CHAPTER 9

ABSCISIC ACID IN PLANT RESPONSE AND ADAPTATION TO DROUGHT AND SALT STRESS

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Abstract: The plant stress hormone abscisic acid (ABA) plays several critical roles in plant response to stress and stress tolerance. ABA is well studied for its roles in the activation of stress-responsive genes and the regulation of guard cell movement. More recently, ABA has also been demonstrated to regulate root adaptation to drought stress. To date, limited success has been achieved in regulating plant ABA action for increasing plant drought tolerance. Revealing the mechanisms of ABA action in stress adaptation will further help the development of hardy crop plants

Keywords: ABA; drought stress; drought tolerance; salt stress; root development

1. INTRODUCTION

Abscisic acid (ABA) is one of the five classic growth regulators that play critical roles in plant growth and development. The chemical identity of this sesquiterpene (C_{15}) was established in the 1960's through the landmark work of several research groups while identifying the compounds responsible for leaf abscission and bud dormancy (reviewed in Zeevaart and Creelman, 1988). It is now known that ABA does not play major roles in these processes for which it was named. ABA does have essential roles in seed maturation and dormancy. However, the critical roles it plays during plant response to environmental stress won its reputation as a 'stress hormone' and as such ABA is central to any discussion of plant adaptation to adverse environmental conditions. This chapter will focus on the role of ABA in plant response and adaptation to drought and salt stresses. Other aspects of ABA biology such as ABA biosynthesis and ABA signal transduction will be discussed only in the context of their involvement in stress response. ABA biosynthesis (Schwartz et al., 2003; Nambara and Marion-Poll, 2005; Taylor et al., 2005) and ABA signal transduction (Leung and Giraudat, 1998; Finkelstein and Rock, 2002; Himmelbach et al., 2003; Christmann et al., 2003; Xie et al., 2006) are discussed in more detail in these recent reviews.

M.A. Jenks et al. (eds.), Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops, 193–221.

2. MAJOR PHYSIOLOGICAL PROCESSES REGULATED BY ABA

The function of ABA in various physiological processes was mainly inferred from studies using exogenous ABA as well as plant mutants defective either in ABA biosynthesis or responses. Other approaches such as examining the spatial and temporal localization of ABA were sometimes also used to speculate about cellular processes that may be regulated by ABA. These approaches may not be able to uncover all functions of ABA. Certain cellular activities may only need such a low level of ABA that even ABA deficient or insensitive mutants can satisfy the needs since all these mutants are leaky (i.e., they either still produce a limited amount of ABA or show some response to ABA). Furthermore, cellular machinery tends to be more sensitive to ABA in case of ABA deficiency (e.g., Xiong et al., 2001). The negative impacts of ABA deficiency in these biosynthetic mutants might have been significantly alleviated. It is thus likely that there are still undiscovered cellular processes that require ABA or that ABA may be essential for plant growth and development. In the latter scenario, complete lack of ABA or ABA signaling (e.g., loss of all ABA receptors) would be lethal. This perhaps is one of the reasons why the ABA receptor(s) was not identified in genetic screens. The significantly reduced vigor of all ABA deficient mutants under normal unstressed conditions also implies that ABA is required for certain cellular processes that may not be related to stress adaptation. Here we will briefly discuss some of the physiological processes that are regulated by ABA.

ABA is widely known for its involvement in seed maturation and dormancy. Seed maturation requires the synthesis of storage proteins and the preparation for desiccation tolerance so that the embryo will remain viable under extreme dehydration conditions as seen with dry seeds (McCarty, 1995; Finkelstein and Rock, 2002; Bentsink and Koornneerf, 2002). ABA activates genes involved in both processes, although seed maturation may require an even lower level of ABA than do dormancy initiation and maintenance. The essentiality of ABA in seed development is witnessed by the observation that once ABA level was suppressed immunologically the embryos will not be able to acquire desiccation tolerance and thus are not viable when desiccated (Phillips et al., 1997). On the other hand, the role of ABA in seed dormancy can readily be seen by the pre-mature (viviparous) germination of maize ABA deficient mutants and by the inhibition of exogenous ABA on seed germination.

Another developmental process that is potentially affected by ABA is the vegetative to reproductive phase transition. It is well known that plants under moderate drought stress will flower earlier (a ‘drought escape’ strategy). However, the relationship of flowering time with drought susceptibility is complex. When using the carbon isotope discrimination ratio ($\delta^{13}C$) as an index of water use efficiency (WUE), it was found that $\delta^{13}C$ is positively correlated with flowering time in several plant species (Araus et al., 2002; Farquhar et al., 1989). However, it is suspicious that late-flowering ecotypes really have a high WUE. Generally, plants (and in particular ecotypes within a plant species, such as Arabidopsis) that are late flowering usually grow more slowly than rapidly flowering plants.

Slow-growing plants consume less water at a given period of time. Since drought stress is well known to promote flowering, one may expect that ABA would promote flowering too. However, Arabidopsis ABA-deficient mutant *aba1* and insensitive mutant *abi1* (Landsberg background) tend to flower earlier under short-day conditions (Martinez-Zapater et al., 1994) while the *aba1/los6* mutant (C24 background) flowered earlier both under long-day and short-day conditions (Xiong et al., 2002). On the other hand, ABA-hypersensitive mutants are either early flowering (*sad1; abh1-2*, C24 ecotype; *chl1*, L. Xiong, unpublished) or late flowering (*hyl1*, Lu and Fedoroff, 2000; *era1*, *fry1*, L. Xiong, unpublished). It is not known whether or not the flowering time changes in these mutants have to do with altered ABA responses. Recently, it was reported that prolonged exogenous ABA treatment in Arabidopsis up-regulates the flowering-suppressing gene *FLC* and delays flowering (Razem et al., 2006). Yet, short-term ABA treatments do not affect *FLC* gene expression or flowering time in Arabidopsis (H. Chen and L. Xiong, unpublished). Nonetheless, the facts that the flowering time regulator FCA can bind to exogenous ABA (Razem et al., 2006) and that ABA is detected in floral primordia (e.g. Peng et al., 2006) suggest that ABA may affect floral development and flowering time regulation.

In vegetative tissues, ABA plays a role in several developmental processes. It was noted that ABA may affect root hair cell patterning through the regulation of *GLABRA (GL) 2* and other cell patterning genes (van Hengel et al., 2004). The role of ABA in root hair patterning and root hair growth may relate to the observation that drought stress and ABA treatments result in abnormal root hair development (Schnall and Quatrano, 1992) (see below). While studying plant response to drought stress, we found that drought stress inhibits the elongation of lateral roots both in artificial growth media and in soil. ABA has similar effects in inhibiting lateral root elongation both in Arabidopsis and in crop plants (Xiong et al. 2006; L. Xiong, unpublished). Observations on osmotic stress and ABA inhibition of lateral root growth were also reported under different experimental conditions (De Smet et al., 2003; Deak and Malamy, 2005). Thus, it is likely that this rhizogenesis process may represent an adaptive response to drought stress, as was demonstrated in recent genetic studies (Xiong et al., 2006; see below).

