Journal of Plant Physiology 171 (2014) 486–496

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Molecular Biology

Abscisic acid and abiotic stress tolerance – Different tiers of regulation

Rajesh Mehrotra[a,b,1], Purva Bhalothia[a,1], Prashali Bansal[a,c], Mahesh Kumar Basantani[d], Vandana Bharti[e], Sandhya Mehrotra[a,*]

[a] Department of Biological Sciences, Birla Institute of Technology & Sciences, Pilani, Rajasthan 333031, India
[b] G_0 Unit, Okinawa Institute of Science and Technology, 1919-1, Onnason, Okinawa, Japan
[c] Cancer Science Institute, National University of Singapore, Singapore, Singapore
[d] Division of Endocrinology, University of Pittsburgh, 200 Lothrop Street, BST E1140, Pittsburgh, PA 15261, USA
[e] Department of Biotechnology, St. Columba's College, Vinoba Bhave University, Hazaribagh, India

ARTICLE INFO

Article history:
Received 10 August 2013
Received in revised form
12 December 2013
Accepted 13 December 2013

Keywords:
ABA signaling
Abiotic stress
Abscisic acid
ACGT *cis* element
Protein phosphatase 2C

SUMMARY

Abiotic stresses affect plant growth, metabolism and sustainability in a significant way and hinder plant productivity. Plants combat these stresses in myriad ways. The analysis of the mechanisms underlying abiotic stress tolerance has led to the identification of a highly complex, yet tightly regulated signal transduction pathway consisting of phosphatases, kinases, transcription factors and other regulatory elements. It is becoming increasingly clear that also epigenetic processes cooperate in a concerted manner with ABA-mediated gene expression in combating stress conditions. Dynamic stress-induced mechanisms, involving changes in the apoplastic pool of ABA, are transmitted by a chain of phosphatases and kinases, resulting in the expression of stress inducible genes. Processes involving DNA methylation and chromatin modification as well as post transcriptional, post translational and epigenetic control mechanisms, forming multiple tiers of regulation, regulate this gene expression. With recent advances in transgenic technology, it has now become possible to engineer plants expressing stress-inducible genes under the control of an inducible promoter, enhancing their ability to withstand adverse conditions. This review briefly discusses the synthesis of ABA, components of the ABA signal transduction pathway and the plants' responses at the genetic and epigenetic levels. It further focuses on the role of RNAs in regulating stress responses and various approaches to develop stress-tolerant transgenic plants.

Introduction

Plants, though continuously exposed and challenged by environmental cues, are capable of surviving by means of adaptations at the molecular, cellular and chromatin level of organization (Kim et al., 2010). Drought, salinity and temperature variations are among the major abiotic stresses, which hamper plant growth and productivity and often cause a series of morphological, physiological and biochemical changes. One such example can be seen in plants adapted to cold conditions, which show up-regulation of genes involved in the synthesis of unsaturated fatty acids, while those exposed to drought show a decrease in the number and surface area of leaves and an increase in root length (Shinozaki et al., 2003). Abscisic acid (ABA) is a widely studied phytohormone, and its role in ameliorating abiotic stress in plants is well established. In plants, ABA is of paramount significance as it plays an important role in mediating host responses to both biotic and abiotic stresses. It not only helps in seed maturation and seed dormancy but also gives desiccation tolerance to the cells during dehydration stress and is hence aptly called as a stress hormone.

ABA: evolutionary significance, synthesis and stress regulatory mechanisms

ABA: ubiquitous presence across different lineages

ABA is present in cyanobacteria, algae, bryophytes, fungi, lichens and higher plants. Out of eleven species of cyanobacteria tested for ABA synthesis under varied stress conditions, four species showed an increase in the level of ABA when subjected to salt stress (reviewed in Wolfram, 2010). Even though the majority of bacterial species does not exhibit ABA synthesis, a study has reported the presence of ABA in endophytic bacteria, which are present in the roots of *Helianthus annuus* under water stress conditions (Forchetti et al., 2007). In the cyanobacterium *Anacystis nidulans*, exogenous ABA has been shown to increase growth (Ahmad et al., 1978;Wolfram, 2010). ABA is believed to have protective functions

Abbreviations: PP2C, protein phosphatase 2C; SNF, sucrose non-fermenting; SnRK2, SNF1-related protein kinase 2.

* Corresponding author. Tel.: +91 1596244183; fax: +91 1596245073.
E-mail address: sandhyamehrotrabits@gmail.com (S. Mehrotra).

[1] Both these authors have contributed equally to the manuscript.

0176-1617/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2013.12.007

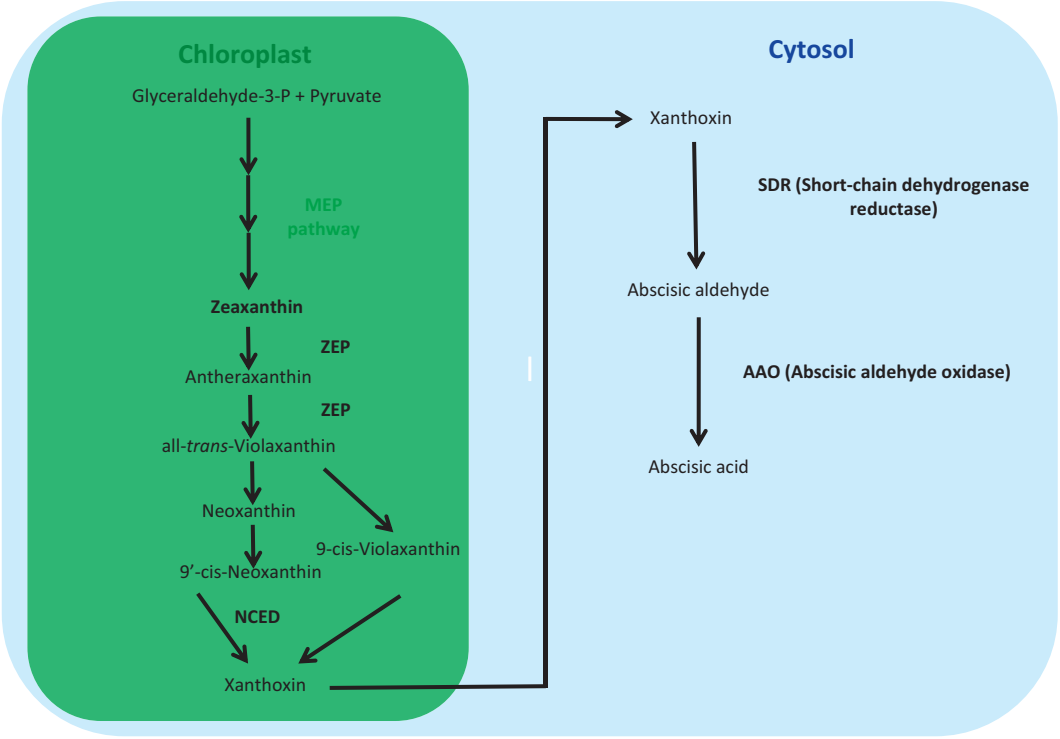

Fig. 1. Cellular synthesis of abscisic acid: ABA biosynthesis occurs partly in chloroplasts, and partly in the cytoplasm. The preliminary steps involve generation of zeathanxin from carotenoids via the MEP pathway. ABA synthesis starts from the breakdown of zeaxanthin to all-*trans* violaxanthin under the catalysis of zeaxanthin epoxidase (ZEP). The all-*trans* violaxanthin, an intermediate product, is catalyzed to xanthoxin in the presence of 9-*cis* epoxycarotenoid dioxygenase (NCED) in the chloroplast which is transported to the cytoplasm. In the cytoplasm, xanthoxin is first converted to abscisic aldehyde by short chain dehydrogenase reductase (SDR) and finally abscisic aldehyde is converted to ABA by the action of abscisic aldehyde oxidase (AAO).

in lower plants as it shields *Chlamydomonas reinhardtii* cells from photoinhibition and oxidative damage caused by salt and osmotic stress (Saradhi et al., 2000). ABA has also been found to regulate opening and closing of stomata in mosses and sporophytes of hornworts elucidating yet another function. More than 100 species of algae have also been reported for the presence of ABA though different algae respond differently to the applied stress (reviewed by Wolfram, 2010). With the evolution of liverworts, mosses and ferns, the endogenous levels of ABA have been observed to rise. However, the quantity of ABA has been shown to be very low in lower classes of plants.

ABA biosynthesis

ABA biosynthesis occurs via the mevalonic-acid independent pathway in plastids ABA, the 15-carbon isoprenoid plant hormone, is synthesized in plastids via the mevalonic acid-independent pathway called the 2-C-methyl-d-erythritol-4-phosphate (MEP) pathway. The 15 carbon atoms of ABA are derived from the cleavage of C_{40} carotenoids originating from the MEP pathway (Nambara and Marion-Poll, 2005). The biosynthesis of ABA starts in the plastids from the C_{40} carotenoid zeaxanthin and ends in the cytosol with the formation of abscisic aldehyde, which in turn, is oxidized into ABA (Seo and Koshiba, 2002). The mechanism of ABA biosynthesis is shown in Fig. 1.

In the plastid: from zeaxanthin to xanthoxin

The first step of the ABA-biosynthetic pathway is the conversion of zeaxanthin to all-*trans*-violaxanthin, catalyzed by zeaxanthin epoxidase (ZEP). Antheraxanthin is formed as an intermediate in this reaction. Thereafter, the conversion of all-*trans*-violaxanthin to 9-*cis*-violaxanthin or 9′-*cis*-neoxanthin occurs. The enzyme(s) involved in this reaction are not known (Seiler et al., 2011). The oxidative cleavage of 9-*cis*-violaxanthin and/or 9-*cis*-neoxanthin, catalyzed by 9-*cis*-epoxy carotenoid dioxygenase (*NCED*), leads to the formation of a C_{15} product, xanthoxin, and a C_{25} metabolite. This reaction is considered to be the rate-limiting step, and *NCED* is the key enzyme in ABA biosynthesis (Tan et al., 1997).

In the cytosol: from xanthoxin to ABA

Xanthoxin is transferred to the cytosol where it is converted to ABA via two enzymatic reactions. In the first step, xanthoxin is converted to abscisic aldehyde by an enzyme belonging to short-chain dehydrogenase/reductase (SDR) family. The gene responsible for this has been identified in *Arabidopsis thaliana*, named *AtABA2* (Gonzalez-Guzman et al., 2002). The final step of ABA biosynthesis is the oxidation of abscisic aldehyde to ABA. This reaction is catalyzed by an abscisic aldehyde oxidase (AAO).

