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A review of salt stress

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ABSTRACT: Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain. The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and ion disequilibrium. In addition, crop plants must be capable of satisfactory biomass production in a saline environment (yield stability). Tolerance and yield stability are complex genetic traits that are difficult to establish in crops since salt stress may occur as a catastrophic episode, be imposed continuously or intermittently, or become gradually more severe and at any stage during development. However, cell biology and molecular genetics research is providing new insight into the plant response to salinity and is identifying genetic determinants that effect salt tolerance. Recent confirmation that many salt tolerance determinants are ubiquitous in plants has led to the use of genetic models, like Arabidopsis thaliana, to further dissect the plant salt stress response. Since many of the most fundamental salt tolerance determinants are those that mediate cellular ion homeostasis, this review will focus primarily on the functional essentiality of ion homeostasis mechanisms in plant salt tolerance. The transport systems that facilitate cellular capacity to utilize Na^+ for osmotic adjustment and growth and the role of the SaltOverly-Sensitive (SOS) signal transduction pathway in the regulation of ion homeostasis and salt tolerance will be particularly emphasized. A perspective will be presented that integrates cellular based stress signaling and ion homeostasis mechanisms into a functional paradigm for whole plants and defines biotechnology strategies for enhancing salt tolerance of crops.

Keywords: Salt adaptation, ion homeostasis, transport determinants, stress signaling

INTRODUCTION

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed

(Flowers and Yeo, 1995). Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability. Water and soil management practices have facilitated agricultural production on soils marginalized by salinity but additional gain by these approaches seems problematic. On the horizon are crop improvement strategies that are based on the use of molecular marker techniques and biotechnology, and can be used in conjunction with traditional breeding efforts (Ribaut and Hoisington, 1998). DNA markers should enhance the recovery rate of the isogenic recurrent genome after hybridization and facilitate the introgression of quantitative trait loci necessary to increase stress tolerance. Molecular marker techniques were used successfully to transfer alleles of interest from wild relatives into commercial cultivars (Tanksley and McCouch, 1997). The basic resources for biotechnology are genetic Determinants of salt tolerance and yield stability. Implementation of biotechnology strategies to achieve this goal requires that substantial research effort be focused to on identify salt tolerance effectors and the regulatory components that control these during the stress episode (Hasegawa et al ,.2000b). Further knowledge obtained about these stress tolerance determinants will be additional resource information for the dissection of the plant response to salinity, which will reveal how plants sense salt stress, transduce signals to mediate a defensive response and define the signal pathway outputs or effectors that accomplish the processes required for stress survival and alleviation, and steady state growth in the saline environment. Molecular genetics and plant transformation advances have made it feasible to assess biotechnological

strategies based on activated signal cascades, engineered biosynthetic pathways, targeted gene or protein expression or alteration of the natural stress responsiveness of genes for development of salt tolerant crops (Hasegawa et al., 2000b; Zhu, 2001). The molecular identities of key ion transport systems that are fundamental to plant salt tolerance are now known (Hasegawa et al., 2000b). More recently, the SOS salt stress signaling pathway was determined to have a pivotal regulatory function in salt tolerance, fundamental of which is the control of ion homeostasis (Hasegawa et al., 2000b; Sanders, 2000; Zhu, 2000). This review will summarize research on plant ion homeostasis in saline environments and present a model that integrates current understanding of salt stress sensing, which leads to the activation of the SOS pathway and the regulation of ion transport systems that facilitate ion homeostasis.

Genetic Diversity for Salt Tolerance in Plants

The extensive genetic diversity for salt tolerance that exists in plant taxa is distributed over numerous genera (Flowers et al., 1986; Greenway and Munns, 1980). Most crops are salt sensitive or hypersensitive plants (glycophytes) in contrast to halophytes, which are native flora of saline environments. Some halophytes have the capacity to accommodate extreme salinity because of very special anatomical and morphological adaptations or avoidance mechanisms (Flowers et al., 1986). However, these are rather unique characteristics for which the genes are not likely to be introgressed easily into crop plants. Research of recent decades has established that most halophytes and glycophytes tolerate salinity by rather similar strategies often using analogous tactical processes (Hasegawa et al., 2000b). The cytotoxic ions in saline environments, typically Na^+ and Cl^-, are compartmentalized into the vacuole and used as osmotic solutes (Blumwald et al., 2000; Niu et al., 1995). It follows then that many of the molecular entities that mediate ion homeostasis and salt stress signaling are similar in all plants (Hasegawa et al., 2000b). In the fact, the paradigm for ion homeostasis that facilitates plant salt tolerance resembles that described for yeast (Bressan et al., 1998; Serrano et al., 1999). The fact that cellular ion homeostasis is controlled and effected by common molecular entities made it feasible to use of model genetic organismal systems for the dissection of the plant salt stress response (Bressan et al., 1998; Serrano et al., 1999; Hasegawa et al., 2000a; Sanders, 2000; Zhu, 2001). Research on the plant genetic model Arabidopsis has increased greatly our understanding of how cellular salt tolerance mechanisms are integrated and coordinated in an organismal context, and are linked to essential phonological adaptations. Since Arabidopsis is a glycophyte, a salt tolerant genetic model will be required to delineate if salt tolerance is affected most by form or function of genes or more by differences in the expression of common genes due either to transcriptional or post-transcriptional control (Zhu, 2000).

