# Mechanisms of Salinity Tolerance

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## Key Words

salt tolerance, salinity stress, sodium toxicity, chloride, stress tolerance

## Abstract

The physiological and molecular mechanisms of tolerance to osmotic and ionic components of salinity stress are reviewed at the cellular, organ, and whole-plant level. Plant growth responds to salinity in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, $Na^+$ or $Cl^-$ exclusion, and the tolerance of tissue to accumulated $Na^+$ or $Cl^-$. Our understanding of the role of the *HKT* gene family in $Na^+$ exclusion from leaves is increasing, as is the understanding of the molecular bases for many other transport processes at the cellular level. However, we have a limited molecular understanding of the overall control of $Na^+$ accumulation and of osmotic stress tolerance at the whole-plant level. Molecular genetics and functional genomics provide a new opportunity to synthesize molecular and physiological knowledge to improve the salinity tolerance of plants relevant to food production and environmental sustainability.

## Contents

# INTRODUCTION

Soil salinity stresses plants in two ways. High concentrations of salts in the soil make it harder for roots to extract water, and high concentrations of salts within the plant can be toxic. Salts on the outside of roots have an immediate effect on cell growth and associated metabolism; toxic concentrations of salts take time to accumulate inside plants before they affect plant function. We discuss the physiology and molecular biology of mechanisms that allow plants to adapt to these stresses.

More than 800 million hectares of land throughout the world are salt affected (31). This amount accounts for more than 6% of the world's total land area. Most of this salt-affected land has arisen from natural causes,

from the accumulation of salts over long periods of time in arid and semiarid zones (107). Weathering of parental rocks releases soluble salts of various types, mainly chlorides of sodium, calcium, and magnesium, and to a lesser extent, sulfates and carbonates (124). Sodium chloride is the most soluble and abundant salt released. The other cause of accumulation is the deposition of oceanic salts carried in wind and rain. Rainwater contains 6–50 mg/kg of sodium chloride; the concentration decreases with distance from the coast. Rain containing 10 mg/kg of sodium chloride would deposit 10 kg/ha of salt for each 100 mm of rainfall per year.

Apart from natural salinity, a significant proportion of recently cultivated agricultural

**Stress:** an adverse circumstance that disturbs, or is likely to disturb, the normal physiological functioning of an individual

land has become saline owing to land clearing or irrigation, both of which cause water tables to rise and concentrate the salts in the root zone. Of the 1500 million ha of land farmed by dryland agriculture, 32 million ha (2%) are affected by secondary salinity to varying degrees. Of the current 230 million ha of irrigated land, 45 million ha (20%) are salt affected (31). Irrigated land accounts for only 15% of total cultivated land, but because irrigated land has at least twice the productivity of rainfed land, it produces one third of the world's food.

Salinity is a soil condition characterized by a high concentration of soluble salts. Soils are classified as saline when the ECe is 4 dS/m or more (131), which is equivalent to approximately 40 mM NaCl and generates an osmotic pressure of approximately 0.2 MPa. This definition of salinity derives from the ECe that significantly reduces the yield of most crops.

Because NaCl is the most soluble and widespread salt, it is not surprising that all plants have evolved mechanisms to regulate its accumulation and to select against it in favor of other nutrients commonly present in low concentrations, such as $K^+$ and $NO_3^-$. In most plants, $Na^+$ and $Cl^-$ are effectively excluded by roots while water is taken up from the soil (89). Halophytes, the natural flora of highly saline soils, are able to maintain this exclusion at higher salinities than glycophytes. For example, sea barleygrass, *Hordeum marinum*, excludes both $Na^+$ and $Cl^-$ until at least 450 mM NaCl (44). It is also not surprising that because salinity is a common feature of arid and semiarid lands, plants have evolved mechanisms to tolerate the low soil water potential caused by salinity, as well as by drought, and so tolerance to osmotic stress is a feature of most glycophytes and halophytes.

Former reviews in this series on plant responses to salinity were published either 20 or more years ago (35, 53, 104) or much more recently (58, 145). The 20-year gap and the recent revival in activity is indicative of the breakthroughs now emerging owing to the application of molecular genetics to increase our understanding of the physiological and molecular mechanisms of salinity tolerance in plants. This recent flurry of activity may also reflect the current excitement in plant science for making practical contributions to food production in the face of increasing salinization of agricultural regions and global climate change (75).

## Aim of This Review

The focus of this review is mechanisms of salinity tolerance at the molecular, cellular, and whole plant levels. The aim is to provide a fundamental biological understanding and knowledge to underpin future applications. The great opportunity for salinity tolerance research now is the ability to marry together new molecular techniques with the body of literature on whole plant physiology. This new opportunity in salinity tolerance research provides exciting prospects for ameliorating the impact of salinity stress on plants, and improving the performance of species important to human health and agricultural and environmental sustainability.

Ultimately, plant function is explained by the operation of genes in cells and tissues to regulate plant growth in coordination with environmental constraints. As such, gene and cell function must always be considered in the context of the whole plant. This is especially so in the case of salinity tolerance, where cell-specific processes are of particular importance. A salt-tolerant cell does not necessarily make a salt-tolerant plant.
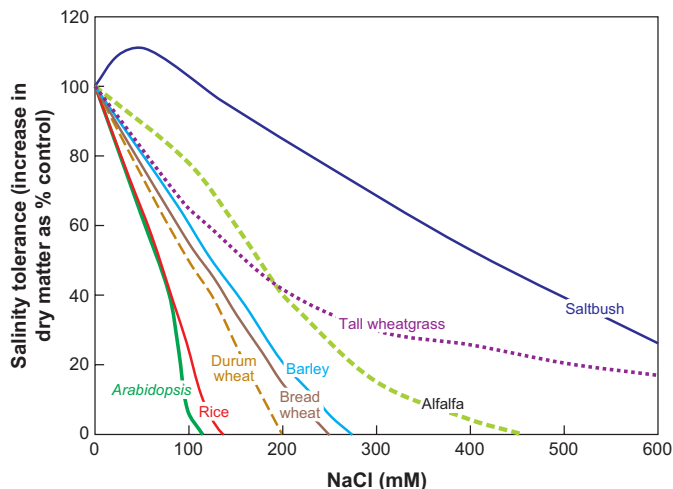
## THE BASES FOR PLANT VARIATION IN TOLERANCE

### Plants Vary in Tolerance

Plants differ greatly in their tolerance of salinity, as reflected in their different growth responses. Of the cereals, rice (*Oryza sativa*) is the most sensitive and barley (*Hordeum vulgare*) is the most tolerant (**Figure 1**). Bread wheat (*Triticum aestivum*) is moderately

**Figure 1**

Diversity in the salt tolerance of various species, shown as increases in shoot dry matter after growth in solution or sand culture containing NaCl for at least 3 weeks, relative to plant growth in the absence of NaCl. Data are for rice (*Oryza sativa*) (6), durum wheat (*Triticum turgidum* ssp *durum*) (19), bread wheat (*Triticum aestivum*) (19), barley (*Hordeum vulgare*) (19), tall wheatgrass (*Thinopyrum ponticum*, syn. *Agropyron elongatum*) (19), *Arabidopsis* (*Arabidopsis thaliana*) (21), alfalfa (*Medicago sativa*) (70), and saltbush (*Atriplex amnicola*) (7).

**Osmotic stress:**
affects growth immediately and is caused by the salt outside the roots

tolerant and durum wheat (*Triticum turgidum* ssp. *durum*) is less so. Tall wheatgrass (*Thinopyrum ponticum*, syn. *Agropyron elongatum*) is a halophytic relative of wheat and is one of the most tolerant of the monocotyledonous species (**Figure 1**); its growth proceeds at concentrations of salt as high as in seawater.

The variation in salinity tolerance in dicotyledonous species is even greater than in monocotyledonous species. Some legumes are very sensitive, even more sensitive than rice (74); alfalfa or lucerne (*Medicago sativa*) is very tolerant, and halophytes such as saltbush (*Atriplex* spp.) continue to grow well at salinities greater than that of seawater (**Figure 1**). Many dicotyledonous halophytes require a

quite high concentration of NaCl (100–200 mM) for optimum growth (35). *Arabidopsis*, when compared with other species under similar conditions of light and humidity (that is, at high transpiration rates), is a salt-sensitive species (**Figure 1**). This sensitive plant may provide limited insights into mechanisms of salinity tolerance unless it is compared with a tolerant relative such as *Thellungiella halophila*. The differences between these two species are highlighted by their responses to 100 mM NaCl under conditions of high transpiration. Continued exposure to 100 mM does not allow *Arabidopsis* to complete its life cycle (116), but has little effect on the growth rate of *Thellungiella* (69).

## Plant Responses Can Occur in Two Distinct Phases Through Time

To understand the physiological mechanisms responsible for the salinity tolerance of these species, it is necessary to know whether their growth is being limited by the osmotic effect of the salt in the soil, or the toxic effect of the salt within the plant. In the simplest analysis of the response of a plant to salinity stress, the reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of $Na^+$ in leaves (**Table 1**).

In the first, osmotic phase, which starts immediately after the salt concentration around the roots increases to a threshold level, the rate of shoot growth falls significantly. The threshold level is approximately 40 mM NaCl for most plants (see definition of salinity above), or less for sensitive plants like rice and *Arabidopsis*. This is largely (but not entirely) due to the osmotic effect of the salt outside the roots. **Figure 2***a* shows the effect on the rate of shoot growth, that is, the rate of increase in shoot dry matter or in leaf area over time. The rate at which growing leaves expand is reduced, new leaves emerge more slowly, and lateral buds develop more slowly or remain quiescent, so fewer branches or lateral shoots form.

