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Identification and study of cis regulatory elements and phylogenetic relationship of *TaSRG* and other salt response genes

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Abstract
Salt stress is the major limiting factor in agriculture that affects on all developmental stages including germination, seedling and vegetative stages. Under salt stress, induce the expression of many plant genes. These genes encode the defending proteins, and regulatory proteins that regulate the stress signal transduction. The TFs interact with cis-elements in the promoter regions of several stress-related genes and thus up-regulate the expression of many downstream genes resulting in imparting abiotic stress tolerance. The identification of cis-regulatory DNA elements responsive to stress is important for studying gene regulation and for biotechnological applications. In this study, we identified cis-regulatory elements and also investigated the phylogenetic relationship of salt response genes of wheat, rice, barley, maize and Arabidopsis. The result of phylogenetic tree showed that TaSRG, LTP and IPK2 genes have located in one branch. The analysis of 5´UTR of salt response genes showed four elements have the most frequencies that including, TATA Box (24.55%), Unnamed-4 (24.55%), CAAT Box (17.43%) and SP1(5.33%), respectively. The TaSRG gene also is a cis elements-rich gene in which TATA Box (5'-TATAAA-3') and CAAT (5'-CAAT-3') Box have most frequencies. The most important identified cis regulatory elements in response to stress are ABRE, G-box, MBS, TGA-element. This results indicated that gene expression control in different checkpoints of cell cycle may be dependent to both the frequency of enhancers and stress responsive cis regulatory elements under abnormal conditions.

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Introduction

Salt stress is the major limiting factor in agriculture and the adverse effect of salt stress is expressed on whole plant level, and appears during all developmental stages including germination, seedling and vegetative stages (chandna *et al.*, 2013). Under salt stress, induce the expression of many plant genes. These gene products can largely be classified into two groups: First group comprises of genes that encode for proteins that defend the cells. Another group of genes stimulated by abiotic stresses includes regulatory proteins that further regulate the stress signal transduction and alter gene expression and hence possibly function in stress response (Agarwal and Jha, 2010). They comprise several transcription factors (TFs) emphasizing the role of various transcriptional regulatory mechanisms in the stress signal transduction pathways, implicated in the regulation of stress signaling and gene expression (Lata *et al*, 2011). The TFs interact with cis-elements in the promoter regions of several stress-related genes and thus up-regulate the expression of many downstream genes resulting in imparting abiotic stress tolerance (Agarwal and Jha,. 2010). Many stress-associated cis-acting regulatory elements that activate transcription in response to salinity, drought, and wounding and pathogen infection have been identified in plants (Ibraheem *et al*, 2010). The short nucleotide motif for this modular code is called the cis-element, which is recognized by factors for transcriptional activation or suppression (Won *et al*, 2009). Cis-regulatory sequences, such as enhancers and promoters, control development and physiology by regulating gene expression (Wittkopp and Kalay, 2012). The identification of cis-regulatory DNA elements responsive to stress is important for studying gene regulation and for biotechnological applications (Agarwal and Jha, 2010).

Based on this finding, the aim of this study is identification cis-regulatory elements and also investigation of phylogenetic relationship of salt response genes of wheat (*Triticum aestivum*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), maize (*Zea mays*) and Arabidopsis (*Arabidopsis thaliana*) as useful information for the prediction of expression and regulation of these genes.

Material and method

The salt response genes in wheat, rice, barley and arabidopsis were identified by scientific resources review. The coding domain sequence (CDS) and 5´-UTR region of this gene were obtained from National Center for Biotechnology Information (NCBI) internet database site (http://www.ncbi.nih.nim.gov). The name of genes, accession number, number of base pairs, CDS region, 5´UTR before ATG were recorded and listed in Table 1. The phylogenetic tree was performed by Molecular Evolutionary Genetics Analysis software (MEGA; version 5) from aligned coding domain sequences. The neighbor-Joining (NJ) method was used to designing the phylogenetic tree. The regulatory region of each salt response gene in Wheat, Rice, Barley, maize and Arabidopsis were scanned for the presence of cis-acting regulatory elements identical with or similar to the motifs registered in Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/cgi-bin/CallMatIE55.htpl) and PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html) .