Cellular Mechanisms of Salt Stress Survival, Recovery and Growth

High salinity causes hyperosmotic stress and ion disequilibrium that produce secondary effects or pathologies (Hasegawa et al., 2000b; Zhu, 2001). Fundamentally, plants cope by either avoiding or tolerating salt stress. That is plants are either dormant during the salt episode or there must be cellular adjust to tolerate the saline environment. Tolerance mechanisms can be categorized as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses. The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which if severe enough can cause growth reduction (Bohnert et al., 1995). Growth cessation occurs when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration begins when the water potential difference is greater than can be compensated for by turgor loss (Taiz and Zeiger, 1998).

The cellular response to turgor reduction is osmotic adjustment. The cytosolic and organellar machinery of glycophytes and halophytes is equivalently Na^+ and Cl^- sensitive; so osmotic adjustment is achieved in these compartments by accumulation of compatible osmolytes and osmoprotectants (Bohnert, 1995; Bohnert; Jensen, 1996). However, Na^+ and Cl^- are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity (Blumwald et al., 2000; Niu et al., 1995). Since plant cell growth occurs primarily because of directional expansion mediated by an increase in vacuolar volume, compartmentalization of Na^+ and Cl^- facilitates osmotic adjustment that is essential for cellular development. Movement of ions into the vacuole might occur directly from the apoplast into the vacuole through membrane vesiculation or a cytological process that juxtaposes the plasma membrane to the tonoplast (Hasegawa et al., 2000b). Then compartmentalization could be achieved with minimal or no exposure of the cytosol to toxic ions. However, it is not clear presently the extent to which processes like these contribute to vacuolar ion compartmentalization. The bulk of Na^+ and Cl^- movement from the apoplast to the vacuole likely is mediated through ion transport systems located in the plasma membrane and tonoplast. Presumably, tight coordinate regulation of these ion transport systems is required in order to control net influx across the plasma membrane and vacuolar compartmentalization. The SOS signal pathway is a pivotal regulator of, at least some, key transport systems required for ion homeostasis (Hasegawa et al., 2000a; Sanders, 2000; Zhu 2000).

Osmolytes and Osmoprotectants

As indicated previously, salt tolerance requires that compatible solutes accumulate in the cytosol and organelles where these function in osmotic adjustment and osmoprotection (Rhodes and Hanson., 1993). Some compatible osmolytes are essential elemental ions, such as K^+, but the majorities are organic solutes. Compatible solute accumulation as a response to osmotic stress is a ubiquitous process in organisms as diverse as bacteria to plants and animals. However, the solutes that accumulate vary with the organism and even between plant species. A major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose), sugar alcohols (glycerol and methylated inositol) and complex sugars (trehalose, raffinose and fructans) (Bohnert and Jensen., 1996). Others include quaternary amino acid derivatives (proline, glycine betaine, β-alanine betaine, proline betaine), tertiary amines 1,4 ,5 ,6-tetrahydro-2-mehyl-4-carboxyl pyrimidine), and sulfonium compounds (choline o-sulfate, dimethyl sulfonium propironate) (Nuccio et al., 1999). Many organic osmolytes are presumed to be osmoprotectants, as their levels of accumulation are insufficient to facilitate osmotic adjustment. Glycine betaine preserves thylakoid and plasma membrane integrity after exposure to saline solutions or to freezing or high temperatures (Rhodes and Hanson, 1993). Furthermore, many of the osmoprotectants enhance stress tolerance of plants when expressed as transgene products (Bohnert and Jensen, 1996; Zhu, 2001). An adaptive biochemical function of osmoprotectants is the scavenging of reactive oxygen species that are by-products of hyperosmotic and ionic stresses and cause membrane dysfunction and cell death (Bohnert and Jensen, 1996). A common feature of compatible solutes is that these compounds can accumulate to high levels without disturbing intracellular biochemistry (Bohnert and Jensen, 1996). Compatible solutes have the capacity to persevere the activity of enzymes that are in saline solutions. These compounds have minimal effect on pH or charge balance of the cytosol or luminal compartments of organelles. The synthesis of compatible osmolytes is often achieved by diversion of basic intermediary metabolites into unique biochemical reactions. Often, stress triggers this metabolic diversion. For example, higher plants synthesize glycine betaine from choline by two reactions that are catalyzed in sequence by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) (Rhodes and Hanson, 1993). Pinitol is synthesized from myo-inositol by the sequential catalysis of inositol-o-methyltransferase and ononitol epimerase (Bohnert and Jensen, 1996).

Ion Homeostasis - Transport Determinants and their regulation

Since NaCl is the principal soil salinity stress, a research focus has been the transport systems that are involved in utilization of Na^+ as an osmotic solute (Blumwald et al., 2000; Hasegawa et al., 2000b; Niu et al., 1995). Research of more than 30 years previously, established that intracellular Na^+ homeostasis and salt tolerance are modulated by Ca^{2+} and high [Na^+] Ext negatively affects K^+ acquisition (Rains and Epstein., 1967). Na^+ competes with K^+ for uptake through common transport systems and does this effectively since the [Na^+] Ext in saline environments is usually considerably greater than [K^+]ext. Ca^{2+} enhances K^+/Na^+ selective intracellular accumulation (Maathuis et al.,1996; Rains and Epstein, 1967).

