REVIEW PAPER

Role of DREBs in regulation of abiotic stress responses in plants

Charu Lata and Manoj Prasad

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi-110067, India

* To whom correspondence should be addressed. E-mail: manoj_prasad@nipgr.res.in

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Abstract

Abiotic stresses such as drought, high salinity, and cold are common adverse environmental conditions that significantly influence plant growth and productivity worldwide. The phytohormone abscisic acid (ABA) plays an important role in physiological and developmental responses as well as in co-ordinating various stress signal transduction pathways in plants. DREBs (dehydration responsive element binding) are important plant transcription factors (TFs) that regulate the expression of many stress-inducible genes mostly in an ABA-independent manner and play a critical role in improving the abiotic stress tolerance of plants by interacting with a DRE/CRT cis-element present in the promoter region of various abiotic stress-responsive genes. This review summarizes recent studies highlighting the role of the DRE-binding family of TFs in the adaptive responses to different abiotic stresses and their structural and functional characters with emphasis on the expression and regulation of DREBs. The practical and application value of DREBs in crop improvement, such as stress tolerance engineering as well as marker-assisted selection (MAS), has also been discussed.

Key words: Abscisic acid, dehydration-responsive element-binding, DRE/CRT, marker-assisted selection, over-expression, transcription factors.

Introduction

As sessile organisms, plants are constantly challenged by a wide range of environmental stresses such as drought, high salt, and temperature change. Growth constraints and stress due to these environmental changes result in reduced productivity and significant crop losses. Drought and salinity together affect more than 10% of arable land, resulting in a more than 50% decline in the average yields of major crops worldwide (Bray et al., 2000). Response to abiotic stresses is a very complex phenomenon as various stages of plant development can be affected by a particular stress and often several stresses simultaneously affects the plant (Chinnusamy et al., 2004). Therefore, the mechanisms underlying stress tolerance and adaptation have long been the focus of intensive research.

Plants usually respond to their changing environment in a complex, integrated way allowing them to respond and adapt to the specific set of conditions and constraints present at a particular time. It involves an array of physiological and biochemical modifications in plants including leaf wilting, reduction in leaf area, leaf abscission, stimulation of root growth, changes in relative water content (RWC), electrolytic leakage (EL), generation of reactive oxygen species (ROS), and accumulation of free radicals which disrupt cellular homeostasis by reacting with lipids, proteins, pigments, and nucleic acids resulting in lipid peroxidation (LP), membrane damage, and the inactivation of enzymes, thus affecting cell viability (Bartels and Sunkar, 2005). Besides this, abscisic acid (ABA), a plant growth regulator and stress hormone, induces leaf stomata closure to reduce water loss through transpiration and decreases the photosynthetic rate in order to improve the water-use efficiency (WUE) of plants. Molecular responses to abiotic stresses, on the other hand, include stress perception, signal transduction to cellular components, gene expression, and, finally, metabolic changes imparting stress tolerance (Agarwal et al., 2006). The genes thus induced by stress not only function in protecting cells...
from stress by the production of important metabolic proteins but also in regulating the downstream genes for signal transduction.

Large-scale transcriptome analysis has revealed that these gene products can broadly be classified into two groups (Bohnert et al., 2001; Seki et al., 2002; Fowler and Thomashow, 2002). One group constitutes genes that encode proteins to protect the cells from the effects of water stress. These genes include those that govern the accumulation of compatible solutes (key enzymes for osmolyte biosynthesis such as proline, betaine, sugars, etc.); passive transport through membranes and energy-requiring water transport systems (water channel proteins and membrane transporters); and the protection and stabilization of cell structures from desiccation and damage by reactive oxygen species (the detoxification enzymes such as glutathione S-transferase, catalase, superoxide dismutase, ascorbate peroxidase, etc.); enzymes for fatty acid metabolism, proteinase inhibitors, ferritin, and lipid-transfer proteins; and other proteins for the protection of macromolecules (LEA (late embryogenesis abundant) protein, osmotin, antifreeze proteins, chaperons, etc.). It has been suggested that introduction or over-expression of genes encoding LEA proteins, proline synthetase or betaine synthetase, etc. can provide tolerance to drought or high salinity in transgenic plants (Cushman and Bohnert, 2000).

A second group of genes activated by abiotic stresses comprises regulatory proteins that further regulate stress signal transduction and modulate gene expression and, hence, probably function in the stress response. They include various transcription factors (TFs) such as myelocytomatosis oncogene (MYC), myeloblastosis oncogene (MYB), basic leucine zipper (bZIP), NAM, ATAF, and CUC (NAC), dehydration responsive element binding (DREB), etc. suggesting the role of various transcriptional regulatory mechanisms in the stress signal transduction pathways; protein kinases [mitogen activated protein (MAP) kinase, calcium-dependent protein (CDP) kinase, receptor protein kinase, etc.]; protein phosphatases and proteinases (phosphoesterases and phospholipase C, etc.) implicated in the regulation of signal transduction and gene expression (Agarwal et al., 2006; Shinozaki and Yamaguchi-Shinozaki, 2007).

The TFs interact with cis-elements in the promoter regions of various stress-related genes to up-regulate the expression of many downstream genes, thus imparting stress tolerance (Agarwal and Jha, 2010). In the Arabidopsis thaliana genome only, nearly 1500 TFs are reported which are thought to be involved in stress-responsive gene expression (Riechmann et al., 2000). Microarray analysis data in Arabidopsis and in several other plants reveal that there are several pathways that independently respond to abiotic stress (in both an ABA dependent and an ABA-independent manner), thus forming a highly complex gene network (Fowler and Thomashow, 2002; Umezawa et al., 2006).

Role of ABA in stress-responsive gene expression

ABA is an important plant hormone that plays a regulatory role in many physiological processes in plants, such as embryo maturation, seed development, seed and bud dormancy, seed germination, root growth, fruit ripening, regulation of stomatal aperture, and the activation of stress-responsive genes (Agarwal and Jha, 2010). Increased levels of ABA are triggered by a variety of environmental stresses such as drought, water stress, salinity, cold, desiccation, heat stress, and wounding. Further, it is also proved that ABA is a major physiological signal that induces drought and high salinity responses (Gomez et al., 1988; Verslues and Bray, 2006; Farooq et al., 2009; Cutler et al., 2010; Hubbard et al., 2010). The action of ABA, therefore, not only involves the regulation of developmental pathways but also controls many stress-adaptation responses such as the activation of genes responsible for osmotic adjustment, ion compartmentalization, root hydraulic conductivity, the regulation of shoot and root growth, limiting transpiration rate and wilting, thus reducing water loss in the plants (Verslues and Zhu, 2005; Pospíšilová et al., 2009). It is also involved in the modification of gene expression, and a number of stress-responsive genes are up-regulated by ABA during osmotic imbalance (Ingram and Bartels, 1996).

