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Review

Regulation of plant stress response by dehydration responsive element binding (DREB) transcription factors

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Plant growth and productivity are greatly affected by environmental stresses such as dehydration, high salinity, low temperature and biotic pathogen infection. Plant adaptation to these environmental stresses is controlled by cascades of molecular networks. The dehydration responsive element binding (DREB) transcription factors, which specifically interact with C-repeat/DRE (A/GCCGAC), play an important role in plant environmental stress tolerance by controlling the expression of many stress related genes. This review specifically focused on the structure characteristics of DREB proteins and their roles in regulating abiotic and biotic stress tolerance in plants. The DREB proteins are also involved in phytohormones signaling pathway such as abscisic acid, salicylic acid, jasmonate acid, ethylene and gibberellic acid. In addition, this review summarized the progress of the genetic engineering of DREB transcription factors in the main crops and model plants.

Key words: Abscisic acid, biotic stress, DREB transcription factor, environmental stress, signaling pathway, transgenic crop.

INTRODUCTION

The environmental stresses such as drought, high-salt, cold and pathogens present a major challenge in our quest for sustainable food production as it reduces the potential yields in crop plants. Under serious condition, these adverse environmental stresses can result in death of plants. Surviving such stresses led plants to acquire mechanisms by which they can sensitively perceive incoming stresses and regulate their physiology accordingly over a long evolutionary scale. Deciphering, the mechanism by which plants perceive environmental signal is of critical importance for the development of rational breeding and transgenic strategies. Recent studies are focused on plant molecular responses and signal transductions when subjected to stress factors. According to the recent reports, many plant genes are induced by biotic and abiotic factors. These inducible genes are classified into functional and regulatory proteins according to the functions of their encoding products. The regulatory proteins are involved in the regulation of signal transduction and gene expression. These regulatory proteins including the most research transcription factors can interact with cis-elements present in the promoter region of various stress-related genes and thus, regulate the expression of many genes resulting in the adaptation to stress factors. Recent researches identified several transcription factors that are important in regulating plant responses to different stresses (Bohnert et al.,

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2001; Seki et al., 2001; Zhu et al., 2001; Agarwal et al., 2006). Dehydration responsive element (DRE)/c-repeat sequence (CRT) core sequence are present in a number of environmental stress responsive genes promoter (Yamaguchi-Shinozaki and Shinozaki, 1994; Baker et al., 1994; Stockinger et al., 1997; Liu et al., 1998). One class of transcription factors that is ethylene responsive factor (ERF) proteins is involved in biotic and abiotic stress response. The transcription factor binding with the DRE/CRT has been named as DRE binding factor (DREB) or c-repeat binding factor (CBF). The dehydration responsive element binding (DREBs) belong to the ERF family of transcription factors consisting of two subclasses, DREB1/CBF and DREB2, which are induced by cold and dehydration, respectively. The DREB protein is part of a plant-specific family of transcription factors that play important roles in regulating the expression of genes (*rd29A*, *kin1* and *erd10*) in response to a variety of abiotic and biotic stresses (Dubouzet et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2005; Agarwal et al., 2006). DREBs contain a conserved apetala2/ethylene response factor (AP2/ERF) domain of approximately 60 amino acids (Stockinger et al., 1997). DREB genes form a large multigene family and can be classified into six small groups named as A-1 to A-6 (Sakuma et al., 2002). The first isolated DREB family member was CRT/DRE-binding factor 1 (CBF1) which response to low temperature and water deficit from *Arabidopsis* (Stockinger et al., 1997). In the last few years, many DREB cDNA*s* have been cloned and characterized in different economic plants, including rice (*Oryza sativa*) (Dubouzet et al., 2003; Tian et al., 2005), soybean (*Glycine max*) (Li et al., 2005), maize (*Zea mays*) (Kizis and Pages, 2002; Qin et al., 2004), cotton (*Gossypium hirsutum*) (Huang and Liu, 2006a, b), *Hordeum vulgare* (Choi et al., 2002; Xu et al., 2009), wheat (*Triticum aestivum*) (Xu et al., 2008), *Populus euphratica* (Chen et al., 2009), and *Caragana korshinskii* (Wang et al., 2010). In worldwide growth and productivity of crops, especially those primary crops such as rice and wheat, always suffer from dehydration, freezing and other stress factors. Major efforts have been made to develop stress-tolerant lines or cultivars of crops using conventional plant breeding. However, the complexity of the tolerance mechanisms, lack of selection criteria and variation in responses of plants at different developmental stages have resulted in only limited success. 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. DREB transcription factors play an important role in plant environmental stress tolerance by controlling the expression of many stress related genes. The roles of the DREB gene involved in abiotic stresses include salt, drought and extreme temperatures. What more, plants also must respond to biotic stress such as pathogen infection by activating a defense mechanism. Interestingly, recent research results showed that, DREB TFs also has an upstream regulatory role in mediating physiology signaling pathways for biotic stress responses. In this article, recent progress on the DREB proteins and their roles in regulating abiotic and biotic stress tolerance in plants was reviewed. 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 the future.

STRUCTURE CHARACTERISTICS OF DREB TFs

DREB transcription factors (TFs) are a class of transcriptt-ion factor belonging to the family of AP2/ERF transcription factor, which is involved in plants response to drought, high salinity, low-temperature and other environmental stresses. DREBs consist of a conserved AP2/ERF domain of approximately 60 amino acids (Figure 1) (Stockinger et al., 1997). According to the structure characteristic of DREB transcription factors, the subfamily of DREB transcription factors can be further divided into six subgroups stem A-1 to A-6 (Sakuma et al., 2002) (Figure 1). DREB members of different subgroups play multiple roles in plants. Amino acid alignment of DREB proteins from varieties of plants show highly sequence similarity in the AP2/EREBP middle domain of the proteins (Figure 1), which are the significant characteristics of plant DREBs. However, here is a lower sequence similarity both in the N-terminal region and C-terminal acidic domain. The subgroups of the DREB proteins, except A-4 and A-5, have a highly conserved motif at the end of the proteins sequences, which are LWSY (A-1), GDDGFSLFxY (A-2), GSIWDxxDPFF (A-3) and KYPSxEIDW (A-6), respectively (Figure 1) (Agarwal et al., 2006; Xiong and Fei, 2006). The middle of DREB transcription factors has a conserved Ser/Thr-rich region adjacent to the AP2/EREBP DNA binding domain. This region may be probably phosphorylated under dehydration conditions (Agarwal et al., 2006; Liu et al., 1998). The proteins of A-1 subgroup of DREB transcription factors are distinctly different from the other five subgroups DREB proteins. It is a response to low temperature and two short polypeptide sequences flank the AP2/EREBP domain on both sides. The nuclear localization signal (NLS) sequence PKK/RPAGRxKFxE-TRHP nestles up to the AP2/EREBP domain in the upstream and the motif DSAWR in the downstream (Jaglo et al., 2001). These "signal sequence" only specific exists in the member of cold-induced DREB A-1 subgroup. This is the striking feature of the A-1 subgroup of DREB proteins (Jaglo et al., 2001). Similar to A-1 DREB proteins, A-2 and A-3 possess a PKK-like NLS sequence RKxPAKGSKKGCMxGKGGPENxx and RKxxxxKGGPx-NxKF snuggle up to the AP2/EREBP domain in the upstream but different to A-1 DREB TFs, they do not have a conserved motif closely the C- terminal AP2 domain.