**Table 1  The effects of salinity stress on plants**

| Effect of stress | Osmotic stress | Stress due to high leaf $Na^+$ (ionic stress) |
|---|---|---|
| Speed of onset | Rapid | Slow |
| Primary site of visible effect | Decreased new shoot growth | Increased senescence of older leaves |

In cereals, the major effect of salinity on total leaf area is a reduction in the number of tillers; in dicotyledonous species, the major effect is the dramatic curtailing of the size of individual leaves or the numbers of branches. Curiously, shoot growth is more sensitive than root growth, a phenomenon that also occurs in drying soils and for which there is as yet no mechanistic explanation (see the following section). The teleological explanation is that a reduction in leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent an escalation in the salt concentration in the soil.
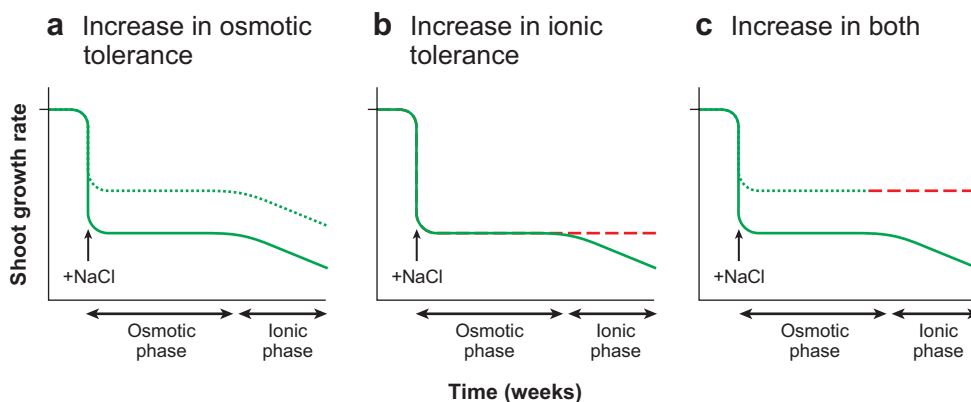
The second, ion-specific, phase of plant response to salinity starts when salt accumulates to toxic concentrations in the old leaves (which are no longer expanding and so no longer diluting the salt arriving in them as younger growing leaves do), and they die. If the rate at which they die is greater than the rate at which new leaves are produced, the photosynthetic capacity of the plant will no longer be able to supply the carbohydrate requirement of the young leaves, which further reduces their growth rate (**Figure 2a**).

The osmotic stress not only has an immediate effect on growth, but also has a greater effect on growth rates than the ionic stress. Ionic stress impacts on growth much later, and with less effect than the osmotic stress, especially at low to moderate salinity levels (**Figure 2a**). Only at high salinity levels, or in sensitive species that lack the ability to control $Na^+$ transport, does the ionic effect dominate the osmotic effect. The effect of increased tolerance to the osmotic stress, with no change in ionic stress tolerance, is shown by the dotted line in **Figure 2a**. A significant genetic variation within species may exist in the osmotic response, but this has not yet been documented. An increase in ionic tolerance takes longer to appear (**Figure 2b**). Within many species, documented genetic variation exists in the rate of accumulation of $Na^+$ and $Cl^-$ in leaves, as well as in the degree to which these ions can be tolerated. An increase in tolerance to both stresses would enable a plant to grow at a reasonably rapid rate throughout its life

**Ionic stress:** develops over time and is due to a combination of ion accumulation in the shoot and an inability to tolerate the ions that have accumulated



**a** Increase in osmotic tolerance

**b** Increase in ionic tolerance

**c** Increase in both

Shoot growth rate

+NaCl

Osmotic phase · Ionic phase

**Time (weeks)**

**Figure 2**

The growth response to salinity stress occurs in two phases: a rapid response to the increase in external osmotic pressure (the osmotic phase), and a slower response due to the accumulation of $Na^+$ in leaves (the ionic phase). The solid green line represents the change in the growth rate after the addition of NaCl. (*a*) The broken green line represents the hypothetical response of a plant with an increased tolerance to the osmotic component of salinity stress. (*b*) The broken red line represents the response of a plant with an increased tolerance to the ionic component of salinity stress (based on Reference 93). (*c*) The green-and-red line represents the response of a plant with increased tolerance to both the osmotic and ionic components of salinity stress.

cycle. This combined tolerance is shown in **Figure 2c**.

For most species, Na$^+$ appears to reach a toxic concentration before Cl$^-$ does, and so most studies have concentrated on Na$^+$ exclusion and the control of Na$^+$ transport within the plant. However for some species, such as soybean, citrus, and grapevine, Cl$^-$ is considered to be the more toxic ion (74, 119). The evidence for this is the association between genetic differences in the rate of Cl$^-$ accumulation in leaves and the plant's salinity tolerance. This difference may arise because Na$^+$ is withheld so effectively in the woody roots and stems that little reaches the leaves, and K$^+$ becomes the major cation. Thus Cl$^-$, which continues to pass to the lamina, becomes the more significant toxic component of the saline solution.

## Three Distinct Types of Plant Response or Tolerance

The mechanisms of salinity tolerance fall into three categories (**Table 2**):

1. Tolerance to osmotic stress. The osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure. A reduced response to the osmotic stress would result in greater leaf growth and stomatal conductance, but the resulting increased leaf area would benefit only plants that have sufficient soil water. Greater leaf area expansion would be productive when a supply of water is ensured such as in irrigated food production systems, but could be undesirable in water-limited systems, and cause the

**Table 2** **Mechanisms of salinity tolerance, organized by plant processes and their relevance to the three components of salinity tolerance**

| Process involved | Candidate genes[a] | Osmotic stress | Ionic stress | |
| --- | --- | --- | --- | --- |
| | | Osmotic tolerance | Na$^+$ exclusion | Tissue tolerance |
| Sensing and signaling in roots | SOS3, SnRKs | Modification of long-distance signaling | Control of net ion transport to shoot | Control of vacuolar loading |
| Shoot growth | ? | Decreased inhibition of cell expansion and lateral bud development | Not applicable[b] | Delay in premature senescence of old (carbon source) leaves |
| Photosynthesis | ERA1, PP2C, AAPK, PKS3 | Decreased stomatal closure | Avoidance of ion toxicity in chloroplasts | Delay in ion toxicity in chloroplasts |
| Accumulation of Na$^+$ in shoots | HKT, SOS1 | Increased osmotic adjustment | Reduced long distance transport of Na$^+$ | Reduced energy spent on Na$^+$ exclusion |
| Accumulation of Na$^+$ in vacuoles | NHX, AVP | Increased osmotic adjustment | Increased sequestration of Na$^+$ into root vacuoles | Increased sequestration of Na$^+$ into leaf vacuoles |
| Accumulation of organic solutes | P5CS, OTS, MT1D, M6PR, S6PDH, IMT1 | Increased osmotic adjustment | Alteration of transport processes to reduce Na$^+$ accumulation | Accumulation of high concentrations of compatible solutes in cytoplasm |

[a]This list is not comprehensive, please see reviews such as Bartels & Sunkar (8), Munns (89), and Zhu (145), as well as the Clickable Guard Cell available at **http://www-biology.ucsd.edu/labs/schroeder/clickablegc.html**
[b]Ions do not accumulate to toxic levels in growing tissues.

soil water to be used up before the grain is fully matured.[1]

2. Na$^+$ exclusion from leaf blades. Na$^+$ exclusion by roots ensures that Na does not accumulate to toxic concentrations within leaves. A failure in Na$^+$ exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves.

3. Tissue tolerance, i.e., tolerance of tissue to accumulated Na$^+$, or in some species, to Cl$^-$. Tolerance requires compartmentalization of Na$^+$ and Cl$^-$ at the cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf. Toxicity occurs with time, after leaf Na$^+$ increases to high concentrations in the older leaves.

**Table 2** summarizes some of the mechanisms relevant to the three components of salinity tolerance, classified by various plant processes.

## Relative Importance of the Three Tolerance Mechanisms

The relative importance of these various processes clearly varies with the species (i.e., the strategy a particular plant species has evolved for responding to the salinity stress), but probably also varies with the length of exposure to the salinity, the concentration of the salt, and possibly the local environmental conditions, notably soil water supply and air humidity, and thus transpiration rate and leaf water potential.

For example, in some conditions a high shoot Na$^+$ may be beneficial by helping the plant maintain turgor. This may become particularly important in drying soils, where access by the plant to other beneficial nutrients (such as N, P, and K) becomes increasingly difficult. A balance probably needs to be struck between the use of Na$^+$ and Cl$^-$ by the plant to maintain turgor and the need to avoid chemical toxicity. Where that balance lies will depend on the species and conditions. This dilemma has been likened to that of Ulysses who had to steer a course through treacherous waters between the twin perils of Scylla and Charybdis (53).

## Methods to Distinguish the Three Tolerance Mechanisms

The two-phase effects of salinity on plants are not obvious if the salinity is high, or if the species is particularly sensitive to Na$^+$. The roots of some species, such as rice, are leaky and Na$^+$ may be taken up apoplastically (48). Then, the first phase, or osmotic effect, might last only a few hours or days at the most before the Na$^+$ levels build up to toxic levels within the leaves (142). However, for most plants in most conditions, the two phases are clearly separated in time (93), which facilitates the experimental separation of the three tolerance mechanisms.

Distinguishing the osmotic effect from the ion-specific effect requires observations over time of the rate of new leaf production and the rate of increase in injury of old leaves. The effect of the osmotic stress is seen as a rapid inhibition of the rate of expansion of young leaves and reduced stomatal conductance of mature leaves. Daily measurements of the length of a growing leaf, or spot measurements of stomatal conductance with a porometer, are good indicators of growth rate.

Ion-specific toxicity is seen as an increase in the rate of senescence of older leaves, due to either high leaf Na$^+$ concentrations or to low tolerance of the accumulated Na$^+$. Leaf Na$^+$ concentration is best measured in a defined leaf of a defined age if the plant was exposed to Na$^+$ at around the time of the

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[1]The focus of this review is on tolerance in agricultural systems, where growth and productivity of annual crops is more important than survival per se. Thus, tolerance to osmotic stress is considered in this review to be the ability to maintain growth. However, in an ecological context, especially for perennial species, survival is often more important than growth, so the emphasis on growth maintenance as an adaptive (beneficial) response is less pronounced.

emergence of that leaf (91, 141). Leaf senescence can be measured nondestructively with a chlorophyll meter or image analysis. Combining rates of senescence in older leaves with measures of leaf $Na^+$ concentration provides an estimate of tolerance to $Na^+$ that has accumulated (tissue tolerance). The use of nondestructive assays, exploiting image analysis, thermography, and hyperspectral reflectance techniques, greatly facilitates the separation of these different types of $Na^+$ tolerance.

