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REVIEW

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Role of DREB transcription factors in abiotic and biotic stress tolerance in plants

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Abstract Abiotic and biotic stresses negatively influence survival, biomass production and crop yield. Being multigenic as well as a quantitative trait, it is a challenge to understand the molecular basis of abiotic stress tolerance and to manipulate it as compared to biotic stresses. Lately, some transcription factor(s) that regulate the expression of several genes related to stress have been discovered. One such class of the transcription factors is DREB/CBF that binds to drought responsive *cis*-acting elements. DREBs belong to ERF family of transcription factors consisting of two subclasses, i.e. DREB1/CBF and DREB2 that are induced by cold and dehydration, respectively. The DREBs are apparently involved in biotic stress signaling pathway. It has been possible to engineer stress tolerance in transgenic plants by manipulating the expression of DREBs. This opens an excellent opportunity to develop stress tolerant crops in future. This review intends to focus on the structure, role of DREBs in plant stress signaling and the present status of their deployment in developing stress tolerant transgenic plants.

Keywords Abiotic stress · Biotic stress · DREBs · Transcription factor

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Introduction

Plants being sessile, their growth and yield are strongly influenced by abiotic stress such as drought, high salt content and temperature change. Environmental stress presents a major challenge in our quest for sustainable food production as it reduces the potential yields as high as 70% in crop plants. Water stress imparted by drought and temperature severity is the most prevalent abiotic stress that limits plant growth and productivity. Plants respond and adapt to these conditions with an array of biochemical and physiological alterations. Multiple signaling pathways regulate the stress responses of plants (Knight and Knight 2001) and there exists an overlap between the patterns of expression of genes that are induced in response to different stress factors (Seki et al. 2001; Chen et al. 2002). Deciphering the mechanisms by which plants perceive environmental signal and its transmission to cellular machinery to activate adaptive responses is of critical importance for the development of rational breeding and transgenic strategies leading to ameliorate stress tolerance in crops. Abiotic stresses are indeed complex stimuli that induce many different yet related attributes (ionic imbalance and osmotic stress), which may provide the cells with unique information. Based on this multiplicity of signaling, possibly there may be multiple primary sensors that perceive the initial stress signal and alter the expression of a large number of genes. Molecular and cellular responses to abiotic stress include perception, signal transduction to cytoplasm and nucleus, gene expression and finally metabolic changes leading to stress tolerance.

Transcriptome analysis using microarray technology (Bohnert et al. 2001; Seki et al. 2001; Zhu et al. 2001) has revealed that genes induced by stress could be categorized into two groups according to the functions of their products. The first group consists of functional proteins such as membrane proteins that maintain water movement through membranes (water channel proteins and membrane transporters); key enzymes for osmolyte biosynthesis (proline, betaine and sugars, etc.); the detoxification enzymes

enabling cellular, physiological or biochemical metabolism to maintain a normal level (glutathione *S*-transferase, hydrolase, catalase, superoxide dismutase and ascorbate peroxidase, etc.); and other proteins for the protection of macromolecules (LEA protein, osmotin, antifreeze proteins, chaperons and mRNA binding protein, etc.). Tolerance to drought or high salinity can be improved by introduction of genes encoding LEA proteins, proline synthetase or betaine synthetase, etc. The second group comprises regulatory protein, i.e. transcription factors (bZIP, MYC, MYB and DREB, etc.), protein kinases (MAP kinase, CDP kinase, receptor protein kinase, ribosomal-protein kinase and transcription-regulation protein kinase, etc.) and proteinases (phosphoesterases and phospholipase C, etc.) involved in the regulation of signal transduction and gene expression. The transcription activation factors interact with *cis*-elements present in the promoter region of various abiotic stress-related genes and thus up-regulate the expression of many genes resulting in imparting tolerance to abiotic stresses. Molecular and genomic analyses display several transcriptional regulatory systems involved in stress responsive induction of genes. The *Arabidopsis* genome encodes for ~1500 transcription factors (Riechmann et al. 2000) of which those involved in stress responsive gene expression are traditionally classified in ABA-dependent and ABA-independent regulatory pathways (Shinozaki and Yamaguchi-Shinozaki 2000; Thomashow 1999; Xiong et al. 2002). According to microarray analysis in *Arabidopsis*, there are several pathways that independently respond to abiotic stress and one such important pathway involves the DREB/CBF regulon (Fowler and Thomashow 2002).

Traditional breeding strategies have generated very few crop varieties with improved stress tolerance (Flowers 2004). Contrary to the classical breeding and marker assisted selection approaches, direct introduction of genes by genetic engineering seems a more attractive and quick solution for improving stress tolerance (Dunwell 2000; Wang et al. 2003). This has been successfully applied to combat scores of pests and for weed abatement. For abiotic stresses, engineering of stress proteins or the enzymes of the biosynthetic pathways associated with stress responses has been evolved as an encouraging method for improving stress tolerance (McCue and Hanson 1990; Bohnert and Jensen 1996; Dixon and Arntzen 1997; Barkla et al. 1999; Blumwald 2000; Hong et al. 2000). Apparently, the introduction of any single gene may not give sustained tolerance to abiotic stresses (Steponkus et al. 1998; Shimamura et al. 2006) and the constitutive expression of these genes by strong constitutive promoter may have serious implications with respect to energy loss and other deleterious effects. Genetic engineering of plants for tolerance to extreme abiotic stresses could be achieved by the regulated expression of stress-induced transcription factors, which in turn would regulate the expression of a large number of relevant downstream genes. Thus, transcription factors are powerful tools for genetic engineering as their overexpression can lead to the up-regulation of a whole array of genes under their control.

