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REVIEW

# Transcription factors in plants and ABA dependent and independent abiotic stress signalling

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## Abstract

Plants face variable environmental stresses that negatively affect plant growth and productivity. The multiplicity of responses is an important aspect of the complexity of stress signalling. Abscisic acid (ABA) is a broad-spectrum phytohormone involved not only in regulating stomatal opening, growth and development but also in coordinating various stress signal transduction pathways in plants during abiotic stresses. The both ABA-dependent and ABA-independent signal transduction pathways from stress signal perception to gene expression involve different transcription factors such as DREB, MYC/MYB, AREB/ABF, NAM, ATAF1,2, CUC and their corresponding *cis*-acting elements DRE, MYCRS/MYBRS, ABRE, NACRS. Genetic analysis of ABA mutants has given insight that ABA-dependent and ABA-independent pathways for osmotic stress and cold stress interact and converge. This review focuses on ABA-dependent and ABA-independent transcriptional components and cascades, their specificity and cross-talk in stress gene regulation.

*Additional key words*: *cis*-element, cross talk, downstream genes, gene regulation, overexpression.

## Introduction

Plants face variable forms of environmental stresses like drought, cold, temperature and soil salinity. Although, plants gradually evolved a remarkable ability to cope with such environmental onslaughts, the stresses nevertheless represent a primary cause of crop loss worldwide. During abiotic stress plants show an array of biochemical and physiological changes. Plant breeding showed that abiotic stress tolerance is governed by multiple loci and thus is multigenic in nature, therefore adapting with variable environmental cues is a highly complex phenomenon. A number of abiotic stress related genes, some transcription factors and regulatory sequences in plant promoters have been studied and characterized. The transcription factors interact with *cis*-elements in the promoter regions of various abiotic stress-related genes and thus up-regulate the expression of many secondary responsive genes resulting in abiotic stresses tolerance. In *Arabidopsis thaliana*, *cis*-elements and corresponding binding proteins, with distinct type of DNA binding domains, such as AP2/ERF (apetala 2/ ethylene responsive factor), basic leucine zipper, HD-ZIP (homeodomain leucine zipper), MYC (myelocytomatosis), MYB (myeloblastosis) and different classes of zinc finger domains, have been identified (Shinozaki and Yamaguchi-Shinozaki 2000, Pastori and Foyer 2002). Genetically engineering the expression of certain transcription factors can greatly influence plant stress tolerance. The transcription factor-based technologies are likely to be a prominent part of the next generation of successful biotechnology-derived crop (Century *et al.* 2008). Some transcription factors follow an ABA-dependent signal transduction pathway, while others

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*Abbreviations*: ABA - abscisic acid; ABF - ABRE binding factor; AP2 - apetala 2; AREBs - ABA responsive element binding protein; ATAF1,2 - *Arabidopsis* transcription factor 1 or 2 like family; CUC - cup-shaped cotyledon; DREB2 - drought responsive element binding protein 2; ERF - ethylene responsive factor; *hos5* - high expression of osmotic responsive genes; NAM - no apical meristem.

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appear ABA-independent. The molecular mechanisms for ABA-dependent and ABA-independent gene regulation are not entirely clear, analysis of the promoters of stress-responsive genes and the isolation of transcription factors that activate these genes suggest that there are distinct regulatory mechanisms for the different pathways. Despite the differences in transcriptional activation, molecular mechanisms indicate that the ABA-dependent and ABA-independent pathways have extensive interactions in controlling gene expression under abiotic stress (Ishitani *et al.* 1997, Xiong *et al.* 1999a). In this review, we discuss the role of ABA in stress signalling and its involvement in different regulatory systems during abiotic stress in ABA-dependent and independent manner.

## Role of ABA in stress signalling

ABA is an important plant hormone and is involved in many aspects of plant growth and development, as embryo maturation, prevention of precocious germination, seed development, seed dormancy, seed germination, stomatal aperture regulation and activation of stress responsive genes. ABA is also known to influence flowering in plants (Tanimoto *et al.* 1985, Saxena *et al.* 2008). In addition to its involvement in developmental pathways, ABA controls many stress adaptation responses, activation of genes involved in osmotic adjustment, ion compartmentation, regulation of shoot versus root growth and modifications of root hydraulic conductivity (Ruggiero *et al.* 2004, Verslues and Zhu 2005). ABA also helps in limiting transpiration rate and controls wilting, thus helps reduce water loss in the plants (Pospíšilová *et al.* 2009). A number of stress-responsive genes are upregulated by ABA (Ingram and Bartels 1996) in desiccation and osmotic imbalance. There is an overlap in the expression pattern of stress genes under cold, drought, high salt and or ABA application. The role of ABA in osmotic stress signalling was studied by ABA biosynthesis mutants in *Arabidopsis* (Finkelstein *et al.* 2002). Several ABA deficient mutants namely *aba1, aba2* and *aba3* have been reported from *Arabidopsis* (Koornneef *et al.* 1998). Without any stress treatment the growth of these mutants was comparable to wild type plants. Under prolong drought conditions the ABA deficient mutants wilt and die and under salt stress they show poor growth.

