

# A study on the regulatory network with promoter analysis for *Arabidopsis DREB*-genes

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## Abstract:

Dehydration response element binding factors (*DREBs*) are one of the principal plant transcription factor subfamilies that regulate the expression of many abiotic stress-inducible genes. This sub-family belongs to AP2 transcription factor family and plays a considerable role in improving abiotic stresses tolerance in plants. Therefore, it is of interest to identify critical cis-acting elements involved in abiotic stress responses. In this study, we survey promoter cis-elements for *ATDREBs* genes (*Arabidopsis thaliana* *DREBs*). Regulatory networks based on *ATDREB* candidate genes were also generated to find other genes that are functionally similar to *DREBs*. The study was conducted on all 20 *Arabidopsis thaliana* non redundant *DREB* genes stored in RefSeq database. Promoter analysis and regulatory network prediction was accomplished by use of Plant CARE program and GeneMANIA web tool, respectively. The results indicated that among all genes, *DREB1A*, *DREB1C*, *DREB2C*, *DREB2G* and *DEAR3* have the most type of diverse motifs involved in abiotic stress responses. It is implied that co-operation of abscisic acid, ethylene, salicylic acid and methyl jasmonate signaling is crucial for the regulation of the expression of drought and cold responses through *DREB* transcription factors. Gene network analysis showed different co-expressed but functionally similar genes that had physical and functional interactions with candidate *DREB* genes.

**Keywords:** Abiotic stress, *Arabidopsis*, *DREB*, Transcription factor, Promoter analysis, Gene network

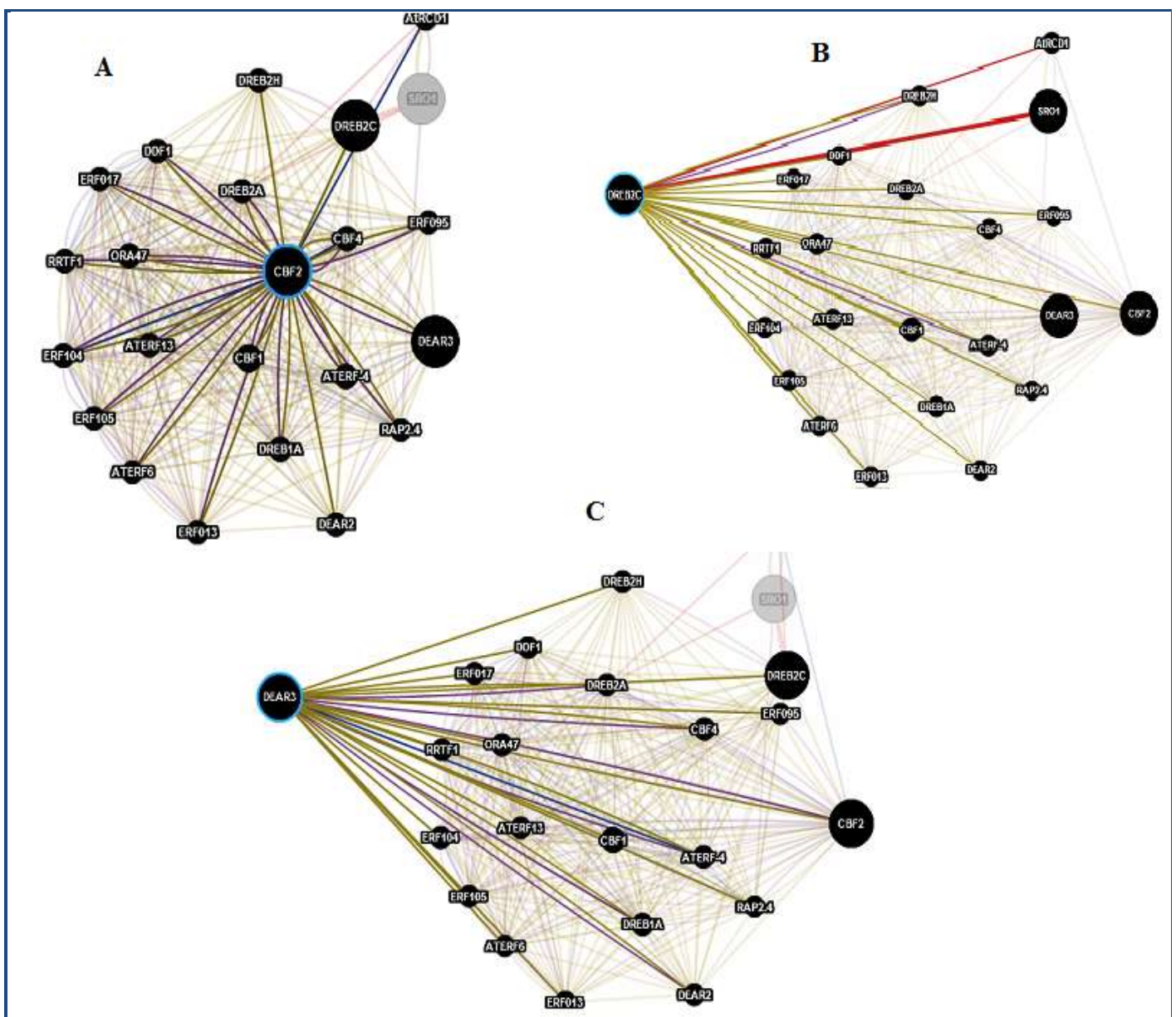
## Background:

