OsRuvBL1b	X58339	part of a light responsive
	TCCACCT	TCCACCT	OsRuvBL2a	Synthetic	part of a light responsive element
	I-box	GTATAAGGCC	OsRuvBL2a	M37328	part of a light responsive element
	MRE	AACCTAA	OsRuvBL2a	U67134	MYB binding site involved in light responsiveness
	TCCC	TCTCCCT	OsRuvBL2a	X61362	part of a light responsive element
	AE-box	AGAAACTT	OsRuvBL2b	L14743	part of a module for light response
	Box I	TTTCAAA	OsRuvBL2b	M21356	Light responsive element
	ATCC motif	CAATCCTC	OsRuvBL2b	S66544	part of a conserved DNA module involved in light responsiveness
	chs-CMA2a	GCAATTCC	OsRuvBL2b	M35515	part of a light responsive element
Development	SKn-1_motif	GTCAT	OsRuvBL1a OsRuvBL2a	X54314	cis-acting regulatory element required for endosperm expression
	CAT	GCCACT	OsRuvBL1b OsRuvBL2a	Synthetic	cis-acting regulatory element related to meristem expression
	5UTR Py-rich stretch	TTTCTTCTCT	OsRuvBL2a	U68071	cis-acting element conferring high transcription levels

Continued....

Abiotic stress Responsive	LTR	CCGAAA	OsRuvBL1a OsRuvBL1b OsRuvBL2a OsRuvBL2b	U63993	cis-acting element involved in low-temperature responsiveness
	GC-motif	CCCCCG	OsRuvBL1b OsRuvBL2a	U09743	enhancer-like element involved in anoxic specific inducibility
	CCGTCC	CCGTCC	OsRuvBL1b	L37357	enhancer-like element involved in anoxic specific deducibility
	HSE	AAAAAATTTC	OsRuvBL2b	X98521	cis-acting element involved in heat stress responsiveness
	ARE	TGGTTT	OsRuvBL2b	U45858	cis-acting regulatory element essential for the anaerobic induction
Biotic Stress responsive	TATCCAT/C	TATCCAT	OsRuvBL2a OsRuvBL2b	M59351	cis-acting regulatory element; associated with G-box like motif; involved in sugar repression responsiveness
	TC-rich repeats	ATTCTCTAAC	OsRuvBL1a	L02124	cis-acting element involved in defense and stress responsiveness
	GCC-box	AGCCGCC	OsRuvBL1b	synthetic	Ethylene responsive factor for PR genes
	box S	AGCCACC	OsRuvBL1b	synthetic	fungal elicitor responsive element
	plant_AP2-like	CGCGCCGG	OsRuvBL1b	X63126	Ethylene responsive element biotic and environmental stress
	Box-WI	TTGACC	OsRuvBL2a	U48863	fungal elicitor responsive element
	W-box	TTGACC	OsRuvBL2a	Synthetic	WRKY TF binding site. Abiotic and Biotic stress responsive
	EIRE	TTCGACC	OsRuvBL2b	X69794	elicitor-responsive element

OsRuvBL2b gene in shoot tissue, showed upregulation in drought, cold and JA treatment and downregulation under ABA treatment in initial hour which resumed in later stages (Fig. 1D i). In root tissue this gene showed upregulation under all the treated conditions (drought, cold, ABA and JA) except salinity stress where the expression was unaltered (Fig. 1D ii). These expression profiles for all genes showed their altered expression in response to various treatments suggested the presence of some CREs specific for these treatments.