Research of the last decade has defined many of the molecular entities that mediate Na^+ and K^+ homeostasis and given insight into the function of Ca^{2+} in the regulation of these transport systems. Recently, the SOS stress-signaling pathway was identified to be a pivotal regulator of plant ion homeostasis and salt tolerance (Hasegawa et al., 2000b; Sanders, 2000). This signaling pathway functionally resembles the yeast cascade that controls calcineurin Na^+ influx and efflux across the plasma membrane (Bressan et al., 1998). Little is known about the mechanistic entities that are responsible for Cl^- transport or the regulation of Cl^- homeostasis (Hedrich., 1994). Ion Transport Systems that Mediate Na^+ Homeostasis H^+ Pumps.H^+pumps in the plasma membrane and tonoplast energize solute transport necessary to compartmentalize cytotoxic ions away from the cytoplasm and to facilitate the function of ions as signal determinants (Maeshima, 2000; Maeshima, 2001; Morsomme and Boutry, 2000; Ratajczak, 2000). The plasma membrane localized H^+ pump is a P-type ATPase and is primarily responsible for the large (pH and membrane potential gradient across this membrane (Morsomme and Boutry, 2000). A vacuolar type H^+ ATPase and a vacuolar pyro phosphatase generate the ∆pH and membrane potential across the tonoplast (Drozdowicz and Rea, 2001; Maeshima, 2001). The activity of these H+ pumps is increased by salt treatment and induced gene expression may account for some of the up regulation (Hasegawa et al., 2000b; Maeshima, 2001). Recently, the plasma membrane H^+ ATPase was confirmed as a salt tolerance determinant based on analyses of phenotypes caused by the semi-dominant aha4-1 mutation (Vitart et al., 2001). The mutation to AHA4, which is expressed predominantly in the roots causes a reduction in root and shoot growth (relative to wild type) of plants that are grown on medium supplemented with 75 mM NaCl. Decreased root length of salt treated aha4-1plants is due to reduce cell length. In NaCl supplemented medium, leaves of aha4-1plants accumulate substantially more Na^+ and less K^+ than those of wild type. It is postulated that AHA4functions in the control of Na^+ flux across the endodermis (Vitart et al., 2001).

Na^+ influx and efflux across the plasma membrane.

Recently, much insight has been gained about Na^+ transport systems that are involved in net flux of the cation across the plasma membrane (Amtmann and Sanders, 1999; Blumwald et al., 2000; Hasegawa et al., 2000b). Transport systems with greater selectivity for K^+ are presumed to facilitate Na^+ "leakage" into cells. Specifically, Na^+ is a competitor for uptake through plasma membrane K^+ inward rectifying channels, such as those that are in the Shaker type family, e.g. AKT1 (Schachtman, 2000). K^+ outward rectifying channels also may facilitate Na^+ influx (Schachtman, 2000). The high affinity K transporter (HKT) from wheat and low affinity cation transporter (LCT) also may be responsible for Na^+ influx across the plasma membrane (Schachtman, 2000; Amtmann and Sanders, 1999; Blumwald et al., 2000). Both HKT and LCT transport Na^+ when expressed in heterologous systems, providing evidence of their function in Na^+ uptake. Wheat HKT1 is a Na^+ H^+ dependent K^+ transporter. Modifications to HKT1 that enhance K^+ transport also reduce Na+ influx and enhance salt tolerance further establishing that Na^+ conductance occurs through this protein (Rubio et al., 1999). Physiological data also implicate nonselective cation (NSC) channels in Na^+ influx (Amtmann and Sanders, 1999). Recently, the properties of HKT proteins from Arabidopsis (Kato et al., 2001; Uozumi et al., 2000; rice Horie et al., 2001; Fairbairn et al., 2000) have been characterized. AtHKT1 is the only member of the Arabidopsis gene family while both rice and eucalyptus have at least two genes. AtHKT1 expression increases NaCl sensitivity of a yeast strain deleted for the plasma membrane Na^+ efflux system (ena1-4Δ) but does not suppress the K^+ deficiency of trk1 trk2mutant cells that have attenuated uptake of this essential cation. However, AtHKT1 expression does suppress the K^+ deficient phenotype of an E. colimutant for which acquisition of the cation is disrupted. Electrophysiological data indicate that AtHKT1 expressed in Xenopus oocytes specifically transports Na^+ and conductance is K^+, H^+ and voltage independent (Uozumi et al., 2000). The in planta function of AtHKT1 as an effector of Na^+ influx has been confirmed recently (Rus et al., 2001). T-DNA insertion AL and deletion mutations of AtHKT1 were identified in a screen for suppressors of NaCl sensitivity of the sos3-1mutant (Liu and Zhu., 1997., Rus et al., 2001). Suppression of sos3-1NaCl sensitivity is correlated with reduced cellular accumulation of Na^+ and capacity to maintain [K^+] int. Together; these results establish that AtHKT1 controls Na^+ influx into plants. It is likely that AtHKT1 is a Na^+ influx system but its function as a regulator of Na^+ and K^+ influx systems cannot be precluded. Since the transcript is expressed predominantly in the roots, AtHKT1 most probably functions in the control of Na^+ into the xylem for export to the shoot (Rus et al., 2001; Uozumi et al., 2000). Rice (Oryza sativaL. Indica) OsHKT1 and OsHKT2 were identified based on sequence similarity with wheat HKT (Horie et al., 2001). OsHKT1and 2transcripts accumulate in response to low K^+ but their steady-state abundance is reduced by treatment with 30 mM NaCl. Yeast complementation and Xenopus oocyte expression data indicate that OsHKT1 functions as a Na^+ influx system like AtHKT1 but OsHKT2 is a Na^+/K^+ symporter. The two Eucalyptus camaldulensis HKT1 (EcHKT1 and 2) orthologs have similar transport characteristics as the wheat protein (Fairbairn et al., 2000). Interestingly, activation of these proteins occurs in response to hypotonic treatment, implicating an osmosensing capacity. The recent identification of sos3-1 hkt1double mutations in Arabidopsis has confirmed the existence of a Na^+ entry system(s) different than HKT1 that functions in planta. Reduction in [Ca] ext to µM concentrations abrogates the capacity for an hkt1 knockout mutation to suppress Na^+ sensitivity of sos3-1. These results indicate the presence of a Ca^{2+} inhibited Na^+ influx system. For the last several years, physiological research has indicated the presence of Ca^{2+} insensitive and sensitive Na^+ conductance in analysis of plant cell patches (Amtmann and Sanders, 1999). The Ca^{2+} sensitive component of Na^+ uptake recently has been attributed to NSC channels (Davenport and Tester, 2000). Energy-dependent Na^+ transport across the plasma membrane of plant cells is mediated by the secondary active Na^+/H^+ antiporter SOS1. Phylogenetic ally, SOS1 is similar to SOD2 of Saccharomyces pombe, NHA1 of S. cerevisiae and NhaA and NhaP of Pseudomonas aeruginosa (Shi et al., 2000).

