Review

Mechanisms of Plant Responses and Adaptation to Soil Salinity

Chunzhao Zhao,[1,2,*] Heng Zhang,[3] Chunpeng Song,[2] Jian-Kang Zhu,[1,4,*] and Sergey Shabala[5,6,*]

Soil salinity is a major environmental stress that restricts the growth and yield of crops. Understanding the physiological, metabolic, and biochemical responses of plants to salt stress and mining the salt tolerance-associated genetic resource in nature will be extremely important for us to cultivate salt-tolerant crops. In this review, we provide a comprehensive summary of the mechanisms of salt stress responses in plants, including salt stress-triggered physiological responses, oxidative stress, salt stress sensing and signaling pathways, organellar stress, ion homeostasis, hormonal and gene expression regulation, metabolic changes, as well as salt tolerance mechanisms in halophytes. Important questions regarding salt tolerance that need to be addressed in the future are discussed.

*Corresponding author:
Email: czzhao@psc.ac.cn (C.Z.),
jkzhu@sibs.ac.cn (J.-K.Z.),
sergey.shabala@utas.edu.au (S.S.)

Introduction

Soil salinity is a worldwide problem that threatens the growth and yield of crops and prevents the sustainable development of modern agriculture. More than one-third of irrigated lands in the world are affected by salinization.[1] The major causes of soil salinity are rising levels of groundwater with high salt content and poor-quality drainage and irrigation systems.[2] All the major staple crops responsible for the bulk of calorie uptake by humans (e.g., rice, wheat, and corn) are glycophytes, which are unable to complete their life cycle when soil NaCl concentrations exceed 200 mM.[3,4] Thus, improving salinity stress tolerance in crops is of paramount importance for global food security. To achieve this goal, it is necessary to understand how high salinity affects the morphological, physiological, biochemical, metabolic, and gene expression properties of plants.

The ability of plants to tolerate high salinity varies between and within species,[4] which enables us to identify gene loci and natural variations that are critical for salt stress tolerance in plants. Fundamental studies in the model plant *Arabidopsis* have revealed many genes that are required for salt stress tolerance, and applications of some of these genes to crops increase their salt stress tolerance. The discovery of the salt overly sensitive (SOS) signaling pathway,[5,6] a major mechanism behind the exclusion of Na+ from the cytosol to the outside of cells, was a milestone in our understanding of how plants deal with salt load. Recent work showed that glycosyl inositol phosphorylceramide (GIPC) sphingolipids may function as salt stress sensors in plants.[7] Research on halophytic plants, which usually reside in high salinity environments, have also been important for us to understand salt tolerance mechanisms in plants. Some halophytes have

[1]Shanghai Center for Plant Stress Biology and CAS Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai 200032, China;
[2]State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, Henan University, Kaifeng 475004, China;
[3]State Key Laboratory of Plant Molecular Genetics, Shanghai Center for Plant Stress Biology, Center of Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai 200032, China;
[4]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA;
[5]International Research Centre for Environmental Membrane Biology, Foshan University, Foshan 528000, China;
[6]Tasmanian Institute of Agriculture, University of Tasmania, Hobart, TAS 7001, Australia

developed special structures, such as epidermal bladder cells that accumulate excessive Na^+ in their vacuoles, which enable them to adapt to high salinity. Recent progress in understanding the mechanisms underlying the salt stress tolerance of halophytes will facilitate the breeding of salt-tolerant crops. This review provides a synopsis of salt stress responses and adaptation in plants and considers the newest developments in the field. These developments include adaptive physiological responses, sensing and signaling pathways, salinity-induced stress on specific organelles, hormonal regulation, ion homeostasis, metabolic changes, and salt tolerance mechanisms of halophytes.

Physiological Responses to Salt Stress

Salinity stress inhibits plant growth and development by imposing several major constraints. The first constraint is an osmotic stress (the lowering of the external water potential) that compromises a plant's ability to take up water. This process triggers several major events in plant tissues. At the macroscopic level, expansion of both root[8] and shoot[9] cells is immediately arrested as a result of a decreased turgor pressure. To deal with this issue, plants must adjust osmotically. Most of the cell turgor in the root is regained within 40–60 min[10] by an increase in the uptake of inorganic ions, and growth is resumed, although at a reduced rate. The latter fact is most likely explained by modification of the composition of the cell wall resulting from the binding of Na^+ to cell wall components.[11] Osmotic stress also results in rapid closure of stomata, which reduces the plant's ability to assimilate CO_2. Rapid closure of stomata can be explained by the rapid drop in xylem pressure (e.g., by 0.05 MPa in maize roots exposed to 100 mM NaCl)[12] that accompanies salinity stress. This drop in xylem pressure occurs within minutes upon stress onset, and the hydraulic signals sensed by roots move at the speed of sound[13] and are transduced almost instantaneously to the shoot, where they are decoded and alter shoot metabolism.[14] Because stomatal guard cells possess a range of mechano-sensitive (stretch-activated) ion channels,[15,16] they could potentially transduce a change in the xylem pressure caused by salinity into altered stomatal apertures.

The second constraint imposed by salinity is ionic imbalance (often called "ionic stress" or "ion toxicity" in the literature). In most cases, this constraint is associated with an excessive accumulation of Na^+ and Cl^- in metabolically active intracellular compartments. Surprisingly, the mechanistic basis of such toxicity is poorly understood. Although it is well known that Na^+ can harm plant metabolism and can potentially kill the plant,[17] the target(s) of Na^+ in the plant is unknown. The most common explanation for Na^+ toxicity is that it has an inhibitory effect on the activities of enzymes. The cytosolic compartment, for example, contains many enzymes involved in primary metabolism, the Calvin cycle, the phenylpropanoid pathway, glycolysis, and polyamine and starch synthesis. Many of these enzymes are controlled by K^+.[18] Given the close similarity between Na^+ and K^+,[19] it is usually accepted that the Na^+ tends to replace K^+ in those enzymatic reactions, but with much less efficiency.[14,18,20] In addition Cheeseman,[17] noted that biomacromolecules occupy 20%–30% of the cytoplasmic volume. Because biomacromolecules are complexed and charged 3D structures, their operation is strongly affected by electrostatic interactions and the ionic strength of the solution, as well as by the presence of both screened and unscreened electrostatic forces.[21] As a result, small hydrated Na^+ ions tend to accumulate in the regions of greater density, while larger K^+ ions tend to be found in the less dense regions. This differential intracellular ion distribution will have an impact on cell operation, as the fixed charge and ionic conditions of the cytosol will inevitably determine the local water relations relevant to the cytoskeleton and proteins.[17]

A related issue is chloride (Cl^-) toxicity. The current notion is that Cl^- exclusion from the shoot is crucial for salt tolerance.[22] This inference is supported by the findings in certain salt-sensitive species that high shoot Cl^- levels are positively correlated with severe physiological dysfunctions.[23,24] However, the negative correlation between shoot Cl^- concentration and plant biomass

recorded for some salt-grown non-halophytes does not hold for halophytes,[23] some of which are capable of accumulating Cl^- at a concentration >1 M without experiencing a major negative effect on plant performance.[25] Researchers have argued that the detrimental effects of Cl^- on plant performance may be not a result of toxicity *per se* but rather from a Cl^--induced deficiency of key macro-nutrients (e.g., N and S), as uptake of NO_3^- and SO_4^{2-} are mediated by the same (non-selective) anion transporters as Cl^-.[23] It therefore appears that the negative effect caused by either Na^+ or Cl^- is not a nutrient toxicity *per se* but instead results from interference with the uptake or metabolism of other essential ions. For this reason, the use of the term ionic imbalance is more suitable and should be used instead of the more popular specific ion toxicity.

Another widely held misconception is related to the timescale imposed by these two constraints. The traditional view is that ionic stress has a slower speed of onset than osmotic stress and operates at a timescale of days if not weeks.[26,27] Although this view does apply to shoot tissues, it does not apply to the plant as a whole. In response to ionic stress, various PCD (programmed cell death) events are observed in roots at a much more rapid timescale. Apoptotic events, such as DNA laddering or cytochrome *c* release, are observed in plant roots within hours of salinity exposure.[28–30] In *Arabidopsis* roots, the level of autophagy (another form of PCD) peaks within 30 min of salt stress.[31] Importantly, these salinity-induced PCD events are Na^+ specific and not related to the osmotic component of salt stress.[32,33] The salinity-induced PCD events in plant roots are significantly reduced or prevented in *Arabidopsis* mutants lacking a gated outwardly rectifying K^+ channel (GORK),[34] suggesting a causal link between Na^+ entry into the cytosol, resulting in membrane depolarization and accompanied by K^+ efflux, and activation of caspase-like proteases and endonucleases that execute PCD.[35,36]

Salinity and Oxidative Stress

Reactive oxygen species (ROS), which function as versatile signals, are rapidly induced by a variety of environmental stresses, including pathogen infection, high salinity, drought, and heat stress.[37] The major ROS in plants include hydrogen peroxide (H_2O_2), superoxide anion ($O_2 \cdot^-$), singlet oxygen ($^1O_2$), and hydroxyl radical ($OH \cdot$). These ROS are mainly produced in the apoplast, chloroplasts, mitochondria, and peroxisomes.[37] Production of ROS in the apoplast is mediated by plasma membrane-localized respiratory burst oxidase homologs (such as AtRbohD and AtRbohF), apoplastic diamine oxidase, peroxidase, and polyamine oxidases.[37] *AtRbohD* and *AtRbohF* genes are both upregulated under salt stress, and simultaneous mutations of these two genes result in hypersensitivity to salt stress.[38] The mechanisms underlying the positive roles of AtRbohD/AtRbohF in salt stress tolerance have been revealed by several studies. Salt-induced production of ROS by AtRbohD/AtRbohF promotes the movement of K^+ into the cytosol and thus reduces Na^+/K^+ ratios.[38] AtRbohF is able to restrict the distribution of Na^+ in xylem sap, and thereby reduces the delivery of Na^+ from roots to shoots via transpiration.[39] AtRbohD mediates the propagation of long-distance signals triggered by a variety of environmental stimuli, including high salinity, wounding, heat, cold, and high-intensity light,[40] suggesting that ROS are required for systemic signaling in plants. AtRbohD/F-mediated production of H_2O_2 at the early stage of salt stress could be a signal that triggers an antioxidative response that reduces the oxidative damage to cells.[41] ROS production in the apoplast contributes to lignin formation under a saline environment.[42]

In shoots, both osmotic stress-induced stomatal closure and accumulation of high levels of Na^+ in the cytosol impair the photosynthetic machinery. As a result, the amount of absorbed light exceeds the demand for photosynthesis, which leads to the formation of ROS in green tissues.[43] There are three major sites of ROS production in chloroplasts: (1) the Mehler reaction in the PSI; (2) $^1O_2$ production by photosystem II (PSII) in the thylakoid membrane resulting from limitation of the electron transport chain between photosystems; and (3) H_2O_2 production at the electron donor side of PSII via incomplete oxidation of water due to the

inhibition of the water-splitting manganese complex.[44,45] Another major source of ROS production in salt-affected plants is mitochondrial respiration. Over-reduction of the ubiquinone pool during salt stress allows electrons to leak from complexes I and III of the mitochondrial electron transport chain to molecular oxygen, which results in $O_2 \cdot^-$ production.[37] The peroxisome is a major site for the production of intracellular H_2O_2.[46] The reduced CO_2/O_2 ratio in plant cells under salt stress promotes H_2O_2 production in peroxisomes through enhanced photorespiration.[47]

