REVIEW ARTICLE

DREB1/CBF transcription factors: their structure, function and role in abiotic stress tolerance in plants

M. AKHTAR[1,3], A. JAISWAL[1], G. TAJ[2], J. P. JAISWAL[1], M. I. QURESHI[3] and N. K. SINGH[1]*

[1]*Department of Genetics and Plant Breeding, and* [2]*Department of Molecular Biology and Biotechnology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar 263 145, India*
[3]*Department of Biotechnology, Faculty of Natural Sciences Jamia Millia Islamia, New Delhi 110 025, India*

Abstract

Drought, high salinity and low temperature are major abiotic stresses that influence survival, productivity and geographical distribution of many important crops across the globe. Plants respond to these environmental challenges via physiological, cellular and molecular processes, which results in adjusted metabolic and structural alterations. The dehydration-responsive-element-binding (DREB) protein / C-repeat binding factors (CBFs) belong to APETALA2 (AP2) family transcription factors that bind to DRE/CRT *cis*-element and regulate the expression of stress-responsive genes. *DREB1*/*CBF* genes, therefore, play an important role in increasing stress tolerance in plants and their deployment using transgenic technology seems to be a potential alternative in management of abiotic stresses in crop plants. This review is mainly focussed on the structural characteristics as well as transcriptional regulation of gene expression in response to various abiotic stresses, with particular emphasis on the role of *DREB1*/*CBF* regulon in stress-responsive gene expression. The recent progress related to genetic engineering of DREB1/CBF transcription factors in various crops and model plants is also summarized.

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Introduction

Plants are exposed to many types of environmental conditions during their life cycle. The extremes of the major environmental conditions, namely soil moisture, salt concentration and temperature, limit the growth, development, productivity and geographical distribution of agricultural crops across the globe and reduce potential crop yields by as much as 70% (Agarwal *et al.* 2006). Every year significant losses occur due to sudden frost and unusual freezing temperatures in winter and late cold spring (Heidarvand and Amiri 2010). Globally, approximately 22% of the agricultural land is saline (FAO 2004). Soil salinity is still increasing due to many factors, including modern agricultural practices, and serious salinization of more than 50% of all arable lands is expected by the year 2050 (Wang *et al.* 2003). Drought is a widespread phenomenon in many regions and expected to increase further (Burke *et al.* 2006). These abiotic stresses result in both general and specific effects on plant growth and development. For example, drought stress retards plant growth due to decline in photosynthesis and nonavailability of nutrients as soil dries. Similarly, salinity leads to physiological dryness. Chilling and freezing temperatures can also cause osmotic stress (Chinnusamy *et al.* 2004). Plants respond to these conditions with an array of morphological, physiological, biochemical and molecular changes, which enable plants to survive and reproduce. When a plant is subjected to abiotic stresses, an assortment of genes with diverse functions are induced or repressed. These proteins could be categorized into two groups (figure 1). The first group include functional proteins namely late embryogenesis abundant (LEA) proteins, antifreeze proteins, molecular chaperones, key enzymes for osmolytes biosynthesis like proline, sugar and sugar alcohols, betaines, detoxification enzymes, water channel proteins and membrane transporters which are directly associated with protection of plants from ill effects of abiotic stress. The second group is comprised of proteins that are regulatory in nature and further regulate signal transduction and stress-responsive gene expres-

*For correspondence. E-mail: narendraksingh2@gmail.com.

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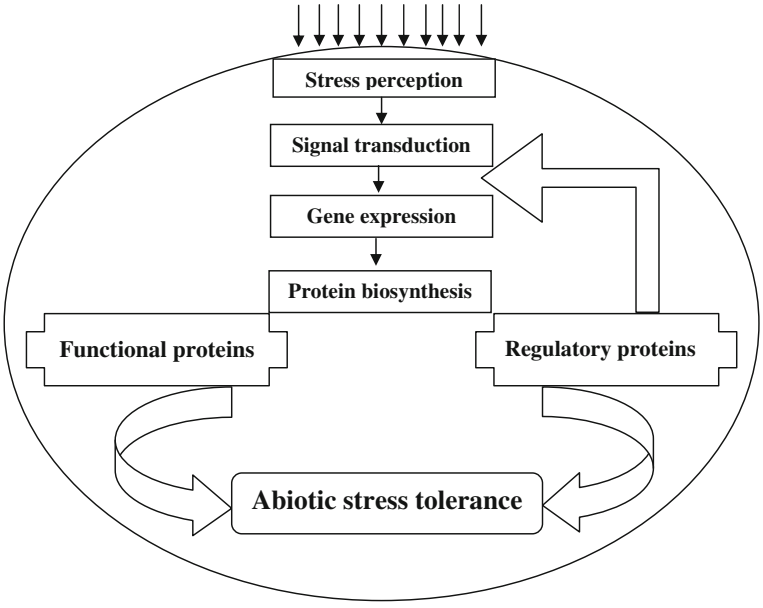

Figure 1. Diagrammatic representation of stress tolerance in adaptive responses of a plant to abiotic stresses. Protein product could be categorized into two parts, namely functional proteins and regulatory proteins. Abiotic stress tolerance is the result of combined effect of both types of proteins.

sion. These include various transcription factors, protein kinases, enzymes involved in phospholipid metabolism, and other signalling molecules such as calmodulin-binding protein and 14-3-3 protein. Analysing and elucidating the function of these genes is very critical for further understanding of the molecular mechanisms governing plant abiotic stress tolerance, and this may help in genetic manipulation of crops for enhanced stress tolerance (Agarwal *et al.* 2006; Shinozaki and Yamaguchi-Shinozaki 2007; Lata and Prasad 2011).

