Plant Cell Rep (2013) 32:985–1006
DOI 10.1007/s00299-013-1414-5

REVIEW

Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress

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Received: 27 December 2012 / Revised: 28 February 2013 / Accepted: 4 March 2013 / Published online: 19 March 2013
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Abstract Salinity, drought and low temperature are the common forms of abiotic stress encountered by land plants. To cope with these adverse environmental factors, plants execute several physiological and metabolic responses. Both osmotic stress (elicited by water deficit or high salt) and cold stress increase the endogenous level of the phytohormone abscisic acid (ABA). ABA-dependent stomatal closure to reduce water loss is associated with small signaling molecules like nitric oxide, reactive oxygen species and cytosolic free calcium, and mediated by rapidly altering ion fluxes in guard cells. ABA also triggers the expression of osmotic stress-responsive (*OR*) genes, which usually contain single/multiple copies of *cis*-acting sequence called abscisic acid-responsive element (ABRE) in their upstream regions, mostly recognized by the basic leucine zipper-transcription factors (TFs), namely, ABA-responsive element-binding protein/ABA-binding factor. Another conserved sequence called the dehydration-responsive element (DRE)/C-repeat, responding to cold or osmotic stress, but not to ABA, occurs in some *OR* promoters, to which the DRE-binding protein/C-repeat-binding factor binds. In contrast, there are genes or TFs containing both DRE/CRT and ABRE, which can integrate input stimuli from salinity, drought, cold and ABA signaling pathways, thereby enabling cross-tolerance to multiple stresses. A strong candidate that mediates such cross-talk is calcium, which serves as a common second messenger for abiotic stress conditions and ABA. The present review highlights the involvement of both ABA-dependent and ABA-independent signaling components and their interaction or convergence in activating the stress genes. We restrict our discussion to salinity, drought and cold stress.

Keywords Abiotic stress · Abscisic acid · Calcium · Cross-talk · Stress signaling

Communicated by P. Kumar.

A contribution to the Special Issue: Plant Hormone Signaling.

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Introduction

As sessile organisms, plants are constantly challenged by a wide range of environmental stresses such as drought, high salt and temperature fluctuations. Drought and salinity together affect more than 10 % of arable land, resulting in a more than 50 % decline in the average yields of major crops worldwide. Drought-induced crop losses have a significant economic impact, which is predicted to increase with global climate change (Marris 2008; Battisti and Naylor 2009). Among abiotic stresses, high salinity is the most severe environmental stress, which impairs crop production on at least 20 % of irrigated land worldwide. In addition, increased salinity of arable land is expected to have devastating global effects, resulting in up to 50 % land loss by the middle of 21st century. The problem of drought is also quite pervasive and economically damaging. Nevertheless, most studies on water stress signaling have focused on salt stress primarily because plant responses to salt and drought are closely related and the

mechanisms overlap. From a practical point, salt stress can be imposed more easily and precisely in laboratory settings. Freezing injury is caused due to ice-crystal formation in the intercellular spaces of plant tissues causing physical disruption of cells at temperature below 0 °C (Thomashow 1999). All these forms of abiotic stresses result in both general and specific effects on plant growth and development, reduced productivity and significant crop losses. The deteriorative effects may include leaf wilting, reduction in leaf area, leaf abscission, changes in relative water content, electrolyte leakage, generation of reactive oxygen species (ROS), and accumulation of free radicals which disrupt cellular homeostasis by reacting with lipids, proteins, pigments and nucleic acids resulting in lipid peroxidation, membrane damage, and inactivation of enzymes, thus affecting cell viability. Tolerance or susceptibility to these abiotic stresses is a very complex phenomenon, in part, because stress may occur at multiple stages of plant development and often multiple stresses simultaneously affect the plant during their development. Not only that, plant breeding experiments 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 (Yamaguchi-Shinozaki and Shinozaki 2006). Hence, the mechanisms underlying stress tolerance and adaptation have long been the focus of intensive research.

In contrast to animals, plants cannot avoid unfavorable circumstances by adjusting their location since they are sessile. When plants encounter stress conditions, they reprogram their cellular processes by triggering a network of long-distance signaling events that start with perception of stress cues, relay of signals to switch on adaptive responses and end with a cellular response, such as gene expression in the nucleus. Genes that normally are silent and activated under stress conditions often are referred as osmotic stress-responsive (*OR*) genes. As a result, differences in stress tolerance between genotypes or different developmental stages of a single genotype may arise from differences in signal perception and transduction mechanisms. The guard cell signaling is of critical importance because it is a key denominator within the plant water budget. Prevention of osmotic stress caused by drought depends upon minimizing stomatal and cuticular water loss and maximizing water uptake (through root growth and osmotic adjustment, OA). During salt stress, OA appears to play a major role in maintaining osmotic homeostasis, while survival during freezing-induced osmotic stress may depend upon prevention or delay of ice nuclei formation. The stress signaling can be divided into three functional categories: ionic and osmotic stress signaling for the re-establishment of cellular homeostasis under stress conditions, detoxification signaling to control and repair stress damages, and signaling to coordinate cell division and expansion. Numerous *OR* genes have been identified using microarray experiments (Kreps et al. 2002; Seki et al. 2002), including the transcription factors (TFs) and regulatory sequences in stress-inducible promoters. The *OR* genes not only function in protecting cells from stress by the production of important metabolic proteins but also in regulating the downstream genes for signal transduction. Large-scale transcriptome analysis has revealed that these gene products can broadly be classified into two groups. One group constitutes genes that govern the accumulation of compatible solutes (key enzymes for osmolyte biosynthesis like proline, betaine, sugars, etc.); passive transport through membranes and energy-requiring water-transport systems (water channel proteins and membrane transporters); and the protection and stabilization of cell structures from desiccation and damage by ROS (the detoxification enzymes such as glutathione S-transferase, catalase, superoxide dismutase, ascorbate peroxidase, etc.); enzymes for fatty acid metabolism, proteinase inhibitors, ferritin and lipid-transfer proteins; and other proteins for the protection of macromolecules [late embryogenesis abundant (LEA) protein, osmotin, antifreeze proteins, chaperons, etc.]. The second group of genes comprises regulatory proteins that further regulate stress signal transduction and modulate gene expression and, hence, probably function in the stress response. They include various TFs, protein kinases [mitogen activated protein kinase (MAPK), calcium-dependent protein kinase (CDPK), receptor protein kinase, etc.]; protein phosphatases and proteinases (phosphoesterases and phospholipase C, etc.) (Agarwal et al. 2006; Shinozaki and Yamaguchi-Shinozaki 2007). The TFs interact with *cis* elements in the promoter regions of various abiotic stress-related genes and thus upregulate the expression of many secondary responsive downstream genes, imparting stress tolerance. The molecular mechanisms regulating gene expression are dependent in part on the structure of chromatin, which determines the accessibility of DNA to the transcriptional machinery. Chromatin-driven mechanisms, including noncoding RNAs, changes in histone variants, histone modifications and DNA methylation are involved in the responses of plants to environmental cues (Chinnusamy and Zhu 2009). Changes in DNA methylation and/or histone modifications associated with altered gene expression under various stresses have been reported in plants. Chemical modifications of the histone amino-terminal tails may recruit specific TFs and/or induce nucleosome remodeling to facilitate transcription by RNA polymerase II.

Because of limited knowledge of stress sensors and because most of the signaling intermediates have not yet been identified, there is also little information regarding cross-talk between different stress signal transduction

pathways in plants. The term 'cross-talk' is used loosely to refer to situations where different signaling pathways share one or more intermediates/components or have some common outputs. Based on the presence of these general and specific abiotic stress tolerance mechanisms, it is logical to expect plants to have multiple stress perception and signal transduction pathways, which may cross-talk at various steps in the pathways. In this review, we aim to highlight this cross-talk mechanism with respect to the universal stress phytohormone abscisic acid (ABA).

Abscisic acid

ABA is an important sesquiterpenoid ($C_{15}H_{20}O_4$) phytohormone whose application to plant mimics the effect of stress condition. ABA is synthesized de novo during dehydration, and degraded during rehydration after dehydration. The endogenous ABA level is triggered in a plant system in response to various stress signals, mainly due to the induction of genes encoding enzymes responsible for ABA biosynthesis from β-carotene (Roychoudhury and Basu 2012). Several ABA biosynthesis genes have been cloned which include zeaxanthin epoxidase (ZEP, Known as *ABA1* in *Arabidopsis*), 9-*cis*-epoxycarotenoid dioxygenase (*NCED*), ABA aldehyde oxidase (AAO3) and MoCo sulfurase (ABA3) (Tuteja 2007). *AtNCED3* is most strongly induced by dehydration and high salinity. Overexpression of *AtNCED3* improved dehydration stress tolerance in transgenic plants and its knockout mutant showed a dehydration-sensitive phenotype. This indicated that AtNCED3 must have an important role in ABA accumulation during dehydration. ABA is catabolized to inactive form by oxidation or conjugation. An enzyme named ABA 8′-hydoxylase, encoded by *CYP707A* gene, regulates the oxidative pathway of ABA catabolism. CYP707A3 is the major ABA-catabolizing enzyme expressed in vegetative tissues, strongly induced by exogenous ABA treatment, dehydration, and rehydration. Various stress signals and ABA share common elements in the signaling pathway and these common elements cross-talk with each other, to maintain cellular homeostasis. ABA controls many stress adaptation responses, activation of *OR* genes involved in OA, ion compartmentation, regulation of shoot versus root growth and modifications of root hydraulic conductivity. ABA also helps in limiting transpiration rate and controls wilting, thus reducing water loss in plants (Agarwal and Jha 2010). The stomatal closure, initiated by ABA, under drought conditions prevents the intracellular water loss and is affected through a complex symphony of intracellular signaling in which nitric oxide (NO) appears to be one component. Exogenous NO induces stomatal closure, ABA triggers NO generation, removal of NO by scavengers inhibits stomatal closure in response to ABA, and ABA-induced stomatal closure is reduced in mutants that are impaired in NO generation (Neill et al. 2008). The main function of ABA seems to be the regulation of plant water balance and osmotic stress tolerance. In addition, ABA is required for freezing tolerance, which also involves the induction of dehydration tolerance genes.