Hormone responsive cis-regulatory elements in *OsRuvB* family

Hormone responsive (HR) CREs are the elements which are regulated by various types of hormones such as auxin, ABA, JA, jasmonic acid etc. such hormone responsive CREs in *OsRuvB* family promoters were CGTCA, TGACG, ABRE, motif IIb, TGA, TCA-element and AuxRR-core elements (Table 1). Most common hormone responsive CRE CGTCA which is involved in methyl jasmonate responsiveness was present in all the members of *OsRuvB* family (Fig. 2 A-D). Second most common HR elements were TGACG (*OsRuvBL1a*, *OsRuvBL1b* and *OsRuvBL2a*) and ABRE (*OsRuvBL1b*, *OsRuvBL2a*, *OsRuvBL2b*) which are responsible for JA and ABA responsiveness respectively (Fig. 2 B-D). Other HREs were present in one or two members of *OsRuvB* family and these are mostly involved in auxin, ABA and SA responsiveness. Jasmonic acid and salicylic acid are considered as biotic stress hormones which are involved in signaling the pathway in response to some biotic attack on plant and presence of CREs responsive to these hormones suggest the role of *OsRuvB* family genes in pest-pathogen attack signaling and resistance mechanism within the plant. Another very important hormone is the ABA which is also called as the stress hormone of plant. Presence of ABA responsive elements in upstream sequence of *OsRuvB* family genes suggested that their expression is regulated by ABA hormone in the presence of stress conditions. It is evident from the study of expression profile of *OsRuvB* family genes that these genes showed differential expression in response to various hormonal treatments (ABA and JA) resulting in upregulation of several genes (Fig. 1 A-D). Presence of HREs in *OsRuvB* genes promoters and their positions with number of copies is shown in supplementary figure 1

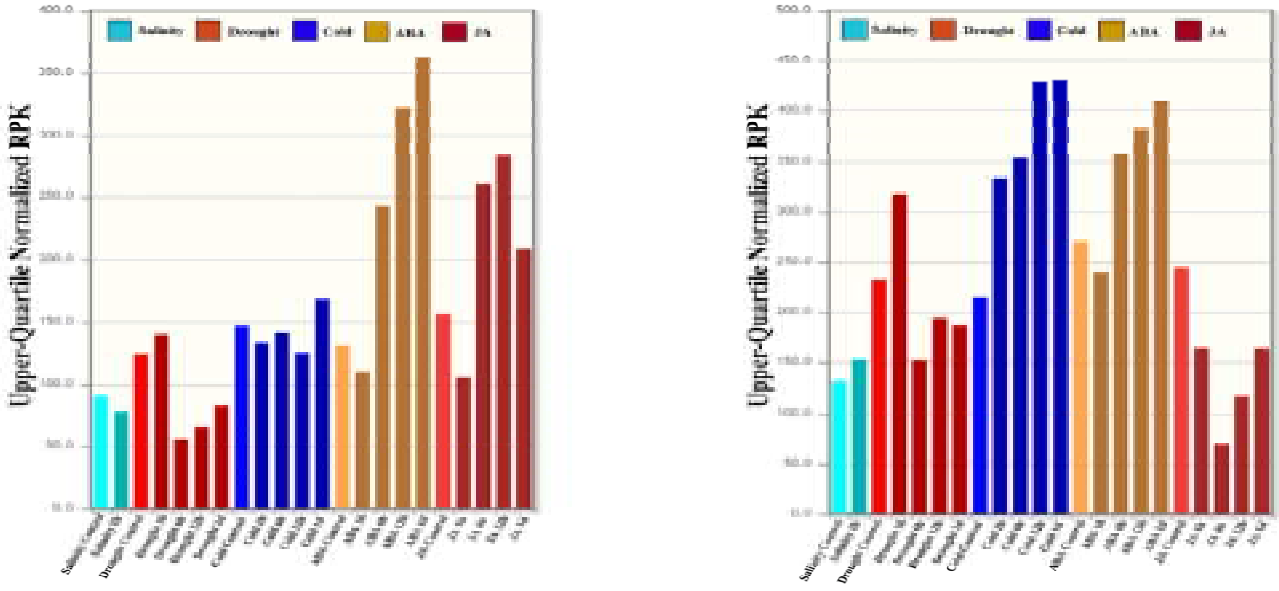

Fig. 1(B)..Expression profile of *OsRuvBL1b* gene in presence of salinity (1h), drought, cold, ABA and JA (1h, 6h, 12h and 1day) in (i) shoot tissue and (ii) root tissue.

(A-D).

Light Responsive cis-regulatory elements in *OsRuvB* family

Light Responsive Elements (LREs) are the elements, which regulate the expression of genes in response to light stimulus. CREs responsible for light stimulus in *OsRuvB* family upstream regions were sp1, CATT, Box 4, AT1, Box1, GATA, chs-Unit1ml, TCCACCT, I-box, MRE, TCCC, AE-box, Box I, ATCC motif and chs-CMA2a. Distribution of these CREs was highly variable and it has been shown in Table 1 and supplementary figure 1 (A-D) for the presence of LREs in respective promoter sequences. Presence of such a wide range