Result and discussion

The result of phylogenetic tree showed that *TaSRG*, *LTP* and *IPK2* genes have located in one branch. The *LEA*, *MYB2*, *HAK1* and *KUP1* genes have close relationship with TaSRG gene (Fig. 1). *TaSRG* gene is a transcription factor that increase salt tolerance and might encode a transcription factor that could be utilised for enhancing stress tolerance in a wide range of plants (HE *et al.*, 2011). The *LTP* and *IPK2* genes also play important role in response to salt stress and abiotic stress. The analysis of 5´UTR of salt response genes have been showed in Fig. 2. The analysis of 5´UTR identified important cis elements. The *TaSRG* gene is the cis elements-rich gene in which TATA *Box* (5'-TATAAA-3') and CAAT (5'-CAAT-3')Box have most frequencies. The both of them have

fundamental role in initiation of transcription process and are found in all the promoter regions of genes (Sadegh Nejad *et al.*, 2013). The location of them which also are closest to the translational start site (ATG) were found to be very close to one another (Ibraheem, *et al.*, 2010).
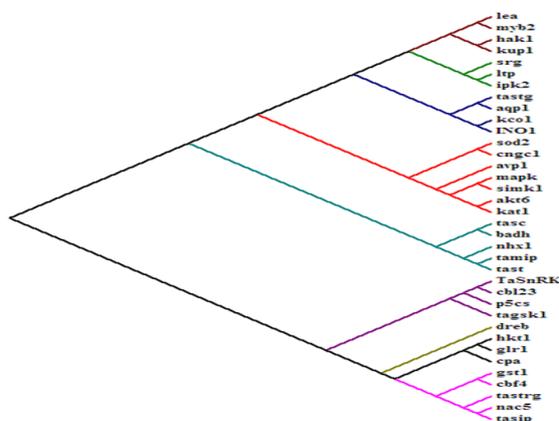
Table 1. The salt response genes with their characteristics.

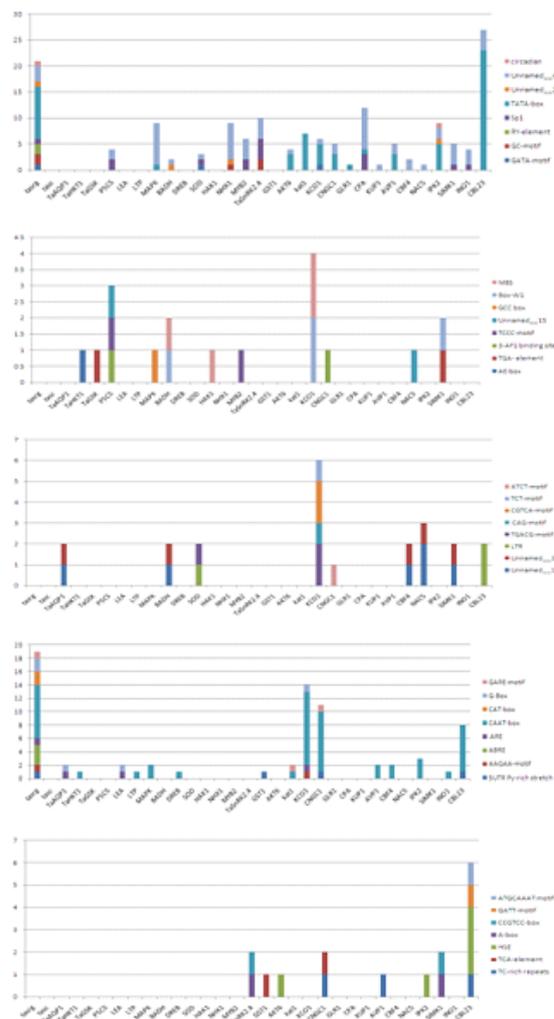| Plant	Genes	Accession Number	Full Length of Gene	CDS region	Length of 5´ UTR
wheat	TaSRG	FJ432082	1797	913-1797	912
wheat	TaSC	AY956330	579	23-488	22
wheat	TaSTG	EF415486	681	1-681	none
wheat	TaAQP1	DQ867075	1227	98-964	97
wheat	TaHKT1	TAU16709	1989	37-1638	36
wheat	TaGSK	AF525086	1573	79-1224	78
wheat	TaMIP	DQ530420	930	18-920	17
wheat	TaST	DQ672341	546	20-457	19
wheat	P5CS	AB193551	2405	83-2233	82
wheat	LEA	X56882	934	25-699	24
wheat	LTP	DQ286561	578	63-410	62
wheat	MAPK	AY881103	2153	85-1821	84
wheat	BADH	AY050316	1726	56-1567	55
wheat	DREB	JQ004969	1286	64-264	63
wheat	SOD2	FJ890987	1058	79-717	78
wheat	HAK1	JF495466	2405	46-2376	45
wheat	NHX1	AY040245	2017	158-1798	157
wheat	MYB2	AY615199	1044	68-901	67
wheat	TaSnRK2.4	GQ384359	1355	77-1168	76
wheat	GST1	AF184059	914	62-700	61
wheat	TaSTRG	EF599631	879	1-879	none
wheat	TaSIP	HM205107	888	1-888	none
arabidopsis	AKT6	NM_128118	3057	118-2784	117
arabidopsis	KAT1	NM_123993	2289	170-2203	169
barley	KCO1	EU007824	1674	437-1590	436
arabidopsis	CNGC1	Y16327	2547	305-2455	304
arabidopsis	GLR1	AY234854	1766	45-1613	44
oryza sativa	CPA	NM_001053568	1391	122-1027	121
oryza sativa	KUP1	AY324878	2655	53-2458	52
arabidopsis	AVP1	NM_001084073	2602	126-2054	125
barley	CBF4	AY785851	873	68-745	67
rice	NAC5	AB028184	1751	106-1095	105
arabidopsis	IPK2	NM_125570	1201	112-1014	111
maize	SIMK1	AY433815	1636	163-1248	162
maize	INO1	AF056326	1665	86-1618	85
arabidopsis	CBL23	NM_102766	2235	414-1862	413