The effect of ABA on stomatal closure is mediated through guard cell depolarization and alterations of guard cell turgor and volume, driven by cation and anion effluxes (Roychoudhury and Paul 2012). ABA induces a large depolarization at the plasma membrane of guard cells that is controlled through (1) the inhibition of the plasma membrane H^+-ATPase, (2) inhibition of the K^+ influx channel, and (3) activation of the slow (S-type) anion channel. This depolarization activates K^+ efflux from the cell, resulting in a net loss of solutes and a decrease in turgor. Cytosolic increases in H_2O_2 concentrations occur in guard cells before exogenous ABA induced stomatal closure. An ABA-dependent increase in the concentration of cytosolic free Ca^{2+} levels blocks inward rectifying K^+ channels, responsible for K^+ influx into guard cells, activates endogenous Ca^{2+} sensitive Cl^- channel, which in turn triggers the intracellular machinery for stomatal closure. Inactivation of the plasma membrane H^+–ATPase by ABA also causes membrane depolarization during stomatal closure (Roychoudhury and Paul 2012). The ABA signal can be relayed to the nucleus of guard cells to bring about alterations in the pattern of gene expression. Stimulation of the ABA-nuclear signaling pathway by exogenous ABA increased the expression of dehydrin-like transcripts in the guard cells of both *Vicia faba* (Shen et al. 1995) and pea (Hey et al. 1997). In *Arabidopsis* guard cells, ABA increased the expression of a water-transport protein encoded by the *AthH2* gene (Kaldenhoff et al. 1996). Exogenous ABA stimulates tissue-specific expression of *OPEN STOMATA 1* (*OST1*) gene in guard cell protoplasts. OST1 is an ABA-activated Ser/Thr protein kinase, closely related to ABA-activated protein kinase (AAPK) of *Vicia faba* (Li et al. 2000). A downstream target of AAPK phosphorylation, identified as a specific RNA-binding protein named AKIP1, within the nucleus of guard cells, can bind to dehydrin mRNA after activation, showing ABA/drought response. AAPK and OST1/SnRK2-type protein kinase (SRK2E) of *Arabidopsis* are ABA-activated SnRKs, which function in stomatal closure (Himmelbach et al. 2003). Protein farnesylation, which enables Rho-like small G-protein ROP10, negatively regulates ABA-mediated stomatal closure. The ROP proteins are also associated with increased H_2O_2 production because of their activation of NADPH oxidases. Lee et al. (2006) have proposed that the activation of inactive ABA pools by polymerized AtBG1 (a β-glucosidase) could be a mechanism by which

plants rapidly adjust ABA levels and respond to changing environmental cues.

ABA-dependent pathways

Many drought and high salinity-inducible *OR* genes respond to ABA in *Arabidopsis* and rice (Kawasaki et al. 2001; Seki et al. 2001, 2002; Rabbani et al. 2003). Complete sequencing of 32,127 full-length cDNA clones from *Oryza sativa* L. ssp japonica cv. Nipponbare identified 200 ABA-responsive genes (Yazaki et al. 2004). The ABA-inducible genes upregulated by salt or drought stress belong to several groups. These genes encode the LEA proteins, enzymes (involved in the biosynthesis of osmolytes, detoxification and general metabolism), transporters (ion transporters, channel proteins and aquaporins), and regulatory molecules like TFs, protein kinases and phosphatases. The most common and widely reported ABA-regulated genes are the *LEA* or *LEA* like genes. Examples of some of these genes include *Osem*, *SalT*, *Rab16* and *Rab25* from rice, *Em* and *Rab15* from wheat, *Rab17* and *Rab28* from maize, *Rab18* from *Arabidopsis*, *HVA1* and *HVA22* in barley, *le16*, *le4* and *TAS14* from tomato, *CDeT27-45* from *Craterostigma plantagineum*, dehydrins from maize and barley, and several other genes from cotton, rapeseed, soybean, carrot, potato, etc. (Roychoudhury et al. 2011). Transgenic tobacco plants overexpressing *Rab16A* from the salt-tolerant rice Pokkali, showed highly inducible gene expression under salinity, water stress and ABA, along with enhanced tolerance to salinity stress (Roychoudhury et al. 2007). Increased tolerance to salinity of indica rice through *Rab16A* gene overexpression has also been recently reported (Ganguly et al. 2012). Most ABA-inducible genes contain a conserved, ABA-responsive, 8 bp *cis*-acting sequence, designated as abscisic acid-responsive element (ABRE, PyACGTGG/TC), with ACGT core in their promoter regions. ABREs were first reported in wheat *Em* gene, which functions in seed during late embryogenesis and in rice *Rab16*, which is expressed in dehydrated vegetative tissues and maturing seeds. The first identified ABRE element, typified by the Em1a element (GGACACGTGGC) of *Em* gene is considered as the strongest ABRE. Comparison of the promoter sequences of the four rice *Rab16* genes revealed two highly conserved motifs, motif I (5′-GTACGTGGC-3′) and motif II (5′-CGG/CCGCGCT-3′), the core of motif II found twice in *Rab16A* (motif IIa and motif IIb) and once each in *rab16B-D*. For ABA-dependent transcription, a single copy of ABRE is not sufficient. Either multiple copies of ABREs may be present in the upstream regions of stress-inducible genes or ABRE(s) acts in conjunction with another GC-rich sequence called the coupling element (CE), usually sharing a CGCGTG consensus. The ABRE and CE, spaced at less than 20 bp distance, together constitute an abscisic acid-responsive complex (ABRC), e.g., CE1, CE3 and motif III constitute an ABA-responsive complex in the regulation of wheat *HVA22*, *HVA1* and *Rab16B* genes (Roychoudhury et al. 2008). Introgression of the two synthetically designed promoters, 4X ABRE (four tandem repeats of Em1a) and 2X ABRC (two copies of Em1a plus two copies of CE1 from *HVA22*), each fused with minimal promoter of CaMV35S, could induce the *gusA* expression in transgenic tobacco in response to high NaCl concentration, water stress or ABA, but not at the constitutive level, proving that they are efficient stress-inducible promoters (Roychoudhury and Sengupta 2009). The above two promoters, together with *Rab16A* promoter, has also been shown to function, in a concentration-dependent manner, as potent ABA-inducible promoters in both vegetative and floral organs of transgenic rice (Ganguly et al. 2011). Most known CEs are similar to ABREs, CE3 functions as a second copy of ABRE in the rice *Osem* promoter. ACGT-containing ABREs and CE3 are functionally equivalent (Hobo et al. 1999). The ABRE and ABRE-like sequences are not the monopoly of *LEA* genes alone. The *Arabidopsis* genes for polyamine biosynthesis, namely *SAMDC1* (S-adenosylmethionine decarboxylase), *SAMDC2*, *SPDS1* (spermidine synthase) and *SPMS* (spermine synthase) were found to contain three, five, four and one ABRE(s), respectively (Alcazar et al. 2006). The TFs, belonging to the basic leucine zipper (bZIP) family, bind mostly to the ABREs/CEs of their target genes and regulate their stress-inducible expression. Several such factors include wheat EmBP-1, rice RITA-1 in developing endosperm, especially in aleurone layer cells, rice OSBZ8, osZIP-1a, 2a and 2b, tobacco TAF-1, *Arabidopsis* TGA-1, ABFs, AREB 1, 2 and 3 (Roychoudhury et al. 2008 and the references therein). The bZIP-ABRE system is an ABA-dependent stress signal transduction pathway. In the *Arabidopsis* genome, 75 distinct bZIP TFs exist and 13 members are classified as a subfamily of AREB/ABFs that contain four conserved domains (Jakoby et al. 2002). *AREB1*, *AREB2* and *AREB3* from *Arabidopsis* all encode bZIP-type proteins. Stress-inducible AREB1 and AREB2 function as transcriptional activators in the ABA-inducible expression of *rd29B*. *Arabidopsis* AREB1/ABF2, AREB2/ABF4 and ABF3 are mainly expressed in response to dehydration and high salinity in vegetative tissues, but not in seeds. Fujita et al. (2005) reported that transgenic *Arabidopsis* plants overexpressing the active form of AREB1 showed ABA hypersensitivity and enhanced drought tolerance. Four ABF (*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). About 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 signaling. The salt tolerance conferred by *GmbZIP* genes from *Glycine max* is dependent on developmental stage and freezing tolerance depends on proline content of the transgenics (Liao et al. 2008). The transgenics of 'group A' bZIP proteins, viz., ABF2/AREB1, ABF3 and ABF4 are hypersensitive to ABA and act as positive regulators of ABA signaling, whereas the GmbZIPs act as negative regulators for ABA signaling. *ABI5* (ABA-insensitive 5) is a member of *Arabidopsis* bZIP TF subfamily whose expression is higher in mature seeds and young seedlings exposed to ABA or dehydration stress. Its expression is promoted by multiple *ABI* gene products including the TFs like ABI3, ABI4 and ABI5 itself and inhibited by closely related ABF3 (Finkelstein et al. 2005). The TFs like OsbZIP23 and TRAB-1 were reported to have nine and five ABREs, respectively (Xiang et al. 2008). G-box homologous sequences were also reported in stress-inducible TFs called NAC [for NAM (no apical meristem), ATAF1, 2, and CUC2 (cup-shaped cotyledon)] from *Arabidopsis*, viz., ACACGTGTCA at position −126 for the ANAC019 promoter, CCACGTGTCA at position −107 for the ANAC055 promoter, and ACACGTGTCG at position −472 for the ANAC072 promoter (Tran et al. 2004). *Arabidopsis rd26* (responsive to dehydration) encodes a NAC protein and is induced not only by dehydration but also by ABA. The 1.5 kbp promoter region of *rd26* contains four ABREs (Fujita et al. 2004). Transgenic plants overexpressing *rd26* were highly sensitive to ABA, whereas *rd26*-repressed plants were insensitive. Microarray analysis showed that ABA- and stress-inducible genes were upregulated in *rd26*-overexpressing plants and repressed in *rd26*-repressed plants, indicating that a *cis*-regulatory factor, the NAC-recognition site, may function in ABA-dependent gene expression under stress conditions. Rice RITA-1 weakly transactivates motif I of *Rab16A*, wheat EmBP-1 regulates transcriptional regulation of *Em* ABRE and rice TRAB-1 controls transcriptional regulation of several genes like *Osem* and *Rab16A* in embryo and vegetative tissues. TRAB-1 has a higher relative affinity to *Em1a* compared with motif A and motif I. Roychoudhury et al. (2008) suggested that OSBZ8 is the master regulatory *trans*-acting factor that simultaneously targets the expression of *Rab16A* and *Osem* in the vegetative tissues of rice. A novel observation was made regarding the binding of OSBZ8, not only with the typical ABRE, i.e., motif I of *Rab16A*, but also with the GC-rich motif IIa, though with a weaker intensity. It was thus suggested that motif IIa probably acts as a CE or functions as a second copy of motif I (ABRE). Earlier, Hobo et al. (1999) also suggested that CE3 acts along with motif A (ABRE) in *Osem* and that both motif A and CE3 specifically bind the same ABRE-binding factor, TRAB-1. The low-temperature-induced protein (lip), a bZIP TF has been isolated from different cereal plants. Rice *lip* is strongly induced by cold (Aguan et al. 1991), 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. 2008). The homeodomain-containing TF, ATHB6 binds to an AT-rich *cis*-acting element CAATTATTG and interacts with ABI1. Transgenic *Arabidopsis* plants that constitutively express *ATHB6* revealed ABA insensitivity in a subset of ABI1-dependent responses. Thus, ATHB6 functions as a negative regulator downstream of ABI1 in the ABA signal transduction pathway. Overexpression of a soybean cold- and ABA-inducible C2H2-type zinc finger protein (SCOF1) in *Arabidopsis* resulted in constitutive expression of stress-responsive genes and freezing tolerance. Although SCOF1 is unable to directly bind to ABRE motifs, it enhances the DNA-binding activity of SGBF1, a bZIP TF, to ABRE of *OR* genes. Thus, SCOF1–SGBF1 appears to regulate the ABA-dependent pathway of *OR* gene expression through ABRE during cold stresses (Kim et al. 2001). *OsABI5* from rice showed transcript upregulation by ABA or high salt and downregulation by drought and cold. Its overexpression enhanced salinity tolerance (Zou et al. 2010). AtERF7 binds to a *cis*-acting element, a GCC-box, and acts as a repressor of GCC-box-mediated transcription. AtERF7 interacts with the protein kinase PKS3, which is a global regulator of ABA responses and can be phosphorylated by PKS3. Overexpression of *AtERF7* in transgenic *Arabidopsis* reduced ABA responses in guard cells and decreased drought tolerance, whereas reductions in *AtERF7* expression caused ABA hypersensitivity in guard cells, seed germination and seedling growth. Maize TFs, DBF1 and DBF2 were found to transactivate the *Rab17* gene. DBF1 was shown to be an activator of the ABA-dependent expression of the *Rab17* gene, whereas DBF2 overexpression reduced promoter activity under either controlled or ABA-induced conditions. Another factor VP1 from maize is unique in the sense that it possesses no specific intrinsic binding to ABRE motifs; rather it is a coactivator that interacts with the associated factors like TRAB-1 by protein–protein interactions, and then binds to the ABRE motif triggered by ABA signal. This interaction stimulates EMBP-1 ability to bind to the *Em* ABRE, supporting a multiple protein interaction model.