Recent research has identified several transcription factors that are important in regulating plant responses to different stresses. Transcription factors often comprise families of related proteins that share a homologous DNA binding domain. The ethylene responsive element binding factors (ERF), basic-domain leucine-zipper (bZIP), MYC, MYB and WRKY binding (WRKY) transcription factors are some of the important families of stress responsive transcription factors.

The WRKY proteins are unique to plants and contain either one or two WRKY domains, a 60-amino acid region highly conserved among the family members. They play a key role in regulating the pathogen-induced defense responses (Dong et al. 2003), abiotic stress responses (Fowler and Thomashow 2002; Seki et al. 2002; Mare et al. 2004) and are involved in various physiological processes, including senescence, trichome development and biosynthesis of secondary metabolites (Eulgem et al. 2000).

The ABA biosynthesis is induced by dehydration and resultant activation of two regulatory ABA-dependent gene expressions (Fig. 1). One is the bZIP/ABRE system and the other is MYC/MYB (Abe et al. 1997; Uno et al. 2000). The ABRE elements contain DNA binding motif of the basic domain/Leu zipper (bZIP structure). The bZIP proteins are involved in UV light, salt, drought (Choi et al. 2000; Uno et al. 2000; Jakoby et al. 2002) and salicylic acid defense signaling pathways (Zhang et al. 1999). Regulatory genes that control anthocyanin biosynthesis belong primarily to the MYB and MYC class of transcription factors (Goodrich et al. 1992). The MYC class of transcription factor has helix-loop-helix and leucine zipper structural

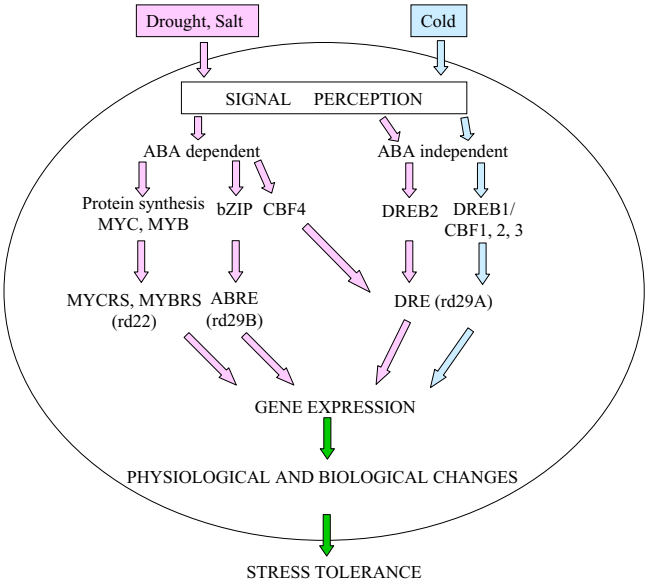

Fig. 1 A schematic representation of cellular signal transduction pathways between stress signal perception and gene expression and the *cis*- and *trans*-elements involved in stress responsive gene expression. DREB1/CBF and DREB2 distinguish two different signal transduction pathways in response to cold and drought stresses, respectively. DRE: drought responsive element, ABRE: abscisic acid responsive binding element, MYBRS: MYB recognition site, MYCRS: MYC recognition site, bZIP: basic-domain leucine-zipper

features (Goodrich et al. 1992). The characteristic feature of the MYB proteins includes a conserved DNA binding domain consisting of two or three imperfect repeats of 51–53 amino acids each (R1, R2 and R3) and regularly spaced tryptophan residues within the repeats. All the plant MYB proteins lack the first repeat. They play an important role in phenylpropanoid metabolism, control of cell shape, signaling pathways responding to plant growth regulators (Martin and Paz-Ares 1997) and in abiotic stress responses (Abe et al. 2003).

Recently, another group of transcription factors known as NAC which are one of the largest families of plant-specific factors (Olsen et al. 2006) have been found to be involved in salinity tolerance. They act downstream of auxin and ethylene signaling pathways in addition to ABA pathway (He et al. 2005). Similarly, the DELLA nuclear proteins are also implicated in the response to many environmental stress signals, including regulation of growth in response to salt (Achard et al. 2006).

One other class of proteins that is unique to plants is called ERF proteins. The ERFs play a vital role in biotic and abiotic stress response. The DREBs (dehydration responsive element binding) are members of the ERF family of transcription factors and follow ABA-independent signal transduction pathway. The two subclasses of DREBs, DREB1 and DREB2 are separately involved in cold and dehydration stress, respectively, and there exists a cross talk between them (Fig. 1).

This review specifically focuses on the DREB proteins and their role in regulating abiotic and biotic stress tolerance in plants.

DREBs and their role in abiotic stress

The dehydration responsive element binding proteins (DREB) are important transcription factors that induce a set of abiotic stress-related genes and impart stress endurance to plants. The DREB transcription factors could be dichotomized as DREB1 and DREB2, which are involved in two separate signal transduction pathways under low temperature and dehydration, respectively. They belong to the ERF (ethylene responsive element binding factors) family of transcription factors. ERF proteins are a sub-family of the APETLA2 (AP2)/ethylene responsive element binding protein (EREBP) transcription factors that is distinctive to plants. There are ~124 ERF proteins in *Arabidopsis* (Riechmann et al. 2000). ERF proteins share a conserved 58–59 amino acid domain (the ERF domain) that binds to two *cis*-elements, the GCC box, found in many PR (pathogens related) gene promoters conferring ethylene responsiveness (Gu et al. 2000), and to the C-repeat CRT/dehydration responsive element (DRE) motif involved in the expression of cold and dehydration responsive genes.