DREB TFs can identify and bind the CRT/DRE cis-acting element (A/GCCGAC) in the promoters and

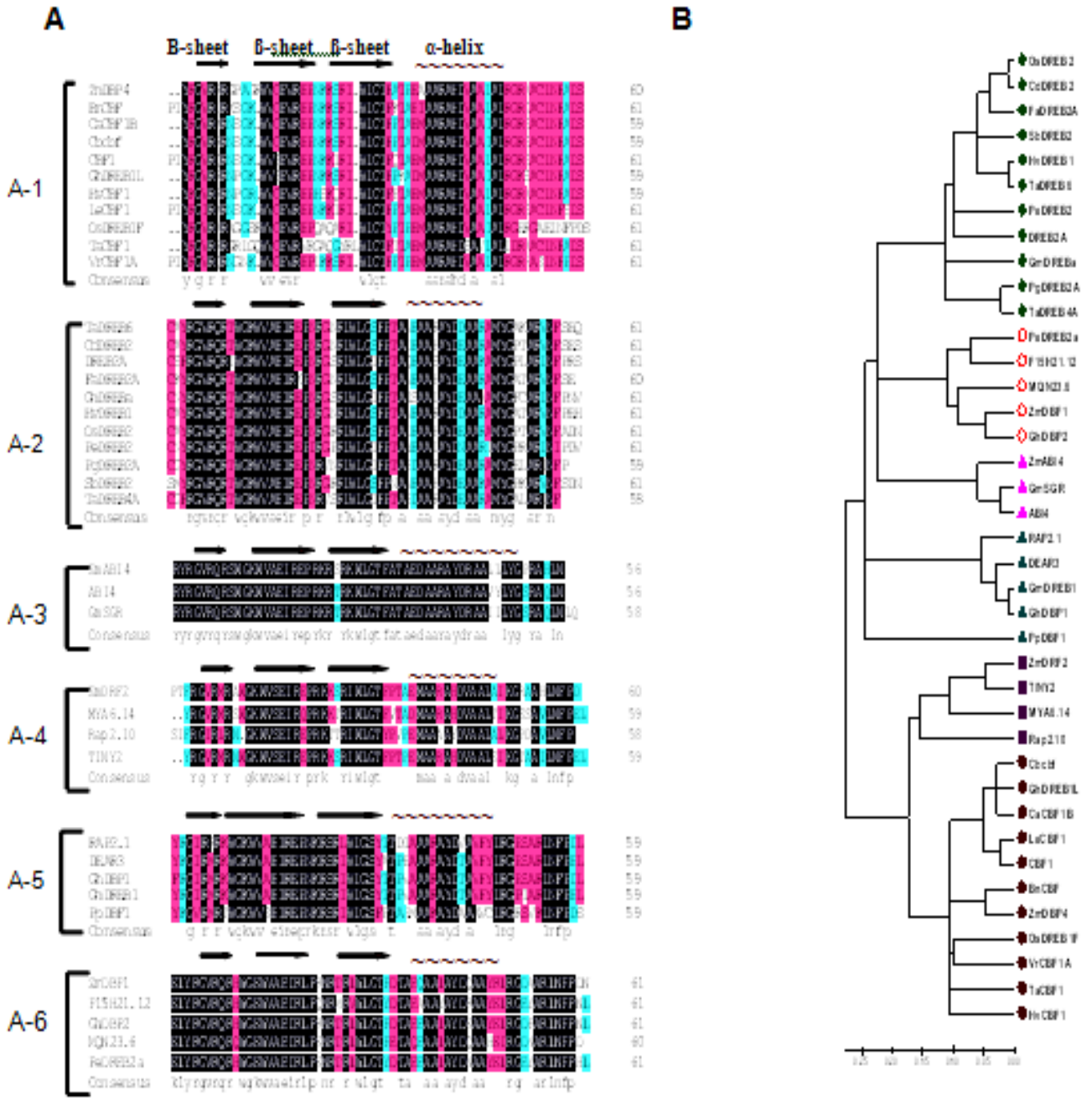

Figure 1. Alignment and phylogenic tree of EREBP/AP2 domain amino acid sequences of DREB TFs. (A) Comparison of the deduced EREBP/AP2 domains of DREB proteins from *A. thaliana*, *Z. mays*, *O. sativa*, *H. vulgare*, *T. aestivum*, *C. bursapastoris*, *C. annuum*, *L. esculentum*, *B. napus*, *G. hirsutum*, *V. riparia*, *G. max*, *C. dactylon*, *T. aestivum*, *S. bicolor*, *F. arundinacea*, *P. glaucum*, *P.patens* and *P. euphratica*. The three-dimensional structure of EREBP/AP2 domains was analyzed by CPHmodels 3.0 Server and Swiss-PDBViewer 4.0.1. Sequences information of *A. thaliana* DREB was taken from the Arabidopsis Information Resource (TAIR). The accession numbers of other plants proteins are as follows: ZmDBP4, NP_001170481.1; BnCBF, AAL38243.1; CaCBF1B, AAQ88400.1; Cbcbf, AAR26 658.1; GhDREB1L, ABD65473.1; HvCBF1, AAX23686.1; LeCBF1, AAK57551; OsDREB1F, AAX23723; TaCBF1, AAL37944.1; VrCBF1A, AAR28671.1; PeDREB2, ABL86587.1; OsDREB2, AAN02487.2; HvDREB1, AAY25517.1; GmDREBa, AAT12423.1; CdDREB2, AAS46285.1; TaDREB6, AAX13289.1; SbDREB2, ABD66654.1; FaDREB2A, CAG30549.1; PgDREB2A, ABB05044.1; TaDREB4A, AAX13282.1; GmSGR, ABS30430.1; ZmABI4, AAM95247.1; ZmDRF2, AAM80485.1; PpDBF1, ABA43687.2; GmDREB1, AAP47161.1; GhDBP1, AAO43165.1; ZmDBF1, AAM80486.1; GhDBP2, AAT39542.1; PeDREB2a and ABU86872.1. (B) A phylogenetic tree of AP2/EREBP domains of different higher plants DREB TFs. Alignment was constructed by cluster W and the neighbor-joining method was used to generate the phylogenetic tree using the MEGA program. The scale under the phylogenetic tree indicates branch lengths. The dark red dots indicate A-1; the green diamonds represent A-2; the pink triangles show the A-3; the plum red squares stand for A-4; the blue triangles display A-5; the red circles represent A-6.

regulate the expression of genes involved in environmental stress in higher plants (Sakuma et al., 2002). OsDREB1A specifically binds DRE-related core binding motif GCCGAC more preferentially than to ACCGAC unlike AtDREB1A, which shows efficient binding to both ACCGAC and GCCGAC (Dubouzet et al., 2003). Seven key residues are involved in highly specific interactions with CRT/DRE element in AP2/EREBP domain. They are four Arg (R) residues, two Trp (W) residues and one Val (V) residue, respectively (Allen et al., 1998). Two important elements YRG and RAYD located within AP2/EREBP domain can bind with promoter sequence or some other interacted proteins (Okamuro et al., 1997). YRG element is the basic hydrophilic area in the N-terminal region of the AP2/EREBP domain, containing 19 to 22 amino acids. Protein three-dimensional analysis showed that the AP2/EREBP domain solution structure consists of three-stranded anti-parallel β-sheets and α-helix running almost parallel to the β-sheet. The first two β-sheets belong to YRG element and the V14 and E19 residues in the second β-sheet play an important role in the binding to the DRE cis-elements (Wang et al., 2008; Sakuma et al., 2002). The mutation experimental results revealed that the E19 residue mutation did not affect the binding between the DREB1A transcription factor and DRE cis-element, but the V14 site mutation led almost to the lost of the activity of DREB1A protein binding to DRE cis-element (Cao et al., 2001). Meanwhile, recent studies have indicated that E19 is not conserved in DREB1 from rice and barley and is instead replaced by valine (Dubouzet et al., 2003). In most OsDREB1-type proteins, valine is found at both the 14th and 19th positions, with the exception of OsDREB1C. The other DREB1-type proteins in monocots (barley, wheat and rye) also have a valine in the 19th position (Agarwal et al., 2006). Moreover, through sequence alignment of DREB-like proteins in *Arabidopsis thaliana* database and other species plants, it was found that the 14th sites were all valine while there will not always be glutamate in the 19th sites, suggesting that V14 plays a more important role in the transcription factors binding to the DRE cis-acting element than E19 (Figure 1). Another element RAYD in the C-terminal of AP2/EREBP domain is 42 to 43 amino acids in length. A highly conserved 18 amino acid core region of RAYD is predicted to form an amphipathic α-helix in the AP2 domains (Okamuro et al., 1997). Research shows that, the conserved A37 in the α-helix of AP2/EREBP domain might play a crucial role in the DNA binding or the stability of the AP2/EREBP domain (Liu et al., 2006). A β-sheet, that is the third one sheet in the AP2/EREBP domain containing WLG motif, belong to RAYD. The main function of RAYD element is to regulate the special binding activity of DREB transcription factors by influencing the conformation of the YRG element or interacting with other proteins (Okamuro et al., 1997).