The classical tools of genetics and plant breeding have already established that abiotic stress tolerance in plants is multigenic and quantitative. Hence, it is difficult to manipulate abiotic-stress-related pathways using modern molecular genetics techniques. Introduction of a single gene, encoding functional proteins like LEA proteins, antifreeze proteins, and molecular chaperons, would confer some degree of tolerance but do not give sustained tolerance to most of the abiotic stresses. However, during the evolutionary process, plants have developed some complex molecular mechanisms probably for their survival under the extreme environmental conditions. In this way, another category of genes namely genes encoding regulatory proteins have emerged. Such genes play important roles in survival of plants under stress situation by serving as master regulator of sets of downstream stress-responsive genes. Thus, expression of many genes responsive to abiotic stresses can be regulated and coordinated by manipulating a single regulatory gene for management of crops under stress conditions (Thomashow 2001; Bhatnagar-Mathur *et al.* 2007; Century *et al.* 2008; Yang *et al.* 2011).

Among the regulatory proteins, transcription factors (TFs) have a central role in activating defence gene expression (Chen and Zhu 2004; Xu *et al.* 2008). The TFs interact with *cis*-acting elements present in the promoter region of various stress-responsive genes and thus activate cascades or whole network of genes that act together in enhancing tolerance towards multiple stresses at a time. This property of TFs makes them an attractive category of genes for manipulation of abiotic stress tolerance. Thus, stress responsive TFs are powerful tools for genetic engineering as their overexpression can lead to either upregulation or downregulation of a whole array of genes under their control. Dozens of TFs are involved in plant stress tolerance, regulating plant responses to different stresses. For example, in *Arabidopsis* more than 1500 genes encode various TFs (Riechmann *et al.* 2000). Most of the stress-related TFs are grouped into several large families, such as AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2, zinc-finger and WRKY (Umezawa *et al.* 2006). Members of a family encode related proteins that share a homologous DNA binding domain. Individual members of the same family respond differently in response to different stress stimuli.

One class of protein that is unique to plants and plays a vital role in biotic and abiotic stress response is the AP2/ERF proteins (Agarwal *et al.* 2006). The AP2/ERF protein coding genes constitute a large superfamily, which has been further divided into three groups namely the AP2, ERF, and RAV families based on their sequence similarities and numbers of AP2/ERF domains (Nakano *et al.* 2006; Lata and Prasad 2011). The DREBs (dehydration responsive

Table 1. Genes of DREB subfamily and their induction.

Group	Gene	Induced by	Binding/recognition sequences
A1	*DREB1A*, *DREB1B*, *DREB1C*	Cold	DRE/CRT
	DREB1D/*CBF4*	ABA, salt and dehydration	DRE/CRT
	DREB1E/*DDF2*	Salinity	DRE/CRT
	DREB1F/*DDF1*	Salinity	DRE/CRT
A2	*DREB2*- subtype 1		
	DREB2A	Dehydration, salinity, heat shock	DRE/CRT
	DREB2B	Dehydration, salinity, heat shock	DRE/CRT
	DREB2C	Heat, salinity	DRE/CRT
	DREB2E	ABA-inducible	DRE/CRT
	DREB2H	Not induced by stress	
	DREB2- subtype 2		
	DREB2D	High salinity	
	DREB2G	Not induced by stress	
	DREB2- subtype 2		
	DREB2F	High salinity	
A3	*ABI4*	ABA-inducible	Coupling-element-1 (CE1)-like sequence, CACCG, and a CCAC motif
A4	*TINY*	Dehydration, cold	DRE/CRT and GCC-box motifs
	HARDY	Weak stress inducibility	DRE/CRT and GCC-box motifs
A5	*DBF1*	Strongly induced by ABA but weakly induced by high salinity, dehydration, cold, salicylic acid and hydrogen peroxide	DRE/CRT
A6	*RAP2.4*	Cold, dehydration, high salinity and heat	DRE/CRT and GCC-box
	RAP2.4A	Redox-regulated	CGCG core of a CE3-like element
	RAP2.4B	Dehydration, high salinity and heat	DRE/CRT and GCC-box

element binding) also referred as CBF (C-repeat binding factor) proteins, belonging to ERF subfamily and play crucial role in plants in response to abiotic stresses and therefore received considerable attention in past decades. Proteins belonging to DREB subfamily were further divided into six small groups termed A-1 to A-6, of these A-1 and A-2 constitutes the two largest groups (Sakuma *et al.* 2002). The *DREB1*/*CBF* genes, belonging to the A-1 subgroup and, *DREB2*-like genes, belonging to the A-2 subgroup, are mainly involved in cold and osmotic stress-responsive gene expression, respectively, and there exists a crosstalk between them. The characteristics and functions of members of the other subgroups in the *DREB* subfamily remain to be studied (table 1).

Arabidopsis genome has six *DREB1*/*CBF* genes, namely *DREB1A*/*CBF3*, *DREB1B*/*CBF1*, *DREB1C*/*CBF2*, *DREB1D*/*CBF4*, *DDF1*/*DREB1F* and *DDF2*/*DREB1E* (Sakuma *et al*. 2002). DREB1/CBF TFs exist in a wide array of plants, in addition to *Arabidopsis*, including plants that are acclimatized to cold, such as *Brassica napus* (Jaglo *et al.* 2001) and barley (Choi *et al.* 2002), and those that are not, such as tomato and rice (Dubouzet *et al.* 2003; Zhang *et al.* 2004), some halophytes like *Atriplex hortensis* (Shen *et al.* 2003), and other plants including perennial rye grass (Xiong and Fei 2006) and soybean (Chen *et al.* 2007). Puhakainen *et al.* (2004) reported existence of DREB/CBF pathways in tree plants that have extremes of cold acclimation capacity. Since then, putative *DREB1*/*CBF* orthologues have been identified in a number of woody plants, including birch (*Betula pendula*) (Welling and Palva 2008), *Eucalyptus* (Navarro *et al.* 2009) and grapes (Xiao *et al.* 2006).