The role of ABA in cold response is not clearly understood. Studies have shown transient increase of ABA in response to chilling treatment (Lang *et al.* 1994) and increased freezing tolerance after exogenous ABA application (Chen *et al.* 1983). However, other studies have shown that ABA accumulation is not observed under cold stress, probably owing to slowing down of the cellular metabolism. Studies in the ABA-deficient mutant *aba1-1* and ABA-insensitive mutants *abi1-1* and *abi2-1*, indicate that low-temperature-regulated gene expression is relatively independent of ABA, whereas drought and salt stress signal transduction is controlled by both ABA-dependent and ABA-independent pathways (Thomashow 1999, Shinozaki and Yamaguchi-Shinozaki 2000). The *hos5* (high expression of osmotic responsive genes) mutants showed enhanced induction of the *RD29A-LUC* transgene and increased osmotic stress but not cold induction by ABA (Xiong *et al.* 1999a). The *los5* (low expression of osmotic responsive genes) mutation reduces the induction of several stress-responsive genes by cold and severely blocks the induction of *RD29A, COR15, COR47, RD22 and P5CS* by osmotic stresses (Xiong *et al.* 2001). The *los5* and *los6* mutants show different responses to cold treatment. In *los6*/*aba1*, the ABA treatment complemented the defect in cold-regulated *RD29A-LUC* expression, whereas the same treatment failed to rescue *los5*/*aba3* (Xiong *et al.* 2002). This suggests that cold signalling requires a function of *LOS5/ABA3*, which is not related directly to ABA biosynthesis. However, it is unclear how *LOS5/ABA3* involved in the cold or ABA regulation of some genes. Recently, Zhou and Guo (2009) reported in *Stylosanthes guianensis* that ABA enhances chilling resistance by inducing antioxidant enzymes, like superoxide dismutase (SOD) and ascorbate peroxidase (APX).

## ABA-dependent and ABA-independent signalling

Dehydration and salt stress activate ABA-dependent and ABA-independent gene expression systems involving ABFs (ABRE binding factor)/AREBs (ABA responsive element binding protein), MYC/MYB, DREB2 (drought responsive element binding) and NAC (NAM, ATAF1,2 and CUC ) transcription factors. The cold stress regulates an ABA-independent pathway through CBF/DREB1 transcription factors. These major transcription factors show differential transcript regulation in response to different stresses (Table 1) and their overexpression resulted in upregulation of large number of genes directly or indirectly linked with stress tolerance in plants (Table 2).

**AREB and ABA-dependent stress signal transduction:** The bZIP-ABRE system is an ABA-dependent stress signal transduction pathway. Many ABA-inducible genes contain a conserved, ABA-responsive *cis*-acting element named ABRE (ABA responsive element PyACGTGGC) in their promoter regions (Grill and Himmelbach 1998). ABREs were first reported in wheat EM gene which functions in seed during late embryogenesis (Guiltnan *et al.* 1990) and in rice RAB16, which is expressed in dehydrated vegetative tissues and maturing seeds (Mundy *et al.* 1990). A coupling element (CE3) is needed to specify the function of ABRE for the expression of ABA

Table 1. Differential transcript regulation of ABA-dependent and ABA-independent transcription factors. Ah - *Atriplex hortensis*, At - *Arabidopsis thaliana*, Ca - *Capsicum annuum*, Dm - *Dendranthema* × *moriforlium*, Dv - *Dendranthema vestitum,* Gm - *Glycine max*, Lp - *Lolium perenne*, Os - *Oryza sativa*, Pg - *Pennisetum glaucum*, Ss - *Saccharum* species, Ta - *Triticum aestivum*, W - common wheat, Zm - *Zea mays*.