The constitutive over expression of DREB1A in transgenic *Arabidopsis* induced strong expression of downstream stress-responsive genes under unstressed conditions, enhancing freezing and dehydration tolerance (Liu et al., 1998). However, constitutive over expression of DREB2A in transgenic *Arabidopsis* was not sufficient for the induction of stress inducible genes. The domain analyses of *Arabidopsis* DREB2A gene revealed the presence of negative regulatory domain in the central region (136 to 165 aa); deletion of this region transforms DREB2A to a constitutive active form DREB2A-AC (Sakuma et al., 2006). Sakuma and colleagues reported the presence of the PEST sequence (RSDASEVTSTSSQSEVCTVETPGCV) in the negative regulatory domain consisting of many phosphorylation target sites for protein kinases such as PKC and CK2 (Sakuma et al., 2006). The PEST sequence acts as signal peptide for protein degradation (Rogers et al., 1986). The phosphorylation of PEST sequence has also been reported to be important for protein degradation (Salmeron et al., 2001). It is suggested that, this region is an inhibitory domain in the normal condition and is modified under salt/drought stress. However, in contrast to *Arabidopsis* no PEST sequence was found in PgDREB2A from *Pennisetum glaucum*; PgDREB-2A is a phosphorprotein. The phosphorylation of PgDREB2A *in vitro* by *P. glaucum* total cell extract occurs at threonine residue(s). The phosphorylated PgDREB2A did not bind to the DREs. The dephosphorylated PgDREB2A could bind specifically to ACCGAC core element of DRE from the *rd29A* promoter. This indicates that, stress induction of genes could occur via post-translational modification by phosphorylation of DREB2A (Agarwal et al., 2007; Agarwal et al., 2010). In addition, except the conserved amino acids that have been mentioned earlier, there are several other invariant amino acid residues within or outside the AP2/EREBP domain. They may play an important role that is not yet known, in the conformation or function of those DREB proteins.

Every DREB TFs has one AP2 domain within the different protein sequences, and it is about 60 amino acids showing high sequence similarity between each other's (Figure 1). With wide studies in the field recently, the AP2 domain was surprisely found in other proteins outside the plant kingdom, for example, in the bacteria, bacteriophage and ciliate which also encode site-specific endonucleases (Wuitschick et al., 2002; Magnani et al., 2004; Wuitschick et al., 2004). Those phenomena may indicate that, the AP2 domain in plants probably originated from bacteria or viruses in horizontal gene transfer through transposition and homing processes (Magnani et al., 2004). Sequence analysis of all the DREB1 genes showed that they are intronless genes and duplication of them may have produced a small multigene family during species evolution (Haake et al., 2002).

THE ROLE OF DREB TFS IN PLANT STRESS RESPONSE

Plants undergo continuous exposure to various stresses

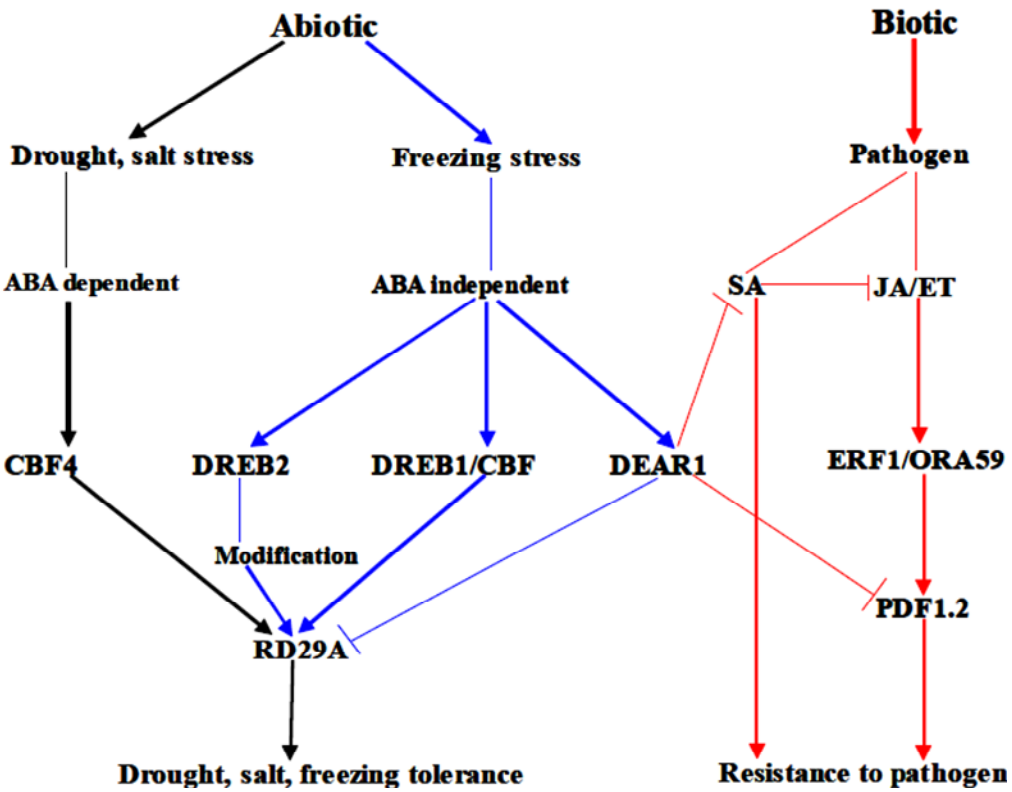

Figure 2. Simplified model for the regulation of abiotic and biotic responsive gene expression.

in their natural environment. In order to survive such a wide variety of stress conditions, ranging from biotic pathogen infection to abiotic low temperature, plants have evolved intricate mechanisms to respond and adapt to these stresses at the molecular, cellular and whole-plant level. Plants perceive the extra-cellular signals at molecular level followed by signal transduction and gene expression and these changes may finally lead to stress tolerance. Both forward and reverse genetic approaches have elucidated genes and gene products that are involved in gene expression and signal transduction of stress tolerance. In the post-genomics era, comprehensive analyses using functional genomics technologies such as transcriptomics, proteomics and metabolomics have increased the understanding of the complex regulatory networks associated with stress adaptation and tolerance. Molecular analysis of stress tolerance may be useful in improving stress tolerance in economic crops using transgenic plant techniques.

Abiotic stress tolerance

Abiotic stress negatively influences survival, biomass production and productivity of crops. Plants being sessile are strongly influenced by abiotic stress such as high salt, drought, freezing and thermotolerance. Drought, salinity and extreme temperatures are the major problems for agriculture because they prevent plants from exploiting their full genetic potential. The DREB protein specifically binds to the dehydration responsive element and is part of a plant-specific family of transcription factors that play important roles in regulating the expression of genes in response to a variety of abiotic stresses (Figure 2). DREB genes form a large multigene family and can be classified into six small groups named as A-1 to A-6 (Sakuma et al., 2002). DREB members of different subgroups play multiple roles in plants. Expressions of the A-1 group genes are induced by low temperature, but not by drought or high salt stress (Liu et al., 1998; Shinwari et al., 1998), while A-2 group genes are regulated by salt and drought, but not by cold (Dubouzet et al., 2003; Liu et al., 1998; Nakashima et al., 2000). However, recent reports have shown some conflicts with respect to these trends. Some A-2 group genes are also induced by ABA or cold (Xu et al., 2008; Chen et al., 2009), indicating crosstalk between different groups. Until now, most reports about DREB/CBFs focused on DREBA1 and A2, while investigation into other groups was limited. TINY2 (A-4), PpDBP1 (A-5), GhDBP1 (A-5) and ZmDBP1 (A-6) were identified as stress-response regulation genes (Kizis and Pages, 2002; Wei et al., 2005; Huang and Liu, 2006b; Liu et al., 2007).

