Chapter 6
Role of DREB-Like Proteins in Improving Stress Tolerance of Transgenic Crops

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1 Introduction

Plants are often exposed to several environmental stresses that adversely affect various stages of their growth and development. It has been estimated that potential yield of annual crops is lost up to 82 % due to abiotic stress every year. Drought and salinity are already spreading worldwide, and are expected to cause serious salinization of more than 50 % of all available productive, arable lands by the year 2050 (Ashraf 1994). In a world where population growth exceeds food supply, plant breeders and biologists need to fully implement the biotechnologies and agricultural practices to overcome these serious issues.

Plants overcome environmental stresses by development of tolerance, resistance or avoidance mechanisms. Tolerance allows an organism to withstand the assault. Resistance involves active countermeasures, while avoidance prevents exposure to the stress. Partly due to their sessile nature, plants have developed sophisticated metabolic responses, various strategies and pathways to tolerate or resist different forms of stress. Plant's tolerance or susceptibility to abiotic stresses is a complex phenomenon. Therefore, intense research has been focused on the mechanisms underlying abiotic stress tolerance and adaptation. Though plants have gradually evolved a remarkable ability to cope with such highly variable environmental onslaughts, the stresses nevertheless represent a primary cause of crop loss worldwide. Therefore, to meet the increasing demands for agricultural commodities it would be imperative to either enhance cultivable land in current use to expand agricultural lands or to create genetically redesigned crops to cope better with the environmental changes.

Drought, cold, and high-salinity stresses generate complex stimuli that have different yet related attributes and may deliver quite different information to the

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plant cells (Xiong et al. 2002). A mild abiotic stress may induce an adaptive response in the plant, allowing it to grow with a greater tolerance to the same or different stresses (Knight et al. 1998; Lang et al. 1994; Mantyla et al. 1995; Siminovitch and Cloutier 1982). The mechanism through which plants perceive environmental signals and transmit them to cellular machinery to generate adaptive response is of fundamental importance to biology. It is the coordinated action of various genes in a pathway that bring about the requisite phenotype and lead to plant tolerance. Plant stress adaptation/tolerance not only involves physiological changes but also the changes at cellular and molecular levels. The ability of the plant to sustain itself under unfavorable environmental conditions determines by the manifestation of a single or a combination of these inherent changes (Farooq et al. 2009).

2 Abiotic Stress Response

Drought, salinity, extreme temperatures, and oxidative stress are often interconnected and may induce similar cellular damage. For example, plants suffer from dehydration under high salinity and drought, as well as low-temperature conditions, all of which cause hyperosmotic stress characterized by metabolic and osmotic imbalance in plants. This leads to turgor loss and closure of the stomata, followed by repression of cell growth and inadequate photosynthesis (Shinozaki and Yamaguchi-Shinozaki 2007). Oxidative stress, which frequently accompanies high temperature, salinity, or drought stress, may cause denaturation of functional and structural proteins and lipids (Smirnoff 1998). As a consequence, these diverse environmental stresses often activate similar cell signaling pathways (Knight et al. 1998; Shinozaki and Yamaguchi-Shinozaki 2000; Zhu 2001, 2002) and cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants, and accumulation of compatible solutes. The switching 'on' of such cellular and molecular responses include perception of stress signal by membrane receptors, which then activate cytoplasmic Ca^{2+} and signaling pathways in cytoplasm and nucleus. This eventually leads to modification in the stress-responsive gene expression and physiological changes (Bressan et al. 1998; Xiong et al. 2002). Also, accumulation of abscisic acid (ABA) plays an important role in abiotic stress signaling and transduction pathways, mediating many responses (Wasilewska et al. 2008). The products of these genes ultimately lead to plant adaptation and/or tolerance and help the plant to survive and surpass the unfavorable conditions. The mechanism by which plants perceive environmental stress signals and transmit to the cellular machinery for activation of adaptive responses is of critical importance. This knowledge can be implied for the development of rational breeding and transgenic strategies leading to alleviate stress tolerance in crops.