Transcription factors interact with cis-acting elements present in the promoter region of various stress-responsive genes and thus, activate cascades or a whole network of genes to enhance tolerance towards multiple stresses at a time [1]. The AP2/ERF family contains unique plant transcription factor subfamilies with a principle role in biotic and abiotic stress responses. Dehydration responsive element binding factors, also known as *DREB* transcription factors, are members of the AP2/ERF family which consist of many important regulatory and stress responding genes [2]. *DREBs*, which are identified in different plant species, induce the expression of functional target genes involved in abiotic stresses. The *Arabidopsis* genome contains different *DREB* genes. Until 2002, it was thought that *Arabidopsis* had 14 *DREB* genes categorized in two different

*DREB1* and *DREB2* subclasses [3]. Since then, other *Arabidopsis DREB* genes such as *DREB3*, *DREB4* and *DREB5* were isolated and studied. In general, it is known that *DREB1* related genes are responsible for cold stress responses, while *DREB2s* regulate genes in drought and salinity signaling pathways [4]. In fact, regulatory network studies show that many of these signal pathways are overlapped [5, 6]. A large regulatory portion of plant genes is located primarily in the 1000 bp upstream the transcriptional start site generally referred to as the gene promoter region [7]. This region consists of specific cis-acting regulatory elements recognized by other transcription factors to regulate gene expression. Cis-acting regulatory elements are specific short DNA sequence motifs of approximately 5-25 bp [8]. Therefore, understanding the cis-acting regulatory region bound by TFs can offer essential

information regarding transcriptional regulatory systems [9]. Moreover, studying *DREB* transcription factor promoters can reveal useful information about genes and signaling networks involved in abiotic stress responses. Web-based databases such as PlantCARE, PLACE, PlantPAN, Softberry and Genomatix Matinspector facilitate the search for promoter motifs and their identification. These databases consist of plant cis-acting regulatory elements and provide access tools for *in silico* analyses of regulatory sequences. Abiotic stresses regulatory networks consist of different regulatory and functional genes that cooperate to induce stress tolerance. *DREBs* have been acknowledged as key regulators in these networks and stimulate other inducible genes. One of the promising ways to identify and functionally analyze genes that cooperate with *DREBs* is to study gene regulatory networks. Recently, many studies for elucidation and annotation of gene functions have

been carried out by use of *in silico* methods. Fortunately, the past decade has seen a revolution in omics technologies that have generated abundant amounts of useful data for *in silico* function predictions [10]. Among the limited plant gene network databases, Genemania is of the most reliable network analyzers. GeneMANIA, is a flexible, user-friendly web interface used for generating hypotheses about gene functions, analyzing gene lists and prioritizing genes for functional assays [11]. Networks are produced from co-expression data from Gene Expression Omnibus (GEO), physical and genetic interactions from BioGRID, and pathways data. This research has focused on *in silico* analyses of *Arabidopsis DREBs* promoters in order to determine chief cis-acting regulatory elements relative to abiotic stress responses. In addition, regulatory network analysis of *At-DREB* genes was carried out to identify *DREB* participant genes in abiotic stresses tolerance.