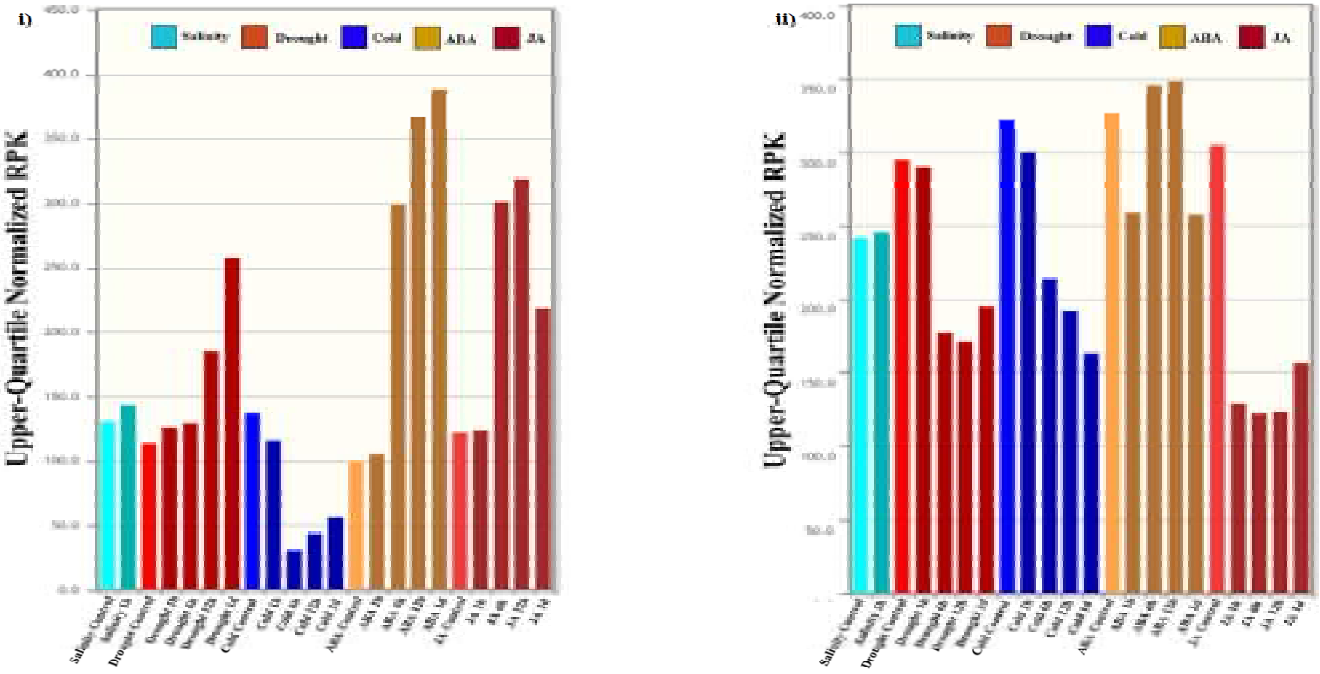

Fig. 1(C). Expression profile of *OsRuvBL2a* gene in presence of salinity (1h), drought, cold, ABA and JA (1h, 6h, 12h and 1day) in (i) shoot tissue and (ii) root tissue.