ABA has also been implicated in plant response to wounding, ozone, light, and pathogens. This may partly result from the fact that these various stress-signaling pathways may share some common components. Some of these stresses may also give rise to osmotic or desiccation stress that indirectly activates the ABA signaling pathways. For example, the hypersensitive response of pathogenesis in plant cells could result in a significant osmotic stress (Wright and Beattie, 2004). On the other hand, the antagonistic role of ABA on disease resistance may have to do with ABA suppression of salicylic acid, jasmonic acid and ethylene signaling (Mauch-Mani and Mauch, 2005).

Perhaps the best known and also the most studied process that ABA is involved in is plant response to abiotic stress such as drought, salt, and cold stress. These different stresses do share some common features. For example, they all induce

dehydration stress to the plant cells. Accordingly, these abiotic stresses all activate ABA biosynthesis to various extents and induce a common set of stress responsive genes.

3. REGULATION OF CELLULAR ABA LEVELS AND THE EXPRESSION OF STRESS-RESPONSIVE GENES

The magnitude of cellular response to ABA is determined by ABA level and ABA sensitivity, and both aspects may involve complex signal transduction processes. Cellular ABA levels are dynamically regulated through biosynthesis/degradation, conjugation/de-conjugation, compartmentalization, and transport. Signal transduction from ABA perception to gene activation involves complex regulatory circuits (network) and multiple components. Here we briefly introduce some of the research on the regulation of ABA levels and ABA activated gene expression. ABA signal transduction will not be discussed here.

3.1. ABA Biosynthesis and Catabolism

In higher plants, ABA is mainly produced by the cleavage of a C_{40} carotenoid precursor that initially occurs in the plastids but the final steps of the pathway occur in the cytosol. This 'indirect' pathway is now well understood and the major enzymes catalyzing these reactions were identified (Schwartz et al., 2003; Nambara and Marion-Poll, 2005). Several of the enzymes in this pathway are encoded by single copy genes in the Arabidopsis genome (e.g., *ZEP*, *ABA3* and perhaps *ABA2*). Surprisingly, null mutations in these single copy genes do not completely block ABA biosynthesis, implying that there are either certain shortcuts in the major pathway that can circumvent these steps or minor pathways that can still produce a limited amount of ABA.

ABA biosynthesis is regulated both by internal developmental cues as well as by environmental stresses. The regulation of the biosynthetic pathway largely occurs at the level of transcriptional regulation of the biosynthetic genes, although regulation at other levels is also possible (Xiong and Zhu, 2003). Furthermore, ABA can self-regulate its own biosynthesis in that all of the ABA biosynthetic genes can be up-regulated to various extents by ABA (Xiong and Zhu, 2003).

The temporal and spatial regulation of ABA biosynthesis constitutes another layer of control of ABA biosynthesis. The tissue-specific regulation of ABA biosynthesis is particularly interesting since it may reveal the sites of ABA action, although ABA and perhaps its precursors as well can be transported over long distance. Among the known ABA biosynthetic genes, several of them appear to be expressed ubiquitously in those major tissues examined (Xiong and Zhu, 2003). However, the short-chain dehydrogenase/reductase gene *ABA2* (González-Guzmán et al., 2002; Cheng et al., 2002) is mainly expressed in vascular tissues of roots, stems, and leaves (Cheng et al., 2002), suggesting that these places are probably the key places of ABA synthesis. Similar expression in vascular tissues and guard cells was also

reported for the ABA aldehyde oxidase 3 (*AAO3*) gene and protein (Koiwai et al., 2004). Using ABA-inducible promoters of the *RD29B* and *AtHB6* genes to drive the expression of the firefly luciferase reporter gene, Christmann et al. (2005) found that these promoters are mainly activated at vascular tissues and guard cells upon drought stress treatment, implying that these sites might be the sites of ABA biosynthesis or ABA response. To determine the actual sites of ABA synthesis and accumulation, more direct approaches to detect ABA *in situ* need to be developed.

ABA can be conjugated with glucose to produce ABA-glucose ester (ABA-GE). This conjugate may not have biological activities similar to ABA. Glycosylation of ABA could thus serve as a regulatory process to inactivate ABA. Nevertheless, ABA can be released from ABA-GE by the hydrolytic enzyme β-glucosidase. These extracellular enzymes in leaves were shown to be able to release ABA from ABA-GE transported from xylem sap (Dietz et al., 2000). The ratio of ABA-GE to free ABA in xylem sap is low, yet ABA-GE can account for the majority of total ABA in older leaves (Weiler, 1980). Like free ABA, the level of conjugated ABA also increases in response to drought and salt stress (see Sauter et al., 2002 and references therein). Thus, conjugated ABA is still originated from de novo biosynthesis and its level may be kept in balance with free ABA.

The significant amount of conjugated ABA accumulated under drought stress may serve important roles in guard cell regulation and stress adaptation. To understand the role of these conjugates in drought stress adaptation requires the identification of the specific glucosidase(s) that hydrolyze these conjugates. Genetic approaches to knock out or over-express these genes can then be used to ascertain the contribution of ABA-GE in stress response and stress tolerance. However, genes encoding ABA-GE glucosidases have not been identified. Plant genomes encode a family of β-glucosidase genes (about 50 in the Arabidopsis genome). It would be interesting to identify the particular glucosidases that regulate ABA levels during drought stress. Proteomics analysis of drought-treated plants often found that the level of β-glucosidase proteins increased (e.g., Riccardi et al., 1998). More alkaline pH in the xylem sap under drought stress may also increase the activity of these enzymes (Sauter et al., 2002). These drought and ABA up- regulated glucosidases are likely candidates for ABA-GE glucosidases.

Upon stress-relief, ABA level can quickly decrease to pre-stress level. Catabolism of ABA is enhanced by both stress-relief signal and by ABA itself. The major enzyme responsible for the early step of the catabolism is ABA 8-hydroxylase (e.g., Krochko et al., 1998). Genes encoding these enzymes were identified (Kushiro et al., 2004; Saito et al., 2004). Regulation of these genes may alter ABA levels and plant drought response.

3.2. Gene Gegulation by ABA

In the decade between late 80‘s to early 90‘s, the discovery that stress and ABA can up-regulate gene expression inspired a great interest in the isolation and characterization of these stress and ABA-responsive genes and their promoter *cis* elements

as well as transcription factors responsible for their activation (e.g., Skriver and Mundy, 1990; Finkelstein and Rock, 2002; Busk and Pages, 1998). To date, genome-wide microarray analysis has made possible to identify all the genes regulated by ABA. Genes regulated by ABA amounted to several hundreds to a few thousands in Arabidopsis dependent on the particular treatment conditions and definition of regulation. Many of these genes are also regulated by drought, salt and low temperature stresses. Thus, relatively few genes are specifically regulated by ABA, implying a common role of ABA in plant stress response. In Arabidopsis, up to 30 percent of the genes can be regulated by abiotic stress (Kreps et al., 2002). The large number of genes up-regulated under stress suggests that a significant re-programming of cellular activities occurs when plants encounter the stress challenge.