Na^+ Vacuolar Compartmentalization.

A Na^+/H^+ antiporter that is energized by the ΔpH across the tonoplast facilitates vacuolar compartmentalization of the cation. The Arabidopsis AtNHX1 was isolated by functional genetic complementation of a yeast mutant defected for the endosomal Na^+/H^+ antiporter yeast (ScNHX1) and has sequence similarity to mammalian NHE transporters (Apse et al., 1999; Gaxiola et al., 1999; Quintero et al., 2000). Transgenic Arabidopsis and tomato plants that over express AtNHX1 accumulate abundant quantities of the transporter in the tonoplast and exhibit substantially enhanced salt tolerance (Apse et al.,1999; Quintero et al., 2000; Zhang and Blumwald, 2001). These results implicate the pivotal function of the AtNHX family in vacuolar compartmentalization of Na^+. Predicted amino acid sequence and topological similarities to AtNHX1 led to the categorization of six loci in Arabidopsisas AtNHX gene family members Yokoi et al. (submitted). Phylogenetic ally, the proteins can be categorized into two subgroups, one containing four (AtNHX1-4) and the other two (AtNHX5 and 6) members. AtNHX2 and AtNHX5 expression suppresses the Na^+/Li^+ sensitive phenotype of a salt sensitive yeast mutant (ena1-4 nha1 nhx1Δ) indicating that both AtNHX2 and AtNHX5 are orthologous to yeast ScNHX1 and AtNHX1. AtNHX2 suppresses the Na^+/Li^+ sensitive phenotype of the yeast

mutant to a greater extent thanAtNHX1. AtNHX1and AtNHX2 is expressed constitutively in shoot and roots. Transcript abundance of AtNHX1and AtNHX2is induced by hyperosmotic stress (NaCl, sorbitol) and this osmotic response is dependent on the hormone abscisic acid (ABA). NaCl but not ABA induces AtNHX5 transcript abundance Yokoi et al. (submitted). Steady-state transcript abundance of AtNHX1, 2and 5is greater in so mutants than wild type Col-0 gl1indicating that the SOS pathway negatively regulates transcriptional expression of these Na^+/H^+ antiporters genes. Yeast complementation and expression profiling data indicate that AtNHX2 and 5, like AtNHX1, are functional salt tolerance determinants. A common hyperosmotic stress signal pathways regulates the expression of AtNHX1 and 2 but a different cascade controls AtNHX5 expression. Post-transcriptional mechanisms that control AtNHX antiporter activation are still not known.

