Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in rice, soybean and Arabidopsis

Low temperature and dehydration affect plant growth and productivity. Many genes respond to both stressors at the transcriptional level, and their gene products function in terms of stress tolerance and response. These genes include key metabolic enzymes, late embryogenesis-abundant (LEA) proteins, detoxification enzymes, chaperones, protein kinases, and transcription factors. The cis-acting elements that function in stress-responsive gene expression have been analyzed to elucidate the molecular mechanisms of gene expression in response to these stresses. The dehydration-responsive element (DRE) containing the core sequence A/GCCGAC is a cis-acting element that regulates cold- and dehydration-responsive gene expression in Arabidopsis (*Arabidopsis thaliana*). The abscisic acid (ABA)-responsive element (ABRE) containing the core sequence ACGTGG/T is a cis-acting element that regulates dehydration- and high salinity-responsive gene expression in Arabidopsis and rice (*Oryza sativa*). ABA-responsive gene expression requires multiple ABREs or an ABRE with a coupling element as a functional promoter.

In this study, oligo microarrays were used to identify cold- and dehydration-responsive genes in rice, soybean and Arabidopsis. The observed frequencies of all ($4^6 = 4096$) hexamer sequences in cold- and dehydration-inducible promoters were compared with standardized promoters to estimate conserved sequences and to determine representative cold- and dehydration-responsive transcriptional pathways in rice, soybean and Arabidopsis.

Microarray analyses were performed using the three species (rice, soybean and Arabidopsis) and the characteristics of identified cold- and dehydration-responsible genes were compared. Transcription profiles of the cold- and dehydration-responsive genes were similar among these three species, showing representative up-regulated (dehydrin/LEA) and down-regulated (photosynthesis-related) genes. All $(4^6 = 4096)$ hexamer sequences in the promoters of the three species were investigated, revealing the frequency of conserved sequences in cold- and dehydration-inducible promoters. A core sequence of the ABRE was the most conserved in dehydration-inducible promoters of all three species, suggesting that transcriptional regulation for dehydration-inducible genes is similar among these three species with the ABRE-dependent transcriptional pathway. In contrast, the highly conserved sequences in cold-inducible promoters of Arabidopsis are different from those of rice and soybean. DRE is the most conserved sequence in cold-inducible promoters of Arabidopsis, but not in those of rice and soybean. The novel sequence and ABRE are the most conserved sequences in cold-inducible promoters of rice and soybean, respectively. In cold-inducible promoters, the conserved hexamer sequences were diversified among these three species, suggesting the existence of diverse transcriptional regulatory pathways for cold-inducible genes among the species.

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	Rice				Soybean				Arabidop			\$
Class	up		down		up		down		up		down	
	С	D	С	D	С	D	С	D	С	D	С	D
Carbohydrate Metabolism	-		-		-	-	-	_	-	_	-	_
Energy Metabolism												
Lipid Metabolism												
Nucleotide Metabolism												
Amino Acid Metabolism												
Metabolism of Other Amino Acids												
Glycan Biosynthesis and Metabolism												
Metabolism of Cofactors and Vitamins												
Metabolism of Terpenoids and Polyketides												
Biosynthesis of Other Secondary Metabolites												
Xenobiotics Biodegradation and Metabolism												
Biosynthesis of Plant Hormones												
Photosynthesis												
Signal Transduction												
Transcription												
Translation												
Epigenetics												
Molecular Chaperone												
DNA Replication Repair												
Helicase												
Ribonuclease												
Ubigutination												
Protease and Peptidase												
Transport												
Detoxification Enzyme												
Dehydrin and LEA												
Others												

Figure 1. Molecular function of cold- and dehydration-responsive genes in rice, soybean and Arabidopsis. Heat maps of 27 up-regulated (red) or down-regulated (blue) molecular function classes indicating the frequency (%) in cold-treated (C) and dehydration-treated (D) plants.



Figure 2. Scatter plots showing Z-scores (y axes) for the observed frequencies of all hexamer sequences (x axes) in cold-inducible (A) and dehydration-inducible (B) promoters in rice, soybean and Arabidopsis compared to standardized promoters. The highly conserved sequences of DRE (blue), ABRE (red), EE (green), G box (purple), T/G box (light blue), CE3 (light green), and novel sequences (black) are shown.