At low concentrations, ROS act as signal molecules to regulate many biological processes, including plant growth and responses to a variety of biotic and abiotic stresses. An excessive accumulation of ROS under saline conditions, however, has detrimental effects on plant tissues. The detrimental effects of ROS are traditionally attributed to their ability to damage key cellular structures, including lipid peroxidation in cellular membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown, and an impairment of enzymatic activities.[48] Before this damage to key cellular structures occurs, however, stress-induced accumulation of ROS disturbs ionic homeostasis in the cells by activating many different types of ROS-sensitive ion channels. H_2O_2-sensitive Ca^{2+}-permeable ion channels have been found in both root epidermal cells[49] and stomatal guard cells.[50] Constitutively expressed inward-rectifying K^+ channels in stomata are also inhibited by H_2O_2.[51] $OH \cdot$, which is generated upon H_2O_2 reduction in cell walls, has a much wider action spectrum and can activate a broad range of non-selective (thus, Na^+-permeable) cation channels[52,53] as well as GORK-like K^+ efflux channels,[34,54] annexin-mediated conductance,[55] and a Ca^{2+}-pump.[54] ROS-regulated ion channels are also present at organellar membranes (e.g., chloroplasts,[56] vacuoles[57]) and alter the operation of these organelles. For example, the salt stress-induced decrease in the photosynthetic performance of chloroplasts is associated with the swelling of thylakoids[58] that results from increased ion fluxes across the thylakoid membrane via H_2O_2-activated ion channels.[56]

To reduce the oxidative stress caused by the accumulation of ROS under high salinity, plants rely on activation of ROS-scavenging machineries. The enhanced tolerance of halophytes to high salinity is to some extent due to an enhanced capacity to maintain ROS homeostasis.[59] The scavenging of excessive ROS under high salinity may be attributed to non-enzymatic antioxidant metabolites, including ascorbate, glutathione, and tocopherols, and to enzymatic agents, such as catalases (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR).[60] Enhancement of these antioxidant systems increases salt stress tolerance in plants. For example, the increased accumulation of total glutathione in peroxisomes, chloroplasts, and mitochondria conferred increased tolerance to salt stress,[59,61] and a reduction in the ascorbate content in the *vtc2-1* mutant is correlated with a reduction in salt stress tolerance.[62] In chloroplasts, SOD catalyzes the transfer of $O_2 \cdot^-$ to H_2O_2, and APX is required for the conversion of H_2O_2 to H_2O.[43] Plants that overexpress the *APX* gene show enhanced tolerance to salt and drought stresses.[63] NCA1, a chaperone protein that is required for the maintenance of the functional state of CAT, positively regulates salt stress tolerance.[64] In mitochondria, alternative oxidase (AOX) and manganese SOD (Mn-SOD) are the major enzymes involved in the detoxification of ROS. Plants with higher activities of mitochondrial AOX1 and Mn-SOD exhibit increased tolerance to salt and drought stress.[65,66]

Salt Stress Sensors

To avoid the damage caused by the high concentrations of salts in soil, plants must have evolved the ability to sense salt stress, to transduce signals to cell interiors, and to adjust cellular traits. Identification of salt stress sensor(s) has been challenging, perhaps because of the functional redundancy of sensors, technical difficulties, or lethality when salt stress sensors are knocked out. Many abiotic stresses, including high salinity, drought, and cold, trigger increases in the cytosolic Ca^{2+} concentration within seconds to minutes.[67,68] For this reason, identification of proteins or other components that are

required for the rapid influx of Ca^{2+} under stress conditions is considered to be a good way to discover stress sensors. Based on this possibility, researchers have conducted genetic screens for mutants that are defective in the activation of Ca^{2+} signaling under a variety of environmental stresses.

Reduced hyperosmolality-induced $[Ca^{2+}]_i$ increase1 (*OSCA1*), which encodes a hyperosmolality-gated calcium-permeable channel, was identified as an osmotic stress sensor.[69] Mutation in the *OSCA1* gene impairs osmotic stress-induced Ca^{2+} signaling in guard cells and root cells, which results in reduced stomatal closure and root growth in response to osmotic stress. The *osca1* mutant does not show altered phenotypes under high salinity, which raises a question about the role of OSCA1 in the sense of high salinity-triggered osmotic stress. Using a similar screening strategy, researchers recently identified *monocation-induced $[Ca^{2+}]_i$ increases 1* (*MOCA1*), which encodes a glucuronosyltransferase that is involved in the biosynthesis of glycosyl inositol phosphorylceramide (GIPC) sphingolipids and that is specifically required for spikes in cytosolic Ca^{2+} in response to ionic stress but not in response to osmotic stress.[7] Mutation in the *MOCA1* gene leads to reduced weight and reduced survival under salt stress. GIPCs were shown to directly bind to Na^+ and regulate the entry of Ca^{2+} into the cytosol.[7] However, which Ca^{2+} channels are involved in this process and how binding of Na^+ to GIPCs activates Ca^{2+} channels remain unknown.

Other proteins that mediate salt-induced Ca^{2+} signaling have also been reported. These proteins include FERONIA (FER), annexin1 (ANN1), and plastid K^+ exchange antiporters (KEAs).[70–72] FER, a member of the *Catharanthus roseus* receptor-like kinase 1-like (*Cr*RLK1L) protein family, directly binds to pectin. Loss of function of *FER* results in reduced salt-induced Ca^{2+} signaling and increased sensitivity to high salinity.[70] ANN1 mediates ROS-activated Ca^{2+} influx in response to increased accumulation of extracellular Na^+. Mutation in the *ANN1* gene leads to increased Na^+ influx and impaired salt-

induced transcriptional and growth adaptation.[71] The KEA1/2 and KEA3 transporters are required for osmotic stress-induced Ca^{2+} response, suggesting that KEA1/2 and KEA3 may function as sensors of osmotic stress.[72]

In addition to sensing salt stress via Ca^{2+} signaling, plants may also sense salt stress by recognizing salinity-induced changes in cellular structures. High salinity rapidly reduces turgor pressure, which is the consequence of osmotic stress-mediated water loss. The reduced turgor pressure can be perceived by mechanosensitive sensors, such as MscS-like (MSL), Midi-complementing activity (MCA), and two-pore potassium (TPK) family proteins.[73] MSL8 is required for the survival of pollens subjected to the hyperosmotic shock of rehydration,[74] and MSL2 and MSL3 are required for the adaptation of plastids to hyperosmotic stress.[75] It is well known that excessive accumulation of ions in the apoplast affects the properties of cell wall components, which are perceived by cell wall-localized glycoproteins or plasma membrane-localized receptor-like kinases.[76] Proteins potentially involved in sensing cell wall changes include hydroxyproline-rich glycoproteins (HRGPs), wall-associated kinases (WAKs), and *Cr*RLK1L family proteins.[76–79] WAKs are able to bind to pectin and Ca^{2+}, and excessive Na^+ may affect these interactions and thus trigger stress signaling.[80] Recent studies indicate that the cell wall-localized leucine-rich repeat extensins LRX3, LRX4, and LRX5, together with secretory peptides RALFs and the receptor-like kinase FER, are involved in sensing and relaying salt stress signals by monitoring the status of cell wall integrity, although the initial sensing of salinity-triggered cell wall changes by the LRXs is still not understood.[79] FER inhibits the proton transport activity of plasma membrane H^+-ATPase (AHA2) and thus regulates pH in the apoplast.[81] FER is also required for the activation of cell wall repair pathways to maintain cell wall integrity under high salinity.[70] FEI1 and FEI2, two leucine-rich repeat receptor kinases (LRR-RKs), are also associated with cell wall integrity sensing. Loss of function of *FEI1* and *FEI2* results in hypersensitivity to high sucrose and high salt.[82,83]

Ionic Stress Signaling

The environmental stimuli-triggered Ca^{2+} influx signal into the cytoplasm can be decoded by diverse Ca^{2+}-dependent proteins, such as calcium-dependent protein kinases (CDPKs), calcineurin B-like proteins (CBLs)/SOS3-like calcium-binding proteins (SCaBPs), CBL-interacting protein kinases (CIPKs)/protein kinases of the SOS2 family (PKS).[67] In *Arabidopsis*, the SOS signaling pathway, which consists of SOS3 and SCaBP8, SOS2, and SOS1, plays a critical role in the regulation of Na^{+}/K^{+} ion homeostasis[5,6,84] (Figure 1). SOS3 and SCaBP8 relay salt-induced Ca^{2+} signals to SOS2 kinase.[84–86] SOS3 activates and recruits SOS2 to the plasma membrane via its interaction with the regulatory domain of SOS2.[5] SOS2 is a serine/threonine protein kinase belonging to the sucrose non-fermenting 1 (SNF1)/AMPK family.[5] Under normal conditions, the kinase activity of SOS2 is inhibited by 14-3-3 and GIGANTEA (GI) proteins. Salt stress promotes the degradation of 14-3-3 and GI, resulting in the release of SOS2 from SOS2-GI/14-3-3 complexes and consequently the activation of SOS2 by SOS3.[87,88] A recent report indicated that PKS5 inhibits the activity of SOS2 by promoting interaction between SOS2 and 14-3-3 proteins; the salt stress-induced Ca^{2+} signal induces the interaction between PKS5 and 14-3-3 and thus releases the inhibition of SOS2.[89] The kinase activity of SOS2 is also regulated by the protein phosphatase 2C ABI2.[90] SOS1 is a plasma membrane Na^{+}/H^{+} antiporter that is required for the extrusion of the excess of Na^{+} out of the cells (i.e., into the rhizosphere via root epidermal cells, or into the xylem via xylem parenchyma cells) and therefore for the alleviation of ionic stress. SOS1 is autoinhibited under normal conditions, and the inhibition is released by the phosphorylation of Ser1044 at the C-terminal domain of SOS1 by SOS2 under salt stress.[91] Notably, *sos* mutants only show a hypersensitive phenotype under high salinity but grow normally under osmotic stress imposed by mannitol or PEG (polyethylene glycol), indicating that the SOS signaling pathway is specifically involved in the response to ionic stress. SCaBP8 also mediates the regulation of Na^{+}/K^{+} ion homeostasis by modulating the activity of the K^{+} channel AKT1, a process that requires SOS2-mediated phosphorylation of SCaBP8.[92,93]

MAPK cascades[94–96] and phosphatidic acid (PA)[97] are also involved in the regulation of the salt stress signaling pathway. Salt stress activates MPK3, MPK4, and MPK6, which contribute to salt stress tolerance in *Arabidopsis*.[94,95,97] The activities of MPK4 and MPK6 under salt stress are induced by MKK2. Phenotypic analysis revealed that plants overexpressing *MKK2* exhibit increased salt stress tolerance, while *mkk2* null mutants are hypersensitive to salt stress.[94] In rice, the *osmkk1*-knockout mutant is hypersensitive to salt stress, which is caused by the impaired activation of *OsMPK4*.[95] Conversely, activation of MKK9 increases sensitivity to salt stress in *Arabidopsis*,[96] suggesting a complex regulatory network among distinct MAP cascades in response to salt stress. Salt stress triggers phospholipase D (PLDα)-mediated production of PA, which in turn regulates the salt stress response by activating MPK6. Activated MPK6 phosphorylates the C-terminal fragment of SOS1 and promotes salt stress tolerance.[97] Mutations in *PLDα1*, *PLDδ*, or *PLDα3* lead to hypersensitivity to salt stress.[98–100] PLD-mediated production of PA is also required for the activation of H^{+}-ATPase[101] and root hair formation under high salinity.[102]