| Family | Gene name | Responsive to ABA | Inducible by | References |
|---|---|---|---|---|
| bZIP | ABF1 | yes | cold | Choi *et al.* 2000 |
| | ABF2 | | salt | |
| | ABF3 | | salt | |
| | ABF4 | | drought | |
| | GmbZIP44 | yes | drought, salt, water stress | Liao *et al.* 2008a |
| | GmbZIP62 | yes | cold, drought, salt | |
| | GmbZIP78 | yes | drought, salt | |
| | GmbZIP132 | yes | cold, drought, salt | Liao *et al.* 2008b |
| | Wlip19 | yes | cold, drought | Kobayashi *et al.* 2008a |
| NAC | ATAF1 | yes | drought | Lu *et al.* 2007 |
| | AtNAC2 | yes | salt | He *et al.* 2005 |
| | AtNAC019 | yes | drought, salt | Tran *et al.* 2004 |
| | AtNAC055 | | | |
| | AtNAC072 | | | |
| | OsNAC6 | yes | cold, drought, salt | Ohnishi *et al.* 2005<br>Nakahsima *et al.* 2007 |
| | SNAC1 | yes | cold, drought, salt | Hu *et al.* 2006 |
| | SNAC2 | yes | cold, drought, salt | Hu *et al.* 2008 |
| | SsNAC23 | yes | low temperature | Nogueira *et al.* 2005 |
| MYB | AtMYB2 | yes | drought, salt | Abe *et al.* 1997 |
| | GmMYB76 | no | salt | Liao *et al.* 2008c |
| | GmMYB92 | no | cold, salt | |
| | GmMYB177 | no | drought, salt | |
| | MYB15 | - | cold | Agarwal *et al.* 2006b |
| | OsMYB3R-2 | - | cold, drought, salt | Dai *et al.* 2007 |
| bHLH | AtMYC2 | yes | drought, salt | Abe *et al.* 1997 |
| CBF/DREB | CBF1, CBF2, CBF3 | no | cold | Gilmour *et al.* 1998<br>Medina *et al.* 1999<br>Jaglo *et al.* 2001<br>Choi *et al.* 2002 |
| | CBF2-1, CBF2-2 | no | cold, drought | Kume *et al.* 2005 |
| | CBF4 | yes | salt | Haake *et al.* 2002 |
| | LpCBF3 | no | cold | Xiong and Fei 2006 |
| | AhDREB1 | - | salt | Shen *et al.* 2003a |
| | Ca-DREBLP1 | no | drought, salt | Hong and Kim 2005 |
| | DREB1A/REB1B | no | cold | Liu *et al.* 1998 |
| | DREB2A | yes | drought, salt | Liu *et al.* 1998 |
| | DvDREB2 | yes | cold, drought, salt | Liu *et al.* 2008 |
| | DmDREBa | yes | cold | Yang *et al.* 2009 |
| | DmDREBb | | | |
| | GmDREB2 | yes | cold, drought, salt | Chen *et al.* 2007 |
| | GmDREBa | yes | cold, drought, salt | Li *et al.* 2005 |
| | GmDREBb | no | cold, drought, salt | |
| | GmDREBc | yes | drought, salt | |
| | OsDREB1A | no | cold, salt | Dubouzet *et al.* 2003 |
| | OsDREB2A | no | drought, salt | |
| | OsDREB1F | yes | cold, drought, salt | Wang *et al.* 2008 |
| | PgDREB2A | - | cold, drought, salt | Agarwal *et al.* 2007 |
| | TaDREB1 | yes | cold, drought, salt | Shen *et al.* 2003b |
| | WDREB2 | yes | cold, drought, salt | Egawa *et al.* 2006 |
| | ZmDREB2 | no | cold, drought, salt | Qin *et al.* 2007 |

induced genes (Shen and Ho 1995). The ABREs core motif, ACGT is present in G-boxes of variety of genes responsive to different environmental and physiological factors, like irradiance (Giuliano *et al.* 1988), auxin (Liu *et al.* 1994), anaerobiosis (McKendree and Ferl 1992), jasmonic acid (Mason *et al.* 1993) and salicylic acid (Qin *et al.* 1994). Recently *cis*-elements other than ABREs related to ABA signalling are also suggested based on the mismatch of cell type-specific enrichment and regulation of gene expression by ABA (Dinneny *et al.* 2008).