The first isolated cDNAs encoding DRE binding proteins were CBF1 (Stockinger et al. 1997), DREB1A and DREB2A (Liu et al. 1998) from *Arabidopsis*. Since then, DREB genes have been isolated from a wide variety of plants (Table 1). Two *DREB1A* homologs (*DREB1B* and *DREB1C*) and one *DREB2A* homolog (*DREB2B*) were isolated from *Arabidopsis* (Liu et al. 1998). Two homologs of *CBF1* (*CBF2* and *CBF3*) have also been identified from *Arabidopsis* (Gilmour et al. 1998; Medina et al. 1999). *CBF1* is identical to *DREB1B*, and its homologs, *CBF2* and *CBF3*, are identical to *DREB1C* and *DREB1A*, respectively. *CBF4*, a close homolog of *CBF/DREB1* has been reported from *Arabidopsis* (Haake et al. 2002). In wheat and barley, a number of *CBF* homologs have been mapped to low temperature QTLs, Fr-2 chromosomal region (Vágújfalvi et al. 2005; Skinner et al. 2005; Miller et al. 2006). In wheat, a functional Fr-A1 allele reportedly plays a significant role in regulating the CBF-mediated *Cor/Lea* gene expression (Kobayashi et al. 2005). Also, a *PgDREB2A* (Accession no. AY829439) gene by cDNA library screening of *Pennisetum glaucum* seedlings has been isolated (unpublished).

Expression of *DREB1* genes is extensively investigated in various crops with regard to different abiotic stresses. However, only a limited number of plant species have been studied for *DREB2* expression (Table 1). It was found that the expression of *AtDREB1* gene is induced by cold, but not by dehydration, or high salt stress (Liu et al. 1998; Shinwari et al. 1998). Similarly, *CBF* genes also showed high expression in response to low temperature treatment and its transcript was detectable after 30 min of exposure to 4°C, and showed maximum expression at 1 h (Medina et al. 1999). The expression of *DREB2A* and its homolog *DREB2B* were induced by dehydration and high salt stress, but not by cold stress (Liu et al. 1998; Nakashima et al. 2000).

It has been found in some studies that the expression of both the *DREB* genes is induced by abiotic stress, however, at different time periods. *AtDREB1A* was induced within 10 min at 4°C and *AtDREB2A* was induced within 10 min under drought or following 250 mM NaCl treatment (Liu et al. 1998). However, *AtDREB1A* and *AtDREB2A* were not induced by exogenous ABA. In rice, *OsDREB1A* and *OsDREB1B* were induced within 40 min after cold exposure and did not respond to ABA treatment. *OsDREB1A* was induced within 5 h after salt treatment whereas *OsDREB1C* showed constitutive expression and the expression of *OsDREB1D* was not detected with or without any stress. *OsDREB2A* was induced within 24 h after dehydration and salt stress (250 mM) and it responded faintly to ABA and cold stress (Dubouzet et al. 2003). Similarly, *PgDREB2A* transcripts were also induced by cold, drought and salt stresses (personal observation). A DREB2-type transcription factor isolated from wheat (*TaDREB1*) was strongly induced by cold but it responded poorly to drought, salinity and ABA (Shen et al. 2003a). Another DRE binding transcription factor, *AhDREB1* from a halophyte *Atriplex hortensis,* was strongly expressed by 200 mM NaCl in roots (Shen et al. 2003b). In hot pepper, *Ca-DREBLP1* was rapidly induced by dehydration, high salinity and to a lesser extent by mechanical wounding but not by cold stress. This expression pattern is quite similar to that of *DREB2A*. However, this gene is still kept in the DREB1 category because of the resemblance in its structural feature to other *DREB1* genes. This corroborates that *Ca-DREBLP1* belongs to a novel

Table 1 DREB genes isolated from different plants and their transcript response to various abiotic stresses

Species	DREB type and accession number	Expression in stress				References
		Cold	Drought	Salt	ABA	
Arabidopsis	CBF1: U77378	Yes	No	–	No	Gilmour et al. 1998
	CBF2: AF074601	Yes	–	–	–	
	CBF3: AF074602	Yes	–	–	–	
Arabidopsis	DREB1A: AB007787	Yes	No	No	No	Liu et al. 1998
	DREB2A: AB007790	No	Yes	Yes	Yes	
Arabidopsis	CBF1: U77378	Yes	No	–	No	Medina et al. 1999
	CBF2: AF062924	Yes	No	–	No	
	CBF3: AF062925	Yes	No	–	No	
Canola	CBF like: AF370733, AF370734	Yes	–	–	–	Jaglo et al. 2001
Wheat	CBF like: AF376136	Yes	–	–	–	
Rye	CBF like: AF370728, AF370729, AF370730	Yes	–	–	–	
Tomato	CBF like: AY034473	Yes	–	–	–	
Tobacco	Tsi1: AF058827	-	–	Yes	–	Park et al. 2001
Barley	CBF3: AF239616	Yes	No	–	No	Choi et al. 2002
	CBF1: AF298230	-	–	–	–	
Arabidopsis	CBF4: AB015478	No	Yes	–	Yes	Haake et al. 2002
Rice	DREB1A: AF300970	Yes	No	Yes	No	Dubouzet et al. 2003
	DREB1B: AF300972	Yes	No	No	No	
	DREB1C: AP001168	*	*	*	*	
	DREB1D: AB023482	No	No	No	No	
	DREB2A: AF300971	No	Yes	Yes	No	
Wheat Xiaoyan54	DREB2: AF303376	Yes	Yes	Yes	Yes	Shen et al. 2003a
Atriplex hortensis	DREB1: AF274033	–	–	Yes	–	Shen et al. 2003b
Bell pepper	DREB1: AY496155	No	Yes	Yes	No	Hong and Kim 2005
Wheat	CBF2-1: AB178166	Yes	Yes	–	No	Kume et al. 2005
	CBF2-2: AB178167	Yes	Yes	–	No	
Soybean	DREBa: AY542886	Yes	Yes	Yes	Yes	Li et al. 2005
	DREBb: AY296651	Yes	Yes	Yes	No	
	DREBc: AY244760	No	Yes	Yes	Yes	