Osmotic Stress Signaling

Whether cellular responses are induced by salinity-induced ion stress or osmotic stress can be determined by comparing plants exposed to high salinity with those exposed to isotonic non-ionic solutions, such as mannitol and PEG. Osmotic stress triggers signaling pathways that promote the biosynthesis and accumulation of compatible osmolytes, which is important for both short-term and long-term osmotic stress tolerance in plants. The increased levels of compatible osmolytes in the cytosol reduce water loss and enhance turgor pressure and cell expansion.[103] Both salt stress and osmotic stress can activate SNF1-related protein kinase 2s (SnRK2s).[104,105] Osmotic stress triggers the activation of
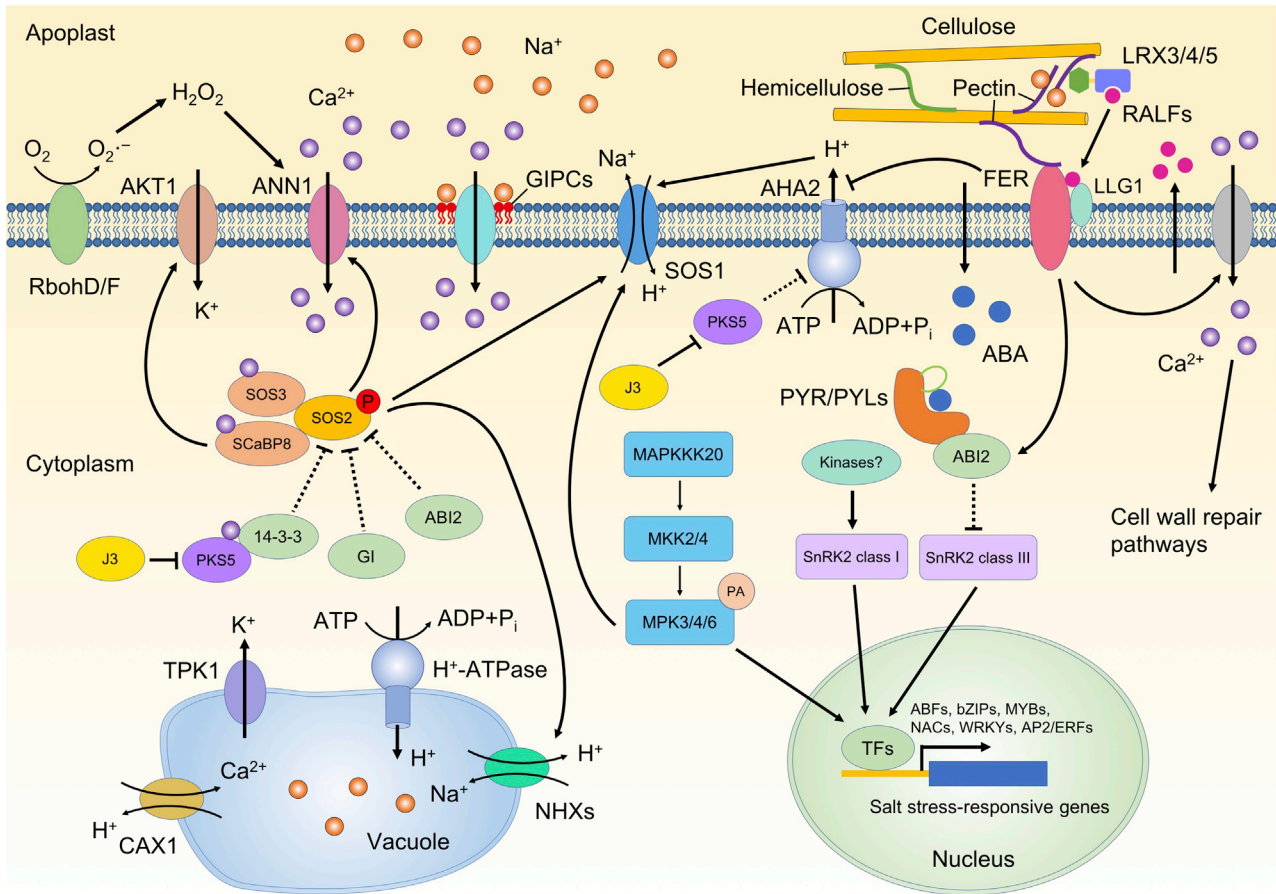
Figure 1 Salt Stress Signaling Pathways

The SOS signaling pathway, consisting of SOS3/SOS3-like calcium-binding protein 8 (SCaBP8), SOS2, and SOS1, is important for sensing salt-induced Ca^{2+} signals and in the regulation of ion homeostasis by extruding excessive Na^+ out of cells. 14-3-3, GI, and ABI2 negatively regulate the kinase activity of SOS2. Ca^{2+}-mediated binding of PKS5 with 14-3-3 releases the inhibition on SOS2. GIPCs act as putative salt stress sensors that directly bind to Na^+ and trigger Ca^{2+} influx via an unknown Ca^{2+} channel. GIPCs-mediated Ca^{2+} influx is required for the activation of the SOS signaling pathway. RbohD/F are involved in the production of ROS at the plasma membrane, and ROS can activate the ANN1-mediated Ca^{2+} signaling pathway. AKT1, which is regulated by SCaBP8, mediates the influx of K^+ to the cytosol under salt stress. MAP kinase cascades, including MAPKKK20, MKK2, MKK4, MPK3, MPK4, and MPK6, are involved in the relay of salt stress signals. Salt stress-induced accumulation of ABA activates subclass III SNF1-related protein kinase 2s (SnRK2s) via the PYR/PYLs-PP2Cs-mediated regulatory module. Subclass I SnRK2s are activated via an ABA-independent pathway under osmotic stress. Activated MPKs and SnRK2s transduce signals to downstream transcription factors, including ABFs, zips, MYBs, NACs, WRKYs, and AP2/ERFs, in the nucleus to induce the expression of stress-responsive genes. In the apoplast, cell wall-localized leucine-rich repeat extensins LRX3, LRX4, and LRX5, together with secreted peptides RALF22/23 and receptor-like kinase FER, function as a module to sense salt stress-induced cell wall changes. FER, RALFs, and LLG1 form a complex at the plasma membrane to trigger Ca^{2+} signaling and consequently activate the cell wall repair pathway. FER also inhibits the activity of AHA2 to regulate apoplastic pH. In the vacuole, NHXs, CAX1, TPK1, and H^+-ATPase are involved in the regulation of ion homeostasis under high salinity. The dashed lines indicate that the negative regulatory roles are released under salt stress.

SnRK2s in both ABA-dependent and -independent manners. SnRK2 kinases are divided into three groups: subclasses I, II, and III.[104,105] Subclass III SnRK2 kinases, including SnRK2.2, SnRK2.3, and SnRK2.6, play an important role in the transduction of ABA signals and in the regulation of downstream gene expression responses by activating AREB/ABF (ABA-responsive element binding factor) transcription factors.[106–108]

Subclass I SnRK2s, including SnRK2.1, SnRK2.4, SnRK2.5, SnRK2.9, and SnRK2.10, are specifically responsive to osmotic stress, but not to ABA.[104,109,110] The molecular mechanisms underlying the specificity of subclass I SnRK2s to osmotic stress are still unclear. It is likely that some early signaling components, such as kinases, can be activated by osmotic stress to trigger the activation of subclass I SnRK2s. Abiotic stress-responsive Raf-like kinases mediate the activation of SnRK2s under osmotic stress in the moss *Physcomitrella patens*,[110] and whether this is also the case in higher plants, such as *Arabidopsis*, needs to be investigated. Phenotypic analysis indicates that the simultaneous disruption of ten *SnRK2s* increases growth inhibition and leaf chlorosis under osmotic stress.[111] Among these SnRK2s, SnRK2.4 and SnRK2.10 are important in the maintenance of root growth and architecture under saline conditions.[105,112] The MKK4-MPK3 and MAPKKK20-MPK6 cascades are required for osmotic stress responses in *Arabidopsis*.[113,114] Loss of function of *MKK4* increases water loss and ROS accumulation under dehydration conditions, and salt-induced activation of MKK4 regulates the activity of MPK3 and increases the expression of abiotic stress-responsive genes *NCED3* and *RD29A*.[114] MAPKKK20 is required for the activation of MPK6 under various abiotic stresses. The plants overexpressing *MAPKKK20* exhibit enhanced tolerance to salt stress.[113]

Salt-Induced Organellar Stresses

Proper functioning of cells requires coordination among different organelles and cellular compartments. The cellular damage caused by abiotic stresses, including high salinity, drought, cold, and heat, can cause stress on various organelles.[115] Endoplasmic reticulum (ER) stress has been widely considered as an important cellular response to stress conditions, and significant advances have recently been made to understand the role of cell wall stress in salt stress tolerance. To attenuate stress-induced organellar stresses, each affected organelle must be able to perceive the stress signals and also to relay the signals to the nucleus.

Cell Wall Stress

The plant cell wall consists of cellulose, hemicellulose, pectins, and many glycoproteins. Cell wall integrity is an important factor that determines plant growth and salt stress tolerance.[70,79] Several mutants that are defective in cell wall integrity are hypersensitive to salt stress. For example, CESA6, which is a core component of the cellulose synthase complex, is required for normal root elongation under salt stress.[116] SOS5, encoding a fasciclin-like arabinogalactan protein (AGP),[117] and SOS6, encoding a cellulose synthase-like protein, are required for root elongation under high salinity.[118] CC1 and CC2 were discovered as companions of CESAs, and both are required for hypocotyl growth under high salinity.[119] MUR4 is a Golgi-localized enzyme involved in the biosynthesis of UDP-arabinose (UDP-Ara). UDP-Ara is involved in the modification of diverse polysaccharides and glycoproteins, which are exported to the apoplast to maintain cell wall integrity. Mutation in *MUR4* leads to reduced root elongation and defective cell-cell adhesion under high salinity,[120] indicating that arabinose modifications are important for the regulation of cell wall integrity in roots under salt stress.