ABA transport

ABA transport is both passive and mediated by ABA transporters

The level of endogenous ABA is maintained by interaction between anabolic and catabolic pathways. The catabolic products like ABA glucosyl ester and phaseic acid are stored in the vacuole or apoplast pool. Under drought stress, ABA is released from its conjugate form, which is facilitated by β-glucosidase, and is transported to guard cells where its accumulation leads to stomatal closure. One such enzyme, AtBG1, a type of β-glucosidase, has been reported in *A. thaliana* (Lee et al., 2006a).

It is not clear if specific transporters facilitate the movement of ABA between cells. It has been shown that ABA transport to the outside of cells in response to pH changes can occur without a specific transporter (Seo and Koshiba, 2011). Similarly, at the site of action, ABA uptake into cells could be mediated by diffusion or by

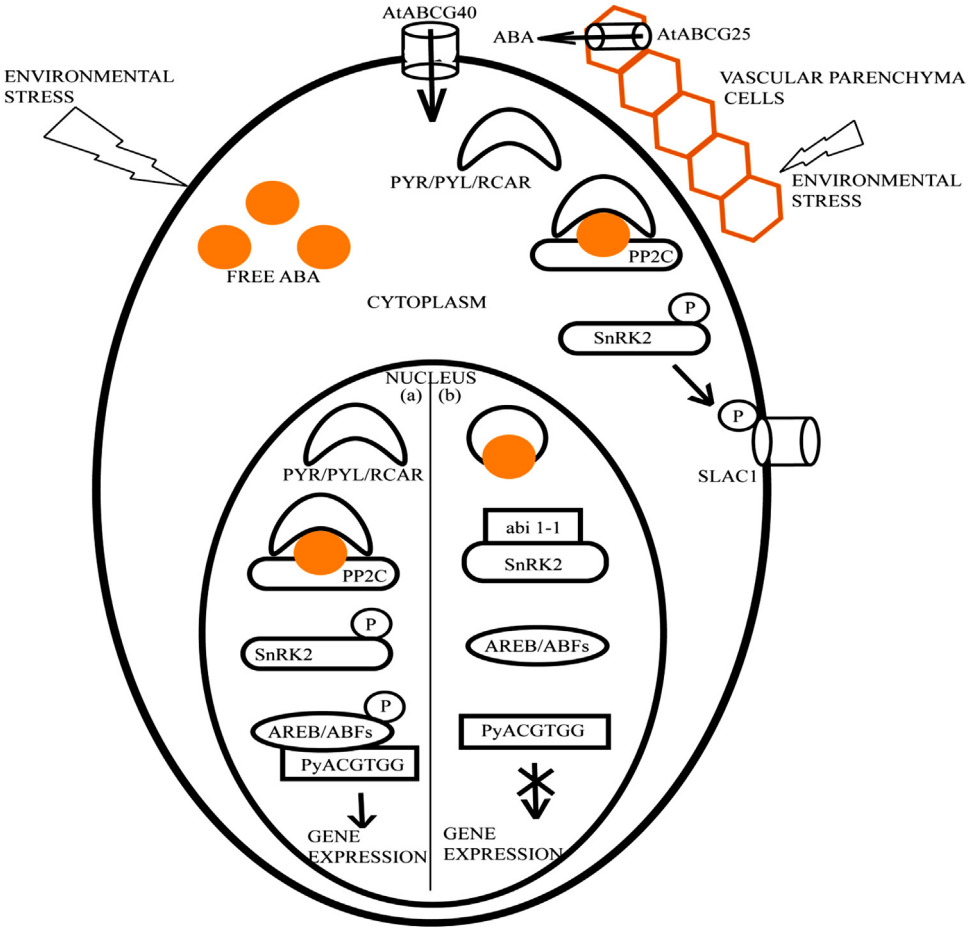

Fig. 2. Brief outline of ABA signaling and its transport "as shown in *Arabidopsis thaliana*": The components involved in ABA signaling include – receptor PYR/PYL/RCAR, a negative regulator PP2C, a positive regulator SnRK2s and transcription factors (AREB/ABFs). The ABA signaling occurs in both, cytoplasm and nucleus. Under abiotic stress conditions (and developmental conditions) the synthesis of ABA increases in vascular parenchyma cells. ABA is transported via AtBCG25 (from parenchyma cells) and AtBCG40 transporters (to target cells). In the presence of ABA, a complex is formed between ABA, receptor and PP2C (ABA-PYR/PYL/RCAR-PP2C) which prevents PP2C in carrying out its inhibitory activity. SnRK2 gets phosphorylated and further activates bZIP transcription factors such as AREB/ABFs by phosphorylation which subsequently induces the expression of target genes. In ABA insensitive condition, ABA insensitive 1-1 (Abi1-1) interacts with SnRK2, hence inhibiting its interaction with ABA-PYR/PYL/RCAR complex and thereby preventing the expression of the downstream target genes.

specific transporters. Therefore, both carrier-mediated and passive ABA transport mechanisms are suggested to exist.

Studies in *A. thaliana* have revealed several ABA transporter genes in vascular tissues belonging to the ATP-binding cassette (ABC) family. One such gene is *AtABCG25*, which codes for an ABA transporting protein (Kuromori and Shinozaki, 2010). It has been suggested that *AtABCG25* mediates ABA export from the inside to the outside of the cell. On the other hand, another transporter *AtABCG40*, expressed in guard cells, has been reported to be responsible for importing ABA from the outside to the inside of cells. Both these genes are reported to be involved in ABA signaling (Kuromori and Shinozaki, 2010).

ABA signaling

ABA plays an important role in various aspects of plant stress responses, embryo maturation, seed dormancy, germination, root branching, stomatal closure, *etc.* A great many studies have attempted to shed light on the mechanisms and core components of ABA signaling (reviewed in Cutler et al., 2010; Umezawa et al., 2010; Liu, 2012). There are three major components of ABA signaling: pyrabactin resistance (PYR)/PYR1-like (PYL)/regulatory component of ABA receptor (RCAR), protein phosphatase 2C (PP2C; a negative regulator) and (sucrose non-fermenting) SNF1-related protein kinase 2 (SnRK2: a positive regulator). These are assembled as a double negative regulatory system and form a signaling complex known as 'ABA signalosome' (Fig. 2).

PP2C is a global negative regulator of ABA signaling

Phosphorylation and de-phosphorylation, mediated by kinases and phosphatases, respectively, help in the transmission of signals from external to internal environment of the cell, and thereby bring about physiological changes.

In *A. thaliana*, 76 genes encode for PP2Cs and are divided into 10 groups (A-J). PP2Cs belong to the PPM family of phosphatases (Mg^{2+}-dependent phosphatases). PP2Cs like ABA insensitive 1 (ABI1), ABI2 (homolog to ABI1), AHG3/PP2CA, ABA-Hypersensitive Germination1 (AHG1), hypersensitive to ABA1 (HAB1) and HAB2 are members of the group A (Saez et al., 2004; Schweighofer et al., 2004; Yoshida et al., 2006). The members of the group A have definite roles in different tissues and organs, and demonstrate tissue-specific expression patterns.

ABA-Insenstive1 (ABI1) and ABI2 were identified in a genetic screen for ABA-insensitive *A. thaliana* mutants. The *abi1-1* and *abi2-1* mutants show ABA insensitivity in various tissues and developmental stages, suggesting that PP2C acts as a negative regulator of ABA signaling (Leung et al., 1997; Rodriguez et al., 1998; Umezawa et al., 2010). The ABA signaling regulators HAB1 and

HAB2 were isolated based on sequence similarity to ABI1 (Saez et al., 2004). AHG1 and AHG3/AtPP2CA were cloned from genetic screens of *A. thaliana* and a yeast complementation test. It has been shown that all of the loss-of-function mutants of group A PP2Cs exhibit significant hypersensitivity, which establishes them as the major negative regulators of ABA signaling (Umezawa et al., 2010). The negative regulatory role of PP2C in ABA signaling is now well founded, and this has been shown in various other plant species. This is further corroborated by the fact that double or triple PP2C knockout mutants show increased ABA hypersensitivity.

Gene expression data and genetic analysis has indicated a dominant role of HAB1, ABI1, ABI2 and PP2CA in ABA signaling pathways in both seeds and vegetative tissues. Recessive *Hab1-1* mutants show enhanced ABA responsive gene expression, enhanced ABA-mediated stomatal closure and ABA-hypersensitivity leading to seed germination and growth inhibition. Conversely, HAB1 reduces ABA sensitivity upon constitutive expression. These results are consistent with the fact that HAB1 negatively regulates ABA signaling (Saez et al., 2004).

Negative regulation of MAPK (MAPK4 and MAPK6) by *A. thaliana* PP2C, AP2C1 (Schweighofer et al., 2004) also potentially links PP2Cs with cold and drought stress responses. A PP2C encoding gene MP2C has been indicated to be responsible for inactivation of stress-activated MAPK (SAMK) pathway, by accumulation of MP2C phosphatase which inhibits further signal transmission (Meskiene et al., 1998) hence confirming the role of PP2Cs as global negative regulators of ABA signaling.

SnRK2 is a positive regulator of ABA signaling

The identification of PP2C indicated that protein phosphorylation events should be important components of the ABA signaling pathway. Many protein kinases were actually found to be involved in ABA signaling. The first SnRK2 cDNA clone (*PKABA1*) was isolated from an ABA-treated wheat embryo cDNA library (Anderberg and Walker-Simmons, 1992). In fava bean, the SnRK2 family was identified as ABA-Activated Protein Kinase (AAPK) (Li et al., 2000) and *A. thaliana* SRK2E/Open STomata1 (OST1)/SnRK2.6 (Yoshida et al., 2002, 2006).

SnRKs are serine/threonine protein kinases grouped under SNF1 family, which also includes yeast's SNF1 kinases and AMPKs from mammals. These are positive regulators of ABA mediated signaling, responsible for the phosphorylation of bZIP transcription factors known to bind *cis*-regulatory motif having ACGT as a core sequence. SnRKs can be classified into 3 sub-divisions: SnRK1, SnRK2 and SnRK3 (Kulik et al., 2011).

The SnRK2 family is the key regulator of plant response to abiotic stress. SnRK2s are monomeric serine/threonine protein kinases. SnRK2s are divided into 3 subgroups based on the affinity toward ABA *viz*. group I, II and III. There are 10 SnRK2 members in *A. thaliana*, SRK2A-SRKJ or SnRK2.1-SnRK2.10. Group I kinases do not respond to ABA, group II does not, or weakly, respond to ABA, and group III strongly responds to ABA. Experimental data suggest functional overlap between group II and group III SnRK2s inactivating bZIP type transcription factor ABA responsive element binding factor/ABA binding factor (AREB/ABF). Group III is believed to be the key regulator in ABA-dependent pathway of gene expression, whereas group II may be involved by a parallel pathway which means that group II SnRK2s could be variants of group III kinases that are not activated or weakly activated by ABA. Phylogenetic studies have also revealed group II as an intermediate during transition of group I to group III (for review refer Hrabak et al., 2003; Kulik et al., 2011).