In the past decades, a number of stress-inducible genes have been identified by transcriptome analyses using microarray technology in several plant species, like *Arabidopsis*, rice, etc. (Bohnert et al. 2001; Seki et al. 2001; Zhu 2001) by several research groups. Several genes that are induced by abiotic stresses have been

classified into two major groups (Bray 1993; Shinozaki and Yamaguchi-Shinozaki 2000; Thomashow 1999). One group encodes for functional proteins such as membrane proteins (membrane channel, transporter proteins, etc.), key enzymes for osmolyte biosynthesis (proline, glycine betain, sugars, etc.), the detoxification enzymes (catalase, hydrolase, superoxide dismutase, etc.), and the proteins for the protection of macromolecules (LEA protein, chaperone, osmotin, etc.). Whilst, other group includes regulatory proteins such as transcription factors (bZIP, MYC, MYB, DREB, etc.), protein kinases (MAP kinase, receptor protein kinase, etc.), proteinases (phosphoesterases, phospholipase C, etc.) that regulate gene expression and signal transduction in stress responses and enzymes involved in phospholipids metabolism and ABA biosynthesis (Chen and Murata 2002; Shinozaki and Yamaguchi-Shinozaki 2007; Yamaguchi-Shinozaki and Shinozaki 2006). Plant engineering strategies for abiotic stress tolerance (Wang et al. 2003) rely on the expression of genes that are involved in signaling and regulatory pathways (Seki et al. 2003; Shinozaki et al. 2003), genes that encode proteins conferring stress tolerance (Wang et al. 2004) or enzymes present in pathways leading to the synthesis of functional and structural metabolites (Apse and Blumwald 2002; Park et al. 2004; Rontein et al. 2002). Many genes encoding enzymes related to functional metabolites are induced by stress. There is a practical limitation of overexpressing multiple genes in a plant in a tissue specific manner to improve stress tolerance. Therefore, early responsive genes that regulate a number of functionally related downstream genes could be attractive targets for engineering stress tolerance since they may regulate quantitative traits. Intuitively, genetic engineering would be a faster way to insert beneficial genes than through conventional or molecular breeding and thus seems to be a viable option to hasten the breeding of "improved" plants. Also, it would be the only option when genes of interest originate from cross-barrier species, distant relatives, or from non-plant sources.

3 Role of ABA in Stress Response

ABA is an important phytohormone that plays a pivotal role in various physiological processes during the plant life cycle, including seed dormancy, germination, and adaptive responses to various environmental stress conditions (Himmelbach et al. 2003; Schroeder et al. 2001; Shinozaki and Yamaguchi-Shinozaki 2000; Zhu 2002). Also, the application of ABA to plant mimics the effect of a stress condition. ABA level is increased in response to various stress signals, particularly when there are changes in the environment that result in cellular dehydration. Accumulation of ABA in leaves induces stomatal closure and inhibits opening (Wilkinson et al. 2001), thereby maintaining plant water potential under conditions of low soil moisture content or high evaporative demand and thus ABA is aptly called as a stress hormone. A rapid and sensitive increase in the ABA production is essential for instant cellular as well as long distance regulations. Also, a rapid ABA bleaching is needed when the stress is relieved.

Water deficit induces activation of genes that encode enzymes for both ABA biosynthesis and hydrolysis of ABA conjugates releasing the active hormone from an inactive pool (Iuchi et al. 2000; Lee et al. 2006; Schwartz et al. 2003; Xiong and Zhu 2003). The phenomenon of ABA biosynthesis in response to osmotic stress is well known (Finkelstein et al. 2002); however, the signaling networks orchestrated by ABA responses are highly complex. ABA is hydroxylated by cytochrome P450-monooxygenase to form unstable hydroxyl-ABA, which is subsequently converted to phaseic acid. This pathway is also regulated by environmental conditions (Kushiro et al. 2004). Also, ABA and hydroxyl-ABA are conjugated with glucose to form inactive ABA-glucose ester (ABA-GE) (Cutler and Krochko 1999) which can be converted into an active form by apoplastic and endoplasmic reticulum β (beta)-glucosidases. Thus, plants have to maintain a proper balance of active and inactive forms of ABA, which is critical for plants growth and development (Chinnusamy et al. 2004).

3.1 ABA Signaling Pathways

Main function of ABA seems to be the regulation of plant water balance and osmotic stress tolerance. Several ABA deficient mutants namely *aba1*, *aba2*, and *aba3* have been reported for *Arabidopsis* (Koornneef et al. 1998). ABA deficient mutants for tobacco, tomato, and maize have also been reported (Liotenberg et al. 1999). Without any stress treatment the growth of these mutants is comparable to wild type plants. Under drought stress, ABA deficient mutants readily wilt and die if stress persists. Under salt stress also ABA deficient mutants show poor growth (Xiong et al. 2001).

