Chapter 22

Role of ABA in Arabidopsis Salt, Drought, and Desiccation Tolerance

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Additional information is available at the end of the chapter

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Abstract

The ability of plants to respond to environmental stimuli is essential to plant survival. Abscisic acid (ABA) is a phytohormone with roles at various stages of plant development. ABA also plays a major role in mediating physiological responses to environmental stresses such as salt, osmotic, and cold stress. Plant responses to environmental stress have been widely studied in the model plant *Arabidopsis thaliana* and ABA signaling mechanisms elucidated. In general, the adaptive responses of plants to various stress conditions can be either ABA-dependent or ABA-independent. Here we focus on the role of ABA in stress signaling and abiotic stress tolerance. We describe the intrinsic mechanisms that confer stress tolerance via ABA, as well as how ABA-regulated gene products play a role in salt and drought tolerance at different stages of the life cycle. In addition, the contribution of ABA to regulation of stomatal aperture and therefore desiccation tolerance will be discussed. Understanding ABA signaling mechanisms in abiotic stress provides avenues for improving plant performance.

Keywords: ABA signaling, salt, drought, dessiccation, Arabidopsis

1. Introduction

Due to their sessile nature, plants cannot avoid environmental stresses, thus they have evolved mechanisms to overcome the detrimental effects of stress. For example, plant endogenous developmental programs are modified such that structural and metabolic changes assist to overcome adverse environmental conditions such as salinity and drought. Failure to adapt to adverse environmental conditions can significantly reduce yield by impacting plant development and productivity. Abiotic stress conditions initiate a number of molecular, biochemical, and physiological changes at both the cellular and whole plant levels [1]. One major biochemical change in response to stress is elevation of abscisic acid (ABA) levels, which in turn triggers

expression of a cascade of stress-responsive genes [2]. Cellular ABA levels are induced by environmental stimuli such as light, water, and salinity stress [3].

The plant hormone ABA has been identified as a key regulator of multiple stresses. In general, the adaptive responses of plants to various stress conditions can be either ABA-dependent or ABA-independent. However, there is no clear boundary between these two pathways and there is lot of crosstalk between the pathways and the components involved. This review will focus on recent advancements in ABA-mediated stress signaling and the role of ABA in abiotic stress tolerance in the model plant *Arabidopsis thaliana*.

2. The phytohormone Abscisic Acid (ABA)

ABA, a sesquiterpenoid ($C_{15}H_{20}O_4$) with a 15-carbon ring (Figure 1), has a variety of biological functions and is found ubiquitously across several kingdoms, including cyanobacteria, sponges, algae, lichens, mosses, and mammals [4-7]. Discovered in the 1960s and initially named dormin or abscissin, ABA is now established as a widely occurring and important plant growth regulator. Although it was initially identified as an abscission-promoting hormone, later scientists discovered that this was partly due to an indirect effect of inducing ethylene biosynthesis [8]. ABA is an important regulator of plant growth, including embryo and seed development, seedling establishment, vegetative and reproductive growth as well as promoting seed dormancy [9,10]. Seed maturation and promotion of dormancy are important in preventing preharvest sprouting. In addition, ABA has the ability to antagonize the germination promoting effects of gibberellin, regulate guard cells, and regulate stress-responsive gene expression under water-deprived conditions. ABA also has a role in plant pathogen responses in a pathosystem-dependent manner [4,5].

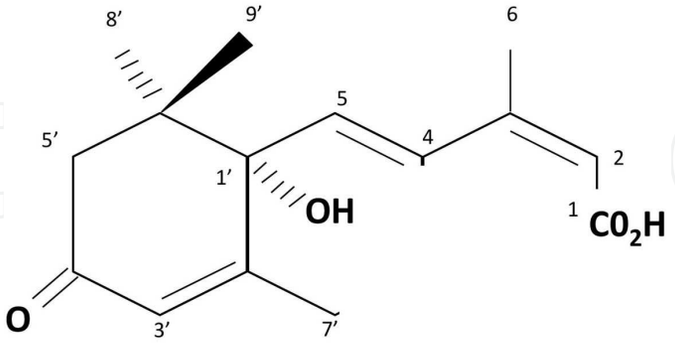

Figure 1. Structure of phytohormone abscisic acid S-(+)-ABA

The molecular structure of ABA has several important features that facilitate its biological functions. The side chain with the two double bonds (Figure 1) and ABA's stereocenter are two such important features. Exposure to UV light changes the conformation from active to inactive form [5].