Structural characteristics of *DREB1*/*CBF* genes

All TFs have a DNA-binding domain containing a short peptide region, called the DNA-binding motif. The amino acid sequences of the DNA-binding domain are conserved within a family. Proteins belonging to CBF/DREB have highly conserved DNA-binding domain called AP2/ERF domain, consisting of ~60 amino acids. This domain is considered plant-specific (Riechmann and Meyerowitz 1998). However, Magnani *et al.* (2004) reported that homologues are present in some cyanobacteria, viruses and bacteriophages, which selectively bind poly(dG)/poly(dC) showing functional conservation with plant AP2/ERF proteins. *DREB*/*CBF* genes containing AP2 domain have been reported from diverse plants species, including both monocots and dicots. Three-dimensional analysis of AP2/ERF domain revealed three-stranded antiparallel β-sheets connected by loops and an α-helix, packed approximately parallel to each other (Allen *et al.* 1998; Lata and Prasad 2011) (figure 2). The amino acids, valine (at position 14) and glutamic acid (19)

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                 β-1       β-2          β-3        Alpha helix
                 ➡         ➡            ➡          αααααααααααααα
CbDREB1A   IYRGVRRRNSGKWVCEVREPNKKTRIWLGTFQTAEMAARAHDVAALALRGRSACLNFADSAWR
NtDREB1A   IYRGVRRRNSGKWVCEVREPNKKTRIWLGTFQTAEMAARAHDVAALALRGRSACLNFADSAWR
AtDREB1B   IYRGVRQRNSGKWVSEVREPNKKTRIWLGTFQTAEMAARAHDVAALALRGRSACLNFADSAWR
AtDREB1A   IYRGVRQRNSGKWVSEVREPNKKTRIWLGTFQTAEMAARAHDVAALALRGRSACLNFADSAWR
AtDREB1C   IYRGVRQRNSGKWVCELREPNKKTRIWLGTFQTAEMAARAHDVAAIALRGRSACLNFADSAWR
ChbCBF     IYRGVRQRNSGKWVCELREPNKKTRIWLGTFQTAEMAARAHDVAAIALRDRSACLNFADSAWR
ApCBF      VYRGVRQRNSGKWVSELREPNKKTRIWLGTFQTAEMAARAHDVAAIALRGRSACLNFADSVWR
EsDREB1    IYRGVRRRNSGKWVCEVREPNKKSRIWLGTFPTAEMAARAHDVAAIALRGRSACLNFADSAWR
Tacold     IYRGVRRRNSGKWVCEVREPNKKSRIWLGTFPTAEMAARAHDVAAIALRGRSACLNFADSAWR
CaDREBLP1 VYRGVRKRNSGKWVCEVREPNKKSRIWLGTFPTAEMAARAHDVAAIALRGRSACLNFADSAWR
BjDREB1B   IYRGVRLRNSGKWVCEVREPNKKSRIWLGTFLTAEIAARAHDVAAIALRGKSACLNFADSAWR
RsCBF1     IYRGVRLRNSGKWVCEVREPNKKSRIWLGTFLTAEIAARAHDVAAIALRGKSACLNFADSAWR
MsDREB1    VYRGVRKRNLDKWVCEMREPNTKTRIWLGTFPTPEMAARAHDVAAMALRGRYACLNFADSMWR
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Figure 2. Multiple amino acid sequence alignment of highly conserved AP2 domains of DREB1 TFs: *Capsella bursa-pastoris* DREB1A (GenBank acc. no. ABM21468), *Nicotiana tabacum* DREB1A (GenBank acc. no. ABD65969), *Arabidopsis thaliana* DREB1B (GenBank acc. no. AB007788), AtDREB1A (GenBank acc. no. AB007787), AtDREB1C (GenBank acc. no. AB007789), *Chorispora bungeana* CBF (GenBank acc. no. AAY21899), *Arabis pumila* CBF (GenBank acc. no. ABA42927), *Eutrema salsugineum* DREB1 protein (GenBank acc. no. AAS00621), *Thlaspi arvense* cold TF (GenBank acc. no. ABV82985), *Capsicum annum* DREB (CaDREBLP1) (GenBank acc. no. AY496155), *Brassica juncea* DREB1B (GenBank acc. no. ABX00639), *Raphanus sativus* CBF1 (RsCBF1) (GenBank acc. no. ACX48435), *Medicago sativa* DREB1 (GenBank acc. no. ABY78835). The three β-sheets and an α-helix are marked above the corresponding sequences. Asterisks indicate positions which have a single, fully conserved residue; colons indicate highly conserved positions; dots indicate weaker-conserved positions; dashes indicate gaps in the amino acid sequences introduced to optimize alignment.