Na$^+$ accumulated in the apoplast may directly bind to cell wall components and affect their chemical properties.[121] Because detecting the changes in cell wall composition is challenging, our understanding of the mechanisms underlying the modifications of cell walls upon exposure to high salinity is limited. Cell wall polymers are negatively charged and are therefore able to reversibly bind to cations.[121,122] Pectin, a major component of the cell wall, is composed of homogalacturonan, rhamnogalacturonan I and II (RGI and RGII), and xylogalacturonan.[123] Boron is required for the cross-linking of RGII,[124] and the cross-linking is coordinated by Ca^{2+}.[125] When present at high levels in the apoplast, Na$^+$ may replace Ca^{2+} in binding to pectins and may thereby interfere with pectin cross-linking,[11] leading to reduced cell expansion.[126] Pectins are synthesized and secreted into the cell wall in a methylesterified form. Pectin methyl esterase (PME)-mediated demethylesterification is an important form of pectin modification.[126] Binding

of Na^+ to the substrate sites of PMEs affects the deme-thylesterification of pectins and thus inhibits cell growth.[11,127] AGPs function as a reservoir of extracellular Ca^{2+}. Excessive Na^+ may free the Ca^{2+} bound in the AGPs and initiate influx of Ca^{2+} into the cytosol.[128] Plants that are defective in the production of AGPs are hypersensi-tive to high salinity.[129,130]

Apoplastic acidification promotes cell expansion. High salinity causes the alkalinization of the apoplast[131] and thus inhibits cell growth. RALF peptides were identified that can cause alkalinization of the apoplast by regu-lating H^+-ATPases at the plasma membrane.[81] High salinity can induce the production of mature RALF pep-tides,[79] suggesting that salt stress-induced alkalinization of the apoplast is probably mediated by RALFs. The ef-fect of apoplastic pH on cell growth is mainly mediated by the regulation of expansin activities.[11] Expansins facilitate cell expansion by loosening cell walls under a variety of environmental stresses, including high salinity and drought stress.[124]

Chloroplast Stress
In addition to functioning in photosynthesis, chloro-plasts also contribute to the biosynthesis of amino acids, vitamins, isoprenoids, fatty acids, and lipids.[132] Dysfunc-tion of chloroplasts caused by environmental stresses can have harmful effects on the physiological, biochem-ical, and metabolic properties of plant cells. High salinity has multiple effects on chloroplasts, including reduced CO_2 uptake due to stomatal closure, reduced photosynthetic efficiency, thylakoid membrane damage, oxidative stress, impaired osmotic and ionic homeosta-sis, and disrupted protein synthesis and turnover.[133] The reduced efficiency of photosynthesis is a major reason for the growth inhibition that occurs under high salinity.[134,135] K^+, as an essential nutrient for plants, is required for the regulation of pH, volume, and elec-tron transport in chloroplast.[56,136] Excessive accumula-tion of Na^+ and Cl^- results in reduced K^+ influx in chlo-roplasts and thus causes ionic, osmotic, and oxidative stresses (see more details in other sections). Transcrip-tomic profiling has indicated that 53 salt-responsive genes encode chloroplast-localized proteins, many of which are important for salt stress tolerance.[133] Most steps of ABA biosynthesis occur in the chloroplast, and ABA biosynthesis-associated proteins, such as ABA1, ABA4, and NCED3, are localized in chloroplasts and are required for salt stress-induced accumulation of ABA.[137,138] *MsK4*, a novel *Medicago sativa* GSK-3-like ki-nase localized in plastid, positively regulates the salt stress response by modulating sugar metabolism.[139] Fad6 and GPAT, two plastid-localized enzymes, facilitate thylakoid membrane fluidity and thereby increase salt stress tolerance by modulating fatty acid meta-bolism.[140,141] Some genes encoded in chloroplasts, such as *RUB* and *RCI*, are associated with the maintenance of PSII activity under high salinity.[142,143]

The signals caused by chloroplast stress can be trans-duced to the nucleus via retrograde signaling path-ways.[132] High salinity-induced production of $^1O_2$, which causes photo-oxidative damage of PSII, acts as one of the retrograde signals.[144] $^1O_2$ can be sensed by EXE-CUTER (EX1), a nuclear-encoded protein localized in the thylakoid membrane of chloroplasts.[145] The stress-induced release of $^1O_2$ promotes the degradation of EX1, the process of which depends on oxidative post-translational modification at the Trp643 residue in the DUF3506 domain of EX1.[144] Besides the EX1-mediated pathway, $^1O_2$ can trigger chloroplast-to-nucleus retro-grade signaling via oxidative products of beta caro-tene.[146] Methylerythritol cyclodiphosphate (MEcPP), a precursor of isoprenoids produced by the plastidial methylerythritol phosphate (MEP) pathway,[147] and phosphonucleotide (3′-phosphoadenosine 5′-phosphate [PAP]),[148] are another two retrograde signaling metabo-lites involved in the transduction of signals from chloro-plast to the nucleus to regulate stress-responsive gene expression.

ER Stress
Biotic and abiotic stresses can cause the accumulation of unfolded or misfolded proteins in the ER, resulting in ER stress. The misfolded proteins can be recognized by a protein quality control system in the ER, which

induces the expression of chaperone genes that are important for protein folding and triggers ER-associated protein degradation (ERAD) and autophagy.[149] Upon exposure to salt stress, the ubiquitinated proteins increase in the ER, and this accumulation activates an ER stress response. The positive role of the ER stress response in salt stress tolerance is supported by the finding that a defect in the HRD3A of the HRD1/HRD3 complex, which is required for the unfolded protein response in the ERAD pathway, confers hypersensitivity to salt stress, and Ca^{2+} and ROS are required for the ERAD-mediated response to salt stress.[150] *UBC32*, which encodes an E2 ubiquitin-conjugating enzyme, is an active component of the plant ERAD compartment. *UBC32* gene expression is highly induced by drought and salt stress, and loss of function of *UBC32* enhances salt stress tolerance via a BR-dependent pathway.[151] ER stress can trigger regulated intramembrane proteolysis under stress conditions. For example, salt stress induces subtilisin-like serine protease (AtS1P)-mediated cleavage of a membrane-localized bZIP transcription factor, AtbZIP17, in the ER. The activated AtbZIP17 translocates to the nucleus where it upregulates the expression of many salt stress responsive genes.[152] S2P stimulates the nuclear localization of bZIP17 and bZIP28 via a process of cleavage. In the nucleus, these two transcription factors induce the expression of chaperone genes and the activation of BR signaling, and finally confer salt stress tolerance.[153]

Mitochondrial Stress

The mitochondrion is an energy-producing organelle that is important for the survival of plants under stress conditions.[154] Abiotic stress-induced perturbation of mitochondrial functions can activate the expression of stress-responsive genes via a mitochondrial retrograde regulation (MRR) or retrograde signaling pathway.[154,155] AOX is a well-studied mitochondrion-localized protein that is responsive to environmental stresses, and *AOX* induction has been used as a marker for MRR in response to stress.[154] In *Arabidopsis*, influx of Ca^{2+} into mitochondrial is required for the induction of *AOX1a* gene expression under salt stress,[156] and ABA INSENSITIVE 4 (ABI4) acts as a negative regulator of *AOX1a* gene expression. Cyclin-dependent kinase E1 (CDKE1), a component of the mediator complex, regulates *AOX1a* gene expression by interacting with SNF1-related kinase 1 (SnRK1/KIN10).[157]

Hormonal Regulation during Salt Stress

Response and adaptation to salt stress require the integration and coordination of multiple phytohormones, including ABA, jasmonic acid (JA), gibberellic acid (GA), ethylene, and salicylic acid (SA) (Figure 2). Among these hormones, ABA is the most involved in the response to diverse abiotic stresses. Osmotic stress imposed on roots results in a very rapid (within several minutes) and massive increase in ABA concentration in both root and leaf tissues.[9,158] ABA is one of the key signaling molecules known to cause stomatal closure.[159] This process involves binding of ABA to PYR/PYL/RCAR receptors.[160,161] Once the receptors bind to ABA, they interact with PP2C phosphatases and inhibit their activity, thus releasing SnRK2s from repression.[160,161] The SnRK2s then activate a range of anion efflux channels[162,163] resulting in a loss of turgor pressure and stomatal closure. Although the role of ABA in salinity-induced stomatal closure is beyond any doubt, the origin of the ABA signal is still debated. For many years, it was thought that ABA is generated in osmotically stressed roots and is then rapidly transported to the shoot with the transpiration stream.[164,165] More recent studies, however, showed that root-to-shoot ABA delivery may be not be required for stress-induced stomatal closure.[166,167] The *NCED3* gene, which encodes the first step of ABA biosynthesis, is expressed predominantly in the vascular parenchyma of leaves[168] and is rapidly upregulated by osmotic stress, and many experiments using reciprocal grafting of ABA-deficient mutants suggest that drought resistance was conferred by the genotype of the scion and not of the rootstock (reviewed in Buckley[166]). In addition, ABA synthesis in roots may require precursors transported from leaves.[169] On the other hand, the stress-induced increase in ABA concentration is several fold
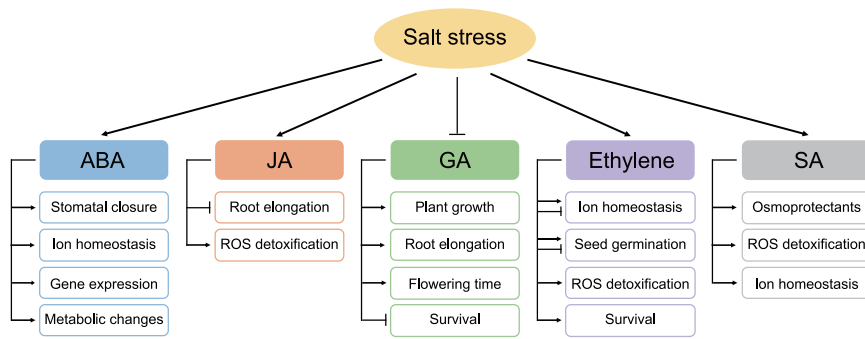
ABA is a major hormone involved in the regulation of salt stress response, including the regulation of stomatal closure, ion homeostasis, salt stress-responsive gene expression, and metabolic changes. JA is required for the inhibition of root elongation and activation of antioxidative enzymes upon exposure to high salinity. Salt stress reduces the accumulation of endogenous bioactive GAs, leading to inhibition of plant growth and root elongation, delay of flowering, and promotion of survival under high salinity. The effect of ethylene on salt stress tolerance acts in a species- or gene-specific manner. The components involved in ethylene biosynthesis or signaling transduction either positively or negatively regulate ion homeostasis and seed germination, but ethylene induces detoxifying machineries and promotes survival under salt stress. SA participates in the accumulation of osmoprotectants, induction of antioxidative enzymes, and improvement of ion homeostasis under salt stress.

higher in roots than in leaves,[158] but the role of ABA production in roots in the plant response to salt stress remains unclear.

A clue to how ABA production in roots may contribute to the salt stress response is provided by the fact that ABA interacts with H_2O_2 in plant systemic responses to stresses.[170] Salinity stress results in a significant accumulation of ROS in plant roots.[171,172] A major source of these ROS is NADPH oxidase, a plasma-membrane-bound enzyme complex from the NOX family.[173] Osmotic stress-induced increase in H_2O_2 production requires NADPH oxidase stimulation by ABA.[174] Also, inhibition of the NADPH oxidase-mediated H_2O_2 production in the root abolishes rapid stomatal closure, resulting in a salt-sensitive phenotype.[172] Thus, salinity-induced increase in root ABA content may be critical for generating an "ROS wave" that triggers stomatal closure.

Increasing evidence has linked the JA pathway to salt stress responses in plants.[175] Transcriptomic studies have revealed that many JA biosynthesis genes are upregulated under salt stress and that the JA signaling pathway is involved in the regulation of salt stress-responsive genes.[176,177] In *Arabidopsis*, the JA signaling pathway is required for the inhibition of root elongation under high salinity.[178] In rice, RICE SALT SENSITIVE3 (RSS3), a nuclear-localized protein, promotes root cell elongation under salt stress by physically interacting with class C basic-helix-loop-helix (bHLH) transcription factors and JASMONATE ZIM-DOMAIN (JAZ) proteins, the latter being the negative regulators of the JA pathway. Loss of function of *RSS3* results in the upregulation of JA-responsive genes.[179] These results support the notion that the JA pathway is required for root growth inhibition under high salinity. Overexpression of the *OsCYP94C2b* gene, which encodes an enzyme that catalyzes the conversion of bioactive JA-Ile to an inactive form, enhances the survival rate of rice under high salinity, demonstrating the negative role of JA in salt stress tolerance. However, opposite phenotypes have also been observed in studies showing that overexpression of the JA biosynthesis gene *TaORP1* or application of exogenous JA enhances salt stress tolerance in wheat, rice, and soybean.[180–182] Together, these results suggest that JA may act as a positive or negative regulator of salt stress response in a spatially and temporally dependent manner.