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2.1. ABA signaling in plants

Although ABA has a broad range of functions in plant growth and development, its main function is to regulate plant water balance and osmotic stress tolerance [11]. Thus, understanding ABA signaling is essential to improving plant performance. Genetic screens in *Arabidopsis thaliana* identified many downstream ABA signaling components. Recent findings in the field of ABA signaling reveal a unique hormone perception mechanism (Figure 2) where ABA binds to the ABA receptors Regulatory Components of ABA Receptor/Pyrabactin Resistance Protein1/PYR-like Proteins (RCAR/PYR1/PYLs). RCAR/PYR/PYL proteins belong to the START-domain superfamily and have soluble ligand-binding properties. RCAR/PYR/PYL receptors are found in the cytoplasm as well as in the nucleus. ABA binding to RCAR/PYR/PYLs leads to inactivation of type 2C protein phosphatases (PP2Cs) such as ABSCISIC ACID INSENSITIVE 1 (ABI1) and its close homolog ABI2 [12]. All 14 members of the RCAR family of proteins bind to ABA and interact with PP2Cs. Except for RCAR7/PYL13, all the other RCAR members are positive regulators of ABA signaling. Among the 80 PP2Cs identified in Arabidopsis, six out of nine clade A PP2Cs act as negative regulators of ABA signaling [13]. These phosphatases and RCAR/PYR1/PYLs function as co-receptors and form a high-affinity ABA-binding site. Inactivation of PP2Cs causes suppression of PP2C-mediated dephosphorylation of Sucrose nonfermenting Kinase-1-Related protein kinase 2s (SnRK2s), which are important positive regulators of ABA signaling. As a result, activated SnRK2s target ABA-dependent gene expression and ion channels [5,11]. Table 1 summarizes the major positive and negative regulatory elements in the ABA signaling pathway. Phosphorylated SnRK2s subsequently phosphorylate ABA-responsive element Binding Factors (ABFs), which are basic leucine zipper transcription factors that bind to ABA-Responsive Elements (ABRE) (PyACGTGG/TC), the major *cis*-element in the promoter region of downstream genes that are induced by ABA [19,20].

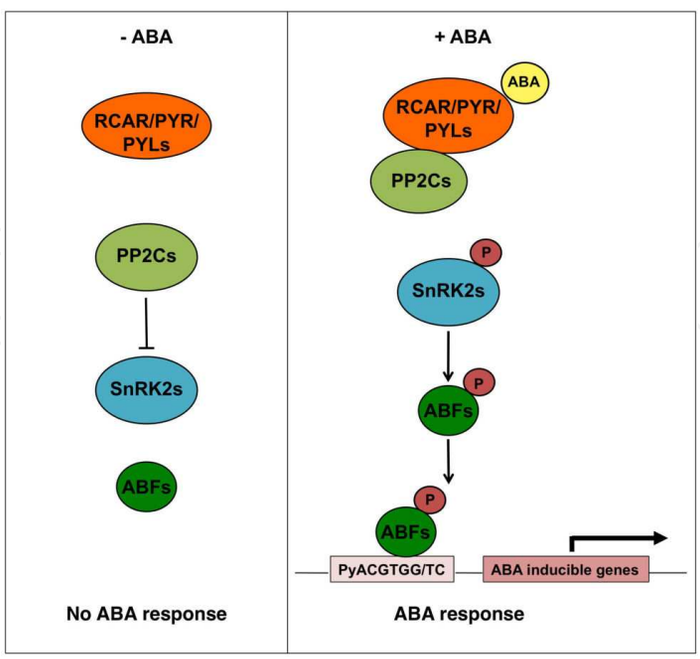

Figure 2. Main components in the core ABA signal transduction pathway

Signaling component	Regulation	Expressed	References
Group A PP2Cs	**Negative regulators**		
ABA INSENSITIVE 1/2 (ABI1/2)		Various tissues and developmental stages	[13-16]
ABA HYPERSENSITIVE GERMINATION 1 (AHG1)			
HYPERSENSITIVE TO ABA 1/2 (HAB1/2)			[17]
SnRK2 subgroup III	**Positive regulators**		
SRK2D/SnRK2.2		Seeds and vegetative tissues	[18]
SRK2I/SnRK2.3		Seeds and vegetative tissues	[18]
SRK2E/OST1/SnRK2.6		Expressed in guard cells and involved in stomatal closure	[15]