ABA-responsive genes fall into a diverse range of functional groups that are categorized in different ways (Ramanjulu and Bartels, 2002; Leung and Giraudat, 1998; Finkelstein and Rock, 2002, Bray, 1997). These may include genes encoding enzymes that function in the production of compatible solutes, antioxidants, and genes encoding peptides with unclear functions as well as genes encoding signal transduction components. Among them, two related groups of ABA regulated genes encoding peptides of unclear functions received intensive research. These are the *COR/RD/LT* group stress-responsive genes and the late embryogenesis abundant (LEA) genes. Although they are expressed at different developmental stages and in different tissues, they share many commonalities and have similar functions: to preserve the cells from dehydration damages. These genes may also be activated by similar mechanisms.

Many of these genes share similar regulatory elements in the promoter regions. Early work identified the ABA-responsive element, ABRE, in the EM genes (encoding LEA proteins) in wheat and rice as a core sequence containing ACGT. This *cis* element is responsible for ABA up-regulation of these EM genes (Marcottee et al., 1989; Guiltinan et al., 1990; Mundy et al., 1990). In addition, coupling elements work together with ABRE in conferring ABA response in these ABA responsive promoters (Shen and Ho, 1995). Other *cis* elements such as MYC and MYB binding sequences are found in other ABA-responsive genes (Shinozaki et al., 2003). In vegetative tissues several basic domain/Leu zipper (bZip) transcription factors (AREB/ABF) that bind to ABRE were later isolated and they confer ABA induction of many stress-responsive genes (reviewed in Finkelstein and Rock, 2002; Rock, 2000; Xie et al., 2005). In contrast, the AP2/ERF transcription factors DREB/CBF that bind to the DRE/CRT element are responsible for dehydration-induced gene expression (Shinozaki et al., 2003). Both the ABRE and DRE/CRT *cis* elements exist in the promoters of many stress- and ABA-responsive genes. Although DRE and ABRE elements have different core sequences and their own binding factors, both pathways collaboratively activate gene expression. Furthermore, stress induction of certain stress-responsive genes often requires ABA (Xiong et al., 2001; Kizis and Pages, 2002; Narusaka et al., 2003). Although the modes of action for many of the ABA and stress-responsive gene products are unknown, overexpression of these ABA-activated

genes or their upstream transcription factor genes was found to confer increased tolerance of the transgenic plants to drought and other abiotic stresses (reviewed in Seki et al. 2003, Bajaj et al., 1999; Chinusamy et al., 2005, Vinocur and Altman, 2005; Umezawa et al., 2006). These experiments demonstrated that these ABA responsive genes do play important roles in plant stress tolerance.

4. ROLE OF ABA IN DROUGHT STRESS RESPONSE AND ADAPTATION

Drought stress is often caused by prolonged water shortage in the soil that could not meet plant transpiration demand. Different plant species may adopt different strategies to deal with drought stress. Researchers sometimes divide plant drought adaptation into several categories (Levitt, 1980): 'drought escape' (shortening life cycle by flowering earlier), 'drought avoidance' (growing deeper roots, depositing leaf wax, and closing stomata), and 'drought tolerance' (production of osmolytes, antioxidants, and other stress-relieving agents). These terms are sometimes confusing and here we loosely define drought tolerance as the ability of plants to withstand water deficit while maintaining appropriate physiological activities.

For a given plant species, one can image that plants will have three ways to deal with drought challenges:to reduce water consumption, to increase water uptake, and to mitigate the negative impacts of water deficit. These tasks are accomplished in several ways. First, guard cell stomatal pores are closed upon drought stress and thus the transpirational water loss is minimized. This is a relatively quick response. Second, an array of stress-responsive genes is activated. The products of these genes function directly or indirectly in drought tolerance (see Section 3.2). Third, in a longer term there are certain developmental changes that may make the plants more adaptive to drought stress. These changes may occur in root development, phase transition, wax deposition, guard cell patterning and perhaps leaf morphology (for newly emerged leaves). ABA, whose level is up-regulated by drought stress (Section 3.1), is either required or is involved in all these processes.

4.1. Guard Cell Regulation

Water potential-driven influx of water in guard cells swells these cells and opens the stomatal pore. Likewise, efflux of water from guard cells shrinks the cells and closes the stomatal pore. Water potentials in these cells in turn are regulated by ion and solute fluxes through respective channels and transporters. The activities of these channels and transporters are regulated by a number of factors such as light, CO_2, and ABA (Schroeder et al., 2001; Luan, 2002; Fan et al., 2004; Hetherington and Woodward, 2003). Thus, the opening and closing of stomata are also controlled by light, CO_2, drought and salt stress. Among them, light and CO_2 regulation of stomata may be independent of ABA. Here we confine our discussion to guard cell regulation by ABA.

ABA regulation of guard cell ion channels is the major basis of ABA regulation of stomatal closing. The dogma of this regulation is that ABA induces a transient increase in cytosolic Ca^{2+} that in turn inhibits plasma membrane proton pumps and inward K^+ channels and also activates anion channels that lead to the release of anions from the guard cells. Anion efflux-induced depolarization activates outward K^+ channels and leads to K^+ efflux as well (reviewed in Schroeder et al., 2001; Fan et al., 2004; Pei et al., 2006). Reduced osmolarity in guard cells thus leads to water efflux and stomata closure. Although the molecular identities of these channels involved in guard cell movement have not been identified, several transporters were shown to affect stomata response to ABA and drought stress (Hosy et al., 2003; Klein et al., 2003). In these processes, ABA induced transient increases of Ca^{2+} was suggested as an early event that regulates subsequent gating of other channels. Since ABA itself has not been suggested to bind to any ion channels and regulate their activities, ABA regulation of ion channels will involve certain intermediate molecules and additional signal transduction processes.

By using various electrophysical, fluorescence imaging, pharmacological, biochemical, molecular, and genetic approaches, several intermediate molecules or second messengers that induce internal Ca^{2+} release in animal cells are also found to mediate ABA-induced Ca^{2+}release in isolated guard cell protoplasts, membrane vesicles, or guard cells. These include inositol phosphates [inositol 1, 4, 5-trisphosphate (IP_3) and inositol 1, 2, 3, 4, 5, 6-hexaphosphate (IP_6)], phosphatidic acid (PA), cADPR (cyclic adenosine 5'-diphosphoribose), NAADP (nicotinic acid adenine dinucleotide phosphate), NO (nitric oxide), H_2O_2, and sphingosphine 1-phosphate (Schroeder 2001; Pei, 2006; Fan et al., 2004). Many of the studies were based on analysis of the currents from unknown channels in isolated protoplasts or membrane vesicles that were treated with exogenous compounds; it is not entirely clear whether all these molecules are the endogenous second messengers in living guard cells (Levchenko et al., 2005). However, the fact that mutations in some of the enzymes involved in the generation of these second messengers were found to affect ABA regulation of stomatal movement and plant drought tolerance suggests that they may indeed play roles in ABA and drought stress responses in planta (e.g., Staxen et al., 1999; Kwak et al., 2003; Zhang et al., 2004). In particular, stomatal regulation requires phosphatidic acid (PA) produced by PLDα1 and PA could bind to ABI1 as well as the heterotrimeric G protein α subunit GPA1 to induce stomatal closure and to inhibit opening in response to ABA (Mishra et al., 2006). Thus, PA may provide a link between ABI1 and GPA1 that are previously demonstrated to regulate stomatal response to ABA (Murata et al., 2001; Wang et al., 2001).