ABRE-like motifs are not involved in the ABA regulation of some stress-inducible genes such as *rd22*. Induction of the dehydration-inducible *rd22* is mediated by ABA and requires protein biosynthesis for its ABA-dependent expression. MYC and MYB recognition sites in the *rd22* promoter function as *cis*-acting elements in the dehydration-inducible expression of *rd22*. The MYC/MYB families of TFs participate in the ABA-dependent pathway for the upregulation of the abiotic stress-responsive genes. The AtMYC2 (rd22BP1) and AtMYB2 proteins bind to CACATG and TGGTTAG *cis*-acting elements, respectively, of the *rd22* promoter of *Arabidopsis* and cooperatively activate this promoter. These two TFs are synthesized after the accumulation of endogenous ABA, indicating that they play roles at a late stage of the plant response to different stresses. 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). The expression levels of *responsive to dehydration29B* (*rd29B*), *DREB2A*, Δ^1*-pyrroline-5-carboxylate synthetase* (*P5CS*), *rd1*, *early dehydration-inducible10* (*erd10*) and *cold-regulated78/responsive to dehydration29A* (*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 (Vannini et al. 2004), the tomato transgenic showed higher tolerance to drought stress but not to cold stress (Vannini et al. 2007), whereas the apple transgenic showed increased drought and cold tolerance (Pasquali et al. 2008).

ABA-responsive 42 kDa kinases phosphorylate the conserved regions of AREB/ABFs, which suggest that ABA-dependent phosphorylation may be involved in activation of AREB subfamily proteins. 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. Activation of AREB1 requires ABA-dependent post-transcriptional modification. ABA-activated 42 kDa kinase phosphorylates Ser/Thr residues of R-X-X-S/T sites in the conserved regions of AREB1. Transgenic plants overexpressing the phosphorylated active form of AREB1 expressed many ABA-inducible genes, including *rd29B*, without ABA treatment (Furihata et al. 2006). Post-translational modification of OSBZ8 through phosphorylation by ATP, casein kinase II (CKII) or cytosolic S_{80} fraction (probably the source of activator or kinase) stimulated its binding to the upstream regions of its target gene *Rab16A* (Roychoudhury et al. 2008), further suggesting the role of phosphorylation event in gene expression during salinity stress. TRAB-1 is activated via ABA-dependent phosphorylation at Ser102 in the N-terminal region. ABA-activated SnRK2 protein kinases directly phosphorylate TRAB-1 in response to ABA. The SAPK8, SAPK9, and SAPK10 (orthologs of SnRK2.2, SnRK2.3 and SnRK2.6) directly phosphorylate TRAB-1 in response to ABA. TRAB-1 gets phosphorylated not only in response to ABA, but also in response to hyperosmotic stress (Kobayashi et al. 2005). These results suggested that the ABA-dependent multisite phosphorylation of TFs regulates its own activation in plants and that phosphorylation- and dephosphorylation-regulated events are important for ABA signaling. The barley TF, HvABI5 binds to the *cis* elements in the promoter region of *HVA1*, an ABA- and stress-responsive gene (Xue and Loveridge 2004). Expression of OsABI5 was induced by ABA and high salinity, but was down-regulated by drought and cold stress in seedlings. Several type-2 SNF1-related protein kinases (SnRK2-type), such as OST1/SRK2E in *Arabidopsis* (Mustilli et al. 2002; Yoshida et al. 2002), were identified as ABA-activated protein kinases and shown to mediate the regulation of stomatal aperture. The genes with an ABRE in their promoters are likely to be regulated by SnRK2s. They are positive regulators of plant responses to water deficit and complement the ABA-dependent kinases in plant defense against environmental stress (Kulik et al. 2011). In *Arabidopsis*, nine of ten SnRK2 are activated by hyperosmolarity and five of nine SnRK2 are activated by ABA (Boudsocq et al. 2004). The regulation of the plant response to ABA via SnRK2 pathways occurs by direct phosphorylation of various downstream targets. ABA-activated SnRK2 protein kinases, including SnRK2.2/SRK2D, SnRK2.3/SRK2I and SnRK2.6/SRK2E, phosphorylate the AREB1 polypeptide. ABA-dependent SnRK2 s from group 3 (SnRK2.2, SnRK2.3, and SnRK2.6) phosphorylate ABFs in vitro at sites crucial for their activity (Furihata et al. 2006; Fujii et al. 2007). Ten SnRK2 protein kinases were identified in rice. All family members are activated by hyperosmotic stress. Three genes of this family are also activated by ABA. Chae et al. (2007) isolated a dehydration-inducible gene (designated OSRK1) that encodes a 41.8-kDa protein kinase of the rice SnRK2 family. OREB1, a rice ABRE-binding factor, was phosphorylated in vitro by OSRK1 at multiple sites of different functional domains.

ABA-independent pathways of stress-responsive gene expression

Whether all or most of the osmotic stress responses are dependent on ABA has been of great interest. This question has often been addressed in the context of stress gene regulation, partly made possible by the availability of ABA-deficient and ABA-insensitive mutants. *Arabidopsis aba1*, *aba2*, and *abi1* and *abi2* mutants have been extensively used for this type of study. The role of ABA in cold responses is still unclear. Only a few years ago, ABA was thought to have a major role in cold responses. Lang et al. (1994) found transiently increased ABA accumulation in response to chilling treatment. However, other studies do not seem to find ABA accumulation under cold stress. It is clear that there is no dramatic ABA synthesis in the cold owing to the general slowdown effect of cold on cellular metabolism. Several studies found that exogenous ABA application increased the freezing tolerance of plants. Furthermore, cold and ABA induce a common set of genes. However, the cold stress induction appears to be completely independent of ABA. Mutations that enhance ABA induction of the *rd29A-luc* transgene also increased osmotic stress but not cold induction (Xiong et al. 1999). Nevertheless, the involvement of ABA in cold acclimation and cold-responsive gene expression cannot be ruled out.

Analyses of many drought-inducible *OR* genes have indicated the existence of ABA-independent signal transduction (Shinozaki and Yamaguchi-Shinozaki 2000). Several water stress-inducible genes, such as *responsive to dehydration29A/low-temperature induced78/cold-regulated78* (*rd29A/lti78/cor78*), *rd17* (*cor47*), *cold-inducible1* (*kin1*), *erd10* and *cor6.6*, are known to be regulated by dehydration and cold stress in one of ABA-independent signaling pathways, in which a *cis*-acting element, termed dehydration-responsive element/cold-responsive element or C-repeat (DRE/CRT), plays an important role (Kasuga et al. 1999; Shinozaki and Yamaguchi-Shinozaki 2000; Agarwal et al. 2006; Nakashima et al. 2009a, b). Many of these drought-induced genes are also responsive to high salinity and cold (Seki et al. 2002), suggesting cross-talk regulation of these environmental stresses. The DRE, with a 9-bp conserved core sequence (5′-TACCGACAT-3′) was first identified in the *rd29A* promoter for ABA-independent expression in response to dehydration and cold stresses (Yamaguchi-Shinozaki and Shinozaki 1994). The ABA independence of DRE-mediated transcription has been supported by several reports. A truncated *rd29A* promoter containing DREs without an ABRE was found to exhibit a loss of induction activity in response to exogenous ABA application, but to retain induction activity under osmotic stress treatments (Narusaka et al. 2003). Moreover, the osmotic stress-responsive transcription of *rd29A* was maintained in ABA-deficient mutant plants. The DRE-related motifs have also been reported in the promoter region of the cold-inducible *wcs120* gene from wheat. Subsequently, the CRT motif and the low-temperature-responsive element (LTRE) were identified in the promoters of cold-regulated genes from *Arabidopsis* such as *kin1*, *kin2* and *rab18*, and were also responsible for the regulation of *BN115* and *WCS120* from *Brassica napus* and wheat, respectively (Baker et al. 1994; Jiang et al. 1996; Ouellet et al. 1998; Saleh et al. 2005). A striking similarity occurs between the DRE and CRT, which share the core sequence CCGAC, suggesting cross-talk and integration of cold and drought stimuli at the promoters of several downstream target genes (Yamaguchi-Shinozaki and Shinozaki 1994; Haake et al. 2002).