Table 1. Major positive and negative regulators of ABA signaling

Therefore, the ABA signaling complex/ABA signalosome is comprised of three major components: (a) RCAR/PYR/PYLs; (b) PP2Cs; and (c) SnRK2s assembled as a double negative regulatory system [7]. In the absence of ABA, PP2Cs dephosphorylate SnRK2s inhibiting kinase activity and thereby preventing downstream gene expression (Figure 2). Several studies showed that these core components are essential for ABA signaling. For instance, Fujita *et al*. [21] showed ABA signaling is completely blocked and *ABF* genes showed reduced expression in the *snrk2.2/2.3/2.6* triple null mutant but not in single or double mutants. In addition, reduced phosphorylation of other bZip transcription factors such as ABSCISIC ACID INSENSITIVE 5 (ABI5), which is a dormancy promoting transcription factor, was also observed [18,22].

In guard cells (Figure 3), ABA binds to the PYR/PYL/RCAR receptor-PP2C complex and blocks its phosphatase activity. Consequently, activated protein kinase SnRK2.6/OPEN STOMATA 1 (OST1) phosphorylates and regulates the key target ion channels, SLOW ANION CHANNEL ASSOCIATED 1 (SLAC1) and K^+ CHANNEL IN ARABIDOPSIS THALIANA 1 (KAT1). SnRK2.6/OST1 acts as a positive regulator of stomatal closure where it activates anion channel SLAC1 and inhibits cation channel KAT1 [23-25].

2.2. ABA-binding proteins and alternate ABA receptors

Identification of putative ABA receptors using forward genetic approaches was not successful for a long time due to genetic redundancy of the genes encoding ABA receptor proteins. However, biochemical approaches leading to purification and analysis of high-affinity ABA-binding proteins have been successful in identification of potential ABA receptor classes [5,26]. Some of these potential ABA receptors are cytosolic while others are on the cell surface,

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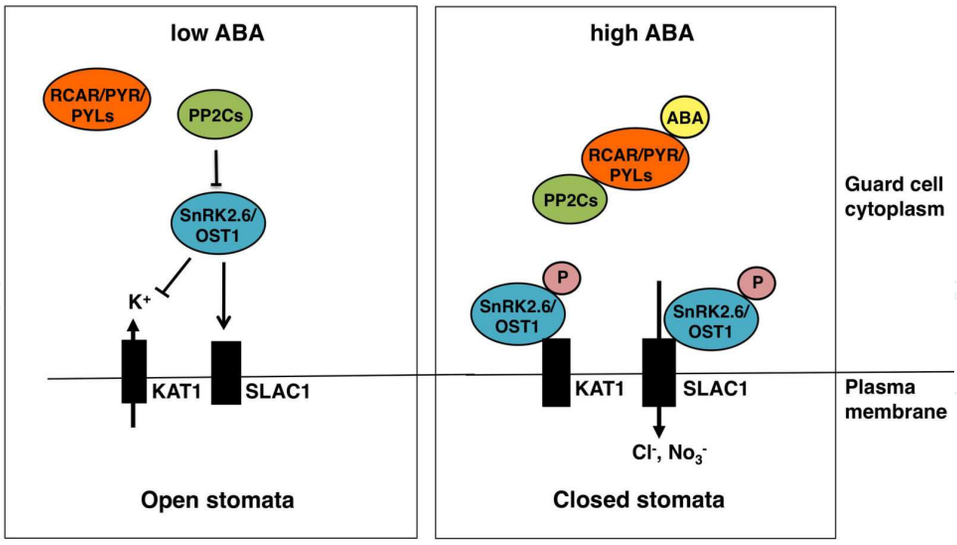

Figure 3. ABA signaling in guard cells

indicating there are extracellular as well as intracellular sites of ABA perception. Studies indicate that there can be multiple ABA receptors at different locations of the cell [27].