Ca^{2+} Signaling and the Activation of the Salt Overly Sensitive (SOS) Signal Transduction Pathway

Jian-Kang Zhu and co-workers identified three genetically linked Arabidopsis loci (SOS1, SOS2and SOS3), which are components of a stress-signaling pathway that controls ion homeostasis and salt tolerance (Hasegawa et al., 2000a; Sanders, 2000; Zhu, 2000). Genetic analysis of Na^+/Li^+ sensitivity established that sos1 is epistatic to sos2and sos3 (Zhu, 2001). These so mutants also exhibit a K^+ deficient phenotype in medium supplemented with µM [K^+] ext and [Ca^{2+}] ext. Na^+ and K^+ deficiency of sos2 and sos3is suppressed with mM [Ca^{2+}]ext (Zhu et al., 1998). Sos1 exhibits hyperosmotic sensitivity unlike sos3and sos2. Together, these results indicate that the SOS signaling pathway regulates Na^+ and K^+ homeostasis and is Ca^{2+} activated. SOS3 encodes a Ca^{2+} binding protein with sequence similarity to the regulatory B subunit of calcineurin (protein phosphatase 2B) and neuronal Ca^{2+} sensors (Ishitani et al., 2000; Liu and Zhu, 1998). Interaction of SOS3 with the SOS2 kinase (Liu et al., 2000) and SOS2 activation is Ca^{2+} dependent (Halfter et al., 2000). The in planta function of SOS3 as a salt tolerance determinant is dependent on Ca^{2+} binding and N-myristoylation (Ishitani et al., 2000). The SOS2 serine/threonine kinase (446 amino acids) has a 267 amino acid N-terminal catalytic domain that is similar in sequence to yeast SNF1 (sucrose nonfermenting) kinase and the mammalian AMPK (AMP-activated protein kinase) (Liu et al., 2000; Zhu, 2000). The kinase activity of SOS2 is essential for its salt tolerance determinant function (Zhu, 2001). The SOS2 C-terminal regulatory domain interacts with the kinase domain to cause auto inhibition. A 21 amino acid motif in the regulatory domain of SOS2 is the site where SOS3 interacts with the kinase and is the autoinhibitory domain of the kinase (Guo et al., 2001). Binding of SOS3 to this motif blocks autoinhibition of SOS2 kinase activity. Deletion of the autoinhibitory domain results in constitutive SOS2 activation, independent of SOS3. Also, a Thr 168 to Asp mutation in the activation loop of the kinase domain constitutively activates SOS2. Genetic and biochemical evidence indicates that components of the SOS signal pathway function in the hierarchical sequence outlined (Hasegawa et al., 2000b; Sanders, 2000; Zhu, 2001). Ca^{2+} binds to SOS3, which leads to interaction with SOS2 and activation of the kinase. Among the SOS signal pathway outputs are transport systems that facilitate ion homeostasis. The plasma membrane sited Na^+ /H^+ antiporter SOS1 is controlled by the SOS pathway at the transcriptional and post transcriptional level (Guo et al., 2001; Zhu, 2001). Recently, functional disruption of AtHKT1was shown to suppress the salt sensitive phenotype ofsos3-1, indicating that the SOS pathway negatively controls this Na^+ influx system (Rus et al., 2001). Also, the SOS pathway negatively controls expression of AtNHX family members that are implicated as determinants in the salt stress response (Yokoi et al., submitted). [Ca^{2+}] ext enhances salt tolerance and salinity stress elicits a transient [Ca^{2+}]cyt increase, from either an internal or external source, that has been implicated in adaptation (Knight et al., 1997; Läuchli, 1990). Data from recent experiments with yeast has provided insight into Ca^{2+} activation of salt stress signaling that controls ion homeostasis and tolerance (Matsumoto et al., 2001). The hyperosmotic component of high salinity induces a short duration (1 min) rise in [Ca^{2+}] cyt that is due substantially to influx across the plasma membrane through the Cch1p and Mid1p Ca^{2+} transport system. The transient increase in [Ca^{2+}] cyt activates the PP2B phosphatase calcineurin (a key intermediate in salt stress signaling controlling ion homeostasis) leading to the transcription of ENA1, which encodes the P-type ATPase that is primarily responsible for Na^+ efflux across the plasma membrane (Nakamura et al., 1993; Mendoza et al., 1994; Matsumoto et al., submitted). The model proposes that the hyperosmotic ally-induced localized [Ca^{2+}] cyt transient activates calmodulin that is tethered to Cch1p-Midp (Elhers et al., 1999; Sanders et al., 1999; Matsumoto et al., submitted). Calmodulin in turn activates signaling through the calcineurin pathway, which mediates ion homeostasis and salt tolerance (Matsumoto et al., submitted). Components of the SOS pathway, either SOS3 or upstream elements, might be associated with an osmotically responsive channel through which Ca^{2+} influx could initiate signaling through the pathway. It is notable that a new elevated [Ca^{2+}] cyt steady state is established in yeast cells, that are maintained in medium supplemented with NaCl, after the hyperosmotic induction of the short duration [Ca^{2+}] cyt transient (Matsumoto et al., submitted). It is likely that the newly established [Ca^{2+}] cyt contributes to cellular capacity for growth in salinity. The vacuolar membrane H^+/Ca^{2+} antiporter Vcx1p and endomembrane localized Ca^{2+} ATPases are pivotal effectors that regulate the amplitude and duration of the [Ca^{2+}] cyt transient (Miseta et al., 1999). The [Ca^{2+}] cyt steady state established in salt containing medium presumably also involves coordination of channel activation that facilitates influx from external and internal sources and energy

dependent transport systems that compartmentalize the divalent caution. It is reasonable to assume that the salt induced [Ca^{2+}] cyt transient detected in plant cells (Knight, 1996) perhaps, a new [Ca^{2+}]cyt steady-state are controlled by the ECA and ACA Ca^{2+} ATPases and CAX1 and 2 transporters which are of Vcx1p (Sze et al., 2000). Nevertheless, Ca^{2+} has at least two roles in salt tolerance, a pivotal signaling function in the salt stress response leading to adaptation and a direct inhibitory effect on a Na^+ entry system.