ABA regulation of stomatal closure may also have Ca^{2+}-independent routes, although these routes are not well characterized. Using florescence dyes and in vivo imaging techniques, Levchenko et al. (2005) reported that cytosolic ABA activation of guard cell anion channels does not involve ABA-induced Ca^{2+}transients, although a basal level of $Ca^{2+}$is required. Likewise, the intermediate molecules of ABA action such as IP_3, IP_6, NAADP, and cADPR were not able to mimic ABA in the activation of the anion channels. It is unclear whether the anion channels responsible

for the currents observed in this study are the same kind of channels referred in previous studies that require transient Ca^{2+} to activate. In previous patch-clamping studies of guard cells, ABA was shown to be able to activate anion channels that contribute to the closure of stomatal guard cells. The signaling pathway for this activation is unclear, but it appears that components requiring farnesylation may negatively regulate the process since in *era1* mutant, which has a mutation in the β subunit of a farnesyl transferase, the activation of S-type anion channels by ABA was enhanced. This facilitates the closure of stomata and leads to increased drought tolerance of the *era1* mutant (Pei et al., 1998).

Stomatal regulation by ABA occurs fairly quickly (with only a few minutes' lag period for channel regulation by exogenous ABA). Thus, protein posttranslational modification is likely the major mode of action for ABA signaling in stomatal regulation. Several protein phosphatases (such as the 2C type and 2A type) and kinases are involved in ABA regulation of guard cells (Finkelstein and Rock, 2002; Xie et al., 2006). ABI1 and ABI2 are well-studied phosphatases affecting guard cell ABA response. The *abi1*-1 mutation impairs ABA-induced K^+ currents (Armstrong et al., 1995). It was shown that ABI1 was impaired in ABA-induced production of reactive oxygen species (ROS) where ABI2 was impaired in steps downstream of ROS production but before the activation of anion channels (Murata et al., 2001). Similar to *abi1*-1, mutations in the OST1 kinase also impair ABA-induced ROS production (Mustilli et al., 2002). Accordingly, guard cells of the *ost1/snrk2* mutants are insensitive to ABA and the mutants exhibited a wilty phenotype (Yoshida et al., 2002 and Mustilli et al., 2002). Interestingly, OST1 was found to interact with ABI1 and the activation of OST1 by ABA was impaired in the dominant *abi1*-1 mutant (Yoshida et al., 2006). These observations suggest that this pair of phosphoproteins may constitute an auto-regulated module in mediating ABA-induced stomata movement.

The control of ion channel gating is a late step of guard cell ABA response. There are other events preceding the gating of these channels. Regulation of membrane proteins including ion channels and transporters often requires vesicle trafficking, targeting and fusion. The swelling and shrinking of guard cells as well as the dynamics of channels and transporters may evoke significant vesicle trafficking. Several components potentially involved in vesicle trafficking have been showed to affect guard cell ABA responses. These include for example, small GTPase (e.g., AtRac1, see Lemichez et al., 2001; Rop10, see Zhang et al., 2001) and SNARE proteins (e.g., OSM1/SYP61 see Zhu et al., 2002; NtSyr1, see Leyman et al., 1999). Some of these GTPases requires prenylation in order to target to the plasma membrane. They are likely the targets of the farnesyl transferases that include the β subunit ERA1 (enhanced response to ABA 1) (Culter et al., 1996). The *osm1/syp61* (osmotic stress-sensitive mutant 1/syntaxin 61) mutant exhibited reduced sensitivity to ABA in guard cell response (but not in seed germination) and increased sensitivity to salt and osmotic stresses (Zhu et al., 2002). In another study, the tobacco syntaxin protein AtSyp1 was found to mediate ABA-induced Cl^- flux in oocytes (Leyman et al., 1999). ABA treatment inactivates the Rho-like

small GTPase AtRAC1/ARAC3/Rop6 and results in the disruption of actin fiber in guard cells. These events precede the stomatal closure induced by ABA. In the ABA-insensitive mutant *abi1*-1, ABA was unable to inactivate AtRAC1 and also failed to reorganize the actin cytoskeleton (Lemichez et al., 2001).

In addition to channel regulation, transcription regulation of genes may also contribute to stomatal regulation. Recently, it was found that a R2R3 type Myb transcription factor AtMYB60 affects stomata regulation (Cominelli et al., 2005). The *myb60* mutant showed constitutively a smaller stomata aperture. Interestingly, the *MYB60* gene was down-regulated by ABA and drought stress, suggesting that regulation of this gene may contribute to ABA and drought-induced stomatal closure. However, in the *myb60* mutant, only a limited number of genes were moderately down regulated. It is unclear how this transcription factor would affect stomata movement.

4.2. ABA Regulation of Drought-Responsive Genes in Drought Tolerance

Drought stress induces the expression of a large number of stress-inducible genes. Many of these genes are also up-regulated by ABA (Section 3.2). The products of these genes may contribute to much of the so-call 'drought tolerance' that emphasizes the ability of cells to tolerate the stress. Drought stress creates several challenges to plant cells. First, it causes an increased production of reactive oxygen species that could be detrimental to cellular membranes and other macromolecules. Second, some proteins may undergo misfolding, aggregating, and denaturation. Third, the low water potential in soil requires the plant cells to lower water potential as well in order to retain and uptake water. Many of the genes that are up-regulated by drought and ABA encode proteins presumably with these functions. For example, some of the drought/ABA up-regulated genes encode enzymes that function in the biosynthesis of compatible solutes (e.g., proline, sugars) that could lower the water potential and facilitate water uptake and retention. Others encode enzymes that can directly detoxify reactive oxygen species. These enzymes include glutathione peroxidase (GPX) (Rodriguez Milla et al., 2003), glutathione S-transferases (GST) (Moon, 2003), superoxide dismutase (SOD), catalase (CAT) (Guan, et al., 2000; Pei et al., 2000), ascorbate peroxidase (APX) and glutathione reductase (GR) (Jiang and Zhang, 2003). Certain stress-responsive genes encode polypeptides that may help to restore the nature structures of abnormal proteins. For proteins that could not be repaired, ABA up-regulated genes that encode various components in the proteolysis pathway (Hoth et al., 2002) will promote the degradation of these unfold proteins to avoid the negative effects of their accumulation on cellular activities.

Although drought alone can activate these stress-responsive genes, ABA can synergistically enhance their expression. This was demonstrated by both exogenous applied ABA and ABA deficient or insensitive mutants. Two possibilities may account for this synergy. One is that the signaling pathways for ABA and drought

stress may act in parallel but may also interact with one another. Another possibility is that the transcription factors that respectively bind to the ABRE and the DRE/CRT elements in the promoters of stress responsive genes may cooperate in gene activation. In any event, the outcome of this interaction results in even higher expression of stress responsive genes that are an advantage to the plants under stress.