2.2.1. ChlH/ABAR (H subunit of the chloroplast magnesium chelatase/ ABA receptor)

The primary function of ChlH is chlorophyll synthesis. ChIH was initially identified as an ABA-binding protein in broad bean (*Vicia faba*) and the Arabidopsis protein was named as ABAR. Later it was found that binding of ABA to ChIH/ABAR depends on the stereochemistry and it specifically binds to only (+) ABA to mediate ABA responses. Although ChIH/ABAR is localized in the chloroplast envelope, it functions as a negative regulator of ABA signaling in the nucleus. The cytosolic C-terminus of the ABAR interacts with WRKY transcription factors (WRKY 18, 40, 60) which act as transcriptional repressors, repressing ABA-responsive gene expression in the nucleus. Binding of ABA with ChlH/ABAR promotes interaction with WRKYs, preventing them from repressing downstream genes such as *ABI5* and *DREB2* [28-31]. Thus, it has been proposed that ChlH mediates nuclear-chloroplast signaling. However, another research group has not been able to reproduce these results using *wrky* loss of function mutants. In addition, barley ChIH does not bind to ABA and ChIH loss of function mutants do not show any impaired ABA responses. Despite its ABA-binding properties in Arabidopsis, it is not confirmed whether ChIH functions as an ABA receptor (reviewed in [5,27]). However, ChlH/ABAR mediates ABA-induced stomatal closure and ABA inhibition of blue-light-mediated stomatal opening. In addition, ChIH/ABAR has a role in ABA-mediated fruit ripening in peach and strawberry (reviewed in [31]).

2.2.2. GTG1/GTG2 (G protein coupled receptor type G protein 1 and 2)

G protein coupled ABA receptors are plasma membrane localized cell surface receptors that are widely expressed in plants. Both GTG1 and GTG2 showed specific and saturable ABA-

binding activity in direct ABA-binding assays. GTGs have GTPase activity and GDP bound GTGs have enhanced ABA-binding ability, which in turn initiates ABA signaling. GTG1/2 bind with GPA1 (G-PROTEIN α SUBUNIT 1), which abolishes its GTPase activity and represses ABA binding. GTP bound GPA1 represses ABA signalling. However, the downstream components of this pathway are not characterized yet [31,32].

2.3. Recent studies on ABA perception and signaling mechanisms

Recent findings that several ABA receptors exist in different parts of the cell provide evidence that ABA is active in a variety of subcellular compartments. ABA synthesis enzymes are present in different compartments, suggesting that ABA synthesis occurs in different parts of the cell and that these ABA levels contribute to overall ABA homeostasis. For example, the ABA biosynthesis enzyme AtABA1 is localized in the chloroplast, whereas AtABA2 is in the cytosol [33,34]. It has also been proposed that ABA produced in cytoplasm, plastids, vacuole, and other subcellular organelles may have different physiological roles initiated by signaling networks via different ABA receptors in each specific compartment [35].

Takeuchi *et al.* [36] identified a potential ABA analog AS6 that can inhibit the activity of PYLs. X-ray crystallography studies showed the structure of ABA facilitates the binding of ABA to PYR/PYL/RCAR receptors and thereby inhibits interaction with PP2Cs. The AS6 ABA analog was able to block PYL-PP2C interaction, indicating that binding of ABA to PYL receptors initiates ABA responses by repressing PP2Cs.

Inhibition of PP2Cs results in autoactivation of SnRK2 kinases and thereby positive regulation of ABA signaling. Recently, the crystal structures of SnRK2.3 and SnRK2.6 were elucidated, providing evidence that kinase activation is a two-step mechanism as well as details of how the ABA signal is transmitted to downstream components [37]. This study also showed that autophosphorylation of SnRK2.6 is more efficient than that of SnRK2.3.

Lumba *et al*. [20] did a comprehensive transcriptomic data analysis in order to generate a mesoscale ABA signaling network. They showed that there are 3 main kinase hubs, MAP3Kδ4, SnRK3.15, and SnRK3.22, that interact with PP2Cs and these kinases act as negative regulators of ABA response, in contrast to the SnRK2s involved in ABA signaling. SnRK3.15 and SnRK3.22 also interact with a large number of transcription factors and may have a role in overall ABA responses in the plant [20].