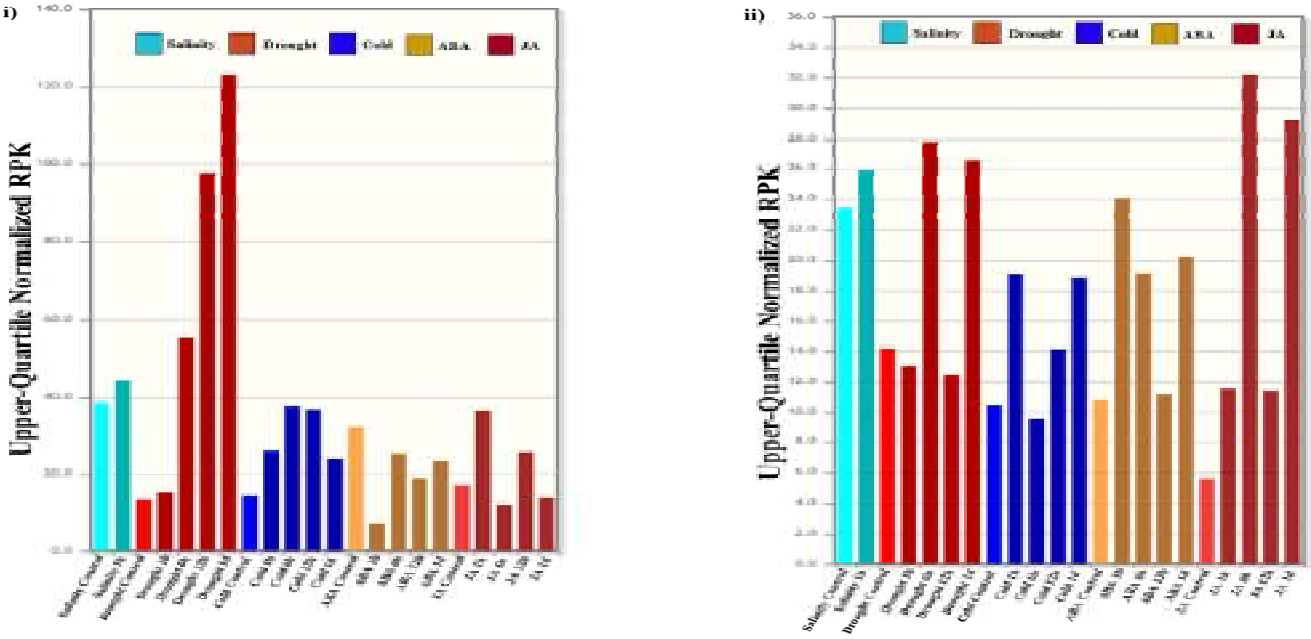

Fig. 1(D). Expression profile of *OsRuvBL2b* gene in presence of salinity (1h), drought, cold, ABA and JA (1h, 6h, 12h and 1day) in (i) shoot tissue and (ii) root tissue.

of LREs in *OsRuvB* family genes suggested the role of these genes in primary metabolic pathways also.

CREs involved in developmental processes

Major CREs involved in developmental processes in plant were SKn-1_motif (required for endosperm expression), CAT (related meristem expression), 5UTR Py-rich stretch (confer high transcription level required in developmental processes) which were present in *OsRuvBL1a*, *OsRuvBL1b* and *OsRuvBL2a* (Table 1 and Fig. 2 A-C). Presence of these development-inducible CREs in *OsRuvB* family genes suggested their critical role in developmental processes in plant development. It has also been observed that gene *OsRuvBL2b* did not have any development specific CRE in its upstream region indicating that it is not involved in normal developmental processes of plant (Fig. 2D).

Abiotic stress responsive CREs in *OsRuvB* family

Major CREs involved in abiotic stress present in *OsRuvB* family were LTR, GC-motif, CCGTCC, HSE and ARE. LTR element which is responsive to low temperature stress was the most common CRE present in all the members of *OsRuvB* family genes upstream region (Table 1). LTR is also responsive in drought like conditions. Expression profile of all the four genes in presence of cold and drought stress showed differential expression in response to the said treatments suggesting the role of LTR element in their regulation of expression. Another CRES GC-motif, CCGTCC and ARE are involved in regulation of expression in response to anoxic and anaerobic condition and present in *OsRuvBL1b*, *OsRuvBL2a* and *OsRuvBL2b*. One of the specific element HSE which is responsive to heat stress was present only in *OsRuvBL2b* suggesting its function in heat stress tolerance in plant.

Biotic stress responsive CREs in *OsRuvB* family

Major biotic stress responsive CREs in *OsRuvB* family were TATCCAT/C (sugar repression responsiveness), TC-rich repeats (defense and stress responsiveness), GCC-box (ethylene responsive factors for PR genes), box S (fungal elicitor responsive element), plant_AP2-like (ethylene responsive element and environmental stress), Box-WI (fungal elicitor responsive element), W-box (WRKY TF binding site for biotic and abiotic stress) and EIRE (elicitor responsive element). These CREs were widely distributed in *OsRuvB* family upstream region as shown in Table 1. It is clearly evident that all the members of this family were induced by biotic stress stimulus, suggesting their role in biotic stress