In ABA-independent pathways during osmotic stress signaling, APETALA2 (AP2)-type TFs called the DREBs transactivate the DRE of stress-responsive genes. The DREB proteins namely, DREB1 and DREB2 are involved in two separate signal transduction pathways under low temperature and dehydration, respectively. The first isolated cDNAs encoding DRE-binding proteins, CBF1 (CRT-binding factor 1), DREB1A and DREB2A were isolated using yeast one hybrid screening (Stockinger et al. 1997; Liu et al. 1998) from *Arabidopsis*. Expression of the *Arabidopsis DREB1/CBF* genes is induced by cold, but not by dehydration and high-salinity stresses, while the *DREB2* genes are generally induced by dehydration, high-salinity, and heat stresses (Shinwari et al. 1998; Nakashima et al. 2000). Sakuma et al. (2002) reported that the DREB family includes three novel DREB1- and six novel DREB2-related genes and that two DREB1-related genes (DREB1D/CBF4 and DREB1F) are induced by high-salinity treatments. DREB1A/CBF3, DREB1B/CBF1 and DREB1C/CBF2 are strongly and transiently induced by low-temperature stresses, but not by dehydration and high-salinity stresses, in an ABA-independent manner (Gilmour et al. 1998; Fowler and Thomashow 2002). DREB2C is inducible by salt, mannitol and cold, but not by ABA. AtDREB1 was expressed within 10 min at 4 °C (Liu et al. 1998). The transcripts of *CBF* genes were detectable after 30 min of exposure to 4 °C with maximum expression at 1 h (Medina et al. 1999). CBF1/DREB1B and CBF3/DREB1A transcripts accumulated after 15 min of cold treatment, while CBF2/DREB1C transcripts accumulated at a slower rate with maximum expression after 2.5 h of cold exposure and then gradually declined (Novillo et al. 2004). The protein products of more than 40 genes, downstream of DREB1/CBF include RNA-binding proteins, phospholipase C, sugar transport proteins, desaturase, carbohydrate metabolism-related proteins, LEA proteins, KIN proteins, osmoprotectant biosynthesis protein and protease inhibitors. Most of those target genes contained the DRE/CRT or

related core motifs in their promoters (Maruyama et al. 2004). The downstream genes also included genes for TFs such as C2H2 zinc finger-type, suggesting the existence of further regulation of gene expression downstream of the DRE/DREB regulon. The product of one such downstream gene, *STZ*, functions as a transcriptional repressor, and its overexpression retards growth and induces tolerance to drought stress. Conserved sequences in the promoter regions of the genes directly downstream of *DREB1A* were analyzed, and A/GCCGACNT was found in their promoter regions between −51 and −450 as a consensus DRE. Vogel et al. (2005) showed that the CBF2/DREB1A-regulated genes included a significant portion of these cold-induced genes. Overexpression of the *Arabidopsis DREB1/CBF* genes in transgenic *B. napus* or tobacco plants induced expression of orthologs of *Arabidopsis* CBF/DREB1-targeted genes and increased the freezing and drought tolerance of transgenic plant. Pellegrineschi et al. (2004) showed that overexpression of DREB1A/CBF3 driven by the stress-inducible *rd29A* promoter in transgenic wheat improved drought stress tolerance. Oh et al. (2005) reported that constitutive overexpression of DREB1A using the CaMV35S promoter in transgenic rice resulted in increased stress tolerance to drought and high salinity. The expression of *Arabidopsis* DREB2A and its homolog DREB2B were induced by dehydration and high salt stress, but not by cold stress and exogenous ABA (Liu et al. 1998; Nakashima et al. 2000). ABA, mannitol and cold treatments had little effect on DREB2C expression but an elevated level of DREB2C mRNA was detected after 250 mM salt treatment (Lee et al. 2010).

Since CBF transcripts begin accumulating within 15 min of plant exposure to cold, it has been proposed that there is a TF already present in the cell at normal growth temperatures that recognizes the CBF promoters and induces CBF expression upon exposure to cold stress. The unknown activator(s) has been called Inducer of CBF Expression (ICE) protein(s). It has been suggested that, upon exposing a plant to cold, modification of either ICE or an associated protein would allow ICE to bind to the CBF promoter and to activate CBF transcription. A gene for a TF, Inducer of CBF Expression 1 (ICE1), encodes a MYC-like basic helix–loop–helix (bHLH) protein that specifically regulates the expression of *CBF3/DREB1A*, but not that of other *CBF/DREB1* genes, indicating that there are different expression mechanisms among the three *CBF/DREB1* genes. The *ice1* mutant plants are impaired in cold acclimation and defective in cold-regulated expression of CBF3 and its target *cor* genes. Zarka et al. (2003) reported two promoter sequences, designated *ICEr1* and *ICEr2* that function cooperatively to induce gene expression under cold conditions. *ICEr1* contains the sequence CACATG, which includes a consensus recognition site for bHLH proteins, viz., CANNTG. Therefore, *ICEr1* is a potential binding site for the ICE1 protein. However, ICE1 does not regulate the expression of *CBF2/DREB1C*. Cold-induced modification of ICE1 protein is necessary for it to act as a transcriptional activator of CBF3 or its target genes *rd29A* and *cor15A* at cold temperatures in planta. Novillo et al. (2004) reported that the *cbf2* mutant, in which the CBF2/DREB1C gene has been disrupted, has higher capacity to tolerate freezing, dehydration and salt stresses. They found that CBF/DREB1-regulated genes showed stronger and more sustained expression in the *cbf2* plants, which results from increased expression of CBF1/DREB1B and CBF3/DREB1A in the mutant. Thus, CBF2/DREB1C functions as a negative regulator of *CBF1/DREB1B* and *CBF3/DREB1A* expression in *Arabidopsis*, indicating complex regulation of *DREB1/CBF* gene expression.

In case of rice, a low-temperature-sensitive cereal, four DREB1/CBF homologous genes, OsDREB1A, OsDREB1B, OsDREB1C and OsDREB1D and one DREB2 homologous gene, OsDREB2A have been isolated (Dubouzet et al. 2003). OsDREB1A (cold/salt-inducible), OsDREB1B (cold-inducible) and OsDREB2B (cold/heat-inducible) are all ABA non-responsive. OsDREB1A and OsDREB1B were induced soon after cold exposure (within 40 min) but do not respond to ABA treatment. OsDREB1A was induced within 5 h of salt stress, whereas OsDREB1C was constitutively expressed during stress. Overexpression of OsDREB1A in transgenic *Arabidopsis* resulted in improved high-salinity and freezing-stress tolerance. A DREB1/CBF-type TF, ZmDREB1A was also identified in maize (Qin et al. 2004). The ZmDREB1A protein was shown to be involved in cold-responsive gene expression, and the overexpression of this gene in *Arabidopsis* resulted in improved stress tolerance to drought and freezing. Skinner et al. (2005) reported that barley, a low-temperature-tolerant cereal, contains a large DREB1/CBF family consisting of at least 20 genes (HvCBFs). OsDREBL accumulated quickly within 30 min in response to low temperature, but not in response to ABA, NaCl and dehydration (Chen et al. 2003). The expression of the *WCBF2* gene from wheat was induced rapidly by low temperature and drought but not by ABA (Kume et al. 2005). OsDREB2A transcript was induced within 24 h of dehydration and 250 mM salt stress but weakly responded to ABA and cold stress (Dubouzet et al. 2003). Expression of *Populus euphratica* PeDREB2 was induced by cold, drought and high salinity, but not by ABA (Chen et al. 2009). However, overexpression of DREB2A in transgenic plants neither caused growth retardation nor improved stress tolerance, suggesting that the DREB2A protein requires post-translational modification such as phosphorylation for its activation. The genes downstream of DREB2A play an important role in drought stress

tolerance, but alone are not sufficient to withstand freezing stress.

Direct hyperosmotic shocks cause rapid water loss from guard cells. For guard cells, osmotic stress signaling may utilize both ABA-dependent and ABA-independent pathways, as both induce stomatal closure by regulating the activities of several ion channels in guard cells. The osmotic stress resulting from variations in air humidity may be temporary and lead to rapid changes in stomatal aperture. A recent study has clearly demonstrated that stomatal regulation by air humidity is an ABA-independent process (Assmann et al. 2000). In the direct, ABA-independent osmosensing pathway, ion channels are the molecular targets of osmotic regulation that may involve cytoskeleton organization.

The role of phosphoinositide-specific phospholipase C (PI-PLC)—a key enzyme in phosphoinositide turnover—in hyperosmotic stress signaling, was analyzed by Takahashi et al. (2001) who noted changes in inositol 1,4,5-trisphosphate [Ins (1,4,5) P_3] content in response to hyperosmotic shock or salinity in *Arabidopsis thaliana* T87 cultured cells. Within a few seconds, a hyperosmotic shock, caused by mannitol, NaCl or dehydration, induced a rapid and transient increase in [Ins (1,4,5) P_3]. However, no transient increase was detected in cells treated with ABA. Neomycin and U73122, the inhibitors of PI-PLC, inhibited the increase in Ins [(1,4,5) P_3] caused by the hyperosmotic shock. A rapid increase in phosphatidylinositol 4,5-bisphosphate [PtdIns (4,5) P_2] in response to the hyperosmotic shock also occurred, but the rate of increase was much slower than that of [Ins (1,4,5) P_3]. These findings indicate that the transient [Ins (1,4,5) P_3] production was due to the activation of PI-PLC in response to hyperosmotic stress. The PI-PLC inhibitors also inhibited hyperosmotic stress-responsive expression of *rd29A* and *rd17* (*cor47*) that are controlled by the DRE/CRT *cis*-acting element but did not inhibit hyperosmotic stress-responsive expression of ABA-inducible genes, such as *rd20*. These observations suggested the involvement of PI-PLC and [Ins (1,4,5) P_3] in an ABA-independent hyperosmotic stress signal transduction pathway in higher plants. [Ins (1,4,5) P_3] thus functions in the induction of *OR* genes containing the DRE/CRT *cis*-acting element in an ABA-independent pathway.

There are several dehydration-inducible genes that do not respond to either cold or ABA treatment, suggesting the existence of another ABA-independent pathway in the dehydration stress response. The ERD1, a NAC family member, which encodes a Clp protease regulatory subunit, ClpD, is upregulated in response to drought, high salinity and dark-induced senescence but not with cold or ABA treatment (Nakashima et al. 1997). Microarray analysis of transgenic plants overexpressing cDNAs of any of the three MYC-like sequence binding proteins, *ANAC019*, *ANAC055* or *ANAC072* revealed that several stress-inducible genes were upregulated in the transgenic plants, and the plants showed significantly increased drought tolerance. However, ERD1 was not upregulated in the transgenic plants. Two different novel *cis*-acting elements, a MYC-like sequence (CATGTG) and a 14-bp *rps1*-like sequence, are involved in induction by dehydration stress. The cDNAs for the TF that binds to the 14 bp *rps1* sequence encode zinc finger homeodomain (ZFHD) proteins. One of these genes, *ZFHD1*, was shown to function as a transcriptional activator in response to dehydration stress. Overproduction of both the NAC and ZFHD proteins increased expression of *erd1*, indicating that both *cis*-acting elements are necessary for expression of *erd1*. The NAC proteins function as transcription activators in cooperation with the ZFHD proteins or alone. The ATAF1 was one of the first NAC domain proteins identified in *Arabidopsis*; it functions as a negative regulator in drought signaling pathways through modulation of *OR* gene expression. The *ataf1* mutant plants expressed high levels of drought-induced *OR* genes, including *cor47*, *erd10*, *kin1*, *rd22* and *rd29A* (Lu et al. 2007). The *SNAC1* gene from rice is induced in the guard cells under drought stress condition. The overexpression of this gene in rice resulted in stomatal closure and drought resistance in drought-stressed field conditions, and also improved salt tolerance (Hu et al. 2006). Another gene encoding the cold-inducible TF, *ZAT12*, was overexpressed under the control of the CaMV35S promoter in *Arabidopsis*. More than 20 cold-inducible genes were upregulated in the transgenic plants and these transgenic plants showed increase in freezing tolerance, indicating that ZAT12 functions in cold-responsive gene expression and plays an important role in cold acclimation.