Although several genes are induced in response to dehydration and cold stress on exogenous ABA treatment (Zhu, 2002; Shinozaki et al., 2003), there are also many genes that do not respond to such treatments (Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2005) suggesting the existence of both ABA-dependent and -independent signal transduction cascades. DRE/CRT is one of the major cis-acting elements which function in ABA-responsive or non-responsive gene expression during abiotic stresses (Nakashima and Yamaguchi-Shinozaki, 2010).

ABA-dependent signalling systems have been described as pathways that mediate adaptation to stress by the activation of at least two different regulons (a cluster of genes controlled by a certain type of TF) can be identified: (i) the AREB/ABF (ABA-responsive element-binding protein/ABA-binding factor) regulon; and (ii) the MYC/MBF regulon (Abe et al., 1997; Busk and Pagès, 1998). On the other hand, ABA-independent regulons are: (i) the CBF/DREB (cold-binding factor/dehydration responsive element binding) regulon; and (ii) the NAC and ZF-HD (zinc-finger homeodomain) regulon (Saibo et al., 2009). In addition, previous studies have identified the existence of both ABA-dependent and -independent pathways of stress response and function through members of the AP2/EREBP (ERF) family of TFs (Yamaguchi-Shinozaki and Shinozaki, 1994; Kizis and Pagès, 2002). Although these different stress response pathways usually function independently, it is possible that some level of cross-talk certainly exists between them (Fig. 1).

Other than the above-mentioned regulons, some other TFs such as WRKY, HARDY, Zinc fingers etc. are also involved in abiotic stress tolerance responses and key regulatory networks in plants (for details see Lata et al., 2011b). However, until now, the best studied group of TFs in abiotic stresses is the DREB genes since it activates the expression of many target genes that are responsible for controlling correlated characters such as osmoprotection...
The dehydration responsive elements

Extensive molecular analyses have revealed the presence of specific cis-elements that mediate the activation of various genes under different environmental stresses. The dehydration responsive element (DRE) with the core sequence 5’-CCGAC-3’ was first identified in the promoter of cold-inducible genes from Arabidopsis (Saleh et al., 2005). This sequence is related to the DRE motif and is essential for the induction of several genes by low temperature. The DRE motif and the low temperature-responsive element (LTRE) were identified in the promoters of cold-regulated genes from Arabidopsis such as kin1, kin2, and rab18, and were also responsible for the regulation of BNI15 and WCS120 from Brassica napus and wheat, respectively (Kurkela and Borg-Franck, 1992; Lang and Palva, 1992; Baker et al., 1994; Jiang et al., 1996; Ouellet et al., 1998). These are activated in an ABA-independent manner during drought and cold stress in ABA-deficient and ABA-insensitive mutants of Arabidopsis. However, it has also been suggested that some DRE/CRT motifs can respond to an ABA-dependent pathway (Haake et al., 2002). The DRE2 of the maize rab17 promoter, for example, is involved in ABA-dependent responses to osmotic stress with a typical core motif of 5’-ACCGAC-3’ and was identified in the embryos and leaves (Busk et al., 1997). Additionally, the DRE1 cis-element (5’-ACCGAGG-3’) has also been identified in the rab17 promoter mediating an ABA-dependent regulation in the embryo, but not in vegetative tissues in response to drought stress (Busk et al., 1997; Busk and Pagès, 1998 Saleh et al., 2005).

Identification, expression, and structural analysis of the DREB transcription factors

The DREB proteins namely, DREB1 and DREB2, involved in two separate signal transduction pathways under low temperature and dehydration, respectively, are important APETALA2 (AP2)/ethylene responsive factor (ERF) plant TFs that induce a set of abiotic stress-related genes. The large AP2/ERF family of TFs in Arabidopsis was characterized on the basis of the number of repetitions and the sequence of the AP2 domain (Sakuma et al., 2002). The 145 members of this family in Arabidopsis were classified into five subfamilies namely, DREB, ERF, AP2, RAV, and others (Table 1). The AP2/ERF superfamily of rice, however, can be divided into three families based on sequence similarity and numbers of domains: AP2, ERF (the ERF subfamily and the CBF/DREB subfamily), and RAV (Nakano et al., 2006).

DREB genes play an important role in the ABA-independent stress-tolerance pathways that induce the expression of various stress-responsive genes in plants. The first isolated cDNAs encoding DRE binding proteins, CBFI (CRT binding factor1), DREB1A and DREB2A were first isolated by using yeast one-hybrid screening (Stockinger et al., 1997; Liu et al., 1998) from Arabidopsis. Since then, numerous DREB genes have been isolated from a number of plants (Table 2). These proteins specifically bind to the DRE sequence and activate the expression of genes driven by it. DREB1/CBF1, DREB1A/CBF3, and DREB1C/CBF2 genes lie in tandem on chromosome 4 of Arabidopsis (Gilmour et al.,

Fig. 1. A schematic representation of stress signal perception and gene expression via ABA-dependent and independent pathways at cellular level in plants (based on well-known concepts).
Table 1. Classification of AP2/ERF family of transcription factors (based on Sakuma et al., 2002).

<table>
<thead>
<tr>
<th>AP2/ERF subfamilies</th>
<th>No. of AP2/ERF domain</th>
<th>Subgroup/domain/motif</th>
<th>No. of genes</th>
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<tbody>
<tr>
<td>AP2</td>
<td>2</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>DREB</td>
<td>1</td>
<td>A-1 to A-6</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Conserved WLG motif</td>
<td>65</td>
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<tr>
<td>ERF</td>
<td>1</td>
<td>B-1 to B-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Conserved WLG motif</td>
<td></td>
</tr>
<tr>
<td>RAV</td>
<td>1</td>
<td>B3 domain</td>
<td>6</td>
</tr>
<tr>
<td>Others (AL070349)</td>
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<td>Lack WLG motif</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>145</strong></td>
</tr>
</tbody>
</table>

1998; Liu et al., 1998). Arabidopsis also contains two DREB2 proteins namely, DREB2A and DREB2B (Liu et al., 1998). DREB1/DREB2-homologous genes have also been isolated from several grasses such as rice, wheat, barley, maize, sorghum, rye, oat, and perennial ryegrass (Nakashima et al., 2009). In important cereal crops like wheat and barley, a number of CBF homologues have been mapped to the Fr-2 chromosomal region (Skinner et al., 2005; Miller et al., 2006). A functional Fr-A1 allele plays an important role in regulating the CBF-mediated Cor/Lea gene expression in wheat (Kobayashi et al., 2005). Shukla et al. (2006) reported the isolation and characterization of a gene (CAP2) from chickpea (Cicer arietinum) encoding a novel AP2-family TF which is relatively small in comparison to most of the well-studied DREB family members. Detailed functional study on small AP2 proteins such as CAP2 and soybean GmDREB may open up new areas in the plant developmental process. Recently, a novel DREB2-like gene, SiDREB2, associated with dehydration stress tolerance, has been isolated from foxtail millet (Setaria italica) (Lata et al., 2011a).