**Figure 1:** Gene networks predicted by GeneMANIA web tool for *DREB1C* (A), *DREB2D* (B), and *DEAR3* (C). Black nodes show *DREB1C*, *DREB2D* and *DEAR3*. The gray nodes show different Genes, co-expressed, physical interacted and shared protein domains with *DREB1C*, *DREB2C* and *DEAR3* respectively.

## Methodology:

### Data collection

All complete mRNA coding sequences of *Arabidopsis thaliana* *DREB* genes were collected from the RefSeq database of NCBI (<http://www.ncbi.nlm.nih.gov>). In order to recognize the upstream promoter region, nucleotide sequences of 1.5 Kbp extending 5' from the genes' translation start site were identified (<http://www.phytozome.net>).

### Tools used for promoter analysis and regulatory network study

1.5 Kbp of the 5' upstream promoter region of each gene was scanned for the presence of cis-acting regulatory elements involved in abiotic stress signaling pathways based on the Plant CARE program ([www.plantcare.com/encyclopedia](http://www.plantcare.com/encyclopedia)). A number of *DREB* genes were nominated for the network study based on containing the most varied motifs involved in abiotic stress tolerance. In order to depict *DREB* regulatory networks and analyze co-expressed genes, the GeneMANIA (<http://www.genemania.org>) web tool was used. Based on this database, an analysis of genes interacting with *DREB* genes and gene function predictions was carried out.

## Results & Discussion:

Accession numbers and definitions of all 20 *Arabidopsis thaliana* *DREB* genes stored in RefSeq database are listed in **Table 1 (see supplementary material)**. According to their definition, all *DREBs* were categorized as *DREB1*, *DREB2*, *DREB3*, *DREB4* and *DREB5* groups for succeeding studies. Promoter analysis was carried out to identify chief cis-acting elements involved in abiotic stress responses. Different functional cis-acting regulatory elements associated with abiotic stress responses were identified in *DREBs* upstream region. These functional motifs are presented in **Table 2 (see supplementary material)**. Nine types of significant motif related to abiotic stress were found in. These cis-acting elements included ABRE, (CGTCA) MeJA response, MBS, HSE, TC rich, (TGACG) MeJA response, TCA, LTR and ERE motifs. The promoter region of *DREB* genes showed variety in motif types. All studied genes contained the ABRE motif in their promoters. ABREs (ABA response elements) showed perception of ABA-mediated osmotic stress signals. The ABA-responsive element (ABRE; PyACGTG/TC) is a well-studied cis-element involved in ABA-induced gene expressions [12, 13]. ABRE-binding Protein/factors (AREB/ABF) have positive effects on the osmotic stress tolerance of plants [14-16]. Although *DREB* genes are assumed to work in ABA-independent signal transduction pathways, presence of ABREs in the promoters of *DREB* genes indicated a role for ABA-signaling in the regulation of *DREB* gene expressions.

Another important stress-signaling observed motif is methyl jasmonate (MeJA), which activates plant defense mechanisms in response to environmental stresses such as drought, low temperature, and salinity [17]. This motif was distributed in the regulatory region of all *DREB* gene promoters except for *DREB2E* and *DREB2F*. *DREB2G* and *DREB2D* possess ERE motif. Having an important role in stress conditions, ERE, or ethylene responsive element, is a conserved motif bind to ethylene-responsive factor (ERF) [18]. In fact, it seems that an integration of abscisic acid, ethylene and methyl jasmonate signaling is crucial for regulating expressions of drought and cold responses through *DREB* transcription factors.