Phosphorylation at the post-translational level and gene response to changes in the environment regulate SnRK2 expression. Members of SnRK2 family are found in algae (Ballester et al., 2008), monocots like rice, sorghum (Kobayashi et al., 2004; Li et al., 2010), maize (Huai et al., 2008) and wheat (Mao et al., 2010; Zhang et al., 2010), and dicots like tobacco (Kelner et al., 2004), soybean, *A. thaliana* (Monks et al., 2001; Boudsocq et al., 2004) and fava bean (Li et al., 2000). Enhanced expression of SnRK2 genes in maize occurs in response to NaCl and cold while heat suppresses it (Huai et al., 2008). Induced expression of SnRK2 genes in soybean occurs in response to hyperosmolarity, and the regulation of expression of some SnRK2s in response to dark-light cycle has also been reported (Monks et al., 2001).

SnRK2 kinases encode a protein of about 40 kDa. The N-terminal of the amino acid sequence is a highly conserved kinase domain, and the C-terminal is a variable regulatory domain with stretches of glutamate (Glu) or aspartate (Asp) which functions in protein interactions. Domain I, a subdomain of C-terminal is independently activated by osmotic stress whereas domain II is ABA dependent and is responsible for activation by ABA and interaction with ABI1 (Yoshida et al., 2010). Group I has a glutamate rich region in domain II, whereas group II/III have aspartate rich regions in this domain, which accounts for their differential response toward ABA activation (Mizoguchi et al., 2010; Yoshida et al., 2010; Fujita et al., 2013).

Three members of SnRK2 subgroup III namely SnRK2D/SnRK2.2, SRK2E/OST1/SnRK2.6 and SRK2I/SnRK2.3 are major regulators of plant response to ABA. SRK2E/OST1/SnRK2.6 is expressed in guard cells leading to stomatal closure in the presence of ABA. Other SnRK2s like SRK2D/SnRK2.2 and SRK2I/SnRK2.3 are expressed in seeds and vegetative tissues. Although partial functional redundancy has been shown for all of them but their importance in regulating water stress as well as ABA-dependent developmental processes cannot be neglected. A triple mutant in *A. thaliana* not only had disrupted stomatal closure but also had down regulated ABA and water stress induced genes resulting in ABA insensitivity and reduced drought tolerance (Yoshida et al., 2002, 2006; Fujita et al., 2009; Fujii and Zhu, 2009; Nakashima et al., 2009a). This mutant also showed up-regulation of jasmonic acid (JA) dependent genes, genes regulating flowering and those related to photosynthesis and tetrapyrrole synthesis. Researchers have also reported down regulation of almost all genes encoding dehydration-responsive late embryogenesis abundant (LEA) proteins and group A PP2Cs under water stress conditions in this mutant indicating the essentiality of these three genes in controlling seed development and dormancy (Nakashima et al., 2009a).

SRK2E/OST1/SnRK2.6 majorly regulates ABA dependent stomatal closure in water deficit conditions. Its mutant is unable to withstand low humidity and shows a wilted phenotype indicating its crucial role in combating environmental stress (Yoshida et al., 2002). It also regulates reactive oxygen species (ROS) production in the cell wall through phosphorylation of the two plasma membrane NADPH oxidases (*RbohD* and *RbohF*) in an ABA-dependent manner leading to stomatal closure (Kwak et al., 2003). Apart from this, OST1 has also been shown to positively regulate stress response genes like *RD29B* and *RD22* (Yoshida et al., 2002).

ABA and osmotic stress activate SRK2E/OST1 mediated stomatal closure by two independent pathways namely ABA and OS-dependent pathways as shown by experiments on ABA-insensitive or ABA-deficient mutants. C-terminal domain II is believed to be responsible for ABA-dependent activation of OST1 while low humidity or OS-mediated (ABA-independent) activation transduces stress signals to induce stomatal closure. Thus, both pathways are required to respond to sudden water stress by causing complete stomatal closure (Yoshida et al., 2006).

SRK2E/OST1 in *Arabidopsis* also interacts with ABI1/ABI2, where both have distinct roles in controlling its activity. Experimental results have indicated two roles of ABI1 in ABA dependent pathway. It acts as a negative regulator of SRK2E in ABA signaling and as a positive regulator in activation of SRK2E in low humidity.

Interaction of SRK2E with ABI1 and activation by ABA requires the presence of domain II, whereas in its absence, SRK2E is activated by low humidity, partially complementing the wilted phenotype of *srk2e*. A proposed explanation is the interaction of domain II with a downstream factor triggered by low humidity leading to stomatal closure. It has also been reported that ABI-1 protein acts as a dominant negative inhibitor, which blocks interactions of downstream proteins with domain II of SRK2E (Yoshida et al., 2006).

SnRK2s and downstream effectors of ABA action

SnRK2s are central components of ABA signaling pathways. The *SnrK2.2/2.3/2.6* triple mutant plants are nearly insensitive to ABA, suggesting that most of the molecular actions of ABA are triggered by the SnRK2s-mediated phosphorylation of substrate protein. Only a few substrate proteins of SnRK2s are known. In an effort to identify additional substrates of SnRK2s, Wang et al. (2013) used quantitative phosphoproteomics to compare the global changes in phosphopeptides in wild type (WT) and *snrK2.2/2.3/2.6* triple mutant seedlings in response to treatment. They reported that ABA could increase the phosphorylation of 166 peptides and decrease the phosphorylation of 117 in WT seedlings. They further reported that with *snrK2.2/2.3/2.6* triple mutant, 84 of the 166 peptides, representing 58 proteins, could not be phosphorylated. These results shed new light on the role of the SnRK2 protein kinases and on the downstream effectors of ABA action.

ABA-PYR/PYL/RCAR receptor

ABA receptors encode STAR domain/Bet V allergen super family proteins. Genome wide studies of *A. thaliana* have revealed presence of 14 highly conserved PYR/PYL/RCAR protein encoding genes out of which 9 are major in interactions with ABI1 (Ma et al., 2009; Park et al., 2009; Hubbard et al., 2010; Nishimura et al., 2010).

It has been reported that the PYR/PYL/RCAR receptor, which is a direct ABA receptor, interacts with PP2C in the presence of ABA, which inhibits phosphatase activity of PP2C and acts as negative regulator. These receptors play an important role and are observed in the case of quadruple mutants *pyr1*, *pyl1*, *pyl2* and *pyl4*, which are insensitive toward ABA. On the other hand, over-expression of RCAR1/PYL1, PYL5/RCAR8 or PYL8/RCAR3 gives more tolerance toward drought stress in *A. thaliana*. Even in case of ABI1 and ABI2, HAB1 activity is inhibited by stereo-specific RCAR1, RCAR3, RCAR8, RCAR11 and RCAR12 receptors in the presence of ABA, but not observed in the absence of receptors (Ma et al., 2009; Park et al., 2009; Nishimura et al., 2010).

PYR/PYL/RCAR interaction with ABI1 can be both ABA-dependent as in case of PYR1 and PYL1-4 or constitutive as shown in case of PYL5-12. Similar results have been obtained for *A. thaliana* (Nishimura et al., 2010). Bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation assays have established PYL5 as an HAB1 antagonist. PYL5 inhibits HAB1, ABI1 and ABI2 functions in an ABA dependent manner activating ABA signaling cascade (Ma et al., 2009; Park et al., 2009).

Resolution of ABA binding with PYR1/PYL1/PYL2 has revealed the presence of a proline cap and a leucine lock. ABA binding leads to closing of the cap, limiting the enzyme within the cavity, and preventing solvent contact while the lock rearranges sealing the lid over ABA. The conformational changes brought about by this binding create an oval site for PP2C interaction further stabilizing the structure. This shields the catalytic site of PP2C leading to its inactivation by PYR/PYL/RCAR (Ma et al., 2009; Nishimura et al., 2009; Park et al., 2009). The crucial role of this cap and lock can be indicated by reduced ABA sensitivity in the mutant. Also disruption of ABA-PYR1 interactions by mutating the residues, which make direct contact with ABA highlights the importance of ABA-induced conformational (Nishimura et al., 2009) changes .

Antoni et al. (2013) showed that ABA-dependent inhibition of PP2Cs by PYR/PYLs is required for the perception of moisture gradient. The *pyl8* mutant showed reduced sensitivity to ABA mediated root growth inhibition. They discovered that this was due to lack of PYL8-mediated inhibition of several group A PP2Cs. It has been demonstrated *in vivo* that PYL8 interacted with at least five PP2Cs, namely HAB1, HAB2, ABI1, ABI2 and PP2CA/AHG3. Consistent with the above observations are the facts that ABA-hypersensitive *pp2c* quadruple mutant showed enhanced hydrotropism, whereas an ABA-insensitive sextuple *pyr/pyl* mutant showed reduced hydrotropic response.

Changes at gene expression level

Onset of drought and salt stress induces the accumulation of ABA, which then acts as a stress signal. ABA regulates expression of many stress-responsive genes grouped as ABA responsive genes. *Cis*-elements such as ABA-responsive element (ABRE) are present up stream of the ABA-dependent stress responsive genes, which have a core sequence PyACGTGGC along with one or two coupling elements (CE) also found upstream of stress-responsive genes. Genes having ABRE sequence belong to G box element. This sequence is target of many members of the bZIP transcription factor family known as ABA-responsive element binding protein (AREB). A major finding is the importance of distance between the ACGT *cis*-elements. Two ACGT *cis* elements separated by five nucleotides have been found to be induced in response to salicylic acid (SA), and similarly motifs separated by twenty-five base pairs are induced in response to ABA (Shen et al., 2004; Mehrotra and Mehrotra, 2010).

In accordance with the basic rules, the probability of occurrence of two ACGT motifs separated by a single nucleotide ($ACGT_{N1}ACGT$) has been found to be more as compared to that of $_{N2}$, $_{N3}$, $_{N4}$ and $_{N5}$ separating nucleotides in *A. thaliana* genome. Statistically, the occurrence of $ACGT_{N1}ACGT$ is 1605 but no biological role has been assigned or reported to $_{N1}$, $_{N2}$, $_{N3}$ and $_{N4}$ so far. Only $_{N5}$ and $_{N25}$ have been found to be associated with biotic and abiotic stress responses (Mehrotra and Mehrotra, 2010; Mehrotra et al., 2012, 2013). A study has also shown that flanking sequences play an equally important role in defining the binding efficiency of bZIP transcription factors (Foster et al., 1994). Nucleotides at +2/−2, +3/−3 and +4/−4 positions define the specificity and affinity for binding of bZIP class of transcription factors. Presence of G at +2/−2 positions (G-box), presence of A/C at +3/−3 (C-box) and presence of G/T at +4/−4 are preferred over other nucleotides. Hence it could be reasonably concluded that two ACGT motifs in close proximity cannot serve as flanking sequences for each other (Foster et al., 1994; Mehrotra et al., 2012).