Drought and soil salinity are among the most damaging abiotic stresses affecting today's agriculture. It is understandable that plants are under periodic water stress because of the unpredictable nature of rainfall. Salt stress

may also occur in areas where soils are naturally high in salts and/or where irrigation, hydraulic lifting of salty underground water or invasion of seawater in coastal areas bring salt to the surface soil that plants inhabit. Plants have evolved mechanisms that allow them to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope with them. Many genes that are induced by abiotic stress have been identified. Although, the signaling pathways responsible for the activation of these genes are largely unknown, transcriptional activation of some stress responsive genes is well understood, owing to studies on the RD29A/COR78/LTI78 (responsive to dehydration/cold-regulated/ low-temperature-induced) gene. The promoter of this gene contains both an ABRE (abscisic acid responsive element) and a DRE/CRT (Yamaguchi-Shinozaki and Shinozaki, 1994). ABRE and DRE/CRT are cis-acting elements that function in abscisic acid (ABA)-dependent and ABA-independent gene expression in response to stress, respectively. Transcription factors belonging to the AP2/ERF family that bind to DRE/CRT were isolated and termed DREB1A/CBF3 (DRE-binding protein/C-repeat-binding factor), DREB1B/CBF1 and DREB1C/CBF2 (Stockinger et al., 1997; Liu et al., 1998). DREB2A and DREB2B transcription factors are induced by dehydration stress and promote the expression of various genes involved in drought stress tolerance (Liu et al., 1998). Transcription of GmDREBa/b was induced by cold, salt and drought in soybean leaves. In the roots, the expression of GmDREBc was upregulated by salt, drought and ABA treatment (Li et al., 2005). Expression of CkDBF (*Caragana korshinskii*) was shown to be upregulated by high salt, dehydration, low temperature and the phytohormone abscisic acid (ABA) (Wang et al., 2010). In different wheat cultivars, the TaDREB1 (*Triticum aestivum L.*) gene is induced by low temperature, salinity and drought (Shen et al., 2003). Expression of the *HvDREB1* (*Hordeum vulgare*) in barley leaves, a member of the A-2 subgroup of the DREB subfamily, was significantly induced by salt, drought and low-temperature. In contrast to most A-2 subgroup members in *A. thaliana*, *HvDREB1* also responded to exogenous ABA (Xu et al., 2009). Expression analysis revealed that, *OsDREB1F* was induced by salt, drought, cold stresses and also ABA, but not by pathogen, wound and H_2O_2 (Wang et al., 2008). The *ZmDBP3*, a member of the A-1 subgroup of the CBF/DREB subfamily, was highly activated by cold and moderately by salt (Wang et al., 2009). The *PgDREB2A* (*P. glaucum*) transcript was up-regulated in response to drought within 1 h of the treatment, whereas the induction was delayed in response to cold and salinity stress. However, during cold stress, the transcript was induced more when compared with drought and salinity (Agarwal et al., 2007). Quantitative real-time polymerase chain reaction amplification (QRT-PCR) experiments showed that, expression level of *DvDREB2A* was significantly affected by heat, low temperature, drought, abscisic acid (ABA) and high salinity treatments (Liu et al., 2008). RNA gel-blot analysis showed that, expression of the *GhDBP1* (Dehydration responsive element binding proteins) (*G. hirsutum*) was mainly induced under osmotic stresses conditions such as drought and high salinity (Huang and Liu, 2006b). Semi-quantitative RT-PCR indicated that, the *GhDBP2* (*G. hirsutum*) transcripts, a member of the A-2 subgroup of the DREB subfamily, were greatly induced by drought, NaCl, low temperature and ABA treatments in cotton cotyledons (Huang et al., 2008).

Low temperatures are major factors limiting the geographical locations suitable for growing crops and horticultural plants and periodically account for significant losses in plant productivity (Thomashow, 1999). Improving the cold hardiness of crop plants is an important goal in agriculture and thus, demands a clear understanding of the cold-stress signal perception and transduction. Toward this goal, many genes induced by cold stress were isolated in plants. Transcriptional control of the expression of the cold regulated (*COR*) genes is a crucial part of the plant response to cold stress. The research carried out in the past few years has been productive in identifying transcription factors that are important for regulating plant responses to cold stress. One class of such factors that was proved to regulate expression of the cold-responsive genes is the DREB1/CBF subfamily of the AP2/EREBP transcription factors. Three DREB1/CBF genes, namely *CBF1* (*DREB1b*), *CBF2* (*DREB1c*) and *CBF3* (*DREB1a*), have been isolated from *Arabidopsis* (Stockinger et al., 1997; Gilmour et al., 1998; Liu et al., 1998). These DREB1/CBFs all contained the AP2/EREBP DNA binding-domain, which can recognize the CRT/DRE. CRT/DRE elements, have a conserved 5-bp core sequence of CCGAC and are essential for the low-temperature responsiveness of many cold-regulated (*COR*) genes, including the *Arabidopsis* gene *COR15a* (Baker et al., 1994), the *Brassica napus* gene *BN115* (Jiang et al., 1996) and the wheat gene *WCS120* (Ouellet et al., 1998). Under normal conditions, neither *CBF* nor *COR* genes are expressed. However, when treated with cold (4°C), the expressions of the *CBF* genes are induced very early, followed by the expression of CBF-regulated target genes. In *Brassica napus*, two groups of DREB transcription factors are present, named group I and II, functioning as trans-active and trans-inactive proteins, respectively. The two groups of genes were both induced by low temperature. Group I DREBs were expressed at the early stage of cold stress to open the DRE-mediated signaling pathway in cold stress, whereas, the trans-inactive group II DREBs were expressed at the later stage to close the signal pathway in a competitive manner (Zhao et al., 2006). Northern analysis showed that, the transcripts of *Os-DREBL* (*O. sativa*) accumulated rapidly (within 30 min) in response to low temperature, but not in response to ABA, NaCl and dehydration treatments (Chen et al., 2003). In plants, transcriptional repressors containing the ERF-associated amphiphilic repression (EAR) motif have

been reported to play important roles in modulating plant stress and defense responses. The latest report showed that AtRAP2.1 is a DREB-type, EAR-motif-containing transcriptional repressor that negatively regulates plant responses to cold and drought stresses. RAP2.1 is transcriptionally activated by drought and cold stresses and binds to the DRE/CRTs in the promoters of *RD/COR* genes, repressing the stress-induced expression of such genes (Dong and Liu, 2010).