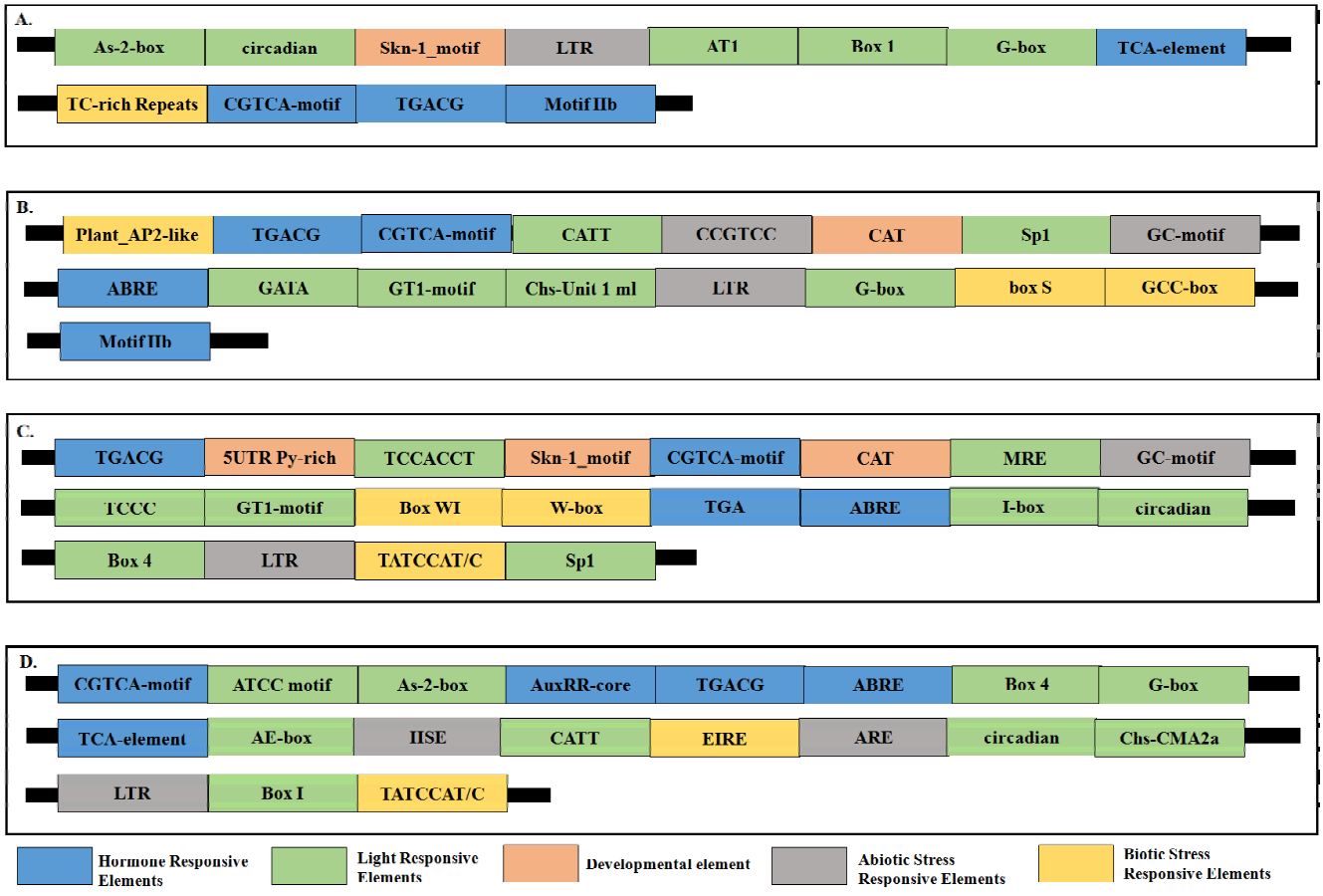

Fig. 2. Diagrammatic representation of cis-regulatory elements presents in *OsRuvB* family promoters involved in hormone response, light response, development, abiotic stress response and biotic stress response. (A) *OsRuvBL1a* gene promoter showing CREs present in promoter region. (B) *OsRuvBL1b* gene promoter showing CREs present in promoter region. (C) *OsRuvBL2a* gene promoter showing CREs present in promoter region. (D) *OsRuvBL2b* gene promoter showing CREs present in promoter region.

resistance in *Oryza sativa* plants.