4.3. Roots Signal Drought to the Shoot

It was thought that the ability of plants to sense water deficit in soil may have to do with ABA production in the roots and its translocation to the leaves where it serves as a signal to close stomata. This has been a subject receiving intensive study and debate (Wilkins and Davies, 2002). Other hormones (auxin, cytokinine, and ethylene), metabolites, various cations and anions, reactive oxygen species as well as pH sometimes are also associated with drought-induced stomatal closure (e.g., Goodger et al., 2005).

4.4. Root Adaptation to Drought Stress

It has been well documented that the growth of roots is generally less inhibited by drought stress relative to that of the shoot (Hsiao and Xu, 2000; Wu and Cosgrove, 2000; Serraj and Sinclair, 2002; Sharp et al., 2004). Thus, plants growing under lower water potential conditions usually have a higher ratio of root to shoot mass (Fisher and Turner, 1978). Here we will confine our discussion to root development under drought stress.

While root growth is more adapted to drought stress than that of the shoot, it is not clear whether root development also has some adaptation to drought stress. Many studies have been conducted to explore the root growth and development of crop plants under drought stress. Due to the genetic complexity of crop cultivars, however, most of these studies did not provide a clear clue about root development under drought stress (see below). In Arabidopsis, several earlier reports had described roots' responses to drought stress. It was reported that in response to drought root hairs become 'bulbous' and 'shortening' (Schnall and Quatrano, 1992) or 'short, tuberized, hairless roots' form (Vartanian et al., 1994). Interestingly, ABA was shown to have similar effects on roots in inducing these alterations. However, it is not known whether these responses give any advantage to plants under drought stress. Thus, whether these responses are adaptive is unclear.

Assuming that roots may play a critical role in drought tolerance, researchers had tried to link root development with drought tolerance in crop plants such as rice, maize, soybean, sorghum, barley, and coffee tree (e.g., Champoux et al., 1995; Nguyen et al., 1997; Maggio et al., 2001; Xu et al., 2001; Lafitte and Courtois, 2002; Sharp et al., 2004; Pinheiro et al., 2005). Many of these studies were intended to identify root traits that could be used in breeding for drought tolerance. In these studies root characteristics (such as dry mass, thickness, length, etc) between

drought tolerant and drought sensitive cultivars were compared and their correlation to drought tolerance was inferred. The conclusions of these studies vary and are often contradictory (Price et al., 2002). Apparently, these correlative studies may have had difficulty in ascertaining whether the differences in root systems or architecture are responsible for or linked to drought tolerance, because the genetic backgrounds of the tolerant and the sensitive strains often are different or unclear. In this regard, quantitative trait locus (QTL) analysis using progenies derived from crosses between drought tolerant and drought sensitive lines may yield more reliable information regarding whether a particular trait has anything to do with the tolerance.

Despite some conflicting results in different QTL analyses, several studies did suggest a connection between root characteristics and drought tolerance (Nguyen et al., 1997; Zhang et al., 2001; Yue et al., 2005). For instance, using a double haploid mapping population derived from a cross between a strain of upland rice (drought tolerant) and a strain of low land rice (drought sensitive), Mu et al. (2003) reported that drought tolerance correlates with longer maximum root length and fewer root numbers. However, with multiple QTLs controlling overall drought tolerance, analysis of variance alone will be difficult to determine the actual contribution of the root traits to overall drought tolerance. In some analyses, the QTL effects were considered pleiotropic rather than direct linkage (Giuliani et al., 2005). Furthermore, it is still not easy to identify genes underlying the QTL loci in crop plants. Therefore, direct genetic study of the relationship between root traits and drought tolerance in a model plant species would be desirable in order to clarify the role of roots in drought adaptation and to reveal novel drought tolerance mechanisms.

To isolate drought tolerance determinants, we searched for possible responses of roots to drought stress in Arabidopsis. It was noticed that under osmotic stress the Arabidopsis root system underwent a characteristic change that had not clearly been described in the literature before we started our work. Whereas the control plants developed a number of lateral roots, those subjected to osmotic stress (by supplementing nutrient media with 50 mM or 75 mM mannitol) failed to develop or were delayed in lateral root development. Importantly, this inhibition of lateral root growth by drought stress was also observed in seedlings growing in soil and was observed in several crop plants (Xiong et al., 2006; L. Xiong, unpublished).

Like many other drought responses, drought inhibition of lateral root development is also partly mediated by ABA. Although exogenous ABA at lower concentrations (0.1 to 1.0 μM) has little effect on the growth of the primary roots (sometimes primary root elongation is stimulated by lower concentrations of ABA, e.g., Xiong et al., 2001), it clearly inhibits lateral root elongation. ABA deficient mutants (*aba1*, *aba2*, and *aba3*) generally tend to have more lateral roots under non-stressful conditions. On agar plates supplemented with mannitol, the magnitude of inhibition of lateral root elongation was reduced in *aba* mutants compared to the wild type, although the mutants still responded to the treatment in reducing lateral

root elongation. These data suggest that the inhibition of lateral root elongation by mannitol is partly mediated by ABA (Xiong et al., 2006). Further evidence to support ABA's role in mediating inhibition of lateral root development is that the inhibition of lateral root elongation by osmotic stress and ABA is significantly compromised in the ABA-insensitive mutant *abi1*-1 (Xiong et al., 2006). During the course of our study, reports on the influence of osmotic stress and ABA on Arabidopsis root development were recently published (De Smet et al., 2003; Deak and Malamy, 2005). These authors also found that ABA and osmotic stress inhibit lateral root development, although the experimental conditions used in these studies are different from ours. In fact, osmotic stress or drought stress inhibition of lateral root growth was also documented in early reports (e.g., van der Weele et al., 2000), although its significance was previously unclear. Our study and those of others thus demonstrate that osmotic stress and drought stress can regulate lateral root development. With these findings, we further hypothesized and subsequently confirmed that the characteristic inhibition of lateral root development by drought/osmotic stress may represent an adaptive response to drought and therefore it is a typical 'drought rhizogenesis' process. This drought rhizogenesis process might be related to the one reported in a previous study on morphological changes of roots observed with soil-grown Arabidopsis (Vartanian et al., 1994). In our follow-up studies, the *dig* (*d*rought-*i*nduced rhizo*g*enesis) mutants defective in drought rhizogenesis were isolated. It was found that those *dig* mutants that exhibit a hypersensitive response to drought or ABA in drought rhizogenesis are more tolerant to drought stress and the insensitive ones are drought sensitive (Xiong et al., 2006). Our genetic studies thus demonstrate that drought rhizogenesis response is closely linked to whole plant drought tolerance and is an adaptive response to drought stress.

Now that drought rhizogenesis has been established as an adaptive response, what would its benefits be to the plants? Under drought or any other abiotic stresses, there is a significant decrease in photosynthesis and, consequently, a reduction in the amount of metabolites and energy. It is imperative for the plants to use this reduced amount of resources to their maximum advantage – usually to survive stresses. Apparently, under drought stress conditions, an urgent need of the plants would be to increase the uptake of water, which is usually more available deep down in the soil. Restriction of the horizontal proliferation of lateral roots in the top soil and allocation of more resources to the growth of primary roots certainly would offer an advantage to the plants by expanding their domains of water supply. Thus, the adaptive response of roots to water deficit by means of drought rhizogenesis is in sharp contrast to their response to nutrient deficiency. Under nutrient starvation conditions, increased proliferation of lateral roots are commonly observed, which may help the plants to increase their exploitation of the topsoil where bioavailable nutrients are more enriched relative to the subsoil.