Expression of the Arabidopsis DREB1/CBF genes is induced by cold, while the DREB2 genes are induced by dehydration, high-salinity, and heat stresses generally (Fig. 2) (Liu et al., 1998; Shinwari et al., 1998; Nakashima et al., 2000). However, CBFA1/DREB1A, DREB1B/DREB2, and DREB1F/DDF1 are induced by osmotic stress, suggesting the existence of cross-talk between the DREB1 and the DREB2 pathways (Haaake et al., 2002; Nakashima et al., 2009).

According to the structure characteristic of DREB TFs, the subfamily of DREB TFs can be further divided into six subgroups from A-1 to A-6 (Sakuma et al., 2002). The DREB TFs contain a highly conserved AP2/ERF DNA-binding domain across the plant kingdom including Arabidopsis, rice, soybean, chickpea, tomato, tobacco, and millets (Lata et al., 2011a). The three-dimensional structure of the Arabidopsis AtERF1 AP2 domain (PDB ID: 1GCC) was solved by a heteronuclear multi-dimensional nuclear magnetic resonance (NMR) technique (Allen et al., 1998). The domain consists of a three-stranded β-sheet and one α-helix running almost parallel to the β-sheet, and it contacts DNA via Arg and Trp residues located in the β-sheet (Magnani et al., 2004). The structure and characteristics largely hold true for AP2 domain-containing proteins from other crops as well. A recent study showed AP2-DNA binding domain of SiDREB2 from foxtail millet sharing the same conserved features (Lata et al., 2011a). Two conserved functional amino acids (valine and glutamic acid) at the 14th and 19th residues, respectively, are present in the DNA binding domain and are thought to be crucial sites for the binding of DREBs to the DRE core sequences (Liu et al., 1998). An alkaline N-terminal amino acid region, which acts as a nuclear localization signal (NLS), and a conserved Ser/Thr-rich region adjacent to the AP2/ERF DNA binding domain are also mostly present. Ser/Thr-rich region is considered to be responsible for phosphorylation of DREB proteins (Liu et al., 1998; Agarwal et al., 2006). The proteins also contain an acidic C-terminal region which is predicted to be functional in trans-activation activity (Stockinger et al., 1997). Most of the positively charged residues are conserved at the N-terminal domain. The NLS ‘PKRPAGRTK-FRETTRP’, a DSAP motif immediately flanking the ERF/AP2 domain and a conserved LWSY motif at the end of the C-terminal are present in most of the DREB1-type proteins (Cong et al., 2008). Similarly, A-2 and A-3 subgroup proteins possess a PKK-like NLS sequence RKKPAGSKGKC-MGKGPPXNXRF almost up to the AP2/ERF domain, however, unlike A-1 subgroup members they do not have a conserved motif close to the C-terminal of the AP2-domain (Zhou et al., 2010). A systematic phylogenetic analysis has been carried out, based on the similarities of AP2 domains in the DREB subfamily proteins isolated from various plant species, using MEGA 4.0 by the Neighbor-Joining method (Fig. 3). The phylogenetic analysis showed that all the six groups within the DREB subfamily can easily be classified on the basis of AP2 domains as suggested by Sakuma et al. (2002). It was also observed that AP2 domains can easily dichotomize monocots from dicots. This functional conservation makes them important targets for crop improvement for abiotic stress tolerance through genetic engineering and plant breeding.

Role of DREB1/CBFs in cold-responsive gene expression

The Arabidopsis DREB1 subgroup consists of six genes (Sakuma et al., 2002). DREB1A/CBF3, DREB1B/CBF1, and DREB1C/CBF2 are strongly and transiently induced by low temperature stresses (Gilmour et al., 1998; Fowler and Thomashow, 2002). Interestingly, it was observed that the activation of CBF1–CBF3 genes in response to low temperature is gated by the circadian clock, suggesting their regulation has aspects in common with the regulation of Arabidopsis chlorophyll a/b-binding (CAB) genes (Fowler et al., 2005). OsDREB1A, OsDREB1B, OsDREB1C, and OsDREB1D, respectively, have been isolated from rice (Dubouzet et al., 2003). A DREB1/CBF-type TF, ZmDREB1A was also identified in maize (Qin et al., 2004).

Competitive DNA binding assays revealed that AtDREB1A protein could bind to both ACCGAC and GCCGAC with the
same efficiency; however, OsDREB1A protein showed preferential binding to GCCGAC compared with ACCGAC (Sakuma et al., 2002; Dubouzet et al., 2003). However, although the Aloe DREB1 can bind to the DRE cis element it may also bind to other cis-elements effectively and, hence, can function in a new cold-induced signal transduction pathway (Wang and He, 2007). A similar result was also observed for OsDREBL which did not bind effectively to the CRT/DRE motif (Chen et al., 2003).

Temporal gene expression studies of DREB/CBF genes in various crops have revealed that these are induced by abiotic stresses particularly low temperature, however, at different time periods (Table 2). For example, AtDREB1 expressed within 10 min at 4°C (Liu et al., 1998). The transcripts of CBF genes were detectable after 30 min of exposure to 4°C with maximum expression at 1 h (Medina et al., 1999). CBF1/DREB1B and CBF3/DREB1A transcripts accumulated after 15 min of cold treatment while CBF2/DREB1C transcripts accumulated at a slower rate with maximum expression after 2.5 h of cold exposure and then gradually declined (Novillo et al., 2004). However, the CBF4 TF in Arabidopsis was rapidly induced during drought and ABA treatment but not by cold stress (Haake et al., 2002). OsDREB1A and OsDREB1E were induced soon after cold exposure (within 40 min) but do not respond to ABA treatment. OsDREB1A was induced within 5 h of salt stress whereas OsDREB1C was constitutively expressed during stress. OsDREB1D expression was not detected with or without any stress (Dubouzet et al., 2003; Agarwal et al., 2006). OsDREBL also accumulated quickly within 30 min

### Table 2. Response of DREB genes to various stresses.

<table>
<thead>
<tr>
<th>DREB TFs</th>
<th>Species</th>
<th>Accession no.</th>
<th>Stress response</th>
<th>References</th>
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<td>AB007787</td>
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<td>At2g40340</td>
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<td>Cold</td>
<td>Gilmour et al., 1998</td>
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</tr>
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<td>StDREB2</td>
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<td>EF490996</td>
<td>Cold, ABA</td>
<td>Yang et al., 2009</td>
</tr>
<tr>
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<tr>
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<td>GJ52205</td>
<td>Drought, Salt, Heat</td>
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in response to low temperature, but not in response to ABA, NaCl, and dehydration (Chen et al., 2003). The expression of the *WCBF2* gene from wheat was induced rapidly by low temperature and drought but not by ABA (Kume et al., 2005). In *Arachis hypogaea*, *PNDREB1* was strongly up-regulated by treatments with low temperature, and also responded to dehydration (Mei et al., 2009). However, Co-*DREBLP1* from hot pepper was rapidly induced by dehydration and high salinity but was not at all affected by cold stress (Hong and Kim, 2005). The expression of *PpDBF1* was also induced by NaCl, cold, drought, and ABA treatments in *Physcomitrella patens* (Liu et al., 2007).