Increased osmotic tolerance and increased tissue tolerance will both lead to an increased ability to maintain growth for a given accumulation of $Na^+$ in the leaf tissue. However, they can be distinguished because of their differential effects on younger versus older tissue. Increased osmotic tolerance will be mainly evident by an increased ability to continue production of new leaves, whereas tissue tolerance will be primarily evident by the increased survival of older leaves (**Table 1**).

Interestingly, the *sos* (*salt overly sensitive*) mutants of *Arabidopsis* were identified from a screen based on the maintenance of root growth in nontranspiring conditions, where the delivery of $Na^+$ to the shoot in the transpiration stream would be low. The *sos* mutant screen might detect mutants that are related to the osmotic component of salinity stress because in the nontranspiring conditions used for the initial screen, the primary effect of salinity would be osmotic. In nontranspiring conditions, salinity tolerance in *Arabidopsis* is unrelated to the extent of shoot $Na^+$ accumulation; however, in transpiring conditions salinity tolerance is related to the extent of shoot $Na^+$ accumulation (86).

In the following three sections, each of the three tolerance mechanisms is discussed in more detail.

## OSMOTIC STRESS TOLERANCE

### Growth

The decreased rate of leaf growth after an increase in soil salinity is primarily due to the osmotic effect of the salt around the roots. A sudden increase in soil salinity causes leaf cells to lose water, but this loss of cell volume and turgor is transient. Within hours, cells regain their original volume and turgor owing to osmotic adjustment, but despite this, cell elongation rates are reduced (21, 42, 97, 142). Over days, reductions in cell elongation and also cell division lead to slower leaf appearance and smaller final size. Cell dimensions change, with more reduction in area than depth, so leaves are smaller and thicker.

For a moderate salinity stress, an inhibition of lateral shoot development becomes apparent over weeks, and over months there are effects on reproductive development, such as early flowering or a reduced number of florets. During this time, a number of older leaves may die. However, production of younger leaves continues. All these changes in plant growth are responses to the osmotic effect of the salt, and are similar to drought responses.

The reduction in leaf development is due to the salt outside the roots. That this reduction is largely due to the osmotic effect of the salt is supported by experiments using mixed salts such as concentrated Hoagland's solution (125), other single salts such as KCl (142), and nonionic solutes such as mannitol or polyethylene glycol (PEG) (121, 142). These different osmotica all have a similar qualitative effect as NaCl on leaf expansion.

However, the salt outside the roots may affect plant growth not only through its effect on osmotic pressure. Sümer and coworkers (121) found evidence for $Na^+$ but not $Cl^-$ toxicity during the first phase of salt stress in maize in innovative experiments with different salts and PEG, via the use of additional PEG to adjust the equimolar solutions to equivalent osmotic pressures. Further, Cramer (20) found evidence for the effect of supplemental $Ca^{2+}$ in the rooting solution affecting rapid responses of leaf elongation rate from working with two maize cultivars of different salinity tolerance. A possible $Na^+$-specific effect associated with the growth

response is discussed below in the section on signaling.

The mechanism that downregulates leaf growth and shoot development under stress is not precisely known. The reduction in leaf growth must be regulated by long distance signals in the form of hormones or their precursors, because the reduced leaf growth rate is independent of carbohydrate supply (90) and water status (42, 90). The reduction occurs in the absence of nutrient deficiency (61) and ion toxicity, as evidenced by very low concentrations of $Na^+$ and $Cl^-$ in expanding cells or tissues that do not correlate with growth rates (38, 61, 62, 94). Changes in wall properties must occur (22), but their exact nature remains unknown. The long distance and local signals regulating these wall properties and expansion rates are still obscure.

Abscisic acid (ABA) plays a central role in root-to-shoot and cellular signaling in drought stress and in the regulation of growth and stomatal conductance (26, 145). However, measurements of ABA in growing zones of barley and maize leaves in saline soil do not support a simple ABA control theory. ABA concentrations in the growing zone of salt-treated barley increase transiently but return to the original low value after 24 h, whereas leaf growth rate is still reduced (39). ABA-deficient mutants in maize and tomato generally have the same leaf growth rates as wild-type in drying soil and saline soil (80, 132), indicating that there is another limiting factor. Gibberellins (GAs) are a good candidate. ABA can inhibit leaf elongation in barley by lowering the content of active GA, as indicated by exogenous treatments with ABA and GA and measurements of endogenous GAs in the elongating zone (P.M. Chandler, M. Maheswari & R. Munns, unpublished data). Accumulating evidence shows that members of a class of negative regulators of growth, the DELLA proteins, mediate the growth-promoting effects of gibberellins in a number of species, and integrate signals from a range of hormones and abiotic stress conditions, including salinity (2). DELLA proteins may be the central coordinators that adapt plant growth to different environments (2).

Root growth is usually less affected than leaf growth, and root elongation rate recovers remarkably well after exposure to NaCl or other osmotica (88). Recovery from a moderate stress of up to 0.4 MPa of mannitol, KCl, or NaCl (i.e., an osmotic shock that does not cause plasmolysis) is complete within an hour (37). Even so, recovery from NaCl concentrations as high as 150 mM can occur within a day (88). In contrast to leaves, these recoveries take place despite turgor not being fully restored (37). This indicates different changes in cell wall properties compared with leaves, but the mechanism is unknown. With time, reduced initiation of new seminal or lateral roots probably occurs, but little is known about this.

## Photosynthesis and Stomatal Conductance

The most dramatic and readily measurable whole plant response to salinity is a decrease in stomatal aperture. Stomatal responses are undoubtedly induced by the osmotic effect of the salt outside the roots. Salinity affects stomatal conductance immediately, firstly and transiently owing to perturbed water relations and shortly afterward owing to the local synthesis of ABA (39). A short-lived increase in ABA is detected in the photosynthetic tissues within 10 minutes of the addition of 100 mM NaCl to barley (39, 40); the rapidity of the increase suggesting in situ synthesis of ABA rather than transport from the roots. However, a new reduced rate of transpiration stabilizes within hours (40) while ABA tissue levels return to control concentrations (39, 40). This stomatal response is probably regulated by root signals in common with plants in a drying soil (26), as evidenced by stomatal closure in salt-treated plants whose water status is kept high by applying a balance pressure (126).

Rates of photosynthesis per unit leaf area in salt-treated plants are often unchanged, even though stomatal conductance is reduced (68).

This paradox is explained by the changes in cell anatomy described above that give rise to smaller, thicker leaves and result in a higher chloroplast density per unit leaf area. When photosynthesis is expressed on a unit chlorophyll basis, rather than a leaf area basis, a reduction due to salinity can usually be measured. In any case, the reduction in leaf area due to salinity means that photosynthesis per plant is always reduced.

Cause-effect relationships between photosynthesis and growth rate can be difficult to untangle. It is always difficult to know whether a reduced rate of photosynthesis is the cause of a growth reduction, or the result. With the onset of salinity stress, a reduced rate of photosynthesis is certainly not the sole cause of a growth reduction because of the rapidity of the change in leaf expansion rates described earlier (22, 39, 97), and also because of the increase in stored carbohydrate, which indicates unused assimilate (90). However, with time, feedback inhibition from sink to source may fine tune the rate of photosynthesis to match the reduced demand arising from growth inhibition (98). Reduced leaf expansion resulting in a buildup of unused photosynthate in growing tissues may generate feedback signals to downregulate photosynthesis.

At high salinity, salts can build up in leaves to excessive levels. Exactly how the salts exert their toxicity remains unknown. Salts may build up in the apoplast and dehydrate the cell, they may build up in the cytoplasm and inhibit enzymes involved in carbohydrate metabolism, or they may build up in the chloroplast and exert a direct toxic effect on photosynthetic processes.

## Oxidative Stress

The reduced rate of photosynthesis increases the formation of reactive oxygen species (ROS), and increases the activity of enzymes that detoxify these species (4, 36, 78). When plants acclimate to a changed environment, they undergo adjustments in leaf morphology, chloroplast pigment composition, and in the activity of biochemical processes that prevent oxidative damage to photosystems. The two processes that avoid photoinhibition owing to excess light are heat dissipation by the xanthophyll pigments and electron transfer to oxygen acceptors other than water. The latter response necessitates the upregulation of key enzymes for regulating ROS levels such as superoxide dismutase, ascorbate peroxidase, catalase, and the various peroxidases (4, 78). The coordinated activity of the multiple forms of these enzymes in the different cell compartments achieves a balance between the rate of formation and removal of ROS, and maintains hydrogen peroxide ($H_2O_2$) at the levels required for cell signaling.

All these ROS detoxifying mechanisms are present naturally in surfeit (4, 36, 78), and are "woven into the regulatory regimes of the chloroplast" (78), to protect the photosystems from photoinhibition that might otherwise occur from the rapidly increasing light loads experienced by leaves under naturally variable situations. If a plant has sufficient capacity to adjust to the instant, large changes in light intensity as the sun emerges from behind a cloud, it has more than enough capacity to adjust to the slower changes in the rate of photosynthesis induced by a saline soil. The only situation in which antioxidants appear to be insufficient is when an oxidative burst is induced. However, this does not occur under abiotic stress, but is confined to pathogen attack, when a massive rise in ROS triggers programmed cell death (4).

Therefore, genetic differences in salinity tolerance are not necessarily due to differences in the ability to detoxify ROS. Many studies have found differences in levels of expression or activity of antioxidant enzymes; these differences are sometimes associated with the more tolerant genotype, and sometimes with the more sensitive genotype. We suggest that differences in antioxidant activity between genotypes may be due to genotypic differences in degrees of stomatal closure or in other responses that alter the rate of $CO_2$ fixation, differences that bring into

play the processes that avoid photoinhibition and for which the plant has abundant capacity. For such basic and important defense mechanisms, the biochemical pathways are complex, interactive, and have built-in redundancy. More than 150 genes make up the complex ROS network in *Arabidopsis* (84). Knowledge of the many possible functions of these genes, and the coordination, degree of redundancy, and cross talk between different branches of the ROS network, is still incomplete (84). Doubt has been expressed that the manipulation of a single gene related to oxidative stress tolerance can enhance the tolerance to any abiotic stress (78). Recently *Arabidopsis* mutants lacking either or both a cytosolic and chloroplastic ascorbate peroxidase ($H_2O_2$ removal enzymes) were found to be actually more tolerant of salinity stress (83), illustrating the plasticity of ROS regulatory pathways, and the redundancy of pathways for ROS regulation and protection.