Plants' responses and acclimation to temperature stress have been precisely characterized by metabolite profiling. A recent metabolome analysis showed that common metabolites play a prominent role for the DREB1/CBF transcriptional network in the cold response pathway (Cook et al., 2004; Maruyama et al., 2009). In *Arabidopsis* and rice, the DREB1/CBF cold-response pathway is one of the well-characterized genetic systems in cold-responsive gene expression and acclimation (Yamaguchi-Shinozaki and Shinozaki, 2006). Metabolome analysis of transgenic *Arabidopsis* overexpressing *DREB1A/CBF3* revealed that, there is a striking similarity between the low-temperature regulated metabolome (monosaccharides, disaccharides, oligosaccharides and sugar alcohols) and by the DREB1A/CBF3 transcription factor (Cook et al., 2004; Maruyama et al., 2009). In particular, the low-temperature-inducible accumulation of galactinol and raffinose is correlated with the expression of the galactinol synthase gene (*GolS3*), which is a direct target of DREB1A/CBF3 (Cook et al., 2004; Maruyama et al., 2009). In addition, through analysis of increased metabolites with those in cold-treated plants, those accumulated in the *DREB1A*-overexpressing transgenic plants and *DREB2A-CA*-overexpressing transgenic plants, *DREB2A* over expression did not increase the level of any low temperature regulated metabolites but increased the tolerance to freezing stress in transgenic plants (Maruyama et al., 2009). It was previously reported that over expression of *DREB2A-CA* in transgenic plants increased their tolerance to dehydration stress, but only slightly increased their tolerance to freezing stress (Yamaguchi-Shinozaki and Shinozaki, 2006). These results indicate that, the increased tolerance to freezing stress in transgenic plants overexpressing *DREB1A* may depend on the accumulation of low-temperature regulated metabolites, especially for sucrose, raffinose, galactinol and myo-inositol.

Recent reports showed that, sunflower HaDREB2 (*Helianthus annuus*) binds to the DRE/CRT element within the *Hahsp17.6G1* (heat stress protein from sunflower) promoter, where it enhances *Hahsp17.6G1* expression through a synergistic interaction with HaHSFA9 (sunflower heat stress factor A9) (Díaz-Martín et al., 2005). Furthermore, a genome-wide *in silico* analysis found that DRE/CRT-containing genes were significantly more likely to be induced by heat shock (Geisler et al., 2006). In transgenic *Arabidopsis* plants, over expression of *Arabidopsis DREB2A-CA* or corn *ZmDREB2A* was found to activate the expression of many heat stress inducible genes such as *HSPs* and *HsfA3* (heat shock factor A3) and their induction resulted in enhanced thermotolerance (Sakuma et al., 2006; Qin et al., 2007). Over-expression of *DREB2C cDNA* enhances thermotolerance in transgenic *Arabidopsis* and up-regulates heat stress related genes via a DRE/CRT (A/GCCGAC) motif in their promoter regions. Yeast one-hybrid assays and *in vitro* electrophoretic mobility shift assays further showed that, DREB2C interacts with two DREs located in the *HsfA3* promoter with a binding preference for the distal DRE2 (Chen et al., 2010). Interestingly, DREB2C could interact with a basic leucine zipper protein ABF2 cooperate to activate the transcription of an ABA-responsive gene (Lee et al., 2010). These results indicate that, the DREB2 subgroup of genes play crucial roles in thermotolerance mechanisms governed by DRE/CRT elements and deduced that DREB2C is a component of the heat stress tolerance response (Lim et al., 2007; Chen et al., 2010). The *SwDREB1* (sweet potato) transcript is induced under various abiotic stress conditions such as dehydration, chilling, salt, methyl viologen (MV) and cadmium (Cd) treatment, whereas, it did not respond to abscisic acid (ABA) or copper (Cu) treatment. The results indicate that, SwDREB1 may be involved in the process of the plant response to diverse abiotic stresses through an ABA-independent pathway (Kim et al., 2008). However, the latest report showed that, LbDREB (*Limonium bicolor*) mediates molecular and physiological responses to copper stress in transgenic tobacco (Ban et al., 2010).

Biotic stress tolerance

Generally, plants in the field are not subjected to only abiotic stress at a time, but also respond to biotic stress such as pathogen infection by activating a defense mechanism known as plant immunity. Recent research results showed that, DREB TFs also has an upstream regulatory role in mediating crosstalk between signaling pathways for biotic and abiotic stress responses (Figure 2). Some recent reports have highlighted the connection between disease resistance and drought tolerance. 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). Over-expression of *Adr1* conferred significant tolerance to drought but not for thermal and salt stress. 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. Meanwhile, the *Arabidopsis DEAR1* (DREB and EAR motif protein 1) gene encodes a protein containing significant homology to the DREB1/CBF

domain and the EAR (ethylene response factor-associated amphiphilic repression) motif. The *DEAR1* transcript responses to both pathogen infection (*Pseudomonas syringae*) and cold treatments. Transgenic *Arabidopsis* overexpressing *DEAR1* (DEAR1ox) showed lesion-like cell death, together with constitutive expression of *PR* genes and accumulation of salicylic acid. DEAR1ox also showed more limited *P. syringae* pathogen growth when compared with the wild-type, consistent with an activated defense phenotype (Tsutsui et al., 2009). However, the DEAR1 protein also could repress DRE/CRT-dependent transcription, which is regulated by low temperature. This means that DEAR1 has an upstream regulatory role in mediating crosstalk between signaling pathways for biotic and abiotic stress responses (Tsutsui et al., 2009). There are five *DEAR1* homologues within the *Arabidopsis* genome that also contain sequences with significant homology to the DREB domain and EAR motif. These genes have been designated as *DEAR2*, *DEAR3*, *DEAR4*, *DEAR5* and *DEAR6* and share 60.2, 53.9, 48.3, 42.9 and 42.1% identity with *DEAR1*, respectively (Tsutsui et al., 2009). These proteins have all been classified into the same DREB/CBF family subgroup (Nakano et al., 2006). The other five genes in this subgroup (*DEAR2* to *DEAR6*) were not transcriptionally induced by pathogen infection, indicating that the biotic response is specific to *DEAR1* (Tsutsui et al., 2009). Microarray database revealed that, *DEAR1* is also induced by infection with *Botrytis cinerea* as well as *P. Syringae* (Tsutsui et al., 2009). Transgenic overexpressing *PgDREB2A* (*P. glaucum*) showed upregulation of dehydrins, heat shock related genes, signal transduction proteins, biotic stress related genes and lipid transfer proteins. However, over-expressing *PgDREB2A* also showed upregulation of *NtERF5* gene which suggests that *PgDREB2A* gene crosstalk with biotic stress signal pathways (Agarwal et al., 2010). The NtERF5 protein, binds weakly to GCC box cis-elements, which mediate pathogen regulated transcription of several PR (pathogenesis related) genes. *NtERF5*-over-expressing plants suppress TMV (tobacco mosaic virus) proliferation, leading to enhanced viral resistance (Fischer and Dröge-Laser, 2004). Over-expression of the *OsDREB1B* led to an enhanced disease resistance against tobacco streak virus TSV in transgenic tobacco plants, apart from tolerance to various abiotic stresses, such as mannitol, NaCl, PEG, drought, methyl viologen, salicylic acid, ABA and cold. The expression levels of pathogenesis related-1 protein gene (*PR1b*) were more in all the transgenic lines over the wild type. Meanwhile, the expression of the GCC-box containing *PR2*, *PR3*, osmotin (*PR5*) and *CHN50* genes was observed in all the transgenic plants in different levels, but not in wild type tobacco plants (Gutha and Reddy, 2008). Interestingly, OsDREB1B induced the GCC-box containing *PR1b*, *PR2*, *PR3*, *PR5* and *CHN50* genes, which were reported as the target genes of various EREBP transcription factors (Ohme-Takagi and Shinshi, 1995). Constitutive expression of *Tsi1* (EREBP transcription factor gene) gene in tobacco induced the expression of disease responsive genes such as *PR1*, *PR2*, *PR3*, *PR4*, *Osmotin* and *SAR8.2* in transgenic plants without any stress. Furthermore, it was also observed that Tsi1 also binds DRE/CRT element, apart from the GCC box. Therefore, it is suggested that the biotic and abiotic signal pathways may interact to activate or repress biotic and abiotic response genes in plants and the Tsi1 protein may have a function. Further, the GCC-box contains GCCGCC core sequence and resembles the DRE/CRT (C/GCCGNC) common core sequence (Park et al., 2001). All these observations suggest that, OsDREB1B activates several target genes containing GCC-box in addition to the genes having DRE/CRT element in their promoters.