Two temperature associated motifs, HSE and LTR, were distributed in different *DREBs*. HSE motifs that bind to heat shock factors (HSFs) are responsible for heat stress tolerance. HSEs have been found to be consistently conserved in the regulatory regions of many heat induced genes [19]. The low temperature responsive element (LTR) is important for the induction of cold regulated genes [20, 21]. Our results indicate that *DREB2* group members possess LTR cis-acting elements in their promoter regions. Thus, from the theoretical point of view, *DREB2* genes can be expressed in cold temperatures. This result is, however, in contrast with previous studies [4] which demonstrated that *DREB2s* had a role in drought and salinity signaling pathways but not cold stress. Nevertheless, to support this finding, more empirical evidence is required. Some *DREB1* and *DREB2* members were found to have an HSE functional motif. Therefore, it can be concluded that both groups can participate in heat stress tolerance. Moreover, the results showed that MBS motifs are located in the promoters. MBS cis-elements have been previously reported to be required for the binding of MYB transcriptional factors required for drought inducible gene expressions [22]. TCA is another motif found in *DREBs* involved in salicylic acid responsiveness. Evidence suggests that salicylic acid plays a significant role in responses to abiotic stress effects such as low and high temperatures. The TC rich motif, present in the studied genes, is a cis-acting, stress responsive element involved in defense. Due to the diversity of cis-acting elements involved in abiotic stress tolerance, five *DREB* genes included *DREB1A*, *DREB1C*, *DREB2C*, *DREB2G* and *DREB3* were selected as candidate genes containing the most diverse motif types related to abiotic stresses. They all have seven different types of Cis element. It can be suggested that these five genes are the most involved transcription factors in abiotic stresses which can be expressed in almost all different signaling pathways related to stresses. Gene network depicting and analyses were hence carried out on these genes. Since the type of cis-acting elements in *DREB1A* and *DREB1C*, *DREB2C* and *DREB2G* were identical, only one was selected. *DREB1C*, *DREB2C* and *DREB3* were selected for gene network studies using GeneMANIA. Network prediction results using GeneMANIA for *DREB1C*, *DREB2C* and *DEAR3* are shown in **(Figure 1)**. The results of co-expressed genes with each of the candidate *DREBs* are presented in **Table 3 (see supplementary material)**. According to physical interaction predictions, only *DREB1C* had physical interactions with *ATRCD1*, *SRO1* genes **(Table 3)**. These two genes are important regulators in stress, hormonal and developmental responses in *Arabidopsis thaliana* [23]. From all input parameters such as shared protein domains, co-expression and physical interactions, 13 distinct functional groups were predicted. All genes in the network were classified according to their function in different groups. The results show that 7 groups including ethylene-activated signaling pathway, response to water, cold acclimation, heat acclimation, response to water deprivation, response to cold and response to heat were involved in abiotic stress responses. Each gene belongs to one or more group(s) **Table 4 (see supplementary material)**. The network contained 23 nodes, involving 8 known *DREB* genes. All functional groups of the genes are shown in **(Table 4)**. The network analysis showed that other *Arabidopsis* *DREB* genes had interactions with the selected *DREB* genes. *DREB1A*, *CBF4*, *DREB2H*, *DEAR2* and *DREB2A* were found to be types of other dehydration responsive element transcription factors that either



had similar domains or co-expressed with *DREB1C*, *DREB2C* and *DEAR3* genes. Therefore, It can be suggested that different *DREB* genes cooperate in abiotic stress responses. The results demonstrate the utility of web based tools for discovering potential cross-talk and interaction between *DREB* genes in abiotic tolerance pathways.

## Conclusion:

This study focused on AtDREB genes and their non-coding regulatory regions by computational analysis. Comparisons among regulatory regions of different AtDREBs accomplished and a number of putative motifs at the 5' regulatory regions were identified. The diversity found among Cis-acting elements in genes implied their different roles in signaling pathways. Most of these transcription factors (ATDREBs) show remarkable activity overlapping that confirmed with regulatory network analysis in this study. Both promoter analysis and genes network study of AtDREBs provide comprehensive information about many other genes which induce the expression of AtDREB genes or have functional collaborations with AtDREBs. In fact, the knowledge of cis-acting regulatory elements make a better understanding of abiotic stresses signaling pathways, in which AtDREB transcription factors are involved. However, since the predicted cis-acting regulatory elements may not precisely correlate with experimental expression data, experimental studies are crucial in order to confirm computational analysis results.

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## Supplementary material:

**Table 1:** Accession numbers and definition of all 20 *Arabidopsis DREB* genes stored in RefSeq database

Accession number (RefSeq)	Definition
NM_106369.1	<i>DREB4A</i>
NM_105820.3	<i>DREB5A</i>
NM_127898.4	<i>DREB3</i>
NM_111939.2	<i>DREB2B</i>
NM_118680.1	<i>DREB1A</i>
NM_118679.1	<i>DREB1C</i>
NM_115620.1	<i>DREB2F</i>
NM_104981.3	<i>DDF2</i>
NM_101133.2	<i>DREB4A</i>
NM_101131.3	<i>DREB1F</i>
NM_001084295.1	<i>DDF2</i>
NM_129595.2	<i>DREB2H</i>
NM_106202.2	<i>DREB2D</i>
NM_129594.2	<i>DREB2C</i>
NM_129390.2	<i>DREB2E</i>
NM_121197.2	<i>DREB3</i>
NM_001036760.1	<i>DREB2A</i>
NM_120623.2	<i>DREB2A</i>
NM_124578.1	<i>DREB1D</i>
NM_121850.1	<i>DREB2G</i>