in the AP2/ERF domain are quite conserved and play a central role in recognition and binding specificity of DRE *cis*-elements (Sakuma *et al.* 2002). However, some studies on rice, wheat, rye and barley demonstrated that E19 is not conserved in DREB1 proteins, and instead it is replaced by valine (Agarwal *et al.* 2006). Therefore, it could be proposed that E19 may not be as important as V14 for the recognition of the DNA-binding sequence in the DREB1 proteins. In addition, other amino acids that facilitate direct contact with DNA for DNA binding activity are arginine (6), arginine (8), trytophan (10), glutamic acid (16), arginine (25) and tryptophan (27) (Allen *et al.* 1998). These amino acids are mostly conserved in DREB1-type TFs. Liu *et al.* (2006) also reported conservation of alanine at the position 37 in AP2/ERF domain, which indicates an important role in binding to DRE element. AP2 domain includes two regions. The first one is the YRG region (YRG element) of 20 amino acids rich in basic and hydrophobic amino acids in the N-terminal stretch. Due to its basic nature, it is proposed to have a role in DNA-binding activity (Okamuro *et al.* 1997). The second region is the RAYD element of about 40 amino acids. Of these, an 18-amino-acid stretch in the C-terminal region is capable of forming an amphipathic alpha helix, and is proposed to mediate protein–protein interaction. In addition, RAYD element also regulates the special binding activity of DREB proteins by influencing the conformation of the YRG element (Okamuro *et al.* 1997). Multiple alignments of different DREB1 proteins revealed the presence of DSAW motif at the end of the AP2/ERF domain and LWSY motif at the end of the C-terminal region. These 'signature sequences' are specifically found in members of A-1 subgroup and distinguish this group from five other subgroups of DREB proteins. Such motifs are conserved in diverse plant species, which indicates its important functional role. In addition to these well-known motifs, two additional putative domains are conserved in most plant CBF homologues (Xiong and Fei 2006). One domain is located downstream of the DSAW motif. The consensus sequence of this domain is A (A/V)xxA (A/V)xxF, with the underlined residues conserved in all known CBF homologues. The other domain is located upstream of LWSY motif, with a consensus sequence of (L/Y)(L/Y)x(N/S)(M/L)A(E/Q)G(M/L)(L/M)xxPP. Interestingly, the CBF homologues from eudicot plants have LLxNM at the beginning of the domain, while the monocot CBF homologues have YYxSL instead. Since, TFs only function in the nucleus, the regulation of their entry into the nucleus is critical to their function. The entry of DREB proteins is mediated by one or two nuclear localization signal (NLS). The DREB1/CBF-type proteins have a basic-amino-acid-rich stretch having consensus sequence PKRPAGRTKFRETRHP as NLS. TFs that have no NLSs enter the nucleus by the way of protein–protein interaction with the TFs that have NLSs. All the *DREB1/CBF* genes posses caboxyl-terminal acidic region. This region is supposed to be a

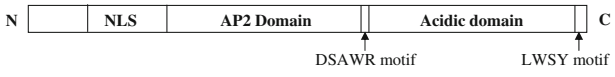

Figure 3. Schematic structure and domains of DREB1/CBF transcription factors.

transcriptional activator motif (Stockinger *et al.* 1997) (figure 3). Sequence analysis of *DREB1* genes showed that they are intronless.

Expression analysis of *DREB1*/*CBF* TFs

Expression of *DREB1*/*CBF* genes under different abiotic stresses has been investigated extensively from a genetically wide variety of plant species. The expression of most of these genes generally displayed a trend of increasing first and then decreasing, with the highest expression achieved between 1 and 4 h under cold stress treatment, in various plant species. In *Arabidopsis*, out of six *DREB1*/*CBF* genes, *CBF3*/*DREB1A*, *CBF1*/*DREB1B* and *CBF2*/*DREB1C* were expressed at high levels under cold stress but not under drought or high-salinity stress (Gilmour *et al.* 1998; Liu *et al.* 1998). The remaining three genes were not expressed at high levels under various stress conditions (Sakuma *et al.* 2002). Similarly, *CBF* genes from different plant species, such as *DREB1* from aloe (Wang and He 2007), *LpCBF3* from rye grass (Xiong and Fei 2006), *PeDREB1* from *Phyllostachys edulis* (Liu *et al.* 2012), *Ptcbfb* from *Poncirus trifoliata* (Wang *et al.* 2009), *OsAP211* from rice (Gao *et al.* 2009), *EguCBFs* from eucalyptus (Navarro *et al.* 2009) showed high expression under low temperature (4°C) treatment. It is well known that expressions of the A-1 group of genes is induced by low temperature, but not by drought or high-salt stress, while A-2 group genes are regulated by salt and drought, but not by cold (Liu *et al.* 1998; Dubouzet *et al.* 2003). However, recent reports have shown some conflicts with respect to these trends. A number of *DREB1*/*CBFs* genes such as *BrCBF* from Chinese cabbage (Jiang *et al.* 2011), *MbDREB1* from apple (Yang *et al.* 2011), *OsDREB1F* from rice (Wang *et al.* 2008), *VviDREB1* from *Vaccinium vitis-idaea* (Wang *et al.* 2010) have been reported which are not only responsive to cold but also to high-salt stress, drought, exogenous ABA treatment. The different abiotic stress signalling pathways are assumed to interact and share some common elements that formed as potential 'node' for crosstalk. These *DREB1*/*CBF* genes may act as cross-point or node connecting several pathways and simultaneously regulate cold, salt, drought and ABA pathways. Interestingly, *OsDREB1B* showed high expression in cold stress as expected, but was also induced by high temperature (Qin *et al.* 2007). Different expression profiles are also reported within the same *CBF* family. For example, in Vitis sp., the *CBF4* gene was mostly induced by cold while the *CBF1*, *CBF2* and *CBF3* genes showed better response to drought compared to cold (Xiao *et al.* 2008). Majority of *CBF*s from different plant species are reported to be significantly upregulated in response to cold stress and perform the important function of cold adaptation. However some genes such as *CrCBF* from *Catharanthus roseus* and *OsDREB1C* from *Oryza sativa* are found to be constitutively expressed under cold stress (Dubouzet *et al.* 2003; Dutta *et al.* 2007). The common features of most *CBF*s are quick and durable response. *AtDREB1A* is induced within 10 min at 4°C and transcript levels of *EguCBF* and *MbDREB1* are detectable within 15 min and 30 min, respectively, under cold stress condition (Liu *et al.* 1998; Navarro *et al.* 2009; Yang *et al.* 2011). *EguCBF1*, *OsDREB1A*/*CBF3* and *ZmDREB1A* are induced by cold within a period of 30 min, 40 min and 60 min, respectively, and remain detectable even at 24 h after exposure to cold stress (Dubouzet *et al.* 2003; Qin *et al.* 2004; Navarro *et al.* 2009).