Coordination of growth and stress tolerance is critical for the survival of plants under unfavorable conditions. GA, as an important hormone that regulates plant growth, has been linked to the regulation of growth under abiotic stress.[183] Treatment of *Arabidopsis* seedlings with salt reduces endogenous bioactive GAs and increases the accumulation of DELLA protein.[184,185] In the quadruple-*della* mutant, salt stress-triggered growth inhibition and delayed flowering are attenuated, and salt

stress-induced death is enhanced,[184] suggesting that DELLA proteins promote the survival of plants by restricting growth under high salinity. Ethylene is also involved in salt stress tolerance in plants. Application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) increases salt stress tolerance,[186] while mutations in ethylene signaling pathway-associated genes, such as *ETR1*, *EIN4*, *EIN2*, and *EIN3*, lead to hypersensitivity to high salinity.[186–189] The positive effect of ethylene on salt stress tolerance is largely mediated via the modulation of ROS-generating and ROS-scavenging machineries.[187,190] The role of SA in salt stress tolerance is represented by its ability to improve the accumulation of osmoprotectants, such as glycine betaine, proline, and polyamines,[191] and to enhance antioxidant enzyme activities under high salinity.[192] Pretreatment of plants with SA also reduces NaCl-induced K^+ efflux and H^+ influx and thus promotes the adaptation of plants to high salinity.[193]

Salt-Responsive Gene Expression and Epigenetic Regulation of Salt Stress Tolerance

Transcription is the first and the most critical step in the regulation of gene expression. Early transcriptome analyses in *Arabidopsis* indicated that salt stress causes differential expression in hundreds to thousands of genes depending on the strength or duration of the treatment.[194,195] Salt stress, osmotic stress, and ABA treatment induce common sets of differentially expressed genes, especially during the early stages of stress treatment.[194,195] The transcriptome is significantly different when salt stress is combined with other types of environmental stresses compared with when salt stress is applied alone, indicating extensive crosstalk between the salt stress signaling pathway and other stress signaling pathways.[196] To date, hundreds if not thousands of salt stress-related transcriptomes have been conducted in many plant species. Members from all of the major transcription factor (TF) families (such as NAC, ERF/AP2, bZIP, MYB, and WRKY) have been found to be involved in the salt stress response. However,

unlike cold acclimation, for which CBF (C-repeat binding factor)/DREB (dehydration responsive element binding protein) TFs function as master regulators, master transcriptional regulators for salt stress have not been identified. CBF1/2/3, belonging to the AP2/ERF (APETALA2/Ethylene-Responsive Factor) family, were first identified for their involvement in low-temperature responses.[197,198] Overexpression of *CBF3/DREB1A* enhances plant tolerance to salinity,[199] and mutation of all three *CBF* genes results in hypersensitivity to salt stress,[200] indicating a positive role of CBFs in plant salt response. ABA mediates transcriptional regulation mainly through the AREB/ABF subfamily of bZIP TFs.[201,202] For example, overexpression of *ABF2* increases plant resistance to multiple stresses, including salt stress.[203] A new class of ABA-responsive TFs named DIG (dynamic influencer of gene expression)/DIL (DIG-like) was also identified, and overexpression of these TFs results in hypersensitivity to high salt or ABA.[204] TFs involved in other hormone signaling pathways also function in the salt stress response. MYC2, a master TF of jasmonate signaling, is a positive regulator of salt tolerance.[181,205] EIN3, a TF that mediates core ethylene signaling, is stabilized by salt treatment and increases plant salt tolerance through the DELLA proteins.[184,187] EIN3 increases salt tolerance partly through two downstream TFs, ERF1 (Ethylene Response Factor 1) and ESE1 (Ethylene and Salt Inducible 1), which directly bind to salt-responsive genes and activate their expression.[189,206] Many other TFs from non-model plant species have also been identified mainly through transcriptome analyses. Although the specific mechanisms by which most of these TFs operate are not clear, their roles in salt tolerance have usually been validated using transgenic approaches in *Arabidopsis* or the original species.[207] It is also important to keep in mind that TFs are highly dynamic and that the transcriptional network functions in a spatially and temporally specific manner. A recent study constructed an ABA-responsive network and defined the hierarchy among 21 ABA-related TFs by associating the *in vivo* binding dynamics of these TFs with time-series transcriptome data.[204] The results revealed that dynamic binding of multiple TFs, compared

with static binding, better predicts changes in gene expression over time.[204] In summary, many TFs are involved in the regulation of salt-induced changes in gene expression. High-throughput sequencing technology facilitates the identification of TFs involved in the salt stress response. Additional research on the dynamics of transcriptional networks is needed, however, to increase systematic understanding and identify key players in transcriptional regulation of the salt stress response.

Genomic DNA in the eukaryotic nucleus is packed into the highly ordered structure of chromatin. Transcriptional regulation inevitably requires dynamic changes in chromatin conformation. Many chromatin modifiers have been identified as regulators of the salt stress response in plants. The nucleosome is the basic unit of chromatin and is typically composed of 147 bp of genomic DNA wrapped around a histone octamer containing two copies of histone H2A, H2B, H3, and H4. The structure of chromatin can be remodeled by ATP-dependent remodelers or covalent modifications. Histones contain flexible N-terminal tails, many residues of which are post-translationally modified by methylation, acetylation, phosphorylation, ubiquitination, etc. In addition, genomic DNA can be methylated. Specific modifications or a combination of modifications can directly change the chromatin conformation or can recruit specific binding proteins that promote changes. For example, acetylation of the histone lysine residue neutralizes the positive charge of the lysine side chain and reduces the interaction between the histones and the negatively charged DNA backbone, resulting in a less-compact chromatin conformation. As a consequence, histone acetylation is usually associated with transcriptional activation. Histone acetylation is catalyzed by histone acetyltransferases (HATs) and is removed by histone deacetylases (HDACs). Multiple HATs and HDACs are involved in the salt stress response. The plant-specific histone deacetylase HD2C interacts with HDA6 and reduces salt tolerance by repressing the expression of ABA-responsive genes such as *ABI1* and *ABI2*.[208] The class I (HDA6/HDA9/HDA19) and class II enzymes (HDA5/14/15/18) of the RPD3 histone deacetylase family play negative and positive roles in plant salinity tolerance, respectively.[209-211] Furthermore, HUB2, a ubiquitin E3 ligase responsible for mono-ubiquitination of histone H2B, increases plant responses to drought and salt.[212,213] In *Arabidopsis*, the NAP1 (Nucleosome Assembly Protein 1) protein, which functions as a histone chaperone for histone H2A and H2B, is a positive regulator of ABA signaling.[214] In addition to factors that modify chromatin composition and/or conformation, various types of noncoding RNAs, including siRNAs (small interfering RNA), miRNAs (microRNA), or lncRNAs (long noncoding RNAs), also contribute to salt stress response. Additional details are provided in other reviews.[215-217]

Certain chromatin modifications such as DNA methylation and histone H3 lysine 27 trimethylation (H3K27me3) can be faithfully transmitted through meiosis or mitosis and are therefore considered epigenetics.[218-220] Epigenetic regulatory mechanisms of salt stress tolerance or abiotic stress tolerance, in general, have drawn substantial attention in the past two decades because they represent attractive mechanisms for plant stress memory, which enables plants to enhance their stress response by remembering previous stresses. This effect is also termed "stress priming." Priming of seeds using sodium chloride, hyperosmotic reagents, or BABA (β-aminobutyric acid) enhances the drought or salinity tolerance of the plants.[221-223] In *Arabidopsis*, mild salt priming of seeds results in genome-wide alteration of H3K27me3, a repressive histone mark typically associated with developmental genes.[223] In addition, multiple studies have reported salt stress-induced changes in DNA methylation across the genome.[224-226] Natural epigenetic variations or DNA methylation defects resulting from mutations in the DNA methylation machinery have been linked to gene expression differences involved in salt tolerance.[227-229] Despite these observations, convincing examples showing salt-induced epigenetic (i.e., heritable) changes that are important for salt stress response have yet to be reported. The stability of the epigenetic mark and the functional
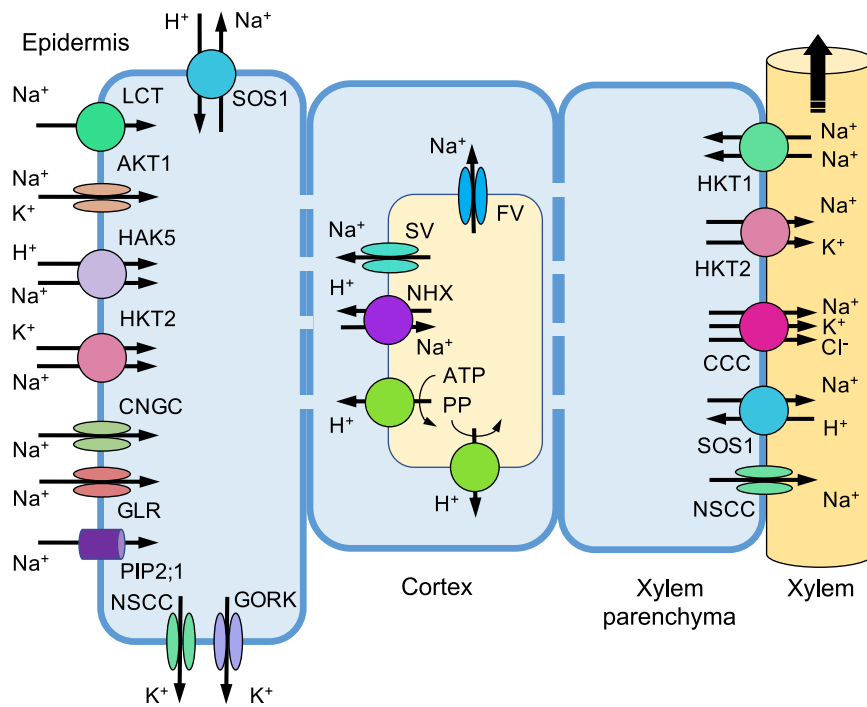
Figure 3 Major Transporters Mediating Na$^+$ Homeostasis in Salinized Root Tissues

The major pathways for Na$^+$ uptake in the root epidermis are glutamate receptor-like (GLRs) channels or cyclic nucleotide-gated (CNGCs) non-selective cation channels and HKT2 high-affinity K$^+$ transporters. Other possible pathways for Na$^+$ uptake may involve AKT1 Shaker-type K$^+$ channels, HAK5 high-affinity K$^+$ transporters, the low-affinity cation transporter LCT1, and PIP2;1 aquaporins. The uptake of Na$^+$ is counterbalanced by active Na$^+$ extrusion via SOS1 Na$^+$/H$^+$ exchangers. Vacuolar Na$^+$ sequestration is conferred by tonoplast-based Na$^+$/H$^+$ exchangers from the NHX family fueled by either H$^+$-ATPase or H$^+$-PPase pumps. Another component of vacuolar Na$^+$ sequestration is efficient control over tonoplast slow- (SV) and fast- (FV) activating ion channels that may allow Na$^+$ to leak back to the cytosol. Passive Na$^+$ loading into the xylem is mediated by non-selective cation channels (NSCCs), and its active loading requires operation of cotransporters such as SOS1, CCC (cation-chloride cotransporters), and HKT2 (K$^+$/Na$^+$ symporter). Na$^+$ withdrawal from the xylem is achieved by HKT1 high-affinity K$^+$ transporters. Salinity-induced K$^+$ loss from the root epidermis is mediated by NSCCs and depolarization-activated outward-rectifying GORK K$^+$ channels.

consequences of stress-induced epigenomic reprograming remain unclear. A recent study of DNA methylome changes in response to phosphate starvation in *Arabidopsis* found that most DNA methylation changes occur after transcriptional changes and are usually transient.[230] Many studies on stress-induced epigenetic changes have been performed in *Arabidopsis*, which has an unusual plant genome with a very low amount of transposable elements. Salt-induced epigenetic changes in other plant species remain to be determined.