cbf1: dreb1b, cbf2: dreb1c, cbf3: dreb1a; (*) constitutive expression; (–) not studied

class of transcription factor in the DREB class (Hong and Kim 2005). The expression of *WCBF2* gene from wheat was induced rapidly by low temperature and drought but not by ABA (Kume et al. 2005). The *CBF4* transcription factor in *Arabidopsis* was rapidly induced during drought stress and ABA treatment but not by cold stress. In ABA-deficient mutant *aba1-1*, the drought induction of *CBF4* expression was dramatically reduced substantiating that ABA biosynthesis is required for the proper drought-induced induction of *CBF4* expression (Haake et al. 2002).

Presently, there is not much information available on the tissue-specific expression of DREBs. Expression of *AtDREB2A* and *AhDREB1* was observed in roots, stems and leaves under normal growth conditions. When exposed to salt stress, *AhDREB1* was highly expressed in roots but less significantly in stems and leaves (Shen et al. 2003b). The transcription of soybean *GmDREBa* and *GmDREBb* was induced by cold, drought and salt in leaves of soybean seedlings. The expression of *GmDREBc* was not significant in leaves but showed high level expression in roots following drought, salt and ABA treatments (Li et al. 2005). Based on the various studies summarized above, it is clear that the DREB proteins are important transcription factors in regulating abiotic stress-related genes and play a critical role in imparting stress endurance to plants. Despite the physiological similarity between the cold and dehydration stresses, it is interesting to note that DREBs group of proteins can distinguish cold and dehydration signal transduction pathways.

Structural analysis of DREBs

The DREB proteins contain an ERF/AP2 DNA-binding domain. The ERF/AP2 domain is quite conserved (Fig. 2a and b) and the transcription factor(s) containing it are widely found in many plants, including *Arabidopsis* (Drews et al.

Fig. 2 Comparison of amino acid alignment of the DREB proteins. ▶ **a** DREB1-type *AhDREB1* (AF274033), *AtDREB1A* (AB007787), *AtDREB1B* (AB007788), *AtDREB1C* (AB007789), *CaDREBLP1* (AY496155), *OsDREB1A* (AF300970), *OsDREB1B* (AF300972), *OsDREB1C* (AP001168), *OsDREB1D* (AB023482). **b** DREB2-type *AtDREB2A* (AB007790), *AtDREB2B* (AB007791), *OsDREB2A* (AF300971), *PgDREB2A* (AY829439), *TaDREB1* (AF303376). The conserved ERF/AP2 domain is underlined and asterisks indicate the conserved valine and glutamic acid among the DREB-related proteins