As far as tissue-specific expression is concerned, only some information is available and in limited plant species only. Previous studies indicated that *DREB1*/*CBF*s are expressed in almost all tissues and organs. However, kinetics is different in various tissues. *CrCBF* is expressed at higher levels in hairy root and callus tissues compared to leaf and root tissues (Dutta *et al.* 2007). Similarly, expression of *OsDREB1F* was higher in panicles and callus than in other tissues (Wang *et al.* 2008). *PtCBFb* was found to be constitutively expressed and upregulated in response to cold stress. The expression was a little higher in leaf and stem, and distinctly increased in root, but there were no expression changes in cotyledon (Wang *et al.* 2009). Interestingly, expression of *MbDREB1* was higher in mature organs than in young tissues, suggesting that the gene may function differently in different organs and at different developmental stages (Yang *et al.* 2011).

Regulation of *DREB1*/*CBF* genes

The *CBF* regulatory network is very complex and best understood in *Arabidopsis*. Regulation of transcription often works through binding of regulatory proteins to the *cis*-acting elements located upstream of functional genes. Promoter analysis of drought-inducible, high-salinity-inducible and cold-inducible genes *RD29A*/*COR78*/*LT178* in *Arabidopsis* revealed a 9 bp conserved sequence (TACCGACAT). This conserved sequence constitutes the drought-responsive element (DRE). It is found in the promoter region of many drought-inducible and cold-inducible genes (Thomashow 1999; Shinozaki and Yamaguchi-Shinozaki 2000). Similar *cis*-acting elements, named C-repeat (CRT) and low-temperature-responsive element (LTRE), both containing an A/GCCGAC motif that forms the core of the DRE sequence, regulate cold-inducible promoters (Stockinger *et al.* 1997; Thomashow 1999). ABRE (ABA-responsive element) and CRT/DRE are the two major *cis*-acting elements that function in ABA-dependent and ABA-independent gene expression, respectively, under abiotic stress conditions (figure 4). DREB1/CBF proteins contain the conserved AP2 DNA-binding domain, which specifically binds to the CRT/DRE sequences and activates the transcription of genes driven by the CRT/DRE sequence. In *Arabidopsis*, three DREB1/CBF proteins are encoded by genes that lie in tandem on chromosome 4 in the order of *DREB1A*/*CBF3,*

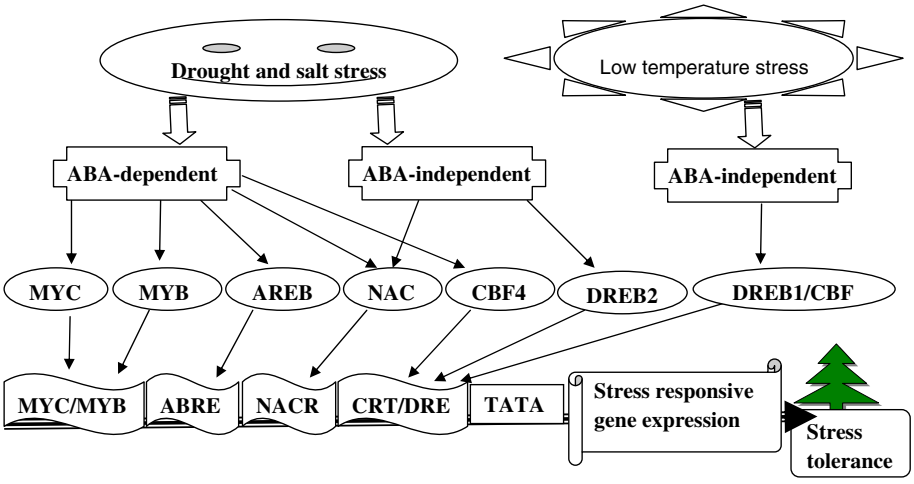

Figure 4. Transcriptional regulatory networks and transcription factors involved in abiotic-stress-responsive gene expression and signal transduction. ABRE functions as a major *cis*-acting element in ABA-dependent pathway. DRE *cis*-acting element is mainly involved in regulation of genes by drought, salt and cold stress under ABA-independent pathways. DREB1/CBF and DREB2 distinguish two different signal transduction pathways. DREB2s are involved in drought and salt stresses but not in cold stress while DREB1/CBF-type transcription factors function in response to cold stress in another ABA-independent pathway. MYC/MYBR, NACR, MYC, MYB and NAC recognition sequences.