*AREB1, AREB2* and *AREB3* from *Arabidopsis* encode bZIP-type proteins. Stress-inducible AREB1 and AREB2 function as transcriptional activators in the ABA-inducible expression of *RD29B* (Uno *et al.* 2000). Four ABFs (*ABF1, ABF2, ABF3, ABF4*) cDNA similar to *AREB1 and AREB2* are reported from *Arabidopsis*. *ABF1* expression is induced by cold, *ABF2* and *ABF3* by high salt and *ABF4* by cold, drought and high salt (Choi *et al.* 2000). The constitutive overexpression of stress-responsive *ABF3* or *ABF4*/*AREB2* resulted in ABA hypersensitivity as well as reduced transpiration rates and enhanced drought tolerance (Kang *et al.* 2002). *ABF2*/*AREB1* is an essential component of glucose signalling, and its overexpression increased tolerance to multiple stresses (Kim *et al.* 2004, Fujita *et al.* 2005). 131 bZIP genes of different groups were identified from soybean. Generally, it is known that group A bZIP proteins are involved in ABA and stress signalling. Recently, it was found that other bZIP-type proteins GmbZIP44, GmbZIP62 and GmbZIP78 belonging to subgroup S, C and G, respectively, are also involved in salt and freezing stress. These proteins bind to GCN4-like motif (GLM, GTGAGTCAT), ABRE (CCACGTGG) and PB-like (TGAAAA) elements with differential affinity and improve stress tolerance in transgenic *Arabidopsis* by upregulating *ERF5*, *KIN1*, *COR15A*, *COR78A* and *P5CS1* and down regulating *DREB2A* and *COR47* (Liao *et al.* 2008a). The salt tolerance conferred by *GmbZIP* genes is dependent on developmental stage and freezing tolerance depends on proline content of the transgenics (Liao *et al.* 2008a,b). The transgenics of group A bZIP proteins ABF2/AREB1, ABF3 and ABF4 are hypersensitive to ABA and act as positive regulators of ABA signalling, whereas the GmbZIPs act as negative regulators for ABA signalling, thereby facilitating the plants to balance ABA signalling and avoid extreme stress responses. *ABI5* (ABA insensitive 5) is a member of *Arabidopsis* bZIP transcription factor subfamily that contains four highly conserved domains in addition to the bZIP binding domain (Jakoby *et al.* 2002). *ABI5* expression is higher in mature seeds and young seedling exposed to ABA or dehydration stress and also its expression is promoted by multiple *ABI* gene products including the transcription factors *ABI3*, *ABI4* and *ABI5* itself (Finkelstein and Lynch 2000, Lopez-Molina *et al.* 2001, Brocard *et al.* 2002) and inhibited by closely related *ABF3* (Finkelstein *et al.* 2005). *OsABI5* from rice showed transcript upregulation by ABA, high salt and down regulation by drought and cold. Its overexpression enhanced salinity tolerance (Zou *et al.* 2008).

The expression of *AREB1* is induced by ABA application, however, its overexpression is not sufficient to activate ABRE-dependent gene expression. According to recent reports, AREB1 and its homologs are phosphorylated *in vitro* or *in vivo* (Kagaya *et al.* 2002), which may be involved in modulation of its activity. Furihata *et al.* (2006) revealed that the ABA-dependent multisite phosphorylation of AREB1 activates ABRE-dependent gene expression. A rice ABF, TRAB1 (transcription factor responsible for ABA regulation) is activated via ABA-dependent phosphorylation. ABA-activated SnRK2 protein kinases directly phosphorylate TRAB1 in response to ABA. TRAB1 gets phosphorylated not only in response to ABA, but also in response to hyper osmotic stress (Kobayashi *et al.* 2005). These studies show that phosphorylation/dephosphorylation regulated events play important role in ABA signalling.

The low temperature induced protein (lip), a bZIP type transcription factor has been isolated from different cereal plants. Rice *lip* is strongly induced by cold (Aguan *et al.* 1993), whereas maize *mlip5* is expressed in response to low temperature, salt stress and exogenous ABA (Kusano *et al.* 1995). Recently a *Wlip19* identified from wheat showed higher expression to cold, drought and ABA treatments. The transactivation study of this gene showed the positive regulation of five wheat *LEA* genes *WDHN13*, *WRAB17*, *WRAB18* and *WRAB19* (Kobayashi *et al.* 2008a).

**MYC/MYB and ABA-dependent stress signal transduction:** The MYC/MYB families of proteins are found in both plants and animals and known to have diverse functions. Members of this family were first identified in the regulation of anthocyanin biosynthesis (Goodrich *et al.* 1992). Both MYC/MYB transcription factors participate in the ABA-dependent pathway for the upregulation of the abiotic stress responsive genes. The DNA-binding domain of plant MYB proteins usually consist of two imperfect repeats of about 50 residues (R2, R3), whereas it contains three repeats (R1, R2 and R3) in animals. However, recently OsMYB3R-2 with three repeats was reported in rice (Dai *et al.* 2007). Different MYB proteins bind to different *cis*-elements in their target gene's promoter. Mammalian MYBs such as C-MYB, A-MYB, and B-MYB bind to the cognate site T/CAACG/TGA/C/TA/C/T (MBSI). Several plant MYB proteins that bind to MBSI will also bind to a second site, TAACTAAC (MBSII) (Romero *et al.* 1998). The AtMYC2 and AtMYB2 proteins bind to CACATG and TGGTTAG *cis*-acting elements, respectively, of the rd22 promoter of *Arabidopsis* and cooperatively activate this promoter (Abe *et al.* 1997). Overexpression of *35S:AtMYC2* and *35S:AtMYB2* and *35S:AtMYC2+ AtMYB2* in *Arabidopsis* induced ABA responsive stress genes. The transgenic showed an ABA-hypersensitive phenotype and increased osmotic stress tolerance (Abe *et al.* 2003). In contrast, *OsMYB3R-2* transgenic plants enhanced tolerance to freezing, dehydration and salt stress and decreased sensitivity to ABA (Dai *et al.* 2007). Liao *et al.* (2008c) identified 156 *GmMYB* genes of which the expression of 43 genes changed on treatment with ABA, salt, drought and/or cold stress. GmMYB76, GmMYB92 and GmMYB177 could bind to the sequence