Perspectives

Database analysis indicates that there are at least seven additional SOS2 isoforms (PKS:Protein Kinase S) and six SOS3 isoforms (SCaBPs:SOS3-like Calcium Binding Proteins) of SOS3. Whether these isoforms also are salt tolerance determinants has yet to be elucidated. One can speculate that these proteins have similar signaling intermediate functions as the prototype proteins but in different cell types or at unique stages of development. Perhaps these isoforms are constituents of signal pathways that respond to different inducers but are still components of the plant response to salt stress. Notwithstanding, it is likely that these proteins have both unique and overlapping functions. It is plausible also that some of these isoforms act as negative regulators of SOS signal transduction by physical interaction with the positive effector or competition for substrates required for signaling. Such positive and negative regulation of signal modulation may constitute a "fine tuning" necessary to achieve the appropriate plant response for stress adaptation and yield stability. Further insight regarding these suggestions may establish how the plant salt stress response is coordinated through gene families. The control system probably is even more complicated since other SOS signal pathway intermediates and outputs and other signaling cascades necessary for salt tolerance may exist. Recent progress in the elucidation of salt stress signaling and effector output determinants that mediate ion homeostasis has uncovered some potential biotechnology tactics that may be used to obtain salt tolerant crop plants, i.e. enhance the yield stability under salinity. Two basic strategies are feasible; regulate the salt stress signal pathway that controls tolerance effectors or modulate effector activity or efficacy. The recent demonstration that a constitutively activated SOS2 kinase can be achieved by deletion of the auto inhibitory domain or by site-specific modifications to the catalytic domain of the protein kinase (Guo et al., 2001) offers an approach to regulate stress signaling that controls ion homeostasis. Constitutive activation of yeast calcineurin in the host or in plants increases salt tolerance by predisposing the plants to survive the stress episode (Mendoza et al., 1996; Pardo et al., 1998). Furthermore, overexpression of AtNHX1 enhances plant salt tolerance, presumably by increasing vacuolar Na^+ compartmentalization that minimizes the toxic accumulation of the ion in the cytosol and facilitates growth in the saline environment (Apse et al., 1999; Zhang and Blumwald, 2001). Perhaps regulating net Na^+ influx across the plasma membrane would enhance salt tolerance efficacy achieved by overexpressing the vacuolar antiporter. Control of net Na^+ flux across the plasma membrane should be achieved by modulating the expression or activation of SOS1 (Na^+ efflux) and/or HKT1 (Na^+ influx). Or, by expressing more efficacious forms of the Na^+ transport proteins. For example, mutant variant forms of HKT1 transport more K^+ at the expense of Na^+ and render greater salt tolerance (Rubio et al., 1999). Promoters that direct tissue and inducer specific regulation of the target genes can condition the expression of the signal intermediates and the effectors. Thus regulation of the numerous salt tolerance determinants can be coordinated for an effective plant response but many of the costs associated with salt tolerance in nature might be minimized because some essential evolutionary necessities can be compensated for by agricultural practices.

REFERENCES

Amtmann A, Sanders D. 1999. Mechanisms of Na^+ uptake by plant cells. In: eds) Advances in Botanical Research. Academic Press, pp.76-114

Apse MP, Aharon GS, Snedden WA, Blumwald E. 1999. Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in Arabidopsis. Science 285:1256-1258

Blumwald E, Aharon GS, Apse MP. 2000. Sodium transport in plant cells. Biochemica et Biophysica Acta 1465: 140-151

Bohnert HJ, Jensen RG. 1996. Strategies for engineering waterstress tolerance in plants. Trends in Biotechnology 14: 89-97.

Bohnert HJ, Nelson DE, Jensen RG. 1995. Adaptations to environmental stresses. Plant Cell 7: 1099-1111

Braun Y, Hassidim M, Lerner H, Reinhold L. 1986. Studies on H^+ translocationg ATPase in plants of varying resistance to salinity. I. Salinity during growth modulates the proton pump in the halophyte Atriplex nummularia. Plant Physiol. 81: 1050-1056

Bressan RA, Hasegawa PM, Pardo JM. 1998. Plants use calcium to resolve salt stress. Trends in Plant Sci. 3: 411-412

Casas AM, Bressan RA, Hasegawa PM. 1991. Cell growth and water relations of the halophyte A triplex nummularia L., in response to NaCl. Plant Cell Rep. 10: 81-84

Davenport RJ, Tester M. 2000. a weakly voltage-dependent, nonselective cation channel mediates toxic sodium influx in wheat. Plant Physiol. 122: 823-34

Drozdowicz YM, Rea PA. 2001. Vacuolar H(+) pyrophosphatases: from the evolutionary backwaters into the mainstream. Trends Plant Sci. 6: 206-11

Ehlers MD, Augustine GJ. 1999. Cell signaling. Calmodulin at the channel gate. Nature399, 105: 107-8

Fairbairn DJ, Liu W, Schachtman DP, Gomez-Gallego S, Day SR, Teasdale RD. 2000. Characterisation of two distinct HKT1-like potassium transporters from Eucalyptus camaldulensis. Plant Mol. Biol. 43: 515-25