DREB1B/*CBF1* and *DREB1C*/*CBF2* (Gilmour *et al.* 1998; Liu *et al.* 1998). These three DREB1 proteins are probably major TFs involved in cold-inducible gene expression (figure 5). In contrast, expression of the *DREB2* genes namely *DREB2A* and *DREB2B*, are induced by dehydration and high-salinity stresses but not by cold stress (Liu *et al.*

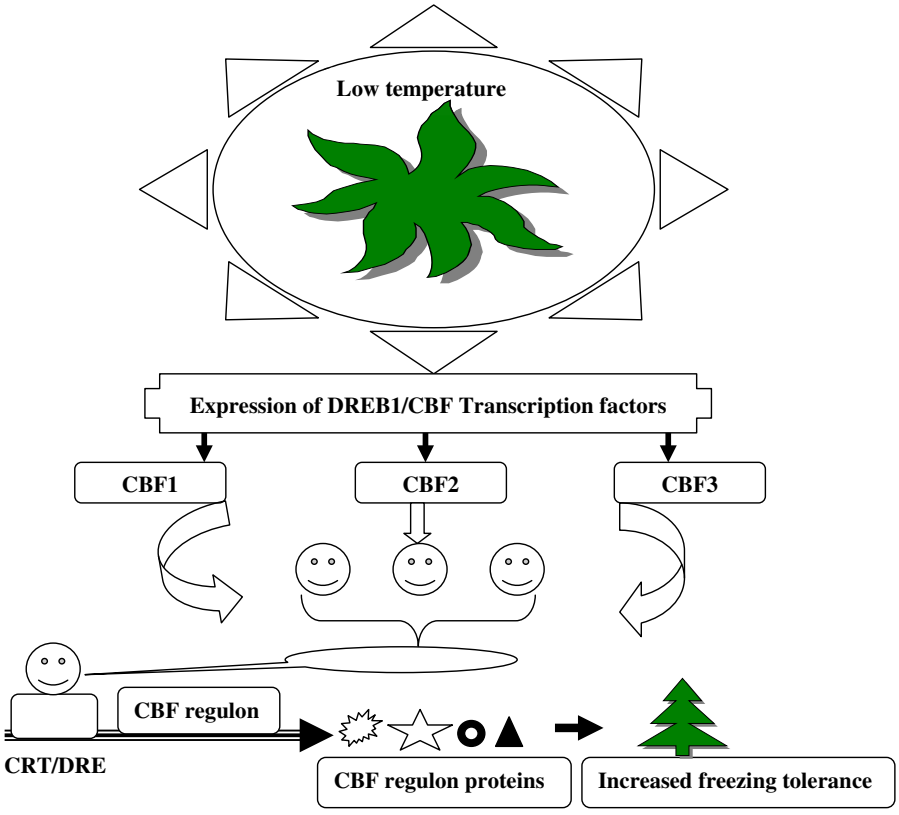

Figure 5. Diagrammatic representation of cold acclimation pathway in *Arabidopsis*. Low-temperature stress leads to induction of CBFs, which in turn bind to CRT/DRE *cis*-acting element of *COR* gene promoters, thereby activating a whole array of genes under their control. The combined action of all the CBF regulon proteins results in an increase in freezing tolerance (Van Buskirk and Thomashow 2006).

1998; Nakashima *et al.* 2000; Lata and Prasad 2011). Interestingly, one of the *DREB1*/*CBF* genes, *CBF4*/*DREB1D*, is inactivated by osmotic stress whereas two other *DREB1*/*CBF* genes, *DDF1*/*DREB1F* and *DDF2*/*DREB1E*, are induced by high-salinity stress, strongly suggesting crosstalk between DREB1 and DREB2 pathways. The CRT/DRE *cis*-acting elements have been identified in promoters of many stress-inducible genes from various plants such as *Brassica napus*, wheat, tobacco and some grasses, suggesting existence of a similar regulatory system in other plants also (Nakashima and Yamaguchi-Shinozaki 2010).

Besides CRT/DRE *cis*-acting element, *ICE1* (inducer of CBF expression), *HOS1* (high expression of somatically responsive genes), and *MYB15* are direct regulators of *DREB1*/*CBF* and their downstream genes. *ICE1* is a transcriptional activator of *CBF3*, and encodes constitutively expressed and nucleus-localized MYB-like basic helix-loop-helix (bHLH) protein, binds specifically to the MYC recognition sequences (CANNTG) on the *CBF3* promoter, but not to a putative MYB recognition sequence, and thereby increases expression of *DREB1A*/*CBF3* gene (Chinnusamy *et al.* 2004). *ICE1* mutant lines are defective in cold-regulated expression of *CBF3* and its target *COR* genes, which leads to a significant reduction in chilling and freezing tolerance (FT) in *Arabidopsis* (Chinnusamy *et al.* 2003). Transgenic lines constitutively overexpressing *ICE1* did not express *CBF3* at warm temperatures but showed upregulation of *CBF3* and its target genes at cold temperatures. Therefore, it has been suggested that, upon exposing a plant to cold stress, modification of either ICE or an associated protein would allow ICE to bind to the *CBF* promoter and to activate *CBF3* transcription (Chinnusamy *et al.* 2004). *HOS1* negatively regulates the expression of *CBF2* and *CBF3*. *HOS1* mutant plant showed superinduction of *CBF*s and their target genes (Chinnusamy *et al.* 2004). It encodes a RING-finger protein, which is localized in the cytoplasm at normal temperature and accumulates in the nucleus upon cold stress and mediates the ubiquitination and degradation of *ICE1* (Lee *et al.* 2001). Thus *HOS1* negatively regulates functioning of *ICE1* in low-temperature adaptation. The next direct regulator of *DREB1*/*CBF* gene expression is *MYB15*. It is also involved in negative regulation of FT by repressing DREB1/CBF activity via binding to the promoter region of *DREB1*/*CBF* genes (Heidarvand and Amiri 2010). ICE1 mutants showed increased level of *MYB15* transcript compared to wild-type, suggesting that ICE1 is a negative regulator of *MYB15* (Chinnusamy *et al.* 2003). It has been proposed that ICE1 physically interacts with *MYB15* and attenuates its expression. Moreover, *DREB1C* negatively regulates *DREB1A* and *DREB1B*, as revealed by analysis of *CBF2* mutant, in which the expression of *DREB1C* was disrupted (Novillo *et al.* 2004). *CBF*/*DREB1* gene expression is also partially controlled by the *CBF*/*DREB1* factors themselves (Novillo *et al.* 2004). Taken together, these studies suggest that all three proteins (DREB1A, DREB1B and DREB1C) function in a regulatory cascade to modulate expression of *DREB1*/*CBF* genes to control plant responses to low temperature.