Several different sets of *cis*- and *trans*-acting factors are known to be involved in stress-responsive transcription. Some of them are controlled by ABA but others are not responsive to exogenous ABA treatment, indicating the involvement of both ABA-dependent and -independent signal transduction cascades for stress-responsive gene expression (Bray 1993; Shinozaki and Yamaguchi-Shinozaki 2000; Thomashow 1999; Xiong et al. 2002). Abiotic stress response involves at least four different regulons in plants. A regulon is a group of genes controlled by a certain type of transcription factor. These regulons are (a) AREB/ABF regulon, (b) MYC/MYB regulon, (c) NAC (NAM/ATAF and CUC) and ZF-HD regulon and (d) DREB/CBF regulon. The former two are ABA-dependent regulons and later two are ABA-independent (Fig. 6.1).

3.2 ABA-Dependent Signaling Cascade

The ABA-dependent pathway may follow either of the two routes, either requiring new protein synthesis or not (Ingram and Bartels 1996; Shinozaki and Yamaguchi-Shinozaki 1997). Route independent of new protein synthesis, includes

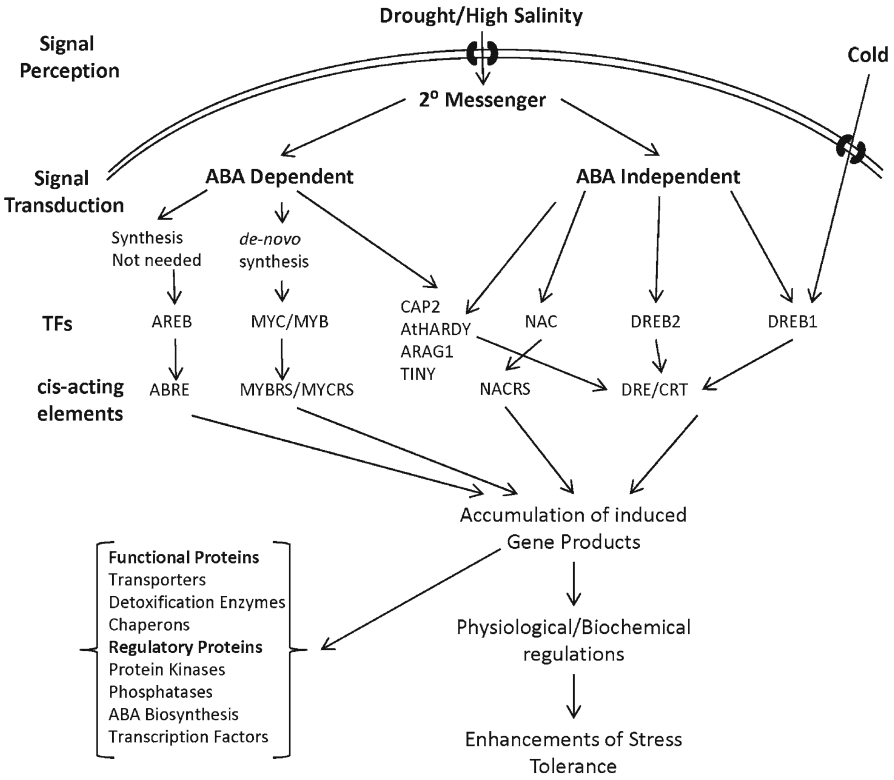

Fig. 6.1 A schematic representation of cellular signaling pathways. Stress signal is perceived by membrane receptors, activating secondary messenger molecules, signal is transduced by a set of transcription factors via two different signaling branches. The *cis*- and *trans*-elements involved in stress-responsive gene expression are also shown. Two different signal transduction pathways were followed by DREB proteins in response to cold and drought stresses. *ABRE* abscisic acid responsive binding element; *DRE*: drought responsive element; *MYBRS* MYB recognition site; *MYCRS* MYC recognition site; *NACRS* NAC recognition sequences