The induction of *DREB/CBF* transcripts is organ-specific and proportional to the length of the stress treatment. *AhDREB1* was highly expressed in roots but less in stems and leaves in response to salt stress (Shen et al., 2003b). *OsDREB1F* was constitutively expressed in almost all the tissues and organs, including young leaves, young roots, mature leaves, mature roots, young panicles, and callus with higher expression in panicles and callus than in the other tissues (Wang et al., 2008). Expression of the *HvDREB1* gene in barley leaves was significantly induced by salt, drought, and low-temperature (Xu et al., 2009). *GmDREBa* and *GmDREBb* were also induced by cold, drought, and salt in leaves of soybean seedlings while expression of *GmDREBc* was high in roots following drought, salt, and ABA treatments (Li et al., 2005).

**Role of DREB2 proteins in drought, salinity, and heat-responsive gene expression**

Among the DRE-binding proteins, the DREB2 subfamily is induced by drought and high-salinity stress indicating their important role in stress-responsive gene expression (Table 2). The *DREB2A* and *DREB2B* were first isolated as cDNAs encoding DRE/CRT-binding protein from *Arabidopsis* (Liu et al., 1998). However, among the eight DREB2-type proteins, *DREB2A* and *DREB2B* were thought to be major transcription factors that function under osmotic stresses (Nakashima et al., 2000; Sakuma et al., 2002). Though *DREB1* genes are widely investigated in many crops in response to different abiotic stresses, the studies did not proceed as rapidly for *DREB2* expression. *DREB2* homologous genes have been isolated from economically important cereal crops also such as rice, wheat, barley, maize, pearl millet, and foxtail millet (Dubouzet et al., 2003; Xue and Loveridge, 2004; Egawa et al., 2006; Agarwal et al., 2007; Qin et al., 2007; Lata et al., 2011a). *DREB2* transcripts were found to be regulated by alternative splicing in barley, wheat, maize, and rice with most of them showing transactivation abilities in yeast or plant cells (Dubouzet et al., 2003; Egawa et al., 2006; Agarwal et al., 2007; Qin et al., 2007). *PgDREB2* from pearl millet was shown to be phosphorylated by total cell extracts and could not bind to DRE/CRT sequence (Agarwal et al., 2007).

The expression of *Arabidopsis DREB2A* and its homologue *DREB2B* were induced by dehydration and high salt stress, but not by cold stress and exogenous ABA (Liu et al., 1998; Nakashima et al., 2000). ABA, mannitol, and cold treatments had little effect on *DREB2C* expression but an elevated level of *DREB2C* mRNA was detected after 250 mM salt treatment (Lee et al., 2010). *OsDREB2A* transcript was induced within 24 h of dehydration and 250 mM salt stress but weakly responded to ABA and cold stress (Dubouzet et al., 2003). A comprehensive analysis of all five *OsDREB2s* from rice revealed that *OsDREB2A* accumulated to the highest levels under the non-stress condition, and its expression was increased slightly by high temperature, drought, and high salinity treatments, but not by low temperature (Matsukura et al., 2010). The *OsDREB2B* transcript level was markedly increased especially after 20 min of high temperature and 24 h of low temperature stress, respectively. The transcript levels of *OsDREB2C* and *OsDREB2E* were low under control conditions and were not strongly induced by the abiotic stresses (Matsukura et al., 2010). Wheat *TaDREB1* and *WDRE2*, maize *ZmDREB2A*, and pearl millet *PgDREB2* are responsive to cold stress as well, whereas foxtail millet *SiDREB2* was not (Dubouzet et al., 2003; Shen et al., 2003a; Egawa et al., 2006; Agarwal et al., 2007; Qin et al., 2007; Lata et al., 2011a). *ZmDREB2A* also responded to high temperature (Qin et al., 2007). Sorghum *SbDREB2* showed induction at 1 h exposure to drought, after which expression gradually dropped to basal levels by 24 h in transgenic rice (Bihani et al., 2011). The transcript level of chickpea *CAP2* increased by dehydration, NaCl, ABA, and

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**Fig. 2.** A typical representation of the induction of abiotic-stress-inducible genes with the CRT/DRE cis-element in their promoters (based on well-known concepts from *Arabidopsis*). Transcription factor modifying enzymes are represented as small triangles. The star corresponds to post-translational modification of DREB2.
Fig. 3. Phylogenetic analysis of AP2/ERF domains of published DREB proteins in NCBI database. The phylogenetic tree was generated by MEGA 4.0 software. Branch lengths indicate distance. A-1 to A-6 indicate the groups proposed by Sakuma et al. (2002). The appended proteins are as follows: Arabidopsis thaliana AtDREB1 (BAA33434), AtDREB1B (BAA33435), AtDREB1C (BAA33436), RAP2.1.
auxin treatments but not by treatments with low temperature, salicylic acid, and jasmonic acid (Shukla et al., 2006). Chrysanthemum *DvDREB2A* was significantly affected by heat, low temperature, drought, ABA, and high salt treatments (Liu et al., 2008). Expression of *Populus eurhacifica* *PeDREB2* was induced by cold, drought, and high salinity, but not by ABA (Chen et al., 2009). The transcript expression of *SbDREB2A* from *Salicornia brachiata* was induced by NaCl, drought, and heat stress (Gupta et al., 2010).

*AtDREB2A* accumulated in roots, stems, and leaves under normal growth conditions (Liu et al., 1998). *DREB2C* expression was observed in mature embryo and the cotyledons of germinating seedlings (Lee et al., 2010). Almoguera et al. (2009) reported that sunflower *HaDREB2* accumulates in all vegetative tissues. Chrysanthemum *DvDREB2A* was expressed in all organs under natural conditions with the highest transcript accumulation in flowers while less accumulation was detected in roots, stems, and young leaves (Liu et al., 2008). *SidREB2*, an A-2 type DREB family gene was expressed in leaves, roots, and young and mature spikelets of foxtail millet suggesting its role in developmental pathways also (Lata et al., 2011a).