Engineering stress tolerance using DREB1/CBF transcription factors

With the rapid advancement in recombinant DNA technology, development of genetically modified plants for improving the value of crops by addition/deletion of selected gene(s) seems to a viable alternative or supplementary option of crop improvement compared to traditional or marker-assisted breeding approaches. This strategy is generally referred to as transgenic breeding approach. In some cases, it would be the only option especially when gene of interest originates from nonplant source or when crossing barrier exists between donor and recipient plants. This approach is also advantageous when only one or a few genes are required to be added or deleted in target plant species. Development of precise and efficient transformation protocols has resulted in efficient generation of transgenic lines in a number of crop species. Thus genetic engineering may prove to be a powerful tool to explore the effect of *DREB1*/*CBF* and to deploy the *DREB*/*CBF* TFs for increasing abiotic stresses tolerance potential of crop plants. Majority of abiotic-stress-related studies have been performed in *Arabidopsis* and in general it has been found that constitutive expression of the *CBF* genes in transgenic *Arabidopsis* plants results in induction of *COR* gene expression and increase in freezing tolerance. Jaglo-Ottosen *et al.* (1998) showed that overexpression of *Arabidopsis CBF1* induces expression of *cor6.6*, *cor15a*, *cor47* and *cor78*, and increased the freezing tolerance of nonacclimated *Arabidopsis* plants. Similar observations were also noted by Kasuga *et al.* (1999) when *Arabidopsis* transformant overexpressing *CBF3* gene, driven by either *35S* CaMV or *rd29A* promoter, showed a marked increase in tolerance to freezing, water stress and salinity stress. Transgenic *Arabidopsis* overexpressing *CBF3* had significant increase in transcript levels of *rd29A*, *rd17*, *cor6.6*, *cor15a*, *erd10*, and *kin1* and showed multiple biochemical changes associated with cold acclimation such as elevated levels of total soluble sugars and proline (Gilmour *et al.* 2000). It is interesting to note that constitutive expression of *Arabidopsis CBF1*, *CBF2*, and *CBF3* in transgenic *B. napus* resulted in the accumulation of transcripts of *Bn28* and *Bn115* without a low-temperature stimulus. *Bn28* and *Bn115* encode orthologues of the *CRT*/*DRE*-regulated cold-responsive genes *cor6.6* and *cor15a*, respectively (Jaglo *et al.* 2001). Thus, components of the *Arabidopsis CBF*/*DREB* cold-responsive pathways are also found in *B. napus*, a close relative of *Arabidopsis*. Recently, Jiang *et al.* (2011) reported that components of *CBF* cold-response pathways are also highly conserved in nonheading Chinese cabbage (*Brassica campestris* ssp. *chinensis*). Genome sequence analyses revealed 10 *OsDREB1*s, and function of these genes has been demonstrated in rice (Dubouzet *et al.* 2003;

Shinozaki and Yamaguchi-Shinozaki 2007). Overexpression of *OsDREB1A* in *Arabidopsis* revealed induction of stress-responsive genes and enhanced tolerance to stress (Dubouzet *et al.* 2003). Similarly, transgenic rice overexpressing *Arabidopsis DREB1* improved drought and chilling tolerance (Ito *et al.* 2006). *ZmDREB1A*, a *DREB1*/*CBF*-type TF from maize, was reported to be involved in cold-responsive gene expression (Qin *et al.* 2004), and overexpression of this gene in *Arabidopsis* resulted in improved tolerance to drought and freezing. Overexpression of *DREB1*/*CBF* homologous genes from barley, perennial rye grass, and wheat in transgenic *Arabidopsis* or tobacco plants resulted in induction of stress-inducible genes downstream of *Arabidopsis DREB1A* under nonstress conditions, and lines exhibited tolerance to drought and freezing (Nakashima *et al.* 2009). All these findings support that TFs function in a similar way in abiotic stress tolerance among dicots and monocots. In most cases, transgenic plants showed severe growth retardation under normal growth conditions. The growth retardation in transgenic tomato overexpressing *DREB1B* was prevented by exogenous application of gibberellic acid (GA), which suggests that hyperaccumulation of CBF1 protein may interfere with GA biosynthesis (Hsieh *et al.* 2002a, b). However, microarray analysis did not detect changes in transcript level of any known GA-related genes in transgenic *Arabidopsis* overexpressing *DREB1A*, *DREB1B* or *DREB1C* (Fowler and Thomashow 2002). The use of stress-inducible promoters has been observed to minimize the negative effects of *DREB* TFs on plant growth and development. Overexpression of chrysanthemum *DREB1* gene, named *DgDREB1A*, in *Arabidopsis* resulted in higher tolerance to freezing and drought. *COR47*, *COR15A* and *RD29A* were found to be induced strongly while expression of *CO* and *FT*, two photoperiod-responsive flowering-time genes, was inhibited (Tong *et al.* 2009). Therefore, plants overexpressing of *CO* and *FT* genes showed delayed flowering but not dwarfism. Delayed flowering is the second most common problem in plants overexpressing a *DREB1* gene. Although literature on this aspect is not enough to clearly justify the point, both dwarfism and delayed flowering are thought to be related to interference in GA metabolism (Achard *et al.* 2008; Magome *et al.* 2008). Overall, these studies revealed that monocots and dicots have similar conserved regulatory system (*DREB* regulon), and these regulons can be used in management of abiotic stresses in plants.