A similar study (Suzuki et al., 2005) considered 5 classes of stress-responsive gene promoters namely ABA-regulated (ABR), ABA-dependent (ABD), VP1-dependent (VPD), VP1 and ABA-dependent (VAA), VP1 or ABA-dependent (VOA). In all these classes, distribution of flanking sequences of ACGT motifs was studied. It has been reported that out of 64 variants, 23 variants were distributed among these 5 stress-responsive promoters. GAC as a flanking sequence of ACGT has been observed in all classes majorly in VAA. Another variant GCCACGT has been dominantly reported in VAA, VPD and in two sub-classes of VP1-dependent class of promoters. Similarly, AACACGT and CCCACGT were reported in ABA-dependent and ABA-regulated gene promoters, respectively. Genes regulated by cold also exhibit the same whereas CCGACGT sequence was found to be predominating in up-regulated cold genes along with ABA and VP1-regulated genes (Suzuki et al., 2005).

Different members of the bZIP class of transcription factors bind to the ACGT core specifically. This binding also depends upon the sequences flanking the core region. Flanking sequences are also

responsible for differential distribution of stress-responsive promoters across the genome, which could be one of the reasons behind the often-observed differences in ABA regulation and/or dependency (Riechmann et al., 2000; Mehrotra and Mehrotra, 2010). The flanking sequences might influence the binding specificity of different members of bZIP class to the ACGT core sequence (Foster et al., 1994; Suzuki et al., 2005).

Transcription factors involved in stress signaling

The interaction between specific transcription factors and their *cis*-elements, causes expression of stress inducible genes. Major transcription factor (TF) families, which are involved in the regulation of abiotic stress responses, are bZIP, MYB, MYC, NAC, ERF and DREB/CBF (C repeat binding factor).

The ABRE-binding (AREB) proteins or ABRE-binding factors (ABFs) encode bZIP transcription factors among which AREB1/ABF2, AREB2/ABF4 and ABF3 are induced by dehydration, high salinity or ABA treatment in vegetative tissues, and are involved in enhanced drought stress tolerance (Riechmann et al., 2000; Yoshida et al., 2010).

AREB1 requires ABA for full activation, and its activity is regulated by ABA-dependent multi-site phosphorylation of the conserved domains by SnRK2. SnRK2 protein kinases phosphorylate serine/threonine residues at R-X-X-S/T sites in the conserved regions of AREB/ABF proteins causing its inactivation which is further promoted by ABA-mediated inactivation of the PP2Cs, known to negatively regulate protein kinases (Nakashima et al., 2009a).

GmbZIP1, a member of AREB subfamily has been identified (Gao et al., 2011) in soybean cv.Tiefeng8. Its expression was found to increase in the presence of ABA, drought, high salt and low temperature. Stomatal closure under stress was induced because of the over expression of GmbZIP1 in transgenic plants, leading to enhanced tolerance to abiotic stresses.

Members of bZIP family, like ABP9, have been found to be associated with enhanced photosynthetic capacity of plants in drought and heat stresses (Zhang et al., 2008). Another bZIP transcription factor, OsABF1, reported (Hossain et al., 2010) in roots of the seedlings and shoots of rice has been found to be involved in abiotic stress responses and ABA signaling. In tomato, a bZIP transcription factor SlAREB1 participates in abiotic stress by regulating oxidative-stress related proteins, LEA proteins and lipid transfer proteins (LTPs) (Orellana et al., 2010).

MYC and MYB also play an important role in ABA signaling by activating some stress-inducible genes like *RD22* (Abe et al., 2003). MYB in *A. thaliana* namely *AtMYB60*, *AtMYB44* and *AtMYB15* have been found to be involved in stomatal closure, and protects against drought and salt stresses.

NAC family studies reveal that cDNAs encoding NAC-like proteins namely ANAC019, ANAC055, ANAC072 bind to ERD1 (early response to dehydration 1) present in the promoter region of stress inducible genes. Enhanced expression of these transcription factors ANAC019, ANAC055, ANAC072 and SNAC1, OsNAC6/SNAC2, ONAC045 leads to tolerance against drought stress in *A. thaliana* and rice vegetative tissues, respectively (Nakashima et al., 2009b). In wheat, *TaNAC69* has been found to be involved in the regulation of drought stress responses (Xu et al., 2011).

AtNAP (a NAC family transcription factor in *Arabidopsis*) and senescence associated gene113 (*SAG113*) (a PP2C family member localized in Golgi apparatus) have been reported to get activated in the presence of ABA. Knock out studies have shown that expression of *SAG113* is dependent on AtNAP (Zhang and Gan, 2012). In the presence of ABA, binding of AtNAP to 9 bp core sequence (CACGTAAGT) present in the promoter of *SAG113* leads to stomatal closure, creating a dehydration condition, which ultimately leads to leaf senescence (Zhang and Gan, 2012).

The central concept involved in ABA signaling is the inhibition of phosphatase activity of PP2C by ABA bound PYR/PYL/RCAR receptors. This leads to the subsequent activation of SnRK2 and phosphorylation of downstream targets such as AREB/ARF basic domain leucine zipper (bZIP), slow (S) anion channels and others, leading to stress induced response in plants (Ma et al., 2009; Park et al., 2009). Activation of ABA-responsive genes requires the ABA responsive element (ABRE). Three members of AREB/ABF family viz., AREB1, AREB2 and AREB3 have been identified to be expressed in response to ABA and water stress. AREBs require ABA for their full activation and show certain level of redundancy (Yoshida et al., 2010). The AREB/ABF encode bZIP transcription factors and belong to group A subfamily (Riechmann et al., 2000). Several late embryogenesis abundant (LEA) genes, group A PP2C genes and transcription factors as downstream targets of AREB/ABF have also been revealed (Yoshida et al., 2010). By generating a triple mutant of *areb1 areb2 abf3*, their role in stress tolerance and regulation of ABRE dependent gene expression in ABA signaling was elucidated. The triple mutant not only shows down regulation of *LEA* genes but also group A PP2Cs such as *AHG1*, *AHG3*, *HAI1*, *HAI2* and *HAI3* (Schweighofer et al., 2004; Yoshida et al., 2010).

The *srk2dei* (same as SnrK2.2/2.3/2.6) triple mutants also exhibit reduced phosphorylation of bZIP transcription factors including ABI5 which indicates that through phosphorylation of ABI5, SRK2D/E/I also plays a role in controlling genetic expression in ABA signaling pathways (Nakashima et al., 2009a).

Epigenetic changes in response to abiotic stress

Chromatin remodeling plays a major role in the regulation of gene expression under stressful conditions and the expression of stress-responsive genes requires them to be free from the confines of the nucleosome organization (Kim et al., 2010). Such histone modifications are mainly carried out by enzymes such as HAT (histone acetyl transferases), HDAC (histone deacetylases), HMT (histone methyltransferases) and HDM (histone demethylases).

Acetylation (ac) and/or methylation (me) of specific lysine residues in histone proteins have been reported in mammals, yeast and plants and H3K9Me3, H3K27me3 and H3K9ac are used as a marker. 28 such sites have also been reported in abiotic stress related genes in *A. thaliana* with the aid of techniques such as mass spectroscopy and chromatin immunoprecipitation (ChIP) (Zhang et al., 2007; Kim et al., 2008, 2010). In case of dehydration stress in *A. thaliana*, changes in nucleosome occupancy have been observed and the levels of methylation or acetylation of histone H3 have also been found to vary (Kim et al., 2008).

Not only genes but also promoters can be constrained by chromatin architecture. PvAlf, a putative seed specific *phas* activator (encodes phaseolin, seed storage protein of *Phaseolus vulgaris*) potentiates the *phas* promoter by repositioning nucleosomes on three TATA boxes initiating remodeling, and ABA further recruits the machinery leading to gene expression. A PvAlf factor has also been reported to assign chromatin switches (SWI/SNF) or in an alternative pathway acetylate or phosphorylate histone tail by histone kinases for transcriptional activation (Li et al., 1999). These findings not only elucidate diverse physiological roles of ABA but also provide a link between ABA and chromatin remodeling.

Histone modifications by acetylation and de-acetylation

Histone acetylation causes relaxation of the chromatin structure. Twenty-six lysine sites that undergo acetylation have been reported which are 9, 14, 18, 23 in H3; lysine 5, 8, 12, 16, 20 in H4; lysine 5 and 9 in H2A; and lysine 5, 12, 15 and 20 in H2B (Turner, 2002).

HATs have been classified into four groups on the basis of sequence homology: GNAT-MYST family, p300/CBP family, $TAF_{11}250$-related family and the nuclear receptor co-activator family. Apart from modifying histones, HDACs and HATs have also been reported to target non-histone proteins like p53, retinoblastoma protein (pRb), tubulin, and c-Myc (reviewed by Pawlak and Deckert, 2007).

Salt stress causes an increase in transcriptional expression through histone acetylation (Waterborg et al., 1989) and drought stress leads to acetylation of H3K23 and H3K27 in a gene specific manner (Kim et al., 2008). High salinity, cold and ABA stress also increase phosphorylation of H3Ser10, phosphoacetylation of H3Ser10 and Lys14 and acetylation of H4 in *A. thaliana* T87 and tobacco BY-2 cell lines (Sokol et al., 2007).

AtGCN5, a HAT protein in *A. thaliana*, which responds to environmental changes such as cold (Stockinger et al., 2001), is dephosphorylated by AtPP2C-6-6 (a PP2C protein) *in vitro* (Servet et al., 2008). However, *atpp2c-6-6* mutants do not show reduction of acetylation levels of H3K14 and H3K27 but the same is not true for *atgcn5* mutants. Also, reduced expression of several ABA stress inducible genes like *RAB18*, *RD29A* (responsive to dehydration 29A), *RD29B* and *COR15A* under high-salinity conditions was observed in *atpp2c-6-6* mutants, whereas in *atgcn5* mutants *RAB18* and *RD29B* genes were up-regulated in unstressed conditions with no effect on genes under high-salinity stress. These results suggest that although AtPP2C-6-6 plays a role in induction of stress-inducible genes, this interaction might not involve AtGCN5.