RELATIONSHIP WITH PHYTOHORMONE SIGNAL PATHWAY

Phytohormone abscisic acid (ABA) is an important regulator of plant growth and development and especially of plant responses to environmental stress such as cold, salinity and drought. Among these regulated physiological responses, the plant hormone ABA plays a central role. ABA is defined as a stress hormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that help plant survival over the stresses. Recently, DREB proteins or DRE/CRT elements involved in ABA-dependent pathway were reported (Egawa et al., 2006; Xu et al., 2008; Wang et al., 2010). So, DREBs control the expression of stress-responsive genes involved in ABA-dependent pathways in both abiotic and biotic stress. *CBF4* gene encodes a protein that is the closest homolog to the CBF1,2,3/DREB1abc transcriptional activators in *Arabidopsis*. In ABA-deficient mutant *aba1-1*, the drought induction of *CBF4* expression is dramatically reduced, indicating that ABA biosynthesis is required for the proper drought-induced induction of *CBF4* expression (Haake et al., 2002). This observation is in agreement with an earlier study that suggests that, the CRT/DRE elements are involved in ABA signal transduction because the ABA-responsive element in the promoter of *COR78a/RD29a* is not sufficient by itself to elicit an ABA response and that proper ABA response requires the presence of a region containing the CRT/DRE elements (Yamaguchi-Shinozaki and Shinozaki, 1994). The *GhDBP3* (*G. hirsutum*) gene, belonging to A-4 group of DREB subfamily, was greatly induced by ABA treatment, apart from response to drought, NaCl and low temperature (Huang and Liu, 2006a). The PpDBF1 (*Physcomitrella patens* DRE-binding factor1) which belongs to the A-5 group of DREB transcription factor subfamily was also induced by phytohormones including ABA (Liu et al., 2007).The *BjDREB1B* (*B. juncea*) transcript was induced by drought, high salt, low temperature,

as well as ABA, suggesting that BjDREB1B acts as a cross point and is simultaneously involved in both ABA-independent and ABA-dependent stress signaling pathways (Cong et al., 2008). In recent literature there was a new focus on the role of ABA in either promoting or suppressing resistance against various pathogens (de Torres-Zabala et al., 2007). For example, recent reports showed that *P. syringae* infection dramatically induced the biosynthesis of ABA. Moreover, genome-wide expression analysis revealed a substantial overlap between ABA and pathogen-responsive genes. Subsequent disease tests with ABA-insensitive and ABA-hyper sensitive mutants revealed enhanced resistance and susceptibility, respectively, indicating that ABA functions to promote virulence. It has been proposed that ABA suppresses the deposition of callose and lignin, both of which reinforce the cell wall to prevent pathogen invasion. Additionally, ABA inhibits the accumulation of SA and the expression of genes involved in basal resistance (de Torres-Zabala et al., 2007; Mohr and Cahill, 2007). Current evidence suggests that, ABA affects disease resistance which is mainly negative by interfering at different levels with biotic stress signaling. The involvement of ABA in primed callose production is one of the few examples of a positive role of ABA in disease resistance. It has become increasingly clear that the previously isolated abiotic signaling network that is controlled by ABA and the biotic network that is controlled by salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are interconnected at various levels. Whether all of the potential connections and shared nodes are actually used for cross-talk remains to be determined. The analysis of this combined network is a difficult task.

Plants adaptation to drought, low temperature and salinity is regulated by the combinatorial activity of interconnected ABA-dependent and ABA-independent signaling pathways. By contrast, the phytohormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play major roles in disease resistance. These biotic stress hormones do not control isolated linear signaling pathways but are part of a complex network of synergistic and antagonistic interactions. Recent evidence suggests the existence of a significant overlap between signaling networks that control abiotic stress tolerance and disease resistance (Figure 2). Salicylic acid is involved in establishing local and systemic disease resistance response of plants after pathogen attack. Although, it is currently unclear if SA level increases during abiotic stress, it has been established that SA and osmotic stress could activate the same mitogen activated protein kinase (MAPK) (Mikolajczyk et al., 2000), indicating that these pathways might share some components. In addition, exogenous application of the SA analogue Benzothiadiazole (BTH) could induce expression of *DREB2A* in *Arabidopsis* (Chini et al., 2004). However, SA might function as a negative regulator of some abiotic response pathways. Lack of SA enhanced *Arabidopsis* germination under salt and osmotic stress (Borsani et al., 2001). The *BjDREB1B* was induced by SA and *BjDREB1B*-expressing transgenic tobacco displayed drought and salt tolerance, indicating that *BjDREB1B* might function as positive regulator in a SA-related abiotic stress pathway (Cong et al., 2008). Transgenic *Arabidopsis* over-expressing *DEAR1* (DEAR1ox) showed up-regulation of SA-dependent *PR* genes (*PR1*, *PR2* and *PR5*) and accumulation of SA content, which demonstrate that the SA-dependent defense pathway was activated. Similar to what has been observed for other *PR* genes, plant defensin (*PDF1.2*) and basic chitinase (*PR3*) were transcriptionally activated in transgenic *Arabidopsis* overexpressing *DEAR1* (Tsutsui et al., 2009). Two members of AP2/ERF super family, ERF1 and ORA59, emerged as principal integrators of the JA and ET signaling pathways (Lorenzo et al., 2003; Pré et al., 2008). The expression of both ERF1 and ORA59 is induced by JA and ET and can be activated synergistically by both hormones. In addition, over-expression of the transcription factor genes ERF1 or ORA59 in the JA-insensitive mutant *coil-1* or ERF1 in the ET-insensitive mutant *ein2* constitutively activated the *PDF1.2* gene, which indicates that, these transcription factors are important nodes of convergence of JA and ET signaling. ERF1 and ORA59 bind to the GCC box in gene promoters and induce the expression of downstream genes, including *PR3* and *PDF1.2* genes (Lorenzo et al., 2003; Pré et al., 2008). Both *PDF1.2* and *PR3* are known to be induced by jasmonate and/or ethylene, implying that DEAR1 is also associated with the jasmonate and/or ethylene signaling pathways. Over-expressing *DEAR1* showed no increase in the transcription of ERF1 and other ERFs, however, ERF9 transcription was strongly repressed. The ERF9 gene is unique within the ERF family as it contains an EAR motif in its protein sequence, which likely functions as a transcriptional repressor and a DRE motif in the promoter region, which is known to be bound by DREB proteins (Huang and Liu, 2006). Consequently, increasing DEAR1 protein levels could repress ERF9 transcription, which then leads to induction of *PDF1.2* and *PR3* due to removal of the transcriptional repression imposed by ERF9. In general, plant defense to *P. syringae* infection is activated by the SA-mediated pathway, whereas the ET/JA-mediated pathway is associated with defense against *B. cinerea* infection. Together with the gene expression patterns observed in the *DEAR1* overexpressor, it can be suggested that DEAR1 functions as a negative transcriptional regulator of both SA- and ET/JA-mediated defense pathways, leading to a broad-range resistance to pathogens (Tsutsui et al., 2009). A melon 1-aminocyclopro pane-1-carboxylate synthase (CMe-ACS2) is a key enzyme in the ethylene biosynthesis pathway in melon. CMe-DREB1 belongs to AP2/ERF transcription factor, which was isolated by a yeast one-hybrid screening method using a GCCGAC sequence (DRE/CRT cis-acting element) in the CMe-ACS2 promoter. The expression levels of *CMe-ACS2* and

CMe-DREB1 in melon leaves were increased by methyl jasmonate (MeJA) treatment (Mizuno et al., 2006). These results show that CMe-DREB1 functions as a transcriptional activator of CMe-ACS2 in the presence of jasmonate. These results also suggest that DREB1 transcription factors are involved in JA/ET cross-talk.