ABA-responsive genes with ABA-responsive element ABRE, (C/T)ACGTG(G/T)C, in their promoter domain (Himmelbach et al. 2003). ABRE is recognized by members of bZip-transcription factor family, AREB/ABF (ABA-responsive element-binding proteins/factors), and activate ABA-induced gene expression. ABA-dependent phosphorylation is required for the activation of AREB/ABF protein. Overexpression of ABF3/ABF4 enhances drought tolerance in *Arabidopsis* (Kang et al. 2002). Similarly ABF2/AREB1 overexpression improved stress tolerance to heat, drought, and oxidative stresses (Kim et al. 2004). Genes involved in the route where new protein synthesis is required for ABA-induced expression have no ABRE in their promoter region, else having *cis*-acting elements with binding affinity to MYC/MYB transcription factor family (Bray 2002; Shinozaki and Yamaguchi-Shinozaki 1997).

3.3 ABA-Independent Signaling Cascade

Existence of an ABA-independent pathway was unveiled when ERD1 (early responsive to dehydration1, the Clp protease regulatory subunit encoding gene) transcript was found to accumulate even before the accumulation of ABA during the dehydration and salinity stress responses in *Arabidopsis* (Nakashima et al. 1997). Promoter analysis of ERD1 gene shows the existence of DNA-binding domains for transcription factors (TFs) belonging to NAC and ZF-HD (zinc-finger homeodomain) family. *Arabidopsis* plants coexpressing these TFs activate the ERD1 gene expression (Tran et al. 2007). NAC family proteins bind specifically to the NAC recognition site (CATGTG) (Tran et al. 2004). OsNAC6 overexpressing transgenic plants are reportedly tolerant to salt and dehydration stress.

During dehydration stress, endogenous ABA began to accumulate 2 h after dehydration stress started and reached maximum in 10 h but rd29A transcript was found to accumulate within 20 min after dehydration and is followed by a secondary induction phase that begins after ~3 h (Yamaguchi-Shinozaki et al. 1992). This differential behavior suggests that the first rapid induction of *rd29A* is not mediated by endogenous ABA but by an ABA-independent pathway. Promoter analysis of rd29A revealed the presence of two *cis*-acting elements, one of which is ABRE, which is responsible for ABA-dependent late induction, and the other is a new *cis*-acting element (TACCGACAT). This new element was named as dehydration-responsive element (DRE)/C-repeat (CRT) and responsible for very early ABA-independent induction of rd29A. Many dehydration and low-temperature stress-inducible genes were found to have DRE in their promoter (Thomashow 1999; Yamaguchi-Shinozaki and Shinozaki 1994). A lot of efforts were made to identify the transcription factor that regulates DRE with the intuition that the DRE-binding protein would be a very early regulatory factor of stress response and, therefore, would be a potential candidate for genetic manipulation. The first DRE-binding protein identified was named CBF (CRT-binding factor) and was found to improve freezing tolerance in the overexpressing non-acclimated plants (Kasuga et al. 1999). Consequently several other TFs that bind and activate DRE/CRT were discovered (Jaglo-Ottosen et al. 1998). Those were commonly named as DRE-binding (DREB) proteins or CBF. One set of DREB genes were found to be induced at low temperature and named DREB1 and other group was found induced by dehydration and salinity and named DREB2 genes. All the DREB proteins have a conserved AP2/ERF (APETALA2/ethylene-responsive element-binding factor) DNA-binding domain (Riechmann and Meyerowitz 1998) of 50–60 amino acids in length.

AP2/ERF gene family is one of the most important multigene families of TFs. Recently, the AP2 domain was reportedly found in other proteins outside the plant kingdom too (Magnani et al. 2004). AP2/ERF family is classified into three groups based on the number of AP2/ERF domains and gene function. Class I members encode a protein containing two ERF/AP2 domains and includes APETALA2 (AP2), AINTEGUMENTA (ANT), and Glossy15 (Elliott et al. 1996; Jofuku et al. 1994; Klucher et al. 1996; Moose and Sisco 1996). Class II proteins have only one

ERF/AP2 domain and include EREBPs, TINY, DREB1/CBF, DREB2, Ptis, EBP, ERF, AtEBP, AtERFS, and ABI4 (Buttner and Singh 1997; Finkelstein et al. 1998; Fujimoto et al. 2000; Ohme-Takagi and Shinshi 1995; Solano et al. 1998; Vergani et al. 1997; Wilson et al. 1996; Zhou et al. 1997). The third class includes RAV1 and RAV2, with two different DNA-binding domains, ERF/AP2. Of all, DREB TFs are presumably the most promising candidates for genetic manipulation for attaining tolerance against drought, high salinity, low-temperature, and other abiotic stresses. This chapter emphasize on the role of DREB- and DREB-like proteins in the stress responses.