Cross-talk

The term cross-talk, as stated earlier can be defined as any instance of two signaling pathways from different stressors that converge. This might take the form of different pathways achieving the same end or of pathways interacting and affecting each other's outcome, including the flux through one pathway affecting another. These might act in an additive or negatively regulatory way or might compete for a target. The concept of cross-talk is vital since in nature, the plants encounter combinations of abiotic stress concurrently or separated temporally. Several abiotic stress pathways share common elements that are potential "nodes" for cross-talk (Knight and Knight 2001). The *rd29A* gene has served as an excellent paradigm of ABA-dependent and ABA-independent gene regulation. It contains both the DRE/C-repeat and ABRE, and can therefore

integrate input stimuli from cold, drought, high salt and ABA signaling pathways. The *rd29A* promoter contains three functional DREs and one ABRE, involved in ABA-dependent and ABA-independent gene expression. Early studies showed that osmotic stress induction of *rd29A* transcript is only partially blocked by *aba1* or *abi1* mutations, thus suggesting both ABA-dependent and ABA-independent regulation. Though ABA does not activate the DRE element, it may be required for full activation of DRE by osmotic stress. Some of the genes regulated by DRE, such as *cor15a*, *rd17/cor47* and *erd10*, *kin1*, *cor 6.6/kin2*, etc., which also contain DRE, are also highly expressed in response to the exogenous application of ABA (Seki et al. 2001; Thomashow 1999). Narusaka et al. (2003) suggested that DRE/DRE-core motif could be a CE of ABRE, and that DRE and ABRE are interdependent in the stress-responsive expression of the *rd29A* gene. In promoters containing both the elements, the ABRE requires the DRE for ABA-induced expression. This suggestion is supported by data from the *srf6* cold acclimation mutant of *Arabidopsis*, which is deficient in the expression of cold-regulated genes that contain a DRE but expresses non-DRE-regulated cold genes normally. In *srf6*, DRE-containing genes also failed to be expressed fully in response to stimulation by ABA, suggesting that there is cross-talk between the ABRE and DRE (Knight and Knight 2001). The studies of a large number of *cos* (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 cross-talk to activate stress-responsive gene expression (Fig. 1). *Arabidopsis los2*, a chilling-sensitive mutant, is impaired in stress-responsive gene expression during cold but not under ABA, salt or osmotic stress treatment. However, no apparent difference in CBF expression was observed between the wild type and *los2*. *Arabidopsis* LOS2 binds to the promoter element of STZ/ZAT10. STZ/ZAT10 expression is induced rapidly and transiently by cold in the wild type, and this induction is stronger and more sustained in the *los2* mutant, suggesting that LOS2 acts as a repressor of STZ/ZAT10 expression during cold stress (Lee et al. 2002). In the *los5/aba3* mutant that is deficient in ABA synthesis owing to a defective molybdenum cofactor, high salt or drought-mediated induction of *rd29A* and other DRE-type genes is virtually abolished (Xiong et al. 2001a, b). *LOS5/ABA3* encodes a molybdenum cofactor sulfurylase that catalyzes the synthesis of active Mo cofactor required for ABA aldehyde oxidase in the last step of ABA biosynthesis. Mutations causing a hyperresponse to cold and ABA reveal step(s) at which cold and ABA signaling pathways interact. It is currently not known whether the points of interaction, viz., $HOS_{cold/ABA}$, $LOS_{NaCl/ABA}$ and $HOS_{NaCl/ABA}$ are downstream or upstream of the *ABI1* and *ABI2* gene products. Xiong et al. (2001a, b) have proposed that activation of DRE by DREB2A and related TFs may require ABA-dependent factor(s). Consistent with this, the full activation of *rd29A* transcription depends upon the synergy between the DRE and ABRE elements. Narusaka et al. (2003) showed that DREB1A and DREB2A bound to the DRE or DRE core, and that two AREB proteins, AREB1 and AREB2, bound to the ABRE of *rd29A*. Thus, the DREB and AREB proteins cumulatively transactivate the *rd29A* promoter–*gus* fusion gene. Wang et al. (2008) reported that besides responding to exogenous ABA treatment, OsDREB1F was induced by drought or salt stresses in rice, suggesting that OsDREB1F was involved in both the stress-responsive and ABA signaling pathways. Overexpression studies with *OsDREB1F* have also shown increased expression of both ABA-independent (*cor15a*, *rd29A*) and ABA-dependent (*Rab18*, *rd29b*) genes. The expression of one of the *CBF/DREB1* genes, viz., *CBF4/DREB1D*, is induced by osmotic stress and the other two CBF/DREB1 genes, DDF1/DREB1F and DDF2/DREB1E, are induced by high-salinity stress, suggesting the existence of cross-talk between the CBF/DREB1 and the DREB2 pathways. The *HOS1* and *HOS2* loci encode signaling components that negatively regulate gene expression specifically in response to cold and not to other stress stimuli (Ishitani et al. 1997, 1998). Conversely, HOS5 reduces the expression in response to ABA and osmotic stresses but not to cold. Molecular genetic analysis of the *hos1* locus of *Arabidopsis* showed that cold signaling components upstream of the CBFs might be regulated by specific ubiquitin-mediated degradation (Lee et al. 2002). The *hos1* mutation results in sustained and super-induction of CBF2, CBF3 and their target regulon genes specifically during cold stress. Therefore, HOS1 negatively regulates the *cor* genes by modulating the expression level of the CBFs. HOS1 is constitutively expressed but drastically down-regulated within 10 min of cold stress and recovers to basal levels by 1 h of cold stress. HOS1 protein is present in the cytoplasm at normal growth temperatures and accumulates in the nucleus upon cold stress. Combined cold and ABA or salt and ABA treatments have been shown to have a synergistic effect on *rd29A-luc* expression (Xiong et al. 1999), in contrast to the reduced levels of expression in response to combined cold and salt treatment. Dependence of ABA-independent pathway during salt stress on ABA is apparent from the reduced salt stress induction of *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 suggested that LOS5 and LOS6 are the key regulators of ABA biosynthesis, *OR* gene expression and stress tolerance. An RNA helicase mutant of

Arabidopsis, *los4* is impaired in the cold-regulated expression of *CBF* genes and their downstream target genes and, consequently, is sensitive to chilling stress (Gong et al. 2002). 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, CDPKs or other ABA-responsive regulatory factors (Merlot et al. 2001). The results of all these studies have thus suggested that ABA-dependent and ABA-independent pathways do not act in a parallel manner, but might converge at several hitherto unexpected points. The convergence points or common components represent transcriptional enhancers and repressors interacting directly or indirectly with both ABRE and DRE/C-repeat and hence mediate synergistic interaction between osmotic stress and ABA response (Fig. 2). If they do, this increases opportunities for coordination between stress signals and ABA in the regulation of gene expression.

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 ABA-independent pathways simultaneously (Chen et al. 2007). The *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. 2008), thereby suggesting that wheat DREB2 might contribute indirectly to the development of abiotic stress tolerance through an increase in ABA sensitivity. These studies highlight that there is a cross-talk during stress signaling, executed by the synergistic effect of ABA and drought/salt stress, for the regulation of *OR* genes.

The signaling pathways for salt, drought, cold and ABA interact and even converge at multiple steps. A nice example of the pathway convergence is provided by the *fiery1* (*fry1*) mutation. This mutant was shown to be impaired in a bifunctional enzyme with inositol polyphosphate 1-phosphatase activities, providing evidence that phosphoinositides function as second messenger in stress and ABA signaling. This mutation increases the amplitude and sensitivity of stress gene induction not only by ABA, but also by salt, drought and cold stresses. An analysis of double mutants between *fry1* and *aba1* or *abi1* indicated that the cold or osmotic stress hypersensitivity in the mutant is not dependent on ABA. *FRY1* encodes an inositol polyphosphate 1-phosphatase that is required for IP_3

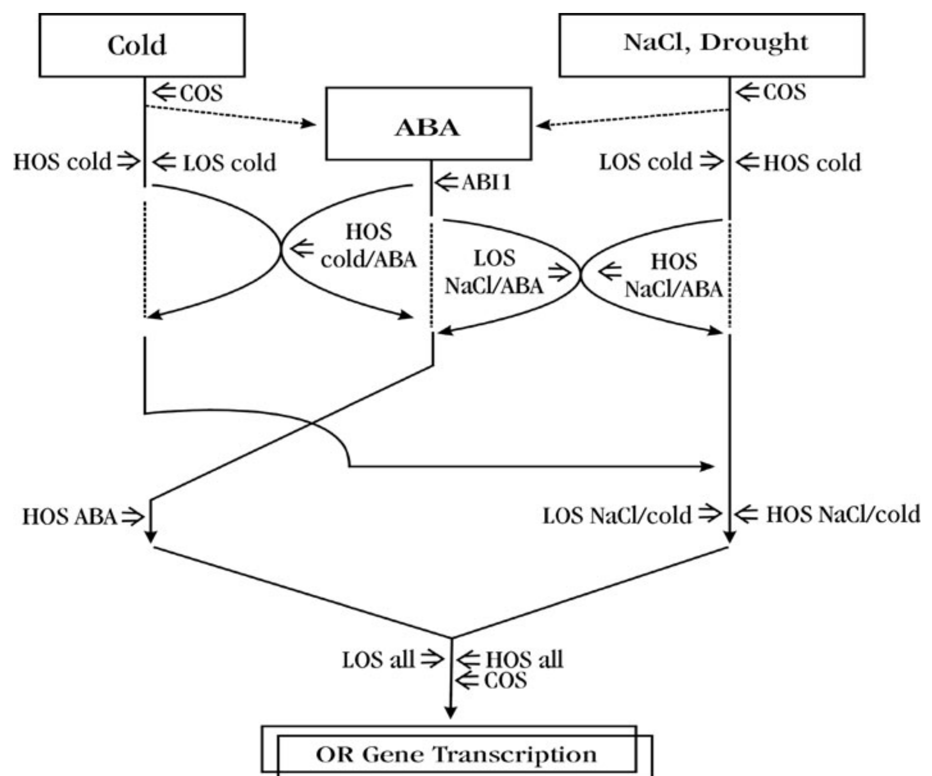

Fig. 1 A schematic representation of cold, osmotic stress and ABA signal transduction. Gene products by the various signaling mutants are shown in proper positions in the pathways. Both the ABA-dependent and ABA-independent pathways interact and converge to activate several *OR* genes. The *dotted lines* represent pathways that are not sufficient by themselves to activate *OR* gene transcription (modified from Ishitani et al. 1997)

