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Limitation of Salt Stress to Plant Growth

Yuncai Hu and Urs Schmidhalter
Technische Universität München, Freising, Germany

I. INTRODUCTION

A. Importance of Salinity in Agriculture

Salinity in soils affects about 7% of the world's total land area (1). Of the cultivated land, 23% is saline. Furthermore, about 17% of the world's cropland is under irrigation, but irrigated agriculture contributes well over 30% of the total agricultural production (2). More importantly, secondary salinization in irrigated lands is of major concern for global food production as well. Currently, about 20% of irrigated land in world average has suffered from secondary salinization and 50% of irrigation schemes are affected. There is also a dangerous trend of a 10% per year increase in the saline area throughout the world (3). In addition, salinity is a problem for agriculture because few crop species are adapted to saline conditions.

B. Definition of Salinity

Salinity as defined herein is the concentration of dissolved mineral salts present in soils (soil solution) and waters. The dissolved mineral salts consist of the electrolytes of cations and anions. The major cations in saline soil solutions consist of Na^+, Ca^{2+}, Mg^{2+}, and K^+, and the major anions are Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^-. Other constituents contributing to salinity in hypersaline soils and waters include B, Sr^{2+}, SiO_2, Mo, Ba^{2+}, and Al^{3+}.

These salinity constituents are reported in units of millimoles per liter (mmol/l) or millimoles charge per liter (mmol charge/l) (meq/l) or milligrams per liter (mg/l) (parts per million [ppm]). The parameters often used to evaluate salinity in soils are electrical conductivity (EC), total dissolved solids (TDS), and osmotic potential (ψ_π).

The relations between these parameters are as follows:

$$\Psi_\pi \text{ (MPa)} \cong -0.036 \times \text{EC (dS/m)}$$
$$\text{TDS (mg/l)} \cong 640 \times \text{EC (dS/m)}$$

On the basis of soil EC of the extract of a saturated soil paste (ECe), sodium ion percentage of soil cation exchange capacity (ESP), and pH of saturated soil paste (pHs), saline soils can be defined as ECe > 4 dS/m, ESP < 15%, and pH < 8.5 (4).

However, this salinity criterion is a relative one because there is substantial difference in salt tolerance among plants.

C. Impacts of Salinity

Salinity not only decreases the agricultural production of most crops, but also, as a result of its effect on soil physicochemical properties, adversely affects the associated ecological balance of the area.

The following are some of the harmful impacts of salinity:

Low agricultural production
Low economic returns due to high cost of cultivation, reclamation, management, etc.
Soil erosion due to high dispersibility of soil
Ecological imbalance due to a change in plant cover from glycophytes to halophytes and marine life forms from fresh water to brackish water
Poor human health due to toxic effect of elements such as B, F, and Se.

D. Causes of Salinity

In principle, elevated salinity level in soils results mainly from two sources: natural and man-made. Salinity in arid and semiarid areas is mainly caused by natural causes, i.e., low precipitation, high level of evaporation, existence of saline parent rock, and hydrological conditions (5). However, salinity also results from mismanaged amelioration systems, poor technique of irrigation, irrigation with salinized water, salt accumulation from high doses of mineral fertilization (5), grazing, and deforestation.

E. Plant Growth Response to Salinity

There is the diversity in salt tolerances between species. In general, the term *halophytes* refers to salt-tolerant plant species. The growth of halophytes (Fig. 1, line A) is optimal at relatively high level of salinity and they are capable of accumulating relatively high quantities of salt in their tissues as mineral nutrients. Most crop plants are nonhalophytes, i.e., glycophytes. Only a few crop species are slightly stimulated by low salinity levels (Fig. 1, line B). The salt tolerance of glycophytes is relatively low (Fig. 1, line C) or their growth is severely inhibited even at low substrate salinity levels (Fig. 1, line D).

Salt tolerance is usually assessed by physiologists as the percentage biomass production in saline versus control conditions over a prolonged period. Dramatic differences are found between plant species. As illustrated in a review by Greenway and Munns (6), after some time in 200 mM NaCl, a salt-tolerant species such as sugar beet might have a reduction of only 20% in dry weight, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might be dead.