4 DRE-Binding Proteins in Stress Responses

In *Arabidopsis*, DREB/CBF-like proteins can be classified into 6 small subgroups (A-1 to A-6) based on similarities in the binding domain. Subgroups (A-1, A-2) include the DREB1/CBF and DREB2 gene families, respectively. The DREB1 family proteins are relatively short and of about 220 amino acids in size while the DREB2-family proteins are of about 330 amino acids in size. They show variation of amino acid sequences in the DNA-binding domains (Liu et al. 1998). Subgroup A-3 has only ABI4, A-4 includes TINY, A-5 contains RAP2.1, RAP2.9, and RAP2.10, and A-6 includes RAP2.4. DREB1/CBF and DREB2 are two independent DREB family members which function via two separate signaling pathways under stress (Liu et al. 1998). DREB1 proteins play major role in cold-induced gene expression and the DREB2 proteins are involved in high-salinity- and drought-induced gene expression. However, for exception, DREB1-related genes DREB1D/CBF4 expression is induced by osmotic stress (Haake et al. 2002) and DREB1F, DREB1E are induced by high-salinity stress (Magome et al. 2004). This suggests the cross-talk between the CBF/DREB1 and the DREB2 pathways. Overexpression of the DREB transcription factors activate a number of downstream genes leading to enhanced abiotic stress tolerance.

4.1 DREB1/CBF Regulates Cold-Inducible Gene Expression

In *Arabidopsis,* three genes encoding DREB1B/CBF1, DREB1A/CBF3, and DREB1C/CBF2 have been mapped on chromosome 4. Transgenic *Arabidopsis* plants overexpressing CBF/DREB1 genes showed improved survival rates under low temperatures (Jaglo-Ottosen et al. 1998; Kasuga et al. 1999). However, the DREB1A overexpression caused growth retardation of transgenic plants under normal growth conditions. Replacing the constitutive *Ca*MV 35S promoter with stress-inducible rd29A promoter minimizes the plant growth defect without compromising with the yield and also imparted tolerance to drought and salinity (Fowler and Thomashow 2002; Kasuga et al. 1999). Similarly, *Medicago*

trancatula DREB1C gene when over expressed into China Rose under *Arabidopsis* rd29A promoter, show normal growth phenotype and enhance freezing tolerance in transgenic plants (Chen et al. 2010). Gene Chip and cDNA microarrays have identified more than 40 genes downstream of DREB1/CBF (Fowler and Thomashow 2002; Maruyama et al. 2004; Seki et al. 2002; Vogel et al. 2005). Categorically these genes belong to LEA proteins, osmoprotectant biosynthesis proteins, RNA-binding proteins, sugar transport proteins, desaturases, carbohydrate metabolism-related proteins, KIN (cold-inducible) proteins, protease inhibitors. These gene products are probably responsible for the stress tolerance of the transgenic plants. Transcription factors like C2H2 zinc-finger-type and AP2/ERF-type, act downstream to CBF/DREB suggesting existence of further regulation of gene expression downstream of the DRE/DREB regulon (Maruyama et al. 2004; Sakamoto et al. 2004). Whilst, CBF3/DREB1 regulates the expression of a number of downstream genes during stress response, it itself is controlled by the Inducer of CBF expression 1 (ICE1) protein, a MYC-type bHLH (basic helix-loop-helix) TF (Chinnusamy et al. 2003). The ICE1 protein is negatively regulated by the higher expression of Osmotically responsive genes 1 (HOS1) protein, a RING E3 ligase, which is responsible for ubiquitination and subsequent degradation of ICE1 protein (Dong et al. 2006). ICE1 ubiquitination can be blocked by SIZ1-dependent sumoylation (Miura et al. 2007). SIZ1 is a SUMO E3 ligase that mediates ICE1 sumoylation which activates and/or stabilizes ICE1 protein, thus facilitating its activity controlling the expression of the CBF3/DREB1A gene. However, ICE1 does not regulate the expression of CBF2/DREB1C. The CBF2 gene is activated by members of calmodulin binding transcription activators, CAMTA (Hua 2009). ICE2, an ICE1 homolog, probably regulates the CBF1 gene (Fursova et al. 2009). Also, CBF/DREB1 is negatively regulated by MYB15 TF. So, different pathways are involved in activation of different DREB1 proteins.