Ion Homeostasis under Salinity

Salinity stress is usually associated with too much NaCl. Because Na$^+$ is considered to be toxic (see the section on physiological responses to salt stress for details), it is hardly surprising that most research concerning salinity stress has been aimed at revealing the mechanisms of Na$^+$ transport and sequestration in plants. This was nicely summarized by Cheeseman,[231] who stated "physiological folklore has elevated sodium toxicity to a belief

in the almost paranoiac avoidance of cytoplasmic Na$^+$". Even though that paper was published more than 30 years ago, Cheeseman's statement still describes the current view.

Plants can use two major ways to prevent accumulation of high levels of Na$^+$ in the cytosol of root cells: (1) Na$^+$ exclusion from root uptake and (2) vacuolar Na$^+$ sequestration. For photosynthetically active mesophyll cells, there are three additional ways: (3) control of xylem Na$^+$ loading; (4) Na$^+$ retrieval from the xylem; and (5) Na$^+$ recirculation from the shoot via the phloem.

Na$^+$ Exclusion from Uptake

Symplastic root uptake of Na$^+$ is potentially mediated by several types of ion transporters (Figure 3). Two major types are non-selective cation channels,[52] either glutamate receptor-like (GLRs) or cyclic nucleotide-gated (CNGCs) channels; and HKT2 high-affinity K$^+$ transporters.[232] Other possible pathways for Na$^+$ uptake may involve AKT1 Shaker-type K$^+$ channels, HAK5

high-affinity K$^+$ transporters,[232,233] and the low-affinity cation transporter LCT1,[234,235] although arguments against their involvement have been presented.[233] PIP2;1 aquaporins (initially identified as plant water channels) were recently added to the list of candidates for root Na$^+$ uptake.[236] The uptake of Na$^+$ is counterbalanced by active Na$^+$ extrusion (Figure 3). The most prominent component in this process is the SOS1 Na$^+$/H$^+$ exchanger,[237] although the involvement of vesicle-mediated Na$^+$ transport cannot be ruled out.[238] It is generally assumed that ~95% of all Na$^+$ taken up by the root is then exported back to the rhizosphere.[26] The reasons for and the consequences of such a futile cycle are discussed in detail elsewhere.[239,240]

Vacuolar Na$^+$ Sequestration

Another way of avoiding excessive Na$^+$ accumulation in the cytosol is to deposit it in the vacuole. The traditional view is that such vacuolar sequestration is conferred by operation of the tonoplast-based Na$^+$/H$^+$ exchangers in the NHX family.[241] Both NHX activity and transcript levels are inducible by salt in glycophytes, and such tonoplast antiporters are constitutive in halophytes.[238,242,243] More recent studies have suggested that NHX exchangers represent only part of the vacuolar Na$^+$ sequestration mechanism. Another equally important sequestration mechanism involves the efficient control of tonoplast leak channels, which enables vacuolar Na$^+$ retention in the tonoplast.[244] Two types of Na$^+$-permeable channels, namely slow- (SV) and fast- (FV) activating ion channels, are present at the tonoplast, and model calculations show that if each cell opened only one SV channel at a specific time, the back-leak fraction would range from 30% to 100%.[244] Thus, to avoid the futile movement of Na$^+$ into and out of the tonoplast, plants can afford to open only a very small percentage (about 0.1%) of all tonoplast channels; this is consistent with experimental observations in salt-tolerant species.[245] Recent studies have reported additional complexity in the relationship between Na$^+$/H$^+$ antiporters and vacuolar Na$^+$ sequestration; the studies showed that NHX antiporters have higher affinity to K$^+$ than to Na$^+$ and thus operate predominantly as K$^+$/H$^+$ exchangers.[246,247] This prompted the suggestion that some other mechanisms and, specifically, enhanced vacuolar trafficking, may also deliver Na$^+$ to the vacuole.[248]

Control of Xylem Na$^+$ Loading

Despite its critical importance for salinity tolerance, whether Na$^+$ loading into the xylem is an active or passive process is still debated. Both active and passive transport systems are probably involved, but their respective roles may differ depending on the length of time since salinity onset.[249,250] Passive Na$^+$ loading is likely to be mediated by non-selective cation channels (NSCC).[251] Consideration of thermodynamics suggests, however, that under most physiologically relevant scenarios, xylem Na$^+$ loading should be an active process (see Shabala[250] for supporting arguments). One of the most likely candidates for such active loading is the Na$^+$/H$^+$ antiporter encoded by the SOS1 gene. Highly abundant in the xylem parenchyma,[237] SOS1 belongs to the cation proton antiporter (CPA) subfamily of proteins. The SOS1 protein is a homodimer and consists of 10–12 transmembrane domains. Class 2 HKT transporters represent another pathway for active xylem Na$^+$ loading. Functionally, HKT2 operates as a K$^+$/Na$^+$ symporter. HKT2 transporters are highly expressed in the stellar root tissues.[252] The depolarization of parenchyma cells under saline conditions[12] favors a passive outward movement of K$^+$ into the xylem, thus creating a driving force for the loading of Na$^+$ into the xylem.[249] Finally, cation-chloride cotransporters (CCC) were found to be preferentially expressed at the xylem/symplast boundary in *Arabidopsis*.[253] These transporters mediate symport of Cl$^-$, Na$^+$, and K$^+$, and given that Cl$^-$ transport into the xylem is thermodynamically passive, they may provide a driving force for Na$^+$ (secondary) active loading into the xylem.[249] Supporting evidence for that view was provided by the pharmacological studies conducted by Zhu et al.,[254] who demonstrated a significant reduction in the magnitude of Na$^+$ efflux from barley root stellar tissue in the presence of bumetanide, a known inhibitor of mammalian CCC.

Retrieval of Na⁺ from the Xylem

Class I high-affinity K^+ transporters have been firmly established to operate in the withdrawal of Na^+ from the xylem sap in various species.[255–259] HKT proteins belong to the HKT/Trk/Ktr-type superfamily of K^+ transporters, which consists of four repeats of transmembrane/pore-loop/transmembrane motifs, similar to the ion-conducting pore-forming units of K^+ channels. Members of class I (HKT1) contain a Ser residue at the first pore-loop domain and are highly selective for Na^+ over K^+.[260] The *Arabidopsis* genome contains only a single copy of the AtHKT1;1 gene, but its halophytic relative *Thellungiella salsuginea* contains three copies of HKT1-type genes.[261] When expressed in *Xenopus laevis* oocytes and yeast, HKT1 transporters show a highly specific Na^+ influx.[259,262] *Arabidopsis hkt1;1* mutants were salt-sensitive compared with the wild type and hyperaccumulate Na^+ in the shoot, but accumulate less Na^+ in the root,[263] and targeted overexpression of the *Arabidopsis* HKT1;5 homolog in *Arabidopsis* and rice increases Na^+ exclusion from the shoot.[264,265] In rice, the OsHKT1;5 locus has been narrowed down as a salt tolerance determinant by QTL (quantitative trait locus) analysis.[257] In wheat, the TmHKT1; 5-A locus derived from a wild wheat relative *T. monococcum* corresponds to the Na^+ exclusion 2 (Nax2) QTL that contributes to the lowering of the Na^+ level in leaves.[266,267]

Na⁺ Recirculation via the Phloem

The Na^+ load in the shoot may also be reduced by its recirculation back to the roots via the phloem.[268] The molecular mechanisms of this process remain elusive, although the HKT1 class of transporters is thought to play a major role.[263,269] The fate of the Na^+ remobilized in the phloem is also unclear. The anatomical structure of the root favors a unidirectional, radial transport of Na^+. Once it passes through the Casparian strip and is loaded into the stele, Na^+ has very little chance of being transported back to the cortex. Hence, if a substantial quantity of Na^+ is returned to the root, it presumably must remain in the rather limited number of parenchyma cells in the stele. Unless sequestered properly in vacuoles, this could cause phytotoxicity and compromise root functions. The export of Na^+ in the phloem could also cause damage to growing leaves and meristematic regions of the shoot, assuming that they are connected to the phloem via sieve tubes.[270] This may explain why salt-tolerant species have much lower rates of Na^+ export in the phloem than salt-sensitive species; for example, the percentage of Na^+ that is exported in the phloem is only ~10% in salt-tolerant barley species[271] but is 50% in salt-sensitive white lupin.[272]

Potassium Retention in the Cytosol

Over the last decade, researchers have found that cytosolic K^+ homeostasis and the ability of various plant tissues to retain K^+ under stress conditions are essential for salinity tolerance (reviewed by Shabala et al.[14] and Shabala and Pottosin[273]). Reported initially for barley roots,[274] a positive correlation between the overall salinity tolerance and the ability of a root tissue to retain K^+ was later expanded to at least a dozen other plant species (reviewed in Wu et al.[18]). Efficient cytosolic K^+ retention is also considered to be a hallmark of halophytes.[275–277] There are at least four physiological reasons why K^+ retention is important under saline conditions. First, a high level of K^+ retention allows the plant to accumulate high amounts of Na^+ in the cytosol without compromising the cytosolic K^+/Na^+ ratio, the ratio that determines cell metabolic competence and, ultimately, the ability of a plant to survive in saline environments. Second, depletion of the cytosolic K^+ pool may activate caspase-like proteases and endonucleases, thus triggering PCD.[34–36] While the physiological role of PCD under saline conditions is still debated,[33,36] the causal relationship between K^+ efflux and stress-induced PCD is beyond any doubt.[18] Third, to maintain normal metabolic activity in the cytosol, plants rely on the vacuolar K^+ pool to replenish the K^+ lost from the cytosol. The vacuolar K^+ buffering capacity in a typical plant cell is estimated to be between 1 and 7 h.[18,244] Thus, unless the cell is able to activate high-affinity K^+-uptake systems within this time frame, depletion of the vacuolar K^+ pool may result in a loss of turgor and collapse of the cell.

Two major pathways mediate salinity-induced K$^+$ loss from the cell.[14,18] One pathway involves the GORK channel, which belongs to the Shaker family of K$^+$ channels and consists of six transmembrane domains (TMDs), a pore helix, and a selectivity filter between the last two TMDs.[278] The GORK channel is highly sensitive to changes in membrane potential (as occur under stress conditions) and is activated upon depolarization.[279] To prevent depolarization-induced K$^+$ leakage, plants must restore (otherwise depolarized) the membrane potential by more active H$^+$ pumping.[280] This comes at a significant ATP cost[281] and may compromise the plant's ability to adapt and grow. The second pathway for salinity stress-induced K$^+$ loss from the cell is via K$^+$-permeable ROS-activated NSCCs.[18] Differential sensitivity of K$^+$-permeable NSCC to various ROS (e.g., H$_2$O$_2$ and ·OH) explains the intraspecific,[275,282] genotypic,[283,284] and tissue-specific[14,49] differences in salinity stress tolerance.