MBSI but with different affinity, the GmMYB92 also showed binding to MRE4 (TCTCACCTA) and mMRE1 (CCGAAAAAAGGAT). The differential binding ability suggests that these genes may regulate different set of downstream genes. The expression levels of *RD29B, DREB2A, P5CS, RD1, ERD10, and COR78/RD29A* were enhanced in the *GmMYB76* transgenic plants, whereas in *GmMYB92* transgenic plants the expression of *DREB2A, RD17, and P5CS* was higher and the expression of *RD29B*, *COR6.6*, *COR15a* and *COR78/rd29A* was lowered. In the *GmMYB177* transgenic plants *RD29B*, *ABI2*, *DREB2A*, *RD17*, *P5CS*, *ERD10*, *COR6.6, ERD11* and *COR78* were upregulated. The *OsMYB4* imparts different level of tolerance depending on the nature of the host plants. *Arabidopsis* transgenic plants overexpressing *OsMYB4* showed increased chilling and freezing tolerance with a dwarf phenotype (Vannini *et al.* 2004), the tomato transgenic showed higher tolerance to drought stress and viral disease but not to cold stress (Vannini *et al.* 2007), whereas the apple transgenic showed increased drought and cold tolerance (Pasquali *et al.* 2008).

**Functions and interactions of transcription factors in an ABA-independent manner:** The ABA-independent stress-responsive gene expression is regulated by DREB proteins that bind to DRE *cis*-elements. DREBs are important plant-specific transcription factors that induce a set of abiotic stress related genes and impart stress tolerance to the plant system. They belong to ERF family of transcription factors unique to plants and contain two subclasses, DREB1/CBF and DREB2 that are induced by cold and dehydration, respectively. DREB genes have been isolated and characterized from wide variety of plants, and their differential transcript regulation and functional analysis is reviewed in Agarwal *et al.* (2006a). The DREB1 and DREB2-type proteins have different binding specificities, therefore, upregulate different set of abiotic stress related genes. The AtDREB1A, AtDREB2A and OsDREB2A proteins bind to both ACCGAC and GCCGAC with same efficiency, however, OsDREB1A showed preferential binding to GCCGAC (Liu *et al.* 1998, Dubouzet *et al.* 2003). In our studies PgDREB2A showed preferential binding to ACCGAC (Agarwal *et al.* 2007), similarly, in AtDREB2A preferential binding to ACCGAC is reported (Sakuma *et al.* 2006a).

A number of downstream genes are activated by the overexpression of the DREB transcription factors leading to enhanced abiotic stress tolerance (Table 2). Overexpression of *AtDREB1A* and *OsDREB1A* upregulated 12 and 10 genes, respectively, which were involved in freezing and dehydration tolerance (Seki *et al.* 2001, Dubouzet *et al.* 2003). Microarray analysis of *AtDREB2A* transgenic plants have shown overexpression of the 21 genes, of these 14 genes were upregulated by drought, 9 of which encode LEA class proteins, which are thought to protect macromolecules, such as enzymes and lipids from dehydration (Sakuma *et al.* 2006a). A number of heat shock related genes (268 and 778 at 0.5 h and 5 h heat shock treatment) were also upregulated by *AtDREB2A* overexpression (Sakuma *et al.* 2006b). Recently, Schramm *et al.* (2008) reported that *HsfA3*, one of the 21 members of *Arabidopsis* Hsf family, is transcriptionally activated during heat shock by DREB2A and regulates a subset of genes encoding Hsps. Microarray analysis of plants overexpressing the *ZmDREB2A* showed upregulation of 44 genes belonging to LEA, heat shock, detoxification, seed proteins and enzymes involved in metabolism, *etc*.

Zhao *et al.* (2006) isolated two groups of low-temperature-responsive DREB genes from *Brassica napus*, expression analysis and the *trans*-active activity of these two groups of genes indicated that they functioned in a competitive manner to regulate the DRE-mediated signalling pathway in response to cold stress. The *trans*-active Group I DREBs were expressed rapidly on exposure to cold stress to switch on the DRE-mediated signalling pathway and when the proteins of Group I reach a certain level the *trans*-inactive Group II were expressed, and they compete with Group I to bind to the DRE elements on the promoter of target genes and decrease their expression, and finally the DRE-mediated signalling pathway is switched off. Using a reverse genetics approach it was shown that *CBF2/DREB1C* acts as a negative regulator of *CBF1/DREB1B* and *CBF3/DREB1A* expression (Novillo *et al.* 2004). The *DREB1/CBF* genes were also regulated by bHLH-type of transcription factor, *ICE1* (Chinnusamy *et al.* 2003), and by Ca$^{2+}$ related processes, because mutations in *CAX1* (encoding a Ca$^{2+}$/H$^{+}$ transporter) and *CBL1* (Ca$^{2+}$-sensor protein) affected expression pattern of *DREB1/CBF* genes (Albrecht *et al.* 2003, Catala *et al.* 2003).