3. ABA in stress signaling

In plants and other organisms, such as algae, cyanobacteria, and fungi, ABA levels tend to increase with exposure to stress, suggesting a potential role of ABA in stress signal transduction [20]. Exogenous ABA application mimics stress conditions in plants and provides a useful means to study the effect of ABA on stress signaling and tolerance [38]. ABA distributes throughout the plant as an inactive glucose sugar conjugate and is converted to the active form by β-glucosidase [4]. ABA acts as an endogenous messenger and salt and drought stress signal

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transmission to initiate downstream gene expression occurs mainly via ABA signaling. However, cold stress signal transduction occurs in an ABA-independent manner via the C-REPEAT/DEHYDRATION RESPONSIVE ELEMENT BINDING FACTORS (CBFs/DREBs) signaling pathway [39].

A large number of ABA-responsive genes have a common *cis*-element called the ABRE element in their promoter regions. An ABRE together with a Coupling Element (CE) results in ABA induction of gene expression [19]. The **A**BA **R**ESPONSIVE **E**LEMENT **B**INDING FACTOR (AREB/ABF) family of transcription factors are the major transcription factors that regulate ABA-induced gene expression. AREB/ABFs are bZIP transcription factors and their expression is induced by ABA and other potential stress conditions [40]. Different ABFs are induced by ABA at different rates. For instance, *ABF2*, *ABF3*, and *ABF4* are induced faster than *ABF1*. Moreover, *ABF1* is induced only by cold stress, whereas *ABF2* and *ABF3* are induced by salt stress. *ABF4* levels are induced by salt, drought, and cold stress, suggesting that distinct ABFs have roles in various ABA-dependent stress-responsive pathways [40].

There are nine Group A bZIP transcription factors implicated in ABA signaling and they are subdivided into two groups based on where they are mainly expressed. The *ABI5*/*AtDPBF* family of genes includes *ABSCISIC ACID INSENSITIVE 5 (ABI5), ENHANCED EM LEVEL (EEL),* and *AREB3* and are expressed in the seed during seed maturation [41]. Other AREB/ABF transcription factors are mainly expressed in vegetative tissues [40].

SnRK2 III is the major subfamily of SnRKs involved in abiotic stress responses. SnRK2 protein kinases phosphorylate AREB/ABFs and regulate their function in ABA-regulated gene expression under stress conditions [10]. SnRK2.6/OST1 is an important regulator of stomatal closure in drought stress. The role of SnRK2.2 and 2.3 is mainly to transmit the ABA signal to inhibit seed germination and seedling growth in response to stress. A decuple *snrk2* mutant in Arabidopsis, which carries mutations for all 10 SnRK2 members, was hypersensitive to osmotic stress and also defective in ABA accumulation and ABA-induced gene expression under osmotic stress, indicating the critical role of SnRK2 kinases in osmotic stress signaling and tolerance. Moreover, the *snrk2.2/3/6* triple mutant had impaired accumulation of proline, which is a compatible osmolite [42].

3.1. ABA and abiotic stress tolerance

In *Arabidopsis thaliana*, about 10% of the genome consists of ABA-regulated genes. Approximately half of these genes are ABA-induced genes and the rest are ABA-repressed. ABA-induced genes code for proteins that confer stress tolerance such as dehydrins, detoxifying enzymes of reactive oxygen species, regulatory proteins (transcription factors, protein kinases, phosphatases) and enzymes required for phospholipid signaling. Genes that are repressed by ABA are mostly related to growth [5]. ABA biosynthesis mutants identified in Arabidopsis [43] and other crop plants [44] wilt and die under prolonged salt and drought stress, suggesting ABA plays an important role in osmotic stress tolerance.