5 DREB2 Protein-Mediated Stress Response Under Osmotic Stress

DREB2 protein subfamily has two main members, DREB2A and DREB2B (Furihata et al. 2006). *Arabidopsis* genome encodes at least six DREB2 homologues other than DREB2A and DREB2B. DREB2A and DREB2B are induced strongly by drought and high salinity, but the others are not (Sakuma et al. 2006a, b). Unlike CBF/DREB1, transgenic plants overexpressing DREB2A did not show growth retardation. Also, overexpression of AtDREB2A and OsDREB2A in *Arabidopsis* was insufficient for stress-inducible gene expression (Dubouzet et al. 2003; Liu et al. 1998). This suggested that some posttranslational modifications might be needed by DREB2A for the activation of stress-inducible gene expression. The amino acid and domain analyses of AtDREB2A gene revealed the presence of a predicted nuclear localization signal in its N-terminal region, a transcriptional activation domain in the C-terminal region between amino acids 254–335, AP2/

ERF domain from amino acids 78–135 and a negative regulatory domain in the central region of the protein (136–165). Deletion of this negative regulatory domain resulted in significant increase of its activity. Sequence analysis shows the presence of a PEST sequence (RSDASEVTSTSSQSEVCTVETPGCV) in this region. The PEST sequence is rich in proline (P), glutamic acid (G), serine (S), and threonine (T). PEST sequence is often associated with proteins of short intracellular half-life, hence PEST sequences are hypothesized to act as a signal peptide for protein degradation (Rogers et al. 1986). The removal of negative regulatory domain containing the PEST sequence transforms DREB2A in a constitutively active form (DREB2-CA) (Sakuma et al. 2006a, b). The DREB2A protein containing the PEST sequence is degraded rapidly by the ubiquitin-proteasome system, whereas DREB2A-CA has a long lifetime in the nucleus. It was recently reported that overexpression of DREB2A-CA gene induces not only drought- and salt-responsive genes but also heat-shock (HS)-related genes (Sakuma et al. 2006a, b). Thus the DREB2A up-regulated genes can be classified into three groups: genes induced by HS, genes induced by drought stress, and genes induced by both HS and drought stress. HS stress induces HS proteins (HSPs). The expression of DREB2A itself was found to be induced by HS transiently and significantly. Microarray analysis of DREB2A-CA overexpressing *Arabidopsis* plants revealed the up-regulation of a number of drought, salt and heat responsive downstream genes even under non-stressed conditions (Sakuma et al. 2006a, b). Surprisingly, it shows the induction of a gene for heat shock factor (HSF), AtHsfA3, along with genes for LEA proteins, dehydrins, and COR15A, which function in acquisition of stress tolerance to drought and high-salinity genes. The heat induction of HSFA3 is directly regulated by the transcription factor DREB2A. Heat-inducible DREB2A can bind to the DRE element in the promoter of HSFA3 under heat shock to induce HSFA3 and further activate HSP expression (Schramm et al. 2008; Yoshida et al. 2008). Overexpression of DREB2C, another CRT/DRE-binding ERF member, enhances thermo-tolerance of transgenic *Arabidopsis* plants (Lim et al. 2007). Taken together, these results indicated that DREB2A plays a critical role in regulating drought- and heat-stress-responsive gene expression (Sakuma et al. 2006a, b).

Though both DREB1A and DREB2A recognize DRE, however, differences were observed between the DREB1A and DREB2A downstream genes. Moreover, some common downstream genes, such as COR15A, COR15B, KIN1, and KIN2, are recognized by both DREB1A and DREB2A, but their expression levels in the DREB2A-CA overexpressing plants were significantly lower than those in the DREB1A transgenic plants (Sakuma et al. 2006a, b). These differences in expression level of the downstream genes between the two DREB proteins explain the reason for the less freezing tolerance of the DREB2A-CA transgenic plants. Extensive promoter analysis of the DREB1A- and DREB2A-up-regulated genes demonstrated that the DREB2A protein could recognize both DRE/CRT variants, A/GCCGACNT and A/GCCGACNA/G/C, but prefers ACCGAC to GCCGAC. DREB1A protein has the highest affinity to the A/GCCGACNT sequence These different binding specificities between DREB1A and DREB2A may explain why these proteins control some different downstream genes.