Flowers TJ, Hajibagheri MA and Clipson NJW. 1986. Halophytes. The Quart. Rev. Biol.61: 313-337

Flowers TJ, Yeo AR. 1995. Breeding for salinity resistance in crop plants: where next? Aust. J. Plant Physiol. 22: 875-884

Gaxiola RA, Rao R, Sherman A, Grisafi P, Alper SL, Fink GR. 1999. The Arabidopsis thaliana proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. Proc. Natl. Acad. Sci. USA 96: 1480-1485

Greenway H, Munns R. 1980. Mechanisms of salt tolerance in no halophytes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 31: 149-190

Guo Y, Halfter U, Ishitani M, Zhu JK. 2001. Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. Plant Cell 13: 1383-400

Halfter U, Ishitani M, Zhu JK. 2000. The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc. Natl. Acad. Sci. USA97: 3735-3740

Hasegawa PM, Bressan RA, Pardo JM. 2000a. the dawn of plant salt to tolerance genetics. Trends in Plant Sci. 5: 317-319

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. 2000b. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 463-499

HassidimM, Braun Y, Lerner H, Reinhold L. 1990. Na^+/H^+ and K^+/H^+ antiport in root membrane vesicles isolated from the halophyte Atriplex and the glycophyte cotton. Plant Physiol 94: 1795-1801

Hedrich R. 1994. Voltage-dependent chloride channels in plant cells: identification, characterization, and regulation of guard cell anion channel. Curr. Topics Membr.42: 1-33

Horie T, Yoshida K, Nakayama H, Yamada K, Oiki S, Shinmyo A. 2001. Two types of HKT transporters with different properties of Na^+ and K^+ transport in Oryza sativa. Plant J 27: 129-38

Ishitani M, Liu J, Halfter U, Kim CS, Shi W, Zhu JK. 2000. SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding. Plant Cell 12:1667-1677

Kato Y, Sakaguchi M, Mori Y, Saito K, Nakamura T, Bakker EP, Sato Y, Goshima S, Uozumi N. 2001. Evidence in support of a four trans membrane-pore-trans membrane topology model for the Arabidopsis thaliana Na^+/K^+ trans locating AtHKT1 protein, a member of the superfamily of K^+ transporters. Proc. Natl. Acad. Sci. USA 98: 6488-6493

Knight H, Trewavas AJ, Knight MR. 1996. Cold calcium signaling in Arabidopsisinvolves two cellular pools and a change in calcium signature after acclimation. Plant Cell 8: 489-503 Läuchli A. 1996. In Calcium in Plant Growth and Development, eds. Leonald, R. T. and Hepler, P. K. The American Society of Plant Physiologists Symposium Series (American Society of Plant Physiologists. Rockvill, MD) 4: 26-35

Knight H, Trewavas AJ, Knight MR. 1997. Calcium signaling in Arabidopsis thaliana responding to drought and salinity. Plant J.12: 1067-78

Liu J, Ishitani M, Halfter U, Kim CS, Zhu JK. 2000. The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proc. Natl. Acad. Sci. 97: 3730-3734

Liu J, Zhu JK. 1997. An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. Proc. Natl. Acad. Sci. USA94: 14960-14964

Liu J, Zhu JK. 1998. A calcium sensor homolog required for plant salt tolerance. Sci. 280, 1943-1945

Maathuis FJM, Verlin D, Smith FA, Sanders D, Ferneáßndez JA, Walker NA. 1996. The

Maeshima M. 2000. Vacuolar $H(^+)$ pyrophosphatase. Biochim.Biophys. Acta1465: 37-51

Maeshima M. 2001. Tonoplast transporters: Organization and function. Annu. Rev. Plant. Physiol. Plant Mol. Biol. 52:469-497

Matsumoto TK, Ellsmore AJ, Cessna SG, Slow P, Pardo JM, Bressan RA, Hasegawa PM. 2001. An osmotically induced cytosolic Ca^{2+} transient activates calcineurin signaling to mediate ion homeostasis and salt tolerance of Saccharomyces cerevisiae. in preparation.

Mendoza I, Quintero FJ, Bressan RA, Hasegawa PM, Pardo JM. 1996. Activated calcineurin confers high tolerance to ion stress and alters the budding pattern and cell morphology of yeast cells. J. Biol. Chem. 271: 23061-7

Mendoza I, Rubio F, Rodriguez Navarro A, Pardo JM. 1994. The protein phosphatase calcineurin is essential for NaCl tolerance of Saccharomyces cerevisiae. J. Biol. Chem. 269: 8792-6

Miseta A, Kellermayer R, Aiello DP, Fu L, Bedwell DM. 1999. The vacuolar Ca^{2+}/H^+ exchanger Vcx1p/Hum1p tightly controls cytosolic Ca^{2+} levels in S. cerevisiae. FEBS Lett451: 132-6

Morsomme P, Boutry M. 2000. The plant plasma membrane H(+)-ATPase: structure,functionand regulation. Biochim. Biophys. Acta1465: 1-16

Nakamura T, Liu Y, Hirata D, Namba H, Harada S, Hirokawa T, Miyakawa T. 1993. Protein phosphatase type 2B (calcineurin)-mediated, FK506-sensitive regulation of intracellular ions in yeast is an important determinant for adaptation to high salt stress conditions. 12:4063-71