## Cellular Signaling

Long-distance signaling of salinity stress to the shoot from the roots, mediated at least in part by ABA, is discussed above in the context of the rapid inhibition of growth upon addition of NaCl. Although this initial response appears similar at the whole plant level with addition of NaCl or isosmotic concentrations of PEG or mannitol (see Growth, above), comparison of cytosolic $Ca^{2+}$ responses in solutions with physiologically realistic ionic composition revealed that responses of roots to addition of NaCl and sorbitol differ (129). Thus, cells in the roots initially must sense both the ionic and osmotic components of the addition of $Na^+$ and then respond rapidly to changes in its external concentration. The responses root cells need to make are necessary not only to maintain their own correct function in the face of the new elevated external $Na^+$, but also for them to signal to the shoot that shoot function must also be altered. In this section, we focus on signaling within root cells, which is likely to be independent of ABA.

Plants respond directly and specifically to the addition of $Na^+$ within seconds (73, 129), yet the mechanism by which plants sense the addition of $Na^+$ and the change in osmotic pressure remains obscure. The extracellular $Na^+$ is either sensed at the plasma membrane, or if it is sensed intracellularly, then it must first cross the plasma membrane. Thus, a plasma membrane protein must either be the sensor or be immediately upstream of the sensor. This gap in our knowledge is surprising given the importance of this first step in the response by a plant to changes in its environment. A similar notable absence of knowledge exists about the molecular basis for turgor sensing.

The first recorded response to an increase in $Na^+$ around roots is an increase in cytosolic free $Ca^{2+}$ ($[Ca^{2+}]_{cyt}$); the extracellular addition of $Na^+$ is apparently able to activate the flux of $Ca^{2+}$ into the cytosol across the plasma membrane and also, interestingly, the tonoplast (71–73, 87, 129). The changes in $[Ca^{2+}]_{cyt}$ are complex, and are modulated by differences in extracellular composition, including $Na^+$ concentration, providing opportunities for information to be encoded by the $[Ca^{2+}]_{cyt}$ changes (129). An additional level of complexity in NaCl-induced $[Ca^{2+}]_{cyt}$ increases has been demonstrated by root cell type–specific expression of aequorin in *Arabidopsis* (71). In response to 220 mM NaCl, the increase in $[Ca^{2+}]_{cyt}$ is lower in the pericycle than in the other cell types (71).

The best-characterized signaling pathway specific to salinity stress likely involves these increases in $[Ca^{2+}]_{cyt}$ (145). In this pathway, the $Na^+$-induced increase in $[Ca^{2+}]_{cyt}$ may be sensed by a calcineurin B-like protein (CBL4), originally identified as SOS3. Although the affinity for $Ca^{2+}$ binding of this protein is unknown, physiologically realistic increases in cytosolic $Ca^{2+}$ likely facilitate the dimerization of CBL4/SOS3 and the subsequent interaction with a CBL-interacting protein kinase (CIPK24, originally identified as SOS2) (55). The CBL4/CIPK24 (SOS3/SOS2) complex

is targeted to the plasma membrane via a myristoyl fatty acid chain covalently bound to CBL4/SOS3 (65), enabling the phosphorylation and thus the activation of the membrane-bound $Na^+/H^+$ antiporter, SOS1 (102, 103, 115).

However, the role of SOS1 in plant salinity tolerance remains uncertain, because reconciliation of its pattern of expression with its function remains incomplete. Measurement of the effects of SOS1 knockout on long-distance transport of $Na^+$ is confounded because most experiments are performed in nontranspiring conditions (86).

Although which aspect of salinity tolerance is contributed to by this pathway remains uncertain, this pathway is likely important for some aspects of salinity tolerance, because *sos* mutants of *Arabidopsis thaliana* are less tolerant to salinity stress than wild-type plants (146).

Many other components of signaling pathways have also been implicated in plant responses to salinity, inferred by a range of approaches such as transcriptomics and reverse genetics. These are reviewed extensively elsewhere (e.g., 18, 137, 145). However, invoking the adaptive relevance of a particular response to $Na^+$ in a plant that is poorly adapted to salinity (*Arabidopsis*) is risky. These approaches could be strengthened by comparing responses in salt-tolerant and salt-sensitive lines—if the response is greater in the tolerant line, this suggests a role in the tolerance, but if the response is smaller, this may indicate the response is not related to the tolerance per se, but is a downstream response to the stress.

Genetic approaches, such as the screening of mutant populations of *Arabidopsis* for altered salinity tolerance (115, 145) and the identification of the genetic alteration causing observed differences in tolerance (12, 64), are essential for identifying significant genes for tolerance. More work is necessary to disentangle the complexities of the myriad signal transduction networks in plants. It is essential that these experiments are performed in physiologically relevant conditions. Future work

may also be able to allow the identification of the different processes that are relevant to particular aspects of salinity tolerance (as summarized in this review).

Signaling pathways identified in salt-tolerant species (e.g., *Thellungiella halophila*) (50, 133, 135) are more likely to deliver results relevant to adaptive, rather than dysfunctional, responses to salinity, than those in the salt-sensitive *Arabidopsis*—unless, of course, screens of *Arabidopsis* are designed to identify salt-tolerant, rather than salt-sensitive, mutants. This is reflected in two components of ionic stress tolerance—ion exclusion and tissue tolerance.

Overall, cells respond to the perceived difference in extracellular $Na^+$ with changes in diverse aspects of function—from biochemistry and gene transcription to physiology, growth, and development. Transcription factors and small RNAs are central in controlling the core aspects of the longer-term plant transcriptional responses, as reviewed in this series and elsewhere; readers are referred to these detailed overviews (123, 139).

## ACCUMULATION OF SODIUM IONS IN SHOOTS

The main site of $Na^+$ toxicity for most plants is the leaf blade, where $Na^+$ accumulates after being deposited in the transpiration stream, rather than in the roots (88). A plant transpires 50 times more water than it retains in leaves (92), so excluding $Na^+$ from the leaf blades is important, even more so for perennial than for annual species, because the leaves of perennials live and transpire for longer. Most $Na^+$ that is delivered to the shoot remains in the shoot, because for most plants, the movement of $Na^+$ from the shoot to the roots in the phloem can likely recirculate only a small proportion of the $Na^+$ that is delivered to the shoot. As such, the processes determining $Na^+$ accumulation in the shoot are primarily the processes controlling the net delivery of $Na^+$ into the root xylem.

The net delivery of $Na^+$ to the xylem can be divided into four distinct components (127):

1. Influx into cells in the outer half of the root;
2. Efflux back out from these cells to the soil solution;
3. Efflux from cells in the inner half of the root to the xylem; and
4. Influx back into these cells from the xylem before the transpiration stream delivers the $Na^+$ to the leaf blade.

## Thermodynamics of $Na^+$ Transport

The thermodynamics of each of these processes for $Na^+$ are illustrated in **Figure 3a**, and the likely molecular mechanisms are shown in **Figure 3b**. The thermodynamic analysis assumes cytosolic $Na^+$ concentrations of 30 mM and an electrical potential of −120 mM, but even if values differ by a factor of two, the principles remain unchanged. For example, at the xylem parenchyma, the efflux of $Na^+$ from the cells would be active even if the xylem $Na^+$ concentrations were nearly ten times lower than cytosolic

$Na^+$ concentrations (owing to the xylem parenchyma cytoplasm potential being 60 mV negative of the potential in the xylem apoplast). With a xylem $Na^+$ free concentration of 10 mM and a potential difference between the xylem parenchyma cell cytoplasm and xylem apoplast of −60 mV, active influx of $Na^+$ into the xylem parenchyma cells would