In vitro studies have shown that GCN5 protein interacts with transcriptional adaptor proteins Ada2a and Ada2b as well (Stockinger et al., 2001). Interaction of GCN5 and ADA with CBF1, which transcribes downstream cold-responsive genes by recruiting ADA/SAGA-like complexes, regulates cold tolerance by mediating chromatin remodeling in target genes. During cold stress, low expression of the cold-induced *COR* genes was observed in mutant for ADA and SAGA histone acetyltransferase in *A. thaliana* (Vlachonasios et al., 2003).

HDACs deacetylate lysine residues resulting in gene inactivation. In eukaryotes, they have been divided into three groups in *A. thaliana* namely RPD3/HDA1, HD2 and SIR2 families. RPD3/HDA1 family is further divided into classes I, II, III (Fu et al., 2007) and IV in rice (Sridha and Wu, 2006). A plant-specific HD2-type HDAC in *A. thaliana* (AtHD2) (Song et al., 2005) has been found to be repressed by ABA and its over expression can modulate ABA stress-responsive genes by regulating stomatal aperture. Over expression of AtERF7 (which represses transcription via its interaction with histone deacetylase HDA19) in transgenic *A. thaliana* plants shows reduced ABA sensitivity of guard cells and elevated water loss through transpiration. These results show that histone deacetylation also regulates guard cell mediated stomatal closure and that ABA regulated stomatal response mediated by chromatin remodeling plays an important role in abiotic stress tolerance.

Another HDAC known as HDA6 in *A. thaliana* has been found to be involved in RNA-directed DNA methylation (Aufsatz et al., 2002) and histone deacetylation in response to stress (Zhou et al., 2005). Low expression of ABA, *DREB2A*, *RD29A* and salt stress marker genes have been observed in those plants which show low expression of *HDA6* during stresses (Chen et al., 2010). Enhanced expression of *HDA19* caused by stress, affecting chromatin modifications by histone deacetylation have also been reported. Studies have revealed that acetylation level of H3K9 is affected in *hda19* mutant plants at seedling stage, which greatly affects gene expression (Zhou et al., 2010).

HOS15 of *A. thaliana* (high expression of osmotically responsive-genes 15) through histone H4 deacetylation represses abiotic stress response genes by encoding a protein complex WD40. *HOS15* mutants have been found to be hyper sensitive to low temperatures (Zhu et al., 2008).

Experimental data has provided evidence for differential roles of different family members of HDACs in regulating processes controlled by plant hormones. Interplay of HDACs and HATs may regulate stress response genes by integrating signaling pathways for ethylene, jasmonic acid, salicylic acid and abscissic acid (Demetriou et al., 2009).

HDACs, HvA22, HvADC2 in barley and HDT701 and HDT702 in rice are induced by ABA and jasmonic acid respectively suggesting functional similarities among monocots for HD2 genes. But on the same hand, repression of HDT genes in rice and AtHD2 in *A. thaliana* (Sridha and Wu, 2006) upon ABA treatment and differential responses of barley genes to ABA may indicate functional differences for HD2 members not only among monocots but between dicots and monocots as well (Demetriou et al., 2009).

Methylation and stress

DNA cytosine methylation represses gene promoters and gene transcription by asymmetric (mCpHpH) and symmetric (mCpGandmCpHpG) methylation. De novo methylation is catalyzed by methyl transferase domains rearranged methylase1 and 2 (DRM1 and DRM2), while enzymes like MET1 (DNMT-1 like enzymes) and plant-specific enzyme chromomethylase3 (CMT3) maintain symmetric CG and CHG methylation respectively (Henderson, 2007; Kim et al., 2010). MET1 and CMT3 may also catalyze new methylation, and maintenance of symmetric methylation may also require DRM1 and DRM2.

Importance of occurrence of methylation was reported in mutant methyl transferase *Dnmt1* as cells, which failed to maintain methylation process lost their viability (reviewed by Madlung and Comai, 2004). Mutant *MET1*, a homologue of *Dnmt1* results in a severe pleiotropic phenotype as observed in plants (Finnegan and Dennis, 1993, Finnegan et al., 1996). Also methylation has been reported to be crucial in retrotransposon suppression (Hirochika et al., 2000). Transposons, which are repressed by methylation, can be activated through demethylation induced by environmental factors. Similar findings have been observed in *Antirrhinum majus*, where cold stress mediated hypomethylation led to transposition of *Tam3* transposon (Hashida et al., 2006).

Histone H3Lys4 tri methylation and histone H3Lys9 and Lys27 di methylation have been correlated with gene activation and silencing respectively (Chinnusamy and Zhu, 2009). Tri methylation of H3K27, a negative histone modification marker was found to decrease in response to cold stress in *A. thaliana* (Kwon et al., 2009).

Methylation of histone H3 has been found to be regulated by mono ubiquitination by H2B mediated by *hub* gene, which positively regulates ABA-induced seed dormancy (Liu et al., 2007). *H2B* deubiquitination by nuclear ubiquitin protease SUP32/UBP26 directs CpNpG and CpNpN methylation leading to gene inactivation. The genome wide map of H3K4 methylation pattern of chromatin during drought stress in *A. thaliana* has been studied (Dijk et al., 2010). In this study, genes with high levels of expression were found to contain nucleosome free regions located upstream of the transcription start site.

H3K9 undergoes either acetylation or mono-di or trimethylation. These epigenetic modifications have been reported to be involved in the regulation of gene transcription and chromatin organization, with varied impact. H3K9 acetylation has been consistently found to be associated with high gene expression/activation while dimethylation of H3K9 is reported to be linked with low gene expression. The balancing mechanism, which occurs between methylation and acetylation of H3K9makes transcription insensitive to variation disruption which affects the

process. A study shows that H3K9 acetylation effect decreases in the presence of H3K27 tri methylation (Zhou et al., 2010).

Chromatin remodeling factors

Chromatin modifying factors remodel or reposition the nucleosome by sliding histone octet in an ATP dependent process. Chromatin modifying factors include switch SWI/SNF complexes, which consist of ATPase SWI/SNF2, a pair of SWI3, SNF5 and SWP73 polypeptides. Plant genomes contain these ATPases of SWI2/SNF2 subgroup chromatin remodeling ATPases, which are called BRAHMA (BRM), SPLAYED (SYD) and SNF5 and FASCIATA (FAS).

Four SWI3-like proteins in *A. thaliana* have been identified namely SWI3A, SWI3B, SWI3C and SWI3D (Sarnowski et al., 2002; Zhou et al., 2003). SWI/SNF remodeling complexes have been found to be involved in hormonal response to abiotic stress conditions (Saez et al., 2008).

Interaction of SWI3 with a functional catalytic domain of HAB1 has been established in the plant cell nucleus by using BiFC and co-immunoprecipitation assays. BiFC assays of tobacco nuclei have also shown interaction of SWI3B with PP2CA, ABI1 and ABI2. Analysis of mutants of *swi3b* alleles, *swi3b-3* and *swi3b-4* showed that SWI3B positively regulates ABA in seeds and vegetative tissues, and also plays a key role in plant growth and development. It has been postulated that SWI3B regulates only a subset of ABA-inducible genes, and shows redundancy for other ABA-responsive genes (Saez et al., 2008).

ABA-insensitive phenotype of *Hab1-1swi3b-3* mutants, but not of *hab1-1* mutants, showed that SWI3B functions downstream of HAB1 in ABA signaling pathways. ChIP assays suggested repression of ABA-induced transcription by HAB1 through direct chromatin interaction, which can be released by subsequent ABA treatment. HAB1 has been found to be present near RAB18 and RD29B promoters, and its presence can be eliminated by ABA treatments. This suggests that SWI3B might anchor HAB1 to a putative SWI/SNF complex targeted to some ABA-responsive promoters and HAB1 regulates its activity by dephosphorylating a component essential for proper functioning of the complex. Given the opposite roles of HAB1 and SWI3B in ABA-signaling pathways, it is also possible that HAB1 by negatively regulating SWI3B modulates its role as a positive regulator (Saez et al., 2008).

In pea, *PsSNF5* gene encoding a homologous SNF5 protein of the SWI/SNF complex was found to be up-regulated by ABA and drought stress conditions. This shows a possible role of PsSNF5 in ABA signaling pathways through changes in chromatin structure (Rio et al., 2007).

In *A. thaliana*, AtCHR12, a SNF2/Brahma-type chromatin remodeling factor has been found to arrest growth of normally active primary bud and primary stem temporarily when subjected to drought and heat stress. Also the growth arrest response was found to be proportional to degree of severity of stress applied (Mlynárová et al., 2007). Han et al. (2012) described the role of *A. thaliana* BRM SWI/SNF2 chromatin remodeling complex components in direct transcriptional repression of ABI5 during post germination development.

The chromo domain helicase-DNA-binding (CHD3) class of SWI/SNF2 has been found to be associated with regulating stress-response genes in plants. *PICLKE* (PKL), which codes for *A. thaliana* CHD3 chromatin remodeling factor together with HDACs, has been implicated to play an important role in ABA signaling (Ogas et al., 1999) and also in methylation of H3K27 (Zhang et al., 2007).

Mutations in DDM1 (decrease in DNA methylation1), an *Arabidopsis* SWI2/SNF2 subfamily member results in methylation depletion by building heterochromatin structures, which are targeted by methyl transferases. However, role of DDM1 in DNA hemimethylation right after DNA replication by recruiting methyl transferases has also been established (Jeddeloh et al., 1999). This establishes a link between chromatin remodeling factors and DNA methylation.

RNAs and abiotic stress

Abiotic stress regulation also occurs at post-transcriptional and post-translational levels. The former involves processing of pre mRNA which starts with splicing of introns and joining of exons. In *A. thaliana*, a gene *STA1*, which codes for nuclear pre-mRNA splicing factor, has been identified under cold stress (Lee et al., 2006b). *Sta1-1* mutant was found to have defective splicing mechanisms as it could not splice introns from *COR15A* pre-mRNA. This unprocessed pre-mRNA showed hypersensitivity toward cold, salt stress and ABA.

Some glycine rich proteins known as RNA binding proteins (RBP) have also been identified to have an important role to play in mRNA splicing. GR-RBP over expressing transgenic plants has been observed to develop more resistance toward cold stress (Kim et al., 2007; Zhu et al., 2007). Up regulation of these proteins was also reported under environmental stress. A similar protein oligouridylate binding protein (UBP1) has been identified in *A. thaliana*. Interaction of UBP1 and RBPs results in formation of UBP associated proteins (UBA) (Lambermon et al., 2000). UBA1 leads to increased accumulation of processed mRNA in protoplasts, which suggests a role of UBA proteins in stabilizing mRNA in cytoplasm as well (Lambermon et al., 2002). A homolog of UBA1 from *Vicia faba*, ABA-activated protein kinase (AAPK)-interacting protein 1 (AKIP1), has been identified, which upon activation by AAPK through ABA dependent phosphorylation stabilizes dehydrin gene mRNA which is involved in cell protection (Li et al., 2000, 2002).