Niu X, Bressan RA, Hasegawa PM, Pardo JM. 1995. Ion homeostasis in NaCl stress environments. Plant Physiol.109: 735-742

Niu X, Narasimhan ML, Salzman RA, Bressan RA, Hasegawa PM. 1993. NaCl regulation of plasma membrane H(+)-ATPase gene expression in a glycophyte and a halophyte. Plant Physiol. 103: 713-8

Nuccio ML, Rhodes D, McNeil SD, Hanson AD. 1999. Metabolic engineering of plants for osmotic stress resistance.Curr. Opin. Plant Biol. 2: 128-34

Pardo JM, Reddy MP, Yang S, Maggio A, Huh GH, Matsumoto T, Coca MA, Paino-D'Urazo M, Koiwa H, Yun DJ, Watad AA, Bressan RA, Hasegawa PM. 1998. Stress signaling through Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin mediates salt adaptation in plants. Proc. Natl. Acad. Sci. USA95: 9681-9686

physiological relevance of Na^+ coupled K^+ transport. Plant Physiol. 112: 1609-1616

Quintero FJ, Blatt MR, Pardo JM. 2000. Functional conservation between yeast and plant endosomal Na^+/H^+ antiporters.FEBS Lett.471: 224-228

Rains D, Epstein E. 1967. Sodium absorption by barley roots. Its mediation by mechanisms 2 of alkali cation transport. Plant Physiol. 42: 319-323

Ratajczak R. 2000. Structure, function and regulation of the plant vacuolar H(+)-translocating ATPase. Biochim. Biophys.Acta1465: 17-36

Rhodes D, Hanson AD. 1993. Quaternary ammonium and terriary sulfonium compounds in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 357-384

Ribaut JM, Hoisington D. 1998. Marker-assisted selection: new tools and strategies. Trends in Plant Sci. 3: 236-239

Rubio F, Schwarz M, Gassmann W, Schroeder JI. 1999. Genetic selection of mutants in the high affinity K^+ transporter HKT1 that define functions of a loop site for reduced Na^+ permeability and increased Na^+ tolerance. J. Biol. Chem.274: 6839-6847

Rus A, Yokoi S, Sharkhuu A, Reddy M, Lee BH, Matsumoto TK, Koiwa H, Zhu JK, Bressan RA, Hasegawa PM. 2001. AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots. Proc. Natl. Acad. Sci. USA. 98: 14150-14155

Sanders D, Brownlee C, Harper JF. 1999. Communicating with calcium. Plant Cell11: 691-706

Sanders D. 2000. Plant biology: The salty tale of Arabidopsis. Curr. Biol. 10: 486-488

Schachtman DP. 2000. Molecular insights into the structure and function of plant K^+ transport mechanisms. Biochimica et Biophysica Acta1465: 127-139

Serrano R, Culiañz-Maciá A, Moreno V. 1999. Genetic engineering of salt and drought tolerance with yeast regulatory genes. Sci Hortic78: 261-269

Serrano R, Kielland-Brandt M, Fink G. 1986. Yeast plasma membrane ATPase is essential for growth and has homology with (Na^+ K^+), K^+ and Ca^{2+} ATPases. Nature319: 689-693

Shi H, Ishitani M, Kim C, Zhu JK. 2000. The Arabidopsisthaliana salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. Proc. Natl. Acad. Sci. USA 97: 6896-6901

Sze H, Liang F, Hwang I, Curran AC, Harper JF. 2000. Diversity and regulation of plant Ca^{2+} pumps: insights from expression in yeast. Annu. Rev. Plant. Physiol. Plant Mol. Bio. l51: 433-62

Taiz L, Zeiger E. 1998. Plant Physiology. Sunderland, Massachusetts: Sinauer Associates, Inc

Tanksley SD, McCouch SR. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science277: 1063-1066

Uozumi N, Kim EJ, Rubio F, Yamaguchi T, Muto S, Tsuboi A, Bakker EP, Nakamura T, Schroeder JI. 2000. The Arabidopsis HKT1gene homolog mediates Inward Na^+ currents in Xenopus laevis oocytes and Na^+ uptake in Saccharomyces. Plant Physiol. 122: 1249-1259

Vitart V, Baxter I, Doerner P, Harper JF. 2001. Evidence for a role in growth and salt resistance of a plasma membrane H^+ ATPase in the root endodermis. Plant J. 27:191-201

Yokoi S, Quintero FJ, Bressan RA, Pardo JM, Hasegawa PM. 2001. Differential expression and function of Arabidopsis thaliana NHX Na^+/H^+ antiporters in the salt stress response. In preparation

Zhang HX, Blumwald E. 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat Biotechnol.19: 765-8

Zhu JK, Liu J, Xiong L. 1998. Genetic analysis of salt tolerance in Arabidopsis: Evidence for a critical role to potassium nutrition. Plant Cell. 10: 1181-1191

Zhu JK. 2000. Genetic analysis of plant salt tolerance using Arabidopsis. Plant Physiol. 124: 941-948

Zhu JK. 2001. Plant salt tolerance. Trends in Plant Sci. 6: 66-71