Crop salt tolerance can be defined as the ability of plants to survive and produce economic yields under the adverse conditions caused by soil salinity. Salt tolerance of agricultural crops is typically expressed in terms of yield decrease associated with soil salinity increase or as relative crop yield on saline versus nonsaline soils (7). Generally, classification of the salt tolerance (or sensitivity) of crop species, forage species, and fruit trees is based on two parameters: the threshold EC and the slope, i.e., percentage of

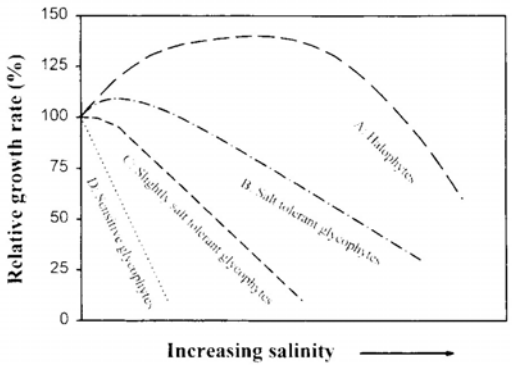

Figure 1 Schematic graph indicating the growth response of halophytes and glycophytes to salinity.

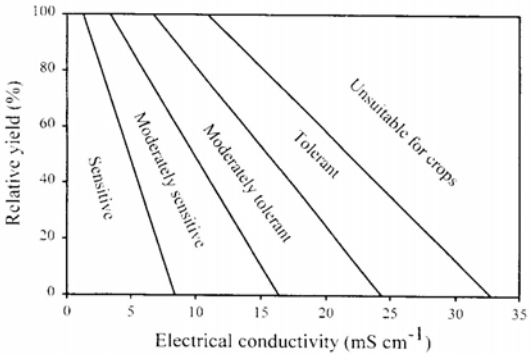

Figure 2 Divisions for crop salinity tolerance classification based on the relationship between relative crop yield and salinity (expressed in terms of electrical conductivity at 25°C). (Adapted from Ref. 7.)

Table 1 Tolerance of Crop Species to Soil Salinity

Crop species	EC saturation soil extract (EC_e) Threshold,[a] $dS\,m^{-1}$	Slope,[b] % per $dS\,m^{-1}$	Tolerance rating
Barley (*Hordeum vulgare*)	8.0	5.0	Tolerant
Sugar beet (*Beta vulgaris*)	7.0	5.9	Tolerant
Bermuda grass (*Cynodon dactylon*)	6.9	6.4	Tolerant
Wheat (*Triticum aestivum*)	6.0	7.1	Moderately tolerant
Tomato (*Lycopersicon esculentum*)	2.5	9.9	Moderately tolerant
Maize (*Zea mays*)	1.7	12.9	Moderately tolerant
Orange (*Citrus sinensis*)	1.7	16.0	Sensitive
Grapevine (*Vitis* sp.)	1.5	22.0	Sensitive
Bean (*Phaseolus vulgaris*)	1.0	19.0	Sensitive

[a]Threshold EC_e (25°C), Maximal soil salinity that does not reduce yield.
[b]Slope, yield reduction per unit increase in EC_e beyond threshold.
EC_e, Electrical conductivity of soil saturation extract.
Source: Adapted from Ref. 8.

yield decrease beyond the threshold. Examples taken from an extensive study area given in Fig. 2 and Table 1. It is evident that barley tolerates relatively high salinity levels in comparison, for example, with bean or grapevine.

There are differences in salt tolerance between cultivars within a crop species. The genetic variability within a species is not only a valuable tool for studying mechanisms of salt tolerance, but also an important basis for screening and breeding for higher salt tolerance.

Plant organs and growth stages may respond to salinity differently, depending on the plant species, cultivars, or environmental factors. Sugar beet, for example, is highly tolerant during most of its life cycle but sensitive during germination. In contrast, the salt sensitivity of rice, tomato, wheat, and barley usually is higher in vegetative stage than in germination (7). In general, the leaf growth is most sensitive to salinity. For example, limitation of salinity to wheat growth may be mainly due to the reduction in leaf growth, as is true also for other grass species (9, 10). A general pattern in the plant response to salinity is that the larger the change in the root/shoot ratio, the greater the effect of salinity on the productivity (11).

F. Solutions for Salinity

Possible solutions can generally be summarized as (a) restriction of salinization by leaching of salts from the root zone, (b) cropping management, and (c) use of tolerant plants.

1. Leaching of Salts from Root Zone

Soil scientists have devised many reclamation methods and management practices to reduce salt stress (4). For reclamation of saline soils, leaching of surface salts has been widely recommended because of normal permeability of those soils; the salts are usually leached below the root zone whenever the amount of water infiltrated exceeds that lost by evapotranspiration. In contrast, in arid and semiarid regions where rainfall is low and irrigation water is saline, it is difficult to achieve adequate leaching (12).