The regulation of lateral root development by ABA under drought stress may result from the interplay of drought and ABA with other hormones such as auxin, ethylene, gibberillic acid and cytokinins, yet current study on this topic is very limited.

4.5. Hydrotropism, Hydraulic Conductivity and Water Uptake

Plant roots have the ability to grow toward soil patches with more available water or grow away from dry soil regions. This hydrotropic response may be important for plants to find water resources. Using existing mutants defective in auxin and ABA biosynthesis or signaling, it was reported that hydrotropism requires ABA since seedlings of ABA deficient mutant *aba1*-1 and ABA-insensitive mutant *abi2*-1 are less responsive to hydrotropic stimuli (Takahashi et al., 2002). On the other hand, auxin insensitive mutants *axr1*-3 and *axr2*-1 showed enhanced hydrotropism (Takahashi et al., 2002). However, another group reported that ABA deficient mutants and ABA insensitive mutants were not defective in hydrotropism (Eapen et al., 2003). Recently, Arabidopsis hydrotropic mutants were isolated (Kobayashi et al., 2003; Eapen et al., 2003). Among the ahydrotropic mutants, some exhibited normal gravitropic responses whereas others were impaired in gravitropism (Kobayashi et al., 2003). In a separate screen, one ahydrotropic mutant *ahr1* showed enhanced gravitropic response. Thus, hydrotropism differs from gravitropism but both responses may interact. Furthermore, the perception of gravity and water availability might share similar mechanisms and auxin may have been recruited in the perception and response to water availability. Future identification of the genes required for hydrotropism may help to reveal the mechanisms of hydrotropism.

Once roots reach the water source, the ability to absorb water depends on the driving force (created by water potential difference across plasma membrane) and the resistance of plant cells to water passage. ABA was shown to transiently activate the expression of certain water channel (aquoporin) genes in a number of plant species (reviewed in Javot and Maurel, 2002). Several experiments showed that exogenous ABA could increase root water conductivity Lp (e.g., Ludewig et al., 1988; Zhang et al., 1995; Quintero et al., 1999; Hose et al., 2000) but how this occurs is unclear. In addition to up-regulation of the aquoporin expression and activity, presumably, ABA may alter root structure and/or decrease water potential and thus would facilitate water uptake. ABA up-regulated gene products may have a significant effect on lowering water potentials. When wheat roots were treated with ABA, there was a significant increase in osmolarity and turgor pressure, although levels of cations were not changed (Jones et al., 1987). This suggests that increased non-ionic solutes after ABA treatments are responsible for the increased osmolarity.

5. ABA IN PLANT RESPONSE TO SALT STRESS

Relative to its well-described functions in drought stress response, less is known about the role of ABA in plant salt stress response. Like drought stress, salt stress also imposes osmotic stress to plant cells and results in the accumulation of toxic compounds such as reactive oxygen species. Therefore, combating osmotic stress and detoxifying toxic compounds are also important for salt tolerance. In these aspects, ABA may have similar functions in plant salt tolerance as in drought

tolerance. Comparison of salt tolerance between plant species or genotypes differing in ABA responses may help to reveal the role of ABA in salt tolerance. Variations in salt tolerance among Arabidopsis ecotypes have been investigated (e.g., Quesada et al., 2002) yet it is unclear whether these differences have anything to do with ABA biosynthesis and responses. In their comparison of the expression profiles of the salt tolerant *Thellungiella halophila* with *Arabidopsis thaliana*, Gong et al. (2005) and Taji et al. (2004) found that the expression level of ABA biosynthetic and ABA responsive genes was higher in *Thellungiella*. Nonetheless, using ABA deficient or ABA response mutants may better address the potential role of ABA in salt tolerance.

Seed germination is highly sensitive to environmental conditions such as water availability, temperature, and salinity. It is known that salt stress enhances ABA biosynthesis which in turn inhibits germination. ABA deficient mutants are thus less inhibited by salt stress during germination. Accordingly, some mutants isolated for their tolerance to salt stress are found to be allelic to ABA deficient or ABA insensitive mutants (e.g., Quesada et al., 2000; Ruggiero et al., 2004). At the vegetative stage, however, ABA deficient mutants are more sensitive to salt stress partly because these mutants are impaired in the activation of stress-responsive genes (Xiong et al., 2001; 2002). Likewise, it is expected that ABA insensitive mutants would be salt sensitive too if ABA signaling is critical to salt tolerance. Nonetheless, the salt sensitivity of adult ABA response mutants was not well studied. Ohta et al. (2004) reported that seedlings of *abi1*-1 and *abi2-1* are more tolerant to salt stress. A similar observation was also reported by Achard et al. (2006). Overall, the effects of mutations in ABA signaling components on plant salt stress response are less obvious than on drought stress response. Measurement of several growth parameters of adult Arabidopsis wild type and *abi1*-1 and *abi2*-1 plants growing under salt stress also did not reveal any differences among these genotypes (Cramer, 2002). Perhaps this has to do with the different impacts of drought stress and salt stress on plants.

While drought stress imposes 'physical' water deficit stress to the plant cells, high salinity in soil creates a 'physiological' water deficit stress. In fact, high external Na^+ often is much more detrimental to glycophytes than the resulting lower water potential. The mechanisms that condition ionic stress tolerance are therefore of predominate importance for plant tolerance to high salinity. Consequently, limiting the uptake, reducing root to shoot transport, increasing the exclusion and compartmentalization of Na^+ are the methods of choice that glycophytic plants may use in dealing with salt stress. This was clearly demonstrated in the genetic study of salt tolerance in Arabidopsis. The most salt sensitive mutants (in terms of primary root elongation and seedling growth) in Arabidopsis are those that are impaired in maintaining ion homeostasis (Zhu, 2000). The importance of regulating Na^+ transporters in salt tolerance was also witnessed in other plants including rice (e.g., Ren et al., 2005). ABA, whose level increases upon salt stress through transcriptional up-regulation of its biosynthetic genes (Xiong and Zhu, 2003), may directly or indirectly modulate ion homeostasis during salt stress.

ABA regulates the expression of some of the transporters involved in salt uptake and compartmentalization. For example, the vacuole localized Na^+/H^+antiporter genes are up-regulated by ABA (Yokoi, et al., 2002; Shi and Zhu, 2002). In addition, protein modification is critical to the function of these transporters. ABA may regulate transporter activities through posttranslational modification of the transporters or their regulators, yet the mechanisms involved in the regulation are unclear at this time. The protein kinase SOS2 can directly phosphorylate and regulate the plasma membrane-localized Na^+/H^+ antiporter SOS1 (Qiu et al., 2002; Quintero et al., 2002) in excluding Na^+ from the cytosol. Interestingly, SOS2 could interact with ABI2, a 2C type phosphatase that negatively regulates ABA signaling (Ohta et al., 2003). Other 2C type phosphatases, for example PP2CA, which may act as a negative regulator of several ABA responses (Kuhn et al., 2006; Yoshida et al., 2006), also interact with K^+ channels (Chérel et al., 2002; Vranova et al., 2001). While K^+ channels in guard cells play critical roles in stomatal opening and closing (Section 4.1), disturbed K^+ homeostasis in roots and other tissues and cell types may contribute to salt sensitivity (e.g., Zhu et al., 1998; Rus et al., 2004). It is thus likely that ABA may play a role in regulating ion transporter activities under salt stress. Nonetheless, this regulation may not be as evident as the regulation of ion channels and transporters in guard cells under drought stress (Section 4.1), although salt stress also quickly induces stomata closure.