**Stress tolerance through over-expressing DREBs**

Functional analyses *in vivo* are important to understand the molecular mechanisms of stress tolerance in plants and also to provide tools that might improve crop productivity. One important way of achieving tolerance to multiple stress conditions is to over-express TFs that control multiple genes from various pathways. In fact, over-expression of several DREB TFs in transgenic plants using various promoters has resulted in plants more tolerant to drought, salt, heat, and freezing stresses (Table 3).

Transgenic *Arabidopsis* plants expressing *DREB1/CBF* or *DREB1/CBF3* under the control of the cauliflower mosaic virus (CaMV) 35S promoter showed strong tolerance to freezing, drought, and high salinity stresses suggesting that DREBs/CBFs target multiple genes (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Kasuga et al., 1999). *DREB1/CBF3* over-expressing lines accumulated osmoprotectants, such as proline and various sugars, under non-stress conditions (Gilmour et al., 2000). Transgenic *Arabidopsis* and rice plants over-expressing *OsDREB1A* were tolerant to low temperatures, high salinity, and drought (Dubouzet et al., 2003; Ito et al., 2006). However, over-expression of both *AtDREB1A* and *OsDREB1A* in *Arabidopsis* caused severe growth retardation under optimal growth conditions. The level of stress tolerance and growth retardation in the transgenic *Arabidopsis* over-expressing *OsDREB1A* was relatively lower compared with *AtDREB1A* which might be due to difference in the number of target stress genes induced (Agarwal et al., 2006).

Over-expression of the *Arabidopsis* DREB1/CBF genes in transgenic *Brassica napus* or tobacco plants induced expression of downstream genes and increased the freezing tolerance of transgenic plants (Jaglo-Ottosen et al., 2001; Kasuga et al., 2004). *LeCBF1-3* (DREB1/CBF homologues) were reported to be present in a tandem array in the tomato genome (Zhang et al., 2004). Out of these three genes only the *LeCBF1* gene was found to be cold-inducible and its constitutive over-expression in transgenic *Arabidopsis* plants induced expression of DREB1/CBF-targeted genes and increased freezing tolerance. On the other hand, over-expression of *CBF1/DREB1B* in tomato has been shown to increase the chilling and drought tolerance of transgenic tomato plants (Hsieh et al., 2002a, b). A constitutive over-expression of either *LeCBF1* or *DREB1A* in transgenic tomato plants, however, did not result in increased freezing tolerance (Zhang et al., 2004).

The over-expression of *DREB1A/CBF3* driven by the stress-inducible rd29A promoter in transgenic wheat improved drought stress tolerance (Pellegrineschi et al., 2004). Similarly, the constitutive over-expression of *CBF3/DREB1A* using the 35S promoter in transgenic rice resulted in increased stress tolerance to drought and high salinity without any growth inhibition or phenotypic aberrations (Oh et al., 2005). The constitutive over-expression of *CBF4* with resultant induction of *cor* genes in *Arabidopsis* displayed higher tolerance for freezing and drought stress (Haake et al., 2002). The 35S:TaDREB1 rice transgenic plants showed a dwarf phenotype, although the same was not observed in the corresponding *Arabidopsis* transgenic plants. Thus, it may be possible that a monocot gene transferred to dicots may not function as effectively as in the monocots (Shen et al., 2003a).

Transgenic tobacco plants over-expressing *PpDBF1* showed higher tolerance to salt, drought, and cold stresses (Liu et al., 2007). The over-expression of the *AhDREB1* gene led to the accumulation of its putative downstream target genes and also conferred a better survival rate to transgenic tobacco plants under salt stress compared with the wild-type plants (Shen et al., 2003b). These results are also consistent with those of other studies (Shen et al., 2003a) in which the over-expression of *DREB1A* genes in *Arabidopsis* and *Brassica napus* resulted in enhanced freezing tolerance, decreased cold sensitivity, and increased stress tolerance in plants.
with the over-expression of OsDREB1F as tolerance to high salinity, drought, and low-temperature is enhanced both in rice and Arabidopsis transgenic plants (Wang et al., 2008).

The over-expression of DREB2A does not result in any phenotypic changes in Arabidopsis transgenic plants, i.e. neither any retardation in growth nor any improved stress tolerance (Liu et al., 1998). This suggested that the DREB2A protein requires post-translational modification such as phosphorylation for its activation (Liu et al., 1998). Busk and Pagès (1998) also reported that phosphorylation may be necessary for the activation of proteins under drought-stress conditions, thus enhancing the DNA-binding activity of several transcription regulators. The central region of DREB2A contains a negative regulatory domain as revealed by its domain analysis using Arabidopsis protoplasts and that the internal deletion of amino acids 136 to 165 makes DREB2A constitutively active. Over-expression of this constitutively active form (DREB2A-CA) resulted in growth retardation in transgenic Arabidopsis plants, up-regulation of many stress-inducible downstream genes, and imparted significant tolerance to drought stress but only slight tolerance to freezing (Sakuma et al., 2006a). The DREB2A-CA-GFP fusion protein showed a stable expression in the nucleus, however, the same was not the case with DREB2A-FL-GFP fusion protein, suggesting that the central region of DREB2A is required for the regulation of its stability (Nakashima et al., 2009).

Although DREB2A and DREB1A were isolated together (Liu et al., 1998), it was found that some of the downstream target genes of DREB2A were different from those of DREB1A (Nakashima et al., 2009). The reason for this may be due to the fact that both DREB2A and DREB1A slightly differ in their DNA-binding specificities. While DREB2A preferentially binds ACCGAC motifs, DREB1A specifically has a high affinity to A/GCCGACNT sequences (Sakuma et al., 2006a). Microarray analysis of transgenic Arabidopsis plants over-expressing DREB2A-CA revealed that its over-expression not only induced drought- and salt-responsive genes but also heat-shock (HS)-related genes. These transgenic plants also showed improved thermotolerance which was significantly decreased in DREB2A knockout plants (Sakuma et al., 2006b). Moreover, it was