**a** Thermodynamics of $Na^+$ and $Cl^-$ transport



**b** Proposed mechanisms of $Na^+$ and $Cl^-$ transport
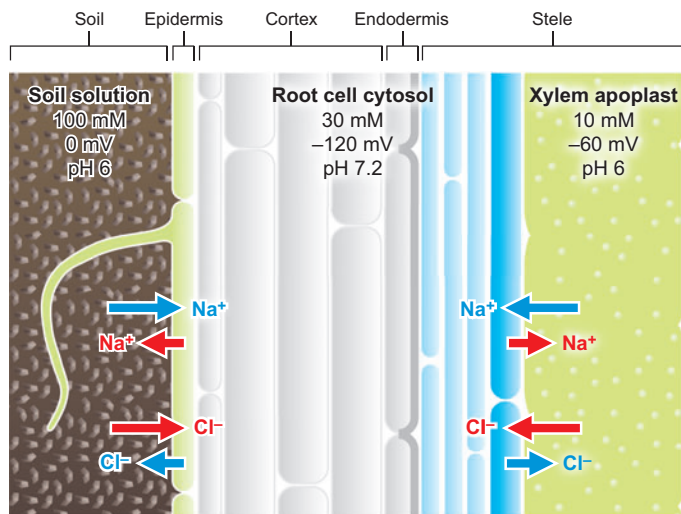


### Figure 3

The thermodynamics and mechanisms of $Na^+$ and $Cl^-$ transport at the soil-root and stelar cell–xylem vessel interfaces in roots. Indicative cytosolic pH, ion concentrations, and voltages are derived from the literature (127, 134). (a) Longitudinal section of wheat root (provided by Dr. Michelle Watt). The cells between the endodermis and the xylem vessel are not labeled, but include pericycle cells and xylem parenchyma (*darker blue*) as well as phloem parenchyma. The stele of dicotyledonous plants is more complex because it includes cambial vascular elements. The thermodynamics of ion movements are indicated by the arrow colors: Active transport is shown as a red arrow, passive transport is shown as a blue arrow. (b) The proposed mechanisms of passive and active $Na^+$ and $Cl^-$ transport at the two interfaces, mediated by ion channels and carriers (uniporters and $H^+$-coupled antiporters and symporters). Abbreviations: SOS1, salt overly sensitive mutant 1; HKT, high-affinity $K^+$ transporter.
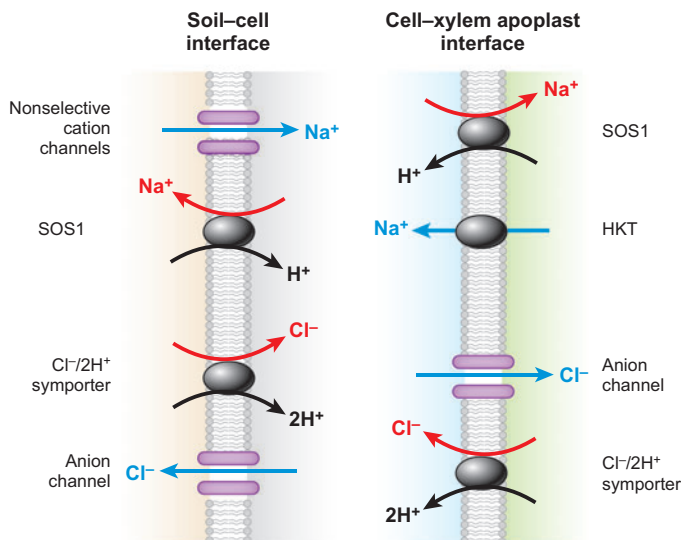
only be necessary with cytoplasmic free $Na^+$ concentrations greater than approximately 100 mM (which, with an activity coefficient of 0.7, is a total concentration of around 140 mM). Another way to look at this is if the cytoplasmic free $Na^+$ were 30 mM and the membrane potential difference were −60 mV, active influx would only be necessary with xylem apoplastic concentrations below 3 mM.

Consideration of the thermodynamics of a $Na^+/H^+$ antiporter is simpler, because the electroneutral exchange this antiporter catalyzes is unaffected by membrane potential. Thus, the direction of $Na^+$ movement is determined simply by the differences in free concentrations of $Na^+$ and $H^+$. A $Na^+/H^+$ antiporter could only work in the opposite direction to that indicated (i.e., it could only pump $Na^+$ into cells) if, for a pH difference of one unit (xylem more acidic), the xylem concentration increased to 10 times that found in the cytoplasm (i.e., to over 300 mM for a cytoplasmic $Na^+$ concentration of 30 mM). Alternatively, if the pH became more alkaline than pH 7.7, then the $Na^+/H^+$ antiporter could pump $Na^+$ into xylem parenchyma cells from a free concentration of 10 mM. These conditions would rarely, if ever, occur, and thus, the

$Na^+/H^+$ antiporter will mostly act to pump $Na^+$ out of cells.

The various processes of $Na^+$ transport are each briefly considered here, but the reader is referred to the more extensive analysis of these processes in Tester & Davenport (127).

## Net Na⁺ Influx Into the Outer Half of Roots

$Na^+$ enters roots passively, via voltage-independent (or weakly voltage-dependent) nonselective cation channels (3, 127) and possibly via other $Na^+$ transporters such as some members of the high-affinity $K^+$ transporter (HKT) family (57, 76). High affinity $Na^+$ influx is also mediated by some members of the HKT transporter family in low salt roots (60), but this is repressed by moderate concentrations of $Na^+$ and so is unlikely to be relevant to salinity tolerance. The identities of the genes encoding nonselective cation channels remain uncertain, although there are several candidates, including cyclic nucleotide–gated channels and ionotropic glutamate receptor–like channels (27).

The main site of $Na^+$ entry in roots is uncertain, although it seems intuitively likely that as water moves across the root cortex toward the stele, ions are removed from this stream into cells, where they are then sequestered in the vacuoles of these cells. This is supported by X-ray microanalysis of roots from rapidly transpiring wheat plants (A. Läuchli, R.A. James, R. Munns, C.X. Huang, & M. McCully, unpublished data), which shows that vacuolar $Na^+$ and $Cl^-$ concentrations decrease across the cortex; vacuolar $Na^+$ and $Cl^-$ concentrations are highest in the epidermis and subepidermis and lowest in the endodermis (**Figure 4**).

Most of the $Na^+$ that enters root cells in the outer part of the root is likely pumped back out again via plasma membrane $Na^+/H^+$ antiporters (127), a process that likely consumes significant energy, given the large fluxes that have been measured. The identities of the genes encoding these $Na^+$ efflux proteins
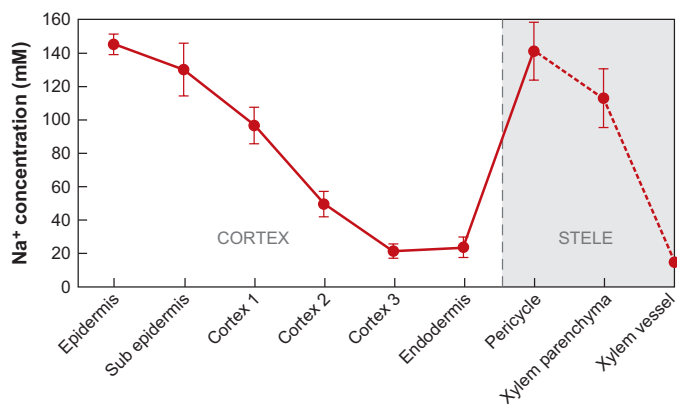


**Figure 4**

Differences in vacuolar concentrations of $Na^+$ across roots of transpiring wheat plants growing in 150 mM NaCl. Concentrations were measured by quantitative and calibrated X-ray microanalysis of snap-frozen sections using a cryo-SEM (scanning electron microscope) method on root tissues 10 cm from the tip (A. Läuchli, R.A. James, R. Munns, C.X. Huang & M. McCully, unpublished data).

are uncertain—in *Arabidopsis*, only two members of the *SOS1* gene family exist, *SOS1* and a currently uncharacterized gene at locus *At1g14660* (95). Plasma membrane $Na^+/H^+$ antiporter activity has been demonstrated for the *Arabidopsis* protein SOS1 (101); although, as discussed above, the levels of expression in the outer half of the mature root are currently uncertain.

Given that active $Na^+$ efflux is required in all cells throughout the plant, it is likely that other genes encoding $Na^+/H^+$ antiporters also exist. Many efflux proteins may be encoded in *Arabidopsis* by the gene at locus *At1g14660* (95), but members of other gene families, particularly the large family of *CHX* genes, may also be important (95). The possibility of other mechanisms for $Na^+$ efflux, such as primary pumping by $Na^+$-translocating ATPases, also needs to be kept in mind (14, 82).

$Na^+$ remaining in the root can be sequestered in vacuoles or transported to the shoot. Compartmentation in vacuoles is achieved by tonoplast $Na^+/H^+$ antiporters such as those belonging to the $Na^+/H^+$ exchanger (NHX) family in *Arabidopsis* (95). There is passive leakage of $Na^+$ back to the cytosol from vacuoles (possibly via tonoplast nonselective cation channels), requiring constant resequestration of $Na^+$ into vacuoles. Constitutive overexpression of *NHX1* or the gene encoding the *Arabidopsis thaliana* vacuolar $H^+$-translocating pyrophosphatase (AVP1), which contributes to the electrochemical potential difference for $H^+$, which energizes the pumping of $Na^+$ into the vacuole, increases both $Na^+$ accumulation and $Na^+$ tolerance in *Arabidopsis*, suggesting that more efficient sequestration may improve tissue tolerance, perhaps by reducing cytosolic $Na^+$ concentrations (5, 45).

## Na+ Loading Into and Retrieval From the Xylem

$Na^+$ moves in the symplast across the endodermis, is released from stelar cells into the stelar apoplast, and then moves to the xylem in the transpiration stream. The plasma membrane $Na^+/H^+$ antiporter, SOS1, is expressed in stelar cells and could be involved in the efflux of $Na^+$ from stelar cells into the xylem. However, this statement needs to be reconciled with the observation that the knockout of this gene causes elevated, not reduced, shoot $Na^+$ levels. The effect of the knockout on $Na^+$ efflux in the outer half of the root may possibly be greater than the effect of the knockout on loading in the inner half of the root.

In another attempt to reconcile the observations, SOS1 has also been implicated in retrieval of $Na^+$ from the xylem (115). However, given the likely difference in pH between the stelar cytosol and apoplast (**Figure 3**), this electroneutral exchange would only be possible with a large (at least an approximately 50-fold) difference in $Na^+$ activity (the apoplast with higher activity), which is extremely unlikely (see section above, Thermodynamics of $Na^+$ Transport).

Increasing evidence exists for the role of some members of the *HKT* gene family in retrieval of $Na^+$ from the xylem. In the *Arabidopsis* root, *AtHKT1;1* is involved in the retrieval of $Na^+$ from the xylem before it reaches the shoot (25, 122). Good evidence is accumulating for a similar function for members of the closely related *HKT1;5* gene family in rice (106) and wheat (12, 24, 66). The candidate gene for the classic $K^+/Na^+$ discrimination (*Kna1*) locus on the long arm of chromosome 4D, described more than 20 years ago by Gorham and colleagues (51) and mapped by Dubcovsky and coworkers (28) and Luo and coworkers (79), is likely an *HKT1;5* gene (12). *Kna1* was associated with a higher leaf $K^+/Na^+$ ratio (mainly determined by the variation in $Na^+$ concentration), and was attributed with providing bread wheat with its superior salinity tolerance over tetraploid wheats (51).

Furthermore, good evidence exists that a closely related gene, *TmHKT1;4-A2*, is the candidate gene for the $Na^+$ exclusion (*Nax1*)

## *AtHKT1;1*, A CASE STUDY OF CONFUSION

The HKT family of proteins comprises a structurally diverse group that separates naturally into two distinct subfamilies (99). This diversity led to early reports of apparently contradictory properties, because the same name used for the first two genes studied, *HKT1*, from wheat and *Arabidopsis*, suggested similar function. Members of the HKT family function as $Na^+/K^+$ symporters and as $Na^+$-selective transporters of both high and low affinity. Subfamily 1 contains low affinity $Na^+$ uniporters.