Besides the major two subfamilies of DREB proteins, much less is known about a lot of small proteins (200aa and less) that bear DREB2-like AP2 domain. In this chapter we summarize these DREB2-like small subgroup proteins.

6 DREB2-Like Small Proteins

DREB proteins play important roles in plant morphology, development, and stress responses. A chickpea cDNA library of dehydration-induced transcripts was constructed and a novel DREB2-like TF, CAP2 was isolated (Shukla et al. 2006). In comparison to most of the well-known DREB2 family members, CAP2 was found to be relatively small (202 amino acids), though it qualifies for being an AP2 transcription factor. Since then, like CAP2, a number of small ORFs containing DREB2-like AP2 domain have been reported in GenBank from different plants. Like DREB2A of *Arabidopsis* and rice (*Oryza sativa*) expression of CAP2 is induced by dehydration and salt but not by cold. But, unlike AtDREB2A, CAP2 transcript was induced by ABA and auxin. Overexpression of CAP2 in transgenic tobacco caused increase in the leaf size and number of lateral roots, also promoted tolerance to salt, osmotic and heat stresses. This suggested that CAP2 is involved in both stress response and development. Another DREB2-like protein ZmDREB2A from *Zea mays* showed enhanced tolerance to drought, high salt and heat stress in the transgenic plants without growth penalty. A novel DREB-like gene, GmDREB2, was isolated from soybean. It has an open reading frame of 159 amino acids. GmDREB2 was classified into A-5 subgroup in DREB subfamily. GmDREB2 gene expression was induced by drought, high salt, and low temperature stresses and ABA treatment. Transgenic *Arabidopsis* plants overexpressing GmDREB2 has activated expression of downstream genes resulting in enhanced tolerance to drought and high-salt stresses. GmDREB2 overexpression did not cause growth retardation (Chen et al. 2007).

Similarly, A-4 subgroup member GhDBP3 (226 amino acids) is an abiotic stress and ABA-induced transcriptional activator (Huang and Liu 2006). A member of A-6 subgroup, ZmDBF1 (222 amino acids) of maize, is also induced by drought, NaCl, and ABA treatments in plant seedlings (Kizis and Pagès 2002). A gain-of-function mutant, *hardy* was identified in a phenotypic screen of an activation tagged mutant collection in *Arabidopsis* with robust roots and dark green leaves (Karaba et al. 2007). The HRD (HARDY) gene belongs to a class of AP2/ERF-like TFs. This gene has an ORF of 184 amino acids. HRD is probably involved in the maturation of inflorescence stage processes that needs tissue protection against desiccation. *Arabidopsis* plants overexpressing HRD gene confers drought and salt tolerance (Verslues et al. 2006).

In rice, a DREB2-like gene was isolated and named as ABA-responsive AP2-like gene (ARAG1). Its ORF is capable of encoding a 225 amino acid protein. Expression of ARAG1 was reportedly up-regulated by drought and ABA treatment as an early stress response as compared with the control. ARAG1-transgenic seeds were able to germinate in the presence of ABA application. It suggests that ARAG1 was involved with the tolerance-associated processes in the seedlings.

7 Conclusion

Taken together, a number of recent reports suggest that unlike DREB genes in A-1 and A-2 subgroups, which are involved only in ABA-independent pathway, these DREB2-like proteins are involved in both ABA-dependent and ABA-independent pathways. They may act as an overlap point and/or might take part separately in both ABA-independent and ABA-dependent pathways. Unlike the authentic DREB genes these DREB2-like genes do not show any growth defect when overexpressed. Some of them promoted growth and development in addition to abiotic stress tolerance when expressed in other plants. Since, little is known about the role of DREB2-like proteins during seed germination and seedling growth, and how they are regulated by ABA; a detailed and comprehensive functional study on these small AP2 proteins may give us an insight in plant developmental process and stress adaptation/tolerance mechanisms. They may also provide an attractive and complementary option for improving a plant's performance under stress conditions and emerge as an important future strategy for facilitating the crop yield in drought-prone environments for sustainable agricultural production.

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