The functions of some of important cis-regulatory elements which are involved in stresses are described: ABRE: cis-acting element (TACGGTC) involved in the ABA responsiveness and drought tolerance, MBS: MYB binding site related to ABA signaling. The recognition sequence is YAACKG (Y = C/T; K = G/T), G-box: G-box cis-element (CACGTG) exists in many gene promoters and has critical effects on plant development, hormone responses, and fungal infections. GARE-motif: This sequence was identified as a gibberellin (GA) response element (GARE).The GARE motif in turn is necessary for gibberellin induction of LEAFY to promote flowering thus hinting at a role of GARE as a factor in GA-regulated signaling pathways that depend on cGMP as second messenger, and TGA-element: The identification of

putative auxin response elements is based mainly on conservation of similar sequence elements found in a variety of genes induced by auxin. Most of these putative auxin response elements have not been tested for functionality, and their direct role in response to auxin remains to be demonstrated. Also, the cis elements have various function including light responsive element, circadian, hormone, specific process and expression regulation. Among cis acting elements of different groups, GAG-motif, GT1-motif, G-Box, Box I and Box 4 involved in different wavelengths of light responsiveness. The circadian element (CIRC) and Skn1_motif which are essential for the control circadian rhythm and endosperm expression, respectively. The upstream sequences of these genes also have cis-regulatory elements that they respond todefence and stress responsiveness (TC-rich- repeats), heat stress responsive element (HSE) and anaerobic induction (ARE). Data obtained from promoter regions for all salt response genes introduced four elements as the most frequency elements in between elements including, TATA Box (24.55%), Unnamed-4 (24.55%), CAAT Box (17.43%) and SP1(5.33%), respectively. The promoter is the sequence where all transcription factors and RNA polymerases can bind and regulate the expression of the gene. Transcription factors will recognize the specific DNA sequence, which is known as cis-regulatory elements and regulate the gene expression in different conditions (Tajrishi and Tuteja, 2011).

Fig. 2. cis-acting regulatory elements identified in the 5′ regulatory sequences of salt stress resistance genes.

The power of bioinformatics and the availability of whole genome sequence has enabled a comprehensive description of transcription factor families in plants. In addition to the core binding sites that seem to be associated with transcription factor families, there may a degree of subtlety in the cis-regulatory elements necessary for transcription factors to facilitate their unique regulatory effects. These gene-specific cis-regulatory regions may function through the recruitment of TF combinations or through a DNA motif consensus that is difficult to determine using conventional methods (Dare *et al.*, 2008).This data indicated that gene expression control in different checkpoints of cell cycle may be dependent to both the frequency of enhancers and stress

Fig. 1. The phylogenetic tree using the CLUSTAL-W (MEGA 5) program. The Neighbor-Joining (NJ) method was used to construct the tree.

responsive cis regulatory elements under abnormal conditions (Sadegh Nejad *et al.*, 2013). cis-Elements play a central role in gene regulation by integrating signals at the DNA level upstream of a target gene (Priest *et al.*, 2009). considering transcription factors as candidate genes in crop improvement programs will give us a clear understanding of abiotic stress related signal transduction events and will eventually lead us to develop crop varieties with superior stress tolerance (Lata et al., 2011).

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