Real-time analysis for functional validation of stresses in selected promoters

Real-time analysis was performed for the expression study of genes *OsRuvBL1a* and *OsRuvBL2a* for the functional validation of CREs involved in stress responsiveness present in 5' upstream region of these genes. This study showed that transcript level of *OsRuvBL1a* and *OsRuvBL2a* increased under cold, ABA and salicylic acid treatments (Fig. 3 A and B) which correlate with the presence of CREs involved in responsiveness of low temperature, ABA treatment and biotic stress in 5' upstream region of these genes (Fig. 3 C and D). Real-time analysis also showed low transcript level under heat, drought and salinity stresses which is again correlate with the absence of CREs responsible for responsiveness to these stresses in promoter of these genes. This pattern of transcript level under various stress conditions and the presence of CREs involve in their responsiveness validate this *insilico* study of 5' upstream region and functional importance of these predicted CREs in abiotic and biotic stresses.

OsRuvB family of genes is a despised family of genes in rice although it belongs to the AAA+ superfamily which is a major family of cellular ATPases involved in multiple cellular functions and pathways. Homologs of *OsRuvB* have been very well studied in other organisms such as *Saccharomyces cerevisiae*, *Homo sapiens* and Drosophila. In plant system there are very few reports on *RuvBL* genes. One study in

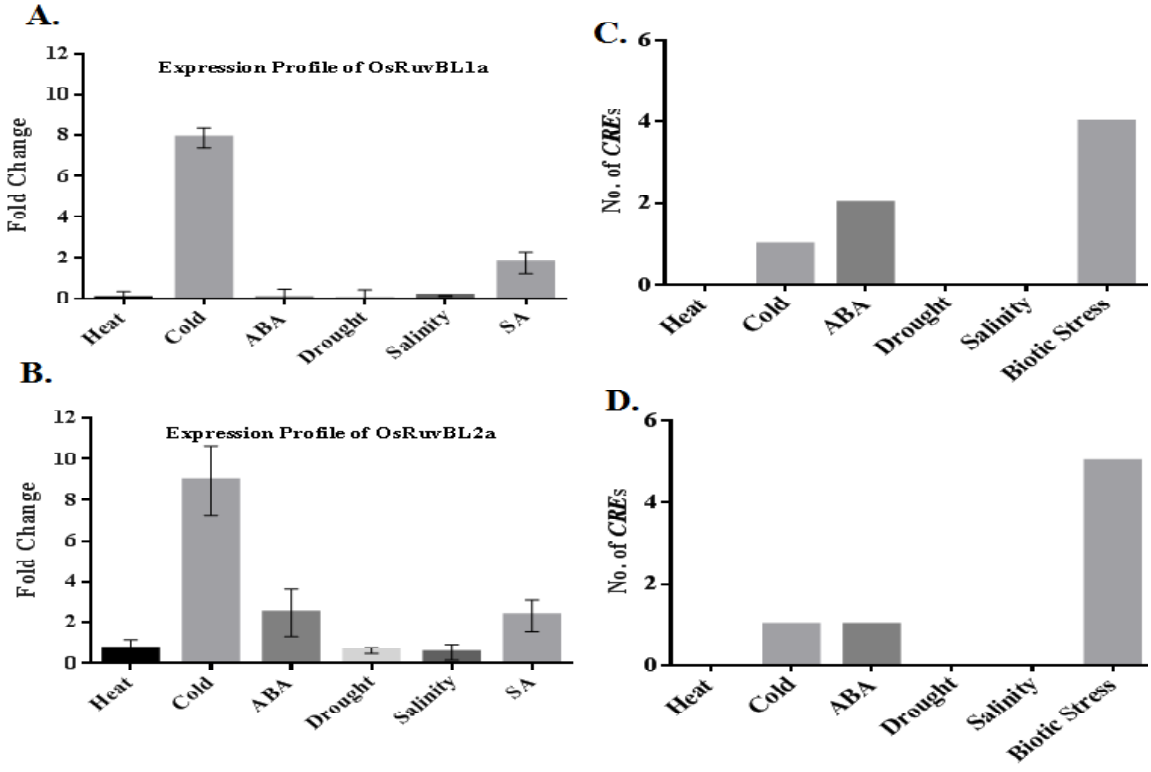

Fig. 3. Functional validation of cis-regulatory elements involved in abiotic and biotic stress responses through real-time analysis. (A) Graph showing fold change in transcript level of *OsRuvBL1a* gene under various stress conditions. (B) Graph showing fold change in transcript level of *OsRuvBL2a* gene under various stress conditions. (C) Graph showing the presence of CREs involved in various stress responses in the promoter region of *OsRuvBL1a* gene. (D) Graph showing the presence of CREs involved in various stress responses in the promoter region of *OsRuvBL2a* gene.