Different patterns of expression within the plant also affect the role of these transporters in net cation uptake to the shoot; expression of a protein that catalyzes influx in the outer half of the root (epidermis and cortex) increases influx into the plant, but an influxer in the stele reduces net influx into the plant (**Figure 3**).

Although the first *HKT* gene identified was from subfamily 2 (114), this group is less well characterized than subfamily 1. The wheat TaHKT2;1 protein functions as a $Na^+/K^+$ symporter when expressed in *Xenopus* oocytes (109), and down-regulation of expression in planta reduces root $Na^+$ accumulation and improves growth in saline conditions (76). In rice, OsHKT2;1 catalyzes high affinity $Na^+$ influx in low salt roots, conditions where $Na^+$ influx is beneficial (60). At higher external $Na^+$ concentrations, OsHKT2;1 is rapidly downregulated, to reduce potentially toxic $Na^+$ influx.

The most studied member of the *HKT1* subfamily is in *Arabidopsis*, which contains a single *HKT* homolog, *AtHKT1;1*. AtHKT1;1 functions as a $Na^+$-selective uniporter when expressed in *Xenopus* oocytes and yeast, but it also complements an *E. coli* $K^+$ uptake deficient mutant and increases its $K^+$ accumulation, suggesting some role in $K^+$ transport (130).

*Athkt1;1* mutants are salt-sensitive compared with wild-type and hyperaccumulate $Na^+$ in the shoot but show reduced accumulation of $Na^+$ in the root (10, 81, 110). Several hypotheses have been advanced concerning the function of AtHKT1;1 in *Arabidopsis*.

Because *hkt1;1* mutations ameliorated the *sos* phenotypes and reduced whole seedling $Na^+$ in the *sos3* background, Rus and coworkers (111) proposed that AtHKT1;1 is an influx pathway for $Na^+$ uptake into the root. However, Berthomieu and colleagues (10) and Essah and coworkers (30) showed that *hkt1;1* mutants do not have lower root $Na^+$ influx and Berthomieu and coworkers proposed instead that AtHKT1;1

*(Continued)*

locus in durum wheat (64), which is associated with $Na^+$ exclusion and a high leaf $K^+/Na^+$ ratio. The protein encoded by this gene retrieves $Na^+$ from the xylem, and has activity in the leaf sheaths as well as in the root (66).

In *Arabidopsis*, the importance of retrieval of $Na^+$ from the xylem as a primary controller of shoot $Na^+$ concentration and plant salinity tolerance is suggested by forward genetic studies that have revealed *AtHKT1;1* as a primary determinant of these parameters (10, 49, 111). It is noteworthy that, to date, no other genes have been revealed from forward genetic screens for altered shoot $Na^+$ concentration. A suppressor screen of *Athkt1;1* plants may usefully reveal other steps in the $Na^+$ transport process.

It should be noted that the *HKT* gene family is quite diverse, which has confused issues in the past (see *AtHKT1;1*, A Case Study of Confusion), and this sequence diversity likely reflects a diversity of function. As such, increased clarity has been provided by dividing the *HKT* gene family into two distinct subfamilies (99). These subfamilies largely, although not exclusively, reflect differences in a likely selectivity-determining amino acid residue in the first so-called 'pore loop' region of the protein, and differences in the cation selectivity. Subfamily 1 members contain an important serine residue, and are largely $Na^+$-selective; subfamily 2 members have the serine replaced by a glycine, and can catalyze the transport of $K^+$ and probably also can catalyze high affinity $Na^+$ influx (60).

## TISSUE TOLERANCE OF SODIUM IONS

At the cellular level, high amounts of $Na^+$ and $Cl^-$ arriving in leaves can be tolerated by anatomical adaptations and intracellular partitioning. Dicotyledonous halophytes exemplify two types of anatomical adaptations: salt-induced increase in cell size due to increases in vacuole volume (succulence), and the excretion of $Na^+$ and $Cl^-$ by salt glands

(modified trichomes) or bladders (modified epidermal cells) (34). Succulence is extremely rare in monocotyledonous species, and salt glands occur in only approximately 15% of monocotyledonous halophytes (T.J. Flowers, personal communication), but in all species, intercellular transport processes can promote partitioning across the leaf.

The effect of salinity on intercellular partitioning of ions has been particularly studied in barley, a cereal known for its ability to tolerate high leaf tissue concentrations of $Na^+$ and $Cl^-$ (19, 67), by measurement of vacuolar concentrations by scanning electron microscope X-ray microanalysis, either in situ (67) or in sap taken from single cells using a microcapillary (41). In salt-treated barley, there is a greater accumulation of $Cl^-$ in epidermal compared with mesophyll cells (41, 63, 67, 77). The converse is true for $K^+$, that is, there is a greater accumulation of $K^+$ in mesophyll compared with epidermal cells (23, 41, 67), but there is no evidence of partitioning of $Na^+$ between different cell types (67).

## Intracellular Compartmentation of $Na^+$

$Na^+$ must be partitioned within cells so that concentrations in the cytoplasm are kept low, possibly as low as 10–30 mM. No direct measurements of cytosolic concentrations in leaves have been reported, but in roots, direct measurements of cytosolic $Na^+$ in salt-treated plants via the use of ion-sensitive microelectrodes indicate cytosolic $Na^+$ concentrations range from 10 to 30 mM (13). In animal cells, cytosolic concentrations are also of this order (9). However, the concentration at which $Na^+$ becomes toxic is not well defined. In vitro studies showed $Na^+$ starts to inhibit most enzymes at concentrations approaching 100 mM (54), although some enzymes are sensitive to lower concentrations (33). The concentration at which $Cl^-$ becomes toxic is even less well defined, but is probably similar to that for $Na^+$ (33). Even $K^+$ starts to inhibit enzymes at concentrations above 100 mM (33, 54).

**(Continued)**

functions in $Na^+$ recirculation from shoots to roots, by loading $Na^+$ from the shoot into phloem and then unloading it into the roots for efflux.

However, Sunarpi and colleagues (122) demonstrated that AtHKT1;1 localizes to the plasma membrane of xylem parenchyma cells in the shoot. They found both reduced phloem $Na^+$ and elevated xylem $Na^+$ in the shoot of *hkt1;1* mutants and proposed that AtHKT1;1 functions primarily to retrieve $Na^+$ from the xylem, at least in the shoot, and that retrieval of $Na^+$ into the symplast has a secondary effect on phloem $Na^+$ levels.

Most recently, Davenport and coworkers (25) used radioactive tracers to dissect the individual transport processes contributing to $Na^+$ and $K^+$ accumulation in intact, transpiring plants to provide the most direct evidence to date that AtHKT1;1 is involved in $Na^+$ retrieval from the xylem.

Results from closely related members of the HKT1 subfamily in rice and wheat are also consistent with a function of AtHKT1;1 in retrieval of $Na^+$ from the xylem. Thus, even though AtHKT1;1 catalyzes $Na^+$ influx into cells, its effect at the level of the whole plant is to reduce net $Na^+$ influx into the shoot.

Hypotheses regarding the role of AtHKT1;1 in $Na^+$ transport have relied mainly on measurements of tissue ion contents, which are the net result of a number of different transport processes, or on disruptive measurements of phloem and xylem contents. These measurements can often be interpreted in many ways.

In addition, many of the experiments have been conducted in plants grown on agar plates (where transpiration is extremely limited). Transpiring conditions have a major influence on $Na^+$ transport and tolerance (86). This is especially important when studying a gene whose function appears to be to remove $Na^+$ from the transpiration stream.

In *Arabidopsis*, although AtHKT1;1 function is now well defined in roots, its function in the shoot remains obscure, and the hypotheses of Berthomieu and colleagues (10) require careful consideration. In rice, functions for the nine *HKT*-like genes identified thus far remain largely unknown. Although *OsHKT1;5* appears to have a similar role to that of the *Arabidopsis* gene, the functions of other members of the gene family may well be quite distinct, as indicated by Horie and coworkers (60). Much more work is required to properly elucidate the functions of this important gene family.

Ideally, Na$^+$ and Cl$^-$ should be largely sequestered in the vacuole of the cell. That this sequestering occurs is indicated by the high concentrations of Na$^+$ found in leaves that are still functioning normally. Concentrations well over 200 mM on a tissue basis are common, yet these same concentrations will completely repress enzyme activity in vitro and are beyond all known direct measurements of cytosolic Na$^+$ in both eukaryotic and prokaryotic cells, other than the extremely halophilic prokaryotes (127). Importantly, enzymes in halophytes are not more tolerant of salt in vitro than the corresponding enzymes in nonhalophytes, suggesting compartmentation of Na$^+$ is an essential mechanism in all plants, rather than a result of the evolution of tolerance of enzymatic functions in plants from saline environments.

Thus, differences in the expression levels of *AtNHX1* or *AtAVP1* may affect the potential to sequester Na$^+$ in vacuoles of the leaves. Increased salinity tolerance of a range of plant species overexpressing *NHX* genes (5, 11, 15, 59, 138, 143, 144) or *AtAVP1* (45) indicates the feasibility of such a mechanism and suggests that this process is important for Na$^+$ tolerance not only in *Arabidopsis* but also across plant species.

Increased efficiency of intracellular compartmentation may explain differences in salinity tolerance between closely related species. This hypothesis is supported by findings of a much greater salt stress–induced Na$^+$/H$^+$ antiporter activity in the salt-tolerant species *Plantago maritima* than in the salt-sensitive species *Plantago media* (118).

Increased vacuolar Na$^+$ concentrations would require a coordinated increase in the osmotic pressure of the other subcellular compartments, including the cytosol, to maintain their volume. This can be achieved by an increase in the concentration of K$^+$ to sub-toxic levels, as well as the concentration of compatible solutes.

## Increased Accumulation of Compatible Solutes

If Na$^+$ and Cl$^-$ are sequestered in the vacuole of a cell, organic solutes that are compatible with metabolic activity even at high concentrations (hence 'compatible solutes') must accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole (35, 136). The compounds that accumulate most commonly are sucrose, proline, and glycine betaine, although other molecules can accumulate to high concentrations in certain species (35, 58, 89).