The mechanism of activation of DREB2-type genes is not well studied, earlier studies have shown that the over-expression of AtDREB2A and OsDREB2A protein in *Arabidopsis* was not sufficient for the induction of target stress inducible genes (Liu *et al.* 1998, Dubouzet *et al.* 2003), and it was assumed that some post-translational modifications, probably phosphorylation and/or dephosphorylation events may be necessary to play a role in activating the expression of stress responsive genes. Furthermore, the removal of a negative regulatory domain containing the PEST sequence (RSDASE VTSTSSQSEVCTVETPGCV) from AtDREB2A, changed it to constitutive active form, capable of upregulating a number of drought, salt and heat-responsive downstream genes. In ZmDREB2A, a PEST sequence is not reported and its overexpression in original form resulted in expression of the abiotic stress tolerance genes (Qin *et al.* 2007). We have shown that stress-inducible *DREB2A* gene from *Pennisetum glaucum* is a phosphoprotein and its phosphorylation negatively regulates its DRE binding activity (Agarwal *et al.* 2007).

Another class of transcription factors, NAC is reported in plants, which regulate both ABA-dependent and independent genes. They are expressed in different tissues at various developmental stages and are involved in many aspects of plant growth and development (Olsen *et al.* 2005). The N-terminal region contains highly conserved NAC [for NAM (no apical meristem), ATAF1, 2, and CUC2 (cup-shaped cotyledon)] domains, which may form helix-turn-helix structure, that specifically binds to target DNA (Aida *et al.* 1997). The C-terminal region of these proteins is a putative transcriptional activation domain and shows high divergence in sequence. The first NAC gene isolated was *NAM* from petunia (Souer *et al.* 1996), which plays a critical role in determining meristem and primordia positions. Recently, NAC genes were also found to be involved in abiotic and biotic stresses (Fujita *et al.* 2004, Tran *et al.* 2004, 2007, Nakashima *et al.* 2007). The *ERD1*, a NAC family member, is upregulated in response to drought, high salinity and dark-induced senescence but not with cold or ABA treatment (Kiyosue *et al.* 1993, Nakashima *et al.* 1997). Promoter analysis of the *ERD1* gene shows that its expression during dehydration depends on the integrity of both 14-bp rps1 sequence and the putative MYC like (CATGTG) sequence (Simpson *et al.* 2003). Three NAC *trans*-acting factors, responsive to drought, high salinity, ABA and MeJA (methyl jasmonic acid) interact with the above mentioned putative *cis*-acting motifs found in the *ERD1* promoter region (Tran *et al.* 2004). The NAC proteins bound specifically to the NAC recognition site (NACRS), which contains the CATGTG motif, both *in vivo* and *in vitro*. The NAC proteins could bind to NACRS even as multimers, heterodimerization might potentiate the transcriptional activity of the NAC proteins (Tran *et al.* 2004). Structural studies of the NAC

Table 2. Abiotic stress tolerance potential of transgenic plants overexpressing ABA dependent and independent transcription factors. Ah - *Atriplex hortensis*, At - *Arabidopsis thaliana*, BN - *Brassica napus,* Gm - *Glycine max*, Os - *Oryza sativa*, W - common wheat, Zm - *Zea mays*, * - microarray analysis.