**Table 2:** Potential abiotic stress responsive cis-acting regulatory elements identified in promoter region of *At DREB* genes

Genes	cis-acting regulatory elements
<b><i>DREB1</i> group</b>	
<i>DREB1A</i> & <i>DREB1C</i>	ABRE, (CGTCA) MeJA response, MBS, HSE, TC rich, (TGACG) MeJA response, TCA
<i>DREB1D</i>	ABRE, TC rich, TCA, (TGACG) MeJA response
<i>DREB1F</i>	ABRE, (CGTCA) MeJA response, MBS, TCA, (TGACG) MeJA response
<b><i>DREB2</i> group</b>	
<i>DREB2A</i> NM-1036760	ABRE, (CGTCA) MeJA response, MBS, HSE, TC rich, (TGACG) MeJA response
<i>DREB2A</i> NM120623	ABRE, (CGTCA) MeJA response, MBS, HSE, TC rich, (TGACG) MeJA response, TCA
<i>DREB2B</i>	ABRE, (CGTCA) MeJA response, HSE, TC rich, (TGACG) MeJA response
<i>DREB2C</i>	ABRE, (CGTCA) MeJA response, LTR, HSE, TC rich, (TGACG) MeJA response, TCA
<i>DREB2D</i>	ABRE, ERE, TC rich, (TGACG) MeJA response, TCA
<i>DREB2E</i>	ABRE, TCA
<i>DREB2F</i>	ABRE, TCA, HSE
<i>DREB2G</i>	ABRE, ERE, HSE, (CGTCA) MeJA response, TC rich, LTR, (TGACG) MeJA response
<i>DREB2H</i>	ABRE, HSE, (CGTCA) MeJA response, LTR, (TGACG) MeJA response
<i>DDF2</i>	ABRE, HSE, (CGTCA) MeJA response, TC rich, (TGACG) MeJA response, TCA
<b><i>DREB3</i> group</b>	
<i>DEAR3</i> NM_121197	ABRE, TC rich, TCA
<i>DREB3</i> NM_127898	ABRE, (CGTCA) MeJA response, TC rich, LTR, HSE, MBS, (TGACG) MeJA response
<b><i>DREB4</i> group</b>	
<i>DREB4A</i> NM_101133	ABRE, TCA, (TGACG) MeJA response, TC rich
<i>DREB4A</i> NM_106369	ABRE, (CGTCA) MeJA response, MBS, TC rich, (TGACG) MeJA response, TCA
<b><i>DREB5</i> group</b>	
<i>DREB5A</i>	ABRE, (CGTCA) MeJA response, HSE, MBS, TCA, (TGACG) MeJA response

**Table 3:** Co-expressed genes with each of the candidate DREBs

DREBs	Co-expressed genes
<i>DREB1C</i>	<i>DREB2H, ATERF4</i>
<i>DREB2C</i>	<i>ERF095, DDF1, DREB2A, ERF017, CBF4, DEAR3, ORA47, RRTF1, ATERF13, ERF104, CBF1, ATERF4, ATERF6, DREB1A, RAP2.4, ERF013</i>
<i>DEAR3</i>	<i>DREB2A, CBF4, ATERF4, DREB1A, DEAR2</i>

**Table 4:** Network gene groups functions. The genes involved in abiotic stress responses are classified in different functional group

<b>Functional groups</b>		<b>Genes</b>
Ethylene-activated pathway	signaling	<i>ATERF6, ATERF13, ATERF4, RAPD2.4, DREB1A, DREB2A, RAPD2.4, CBF1, DDF1</i>
Response to water		
Cold acclimation		<i>CBF1, CBF2, DREB1A</i>
Heat acclimation		<i>DREB2C, DREB2H, DREB2A</i>
Response to water deprivation		<i>DREB2A, DDF1, CBF1, DREB1A, RAPD2,4</i>
Response to cold		<i>CBF1, CBF2, DREB1A, DDF1, RAPD2,4</i>
Response to heat		<i>DREB2A, DREB2H, DREB2C, DDF1</i>