a

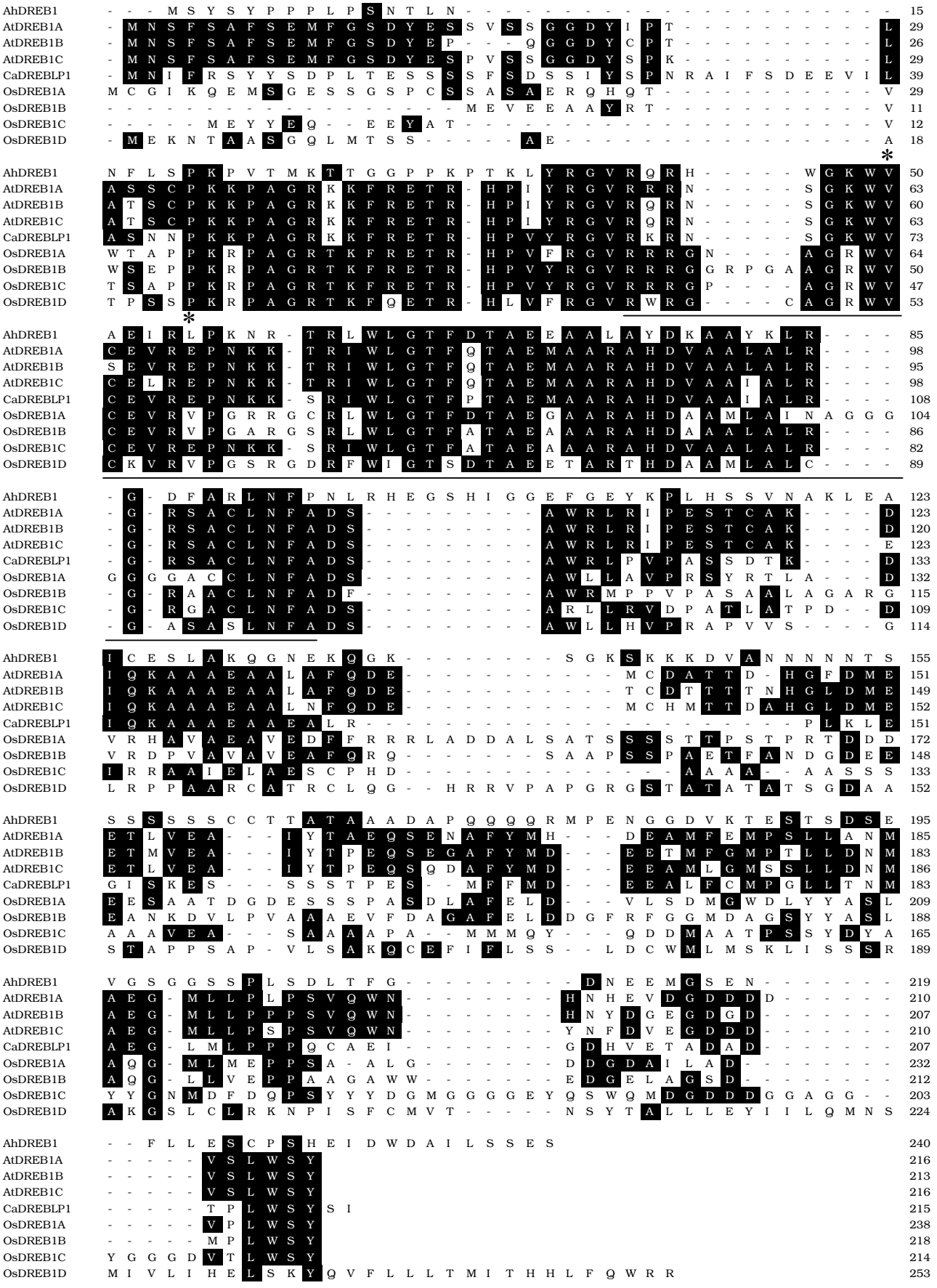
AhDREB1
AtDREB1A
AtDREB1B
AtDREB1C
CaDREBLP1
OsDREB1A
OsDREB1B
OsDREB1C
OsDREB1D

Fig. 2 Continued

1991; Leon-Kloosterziel et al. 1994; Elliot et al. 1996; Wilson et al. 1996; Klucher et al. 1996; Okamuro et al. 1997), tomato (Zhou et al. 1997), tobacco (Ohme-Takagi and Shinshi 1995), rice (Sasaki et al. 1994; Weigel 1995) and maize (Moose and Sisco 1996). Amino acid alignment of different DREB proteins shows high sequence similarity in the nuclear localization signal at the N-terminal region and some similarity in the C-terminal acidic domain (Fig. 2a and b). In the ERF/AP2 domain, the two amino acids, 14th valine and 19th glutamic acid play crucial role in the determination of DNA-binding specificity (Liu et al. 1998; Cao et al. 2001; Sakuma et al. 2002). A conserved Ser/Thr-rich region that is present adjacent to the ERF/AP2 domain is considered to be responsible for phosphorylation of DREB proteins (Liu et al. 1998). The DREB1/CBF1-type NLS consensus PKRPAGRTKFRETRHP distinguishes these proteins from other ERF/AP2 proteins. The DSAW motif at the end of the ERF/AP2 domain and LWSY motif at the end of the C-terminal are conserved in most of the DREB1-type proteins. Phylogenetic relationship among some of the reported DREB-type proteins is shown in Fig. 3.

Identification and involvement of *cis*-acting element DRE in abiotic stress

Yamaguchi-Shinozaki and Shinozaki (1993) analysed the fusion construct of *rd29A* promoter and *GUS* (β-