| Gene | Increased tolerance to | ABA senstivity (+ or -) | Upregulated genes | References |
|---|---|---|---|---|
| ABF2 | drought, salt, freezing | (+) or (-) | 7 | Kim *et al.* 2004 |
| ABF3 | drought, chilling, freezing, heat | (+) | 7 | Kang *et al.* 2002 |
| ABF4 | drought, freezing, heat | (+) | 6 | |
| GmbZip44, 62, 78 | salt, freezing | (+) | 7, 7, 7 | Liao *et al.* 2008a |
| GmbZIP132 | salt at germination stage | | 6 | Liao *et.al.* 2008b |
| Wlip19 | freezing, osmotic stress | | | Kobayashi *et al.* 2008a |
| AtMYB2/ AtMYC2 | osmotic stress | (+) | 40* | Abe *et al.* 2003 |
| GmMBY76 | salt, freezing | | 6 | Liao *et al.* 2008c |
| GmMBY92 | | | 3 | |
| GmMBY177 | | | 9 | |
| OsMYB3R2 | cold, drought, salt | (-) | 3 | Dai *et al.* 2007 |
| MYB15 | reduced freezing tolerance | | | Agarwal *et al.* 2006b |
| ANAC019 | drought | | 8 | Tran *et al.* 2004 |
| ANAC055 | | | 9 | |
| ANAC072 | | | 22 | |
| OsNAC6 | dehydration, salt | | 163* | Nakashima *et al.* 2007 |
| SNACI | drought, salt | | 91 | Hu *et al.* 2006 |
| SNAC2 | cold, drought, salt | (+) | | |
| AtCBF1 | freezing | | 2 | Jaglo *et al.* 2001 |
| | | | 1 | Hsieh *et al.* 2002 |
| | | | 2 | Xiong and Fei 2006 |
| AtCBF3 | freezing | | 4 | Gilmour *et al.* 2000 |
| AtCBF4 | freezing, dehydration | | 2 | Haake *et al.* 2002 |
| BNCBF5, BNCBF17 | freezing | | 16* | Savitch *et al.* 2005 |
| AtDREB1A | freezing, dehydration | | 12 | Liu *et al.* 1998; Seki *et al.* 2001 |
| | freezing, dehydration | | 4 | Kasuga *et al.* 2004 |
| | drought | | 1 | Zhao *et al.* 2007 |
| AhDREB1 | dehydration, salt | | 2 | Shen *et al.* 2003a |
| AtDREB2A | dehydration, salinity | | 483* | Sakuma *et al.* 2006a |
| AtDREB2A | heat stress | | 778* | Sakuma *et al.* 2006b |
| GmDREB2 | dehydration, salt | | 2 | Chen *et al.* 2007 |
| OsDREB1A | freezing, dehydration, salt | | 10* | Dubouzet *et al.* 2003 |
| OsDREB1F | salt, drought, cold | | 4 | Wang *et al.* 2008 |
| OsDREB1G<br>OsDREB2B | drought | | | Chen *et al.* 2008 |
| WDREB2 | freezing, osmotic stress | (+) | | Kobayashi *et al.* 2008b |
| ZmDREB2A | drought, heat stress | | 44* | Qin *et al.* 2007 |

domain suggest dimerization of the NAC proteins through conserved interactions (Ernst *et al.* 2004). Also the co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the *ERD1* gene in *Arabidopsis* (Tran *et al.* 2007). *RD26*, one of the NAC proteins, is probably involved in a novel ABA-dependent stress-signalling pathway (Fujita *et al.* 2004). *Arabidopsis RD26*, is induced not only by drought but also by high salinity, ABA and MeJA. The *RD26* over-expressing plants were highly sensitive to ABA and upregulated ABA- and stress-inducible genes, whereas the ABA insensitive *RD26*-repressed plants repressed the stress-inducible genes (Fujita *et al.* 2004). Another stress-responsive NAC gene from rice *OsNAC6*, a member of ATAF subfamily (Kikuchi *et al.* 2000, Ooka *et al.* 2003), has been reported to mediate responses to cold, high salinity, drought, ABA and JA (Nakashima *et al.* 2007, Ohnishi *et al.* 2005). Many abiotic and biotic stress-responsive genes were upregulated in the *OsNAC6* transgenic plants, and the transgenics were tolerant to dehydration, high salt stresses and even showed slightly improved tolerance to blast disease (Nakashima *et al.* 2007). The ATAF1 was one of the first NAC-domain proteins identified in *Arabidopsis,* it functions as a negative regulator in drought signalling pathways through modulation of osmotic stress-responsive gene expression. *ataf1* mutants lines showed a seven times higher recovery rate than wild-type plants following drought treatment. Northern blot analyses indicated that *ataf1* mutant plants expressed high levels of drought-induced stress-responsive genes, including *COR47* (also known as *RD17*), *ERD10*, *KIN1*, *RD22* and *RD29A* (*COR78* or *LTI78*) (Lu *et al.* 2007).

*SNAC1* gene from rice is induced in the guard cells under drought stress condition (Hu *et al.* 2006). The overexpression of this gene in rice resulted in stomatal closure and drought resistance in drought-stressed field conditions and improved salt tolerance (Hu *et al.* 2006). Recently, plants over expressing the *SNAC2* transcription factor from upland rice IRAT109 showed significantly improved tolerance to cold, salinity and dehydration stresses (Hu *et al.* 2008).

A group of membrane bound NAC transcription factors (designated NTLs) are reported to be closely linked with environmental stresses (Kim *et al.* 2007). The NTL proteins are released from the membranes by proteolytic cleavage, possibly in response to stress conditions, and are transported into the nucleus, where they regulate expression of stress responsive genes. Recently, Kim *et al.* (2008) reported that a salt-inducible NTL member, *NTL8*, regulates gibberellic acid (GA)-mediated salt signalling in seed germination. High salinity reduces GA biosynthesis by repressing GA biosynthetic genes (Magome *et al.* 2004), which in turn induces the *NTL8* gene. The expression of *NTL8* during salt stress prevents seed germination, thereby indicating that GA signalling may also play a negative role in seed germination. This may lead to physiological adaptation for successful plant propagation under high salinity.