Arabidopsis thaliana showed the involvement of *RuvBL* gene in meristem development and responsive to biotic stress stimulus (Holt et al., 2002). In rice *OsRuvBL2* gene (Wang et al., 2011) showed higher expression in pollen cells, interact with a Calcium Dependent Protein Kinase (OsCPK26). Involvement of *OsCPKs* kinases in biotic and abiotic stress and its interaction with *OsRuvBL2* in downstream pathway suggests the role of *RuvBL2* gene in pollen development (Wang et al. 2011). Gene regulation and expression profile study indicated the importance of such gene families, which can be a point of attraction for plant biologist. This study involves expression profiling, *insilico* prediction and functional validation of CREs in 5' upstream region of *OsRuvB* gene family involved in various stress responses and developmental processes.

Major classes of CREs present in *OsRuvB* family genes are hormonal responsive (HRE), light responsive (LRE), developmental, abiotic stress responsive (ASRE) and biotic stress responsive (BSRE). Methyl jasmonate (MeJA), auxin, ABA and jasmonic acids (JA) are the major hormones which affect the expression of *OsRuvB* family genes. Maximum number of HREs were predicted for MeJA as CGTCA and TGACG. HRE (CGTCA) is present in all the genes of *OsRuvB* family but another HRE (TGACG) is present in all genes except *OsRuvBL2b*. In Nicotiana, MeJA act as an inducer of herbivore resistance (Wu et al., 2008). Microarray expression profiling for *OsRuvB* family genes showed upregulation of genes responsive to JA treatment (Fig. 1 A-D). CREs from corresponding microarray data evidenced the involvement of *OsRuvB* family genes in JA regulated biotic stress signaling. ABA is the important abiotic stress hormone also influencing the expression of genes of *OsRuvB* family by upstream sequences e.g. ABRE in *OsRuvBL1b, OsRuvBL2a* and *OsRuvBL2b* and motif IIb in *OsRuvBL1a* and *OsRuvBL1b*. Microarray expression profiling of all the genes under ABA treatment also highlighted the upregulation in either

shoot/root or in both the tissue types. The next HRE is auxin responsive elements, for auxin TGA are present in *OsRuvBL1b* and *OsRuvBL2a* and AuxRR-core present only in *OsRuvBL2b*. Auxin plays substantial role in growth and development of plant and is a new candidate for biotic and abiotic stress regulation (Ghanashyam and Jain, 2009; Sharma et al., 2015). As both subfamilies of *OsRuvB* (1 and 2) contain auxin responsive elements in their 5' upstream sequence supported the essential role of *OsRuvB* family genes in stress management. Salicylic acid hormone, may give cue to the plant about biotic attack, as the role of SA in induced systemic resistance in plants against the biotic stress is suggested by many groups (Zhang et al., 2002; Saikia et al., 2003). *OsRuvBL1a* gene contain HRE (TCA-element) responsive to salicylic acid (SA) suggest its role in systemic acquired resistance.

Role of light in plant is a well-known concept with light involved in the regulation of chloroplast development (Gray et al., 2003) by the induction of chloroplast specific genes and genes associated with photosynthesis (Kobayashi et al., 2014). Major LREs present in upstream sequence of *OsRuvB* family genes are G-box, circadian, as-2-box, GT1-motif, Sp1, CATT, Box 4, AT1, Box1, GATA, chs-Unit 1 ml, TCCACCT, I-box, MRE, TCCC, AE-box, Box I, ATCC motif and chs-CMA2a. GATA (Argüello-Astorga and Herrera-Estrella, 1998), GT1-motif (Chattopadhyay et al., 1998), G-box (Giuliano et al., 1988) and I-box are light responsive elements and are involved in light-mediated transcriptional activity. In addition to this, GATA and I-box are also involved in light regulated light harvesting complexes and small unit of *RUBISCO* (Grob and Stüber, 1987). It supports the role of light perception in sugar metabolism (Ibraheem et al., 2010). Presence of these LREs in *OsRuvB* family promoter region supported the involvement of these genes in primary metabolic pathway of sugar synthesis via photosynthesis and may also have substantial role in developmental and cellular processes. Circadian is the major LRE present in *OsRuvB* family promoter region which plays key role in light responsiveness and circadian rhythm maintenance of plants. Majority of plants genes involved in photosynthesis, photo protection, cold protection, starch mobilization and photoreception have this LRE in their promoter sequences (Wang et al., 2012; Wang et al., 2012). Presence of circadian LRE in *OsRuvB* family promoter sequences suggested their role in photo protection.