Over-expression of *AtDREB1A* transcription factor causes a dwarf phenotype, which is similar to the phenotype of gibberellic acid (GA) deficient mutants, suggesting that AtDREB1A activator might interfere in GA biosynthesis. GA is well known to be associated with internode length (Busov et al., 2003). Exogenous GA3 treatment could restore the dwarf phenotype in *AtDREB1B*-expressing tomato and *AtDDF1/DREB1F* expressing *Arabidopsis* (Hsieh et al., 2002; Shen et al., 2003). However, recently reports showed that *AtDREB1A*-expressing tobacco plants did not show any reversal of growth retardation by GA3 treatment (Kasuga et al., 2004). The expression of *NtGA3ox* (oxygenases) and *NtGA2ox* increased while *NtGA20ox* seemed slightly decreased in *AtDREB1A*-overexpressing plants or under salt stress in wild-type tobacco plants. Exogenous GA3 treatment did not restore the dwarf phenotype except for the enlarged leaf area and the lengthened petiole. These results imply that hyper accumulation of AtDREB1A protein and salt stress might share similar mechanisms to regulate the expression of GA dioxygenases and interfere with GA metabolism (Cong et al., 2008). These reports may also suggest that, different DREB-like transcription factors and the different transformed plants might together contribute to different phenotypes related with plant growth. On the other hand, the metabolism of other phytohormones, such as cytokinins and auxins, might be influenced in 35S-*AtDREB1A* transgenic tobacco plants. Tamaoki and colleagues reported that cytokinin levels were notably increased and IAA was reduced in the leaves of the *NTH15*-expressing transgenic tobacco plant, which exhibited the dwarf phenotype and malformed leaves (Tamaoki et al., 1997). Measurement of phytohormone contents in the transgenic tobacco plants overexpressing *AtDREB1A* would help to further explore the dwarf mechanism. Recently, a C2H2 zinc finger transcription factor named STZ was found to be upregulated by AtDREB1A and to suppress the expression of many genes related to photosynthesis and carbohydrate metabolism in transgenic *Arabidopsis* plants (Maruyama et al*.,* 2004). The identification and analysis of these downstream genes will help to elucidate the dwarf molecular mechanism resulting from overexpressing *AtDREB1A*.

GENETIC ENGINEERING OF TOLERANCE TRAITS IN CROPS

The great challenge of food security being faced these years in the world, has directed plant scientists towards gene revolution after green revolution due to advances in biotechnology. In fact the gene revolution, involves modification of qualitative and quantitative traits in an organism by transferring desired genes from one species to another. This strategy is referred to as the transgenic approach. In contrast to classical breeding, the transgenic approach allows the incorporation of only the specific cloned genes into an organism and restricts the transfer of undesirable genes from donor organism. Through this approach, pyramiding of genes with similar effects can also be achieved. Rapid advance in recombinant-DNA technology and development of precise and efficient gene-transfer protocols have resulted in efficient transformation and generation of transgenic lines in a number of crop species (Gosal et al., 2009). During the last two decades, many abiotic stress inducible genes have been cloned and characterized from different plant species, including DREB TFs. DREB/CBF genes have been over expressed in rice, wheat, *Paspalum notatum* and tobacco, resulting in improved stress responses under various stress conditions and increased expression of downstream DREB target genes under normal conditions. However, for the expression of these genes, there is a need to identify suitable promoters. Most of the stress-specific promoters are cis-acting elements that are recognized by the appropriate transcription factors. Efforts have been made to identify and characterize stress-induced promoters, particularly those induced by anaerobic conditions, low or high temperatures or salt stress (Busk and Pages, 1998; Grover et al., 2001). For example, a gene *DREB1A* was expressed in *A. thaliana*, which was operated by either the constitutive cauliflower mosaic virus (CaMV) 35S or the stress-inducible *rd29A* promoter (Kasuga et al., 1999). It was observed that, plants expressing *DREB1A* constitutively exhibited morphological abnormalities under non-stress conditions but, on the other hand, plants expressing the *DREB1A* under the control of *rd29A* promoter were vigorous and highly tolerant to abiotic stress (Kasuga et al*.,* 2004). *DREB1A/CBF3*, *DREB1B/CBF1* and *DREB1C/CBF2* are strongly and transiently induced by low temperature stresses. Transgenic *Arabidopsis* plants expressing *DREB1B/CBF1* or *DREB1A/CBF3* under the control of the CaMV 35S promoter show strong tolerance to freezing, drought and high salinity stresses (Jaglo-Ottosen et al., 1998; Liu et al., 1998). The transgenic *Arabidopsis* plants over expressing *AtCBF1/DREB1B* under CaMV35S promoter showed enhanced freezing tolerance and higher expression of *COR* genes (Jaglo-Ottosen et al., 1998). Over-expression *AtDREB1A/CBF3* transcrip-tion factor in tobacco improved the drought and low temperature stress tolerance, apart from increasing the accumulation of the group 2 LEA proteins (Kasuga et al., 2004). From these reports, it can be generalized that production of transgenic plants with DREB genes is useful for improvement of tolerance of environmental stresses in a number of species. However, constitutive expression of these genes retards plant growth. Development of

transgenic plants with stress inducible promoters along with DREB genes or regulation of expression of DREB genes by stress-inducible promoters can induce stress tolerance and minimize the adverse effects of stress on growth (Ashraf et al., 2008).

Among all the crop plants, rice is not only an important staple food crop providing sustenance to more than 50% of the world population, but also is a model monocot genetic system. As the DREB transcription factors are proving to be important in the stress tolerance in model plants like *Arabidopsis*, it is very important to analyze the DREBs and their regulation even in rice not only to understand the molecular mechanisms of stress tolerance in monocots, but also to use them in genetic engineering of plants for improved stress tolerance. Several *OsDREB* genes have been isolated and their functions analyzed (Chen et al., 2003; Dubouzet et al., 2003; Oh et al., 2005; Ito et al., 2006). Dubouzet et al. (2003) isolated rice homologues for *DREB1/CBF* and *DREB2*, four *OsDREB1s* and one *OsDREB2* from rice genomic sequences and found that they induced strong expression of stress responsive genes in transgenic *Arabidopsis* plants, resulting in increased tolerance to high-salt and freezing stresses. Overexpression of *OsDREB1A* in *Arabidopsis* revealed that, this gene has a similar function in inducing stress tolerance. From this, it can be generalized that similar transcription factors function in dicotyledonous and monocotyledonous plants. Ectopic expression of *OsDREB1A* in *Arabidopsis* imparted high salt and freezing stress tolerance and also activated the over expression of several stress inducible genes in slightly growth retarded transgenic plants. CaMV 35S-*OsDREB1A Arabidopsis* seedlings showed higher survival rate over the wild type under high salt and freezing stresses (Dubouzet et al., 2003). Unlike other DREB transgenic plants, rice transgenic plants constitutively expressing *AtDREB1A/CBF3* under the influence of ubiquitin promoter did not show any growth retardation. Moreover, transgenic rice plants have shown elevated levels of tolerance to drought and high salt stresses, but very low level of tolerance to low temperature stress (Oh et al., 2005). Recently, Oh et al. (2005) developed transgenic rice plants that constitutively expressed *AtDREB1A*. The transgenic rice overexpressing *DREB1A* was tolerant to drought and high salinity, but had a low level of tolerance to freezing stress. In rice, *DREB/CBF* genes have been induced in response to cold, drought and high salt-stresses, but the expression of *DREB1A* and *DREB1B* genes have been observed only under cold stress (Dubouzet et al., 2003; Ito et al., 2006). Ito et al. (2006) observed that, transgenic rice plants overexpressing the *OsDREB1* or *DREB1* genes not only showed growth retardation under normal growth conditions but also improved tolerance to drought, high-salt and low-temperature stresses. These transgenic rice plants accumulated elevated levels of osmoprotectants such as free proline and various soluble sugars that have a role in stress tolerance. Constitutive expression of the *OsDREB1B* cDNA in tobacco leads to a marked increase in tolerance to several stresses in transgenic plants without any growth inhibition. Under osmotic stress, transgenic plants show higher biomass accumulation, rapid root growth, higher free radical scavenging activity and membrane stability. *OsDREB1BI* gene which was over-expressed in transgenic *Arabidopsis* revealed freezing and heat tolerance (Qin et al., 2007). In addition, transgenic plants overexpressing *OsDREB1B* show improved tolerance to tobacco streak virus and *OsDREB1B* activates the expression of several ethylene responsive *PR* genes in transgenic plants (Gutha and Reddy, 2008). Over-expressing of *OsDREB1F* in transgenic *Arabidopsis* showed that, besides activating the expression of *COR* genes which contain DRE/CRT element in their upstream promoter regions, the expression of *rd29B* and *RAB18* genes were also activated and then increased the salt, drought and low temperature tolerance (Wang et al., 2008). Transgenic rice plants analysis revealed that, over-expression of *OsDREB1G* and *OsDREB2B* in rice significantly improved their tolerance to water deficit stress, while over-expression of *OsDREB1E* could only slightly improve the tolerance to water deficit stress, suggesting that the *OsDREBs* might participate in the stress response pathway in different manners (Chen et al., 2008).