## Cross talk between ABA-dependent and ABA-independent signal transduction pathways

The understanding of molecular mechanisms for ABA-dependent and ABA-independent gene regulation is based on analysis of the promoters of stress-responsive genes and the isolation of transcription factors that activate these genes. The studies of a large number of *co*s (constitutive expression of osmotically responsive genes), *los* (low expression of osmotically responsive genes) and *hos* (high expression of osmotically responsive genes) mutants, show that ABA-dependent and ABA-independent pathways crosstalk to activate stress genes expression. The DREB and AREB proteins cumulatively transactivate the *rd29A* promoter-GUS fusion gene (Narusaka *et al.* 2003). The *rd29A* promoter has both DREs (three) and ABREs (one). The ABA-independent DREB genes are expressed rapidly (within 20 min) in response to dehydration, cold and salinity stress (Yamaguchi-Shinozaki and Shinozaki 1993, 1994), but not by ABA as ABA biosynthesis gets induced in 2 h of dehydration and high salinity stresses (Kiyosue *et al.* 1994). In contrast, the ABRE functions in induction of *rd29* after the accumulation of ABA under dehydration and high salt. Since both DRE and ABRE mediate the expression of *rd29* gene, therefore, an interaction is needed to maintain its level during different stress. Overexpression studies with *OsDREB1F* have also showed increased expression of both ABA-independent (*COR15a*, *rd29A*) and ABA-dependent (*RAB18*, *rd29b*) genes (Wang *et al.* 2008).

Genetic analysis using *RD29A-LUC* as a molecular marker has shown that ABA-dependent and ABA-independent signalling pathways have extensive connections between them (Ishitani *et al.* 1997, Xiong *et al.* 1999a,b). A dependence of ABA-independent pathway during salt stress on ABA is apparent from the reduced salt stress induction of a *RD29A*-LUC transgene in *los5 and los6* mutants, which was restored when ABA was given simultaneously with salt stress, indicating that exogenous ABA complements the reduced salt induction phenotype. This suggests that *LOS5*, *LOS6* are key regulator of ABA biosynthesis, stress-responsive gene expression, and stress tolerance. Previously, Liu *et al.* (1998) reported requirement of post-translational modifications, probably phosphorylation/dephosphorylation for activating DREB2A. These post-translational modifications may require cofactors, which are dependent on ABA-regulated molecules such as ABI1, ABI2, $Ca^{2+}$ dependent protein kinases, or other ABA-responsive regulatory factors (Leung *et al.* 1997, Finkelstein and Lynch 2000, Merlot *et al.* 2001).

Sreenivasulu *et al.* (2006) reported gene expression pattern by macroarray of 10 000 seed-expressed sequences in maternal tissue (mainly pericarp) and filial endosperm and embryo during barley seed development from anthesis until late maturation. In the embryo, ABA seems to influence the acquisition of desiccation tolerance *via* AREBs, but the data also suggest the existence of an ABA-independent but interactive pathway acting via the DREB2A. In soybean, the expression pattern of the *GmDREB2* gene suggests that it acts as an overlap point and might take part in both ABA-dependent and independent pathways, simultaneously (Chen *et al.* 2007). In *WDREB2* expression is responsive to exogenous ABA treatment (Egawa *et al.* 2006), whereas tobacco transgenics of this gene were hypersensitive to exogenous ABA during post germination growth compared with wild-type tobacco (Kobayashi *et al.* 2008b), thereby suggesting that wheat DREB2 might contribute indirectly to development of abiotic stress tolerance through an increase in ABA sensitivity. These studies highlight that there is a crosstalk during stress signalling, executed by the synergistic effect of ABA and drought/salt stress, for the regulation of stress responsive genes.

## Conclusion

Abiotic stress signal transduction pathways from signal perception to gene expression involve different *cis* and *trans*-acting elements. The basic leucine zipper factors AREB/ABF and MYC/MYB proteins activate the major ABA-dependent stress response through different ABREs and MYCRS and MYBRS. The DREB proteins activate the stress response through DREs in ABA-independent manner. NAC play a role through NACRS of *ERD1* and other promoters in both ABA-independent and ABA-dependent pathways, respectively. However, the ABA-dependent and ABA-independent pathways act in parallel and also interact, thereby providing added coordination between stress signals and ABA in the regulation of stress-responsive genes. Recently, *cis*-elements other than ABREs related to ABA signalling are suggested based on the mismatch of cell type-specific enrichment and regulation of gene expression by ABA. Microarray data on transcriptome changes has revealed numerous new stress responsive genes. However, the basic functional architecture for molecular responses, interactions and cross talk towards abiotic stress needs to be studied. Study of large number of ABA-dependent and independent transcription factors and their downstream binding elements can give better understanding of the molecular basis of stress tolerance, which involves complex network of genes operating in co-ordination.

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