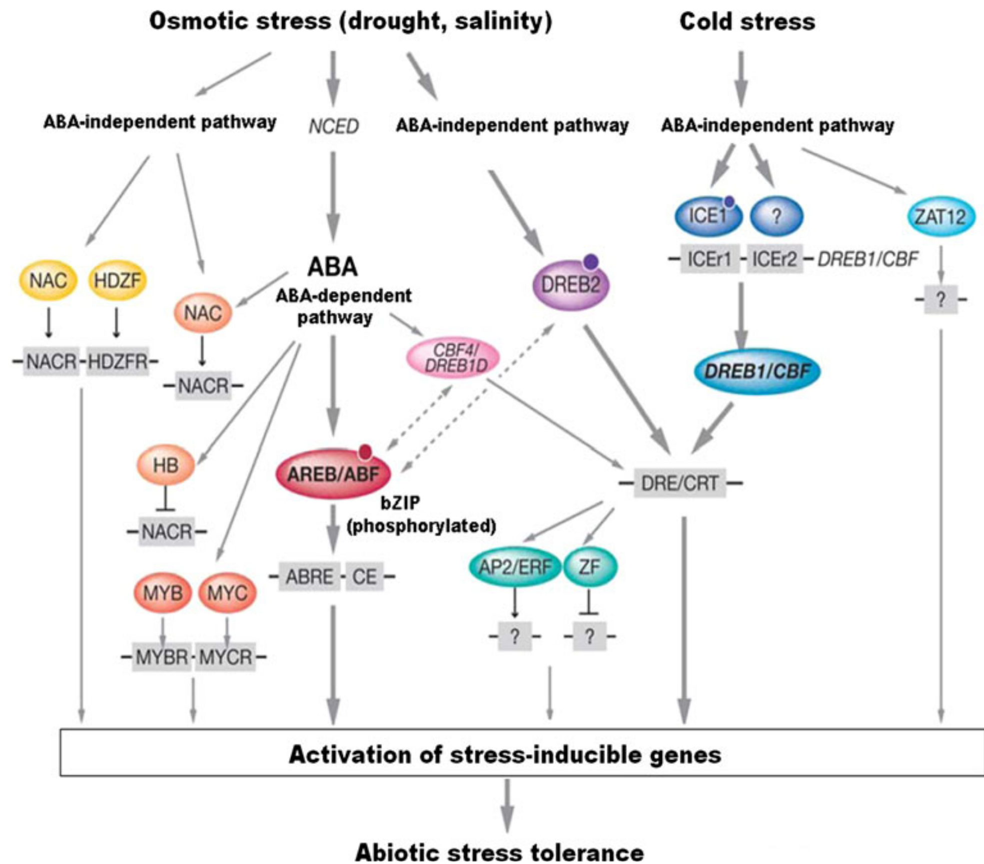

Fig. 2 Cross-talk network among *cis*-acting elements and TFs in ABA-dependent and ABA-independent pathways during abiotic stress. Modification of TFs in response to stress signals for their activation through phosphorylation is shown through *small filled circles*. *Thick gray arrows* represent the major signaling pathways which in turn regulate many downstream genes. *Broken arrows* indicate protein–protein interactions (modified from Yamaguchi-Shinozaki and Shinozaki 2006)

turnover. In response to ABA, wild-type plants accumulated IP_3 transiently. The IP_3 accumulation in *fry1* mutants in response to ABA was more sustained and reached higher levels. IP_3 being a second messenger mediates not only ABA but also salt, drought or cold stress regulation of gene expression. ABA and stress signaling is desensitized by IP_3 turnover. The *fry1* mutant plants are less tolerant to salt, drought or freezing stress despite the enhanced expression of stress genes in the mutant (Zhu 2002).

It has also been suggested that some DRE/CRT motifs can respond to an ABA-dependent pathway (Haake et al. 2002). The DRE2 of the maize *rab17* promoter, for example, is involved in ABA-dependent responses to osmotic stress with a typical core motif of 5′-ACCGAC-3′ and was identified in the embryos and leaves. Additionally, the DRE1 *cis* element (5′-ACCGAG-3′) has also been identified in the *rab17* promoter mediating an ABA-dependent regulation in the embryo, but not in vegetative tissues in response to drought stress (Busk and Pages 1998; Saleh et al. 2005). As exemplified by ZmABI4, a DREB protein which shows ABA-induced expression, it binds to CE1 and acts as a CE of the ABRE in maize (Niu et al. 2002). The expression of gene for DREB1-related TF, in *Arabidopsis*, *CBF4*, is upregulated by drought and ABA, but not by cold stress. Overexpression of CBF4 in *Arabidopsis* resulted in constitutive expression of CRT/DRE-containing *OR* genes and enhanced tolerance to drought and freezing stresses (Haake et al. 2002). Consistently, overexpression of DREB1D/CBF4, which is an ABA-responsive gene of *Arabidopsis*, activates expression of drought- and cold-related downstream genes that contain the DRE/CRT *cis* element (Knight et al. 2004). Overexpression of *DBF2*, a *DREB* gene of maize, represses not only the basal promoter activity of its downstream gene, but also the effect of ABA (Kizis and Pages 2002). Among the 17 downstream genes of DREB2A, 12 carry both the DRE/CRT and ABRE *cis* elements (Maruyama et al. 2004; Sakuma et al. 2006). Zhao et al. (2010) reported that *ABA-responsive AP2-like gene1* (*ARAG1*) in rice encodes a DREB-like protein containing the characterized AP2 DNA-binding domain, which was identified in other DREB proteins, such as *Arabidopsis* DREB1 and DREB2, rice OsDREBs and maize DBF2, and can bind to the DRE/CRT *cis* element of *OR* genes. The three ABRE elements identified in the promoter region of *ARAG1* suggested its involvement in ABA-dependent pathway. Drought stress and ABA treatment increased the transcript levels of the gene rapidly. *ARAG1* knockdown line was hypersensitive to ABA application during seed germination and seedling growth. These results demonstrate that some DREBs are

involved in both ABA signaling and stress-responsive pathways.

Osmotic stress induces the biosynthesis of ABA, whose increased levels subsequently exert a positive feedback on its own biosynthetic pathway. The 9-*cis*-epoxycarotenoid cleavage reaction by NCED is the key regulatory step in NaCl-induced ABA biosynthesis. Strong induction by NaCl of NCED3 was still observed in severe ABA-deficient mutants, pointing to an ABA-independent induction pathway for NCED3 that is NaCl-dependent. Therefore, in the absence of the ABA-mediated positive feedback on ABA biosynthesis, the ABA-independent pathway makes a major contribution to the induction of key ABA biosynthetic genes, such as NCED3, AAO3 and ABA1 (Barrero et al. 2006). It was suggested that both ABA-dependent and ABA-independent pathways govern the induction of NCED3, AAO3 and ABA1 in response to salt stress.

Emerging evidence suggests that the molecular mechanisms driving the responses of plants to environmental stresses are associated with specific chromatin modifications. ABA-induced stomatal closing is mediated by chromatin remodeling, involving histone H4 deacetylation by activating deacetylases (Sridha and Wu 2006; Zhu et al. 2008). Ding et al. (2011) have demonstrated that the *Arabidopsis* trithorax-like factor ATX1, a histone methyltransferase that trimethylates histone H3 at lysine 4 (H3K4me3), is involved in dehydration stress signaling in both ABA-dependent and ABA-independent pathways. The loss of function of ATX1 results in decreased germination rates, larger stomatal apertures, more rapid transpiration, and decreased tolerance to dehydration stress in *atx1* plants. This deficiency is caused in part by reduced ABA biosynthesis in ATX1 plants resulting from decreased transcript levels from *NCED3*, which encodes the rate-limiting enzyme controlling ABA production. Dehydration stress increased ATX1 binding to NCED3, and ATX1 was required for the increased levels of *NCED3* transcripts and nucleosomal H3K4me3 that occurred during dehydration stress. By upregulating NCED3 transcription and ABA production, ATX1 influenced ABA-regulated pathways and genes. Four ABA-dependent genes (*rd29A*, *rd29B*, *rd26* and *ABF3*) were induced by dehydration stress as well as by treatment with ABA. During dehydration stress, transcript levels from these genes were reduced in ATX1 plants, indicating that ATX1 was required for wild-type levels of transcript production from these genes. Treatment with exogenous ABA induced *rd29A* and *rd29B* transcripts in ATX1 plants to levels similar to those induced by ABA in WT plants, whereas those of *rd26* and *ABF3* were partially restored. ATX1 also affected the expression of ABA-independent genes, implicating ATX1 in diverse dehydration stress response mechanisms in *Arabidopsis*. The four ABA-independent genes (*cor15A*, *ADH1*, *ABF2* and *CBF4*) only weakly responded to ABA, relative to their induction during dehydration stress. Three of the genes in this group showed a strong ATX1-dependent induction upon dehydration stress, producing fewer transcripts than in wild-type plants, whereas ABF2 showed only a modest dependence on ATX1. The ABA-dependent (*rd29A*, *rd29B*, *rd26* and *ABF3*) as well as the ABA-independent (*cor15A*, *ABF2*, *CBF4* and *ADH1*) dehydration-inducible genes displayed reduced transcript levels in *atx1* mutants during dehydration stress. These results indicated that ATX1 was required for inducing transcript levels from both groups of genes during a dehydration stress response.

Role of calcium in cross-talk mechanism

In plant cells, Ca^{2+} serves as a second messenger during abiotic stress signaling. Ca^{2+} is a major point of signaling cross-talk because it can be elicited by numerous abiotic stress cues (Fig. 3). A number of studies have demonstrated that ABA, cold, drought and high salt conditions result in a rapid increase in Ca^{2+} levels in plant cells (Sanders et al. 2002; Knight and Knight 2000; Rudd and Franklin-Tong 2001). In addition, studies have shown that cellular Ca^{2+} changes precede the activation of ABA, cold and *OR* genes (Sheen 1996; Wu et al. 1997; Thomashow 1999; Knight and Knight 2001). Specific Ca^{2+} signatures (e.g., oscillations) have been implicated in numerous physiological processes, such as stomatal regulation where oscillations in cytosolic Ca^{2+} led to stomatal closure. The Ca^{2+} signature changes depending on the particular stress, the rate of stress development, previous exposure to stress conditions and the tissue type (Kiegle et al. 2000).