Abiotic stresses like temperature have also been found to be regulated by specific small RNAs. In *A. thaliana*, 24-nt *SRO5-P5CDH* nat-siRNA cleaves mRNA and thereby down regulates the expression of *P5CDH* leading to increased proline accumulation and hence increased salt tolerance (Borsani et al., 2005). Microarray analysis has also revealed that abiotic stresses, particularly ABA play an important role in RNA-mediated DNA methylation. However, further studies are required to elucidate the exact mechanism. *A. thaliana RDR2* has been reported to be involved in transcriptional gene silencing (Chan et al., 2004). Epigenetic changes through chromatin remodeling or DNA methylation can also be induced by non-coding RNAs (Bond and Finnegan, 2007).

Abiotic stresses including ABA have been reported to enhance transcription of miR402, which regulates Demeter-like protein 3 (DML3), a DNA glycosylase domain protein encoded by At4g34060. It has been hypothesized that miR402 under ABA induction reduces the transcript level of DML3 resulting in gene repression through methylation (Sunkar and Zhu, 2004; Chinnusamy et al., 2008).

Discussion

ABA seems to be ubiquitously distributed from cyanobacteria to plants. In many groups, where 9-*cis*-neoxanthin is missing, ABA is likely to be synthesized directly via farnesyl diphosphate. Several recent reports suggest that algal ABA may have protective role against oxidative stress (Yoshida et al., 2003), although a clear role has not been described. The possibility that the complete biochemical pathway to form and metabolize ABA was invented in bacteria, cyanobacteria and algae cannot be ruled out, although it may not have been adding to fitness advantage (Wolfram, 2010). During the course of evolution, when organisms such as submerged living liverworts started to occupy terrestrial habitats, ABA must have gained an important role as a stress hormone. The ability to tolerate drought has been one of the critical steps in the evolution

of land plants. Liu et al. (2008) has shown that *Selaginella* exhibits ABA responsive mechanisms with a subset of core components. This means that ABA signaling pathway was established in bryophyte. Bryophytes occupy an intermediate position between aquatic and land plants and the establishment of ABA signaling mechanism was of great advantage in achieving drought tolerance.

The central principle involved in ABA signaling is the inhibition of phosphatase activity of PP2C by ABA bound PYR/PYL/RCAR receptors. PYR/PYL/RCAR-PP2C-SnRK2 pathway is highly redundant, suggesting that combination of various members of the complex have distinctive roles in ABA signaling among different cells, tissues and organs. To elucidate the finer details of ABA signal transduction, further functional analyses of each combination are required. Many direct substrates or interacting proteins of PP2C or SnRK2 have been discovered and many others need to be unraveled. To fully understand the ABA signaling in plants, screening of SnRK2 substrates is still a major challenge. Global changes in phosphopeptides in WT and SnrK2.2/2.3/2.6 triple mutant seedlings in response to ABA treatment have been analyzed by Wang et al. (2013) with the use of qualitative phosphoproteomics. It was observed that ABA could increase the phorphorylation of 166 peptides and decrease the phosphorylation of 117 peptides in WT seedlings, whereas 84 of the 166 peptides representing 58 proteins could not be phosphorylated by the same. These results shed new light on the role of the SnRK2 protein kinases and on the downstream effectors of ABA action.

Once a plant detects the onset of stress, TFs characteristically respond by inducing the expression of a cascade of downstream targets. However, their activation is in part also dependent on their chromatin structure, which is largely determined by epigenetic means (Boyko and Kovalchuk, 2008; Bilichak et al., 2012). There exists a very clear time lag between transcriptional activation, DNA methylation and histone modification. Song et al. (2012) have shown in soybean that a promoter was demethylated within 1 h of the imposition of salt stress. However, there was no evidence for the TF's activation before 3 h. Similar time lags have been noted for the building of H3K4me3 within the coding regions of *A. thaliana* drought related genes *rd29A* and *rap2.4* during stress (Kim et al., 2008) suggesting the events of transcriptional activation, DNA methylation and histone modification are not simultaneous.

Future perspectives

A good number of genes have been documented which encode for enzymes conferring tolerance to abiotic stress. Transgenics of rice, oat, *etc*., have been engineered to express osmo-protectants such as glycine betaine, proline, polyamines and several other genes encoding enzymes involved in abiotic stress responses (Amudha and Balasubramani, 2011). Constitutive expression of group3 LEA proteins has resulted in improved growth characteristics and drought tolerance. HVA1 (group3 LEA) gene from barley (Xu et al., 1996) a PMA1959 (group1 LEA) from wheat (Cheng et al., 2002) have been reported to confer drought and salt stress tolerance.

To eliminate adverse effects of ROS generated under environmental stress, detoxifying genes have been incorporated in plants. These genes express anti-oxidant proteins like glutathione peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase, *etc*. that scavenge the ROS thereby enhancing tolerance to oxidative stress. Over-expression of superoxide dismutase (SOD), particularly in mitochondria and chloroplast, has been reported to have a profound effect on the relative tolerance of transgenic plants to stress conditions. Apart from this, transformation of plants with TFs like MYC, MYB, bZIP has also been proved to show a dramatic improvement in the performance of plants (Amudha and Balasubramani, 2011).

Early approaches were targeted on stress responsive genes for creation of transgenic plants. But continuous expression of stress inducible genes is a highly energy intensive process which casts a heavy burden on the plant machinery subsequently compromising the quality and quantity of the yield. This can be overcome by designing an inducible promoter where expression is under the control of an inducer. With improvements in sequencing techniques, advent of whole genome sequencing and construction of databases like PLACE, TRANSFAC, PlantCARE, JASPER, *etc*., the task of promoter designing and analysis has become easier. Strategies have been developed to design tissue specific promoters, which direct gene expression in particular tissues only (Mehrotra et al., 2011).

Synthetic promoters have found great application in plant biotechnology particularly in studies related to abiotic stress responses. A minimal expression cassette (Pmec) and transcriptional activation module (TAM) were designed which contain many *cis* motifs responding to biotic and abiotic stresses and growth and development (Sawant et al., 1999). These motifs show not only self-activation but also synergistic activation. Using this concept, a synthetic bidirectional expression module was made by arranging the positions of Pmec and TAM, Pmec being placed on the 5′ and 3′ end of TAM (Chaturvedi et al., 2006).

More recently, synthetic promoters have been produced for stringent regulation. However, the challenge of homology based gene silencing (HBGS) is yet to be met. On similar lines, bidirectional promoters have been developed which not only overcome the risk posed by synthetic promoters but also reduce the quantity of foreign DNA being introduced in the organism (Mehrotra et al., 2011).

Conclusion

Advancements in systemic computational and molecular biology have led to a deeper understanding of the underlying mechanisms of the involvement of ABA in stress tolerance; though a lot is yet to be discovered. The physiological changes that lead to adaptive responses are far more advanced in plants as compared to animals. An understanding of ABA signal transduction pathways and epigenetic modifications can be further exploited to produce better transgenic plants that can withstand adverse climatic conditions. Genetic regulation through *cis* engineering can be achieved by designing promoters targeted at tissue specific expression through which plant dynamics can be further explored.

Acknowledgements

This work is supported by the project of Dr. Sandhya Mehrotra and Dr. Rajesh Mehrotra funded by University Grants Commission, New Delhi, Government of India. The authors are grateful to the administration of Birla Institute of Technology and Sciences, Pilani, Rajasthan, for providing logistic support. The authors are also thankful to UGC/BSR for providing financial support to Purva Bhalothia.

References

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Shinozaki KY. *Arabidopsis* AtM.Y.C2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 2003;15:63–78.

Ahmad MR, Saxena PM, Amla DV, Shyam R. Effect of abscisic acid on the blue green algae *Anacystis nidulans* and *Nostoc muscorum*. Beitr Biol Pflanzen 1978;54:33–40.

Amudha J, Balasubramani G. Recent molecular advances to combat abiotic stress tolerance in crop plants. Biotechnol Mol Biol Rev 2011;6:31–58.

Anderberg RJ, Walker-Simmons MK. Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinase. Proc Natl Acad Sci U S A 1992;89:10183–90.

Antoni R, Gonzalez-Guzman M, Rodriguez L, Peirats-Llobet M, Pizzio AG, Fernandez AM, et al. Pyrabactin resistance1-likeplays an important role for the regulation of abscisic acid signaling in root. Plant Physiol 2013;161:931–40.

Aufsatz W, Mette MF, Van der Winden J, Matzke M, Matzke AJ. HDA6, a putative histone deacetylase needed to enhance DNA methylation induced by double-stranded RNA. EMBO J 2002;21:6832–40.

Ballester DG, Pollock SV, Pootakham W, Grossman AR. The central role of a SnRK2 kinase in sulfur deprivation responses. Plant Physiol 2008;147:216–20.

Bilichak A, Ilnystkyy Y, Hollunder J, Kovalchuk I. The progeny of *Arabidopsis thaliana* plants exposed to salt exhibit changes in DNA methylation, histone modifications and gene expression. PLoS ONE 2012:7e30515.

Bond DM, Finnegan EJ. Passing the message on: inheritance of epigenetic traits. Trends Plant Sci 2007;12:211–20.

Borsani O, Zhu J, Verslues PE, Sunkar R, Zhu JK. Endogenous siRNAs derived from a pair of natural *cis*-antisense transcripts regulate salt tolerance in *Arabidopsis*. Cell 2005;123:1279–80.

Boudsocq M, Barbier-Brygoo H, Lauriere C. Identification of nine SNF1-related protein kinase 2 activated by hyperosmotic and saline stresses in *Arabidopsis thaliana*. J Biol Chem 2004;279:41758–60.

Boyko A, Kovalchuk I. Epigenetic control of plant stress response. Environ Mol Mutagen 2008;49:61–72.

Chan SW, Zilberman D, Xie Z, Johansen LK, Carrington JC, Jacobsen SE. RNA silencing genes control de novo DNA methylation. Science 2004;303:13–36.

Chaturvedi CP, Sawant SV, Kiran K, Mehrotra R, Lodhi N, Ansari SA, et al. Analysis of polarity in the expression from a multifactorial bidirectional promoter designed for high-level expression of transgenes in plants. J Biotechnol 2006;123:1–12.

Chen LT, Luo M, Wang YY, Wu KQ. Involvement of *Arabidopsis* histone deacetylase HDA6 in ABA and salt stress response. J Exp Bot 2010;61:3345–50.

Cheng Z, Targolli J, Huang X, Wu R. Wheat LEA genes, PMA80 and PMA1959, enhance dehydration tolerance of transgenic rice (*Oryza sativa* L.). Mol Breed 2002;10:71–82.