While about one quarter of salt stress-regulated genes are specifically regulated by salt stress (Ma et al., 2006), most others can also be regulated by drought and cold stress as well. Salt stress induction of at least a subset of these genes is ABA-dependent. Early studies suggested that the transcript levels of stress-responsive genes are lower in both *aba* and *abi* mutants when treated with salt or osmotic stress, although controversy also existed in the literature (see references cited in Xiong et al., 2001). Since the *LOS5* and *LOS6* genes are required for salt induction of stress responsive genes and they encode the ABA biosynthetic enzymes ABA3 (molybdenum cofactor sulfurase) and ABA1 (zeaxanthin epoxidase), respectively, this demonstrates that ABA is indeed required for stress induction of these common stress responsive genes. However, it should be noted that there are other stress-responsive genes whose expression may be independent of ABA.

6. ABA INTERACTION WITH OTHER HORMONES IN PLANT STRESS RESPONSE

The growth and development of plants, like that of animals, is regulated by a diverse set of growth hormones. Plants are distinct from animals in that they cannot move and therefore they have evolved more robust mechanisms to deal with adverse environmental conditions. Stress responses in plants evoke a wide array of genes and intensive signaling pathways. Under abiotic stress, the stress hormone ABA works together with other phytohormones to adjust growth and development programs so that the plants may be better adapted to the adverse conditions. Under these conditions, plant growth will generally be slowed down as a result of reduced

synthesis and signaling of growth promoting hormones (e.g., auxin, gibberrelin, and cytokinine) and increased synthesis of growth inhibition hormones (ABA and ethylene). Among these hormones, the interaction of ethylene and ABA in abiotic stress response was the most studied.

Under drought and salt stress, ethylene production increases (McMichae et al., 1972; Apelbaum and Yang, 1981) because of the activation of the biosynthetic genes and enzymes (e.g., Liu and Zhang, 2004). Increased accumulation of ethylene under abiotic stress may inhibit plant growth. It was thought that ABA may restrict the production of ethylene and thus could promote growth under abiotic stresses (Sharp, 2002). On the other hand, ethylene may promote ABA biosynthesis under drought stress. It was suggested that ethylene-induced ABA production may contribute to the inhibition of auxin-related herbicides on plant growth. This is because high levels of IAA or synthetic auxin (such as 2, 4-D) induce ethylene production (e.g., Burg and Burg, 1965), which in turn promotes ABA biosynthesis (Hansen and Grossmann, 2000). However, it is unclear whether indigenous auxin could induce ABA biosynthesis via enhanced ethylene production. The auxin maxima in plant tissues do not appear to completely overlap with the sites of ABA biosynthesis. Furthermore, auxin biosynthesis and signaling may be generally compromised under drought stress, although detailed experimental evidence in this aspect is lacking. Thus, auxin may not play a major role in the interaction of ABA and ethylene under drought stress.

Enhanced production of ethylene may be beneficial for plants under abiotic stress. For example, senescence of old leaves induced by ethylene will remobilize nutrients from these leaves and reduce transpirational water loss of the plant as a whole. When the ethylene biosynthetic gene ACS (1-aminocyclopropane-1-carboxylic acid synthase) was knocked out in maize, the leaves of the mutant plants exhibited delayed senescence and had high levels of chlorophyll and high CO_2 fixation rate. However, these leaves also had a high transpiration rate (Young et al., 2004). So it is expected that these mutant plants will delay leaf senescence (a 'Stay-Green' trait) but may use up the available water in soil more quickly. Thus, 'Stay Green' is beneficial for the plants only when water will be available imminently. Perhaps because of these reasons, the soybean stay-green mutations actually increased drought susceptibility (Luquez and Guiarmet, 2002) although it was previously reported that the Stay-Green trait in sorghum and rice might contribute to increased biomass production under drought stress (e.g., Borrell et al., 2000).

In addition to the biosynthesis, ethylene responsiveness may be regulated by drought or salt stress too. The ethylene receptor ETR1 was down-regulated by salt stress at transcription and protein levels (Zhao and Schaller, 2004). This would result in increased sensitivity to ethylene (ethylene receptors negatively regulate the downstream signaling) and perhaps would offer some advantages to plants under stress. When the ethylene receptor NTHK1 was overexpressed in tobacco (so that the downstream ethylene signaling pathway would be suppressed), the transgenic plants were found to have a higher Na^+/K^+ ratio and were more sensitive to salt stress (Cao et al., 2006). Similarly, the ethylene insensitive mutant *ein3* was more sensitive

to salt stress whereas the constitutive ethylene response mutant *ctr1* was salt-tolerant during the early seedling stage (Achard et al., 2006). Interaction between ABA and ethylene under abiotic stress is also suggested by the fact that certain transcription factors responsible for the activation of ABA/stress-responsive genes and ethylene-responsive genes are of a similar class and may be subject to similar regulations. The ethylene responsive factor binding protein (ERF/EREBP) and the CBF/DREB class of transcription factors may cross-activate stress responsive genes (e.g., Fujimoto et al., 2000). Some ERF proteins act as transcription repressors regulating ethylene and ABA responses (Yang et al., 2005; Song et al., 2005). Accordingly, regulating these ERF transcriptional regulators may result in altered drought and salt stress sensitivity (e.g., Yang and Wu, 2005; Song et al., 2005; Zhang et al., 2005).

The antagonism between ethylene and ABA was also found in other stress response processes. Ethylene inhibits ABA-induced stomatal closure and reduces the induction of the ABA-induced gene *Rab18* (Tanaka et al., 2005). Yet, how this was achieved is unclear. It was noted that the *ABI1* and *ABI2* genes were highly up-regulated by ethylene (De Paepe et al., 2004). These negative regulators of ABA signaling may thus reduce ABA responses. In previous studies, it was found that ethylene and ABA play antagonizing roles in controlling seed germination (Beaudoin et al., 2000; Ghassemian et al., 2000). Ethylene and ABA may also interact in regulating drought rhizogenesis (Section 4.4). The ethylene insensitive mutant *ein2* is hypersensitive to ABA in drought rhizogenesis, implying a role of ethylene in regulating cell fate and cell elongation in response to abiotic stress (L. Xiong, unpublished data). In addition, *ein2* also exhibited reduced lateral root development in the absence of exogenous ABA.