Drought and high salinity generate osmotic stress in plant cells. Endogenous ABA levels are elevated in response to osmotic stress, which in turn coordinates the plant's response to

reduced water availability. In addition, seed maturation and post-germinative growth creates cellular dehydration stress, which again results in accumulation of ABA in cells [45]. The role of ABA in drought and salt stress is twofold: water balance and cellular dehydration tolerance. Water balance is achieved through guard cell regulation and the latter role by induction of genes that encode dehydration tolerance proteins in nearly all cells. ABA accumulation is induced by osmotic stress and this is as a result of activation of ABA biosynthesis as well as inhibition of ABA degradation [46]. Thus, ABA-mediated adaptive stress responses of plants to environmental stimuli occur via ABA-responsive gene expression and regulation of stomatal pore size. ABA-responsive gene expression involves various transcription factors, ABA receptors, secondary messengers, protein kinase/phosphatase cascades, and chromatin remodeling factors [45].

Both drought stress and salinity stress upregulate osmotic stress responsive genes that are ABA-inducible. Most of the high-salinity-induced genes are also induced by drought, suggesting there is overlap between salt and drought stress tolerance mechanisms [6]. A large number of transcription factors are induced by multiple stress conditions. AREB1/ABF2, AREB2/ABF4, ABF3, and MYB41 are some of the main transcription factors that are induced by both salt and drought in vegetative tissues [45].

Drought and salt stress results in osmotic imbalance; thus, salt and drought stress tolerance mechanisms aim at restoring cellular homeostasis. These mechanisms are adaptive responses that create either stress tolerance or avoidance of stress conditions. Modifications in metabolic pathways, synthesis of new proteins, changes in ion uptake, and free radical scavenging are some of the stress responses at the cellular level, immediately followed by stress signal transduction [47]. High ABA levels in cells result in synthesis of storage proteins, desiccation tolerance, and dormancy via inhibition of seed germination [22]. In the plant as a whole, key adaptive responses include induction of stomatal closure as well as control of seedling growth and lateral root formation. While the balance between ABA and auxin levels slightly affects primary root growth, ABA represses lateral root formation while auxin promotes it [4,48].

Inhibition of seed germination under abiotic stress is another function of ABA. Seed germination occurs when there is a balance between germination-promoting gibberellin and dormancy-promoting ABA. During late stages of maturation, seeds accumulate ABI5 which in turn activates transcription of LATE EMBRYOGENESIS ABUNDANT (LEA) proteins. LEA proteins confer osmotolerance to the embryo. ABA is necessary for activation of ABI5 via SnRK2.2 and SnRK2.3 phosphorylation of ABI5 [49,50]. When seeds are in unfavorable environmental conditions, elevated endogenous ABA levels results in ABI5 accumulation, preventing seeds from germinating.

3.1.1. ABA and salt tolerance

Salt stress severely impacts plant growth by affecting metabolic processes and photosynthetic efficiency. NaCl initially induces osmotic stress and eventually accumulation of both Na^+ and Cl^- ions generates ionic stress [51]. However, some responses are salt-specific and distinct from responses to osmotic stress (reviewed in [52]). High salinity in the soil is first sensed by the plant roots. Salt and drought stress induce a rapid increase in cytosolic Ca^{2+} levels in the root

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cells. Ca^{2+} acts a second messenger, inducing salt- and drought-responsive genes [53,54]. Hyperosmotic stress is coupled with Ca^{2+} signaling and Reactive Oxygen Species (ROS) signaling, thereby inducing a cascade of signaling events, which results in downstream gene expression [52].

Biochemical and molecular mechanisms of salt tolerance in plants include exclusion of salt ions, production of suitable osmolytes, changing the structure of the membranes to control ion uptake, and induction of enzymes that produce antioxidants and phytohormones. To manage salt or drought stress, cellular ABA levels increase dramatically. The plant cuticle has been shown to mediate stress signaling as well as ABA biosynthesis and signaling. In addition to its primary function, providing mechanical support to the cell wall and plasma membrane, the cuticle has been implicated in osmotic stress regulation. CED1 (9-CIS EPOXYCAROTENOID DIOXYGENASE DEFECTIVE 1) is an essential protein in cuticle biogenesis. *ced1* mutants are sensitive to osmotic stress, as they are unable to induce ABA biosynthesis in response to osmotic stress [55].