<table>
<thead>
<tr>
<th>DREB gene</th>
<th>Transgenic plants</th>
<th>Stress response</th>
<th>References</th>
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found that transient induction of the *DREB2A* occurred rapidly by HS stress, and that the sGFP-DREB2A (synthetic green fluorescent protein-DREB2A) protein accumulated in nuclei of HS-stressed cells. It was also observed that *DREB2A* up-regulated genes were down-regulated in *DREB2A* knock-out mutants under stress conditions (Yamaguchi-Shinozaki and Shinozaki, 2009). Over-expression of *DREB2C* was also found to induce the expression of many HS stress-inducible genes, ensuring thermotolerance of transgenic *Arabidopsis* (Lim et al., 2007). Recently it has been shown that *HsfA3* (an HS TF) regulates expression of many heat-inducible genes downstream of the *DREB2A* stress-regulatory system and functions in acquiring thermo-tolerance under the control of the *DREB2A* transcriptional cascade (Schramm et al., 2008; Yoshida et al., 2008). Recent analyses of genetic responses involved in plant acclimation to high temperature have also indicated that *AtDREB2B*, together with *AtHsfA3*, are the only two TFs that were specifically induced in thermotolerant lines of *Arabidopsis*. Therefore, it appears that *AtDREB2A* and *AtDREB2B* could have some functional redundancy in thermo-tolerance, as mutants for either did not show a defect in heat acclimation (Schramm et al., 2008); however, *dreb2a-1* mutant plants, showed reduced basal thermotolerance when directly treated at 46 °C for 45 min (Larkindale and Vierling, 2008). Taken together, it can be concluded that DREB2 regulons function in both osmotic and heat stress responses.

The over-expression of rice *OsDREB2A* does not result in any phenotypic changes in transgenic *Arabidopsis*, but over-expression of wheat *WDREB2* and maize *ZmDREB2A* caused such changes in transgenic *Arabidopsis* and tobacco (Dubouzet et al., 2003; Kobayashi et al., 2007; Qin et al., 2007). Transgenic *Arabidopsis* plants over-expressing maize *ZmDREB2A* were dwarf, and exhibited improved drought and heat stress tolerance. Microarray analysis of these plants revealed that 28 of 44 up-regulated genes were common with transgenic *Arabidopsis* over-expressing *DREB2A-CA* (Qin et al., 2007). Over-expression of *OsDREB2B* in transgenic *Arabidopsis* showed enhanced expression of *DREB2A* target genes and improved drought and heat-shock tolerance (Matsukura et al., 2010). Transgenic tobacco plants over-expressing *PgDREB2A* exhibited enhanced tolerance to both hyperionic and hyperosmotic stresses. The transgenics also showed higher expression of downstream genes *NiERD10B*, *HSP70-3*, *Hs18p*, *PLC3*, *AP2* domain TF, *THT1*, *LTP1*, *NtHsf2*, and pathogen-regulated (*NiERF5*) factors with different stress treatments (Agarwal et al., 2010).

Recently Bihani et al. (2011) reported that the constitutive expression of sorghum *ShDREB2* driven by the CaMV 35S promoter led to pleiotropic effects in rice and these transgenics did not set seed. However, the *rd29A:ShDREB2* rice plants set seed and the transgenics showed a significantly higher number of panicles compared with the wild-type rice plants. Phenological and agronomic traits were also not affected in *rd29A:ShDREB2* transgenic rice (Bihani et al., 2011). Over-expression of sunflower *HaDREB2* in seeds did not enhance longevity in transgenic tobacco. The constitutively over-expressing *HaDREB2* could not increase thermo-tolerance in seedlings or lead to the accumulation of HSPs at normal growth temperatures. By contrast, when *HaDREB2* and *HaHsfA9* (sunflower Heat Shock Factor A9) were over-expressed together, positive effects on seed longevity were observed, apart from those observed with over-expression of *HaHsfA9* alone (Almoguera et al., 2009). Over-expression of *CAP2* in tobacco improved growth and development, and tolerance to dehydration and salt stress of the transgenic plants (Shukla et al., 2006). Surprisingly, expression of *CAP2* cDNA in yeast (*Saccharomyces cerevisae*) also enhanced heat tolerance, with increased expression of gene for heat shock factor 1 (*Hsf1*) and its target yeast heat shock protein 104 (*Hsp 104*) suggesting strong evolutionary conservation of the stress response mechanisms (Shukla et al., 2009). In another interesting study it was reported that the recombinant *E. coli* cells expressing *SbDREB2A* exhibited better growth in basal LB medium as well as in medium supplemented with NaCl, PEG, and mannitol. The improved growth in recombinant *E. coli* cells could be due to the regulation of stress-regulated functional genes by this TF and certain interactions with transcriptional network in the bacterial cells, thus providing stress tolerance (Gupta et al., 2010).

These observations suggest that the DREB proteins are important TFs in regulating abiotic stress-related genes and play a crucial role in imparting stress tolerance to plants. The *DREB1* and *DREB2* regulons can thus be used to improve the tolerance of various kinds of agriculturally important crop plants to drought, high-salinity, and freezing stresses by gene transfer.

**Mechanisms of DREB gene regulation and stress tolerance in plants**

In the earlier sections, it was discussed that DREB genes are involved in abiotic stress responses and impart stress endurance to plants, but the physiological and biochemical bases of such responses and their activation mechanisms are still a matter of investigation. In *Arabidopsis* a number of regulatory genes which are involved in CBF cold-responsive pathway have been isolated and analysed by combining genetic and molecular approaches (Fig. 2). The gene products which may function directly in transcription are: higher expression of osmotically responsive genes1 (*HOS1*), a negative regulator of *CBFs*; *FIERY2* (*FRY2*), a transcriptional repressor; and low expression of osmotically responsive genes2 (*LOS2*), a positive regulator in the pathway (Yang et al., 2005). A putative *MYC ICE1* (Inducer of CBF expression 1)-like TF may play an important role in activating *CBF1/DREB1B*, *CBF2/DREB1C* and *CBF3/DREB1A* (Chinnusamy et al., 2003). Its activation requires cold-induced phosphorylation, and may be regulated by *HOS1* which targets the ICE1 protein for ubiquitination and subsequent degradation (Dong et al., 2006; Saibo et al., 2009). Further, a SIZ1 (a SUMO E3 ligase)-dependent sumoylation can block ubiquitination of
ICE1 (Miura et al., 2007), where sumoylation is a process that conjugates SUMO (small ubiquitin-related modifier) to a protein substrate. This alteration activates/stabilizes ICE1 protein, which facilitates its activity to control the expression of the CBF3/DREB1A gene (Saibo et al., 2009). The DREB1/CFB genes were also found to be regulated by Ca2+-related processes as mutations in CAX1, a Ca2+/H+ transporter, and CBL1, a Ca2+ sensor, affected the expression of DREB1/CFB genes (Albrecht et al., 2003, Catala et al., 2003). Using a reverse genetics approach, Novillo et al. (2004) showed that CBF2/DREB1C acts as a negative regulator of CBF1/DREB1B and CBF3/DREB1A gene expression as cff mutants were tolerant to drought, salinity, and freezing stresses. Another negative regulator of CBF/DREB genes is MYB15 which interacts with the promoter regions to repress their expression (Agarwal et al., 2006).