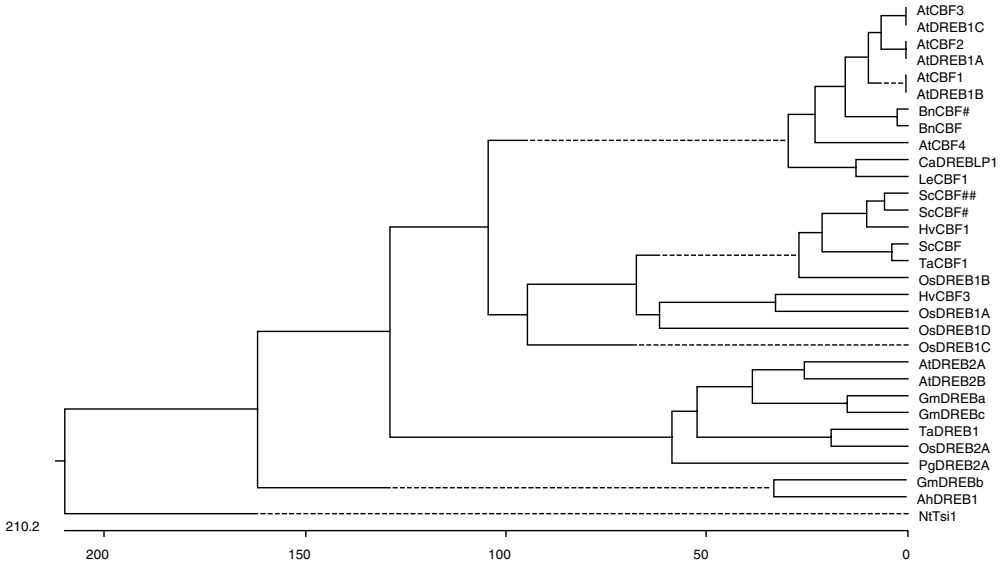

Fig. 3 Relationships among some DREB proteins as illustrated by Tree View produced by DNA STAR, ClustalW. Scale indicates branch length. *AhDREB1* (AF274033), *AtCBF1* (U77378), *AtCBF2* (AF062924), *AtCBF3* (AF062925), *AtCBF4* (AB015478) *AtDREB1A* (AB007787), *AtDREB1B* (AB007788), *AtDREB1C* (AB007789), *AtDREB2A* (AB007790), *AtDREB2B* (AB007791), *BnCBF* (AF370733), *BnCBF#* (AF370734), *CaDREBLP1* (AY496155), *GmDREBa* (AY542886), *GmDREBb* (AY296651), *GmDREBc* (AY244760), *HvCBF1* (AF298230), *HvCBF3* (AF239616), *LeCBF1* (AY034473), *NtTsi1* (AF058827), *OsDREB1A* (AF300970), *OsDREB1B* (AF300972), *OsDREB1C* (AP001168), *OsDREB1D* (AB023482), *OsDREB2A* (AF300971), *PgDREB2A* (AY829439), *ScCBF* (AF370728), *ScCBF#* (AF370729), *ScCBF##* (AF370730), *TaCBF1*(AF376136), *TaDREB1* (AF303376)

glucuronidase) reporter gene in *Arabidopsis* and tobacco plants to study the nature of the *cis*-acting elements under abiotic stress in an ABA-independent manner. The *GUS* reporter gene driven by the *rd29A* promoter was induced at significant levels in transgenic *Arabidopsis* by dehydration, low temperature, high salt or ABA. The base substitution analysis of the promoter region of *rd29A* gene revealed that a 9-bp conserved sequence, TACCGACAT (DRE, dehydration responsive element), is essential for the regulation of *rd29A* induction by dehydration or cold (Yamaguchi-Shinozaki and Shinozaki 1994). Protein factors from nuclear extracts of salt stressed and unstressed *Arabidopsis* plants that specifically bind to DRE in gel shift assays were designated as DRE binding factor-1 (DRBF-1, Yamaguchi-Shinozaki and Shinozaki 1994). It is in fact these studies that resulted in the discovery of the DREB transcription factors.

The DNA binding specificity of AtDREBs and OsDREBs to the *cis*-acting element DRE, was studied by gel mobility shift assay. AtDREB1A and OsDREB1A proteins bound to the wild-type sequence containing A/GCCGAC; however, the binding was not observed with the mutated sequences. Similar results were observed with AtDREB2A and OsDREB2A proteins (Liu et al. 1998; Dubouzet et al. 2003). Competitive DNA binding assays showed that AtDREB1A binds to both ACCGAC and GCCGAC with the same efficiency; however, OsDREB1A protein showed higher preference to GCCGAC compared to ACCGAC (Sakuma et al. 2002; Dubouzet et al. 2003). In contrast, AtDREB2A and OsDREB2A proteins bound to both ACCGAC and GCCGAC with equal efficiency (Sakuma et al. 2002; Dubouzet et al. 2003). It was found that in AtDREB2A, OsDREB2A and AtDREB1A proteins the 14th valine and 19th glutamic acid are conserved in the ERF/AP2 domain. In OsDREB1-type proteins, valine is conserved at both 14th and 19th position except in OsDREB1C, where glutamic acid is conserved at 19th position. The other DREB1-type proteins in monocots (barley, wheat and rye) also have a conserved valine in the 19th position. The conserved nature of DREB2-type protein suggests that these have similar binding specificity in different plants.

The DREBs follow an ABA-independent signal transduction pathway

Drought is one of the most severe environmental stresses and affects almost all the plant functions. Abscisic acid (ABA) is produced under water stress and plays important role in tolerance against drought. Most of the drought stress inducible genes are also induced by ABA (Shinozaki and Yamaguchi-Shinozaki 1997, 2000). However, in *aba* (ABA-deficient) or *abi* (ABA-insensitive) *Arabidopsis* mutants a number of other genes were induced by drought, salt and cold. This suggests that some of the genes do not require ABA for their expression under drought, salt and cold conditions (Thomashow 1994; Yamaguchi-Shinozaki and Shinozaki 1994). These genes included *rd29A*/*lti78*/*cor78*, *kin1*, *cor6.6*/*kin2* and *cor47*/*rd17* (Nordin et al. 1991, 1993; Kurkela and Borg-Franck 1992; Horvath et al. 1993; Yamaguchi-Shinozaki and Shinozaki 1993; Iwasaki et al. 1997).

In an earlier study, it was shown that CRT/DRE elements are involved in ABA signal transduction. However, the ABA-responsive element in the promoter of *cor78a/rd29A* requires the presence of CRT/DRE elements (Yamaguchi-Shinozaki and Shinozaki 1994). It was later shown that the DREBs, except *CBF4*, are ABA-independent. The ABA-independent cold and drought responsive gene expression is regulated by CBF/DREB1 and DREB2 proteins, respectively (Gilmour et al. 1998; Shinwari et al. 1998; Medina et al. 1999; Nakashima et al. 2000). The *CBF4* transcription factor is ABA responsive and involves CRT/DRE elements in ABA-dependent pathway (Fig. 1).