Cellular Ca^{2+} level is upregulated by ABA. The elevation of Ca^{2+} in guard cells is sufficient to induce part of ABA response. The Ca^{2+}-dependent mechanisms are responsible for 70 % of stomatal closure (Siegel et al. 2009), and several Ca^{2+}-associated proteins are required for normal stomatal closure. Buffering Ca^{2+} to a low level in guard cells blocks part of the ABA response in guard cells (Lemitri-Chlieh et al. 2000). The abiotic stress-induced activation of many ABA biosynthetic genes such as *ZEP*, *NCED*, *AAO* and molybdenum cofactor sulphurase (*MCSU*) appeared to be regulated through Ca^{2+}-dependent phosphorylation pathway. The accumulation of ABA has a feedback effect as they stimulate the expression of ABA biosynthetic genes through Ca^{2+}-signaling pathway and also activate the ABA catabolic enzymes to degrade the ABA (Tuteja 2007). Studies have also demonstrated clearly that some of the ABA signaling events in guard cells are Ca^{2+} independent. After ABA application, Ca^{2+} accumulates in the cytoplasm due to activation of several possible pathways. One is the plasma membrane Ca^{2+} channels. A

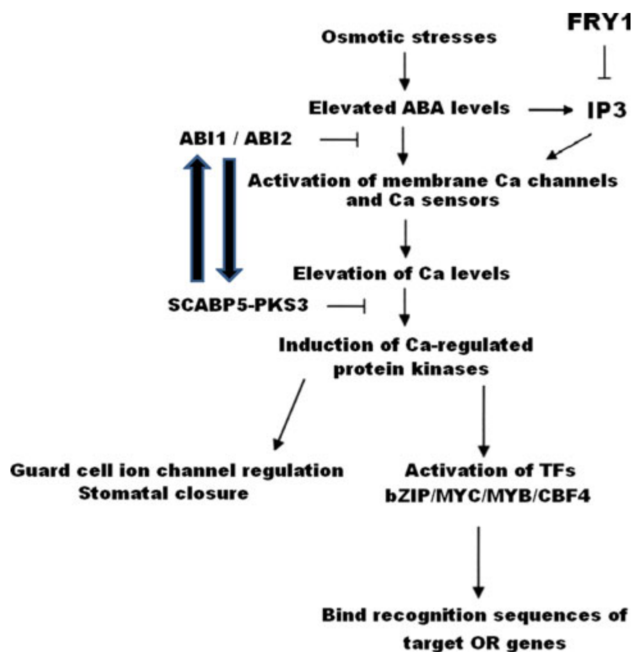

Fig. 3 Calcium as a second messenger during abiotic stress signaling. ABA-dependent abiotic stress signaling is mediated in part through IP_3 and Ca^{2+}. FRY1 negatively regulates IP_3 levels. ABA-induced Ca^{2+} signaling is negatively regulated by the ABI1/2 protein phosphatase 2Cs and the SCaBP5/PKS3 complex

non-selective cation channel was first shown to parallel ABA-induced Ca^{2+} elevation in guard cells. ABA induces production of H_2O_2. If H_2O_2 production is blocked by an inhibitor, ABA induction of the Ca^{2+} channel or stomatal closure is also blocked, implicating H_2O_2 production in the ABA signaling pathway between ABA and Ca^{2+} elevation. The accumulation of proline, a major osmolyte can be mediated by both ABA-dependent and ABA-independent signaling pathways. The role of Ca^{2+} in ABA-dependent induction of *P5CS* gene during salinity stress has been reported. It is also suggested that phospholipase D (*PLD*) along with ABA and Ca^{2+} acts as a negative regulator of proline biosynthesis in *Arabidopsis*. As a second messenger, Ca^{2+} transmits the primary signal downstream in the pathway into cellular responses (such as gene expression), most likely through Ca^{2+} regulated proteins that include Ca^{2+} sensors and their targets. The major Ca^{2+} sensors in plants include calmodulin (CaM) (Luan et al. 2002), CDPKs (Sanders et al. 2002), and the more recently discovered calcineurin B-like proteins (CBLs) (Luan et al. 2002). CBLs specifically target a family of protein kinases referred to as CIPKs (CBL-interacting protein kinases). To date, at least 10 *CBL* genes and 25 *CIPK* genes have been identified from *Arabidopsis*. Among them, *SOS3/SOS2* (*CBL4/CIPK24*) was shown to play a role in ionic homeostasis in *Arabidopsis* (Xiong and Zhu 2002). Loss of function in either SOS3 (CBL4) or its interacting kinase SOS2 (CIPK24) renders plants hypersensitive to high salt conditions as a result of impaired ionic homeostasis. One study showed that a constitutively active form of CDPK is sufficient to activate an ABA- (and stress-) responsive gene promoter in maize leaf cells (Sheen 1996). Another study showed that overexpression of a CDPK leads to the expression of *OR* genes under normal conditions and increased stress tolerance in rice (Saijo et al. 2000). Several studies point to the importance of Ca^{2+}-regulated protein kinases and phosphatases that may mediate Ca^{2+} function in guard cells and in other plant cells. Two studies suggest that CDPK plays a role in guard cell ion channel regulation (Pei et al. 1996; Li et al. 1998). The guard cells of *Arabidopsis cpk3/cpk6* double mutants showed impaired Ca^{2+}-induced activation of S-type anion currents and reduced sensitivity to ABA regulation of these channels. CPK6 and CPK3, the two CDPKs thus play important functions in guard cell ion channel regulation. CPK3 kinase activity was induced by salt and other stresses after transient overexpression in *Arabidopsis* protoplasts, but endogenous CPK3 appeared to be constitutively active in roots and leaves in a strictly Ca^{2+}-dependent manner (Mehlmer et al. 2010). The guard cell anion channel SLAC1 is regulated by CPK21 and CPK23 with distinct Ca^{2+} affinities (Geiger et al. 2010). *AtCPK23* is believed to be serving as a negative regulator of abiotic stress signaling as observed from the *cpk23* mutant, which has improved tolerance to drought and salt stress (Ma and Wu 2007). CPK10, the *Arabidopsis* CDPK, by interacting with a heat shock protein, HSP1, plays important roles in ABA- and Ca^{2+}-mediated regulation of stomatal movements. Induction of stomatal closure and inhibition of stomatal opening by ABA and Ca^{2+} were impaired in the *cpk10* mutants. ABA and Ca^{2+} inhibition of the inward K^+ channels in stomatal guard cells were impaired in the *cpk10* and *hsp1* mutants. The CPK10 overexpression lines displayed enhanced tolerance to drought stress (Zou et al. 2010). AtCPK30 are known to activate an ABA-inducible and stress-related promoter (Sheen 1996). ABF4 was phosphorylated at Ser110 by AtCPK32, AtCPK10 and AtCPK30. Overexpression of *AtCPK32* confers hypersensitive response to ABA and salt stress conditions. The expression of certain ABA-responsive genes such as *Rab18*, *rd29A* and *rd29B* was also upregulated in the overexpression lines, indicating the function of AtCPK32 as a positive regulator of ABA-mediated stress signaling pathway (Das and Pandey 2010). Two more CDPKs from *Arabidopsis* that include CPK4 and CPK11 have also been shown to be positively regulating ABA signal transduction pathway (Zhu et al. 2007). The *cpk4* and *cpk11* loss-of-function mutants displayed an ABA-insensitive phenotype with respect to seed germination, seedling growth and root growth. These mutants also showed reduced sensitivity to ABA in terms of stomatal

closure and had bigger stomatal aperture upon ABA treatment in comparison to the wild type. An opposite phenotype could be seen in the overexpression lines that showed increased sensitivity to ABA in terms of stomatal closure, seed germination, seedling and root growth. ABF1 and ABF4 are phosphorylated by CPK4 and CPK11, which were reduced to a greater extent in the *cpk4* and *cpk11* mutant lines. Li et al. (1998) revealed phosphorylation of KAT1, a putative inward K^+ channel in guard cells, by a CDPK-like activity.

A *CaM* gene has been implicated as a negative regulator of stress- and ABA-induced gene expression, because overexpression of this *CaM* gene caused a reduced level of gene induction (Townley and Knight 2002). CIPK3, a Ser/Thr protein kinase that associates with a CBL, regulates ABA response during seed germination and modulates gene expression induced by cold, salt and ABA treatment in *Arabidopsis*. The expression of the *CIPK3* gene itself is responsive to ABA and stress conditions, including cold, high salt and drought. Disruption of *CIPK3* altered the expression pattern of a number of stress gene markers in response to ABA, cold, and high salt. Interestingly, gene induction by drought and hyperosmotic stress was not affected by disruption of *CIPK3* function, suggesting that CIPK3 regulates specific or selective pathways in response to abiotic stress and ABA. Because both salt and drought have a common ABA-dependent pathway and CIPK3 functions only in salt-induced expression, CIPK3 appears to regulate an ABA-independent pathway of salt stress but is involved in the pathway initiated by exogenous ABA application. CIPK3 acts as a molecular link between stress- and ABA-induced Ca^{2+} signal and gene expression in plant cells. Because the cold signaling pathway is largely independent of endogenous ABA production, CIPK3 represents a cross-talk "node" that mediates the interaction between the ABA-dependent and ABA-independent pathways in stress responses. It is noteworthy that CIPK3 function appears to be most important in the cold induction of gene expression. Of the marker genes examined (*rd29A* and *kin1/kin2*), the induction was delayed most dramatically under cold conditions in the *cipk3* mutant plants. The cold-induced expression of *rd29A* and *kin1/kin2* genes consists of two components, the early phase and the late phase. Only the early-phase component may involve CIPK3 function. Another report establishes the function of CIPK15/PKS3, which interacts with CBL1 as a negative regulator of ABA signaling pathway (Guo et al. 2002). In this study, silencing of both *CBL1* and *CIPK15* resulted in ABA-hypersensitive phenotype. Also, CIPK15 was found to be interacting with PP2C phosphatases, ABI1 and ABI2. Thus, CIPKs together with CBLs could have an opposite effect on ABA signaling as compared to CDPKs, which promote or serve as a positive modulator of ABA signaling. It is also suggested that post-translational modification, such as phosphorylation of DREB1A/CBF3 may involve CIPK3 action (Kim et al. 2003).

Recent studies have implicated Ca^{2+} channels in the regulation of cytosolic Ca^{2+}. Membrane fluidity and reorganization of the cytoskeleton are essential for cold-induced cytosolic Ca^{2+} oscillations in alfalfa and *Brassica napus*, respectively (Orvar et al. 2000; Sangwan et al. 2001). The cADPR-gated Ca^{2+} channels are involved in ABA-induced expression of cold-regulated genes in tomato (Wu et al. 1997) and *B. napus* (Sangwan et al. 2001). IP_3-gated Ca^{2+} channels have been implicated in dehydration- and salt stress-induced cytosolic Ca^{2+} elevations (DeWald et al. 2001; Takahashi et al. 2001). Genetic analysis of the *FRY1* locus of *Arabidopsis* suggested the involvement of IP_3 (which in turn is expected to generate cytosolic Ca^{2+} oscillations) in ABA, salt and cold stress signaling (Xiong et al. 2001a, b). Although the involvement of a heterotrimeric GTP-binding G-protein has been demonstrated in ABA signal transduction during guard cell regulation (Wang et al. 2001), the role of G-protein-associated receptors or classical stress sensors in eliciting Ca^{2+} signatures during abiotic stress has yet to be shown in plants. Specificity and/or cross-talk in Ca^{2+} signaling may depend on the magnitude, duration and subcellular localization of the Ca^{2+} oscillation as well as Ca^{2+} sensor diversity. Experimentally induced increases in cytoplasmic Ca^{2+} (via microinjection, the use of Ca^{2+} ionophores or Ca^{2+} channel agonists/antagonists) have been shown to mediate hormone/stress responses (Sheen 1996; Wu et al. 1997; Orvar et al. 2000; Sangwan et al. 2001).