Potassium as a Second Messenger

In addition to being an essential nutrient, K$^+$ functions in signaling.[285,286] When plants deal with energy crises (as they do under saline conditions[240]), they must use a large fraction of available ATP for defense, at the expense of "business as usual" metabolism. Given that many metabolic enzymes require K$^+$,[18,287] transient cytosolic K$^+$ efflux is thought to operate as a "metabolic switch" that inhibits energy-consuming anabolic reactions and saves energy for adaptation and repair, which may give species a competitive advantage under the energy-limiting conditions imposed by salinity.[35,286] At the same time, the amount of K$^+$ lost for signaling purposes should not compromise the plant's nutritional demand for this element. This dilemma is resolved by the transient nature and high tissue specificity of stress-induced K$^+$ efflux.[14] In addition, different plant species may display distinct salt stress-induced K$^+$ flux "signatures,"[281] prompting analogies with cytosolic Ca^{2+} signaling.[67] In the latter case, an array of protein kinases and calcium-binding proteins decode transient Ca^{2+} spikes.[288] It remains to be determined whether a similar mechanism characterizes K$^+$ signaling.

Salt Stress-Induced Metabolite Changes

A major strategy used by plants to maintain a low intracellular osmotic potential under high salinity is the accumulation of compatible osmolytes,[26,103] including proline, hydroxyproline, glycine betaine, sugars, polyamines, and proteins from the late embryogenesis abundant (LEA) superfamily.[289–291] Among these metabolites, proline plays a dominant role in osmotic adjustment under salt stress.[292] The accumulation of proline under osmotic stress can be achieved both by activation of the proline biosynthesis pathway and by inactivation of the proline catabolic pathway.[293] Proline is biosynthesized mainly via reductions of glutamic acid by two successive enzymes: P5C synthase (P5CS) and P5C reductase (P5CR).[294,295] P5CS is a rate-limiting enzyme in proline biosynthesis, and its activity is mainly controlled at the transcriptional level.[295] Both *P5CS* and *P5CR* genes are upregulated under high salinity, which enables the accumulation of proline and subsequent salt stress tolerance.[295] In the catabolic pathway, proline can be converted to glutamate via the reactions catalyzed by proline dehydrogenase (PDH) and P5C dehydrogenase (P5CDH).[296] The negative role of PDH in salt stress tolerance is supported by the fact that plants with lower transcript levels of *PDH* have higher salt stress tolerance.[297,298] Apart from its role in osmotic adjustment, proline acts to stabilize proteins and membrane structures, as well as an ROS scavenger that attenuates oxidative stress under high salinity.[299–301]

GB is another major metabolite that is increased under salt stress and is associated with adjustment of osmotic potential under dehydrating conditions.[302,303] Choline monooxygenase (CMO) and NAD$^+$-dependent betaine aldehyde dehydrogenase (BADH) are required for the biosynthesis of glycine betaine from choline via two oxidation reactions. Glycine betaine is also important for protecting enzymes, stabilizing membranes, and reducing oxidative stress under stress conditions.[304] Trehalose is a non-reducing disaccharide of glucose that helps protect plants against abiotic stress.[305]

Application of trehalose reduces ROS accumulation, which in turn alleviates oxidative stress under high salinity.[305,306] Transgenic rice plants that overexpress *OsTRE1* have enhanced salt stress tolerance.[307] Other sugars, such as glucose, fructose, and fructans, are also involved in the osmotic adjustment under drought and salt stress.[193] Polyamines are organic amines, which include putrescine, spermidine, and spermine.[308,309] Application of exogenous polyamines or the engineering of plants with increased levels of polyamines increases tolerance to salinity and to other abiotic stresses.[310]

Myo-inositol metabolism is important for the relay of specific signals to the cell. Several myo-inositol-derived inositol phosphates have been shown that are required for the response to environmental stresses. Inositol trisphosphate (IP3) is rapidly induced upon salt stress and ABA treatment.[311,312] In plants, IP3 is associated with the release of Ca^{2+} from vacuolar vesicles and thus relay of environmental stress signals. Inositol 1,3,4-trisphosphate 5/6-kinase (ITPK) catalyzes the conversion of IP3 to inositol tetrakisphopsphate (IP4). In rice, disruption and overexpression of the *OsITPK2* gene both lead to increased sensitivity to salt and drought stress,[313] which implies that the homeostasis between IP3 and IP4 is important for stress tolerance.

Salinity Tolerance in Halophytes

According to the *Dictionary of Botany*, halophytes are "plants that are adapted to live in soil containing a high concentration of salt;" many other definitions of halophytes are available in the literature. Despite these differences in definition, all authors agree that the physiological, anatomical, or genetic traits do not fundamentally differ between halophytic species and domesticated (non-halophytic) crop species.[314] The major difference is that halophytes are simply much more efficient in implementing these traits.[238] Some traits, however, can be considered as hallmarks of halophytic species, and these are briefly summarized in the following sections.

Succulence

Succulence is an important adaptive strategy that accumulates excessive salt and conserves water in saline-grown plants. This trait is not unique to halophytes, because increased leaf succulence also occurs in glycophytes,[315] although to a much lesser extent than in halophytes. In halophytes, a specialized parenchymatous tissue beneath the photosynthetically active chlorechymatous tissue functions in both salt storage[316] and water storage.[317] Succulence is mostly characteristic of dicot species belonging to the Chenopodioideae and Salicornioideae.[318] The mechanistic basis of development of succulent structures in plants is linked to endo-polyploidy or endoreduplication,[319] although specific details are still emerging. An increase in the activity of aquaporins increases succulence in some halophyte species,[320] suggesting that a turgor-driven mechanism may also be involved. The molecular mechanisms mediating salt deposition in succulent storage tissues remain elusive. A recent study found that storage parenchyma cells in the succulent halophyte *Carpobrotus rosii* act as Na^+ sinks and have both a higher Na^+ sequestration ability and a higher K^+ retention ability than mesophyll cells;[316] the latter trait was indicated by a higher rate of H^+-ATPase operation and higher non-enzymatic antioxidant activity in this tissue. Also, storage parenchyma cells have a constitutively lower number of open SV vacuolar channels and the ability to downregulate activity of FV vacuolar channels. Because both SV and FV channels represent major pathways for Na^+ leakage into the cytosol, the efficiency of salt load sequestration in parenchymatous tissues depends on the ability of the plant to control this process.

Salt Glands and Bladders

Salt glands are present in more than 50 halophyte species from 14 families[321] and are believed to have evolved independently at least 12 times. At the functional level, two types of salt glands can be distinguished: those that directly secrete salts to the surface of the leaf (exo-recretohalophytes), and those that collect salt in the vacuole of a specialized bladder cell (endo-recreto-halophytes).[321,322] Structurally, salt glands are divided
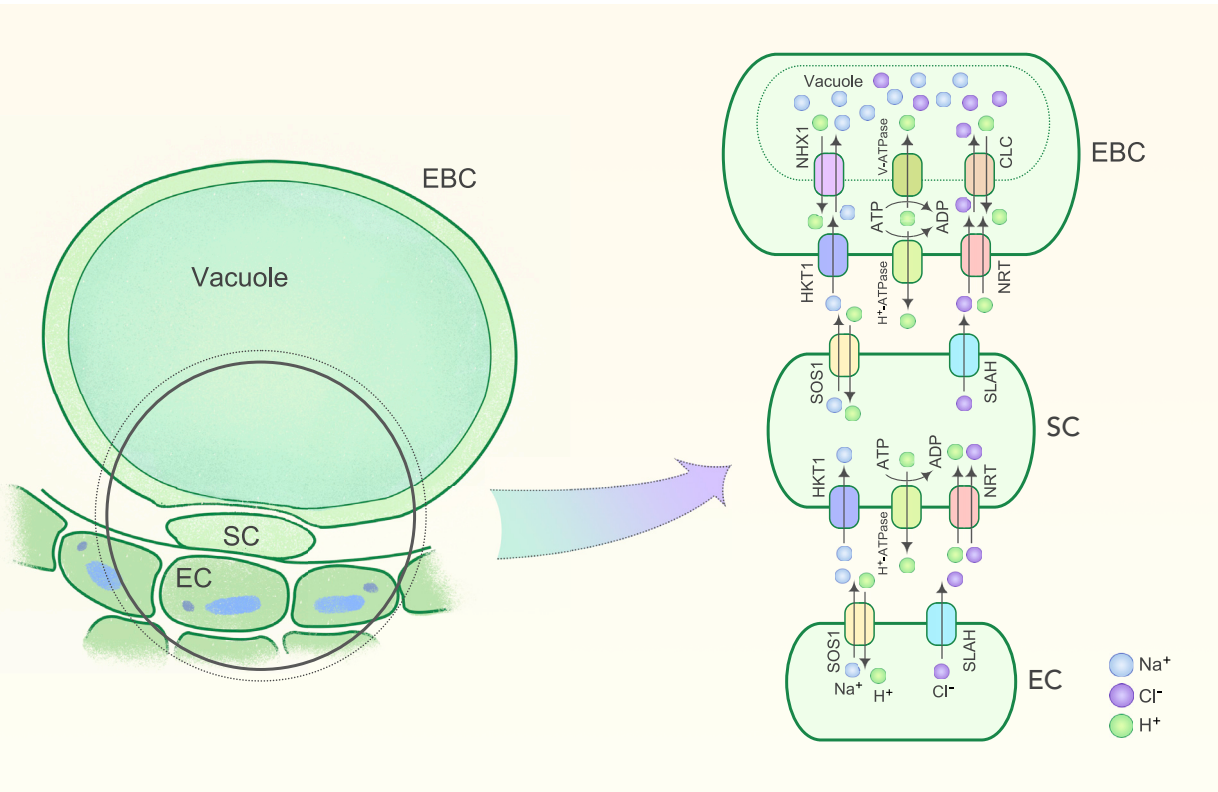
Figure 4 A Proposed Model for the Mechanism of Salt Sequestration in Epidermal Bladder Cells
Left part of figure shows the morphology of an epidermal cell (EC), stalk cell (SC), and epidermal bladder cell (EBC). Right part of the figure presents ion transport systems in the EC, SC, and EBC. SOS1 (Na^+/H^+ plasma membrane exchanger) and HKT1 (high-affinity potassium transporter) transport Na^+, while SLAH (Cl^- permeable anion channel) and NRT (Cl^-/H^+ co-transporter) transport Cl^- from EC to EBC. In the EBC, NHX1 (tonoplast-based Na^+/H^+ exchanger) and CLC (anion channel) are required for the sequestration of excessive Na^+ and Cl^- in the vacuole. Plasma membrane-localized H^+-ATPase and vacuolar ATPase (V-ATPase) are essential for the generation of proton gradients and membrane potential that drive the transport of Na^+ and Cl^- from the EC to the vacuole of the EBC.

into four groups: (1) highly vacuolated unicellular secretary cells (e.g., those in *Porteracia* species); (2) two-celled excretory structures (those in graminoids); (3) multicellular (up to 40 cells) glands found in some graminoids and several dicot families; and (4) a structure consisting of a stalk cell and an epidermal bladder cell (e.g., those in quinoa or *Atriplex* species) (Figure 4).[238,323] Salt glands in the first three groups, which are characteristic of exo-recretohalophytes, release salt periodically. NaCl is thought to be transported into the salt gland through both the plasmodesmata and the regions that are not covered by the cuticle.[323] The salt secretion *per se* involves membrane-bound transport proteins. Several such transporters were postulated to exist in salt glands of various halophyte species.[324] None of them, however,

has been characterized at the molecular and functional level.