Structural studies have shown that the DREB family of transcription factors has unique conserved regions in them that allow them to interact with a series of downstream genes in an ABA-independent fashion. The involvement of DRE in ABA-dependent regulation of stress response suggests a further interaction or a cross talk between the ABA-dependent and ABA-independent signal transduction pathways. This interaction highlights co-ordination between the stress signals and ABA in the regulation of various stress-induced genes.

Engineering stress tolerance by overexpressing DREBs

Although transformation with individual genes has been shown to confer some degree of tolerance in transgenic plants, it is felt that regulated expression of more genes via overexpression of transcription factors can lead to sustained tolerance. Therefore, it is important to enhance regulatory ability of an important transcription factor that can activate the expression of many target genes controlling correlated characters. In fact in many studies overexpression of stress inducible DREB transcription factor was found to activate the expression of many target genes having DRE elements in their promoters and the resulting transgenic plants showed improved stress tolerance (Table 2). The expression of *35S:AtDREB1A* and *35S:OsDREB1A* in transgenic *Arabidopsis* led to an enhanced freezing and dehydration tolerance; however, these plants showed severe growth retardation under normal growth conditions (Liu et al. 1998; Kasuga et al. 1999; Dubouzet et al. 2003). The level of stress tolerance and growth retardation in the *35S:OsDREB1A* transgenic *Arabidopsis* was relatively lower than that in the *35S:AtDREB1A* transgenic *Arabidopsis*. This might be due to the difference in the number of target stress genes induced. cDNA microarray analysis of *35S:AtDREB1A* transgenic plants revealed that 12 genes had twofold higher expression level than in the wild-type (Liu et al. 1998). Out of these 12 genes, six are known as stress-related genes: *rd29A, kin1, cor6.6/kin2, cor15a, cor47/rd17* and *erd10*. The other six genes showed sequence identity with putative cold acclimatization protein, *DC1.2* homolog, enolase, cysteine proteinase inhibitor and *erd4* cDNA. All these gene products may function in stress tolerance in plants. *35S:OsDREB1A* transgenic showed twofold higher expression of six genes, namely, *cor15a*, *FLO5-21-F13*, *rd29A*, *rd17*, *AtGolS3* and *FLO5-20-N18* (Liu et al. 1998; Kasuga et al. 1999; Dubouzet et al. 2003). The *kin1*, *kin2* and *erd10* genes up-regulated by *AtDREB1A* were also identified for *OsDREB1A*. The *35S:TaDREB1* rice transgenic plants showed dwarf phenotype while in the corresponding *Arabidopsis* transgenic plants growth retardation was not reported. It was axiomatic that a monocot gene transferred to dicots may not function effectively as it did in the monocot (Shen et al. 2003a). The *35S:AhDREB1* gene conferred better survival rate to transgenic tobacco plants under salt stress as compared to the wild-type plants (Shen et al. 2003b).

A number of studies have been done on the constitutive expression of the *CBF* genes. Transgenic *Arabidopsis* overexpressing *CBF* showed induction of *cor* gene expression and an increase in freezing tolerance without being ex-

Table 2 Stress response of transgenic plants overexpressing DREBs

Gene	Transgenic plants	Performance of transgenic plants	References
AtCBF1	*Arabidopsis*	Freezing tolerance	Jaglo-Ottosen et al. 1998
AtDREB1A	*Arabidopsis*	Freezing and dehydration tolerance	Liu et al. 1998
AtDREB2A	*Arabidopsis*	–	
AtCBF3	*Arabidopsis*	Freezing tolerance	Gilmour et al. 2000
AtCBF1	Canola	Freezing tolerance	Jaglo et al. 2001
Tsil1	Tobacco	Osmotic stress tolerance	Park et al. 2001
AtCBF4	*Arabidopsis*	Freezing and dehydration tolerance	Haake et al. 2002
AtCBF1	Tomato	Freezing tolerance	Hsieh et al. 2002b
OsDREB1A	*Arabidopsis*	Freezing, dehydration and salt tolerance	Dubouzet et al. 2003
OsDREB2A	*Arabidopsis*	–	
TaDREB1	*Arabidopsis*	–	Shen et al. 2003a
	Rice	–	
AhDREB1	Tobacco	Dehydration and salt tolerance	Shen et al. 2003b
AtDREB1A	Tobacco	Freezing and dehydration tolerance	Kasuga et al. 2004
BNCBF5, BNCBF17	Canola	Freezing tolerance	Savitch et al. 2005
AtDREB2A	*Arabidopsis*	Dehydration tolerance	Sakuma et al. 2006

(–) Transgenic plants not studied for stress tolerance

posed to a low temperature stimulus (Jaglo-Ottosen et al. 1998; Liu et al. 1998; Kasuga et al. 1999; Gilmour et al. 2000). Constitutive expression of heterologous *Arabidopsis CBF1* in canola (*Brassica napus*, Jaglo et al. 2001) and tomato (Hsieh et al. 2002b) conferred freezing tolerance. The dwarf phenotype of transgenic tomato was overcome by exogenous GA_3 (gibberellic acid) treatment. However, the GA_3 treated plants still exhibited chilling tolerance as compared to the wild-type plants (Hsieh et al. 2002b). The dwarf phenotype of transgenic *Arabidopsis* and tobacco plants overexpressing *35S:AtDREB1A* gene did not change by GA_3 treatment. Hence, the dwarf phenotype in these plants may not be due to interference of GA biosynthesis but due to some other mechanism, which is different from the mechanism reported in transgenic tomato (Hsieh et al. 2002a, b). The constitutive overexpression of *CBF4* with resultant induction of *cor* genes in *Arabidopsis* displayed higher tolerance for freezing and drought stress (Haake et al. 2002). This indicated that both cold and drought signal transduction pathway involved the CRT/DRE elements.