Phytohormones may act together with or independently of ABA in regulating stomata movement (reviewed in Dodd, 2003). Exogenous IAA can stimulate stomatal opening and suppress the inhibition of ABA on the opening. Auxin-induced stomatal opening may result from auxin-induced ethylene production since inhibition of ethylene biosynthesis can inhibit IAA-induced stomatal opening (Merritt et al., 2001). Exogenous ABA alkalinizes the cytosol whereas IAA can acidify the cytosol (Gehring et al., 1990). The pH changes induced by IAA or ABA may regulate ion channels and ion flux and thus stomata movement. Evidence has suggested that cytosolic alkalization occurs before ABA-induced ROS production and stomata closure (Suhita et al., 2004). In some of these studies on hormonal regulation of stomata movement, hormone biosynthesis or response mutants were not always used and epidermal strips were the major materials used to observe stomatal regulation. Further investigation of the role of non-ABA hormones in regulating stomata movement is needed. Because of the significant accumulation of ABA within or in the vicinity of the guard cells during drought stress, however, ABA should be the prevailing hormone in regulating stomata under drought stress.

In summary, increased functions of growth inhibitory hormones and simultaneously reduced functions of growth promoting hormones contribute to the retarded plant growth under drought or salt stress. This may represent an adaptive strategy

for the plants to survive the stress at the expense of their growth. This strategy may work well for a population as a whole to survive a drought spell: retarded plants will use less water resource and will be able to recover growth when the next rainfall comes. To genetically abrogate this response may temporarily enhance plant growth but may not result in increased productivity of a population if water will not be available at later stages of plant development.

7. HARNESS ABA AND ITS SIGNALING FOR THE REGULATION OF PLANT DROUGHT AND STRESS TOLERANCE

Since ABA plays such important roles in drought and salt adaptation, it is possible that by manipulating ABA levels or ABA sensitivity one may be able to obtain stress tolerant crop plants. Before the isolation of ABA biosynthetic genes, crop breeders had tried to use ABA levels as a trait in breeding for drought resistant crops. Theoretically, a higher ABA level under drought stress may confer increased drought tolerance. However, it should be born in mind that ABA biosynthesis and catabolism are drought-stress regulated. Thus, drought sensitive plants may in fact experience a higher degree of stress and thus may lead to increased production of ABA (Xiong and Zhu, 2003). Therefore, the causal relation between internal ABA levels and drought susceptibility may be complex. Accordingly, both positive (Samet et al., 1980; Henson et al., 1981) and negative (Ilahi and Dorffling, 1982; Quarrie and Jones, 1979; Wang and Huang, 2003) correlations between leaf ABA content and drought tolerance were found (reviewed in Quarrie, 1991). Since the accumulation of ABA in leaves does not necessarily correlate with stomatal closure even within the same plant (Jia and Zhang 1999), variations in leaf ABA levels may not be able to account for differences in drought tolerance among cultivars of diverse genetic backgrounds. Thus, the correlation between ABA and drought tolerance among different plant species or cultivars within species is pleiotropic at the best (Giuliani et al., 2005). Without some certainty as to the contribution of ABA levels to drought tolerance, breeding ABA levels using conventional approaches for enhancing drought tolerance may not work well. Pertinent to breeding ABA levels, there are also other breeding programs using various traits that appear to correlate with drought resistance of crop plants. Considering the genetic diversity of many crop plants and the complex mechanisms of drought tolerance, it may be advisable to use yield performance as the sole trait in the breeding for drought tolerance.

With the identification of the ABA biosynthetic genes, it becomes feasible to use these genes in enhancing ABA production and, potentially, plant stress tolerance. To maximize the possibility of bursting ABA production, it is ideal to enhance the expression of the rate-limiting enzyme in the biosynthetic pathway. Researchers have suggested that the cleavage step is rate-limiting (Schwatz and Zeevaart, 2003). Therefore, initial efforts to regulate ABA biosynthesis were mainly focused on the *NCED3* gene. It was reported that overexpression of this gene in Arabidopsis and in tobacco resulted in increased ABA production and enhanced drought and salt

tolerance (Iuchi et al., 2001; Qin and Zeevaart, 2002). On the other hand, it was proposed that ABA biosynthetic genes might be subject to self-regulation (Xiong and Zhu, 2003). In this scenario, up-regulation of any of the biosynthetic genes would result in increased ABA biosynthesis to various extents. In consistence with this idea, overexpressing the *ZEP* gene, which is the most abundantly expressed among all these known ABA biosynthetic genes, and *ABA3/LOS5* either resulted in enhanced ABA responses in stress gene induction or increased drought tolerance (Xiong et al., 2002; unpublished). Similarly, regulation of *ZEP* in tobacco also results in increased ABA accumulation and enhanced seed dormancy (Frey et al., 1999). In addition to the regulation of biosynthesis, reduced ABA catabolism and conjugation will also result in enhanced ABA accumulation and potentially will increase drought tolerance of the plants (Section 3.1).

Another approach to enhance drought tolerance is to increase the sensitivity of plant cells to ABA. Since Arabidopsis mutants with altered sensitivity to ABA are available (Finkelstein and Rock, 2002), regulating the expression level of these genes may confer altered drought tolerance in transgenic plants. One example is that suppression of the *ERA1* gene in canola resulted in increased sensitivity to ABA and increased drought tolerance and better yield under mild drought stress (Wang et al., 2005). Because the pathway for ABA signal transduction involves far more components than those in the ABA biosynthesis pathway, there would be many opportunities to regulate ABA responses to control stomata and whole plant response to drought and salt stress. Many successful laboratory studies were reported in enhancing drought or salt tolerance by expressing these signal transduction components. These components include putative receptors/sensors, G-protein subunits, second messenger producers, protein kinases (including Ca^{2+}-dependent protein kinases, MAP kinase components), and transcription factors. In other experiments, stress-inducible genes are directly regulated. Research on the enhancement of stress tolerance by regulating stress responsive genes and stress signaling components has been intensively reviewed elsewhere (Bajaj et al., 1999; Chinusamy et al., 2005; Vinocur and Altman, 2005; Umezawa et al., 2006).

Despite many successful examples in enhancing stress tolerance using transgenic techniques, currently there has been no field application of these techniques. In fact, very few field trials of these transgenic plants (Wang et al., 2005) were conducted. In field conditions, the effectiveness of the transgenic plants in improving drought tolerance may also vary considerably (Bahieldin et al., 2005). A concern about public acceptance of genetically modified crops may not be the only reason for the lack of field application of the above laboratory research. Current transgenic techniques can also be improved. For example, overexpression of stress responsive genes often impairs plant growth under normal conditions. This negative effect could be reduced by using inducible promoters. However, certain stress inducible promoters (such as the *RD29A* promoter) are also regulated by other environmental factors (such as light, circadian rhythm, and mechanical stress). In the field condition, the transgene may still be turned on even if there is no drought stress. Therefore, one important task in the future is to develop artificial promoters that

can be specifically turned on by drought or other designated stresses. Furthermore, the transgenes should be expressed in the specific tissue or cell types (e.g., guard cells) where they are supposed to function. In these ways, the negative effect of their genetic manipulations may be minimized.

ACKNOWLEDGEMENTS

The author would like to thank Christine Ehret for critical reading of the manuscript. Work in the author's laboratory has been supported by the National Science Foundation and the United States Department of Agriculture.

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