ABA regulates root growth and architecture in plants under stress. Duan *et al*. [56] showed that salt has a strong inhibitory effect on lateral root growth, while primary roots are less sensitive to salt stress. They also showed that endogenous ABA signaling affects root system architecture under stress conditions using ABA biosynthesis mutants (*aba1, aba2*) as well as signal transduction mutants such as *abi1*. Salt stress results in elevated levels of ABA exclusively in lateral root cells and induces a quiescent period in postemergence lateral roots. Lateral roots in a quiescent stage form a thick, well-developed Casparian strip, which acts as a barrier to reduce diffusion of Na^+ ions through the endodermis. In the presence of Na^+ ions, endodermal cells activate ABA signaling and arrest growth so that lateral roots do not elongate into high saline environments. Therefore, ABA is an important signaling molecule in suppressing lateral root growth during salt stress [56].

ABA regulates expression of many salt-stress-responsive genes via transcription factors that are elevated in response to salt. For instance, ABF2/AREB1, ABF3, ABF4/AREB2, ABRE BINDING PROTEIN 9 (ABP9), and MYC/MYB, WRKY, and APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) are some of the salt-stress-responsive transcription factors that enhance stress tolerance. A recent study showed that the PYL8/RCAR3 ABA receptor has a role in ABA-mediated inhibition of primary root growth and also recovery of lateral root growth on exposure to ABA. PYL8/RCAR3 combines the action of ABA and auxin through direct interaction with MYB transcription factors during growth recovery of postemergence lateral roots [48,57].

There are proteins in the cell that are produced in an ABA-dependent manner that have a role in osmotic tolerance. For example, ABI5 activates transcription of LEA proteins. LEA proteins are highly hydrophilic small proteins shown to have an osmoprotectant role against cellular dehydration during late embryogenesis. LEA proteins also have a role in salt stress tolerance [58]. Due to their hydrophilic nature, LEA proteins can sequester ions accumulating in the cell, as well as act as chaperones and retain water molecules to prevent protein aggregation and inactivation of cellular enzymes [59]. In Arabidopsis, 51 LEA proteins have been identified that belong to nine different groups [60]. Jia *et al.* [61] showed overexpression of AtLEA14,

which belongs to the LEA group 2 proteins, overactivates salt-stress-inducible genes such as *RD29B*, which encode dehydration protective proteins, and subsequently confers salt tolerance in Arabidopsis.

In addition, ABA has been implicated in histone H3 acetylation and methylation, thereby regulating stress-inducible gene expression at the epigenetic level. Chen *et al.* [62] showed that histone modifications by HISTONE DEACETYLASE 6 (HDA6) are involved in inhibition of seed germination, salt stress responses, and ABA- and salt-mediated gene expression in Arabidopsis.

3.1.2. ABA and drought tolerance

Drought is lack of water in the soil. Drought stress in plants arises due to water deficit conditions and results in removal of water from the cell membranes, disrupting the lipid bilayer structure. In addition, protein denaturation and accumulation of cellular electrolytes results in disruption of cellular metabolism [63]. Therefore, drought causes osmotic stress, and osmotic stress causes dehydration and inhibition of water uptake in plants. ABA accumulates under osmotic stress conditions and plays an important role in the stress response and tolerance of plants. In addition to autoactivation of SnRK2s by inhibition of PP2Cs in the ABA signaling cascade, hyperosmotic stress activates SnRK2s [64]. SnRK2 kinases are a major component of the osmotic stress signaling pathway. The Arabidopsis triple mutant *snrk2.2, snrk2.3, snrk2.6* shows severe drought intolerance and ABA-insensitivity [42]. Also ABF2, ABF3, and ABF4 act as transcriptional activators in mediating ABRE-dependent ABA signaling, which confers drought tolerance in vegetative tissues [40].

ABA induces expression of many transcription factors as well as genes that encode enzymes in the synthesis of osmoprotectants [65]. Osmolytes are compatible solutes such as amino acids (proline), sugar alcohols (mannitol, pinitol), and other sugars that accumulate without disrupting the function of proteins. Osmolytes make an osmotic adjustment facilitating a favorable water potential gradient and promote stress tolerance [66].

Dehydrins and LEA-like proteins act as cellular chaperones that protect cellular membranes and macromolecules in the cell [2]. During seed maturation seeds undergo dehydration stress. LEA proteins accumulate in the embryo as a result of osmotic stress and their functions include protection of enzymes, lipids, and mRNAs from dehydration. LEA proteins have been found to protect mitochondrial membranes from damage. LEA proteins are produced in an ABA-dependent and ABA-independent manner under osmotic stress [47,58].