Apart from the main crop rice, genetic engineering of DREB TFs was also developed in other economic crop. Constitutive over expression of *CBF1* in tomato led to enhanced catalase activity and reduced H_2O_2 accumulation in transgenic plants signifying that CBF1 has a role in oxidative stress tolerance in plants (Hsieh et al., 2002a). CBF1 also imparted dehydration stress tolerance to tomato transgenic plants (Hsieh et al., 2002b). The drought and salt tolerances of T1 transgenic wheat with ubi-*GmDREB* or rd29A-*GmDREB* were improved when compared with wild type. The result suggested that, both ubiquitin and *rd29A* promoters could effectively drive the expression of the *GmDREB* gene and enhance drought and salt tolerance of T1 wheat plants (Gao et al., 2005). Over-expression of *HvDREB1* activated a downstream gene, *rd29A* under normal growth conditions and led to increased tolerance to salt stress in *Arabidopsis* plants (Xu et al., 2009). Over-expression of *ZmDBP3* improved drought and cold stress tolerance in transgenic *Arabidopsis* plants (Wang et al., 2009). Over-expression of *CkDBF* in transgenic tobacco plants resulted in higher tolerance to high salinity and osmotic stresses and induction of a downstream target gene under normal conditions (Wang et al., 2010). Over-expression *TsCBF1* gene from a dicotyledonous halophyte *Thellungiella halophila* into the monocotyledonous crop maize (*Zea mays L.*) showed improved drought tolerance with higher relative water content, higher solute accumulation and less cell damage when compared with wild-type (WT) plants. Most importantly, they showed shorter anthesis-silking interval

(ASI) and produced much higher grain yield than WT under drought stress (Zhang et al., 2010). Constitutive over-expression (double 35S) of TaDREB2 and TaDREB3 (wheat grain) in wheat and barley plants showed improved survival under severe drought conditions relative to nontransgenic controls (Morran et al., 2010). Together these studies have demonstrated the potential use of DREBs as candidate genes in imparting stress tolerance capabilities to transgenic plants.

CONCLUSION

Environmental stresses represent a major constraint to meeting the world's food demand. To meet the increasing demands for plant-based agricultural commodities, it would be imperative to enhance productivity of land in current use, expand agriculture to marginal lands and redesigning of crops to cope with abiotic and biotic stress. Constitutive expression of DREB genes in plants resulted in the expression of numerous stress responsive genes even under non-stress conditions and made the transgenic plants resistant to cold, drought, high salt and pathogen stresses. In this review, the structure characteristics of DREB TFs belonging to the AP2/ERF superfamily and its roles in abiotic and biotic stress tolerance were summarized and meanwhile, the genetic engineering of DREB TFs in economic crops was also concluded. However, the development of stress-tolerant transgenic plants is still at an early stage but may become increasingly more effective as better knowledge of the complex mechanisms involved in plant salt tolerance is acquired. With the development of plant molecular biology and gene-transfer technology to plants, new strategies to develop stress-tolerant transgenic plants via genetic manipulation have attracted more attention from researchers. It has provided a good perspective on how genetic engineering can be used to cultivate salt tolerant lines/cultivars, in particular with regard to the following: (1) DRE-binding proteins are a growing subfamily of AP2/EREBP factors with important roles in directing changes in gene expression during stress. While DREB proteins appear to function mostly in the context of plant responses that are independent of ABA, recent findings support a more complex function in ABA, JA, ET, SA and GA pathways as well. The challenge now, is to understand the relative roles of DRE-binding proteins in different phytohormones pathways, their coordinated regulation and the cross-talk with other signaling. In the longer term, these studies should assist the manipulation of plants in order to improve their stress tolerance and the productivity of crops; (2) furthermore, the rapid expansion in knowledge on genomics and proteomics will undoubtedly accelerate the transgenic and molecular breeding approaches. Remarkable technical advances in transcriptomics and metabolomics are available to clarify the molecular configuration in response to abiotic and biotic stress. 'Omics' analyses are crucial to understand the whole processes of molecular networks in response to stress. It is important to elucidate the functions of newly identified stress-responsive protein-coding and non-coding RNAs to understand the complex stress responses of plants. Integrated metabolome and transcriptome analyses have revealed that, many important metabolic pathways are regulated at the transcriptional level. However, there are also many metabolic pathways that are not regulated at the transcriptional level (Kaplan et al*.,* 2007), but at a post-transcriptional level. Post-transcriptional mechanisms based on alternative splicing and RNA processing, as well as RNA silencing define the actual transcriptome supporting the stress response. Beyond protein phosphorylation, other post-translational modifications like ubiquitination and sumoylation regulate the activation of pre-existing molecules to ensure a prompt response to stress. In addition, cross-connections exist among these mechanisms, clearly demonstrating further and superimposed complexity levels in response to environmental changes (Mazzucotelli et al., 2008). It has been shown that, some siRNAs are stress-inducible and they affect transcriptional and translational processes including alternative splicing. In addition, metabolites not only have functional roles in stress tolerance but also act as signaling molecules (Verbruggen et al*.,* 2008). Limited knowledge of stress-associated metabolism is still a major gap in the understanding of stress tolerance in many plant species. Therefore, comprehensive profiling of stress-associated metabolites, combined with stress metabolomics of major crop plants will be a key factor in molecular breeding for tolerance. Genetic regulation and epigenetic regulation, including changes in nucleosome distribution, histone modification, DNA methylation and npcRNAs (non-protein-coding RNA) play important roles in stress gene networks. Transcriptomics, metabolomics, bioinformatics and high-through-put DNA sequencing have enabled active analyses of regulatory networks that control stress responses. Such analyses have markedly increased the understanding of global plant systems in responses and adaptation to stress conditions (Urano et al*.,* 2010). Integrated omics analyses are necessary to identify the broad function of metabolite regulatory networks during responses to abiotic stresses. Only then, we can better fine tune biotic and abiotic stresses so as to suit the climatic needs; (3) most transgenic plants had no obvious phenotypic variations and grew normally at both the seedling and mature plant stages. The *TsCBF1* gene has characteristics of DREB/CBF transcription factors. Since the *TsCBF1* gene from the dicotyledonous *T. halophila* was constitutively expressed in the monocotyledonous maize without adversely affecting growth, it is postulated that *CBF1* genes from dicotyledons can be transferred into monocotyledonous plants to activate expression of downstream genes without resulting in growth retardation or difference in *TsCBF1* transactivation activity when compared with *Arabidopsis* CBF. It may be a good

idea to breed new varieties with improved abiotic tolerance by introducing DREB genes from dicotyledonous plants into cereals, but more studies are needed to confirm this postulation (Zhang et al., 2010). However, it is still necessary to further elucidate the mechanism of transgenic populations with constitutive over-expression which probably showed slower growth, delayed flowering and lower grain yields relative to the nontransgenic controls.

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