The constitutive homologous overexpression of two *Brassica CBF/DREB1* genes (*BNCBF5* and *BNCBF17*) resulted in increased freezing tolerance, photochemical efficiency and photosynthetic capacity. Accumulation of mRNA for *GLK1* and *GLK2* like transcription factors involved in chloroplast photosynthetic development, chloroplast stroma cyclophilin *ROC4* (*AtCYP20-3*), β-amylase and triose-P/Pi translocator suggested that chloroplast photosynthetic development and carbon partitioning might be affected by *CBF/DREB1* overexpression in *Brassica* (Savitch et al. 2005).

Expression of *DREB* genes is also affected by the members of same DREB family. Using a reverse genetics approach it was shown that *CBF2/DREB1C* acts as a negative regulator of *CBF1/DREB1B* and *CBF3/DREB1A* expression. A *cbf2* mutant, in which *CBF2/DREB1C* gene was disrupted, showed higher capacity to tolerate freezing than the wild-type plants and they were also more tolerant to dehydration and salt stress (Novillo et al. 2004). The *DREB/CBF* were also regulated by bHLH-type of transcription factor, *ICE1* (Chinnusamy et al. 2003), and also by Ca^{2+}-related processes because mutations in *CAX1* (Ca^{2+}/H^{+}) and *CBL1* (Ca^{2+}-sensor protein) affected expression pattern of *DREB/CBF* genes (Albrecht et al. 2003; Catala et al. 2003).

The growth retardation observed on overexpression of *AtDREB1A* using *35S CaMV* constitutive promoter was overcome using abiotic stress inducible promoter *rd29A* in transgenic *Arabidopsis* (Kasuga et al. 1999) and tobacco (Kasuga et al. 2004). This proved that overexpression of *DREB1* with stress inducible *rd29A* promoter was useful for improving drought, cold and salt stress without any penalty on growth. While this is being generally argued that overexpression of transcription factors should be done using stress regulated promoters, it was recently shown that constitutive overexpression of *CBF3* and *ABF3* in rice led to the development of transgenic rice with elevated tolerance to drought and salinity without any growth inhibition or any phenotypic aberrations (Oh et al. 2005). In fact Liu et al. (1998), who overexpressed the *AtDREB2A* gene in *Arabidopsis* for the first time, did not find growth retardation in the transgenic plants. Of course, they did not find any improvement in tolerance either. Therefore, it was presumed that some post-translational modification was necessary. Recently, Sakuma et al. (2006) have shown that overexpression of active form of *AtDREB2A* (without negative regulatory domain, i.e. deletion of a region between residues 136 and 165) up-regulates the downstream drought inducible genes and improves drought stress tolerance of *Arabidopsis*. However, the mechanism of *AtDREB2A* activation is still elusive.

Involvement of DREBs in biotic stress

Generally, plants in the field are not subjected to only a single stress at a time, but they face numerous stresses collectively, whether it is biotic or abiotic. Some recent reports have highlighted the connection between disease resistance and drought tolerance. Inoculation of *Arabidopsis* plants with growth promoting rhizobacteria enhanced protection against both *Erwinia carotovora* and dehydration stress (Timmusk and Wagner 1999). Also distinct abiotic stresses induced the expression of antifungal protein cystatin in *Castanea sativa* (Pernas et al. 2000). Furthermore, the ABA-independent dehydration responsive signaling pathways marked by *DREB2A* were found to cross talk with *adr1* (activated disease resistance 1) activated signaling pathways (Chini et al. 2004). Constitutive or conditional enhanced expression of *ADR1* conferred significant tolerance to drought but not for thermal and salt stress. Northern analysis of hemizygous and homozygous *adr1* lines revealed that *DREB2A* expression was up-regulated, whereas *DREB1A*, *rd29A* or *rd22* remain unaffected. In *ADR1* plants *DREB2A* expression was SA-dependent, since ROIs are also reported to signal *DREB2A* expression (Desikan et al. 2001). Therefore, *DREB2A* expression might have resulted from SA-amplified ROI synthesis, which suggests redox control of *DREB2A* expression.

Microarray analyses of plants containing a conditional *adr1* allele demonstrated that a significant number of drought responsive genes were up-regulated (Chini et al. 2004). Hence, there may be significant overlap between biotic and abiotic stress signaling.

This review summarizes that DREBs are important transcription factors regulating stress responsive gene expression through DRE/CRT *cis*-elements and its DNA binding domain. They play a crucial role in providing tolerance to multiple stresses and display overlapping responses to different stress conditions. DREBs control the expression of stress-responsive genes via ABA-independent pathways in both abiotic and biotic stress. The highly conserved domains in DREB proteins are important for their specific biological functions and identifying such critical domains will help in achieving efficient crop improvement strategies by genetic engineering. The DREBs can be used to produce transgenics with higher tolerance to drought, high salt and/or cold stress in combination with different promoters. Recruitment of stress-induced promoters along with tran-

scription factors can be more effective in stress tolerance without compromising yield. However, the end results will depend on the genotype of the host plant which is to be manipulated.

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