Under moderate water stress conditions plant root growth has to be maintained in order to keep the plants alive. ABA accumulates under moderate water stress and mediates auxin transport in the root tip, which enhances the proton pumps in the plasma membrane. Proton secretions in the root tip play an important role in primary root growth and root hair development under moderate drought stress [67].

Based on the critical water level, drought tolerance is considered to be mechanisms that confer tolerance to moderate dehydration. Further dehydration requires desiccation tolerance mechanisms in order to restore the ability of cells to rehydrate successfully [68].

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3.1.3. ABA and desiccation tolerance

Water loss results in a change in turgor pressure that affects the cell walls. Desiccation tolerance is defined as evolution of cell walls that can withstand extensive water loss without damaging its structure or polymer organization. Desiccation tolerance mechanisms aim to restructure the cells walls and maintain normal growth under water stress conditions [69].

Regulation of the stomatal pore is crucial in adapting plants to abiotic stress by reducing extensive water loss. Stomatal opening and closing occurs as a result of turgor pressure differences in the surrounding guard cells [47]. In response to water stress, ABA concentration is increased in the guard cell cytoplasm and apoplast, which results in a decrease in the turgor pressure due to activation of the K^+ outward rectifying channel and inhibition of the K^+ inward rectifying channel (KAT1 and 2). ABA also induces the anion channel SLAC1 resulting in release of anionic organic acids from the vacuole to the cytoplasm [70]. Reduced turgor pressure initiates closure of stomata as a mechanism of minimizing water loss from the plant. ABA levels rise in leaves immediately following water stress. CHLH/ABAR has been proposed as the chloroplast ABA receptor that links ABA signaling within the chloroplast with ABA signaling in the nucleus. Overexpression of CHLH promotes stomatal closure and thereby dessication tolerance [71].

SnRK2 OPEN STOMATA 1 (OST1) is a key SnRK2 protein kinase involved in regulation of the stomatal aperture by movement of guard cells during ABA signaling [72]. OST1 is activated by ABA, low humidity, and osmotic stress and is an important kinase found in guard cells preventing rapid water loss. Loss of function mutants of SnRK2 do not exhibit ABA-mediated stomatal closure activity and showed a wilty phenotype under dehydration stress conditions [72,73]. Also SnRK2.6/OST1 physically interacts with ABI1 and ABI2. ABI1 is required for ABA-dependent activation of OST1 and both ABI and ABI2 are required for osmotic-stress-induced activation of OST1 [15]. Thus, SnRK2.6/OST1 acts as a positive regulator in ABA-induced stomatal closure. Moreover, Yoshida *et al.* [73] showed that OST1 also positively regulates stress-responsive genes such as *RD29B* and *RD22*.

Reactive Oxygen Species (ROS) have also been identified as secondary messengers in ABA signaling in guard cells. In Arabidopsis, two partially redundant guard cell expressed NADPH oxidase catalytic subunit genes, *AtRbohD* and *AtRbohF*, were found to be involved in ABA signaling in guard cells, ABA-induced stomatal closure and ROS production, ABA activation of Ca^{2+} permeable channels in the plasma membrane of guard cells, and increasing cytosolic Ca^{2+} levels in response to ABA. Thus, these two genes act as positive regulators of ABA signal transduction [74]. Sirichandra *et al.* [72] provided biochemical evidence that OST1 protein kinase physically interacts with AtRbohF NADPH oxidase and phosphorylates it.

4. Conclusions

ABA has a wide range of functions from plant development to biotic and abiotic stress signaling and tolerance. The primary functions of ABA in salt, drought, and desiccation

tolerance act via inhibiting seed germination, altering root architecture, and inducing stress-responsive genes as well as gene products that act as osmoprotectants. ABA signaling cascades and stress tolerance mechanisms studied in Arabidopsis provide insight into application of stress tolerance strategies to commercial crops. While ABA is not the only plant hormone involved in stress responses, many of these responses occur in an ABA-dependent manner, indicating the importance of ABA in plant stress response and tolerance.

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