Salt glands in the fourth group, which are characteristic of endo-recretohalophytes, deposit the salt load in a bladder-like epidermal outgrowth called the epidermal bladder cell (EBC). Because EBCs are large (typically 100–200 μm in diameter),[325,326] each EBC has a volume that is three orders of magnitude larger than that of a mesophyll cell or an epidermal cell; the large volume facilitates external salt storage, away from metabolically active photosynthetic tissues.[327] EBC density is higher in younger leaves than in older leaves.[326] During ontogeny, EBCs increase in size and operate as salt depots. Once an EBC accumulates a certain threshold

quantity of salt, it ruptures and releases the salt into the external environment.[328] The causal link between EBCs and salinity stress tolerance was demonstrated when the mechanical removal of bladder cells in quinoa resulted in a salt-sensitive phenotype.[329] The molecular identities of key transporters involved in accumulation of Na^+ and Cl^- in EBCs in quinoa have recently been postulated[330] and characterized at the functional level.[331] Salt bladders may also function as a secondary epidermis to reduce water loss and prevent excessive UV damage in addition to functioning as reservoirs for the storage of water and various metabolic compounds.[332,333]

Osmotic Adjustment

Plants may achieve osmotic adjustment via two major avenues, i.e., by *de novo* synthesis of organic osmolytes and by increased uptake of inorganic ions. Because the production of organic osmolytes has a very high carbon cost,[334,335] plants that use this strategy may be able to survive stress conditions but may grow poorly. The carbon cost of osmotic adjustment via inorganic ion uptake is an order of magnitude lower than that via organic osmolyte synthesis[334,336] and is generally preferred, assuming that the problem of Na^+ toxicity is resolved. This is the case for halophytes.[250,314,337] Among inorganic osmolytes, K^+ is the most abundant in the cell and non-toxic so is preferred. However, K^+ uptake under saline conditions has an additional cost,[281] while Na^+ is present in the soil in high concentration and thus can be taken passively. Thus, halophytes rely on Na^+ as a "cheap osmoticum" to maintain cell turgor pressure (hence, elongation growth and stomatal operation). This trait is complemented by the superior ability of halophytes to safely sequester toxic Na^+ and Cl^- ions in the vacuole and to thereby maintain their cytosolic and organellar concentrations below toxic levels (as discussed in previous sections). The osmotic potential of the cytosol is then adjusted by an increased accumulation of organic osmolytes to match that of the vacuole. As the volume of the cytosol is only ~10% of the total cell volume, such a strategy is energetically more favorable than one in which the cell deposits large amounts of organic osmolytes into its vacuole in order to maintain its turgor.

Leaf Photochemistry

Both PSII and PSI are inactivated by increasing NaCl levels in the cytosol.[276,338] The dark reactions of photosynthesis are also affected by salinity,[339] with many key photosynthetically related enzymes being inactivated.[134] Such inactivation, however, appears to differ between halophytic and glycophytic species. The transport of some key metabolites such as pyruvate, ascorbate, and phosphate into chloroplasts in halophytes requires the presence of Na^+.[134] Chloroplastic fructose 1,6-bisphosphatase (FruP$_2$ase) from the halophyte *Porteresia coarctata* was less sensitive to NaCl *in vitro* compared with its domesticated relative *Oryza sativa* (cultivated rice);[340] this difference was attributed to mutations in some amino acid residues in the FruP$_2$ase gene.[341] Some halophytes require high concentrations of Cl^- to enhance electron transport and oxygen evolution during salt stress;[134] they also demonstrate a higher extent of attachment of Rubisco activase to the thylakoid membrane.[342,343] The sensitivity of grana unstacking to the ionic strength of the stroma also differs significantly between glycophytes and halophytes.[344,345] Salinity also has a greater effect on rETR and maximal photochemical efficiency of PSII in isolated chloroplasts of glycophytes than of halophytes.[276] There seems to be some important differences in the structure of the PSII complex between halophytes and glycophytes, with PsbQ protein (one of the extrinsic proteins mediating binding affinity of Cl^- to the Mn-Ca-Cl complex) being completely absent in halophytes.[345,346]

Stomatal Patterning and Operation

The low soil-water potential imposed by salinity causes a marked decline in stomatal conductance (G_s); the physiological rationale behind this reduction is the plant's attempt to minimize water loss under the conditions of reduced water availability ("physiological drought") imposed by salinity. This reduction in G_s comes, however, with a reduction in net CO_2 assimilation, and therefore a reduction in plant growth. To understand how this problem is solved by halophytes, consider that G_s may be reduced by a decrease in stomatal aperture or stomatal density.[250] Halophytes seem to

be highly efficient in controlling both stomatal aperture and density. Stomatal aperture is controlled by many environmental and internal signals; among these, ABA and H_2O_2 are the most important. Salt stress-induced ABA production is highly dynamic, with peak ABA levels detected within 15 min of salinity exposure in plant roots and within 30 min in leaves.[347] However, while all crop species respond to salinity by a rapid increase in the xylem sap ABA content,[348–350] this seems not to be the case for halophytes.[351,352] It also appears that the basal leaf ABA content (i.e., content under non-saline conditions) is much lower in halophytes than in glycophytes[353] and is increased only slightly in salt-grown plants, while in glycophytes, salinity stress results in a 2- to 3-fold increase in leaf ABA content. It was suggested that modulation of the stomatal aperture by altering ABA levels operates over a much lower concentration range in halophytes than in glycophytes.[353] Another difference concerns the stress-induced increases in ROS levels, which are accelerated in halophytes compared with glycophytes.[354] Consequently, the ROS sensitivity of the guard cell channels and antioxidant systems should differ between halophytes and glycophytes.[353] Another striking difference comes from the ability of halophytes to substitute K^+ by Na^+ in stomatal operations.[355]

Leaves can lose water even when stomata are fully closed. This process, which is termed residual transpiration, is controlled by several factors, one of which is stomatal density. Stomatal density is positively correlated with G_s,[356] and it was argued that a reduction in stomatal density may represent a fundamental mechanism by which plants can optimize water-use efficiency (WUE).[250] Consistent with this idea are observations by Franks et al.,[357] who found that WUE was increased by ~20% in *Arabidopsis* mutants that had a reduced stomatal density as a result of overexpressing the *EPF2* (epidermal pattering factor) gene. Salinity causes a marked (about 30%) decrease in stomatal density in quinoa,[250,326,358] and a negative correlation between stomatal density and salt tolerance has been reported for many other halophytic species (reviewed in Shabala[250]).The stomatal lineage is dynamic and flexible,

altering stomatal production in response to environmental change, with numerous transcriptional regulators, cell-to-cell signaling, and polarity proteins involved.[359,360] An increased understanding of this process in halophytes could suggest ways to increase WUE (and therefore overall salinity stress tolerance) in crops.

Salt Cress as Model Halophytic Organisms

More than 15 years ago, two close relatives of *Arabidopsis* from the genus of *Thellungiella* were proposed as model halophytes for molecular genetics studies, because of their ease of genetic transformation, short life cycle, and relatively small genome.[361,362] One of them is *Schrenkiella parvula* (formerly known as *Eutrema parvulum* and before that as *Thellungiella parvula*), and the other one is *Eutrema salsugineum* (formerly known as *T. salsuginea*). Consistent with the notion that the same molecular machinery for salt tolerance operates in both glycophytes and halophytes, loss-of-function mutants of the *SOS1* homolog in *E. salsugineum* lost halophytism.[363] The genomic sequences for both *E. salsugineum* and *S. parvula* have been reported. Although both genomes are diploid and contain a similar number of genes, they differ significantly in their content of repeat/transposable elements (TEs).[261,364,365] About 50% of the *E. salsugineum* genome is composed of TEs, and it remains to be investigated whether these TEs contribute to the evolution of its salt tolerance traits. Certain physiological traits are associated with high salt tolerance in salt cress, including high accumulation of the osmoprotectant proline, duplication or increased expression of salt stress-associated genes, swollen roots with additional layers of endodermis and cortex, and efficient management of the Na^+/K^+ ratio.[366,367] In particular, the expression of *SOS1* and the vacuolar H^+-pyrophosphatase VP1 are increased in response to high salinity in *E. salsugineum*.[363,368] EsSOS1 is under positive selection and operates more efficiently than SOS1 from *Arabidopsis*.[369] Interestingly, a single amino acid substitution determines the ion selectivity of EsHKT1;2, which prefers K^+ over Na^+ and confers salt tolerance in *Arabidopsis*.[370] These findings indicate that evolution acts on

Future Perspective

Soil salinity will continue to threaten crop production and food security in the future. Cultivation of salt-tolerant crops is the most effective way to overcome this environmental problem. In the last three decades, extensive efforts have contributed to our understanding of the mechanisms of salt stress tolerance in plants. However, application of this fundamental knowledge to improve salt stress tolerance of crops in the field is a slow and challenging process. With the aid of gene editing technologies and advances in efficient genetic transformation in different species, improvement of salt stress tolerance of crops will become more feasible. Halophytes have developed special molecular mechanisms or special cellular structures to tolerate high concentration of salts. Growing halophytes in saline soils can greatly increase the area of arable land and also improve salt conditions in the soil. Knowledge of the molecular mechanisms underlying the formation and function of salt tolerance structures, such as EBCs, in halophytes can be potentially applied in crops to improve their capacity to accumulate high concentrations of ions.

Identification of salt stress sensor(s) is one of the most important questions concerning the molecular mechanisms of the salt stress response in plants. The discovery of GIPCs as potential monovalent-cation sensors[7] opens a road to understand how plants sense salt stress at the plasma membrane. However, Na^+ can accumulate both in the apoplast and in the cytosol, which implies that Na^+ can be sensed not only at the plasma membrane but can also be perceived in different organelles, such as chloroplasts, mitochondria, and the ER. Most likely, the signals from different organelles need to be integrated and coordinated to achieve optimized cellular responses to salt stress. In the future, salt stress-induced organellar stress and the crosstalk between different organelles need to be further studied. The cell wall, as a front line that is directly exposed to stress, is involved in the sensing of salt stress. The status of cell wall integrity under salt stress is crucial for plants to determine the strength and duration of salt stress response. Currently little is known about the salt-induced cell wall changes and the mechanisms underlying the sensing and repair of cell wall integrity under salt stress. Engineering of crops with enhanced cell wall biosynthesis may not only increase the biomass but also improve the tolerance to a variety of environmental stresses. At the transcriptional level, it is critical to understand the expression of a specific gene in a strict tissue- and cell-based context and also study the gene regulatory network at the whole-plant level. The molecular mechanisms underlying root-to-shoot signaling need to be further elucidated. It has been shown that multiple phytohormones are involved in the response to high salinity. Systemic studies on the crosstalks between different hormones in response to salt stress need to be emphasized in the future.

Declaration of interests

The authors declare no competing interests.

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