OsRuvB family genes promoter sequences consist following CREs: SKn-1_motif, CAT and 5UTR Py-rich stretch (Rahman and Samian, 2014) which are involved in seed specific expression of proteins, meristem specific expression and other development processes, respectively. Skn-1_motif is recognized by Storage Protein Activator transcription factor (SPA) which is involved in the storage protein in seed stage, and was first identified in *Caenorhabditis elegans* (Juhász et al., 2011). CAT is the element involved in meristem specific expression of gene (Ibraheem et al., 2010). Earlier study in Arabidopsis *RuvB* homolog gene showed necessity of its expression for the maintenance of shoot meristem (Holt et al., 2002). Presence of CAT meristem responsive element in *OsRuvB* family genes promoter suggested their role in maintenance of meristem by regulating the cell cycle progression.

CREs responsive to multiple abiotic stress stimuli are present in *OsRuvB* family genes promoter sequences. Major CREs are LTR, GC-motif, CCGTCC, HSE and ARE. LTR, ABRE and W-box are cold and drought stress responsive elements (Ciolkowski et al., 2008; You et al., 2015; Liu et al., 2016) and their presence in promoter region of *OsRuvB* family genes suggested the role of these genes in stress response mechanism in plants. Other abiotic stress responsive elements such as HSE highly specific elements involved in heat stress response (Guha, 2002) and ARE antioxidant responsive element (Nguyen et al., 2003) is involved in sensing the ROS and antioxidant fluctuations in cell. Presence of these response elements in *OsRuvB* family promoters regulate their expression under various environmental stresses as is evident from the microarray expression profile of these genes in shoot and root tissues for various stress treatments (Fig. 1 A-D). Promoter regions of *OsRuvB* family genes also contain a wide range of biotic stress responsive elements such as TATCCAT/C involved in sugar repression responsiveness (Toyofuku et al., 1998; Gupta and Kaur 2005), TC-ric; repeats involved in defense (You et al., 2015; Wang et al., 2016), GCC-box (Fujimoto, 2000; Brown, 2003) and plant_AP2-like involved in ethylene response for pathogen resistance (PR) genes and other environmental stimuli. Box-WI and box S are involved in fungal elicitor responses (Rushton, 2002) and W-box is the binding site for WRKY TF which was reported to play role in biotic and abiotic stress conditions

(Ciolkowski et al. 2008; Hernandez-Garcia and Finer, 2014). Studies carried out in Arabidopsis showed the involvement of *RuvB* genes in pathogen response in combination with R-genes (Holt et al., 2002). In addition to *insilico* prediction of CREs present in promoter region, this study also focused on functional validation of these CREs to narrow down the search of genes provide abiotic and biotic tolerance to the plants. The correlation of microarray expression profiling and real-time analysis with the presence and absence of related CREs strengthen the credibility and prediction of *insilico* identification of cis-acting regulatory elements (Passricha et al., 2016). Presence of all these major environmental stress responsive elements in promoter region of *OsRuvB* family genes in addition to the microarray study for expression profiling of these genes suggested the importance of these genes in stress tolerance mechanism in plants and a detailed study is required on this family to extract as much information and benefit from this family in stress management and crop improvement as possible. Rice is a model crop and still a wide range of genes are not yet characterized. In this era of global climatic change, we are in need of such genes which provide biotic and abiotic stress tolerance to plants. This study helps in narrowing down the probable target genes which can be used for the agricultural crop improvement.

CONCLUSION

Regulation of gene expression is the key concept of differentiation in multicellular organisms and there are multiple regulation processes to maintain a homeostasis in higher organism. Transcriptional regulation at the promoter level is regulated by cis-regulatory elements (CREs), the binding sites for specific transcription factors to initiate the transcription process. Study of CREs in promoter sequences of genes of *OsRuvB* family gave a detailed insight of various mechanisms which involve *OsRuvB* family members. Study of these different classes of CREs and expression profile of *OsRuvB* family genes strongly supports the role of these genes in environmental stress tolerance (biotic and abiotic) and also in various primary metabolic and developmental process in rice. Further research in this area is needed which may help in deciphering the exact pathways followed by these genes for crop improvement and stress management in rice.

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