A large number of studies have been performed on characterization and expression of *CBF* genes from different plant species using *Arabidopsis* and other model plants like rice, and tobacco. However, development of *DREB*-transformed transgenic plants of important cultivated crops with strong tolerance to various abiotic stresses is still in experimental phase. Plant dwarfism and abnormal phenotype are two important aspects which limit the practical use of *DREB* genes. Yang *et al.* (2011) reported a *DREB1*-like gene designated as *MbDREB1* from dwarf apple. Transgenic *Arabidopsis* overexpressing *MbDREB1* showed increased tolerance to cold, drought, and high-salt stresses. Interestingly, overexpression of *MbDREB1* did not cause dwarf phenotype, grown either on MS medium or in soil. Similar observations were noted in a number of experiments in which transgenic plants exhibited neither growth retardation nor visible phenotypic alterations despite constitutive expression of transgenes. Gutha and Reddy (2008), based on their results, concluded that the growth abnormalities observed in many *DREB* transgenic plants are not a universal phenomenon, but may be specific to the source of the gene, promoter, host plant, growth stage of the transgenic plant, and the set of target genes. Shen *et al.* (2003) also opined a similar hypothesis. They reported that overexpression of *TaDREB1* from *Triticum aestivum* in rice led to dwarf phenotype, whereas overexpression in *Arabidopsis* resulted in normal phenotype. Some recent examples where *DREB*/*CBF*-gene-based transgenic plants exhibited promising results are summarized in table 2.

Table 2. Examples of abiotic-stress-tolerant transgenic plants overexpressing *DREB1*/*CBF*-type transcription factors.

Gene	Source of gene	Transgenic plants	Stress responses	Reference
MbDREB1	Apple	*Arabidopsis*	Drought, salt, cold tolerance	Yang *et al.* (2011)
DREB1A/*CBF3*	*Arabidopsis*	*Lolium perenne*	Drought and freezing tolerance	Li *et al.* (2011)
MtDREB1C	*Medicago truncatula*	*M. Trucatula*, China rose	Freezing tolerance	Chen *et al.* (2010)
TsCBF1	*Thellungiella halophila*	Maize	Drought tolerance	Zhang *et al.* (2010)
DgDREB1A	Chrysanthemum	*Arabidopsis*	Drought and freezing tolerance	Tong *et al.* (2009)
HsDREB1A	Wild barley (*Hordeum spontaneum*)	Bahiagrass (*Paspalum notatum*)	Drought and salt tolerance	James *et al.* (2008)
OsDREB1F	Rice	Rice, *Arabidopsis*	Drought, salt and cold tolerance	Wang *et al.* (2008)
OsDREB1G	Rice	Rice	Drought tolerance	Chen *et al.* (2008)
*OsDREB1B**	Rice	Tobaco	Oxidative, drought, freezing tolerance	Gutha and Reddy (2008)
DREB1A	*Arabidopsis*	Chick pea	Drought tolerance	Bhatnagar-Mathur *et al.* (2007)

*Also showed biotic stress tolerance.

Conclusion and perspectives

Environmental stresses are serious threats to crop productivity especially in rain-fed agriculture. Many plant genes are regulated in response to abiotic stresses and their gene products function in stress response. Such genetic systems are thought to be very important in increasing tolerance of plants to abiotic stresses as well as in management for successful crop cultivation. However, understanding the molecular mechanisms of plant responses to these stresses is critical for manipulation of associated pathways to improve stress tolerance. The multigenic inheritance of abiotic-stress, response makes it quite difficult to manipulate many genes with modern genetic engineering tools. The complex inheritance further restricts genetic modification of plants based on a single gene to achieve satisfactory level of tolerance. Expression of many stress-responsive genes is regulated by TFs under abiotic stress conditions. Cloning and characterization of one group of TFs, the DREB1/CBF proteins, from different plant species revealed changes in gene expression profiles and tolerance to abiotic stresses. DREB/CBF proteins are therefore important TFs in plants which regulate expression of various stress-responsive genes, generally in an ABA-independent manner through binding to *DRE*/*CRT* *cis*-elements and help plants to sustain single or multiplicative effects of different abiotic stresses. Genetic modification of plants using *DREB1*/*CBF* genes anchored with either constitutive or stress-responsive or tissue-specific and stage-specific promoters can help in enhancing tolerance of crop plants against abiotic stresses. However, functional analysis of *DREB1*/*CBF*'s target genes and DRE/CRT *cis*-elements in detail can give better understanding of the molecular basis of stress tolerance. There is still only fragmentary knowledge of abiotic-stress signalling pathways. Thus, in-depth research on functional architecture of complex regulatory networks, including their interactions and crosstalks towards abiotic stress, is required for practical exploitation of *DREB1*/*CBF* TFs in plant abiotic stress management. Further, combinations of *DREB1*/*CBF* TFs and promoters from different sources should be used for minimizing the associated negative effects on plants and increasing the level of abiotic stress tolerance.

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