Chinnusamy V, Zhu JK. Epigenetic regulation of stress responses in plants. Curr Opin Plant Biol 2009;12:133–40.

Chinnusamy V, Gong Z, Zhu JK. Abscisic acid-mediated epigenetic processes in plant development and stress responses. J Integr Plant Biol 2008;50:1187–90.

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 2010;61:651–60.

Demetriou K, Kapazoglou A, Tondelli A, Francia E, Stanca MA, Bladenopoulos K, et al. Epigenetic chromatin modifiers in barley: I. Cloning, mapping and expression analysis of the plant specific *HD2* family of histone deacetylases from barley, during seed development and after hormonal treatment. Physiol Plant 2009;136:358–60.

Dijk van K, Ding Y, Malkaram S, Riethoven JJM, Liu R, Yang J, et al. Dynamic changes in genome wide histone H3 lysine 4 methylation patterns in response to dehydration stress in *Arabidopsis thaliana*. BMC Plant Biol 2010;10:238–40.

Finnegan EJ, Dennis ES. Isolation and identification by sequence homology of a putative cytosine methyltransferase from *Arabidopsis thaliana*. Nucleic Acids Res 1993;21:2383–90.

Finnegan EJ, Peacock WJ, Dennis ES. Reduced DNA methylation in *Arabidopsis* results in abnormal plant development. Proc Natl Acad Sci USA 1996;93:8449–50.

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G. Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 2007;76:1145–50.

Foster R, Izawa T, Chua NH. Plant bZIP proteins gather at ACGT elements. FASEB J 1994;8:192–200.

Fu W, Wu K, Duan J. Sequence and expression analysis of histone deacetylases in rice. Biochim Biophys Acta 2007;356:843–50.

Fujii H, Zhu JK. *Arabidopsis* mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction and stress. Proc Natl Acad Sci USA 2009;19:8380–90.

Fujita Y, Nakashima K, Yoshida T, Katagiri T, Kidokoro S, Kanamori N, et al. Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in *Arabidopsis*. Plant Cell Physiol 2009;50:2123–30.

Fujita Y, Yoshida T, Shinozaki KY. Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. Physiol Plant 2013;147:15–27.

Gao SQ, Chen M, Xu ZS, Zhao CP, Li L, Xu H, et al. The soybean GmbZIP1 transcription factor enhances multiple abiotic stress tolerances in transgenic plants. Plant Mol Biol 2011;75:537–40.

Gonzalez-Guzman M, Apostolova N, Belles JM, Barrero JM, Piqueras P, Ponce MR, et al. The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin to abscisic aldehyde. Plant Cell 2002;14:1833–40.

Han SK, Sang Y, Rodrigues A, Wu MF, Rodriguez PL, Wagner D. The SWI2/SNF2 chromatin remodeling ATPase BRAHMA represses abscisic acid responses in the absence of the stress stimulus in *Arabidopsis*. Plant Cell 2012;24:4892–900.

Hashida SN, Uchiyama T, Martin C, Kishima Y, Sano Y, Mikami T. The temperature-dependent change in methylation of the *Antirrhinum* transposon Tam3 is controlled by the activity of its transposase. Plant Cell 2006;18:104–10.

Henderson IR, Jacobsen SE. Epigenetic inheritance in plants. Nature 2007;447:418–20.

Hirochika H, Okamoto H, Kakutani T. Silencing of retrotransposons in *Arabidopsis* and reactivation by the *ddm1* mutation. Plant Cell 2000;12:357–60.

Hossain MA, Lee Y, Cho JI, Ahn CH, Lee SK, Jeon JS, et al. The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. Plant Mol Biol 2010;72:557–60.

Hrabak EM, Chan CWM, Gribskov M, Harper JF, Choi JH, Halford N, et al. *Arabidopsis* CDPK-SnRK superfamily of protein kinases. Plant Physiol 2003;132:666–70.

Huai J, Wang M, He J, Zheng J, Dong Z, Lv H, et al. Cloning and characterization of the SnRK2 gene family from *Zea mays*. Plant Cell Rep 2008;12:1861–70.

Hubbard KE, Nishimura N, Hitomi K, Getzoff ED, Schroeder JI. Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging questions. Genes Dev 2010;24:1695–700.

Jeddeloh JA, Stokes TL, Richards EJ. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 1999;22:94–7.

Kelner A, Pekala A, Kaczanowski S, Muszynska G, Hardie DG, Dobrowolska G. Biochemical characterization of the *Nicotiana tabacum* 42-kD protein kinase activated by osmotic stress. Plant Physiol 2004;136:3255–60.

Kim JM, To TK, Ishida J, Morosawa T, Kawashima M, Matsui A, et al. Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. Plant Cell Physiol 2008;49:1580–90.

Kim JM, To TK, Nishioka T, Seki M. Chromatin regulation function in plant abiotic stress responses. Plant Cell Environ 2010;33:604–10.

Kim JY, Park SJ, Jang B, Jung C-H, Ahn SJ, Goh CH, et al. Functional characterization of a glycine-rich RNA-binding protein 2 in *Arabidopsis thaliana* under abiotic stress conditions. Plant J 2007;50:439–40.

Kobayashi Y, Yamamoto S, Minami H, Kagaya Hattori T. Differential activation of the rice sucrose non fermenting1-related protein kinase 2 family by hyper-osmotic stress and abscisic acid. Plant Cell 2004;16:1163–70.

Kulik A, Wawer I, Krzywinska E, Bucholc M, Dobrowolska G. SnRK2 protein kinases-key regulators of plant response to abiotic stresses. OMICS: J Integr Biol 2011;15:859–60.

Kuromori T, Shinozaki K. ABA transport factors found in *Arabidopsis* ABC transporters. Plant Signal Behav 2010;5:1124–30.

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, et al. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO J 2003;22:2623–30.

Kwon CS, Lee D, Choi G, Chung W. Histone occupancy-dependent and -independent removal of H3K27 trimethylation at cold-responsive genes in *Arabidopsis*. Plant J 2009;60:112–20.

Lambermon MHL, Simpson GG, Kirk DAW, Hemmings-Mieszczak M, Klahre U, Filipowicz W. UBP1, a novel hn RNP-like protein that functions at multiple steps of higher plant nuclear pre-mRNA maturation. EMBO J 2000;19:1638–40.

Lambermon MH, Fu Y, Wieczorek Kirk DA, Dupasquier M, Filipowicz W, Lorković ZJ. UBA1 and UBA2, two proteins that interact with UBP1, a multifunctional effector of pre-mRNA maturation in plants. Mol Cell Biol 2002;22:4346–50.

Lee BH, Kapoor A, Zhu J, Zhu JK. STABILIZED1, a stress-upregulated nuclear protein, is required for pre mRNA splicing, mRNA turnover, and stress tolerance in *Arabidopsis*. Plant Cell 2006a;18:1736–40.

Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, et al. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. Cell 2006b;126:1109–10.

Leung J, Merlot S, Giraudat J. The *Arabidopsis* abscisic acid-insensitive2 (*ABI2*) and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell 1997;9:759–60.

Li G, Bishop KJ, Chandrashekharan MB, Hall TC. β-Phaseolin gene activation is a two-step process: PvALF-mediated chromatin modifications followed by abscisic acid-mediated gene activation. Proc Natl Acad Sci USA 1999;96:7104–10.

Li J, Wang XQ, Watson MB, Assmann SM. Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. Science 2000;287:300–10.

Li J, Kinoshita T, Pandey S, Ng CK, Gygi SP, Shimazaki K, et al. Modulation of an RNA-binding protein by abscisic-acid-activated protein kinase. Nature 2002;418:793–800.

Li LB, Zhang YR, Liu KC, Ni ZF, Fang ZJ, Sun QX, et al. Identification and bioinformatics analysis of SnRK2 and CIPK family genes in sorghum. Agric Sci China 2010;9:19–30.

Liu MS, Chien CT, Lin TP. Constitutive components and induced gene expression are involved in the desiccation tolerance of *Selaginella tamariscina*. Plant Cell Physiol 2008;49:653–60.

Liu Y. Roles of mitogen-activated protein kinase cascades in ABA signaling. Plant Cell Rep 2012;31:1–12.

Liu Y, Koornneef M, Soppe WJJ. The absence of histone H2B monoubiquitination in the *Arabidopsis* hub1 (rdo4) mutant reveals *a role for chromatin* remodeling in seed dormancy. Plant Cell 2007;19:433–40.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, et al. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 2009;324:1064–70.

Madlung A, Comai L. The effect of stress on genome regulation and structure. Ann Bot 2004;94:481–90.

Mao X, Zhang H, Tian S, Chang X, Jing R. TaSnRK2.4, an SNF1-type serine/threonine protein kinase of wheat (*Tricitum aestivum* L.), confers enhanced multistress tolerance in *Arabidopsis*. J Exp Bot 2010;61:683–90.

Mehrotra R, Mehrotra S. Promoter activation by ACGT in response to salicylic and abscisic acids is differentially regulated by the spacing between two copies of the motif. J Plant Physiol 2010;167:1214–20.

Mehrotra R, Gupta G, Sethi R, Bhalothia P, Kumar N, Mehrotra S. Designer promoter: an artwork of *cis* engineering. Plant Mol Biol 2011;75:527–30.

Mehrotra R, Sethi S, Zutshi I, Bhalothia P, Mehrotra S. Patterns and evolution of ACGT repeat *cis*-element landscape across four plant genomes. BMC Genomics 2013;14:203–10.

Mehrotra R, Yadav A, Bhalotia P, Karan R, Mehrotra S. Evidence for directed evolution of larger size motif in *Arabidopsis thaliana* genome. Sci World J 2012., http://dx.doi.org/10.1100/2012/983528.

Meskiene I, Boegre L, Glaser W, Balog J, Brandstoetter M, Zwerger TK, et al. MP2C, a plant protein phosphatase 2C, functions as a negative regulator of mitogen-activated protein kinase pathways in yeast and plants. Proc Natl Acad Sci USA 1998;95:1938–40.

Mizoguchi M, Umezawa T, Nakashima K, Kidokoro S, Takasaki H, Fujita Y, et al. Two closely related subclass II SnRK2 protein kinases cooperatively regulate drought-inducible gene expression. Plant Cell Physiol 2010;51:842–50.

Mlynárová L, Nap JP, Bisseling T. The SWI/SNF chromatin-remodeling gene *AtCHR12* mediates temporary growth arrest in *Arabidopsis thaliana* upon perceiving environmental stress. Plant J 2007;51:874–80.

Monks DE, Aghoram K, Courtney PD, DeWald DB, Dewey RE. Hyperosmotic stress induces the rapid phosphorylation of a soybean phosphatidylinositol transfer protein homolog through activation of the protein kinases SPK1 and SPK2. Plant Cell 2001;13:1205–10.

Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, et al. Three *Arabidopsis* SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2. 6/OST1 and SRK2I/SnRK2. 3, involved in ABA signaling are essential for the control of seed development and dormancy. Plant Cell Physiol 2009a;50:1345–50.

Nakashima K, Ito Y, Yamaguchi-Shinozaki K. Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiol 2009b;149:88–95.

Nambara E, Marion-Poll A. Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 2005;56:165–70.

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, et al. Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. Science 2009;326:1373–80.

Nishimura N, Sarkeshik A, Nito K, Park S, Wang A, Carvalho P, et al. PYR/PYL/RCAR family members are major *in-vivo* ABI1 protein phosphatase 2C-interacting proteins in *Arabidopsis*. Plant J 2010;61:290–300.

Ogas J, Kaufmann S, Henderson J, Somerville C. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc Natl Acad Sci U S A 1999;96:13839–40.

Orellana S, Yañez M, Espinoza A, Verdugo I, González E, Lara SR, et al. The transcription factor SlAREB1 confers drought, salt stress tolerance and regulates biotic and abiotic stress-related genes in tomato. Plant Cell Environ 2010;33:2191–200.

Park SY, Fung P, Nishimura N, Jensen DR, Hiroaki F, Zhao Y, et al. Abscisic acid inhibits PP2Cs via the PYR/PYL family of ABA binding START proteins. Science 2009;324:1068–70.

Pawlak S, Deckert J. Histone modifications under environmental stress. Biol Lett 2007;44:65–73.

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, et al. *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 2000;290:2105–10.

Rio G, Gagete AP, Castillo J, Berbel A, Franco L, Rodrigo I. Abscisic acid and desiccation-dependent expression of a novel putative *SNF5*-type chromatin-remodeling gene in *Pisum sativum*. Plant Physiol Biochem 2007;45:427–30.

Rodriguez PL, Benning G, Grill E. ABI2, a second protein phosphatase 2C involved in abscisic acid signal transduction in *Arabidopsis*. FEBS Lett 1998;421:185–90.

Saez A, Apostolova N, Gonzalez-Guzman M, Gonzalez-Garcia MP, Nicolas C, Lorenzo O, et al. Gain of function and loss of function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signaling. Plant J 2004;37:354–60.

Saez A, Rodrigues A, Santiago J, Rubio S, Rodriguez PL. HAB1-SWI3B interaction reveals a link between abscisic acid signaling and putative SWI/SNF chromatin-remodeling complexes in *Arabidopsis*. Plant Cell 2008;20:2972–80.

Saradhi PP, Suzuki I, Katoh A, Sakamoto A, Sharmila P, Shi DJ, et al. Protection against the photo-induced inactivation of the photosystem II complex by abscisic acid. Plant Cell Environ 2000;23:711–20.

Sarnowski TJ, Swiezewski S, Pawlikowska K, Kaczanowski S, Jerzmanowski A. AtSWI3B, an *Arabidopsis* homolog of SWI3, a core subunit of yeast Swi/Snf chromatin remodeling complex, interacts with FCA, a regulator of flowering time. Nucleic Acids Res 2002;30:3412–20.

Sawant SV, Singh PK, Gupta SK, Madanala R, Tuli R. Conserved nucleotide sequence in highly expressed genes in plants. J Genet 1999;78:1–8.

Schweighofer A, Hirt H, Meskiene I. Plant PP2C phosphatases: emerging functions in stress signaling. Trends Plant Sci 2004;9:236–40.

Seiler C, Harshavardhan VT, Rajesh K, Reddy PS, Strickert M, Rolletschek H, et al. ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. J Exp Bot 2011;62:2615–20.

Seo M, Koshiba T. Complex regulation of ABA biosynthesis in plants. Trends Plant Sci 2002;7:41–8.

Seo M, Koshiba T. Transport of ABA from the site of biosynthesis to the site of action. J Plant Res 2011;124:501–10.

Servet C, Benhamed M, Latrasse D, Kim W, Delarue M, Zhou DX. Characterization of a phosphatase 2C protein as an interacting partner of the histone acetyltransferase GCN5 in *Arabidopsis*. Biochim Biophys Acta 2008;1779:376–80.

Shen QJ, Casaretto JA, Zhang P, Ho THD. Functional definition of ABA-response complexes: the promoter units necessary and sufficient for ABA induction of gene expression in barley (*Hordeum vulgare* L.). Plant Mol Biol 2004;54:111–20.

Shinozaki K, Yamaguchi-Shinozaki K, Seki M. Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 2003;6:410–20.

Sokol A, Kwiatkowska A, Jerzmanowski A, Prymakowska-Bosak M. Up-regulation of stress-inducible genes in tobacco and *Arabidopsis* cells in response to abiotic stresses and ABA treatment correlates with dynamic changes in histone H3 and H4 modifications. Planta 2007;227:245–50.

Song CP, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, et al. Role of an *Arabidopsis* AP2/EREBP-type transcription al repressor in abscisic acid and drought stress responses. Plant Cell 2005;17:2384–90.

Song Y, Ji D, Li S, Wang P, Li Q, Xiang F. The dynamic changes of DNA methylation and histone modifications of salt responsive transcription factor genes in Soybean. PLoS ONE 2012;7:1–11.

Sridha S, Wu K. Identification of *AtHD2C* as a novel regulator of abscisic acid responses in *Arabidopsis*. Plant J 2006;46:124–30.

Stockinger EJ, Mao Y, Regier MK, Triezenberg SJ, Thomashow MF. Transcriptional adaptor and histone acetyl-transferase proteins in *Arabidopsis* and their interactions with CBF1, a transcriptional activator involved in cold-regulated gene expression. Nucleic Acids Res 2001;29:1524–30.

Sunkar R, Zhu JK. Novel and stress-regulated micro RNAs and other small RNAs from *Arabidopsis*. Plant Cell 2004;16:2001–10.

Suzuki M, Ketterling MG, McCarty DR. Quantitative statistical analysis of *cis*-regulatory sequences in ABA/VP1- and CBF/DREB1-regulated genes of *Arabidopsis*. Plant Physiol 2005;139:437–40.

Tan BC, Schwartz SH, Zeevaart JA, McCarty DR. Genetic control of abscisic acid biosynthesis in maize. Proc Natl Acad Sci U S A 1997;94:12235–40.

Turner BM. Cellular memory and the histone code. Cell 2002;111:285–90.

Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K, et al. Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol 2010;51:1821–30.

Vlachonasios KE, Thomashow MF, Triezenberg SJ. Disruption mutations of *ADA2b* and *GCN5* transcriptional adaptor genes dramatically affect *Arabidopsis* growth, development, and gene expression. Plant Cell 2003;15:626–30.

Wang P, Xue L, Batelli G, Lee S, Hou Y, Van Oosten MJ, et al. Quantitative phosphoproteomics identifies SnRK2 protein kinase substrates and reveals the effectors of abscisic acid action. Proc Natl Acad Sci U S A 2013;110:11205–10.

Waterborg JH, Harrington RE, Winicov I. Differential histone acetylation in alfalfa (*Medicago sativa*) due to growth in NaCl: responses in salt stressed and salt tolerant callus cultures. Plant Physiol 1989;90:237–40.

Wolfram H. The evolution of abscisic acid (ABA) and ABA function in lower plants, fungi and lichen. Funct Plant Biol 2010;37:806–10.

Xu D, Duan X, Wang B, Hong B, Ho THD, Wu R. Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 1996;110:249–50.

Xu GP, Way HM, Richardson T, Drenth J, Joyce PA, McIntyre CL. Overexpression of *TaNAC69* leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. Mol Plant 2011;4:697–700.

Yoshida K, Iigarashi E, Mukai M, Hirata K, Miyamoto K. Induction of tolerance to oxidative stress in the green alga, *Chlamydomonas reinhardtii*, by abscisic acid. Plant Cell Environ 2003;26:451–60.

Yoshida R, Hobo T, Ichimura K, Mizoguchi T, Takahashi F, Aronso J, et al. ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in *Arabidopsis*. Plant Cell Physiol 2002;43:1473–80.

Yoshida R, Umezawa T, Mizoguchi T, Takahashi S, Takahashi F, Shinozaki K. The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates ABA and osmotic stress signals controlling stomatal closure in *Arabidopsis*. J Biol Chem 2006;281:5310–20.

Yoshida T, Fujita Y, Sayama H, Kidokoro S, Maruyama K, Mizoi J, et al. AREB1, AREB2 and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. Plant J 2010;61:672–80.

Zhang H, Mao X, Wang C, Jing R. Over-expression of a common wheat gene TaSnRK2.8 enhances tolerance to drought, salt and low temperature in *Arabidopsis*. PLoS ONE 2010;5:16041–50.

Zhang K, Gan SS. An abscisic acid-AtNAP transcription factor-SAG113 protein phosphatase 2C regulatory chain for controlling dehydration in senescing *Arabidopsis* leaves. Plant Physiol 2012;158:961–70.

Zhang K, Sridhar VV, Zhu J, Kapoor A, Zhu JK. Distinctive core histone post-translational modification patterns in *Arabidopsis thaliana*. PLoS ONE 2007., http://dx.doi.org/10.1371/journal.pone.0001210.

Zhang X, Wollenweber B, Jiang D, Liu F, Zhao J. Water deficit and heat shock effects on photosynthesis of a transgenic *Arabidopsis thaliana* constitutively expressing *ABP9*, a bZIP transcription factor. J Exp Bot 2008;59:839–40.

Zhou C, Miki B, Wu K. CHB2, a member of the SWI3 gene family, is a global regulator in *Arabidopsis*. Plant Mol Biol 2003;52:1125–30.

Zhou C, Zhang L, Duan J, Miki B, Wu K. Histone deacetylase 19 is involved in jasmonic acid and ethylene signaling of pathogen response in *Arabidopsis*. Plant Cell 2005;17:1196–200.

Zhou JL, Wang XF, He K, Charron JBF, Elling AA, Deng XW. Genome-wide profiling of histone H3 lysine acetylation and dimethylation in *Arabidopsis* reveals correlation between multiple histone marks and gene expression. Plant Mol Biol 2010;72:585–90.

Zhu J, Dong HC, Zhu JK. Interplay between cold-responsive gene regulation, metabolism and RNA processing during plant cold acclimation. Curr Opin Plant Biol 2007;10:290–300.

Zhu J, Jeong J, Zhu Y, Sokolchik I, Miyazaki S, Zhu JK, et al. Involvement of *Arabidopsis* HOS15 in histone deacetylation and cold tolerance. Proc Natl Acad Sci U S A 2008;105:4945–50.