Regulation of cellular ion homeostasis during salinity stress is critical for plant salt tolerance. The identification of the SOS pathway in *Arabidopsis* and molecular analysis of *sos* mutants of *Arabidopsis* led to the identification of components (SOS1, SOS2 and SOS3) of a pathway that transduce a salt stress-induced Ca^{2+} signal to reinstate cellular ion homeostasis (Fig. 4). SOS3 is a Ca^{2+} sensor essential for transducing the salt stress-induced Ca^{2+} signal and for salt tolerance in *Arabidopsis*. SOS3 encodes a Ca^{2+}-binding protein and a loss-of-function mutation that reduces the Ca^{2+}-binding capacity of SOS3 (*sos3-1*) renders the mutant hypersensitive to salt (Ishitani et al. 2000); a defect that can be partially rescued by high levels of Ca^{2+} in the growth medium (Liu and Zhu 1998). The SOS2 encodes a Ser/Thr protein kinase with an N-terminal kinase catalytic domain similar to SNF1/AMPK, and a unique C-terminal regulatory domain (Liu et al. 2000). The *sos2* mutant is also hypersensitive to NaCl stress. In the presence of Ca^{2+}, SOS3 activates the SOS2 kinase (Halfter et al. 2000). SOS1 encodes a plasma membrane Na^+/H^+ exchanger (antiporter) during salt stress; upregulation of *SOS1* transcript levels is diminished in the *sos3* and *sos2*

mutants (Shi et al. 2000). The antiport activity of SOS1 is regulated by the SOS2 kinase, which was found to phosphorylate SOS1 directly. The constitutive myristoylation of SOS3 allows it to recruit SOS2 to the plasma membrane, thus assisting in bringing SOS2 to its target SOS1 (Quintero et al. 2002). The Na^+ efflux from root cells experiencing salinity stress may be achieved by the activation of SOS1 by the SOS3/SOS2 complex and that SOS1 probably also retrieves Na^+ from the xylem, thereby preventing excess Na^+ accumulation in the shoot (Shi et al. 2002, 2003). The SOS3/SOS2 kinase complex may prevent Na^+ influx by inactivating the HKT1 protein or downregulating *HKT1* gene expression during salt stress (Zhu 2002). In addition, the activity of vacuolar Na^+/H^+ antiporters may also be activated by SOS3/SOS2 (Qiu et al. 2004). Genetic studies to knock-down SOS3-like Ca^{2+}-binding proteins (SCaBP) and SOS2-like protein kinases (PKS) showed that *scabp5* and *pks3* mutants are impaired in their response to ABA and that the SCaBP5/PKS3 complex specifically senses and transduces ABA-specific Ca^{2+} signals (Guo et al. 2002). ABA-induced expression levels of the cold and drought-responsive genes *cor47*, *cor15a* and *rd29A* were substantially higher in the *scabp5* and *pks3* mutants, when compared with their expression in the wild type, and these mutants expressed higher levels of *cor47* and *cor15a* even without exogenous ABA. In the *scabp5pks3* double mutant, ABA sensitivity was not additive supporting a role for these two proteins in the same ABA signaling pathway. SCaBP5 and PKS3 are specific to ABA-induced Ca^{2+} signaling, as *scabp5* and *pks3* were defective in their response to ABA but not in their response to salt and cold stresses (Guo et al. 2002). SCaBP5 physically interacts with PKS3 which in turn strongly interacts with the 2C-type protein phosphatase ABA-insensitive 2 (ABI2) and to a lesser extent with the homologous ABA-insensitive 1 (ABI1) protein. SCaBP5/PKS3 and ABI2 form part of a Ca^{2+}-responsive negative regulatory loop controlling ABA sensitivity.

The studies on mitogen activated protein kinase (MAPK) cascades also provide strongest evidence for cross-talk during abiotic stress signaling. Signals perceived by the 60 MAPKKKs in *Arabidopsis* have to be transduced through 10 MAPKKs to 20 MAPKs which offer scope for cross-talk between different stress signals. Members of MAPK cascades are activated by more than one types of stress, which suggests that MAPK cascades act as points of convergence in stress signaling. Sheen (1996) has shown that a constitutively active form of an *Arabidopsis* CDPK (*AtCDPK1*) activated the expression of a barley ABA-responsive promoter fusion (*HVA1*::*luc*). Ectopic expression of CDPK induced the expression of a rice ABA-responsive gene, *Rab16* (Saijo et al. 2000) and *HVA1* in the maize protoplasts (Sheen 1996). Overexpression of the catalytic domain of ABI1 inhibited ABA- and AtCDPK1-induced *HVA1* transcription. These results suggested that the upregulation of *Rab16* and *HVA1* may be mediated by CDPKs, which are under the negative control of ABI1. Mutations in *CAX1* (encoding a Ca^{2+}/H^+ transporter) and *CBL1* affected the expression pattern of *DREB1/CBF* genes (Albrecht et al. 2003; Catala et al. 2003). Thus, Ca^{2+} is a ubiquitous second messenger which controls abiotic stress through cross-talk with ABA-dependent as well as ABA-independent pathways.

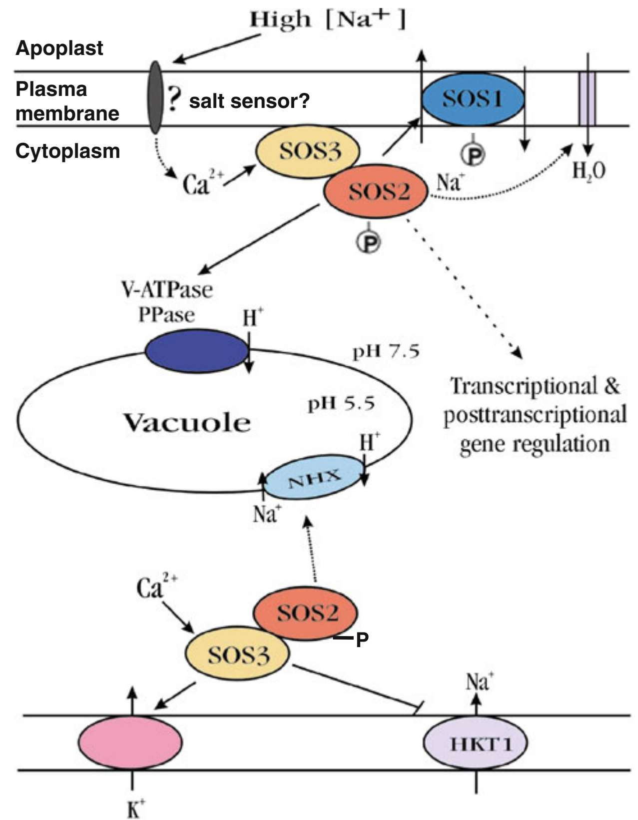

Fig. 4 Regulation of ion homeostasis by Ca^{2+}-dependent SOS pathway during salinity stress. Salt stress-induced Ca^{2+} signals are perceived by SOS3, which activates the SOS2 kinase. The SOS3/SOS2 kinase complex regulates cellular Na^+ levels by stimulating Na^+ transport out of the cytoplasm (by increasing the expression and activity of SOS1) and by restricting Na^+ entry into the cytosol (viz., inhibiting HKT1 activity). The NHX (vacuolar Na^+/H^+ exchanger) also contributes to Na^+ ion homeostasis by transporting Na^+ from the cytoplasm into the vacuole

Conclusion and future perspectives

Understanding the molecular mechanism of plant responses to abiotic stresses such as, drought, salinity and cold is very important as it helps in manipulating plants to improve stress tolerance and productivity. Several abiotic stress signaling pathway components have been identified based

on mutant analysis or their stress induction, ability to complement the growth of relevant yeast mutants and ectopic expression studies (Roy Choudhury et al. 2012). Pioneering work on *rd29A* and *rd29B* has led to the identification of both ABA- and drought-responsive motifs that are essential for their activation by specific stresses. The target promoters (for e.g., of *rd29A*) are often promiscuous in that they contain a combination of motifs that would permit signal cross-talk directly at the level of target gene transcription. The DRE/CRT functions in the early process of *OR* gene expression, whereas ABRE functions after the accumulation of ABA during dehydration and high-salinity stress response. Timing of stress-responsive gene expression is regulated by a combination of TFs and *cis*-acting elements in stress-inducible promoters. The bZIP 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. Functional analysis of DREB TFs will provide more information on the complex regulatory networks involved in abiotic stress responses and the cross-talk between different signaling pathways. NAC plays a role through NACRS of *erd1* and other promoters in both ABA-independent and ABA-dependent pathways, respectively. Genetic analysis has been successfully exploited to define the SOS pathway of ion homeostasis during salt stress. Some of the events involved in the regulation of freezing tolerance by the ICE1/CBF pathway and osmotic stress tolerance by ABA-dependent and ABA-independent pathways are becoming clearer. 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 *OR* genes. Recently, *cis*-elements other than ABREs related to ABA signaling are suggested based on the mismatch of cell type-specific enrichment and regulation of gene expression by ABA. However, there is still only a fragmentary view of abiotic stress signaling pathways. A lot of effort is still required to uncover in detail of each products of gene induced by ABA and their interacting partners. Determination of the upstream receptors or sensors that monitor the stimuli as well as the downstream effectors that regulate the responses is essential, which will also expedite our understanding of ABA-mediated stress signaling mechanisms in plants. The basic functional architecture for molecular responses, interactions and cross-talk toward abiotic stress needs to be studied. Study of a large number of ABA-dependent and ABA-independent TFs and their downstream binding elements can give better understanding of the molecular basis of stress tolerance, which involves complex network of genes operating in coordination. Forward and reverse genetic approaches will continue to be imperative to dissect these complex pathways. Although conventional genetic screens have yielded valuable insight into stress signal transduction, this approach may ultimately be limited because of the functional redundancy of components within the signaling pathways. Reporter gene-based molecular screens offer a systematic way to identify the upstream signaling components that control subsets of responses, which may not manifest as visible tolerance phenotypes. Recently, RNA interference or mRNA degradation were suggested to function in *OR* gene expression. The role of endogenous micro RNA in regulating the ABA-induced stress tolerance will be further helpful in our better understanding of the mechanism of stress tolerance. Thorough characterization of mutant phenotypes will provide an indication whether a signaling component functions in a specific pathway or is involved in multiple pathways. Cell biological analysis of spatial and temporal expression patterns, combined with biochemical characterization of the components, particularly identification of signaling complexes, will be required to firmly establish specificity or cross-talk of the signaling pathways. In conclusion, cross-talk between stress signal transduction pathways will be an important subject in the near future as it will enable more flexible responses of plants to abiotic stresses.

Acknowledgments Financial support from Science and Engineering Research Board (SERB), Government of India through the research grant (SR/FT/LS-65/2010) to Dr. Aryadeep Roychoudhury is gratefully acknowledged.

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