In many halophytes, proline or glycine betaine occur at sufficiently high concentrations in leaves (over 40 mM on a tissue water basis) to contribute to the osmotic pressure (over 0.1 MPa) in the cell as a whole (35). In glycophytes, the concentrations of compatible solutes that accumulate are not so high, on the order of 10 mM, but if partitioned exclusively to the cytoplasm, they could generate a significant osmotic pressure and function as an osmolyte. At low concentrations, these solutes presumably have another role, perhaps in stabilizing the tertiary structure of proteins, and function as osmoprotectants (108). An osmolyte role has been suggested for glycine betaine accumulation in maize; comparison of near-isogenic maize lines with contrasting glycine betaine accumulation showed that lines that were homozygous for the *Bet1* (glycine betaine accumulation) gene had a 10%–20% higher biomass under saline conditions (113).

Accumulation of these compatible solutes, such as proline and mannitol, also occurs under drought stress and sometimes under other stresses that reduce growth, such as low temperature. Many studies of genes controlling the synthesis or metabolism of these solutes have indicated their essential role in tolerance to abiotic stresses (16, 56, 108). For example, the lower expression of a gene encoding proline dehydrogenase (*PDH*) may contribute to the higher salt tolerance of *Thellungiella*

*halophila* compared with its salt-sensitive relative *Arabidopsis thaliana* (69). Enhancement of mannitol accumulation in *Arabidopsis* by overexpression of a mannose-6-phosphate reductase from celery caused substantial and sustained increases in growth rate and photosynthesis in saline treatment but not drought, suggesting that mannitol protects the chloroplasts against salt (116). The transgene had no effect on growth in control conditions (116). This is noteworthy, because most reports of transgenic alterations in levels of enzymes that catalyze rate-limiting steps describe plants whose growth is significantly reduced. This may be because uncontrolled accumulation of the solutes perturbs other metabolic pathways, diverting substrates from essential processes such as protein synthesis and cell wall synthesis.

Compatible solute synthesis comes with an energy cost and hence involves a potential growth penalty. In leaf cells, approximately seven moles of ATP are needed to accumulate one mole of NaCl as an osmoticum, whereas the amount of ATP required to synthesize one mole of an organic compatible solute is an order of magnitude higher (105). The ATP requirement for the synthesis or accumulation of solutes has been estimated as 3.5 for $Na^+$, 34 for mannitol, 41 for proline, 50 for glycine betaine, and approximately 52 for sucrose (105). These values assume a production of 0.5 mole of ATP per photon and nitrate as the source of N. The synthesis of these compounds occurs at the expense of plant growth, but may allow the plant to survive and recover from the presence of high external concentrations of salt.

Tolerance of leaf tissue to high $Na^+$ concentrations is clearly an adaptive mechanism, as exemplified by most halophytes (34) and glycophytes such as barley, which can tolerate at least 400 mM $Na^+$ in leaf blades (67). The high $Na^+$ and the accompanying $Cl^-$ allows barley to osmotically adapt and to maintain turgor in the face of high soil salinities. This is the "cheapest" form of osmotic adaptation. The mechanism of $Na^+$ exclusion enables the plant to avoid or postpone the problem of ion toxicity, but unless the exclusion of $Na^+$ is compensated for by the uptake of $K^+$, it creates a greater demand for organic solutes for osmotic adjustment. The synthesis of organic solutes jeopardizes the energy balance of the plant. Thus, the plant must steer a course through ion toxicity on the one hand, and turgor loss on the other, in analogy to the Scylla versus Charybdis dilemma faced by Ulysses.

## OBSERVATIONS IN WHICH SALINITY TOLERANCE IS CLEARLY INDEPENDENT OF TISSUE SODIUM ION CONCENTRATIONS

A negative correlation between salinity tolerance and $Na^+$ accumulation in leaves is often seen when comparing different genotypes within a species (88, 127), but this is not the case when comparing different species, such as wheat and barley. **Figure 5** illustrates the relationship between salinity tolerance and leaf $Na^+$ concentration found within a species, in
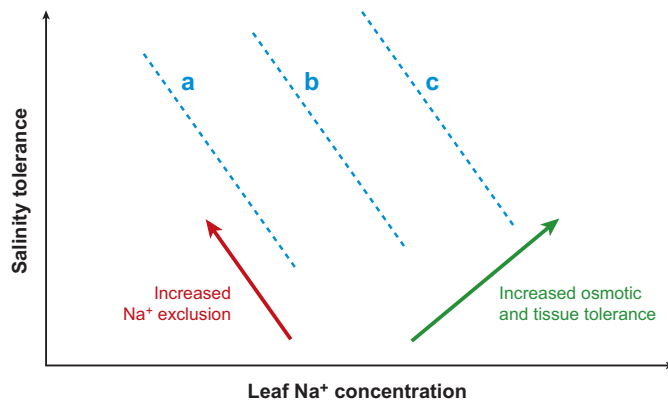
**Figure 5**

Hypothetical relationships between salinity tolerance and leaf $Na^+$ concentration for three different species, denoted by a, b, and c for rice, durum wheat, and barley. Within most species, there is a negative correlation between salinity tolerance and shoot $Na^+$ concentration, as in rice (141) and durum wheat (91) and, with less conviction, in barley (19). A larger intercept on the x-axis indicates an increased tolerance to the osmotic pressure of the soil solution or to high internal concentrations of $Na^+$ or $Cl^-$.
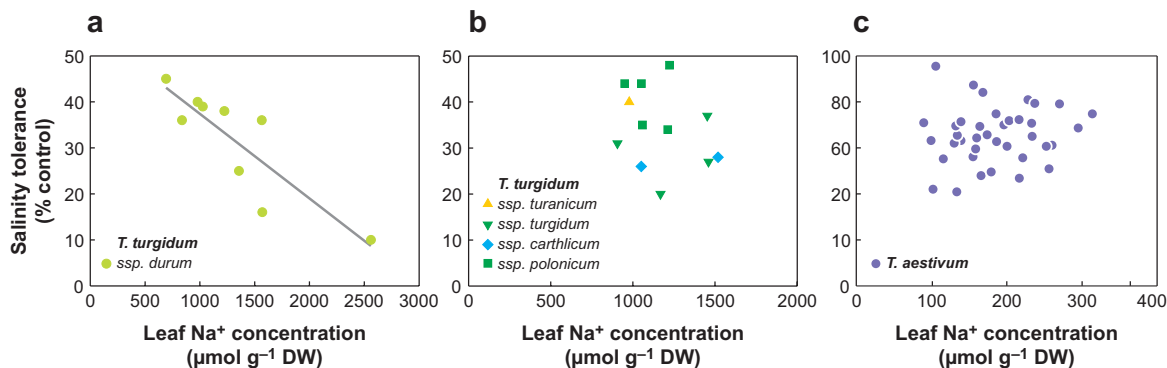
**Figure 6**

Relationships measured between salinity tolerance (biomass in salt as a % of biomass in control conditions) and leaf $Na^+$ concentration in different wheat species. (*a*) Negative relationship for durum wheat (91). (*b*) Lack of relationship for other tetraploid wheats (91). (*c*) Lack of relationship for bread wheat (46).

this case rice (141), durum wheat (91), and the *Hordeum* genus, including barley (44); the figure also shows that this relationship shifts for different species. This shift can reflect differences in tissue tolerance of $Na^+$ between different species, differences in tolerance of $Cl^-$, or differences in tolerance of the osmotic pressure of the soil solution.

Although $Na^+$ exclusion is often a primary determinant of variability in salinity tolerance within a species (**Figure 6a**), many exceptions to this generalization exist, such as within certain subspecies of tetraploid wheat, *Triticum turgidum* (**Figure 6b**), in which differences in salt tolerance do not correlate with differences in $Na^+$ exclusion. One study observed a lack of correlation within bread wheat (*Triticum aestivum*) (**Figure 6c**), possibly because at the moderate salinity of 100 mM NaCl, the leaf $Na^+$ concentration was below the toxic level. The genetic variation in $Na^+$ exclusion may contribute to greater salinity tolerance only in highly saline soil that causes higher leaf $Na^+$ accumulation. We conclude that $Na^+$ exclusion remains an important factor, and increasing $Na^+$ exclusion by conventional or transgenic methods could increase salinity tolerance, but these results indicate other mechanisms may be important in many species, especially at high salinity.

## Mechanisms of Salinity Tolerance Other than $Na^+$ Exclusion

In addition to tissue tolerance mechanisms discussed above, other mechanisms of salinity tolerance unrelated to $Na^+$ exclusion could also be important in these plants.

**Osmotic tolerance.** The relative importance of variation in osmotic tolerance remains unknown for most species, which likely reflects the relative difficulty of quantifying this parameter. A close association likely exists between osmotic tolerance and tissue tolerance of $Na^+$, because genotypes that tolerate high internal $Na^+$ concentrations in leaves by compartmentalizing it in the vacuole should also be more tolerant of the osmotic stress owing to their elevated osmotic adjustment. However, this speculation remains to be tested.

**$K^+$ accumulation in cytoplasm.** The concentration of $K^+$ in the cytoplasm relative to that of $Na^+$ may be a contributing factor to salinity tolerance. In *Arabidopsis*, an additional supply of $K^+$ alleviated the phenotype of the *sos* mutants (145), which may be due to an increase in cytoplasmic $K^+$ concentrations. In barley, Shabala and colleagues (17) found a

negative correlation between $Na^+$-activated net $K^+$ efflux in 3d-old seedlings and salinity tolerance of mature barley plants. This phenomenon may be related to root $K^+$ status, although a strong relationship between leaf $K^+$ concentrations and salinity tolerance has not been found.