The mechanisms by which CBF/DREB1 gene expression in response to low temperature has also been elaborated by Zhao et al. (2006) in Brassica napus providing a new perspective to the regulation mechanisms of the DRE-mediated signalling pathway in cold-stress responses. They have isolated two groups of DREB-like genes namely; Group I and Group II which were induced by low temperature, but Group I expressed earlier than that of Group II. The Group I DREBs could specifically bind to the DRE cis-acting element to activate the downstream genes in Brassica, while Group II DREBs were transcriptionally inactive but retained the ability to bind DRE sequence. Interestingly, the DRE binding ability of the two groups was similar, as revealed by fluorescence quenching assays. The genes of both these groups worked in a competitive manner, where Group II could suppress the trans-activation activity of Group I DREB in a concentration-dependent manner, strongly suggesting that the Group I DREBs express at the early stage of cold stress to switch-on the DRE-mediated signalling pathway, whereas the Group II DREBs express at the later stage of cold stress to switch-off this pathway. Thus the low-temperature response through the CBF/DREB regulon is a tightly regulated mechanism to ward off any negative effects in plants. As a matter of fact, their uncontrolled expression in certain conditions may lead to dwarf phenotypes and reduced yields as well (Saibo et al., 2009).

However, until now, the mechanism of activation of DREB2-type genes is not well-studied. It is assumed that not only transcriptional regulation but post-translational modification like phosphorylation may be necessary for the activation of this class of proteins under stress conditions (Fig. 2). It was also evident from the fact that the over-expression of AtDREB2A and OsDREB2A could not induce target stress-inducible genes (Liu et al., 1998; Dubouzet et al., 2003). The presence of a conserved serine/threonine-rich region adjacent to the AP2/ERF domain may act as a putative site for phosphorylation as mentioned earlier. A negative regulatory domain has been identified in the AtDREB2A under normal conditions, deletion of which makes the protein not only constitutively active under stress conditions but also capable of up-regulating a number of drought, salt or heat-stress-responsive downstream genes (Sakuma et al., 2006a). The authors have suggested the presence of a PEST sequence (RSDASEVTSTSSQSEVCT-VETPGCV) in this negative regulatory domain that contained many phosphorylation target sites for protein kinases like PKC and CK2. Rogers et al. (1986) suggested that the PEST sequence generally acts as a signal peptide and its phosphorylation is necessary for protein degradation (Salmeron et al., 2001). In contrast to Arabidopsis DREB2A protein, maize and pearl millet DREB2A do not contain any PEST sequence (Agarwal et al., 2007; Qiu et al., 2007). An in vitro ubiquitination assay revealed that the DRIPs (DREB2A-interacting protein, C3HC4 RING domain-containing proteins namely DRIP1 and DRIP2) mediate the degradation of DREB2A. The DRIP proteins also function as E3 ubiquitin ligases and are thus capable of mediating DREB2A ubiquitination (Qiu et al., 2008). Over-expression of full-length DREB2A protein was more stable in drip1 than in the wild-type background suggesting DRIP1 and DRIP2 as novel negative regulators in drought-responsive gene expression by targeting DREB2A to 26S proteome proteolysis (Nakashima and Yamaguchi-Shinozaki, 2010).

DREBs are one of the important genes for crop improvement either through engineering stress tolerance or through crop breeding strategies since it is the major TF that binds to the cis-acting elements of most of the osmotic stress-inducible genes responsible for providing osmotic tolerance to the plants under stress conditions (Hussain et al., 2011). As revealed by microarray analyses, most of these target stress-inducible genes contained the conserved DRE or DRE-related core motifs in their promoter regions (Maruyama et al., 2004). Both cDNA and Gene Chip microarrays have revealed more than 40 target genes of DREB1/CFB including TFs, phospholipase C, LEA proteins, KIN (cold-inducible) proteins, sugar transport proteins, desaturase, carbohydrate metabolism related proteins, osmoprotectant biosynthesis proteins, and protease inhibitors known to function in stress response and are thought to be responsible for the observed stress tolerance of the transgenic plants (Fowler and Thomashow, 2002; Maruyama et al., 2004). A cDNA microarray analysis of transgenic Arabidopsis plants over-expressing AtDREB1A revealed that 12 genes expressed 2-fold higher than in the wild-type (Liu et al., 1998), out of which six were stress-related genes namely, rd29A, kin1, cor6.6kin2, cor15a, cor47rld17, and erd10. While the other six genes showed similarity to acclimatization protein, DLI.2, enolase, cysteine proteinase inhibitor, and erd4. A similar study on transgenic Arabidopsis over-expressing OsDREB1A showed 2-fold higher expression of six stress-related genes namely, cor15a, FLO5-2113, rd29A, rd17, AtGolS3, and FLO5-20-N18 (Kasuga et al., 1999; Dubouzet et al., 2003). Both the studies suggested that products of these genes may function in stress tolerance in plants. Several such studies have also revealed that the over-expression of DREB genes driven either by the CaMV 35S or the rd29A promoter led to the accumulation of stress-inducible putative downstream genes such as LEA proteins and heat-shock-related proteins.
genes, thus providing enhanced stress tolerance to plants (Shen et al., 2003a; Oh et al., 2005; Bhatnagar-Mathur et al., 2006; Sakuma et al., 2006a, b; Schramm et al., 2008). The accumulation and activation of these genes have been thought to adapt the plants to stress conditions. Furthermore, elevated contents of osmoprotectants such as free proline, and various soluble sugars like sucrose and raffinose, and metabolites like galactinol and myo-inositol in the over-expressor transgenic plants were also detected, suggesting that the enhanced stress tolerance of the transgenic lines were because of the prompt accumulation of these substances compared with wild type/control plants (Gilmour et al., 2000; Shiqing et al., 2005; Ito et al., 2006).

Since several previous studies have revealed that over-expressing DREBs/CFBs enhances the expression of downstream target genes, especially those that encode for LEA proteins, including dehydrins and COR proteins, a comparison of stress tolerance initiated by DREBs and those initiated by LEA proteins would be particularly interesting at this stage. As the mechanism of stress tolerance initiated by DREBs has already been discussed in this section, the focus here is on the stress tolerance initiated by LEA protein genes. LEA genes are associated with water or cold stress in plants and are active in tissues containing high ABA levels (e.g. seeds) (Tunnacliffe and Wise, 2007). Transgenic rice plants expressing barley HVA1, a Group 3 LEA gene, showed enhanced tolerance to water and salt stress. The transgenics were able to maintain relatively high levels of RWC and suffered less EL from cells suggesting that HVA1 protein was able to protect cell membranes from damage during osmotic stress (Rohila et al., 2002; Babu et al., 2004). Barley HVA1 was also able to confer better growth and higher WUE to transgenic wheat plants (Sivamani et al., 2000). Similar protection against dehydration stress was conferred to transgenic rice expressing Group I and II LEA protein genes from wheat (Cheng et al., 2002). It has also been found that DREB genes regulate the expression of specific LEA genes like COR44B in wheat (Morran et al., 2011). This may be possible due to the fact DREBs/CFBs could possibly regulate the activity of other downstream TFs, which may then regulate the specific expression of LEA genes. Therefore, a change in the expression level of a single DREB/CFB gene could regulate expression levels of other TFs, which, in turn, could lead to the activation of several downstream target genes, thus conferring stress tolerance to plants.

Hence, it can be concluded that DREB genes are the central regulator of abiotic stress responses and tolerance in plants exposed to adverse conditions. Engineering DREBs would regulate the expression of many target osmotic stress-inducible genes as well as up-regulate a group of indigenous stress-responsive pathways that collectively would produce physiological and biochemical adaptations in plants, enabling them to adapt and acclimatize to osmotic stresses. Thus, as a whole, the tolerance level of plants would be enhanced if DREBs are genetically engineered compared with any other stress-inducible genes, making them the popular targets for genetic engineering and crop improvement. In our own laboratory it was recently found that SidDREB2, a DREB2-like gene from foxtail millet is a major QTL for dehydration stress tolerance as it contributes to more than one-quarter of the variation in LP which is an important biochemical marker for oxidative stress in plants (discussed in next section; C Lata, M Prasad, unpublished data).

Role of DREBs in crop improvement through marker-assisted selection

Marker-assisted selection (MAS) provides a strategy for accelerating the process of conventional crop breeding. The tagging of useful genes, such as those responsible for conferring resistance to plants, the synthesis of plant hormones, and abiotic stress, namely drought and salinity tolerance, is a major target for improving crop growth and productivity (Lopez et al., 2003). Plant improvement, either by natural selection or plant breeding has always been based upon creating, evaluating, and selecting the appropriate combination of alleles (Svetlana et al., 2007). Hence, with the use of molecular markers it is now easy to trace valuable alleles either in segregating or natural populations. The efficiency of MAS is influenced by several complex factors, such as recombination between the marker and the candidate gene, a low level of polymorphism between the parents with contrasting traits, and a lower resolution of QTL due to environmental interactions. Marker-assisted breeding is a rapid and accurate method for introgressing any gene from a donor line into the genomic background of a recipient line, providing a very effective tool for marker-assisted backcross (MABC) breeding (Collard and Mackill, 2008). A schematic representation of MABC scheme is shown in Fig. 4. Among the various DNA-based markers, SNPs can serve as a powerful tool for MAS and map-based cloning since they are highly stable markers and often contribute directly to a phenotype (Anderson and Lübberstedt, 2003; Kim et al., 2005). Among the several SNP genotyping methods, allele-specific PCR largely fulfils the basic requirements for MAS because of its simplicity, user friendliness, cost effectiveness, and reproducibility. Introduction of additional mismatch bases has played an important role in enhancing the specificity of this technique for an accurate discrimination of different alleles in MAS (Drenkard et al., 2000).

Some of the recent studies have shown the importance of DREB TFs in marker-aided breeding and crop-improvement strategies. Functional markers in common wheat (Triticum aestivum) were designed based on genome-specific primers for each of the orthologous Dreb1 loci on chromosomes 3A, 3B, and 3D to represent locus-specific differences, and the Dreb-B1 locus was mapped on the long arm of chromosome 3B (Wei et al., 2009). This genetic mapping of Dreb-B1 on chromosome 3B may be useful in wheat breeding programmes aimed at improving its drought tolerance. In another study, drought-tolerant alleles developed for DREB2 in common wheat produced clear, strong, reproducible signals, which were easy to score in a segregating population (Bibi et al., 2010). In our laboratory, a synonymous SNP at the 558th bp position

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(A/G transition) has been identified in the SiDREB2 gene from dehydration-tolerant and -sensitive cultivars in foxtail millet (Lata et al., 2011a). The dehydration-tolerant cultivars had an ‘A’ while the sensitive cultivars had a ‘G’ allele. Based upon this sequence analysis, locus-specific (LSM) and allele-specific markers (ASM) were developed (Lata et al., 2011a) and the association was studied further in an additional 61 and 101 dehydration-tolerant and -sensitive cultivars, respectively (C.Lata, M. Prasad, unpublished data). It is suggested that the ASM is tightly linked with LP, as the SiDREB2-associated trait contributed to 27.2% of the total variation in LP among the 170 cultivars (C. Lata, M. Prasad, unpublished data; and data not shown). The fact that the above ASM controlled more than one-quarter of the total variation in LP, it can be considered as a major QTL for dehydration tolerance in foxtail millet. Further, the ASM is part of the candidate gene, thus it eliminates the main disadvantage of MAS and, with the help of this marker, dehydration-tolerant cultivars can be selected at any stage. Furthermore, allele mining is a technique that exploits the DNA sequences of one genotype to isolate useful and valuable alleles from related genotypes. Such studies could also provide the foundation for plant breeding and translational genomic approaches (Latha et al., 2004). Therefore, the SiDREB2-ASM would facilitate allele-mining of foxtail millet germplasm resources, thus leading to the identification, utilization, and introgression of newer alleles in crop improvement.

**Conclusions**

Understanding the molecular mechanisms of plant responses to abiotic stresses such as, drought, salinity, heat, and cold is very important as it helps in manipulating plants to improve stress tolerance and productivity. In response to these stresses, many genes are regulated mainly by TFs and their gene products function in providing stress tolerance to plants. One such class of the TFs is DREB/CBF that binds to DRE cis-acting elements. In this review, we have summarized that DREB genes are important plant TFs that regulate various stress-responsive gene expression. They play a key role in providing tolerance to multiple stresses, generally in an ABA-independent manner through DRE/CRT cis-elements and the AP2/ERF DNA binding domain. The DREBs can be genetically engineered to produce transgenics with higher tolerance to drought, salinity, heat, and cold using different promoters. Functional analysis of DREB TFs will provide more information on the complex regulatory networks involved in abiotic stress responses and the cross-talk between different signalling pathways during the adaptation of plants to various abiotic stresses. In addition, considering DREBs as candidate genes and developing proper functional markers, which could eventually be used for MAS and allele-mining in breeding programmes, will lead us to develop crop varieties superior in stress tolerance by genetic manipulation.

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