**$Cl^-$ tolerance.** The question is often asked: "Why focus only on $Na^+$, why not also consider $Cl^-$?" This question relates particularly to species that accumulate high concentrations of $Cl^-$ and not $Na^+$ in leaves, such as soybean, woody perennials such as avocado, and those species that are routinely grown on $Cl^-$-excluding rootstocks such as grapevines and citrus. For these species, $Cl^-$ toxicity is more important than $Na^+$ toxicity. However, this statement does not imply that $Cl^-$ is more metabolically toxic than $Na^+$, rather these species are better at excluding $Na^+$ from the leaf blades than $Cl^-$. For example, $Na^+$ does not increase in the leaf blade of grapevines until after several years of exposure to saline soil, then the exclusion within the root, stem, and petiole breaks down, and $Na^+$ starts to accumulate in the leaf blade, whereas leaf blade $Cl^-$ concentrations increase progressively (100). Thus, $Na^+$ may be a more toxic solute, but because the plant is managing the $Na^+$ transport better than $Cl^-$ transport, $Cl^-$ becomes the potentially more toxic component.

Many studies have been undertaken to determine whether $Na^+$ is more or less toxic than $Cl^-$. The use of different salts has produced only equivocal results, because of the difficulty in changing the external concentration of one ion versus another without changing the osmotic pressure of the external solution or the rate of uptake of other ions. The most convincing approaches to test the toxicity of $Na^+$ versus $Cl^-$ are genetic approaches. Between different species of wheat, genetic variation in salinity tolerance correlates with leaf $Na^+$ accumulation but not $Cl^-$ accumulation (51, 52). However, genetic variation in salinity tolerance correlates with leaf $Cl^-$ ac-

## THE THERMODYNAMICS AND MECHANISMS CONTROLLING $CL^-$ TRANSPORT

Mechanisms of $Cl^-$ transport are shown in **Figure 3**. In most situations, $Cl^-$ influx requires energy and is probably catalyzed by a $Cl^-/2H^+$ symporter (32, 112), although Skerrett & Tyerman (117) have shown that passive uptake could occur in saline conditions if the membrane potential is depolarized and cytosolic $Cl^-$ is low (less than 20 mM). The cytosolic $Cl^-$ concentration is likely in the range of 10 to 20 mM, but may be higher in saline conditions. Felle (32) showed that the cytoplasmic concentration doubled (from 15 to 33 mM) within minutes of increasing the external $Cl^-$ concentration from 0 to 20 mM. Given the uncertainties surrounding the thermodynamics, useful speculation on the role of net influx processes in salinity tolerance is difficult. Nevertheless, if $Cl^-$ influx is active, and thus efflux is passive, the opening of a $Cl^-$-permeable channel in nonsaline conditions would favor the passive efflux of $Cl^-$. Thus, activation of a $Cl^-$-permeable channel in saline conditions could be useful for reducing the net influx of $Cl^-$. Yamashita and coworkers (140) observed an increase in $Cl^-$ permeability of protoplasts isolated from barley roots after plants were pretreated with 200 mM NaCl, supporting such a role for $Cl^-$ channels. Comparisons of $Cl^-$ transport in lines with different levels of $Cl^-$ accumulation in the shoot would reveal the significance of different transport processes in whole plant accumulation.

cumulation in citrus (119). $Cl^-$ influx is likely active. See The Thermodynamics and Mechanisms Controlling $Cl^-$ Transport.

$Cl^-$ loading into the xylem is most likely a passive mechanism mediated by anion channels such as those characterized by Gilliham & Tester (47). These channels are downregulated by ABA, which may serve to limit $Cl^-$ transfer to the shoot in saline conditions. Radioactive tracer studies have shown that net $Cl^-$ loading into the root xylem is lower in grapevine genotypes that have lower shoot $Cl^-$ accumulation (J. Tregeagle, M. Tester & R. Walker, unpublished results). The control of $Cl^-$ transport to shoots may be due to reduced loading of $Cl^-$ via anion channels, but may also be due to increased active retrieval

of $Cl^-$ from the xylem stream. Sites of tissue $Cl^-$ accumulation indicate that $Cl^-$ can be retrieved from the xylem in petioles, woody stems, and roots.

Results from biochemical approaches (study of the effects of different salts on protein synthesis or enzyme activity) have been equivocal, as have attempts to estimate $Cl^-$ concentrations in the cytoplasm or organelles such as chloroplast and mitochondria. Yet tissue concentrations as high as 400 mM are tolerated by most species, and even the sensitive species like citrus can tolerate tissue concentrations of 250 mM, so $Cl^-$ must be compartmentalized in the vacuole. The thermodynamics and mechanisms of $Cl^-$ transport at the tonoplast are largely unknown, and differences in properties between tolerant and sensitive lines are regrettably obscure.

## APPROACHES FOR FUTURE STUDIES

### The Importance of Cell Type–Specific Processes

Gene expression studies using constitutive promoters provide limited biological information compared with the use of inducible promoters (120) or cell type–specific promoters (127, 128). The choice of promoters can significantly affect the results from a transgenic manipulation. The constitutive expression of genes encoding compatible solutes often inhibits plant growth, as shown by stunted growth and sterility of lines with higher concentrations of mannitol (1). Expression that is inducible upon plant stress should have little effect on growth in control conditions, but can increase tolerance to the applied stress, as shown for trehalose accumulation in rice (43). In a similar vein, constitutive expression of *AtHKT1;1* causes increased shoot accumulation of $Na^+$ and reduced salinity tolerance, whereas expression specifically in the stele of mature roots has the opposite effect (85).

## Relevant Growth Conditions and Salinity Treatments

There are several easily adopted methods of growing plants that could greatly facilitate the interpretation of results, comparisons between experiments in different laboratories, and the relevance of experiments to field situations.

The time of exposure to salinity and the severity of the salt treatment determine the physiological and molecular changes that are observed. Metabolomics and transcriptomics studies produce different answers depending on the tissue examined and whether the plant is growing or dying. Whether the plant is transpiring or not is also important, as shown for the HKT gene family (See *AtHKT1;1*, A Case Study of Confusion). A high-salt treatment for a sensitive plant like *Arabidopsis* will induce changes predominantly associated with senescence; however, a low-salt treatment may not result in discernable changes in gene expression and metabolite levels. Finding the right balance can be difficult. For application to the agricultural context, experiments should focus on growth and reproductive yield, rather than survival. Tolerance of extreme stress is of ecological relevance to perennial species, but is generally not relevant to annual species.

Osmotic effects could be distinguished from ionic effects by analyzing growing tissues for the osmotic effect, and analyzing older transpiring leaf blades for the ionic effect (**Table 1**). Short times of exposure may be useful for signaling studies; however, it is important to recognize that transient cell shrinkage and recovery of volume occur after a salt shock, and to relate measurements to the new steady state reached.

Addition of $Na^+$ or any electrolyte reduces $Ca^{2+}$ activity in solutions. If $Ca^{2+}$ activity is not maintained by addition of $Ca^{2+}$ with the $Na^+$, uncertainty remains about whether the effects of $Na^+$ addition are due to the increase in $Na^+$ or the decrease in

available $Ca^{2+}$. Thus, salt treatments need to include supplemental $Ca^{2+}$ to maintain stable $Na^+/Ca^{2+}$ ratios, or constant $Ca^{2+}$ activity (as calculated using programs such as Geochem (96) or MINTEQ (**http://www.lwr.kth.se/English/OurSoftware/vminteq/**). Consideration should also be given to the addition of silicon to solutions (29, 48) as sodium silicate (or 'liquid glass', mainly $Na_2SiO_3$), taking care to adjust the pH after its addition.

Without a good understanding of the physiology involved and the phenotype to measure, complemented by the discovery of key genes in model systems, the recent fast progress on control of shoot $Na^+$ in rice and wheat would not have been possible. Elucidating more basic physiology and the molecular genetics of other aspects of salinity responses (notably osmotic tolerance) will facilitate the generation of further applications in major crops.

## SUMMARY POINTS

1. Plant responses to salinity occur in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves.

2. Plant adaptations to salinity are of three distinct types: osmotic stress tolerance; $Na^+$ exclusion; and tissue tolerance, i.e., tolerance of tissue to accumulated $Na^+$, and possibly $Cl^-$.

3. Our understanding of $Na^+$ exclusion from leaves and the role of the HKT gene family is increasing, although the molecular bases for many other transport processes remain obscure.

4. The salt overly sensitive (SOS) signal transduction pathway is clearly important in salinity tolerance, although the mechanism of action at the whole plant level remains to be established.

5. Osmotic tolerance and tissue tolerance both increase the ability to maintain growth for a given accumulation of $Na^+$ in the leaf tissue. Increased osmotic tolerance is evident mainly by the increased ability to continue production of new leaves, whereas tissue tolerance is evident primarily by the increased survival of older leaves.

6. $Na^+$ sequestration and compatible solute synthesis are important processes for tissue tolerance. Mechanisms of osmotic tolerance remain unknown.

7. To benefit more from the new genomics approaches, molecular studies with plants grown in physiologically realistic conditions are needed.

## FUTURE ISSUES

1. Significant breakthroughs have been made on the mechanisms and control of $Na^+$ accumulation by the high-affinity $K^+$ transporter (*HKT*) gene family and the importance of the intraplant management of $Na^+$. Nevertheless, large gaps remain in our knowledge of $Na^+$ transport, notably the control of phloem transport, the identity of the genes encoding nonselective cation channels responsible for the initial entry of $Na^+$ into plants, and the role of other solutes in salinity tolerance, including $K^+$ and $Cl^-$.

2. Molecular processes that control $Na^+$ compartmentalization in vacuoles have received much attention, but other essential processes in tissue tolerance of $Na^+$ and $Cl^-$ and osmotic adjustment remain relatively unknown.

3. Signaling pathways at the intracellular level have been well described, but long-distance signaling requires more attention. How do the leaves know the roots are in saline soil, when so little salt is delivered in the xylem to the leaves? Yet, the leaf growth rate and stomatal conductance are reduced in proportion to the concentration of salt in the soil solution, and not in proportion to the salt concentration in the xylem or the leaves.

4. Forward genetic approaches will provide significant breakthroughs in the coming years, and the use of genomics to address fundamental questions regarding, for example, the basis for the high tissue tolerance of barley, will provide us with new dimensions of understanding of salinity tolerance. Complementing genomics with phenomics to design screens for the other aspects of salinity tolerance, notably the osmotic component, will also open exciting new avenues of research.

## DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

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