2. Irrigation Techniques

Salinization of irrigated agriculture can be prevented by better irrigation practices such as adoption of partial root zone drying methodology and drip or microjet irrigation to optimize use of water.

3. Nutrient Management

Salinity causes nutrient imbalance, resulting from the effect of nutrient availability in growth medium or an increase in the requirement of the plant for essential elements (e.g., N, K^+, and Ca^{2+}). Nutrient management should take account of the following (a) under low salt stress, nutrient deficiency limits plant growth to a greater extent than salinity; a positive interaction or increased salt tolerance results; (b) under moderate salinity, nutrient deficiency and salinity may equally limit plant growth, and no interaction occurs; and (c) under high salinity, salinity limits growth to a greater extent than nutrient deficiency.

4. Cropping Management

Farming systems can change to incorporate perennials in rotation with annual crops (phase farming), in mixed plantings (alley farming, intercropping), or in site-specific planting (precision farming). In precision farming, areas of high production can also be identified, and these sites can be planted with cultivars of high vigor that use water effectively during the growing season and consume most of the available soil water. Phase farming, in which several years of pasture are rotated with several years of crop, can make use of deep-rooted pasture plants to dry the deep subsoil, thereby creating a buffer zone to hold any water that escapes the crops (13).

5. Salt-Tolerant Crops

Although the use of some management options can ameliorate yield reduction under salinity stress, implementation is often limited because of cost and availability of good water quality or water resource. Using the salt-tolerant crops is one of the most important strategies to solve the salinity problem. To increase the plant salt tolerance, there is a need for understanding of the mechanisms of salt limitation on plant growth and the mechanism of salt tolerance at the whole-plant, organelle, and molecular levels.

II. LIMITATION OF SALINITY TO PLANT GROWTH

Under saline conditions, soils contain extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}, and Cl^-/NO_3^-. The growth inhibition due to salinity may be caused primarily by the osmotic stress, ionic effect, and ionic imbalance, acting on biophysical and/or metabolic components of expansive growth (14). These components may be described more elaborately as (a) the average decrease in soil water potential as a function of time and effective root zone area; (b) the toxic effects of ions as a function of time, salt concentration, and composition; and (c) the limitation of nutrients as a function of specific ratios between nutrients and competitive ions (i.e., Na^+/K^+, Na^+/Ca^{2+}, Cl^-/NO_3^-) (Fig. 3).

A conceptual model illustrating the relations among salinity effects (osmotic, ion toxicity, and ionic imbalance) and transduction (assimilate

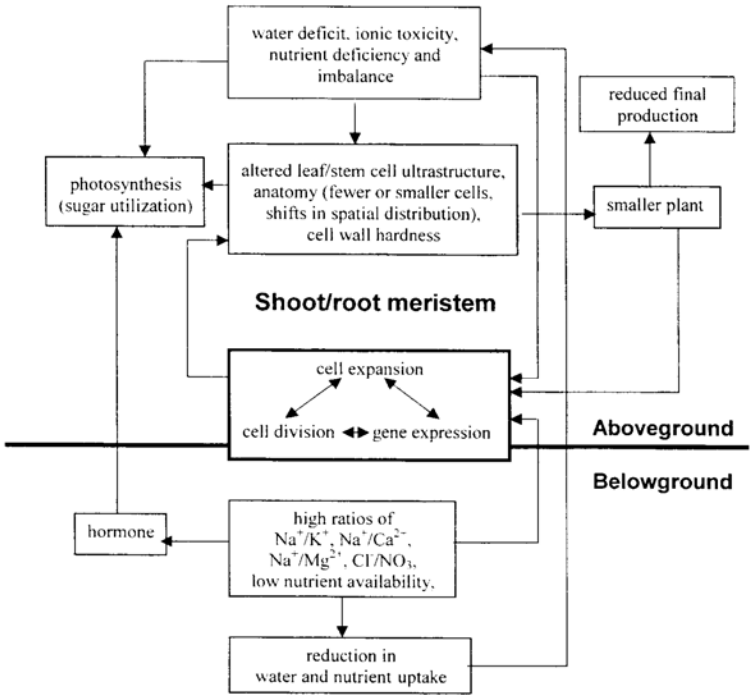

Figure 3 Conceptual model indicating the limitation of salinity to plant growth.

partitioning, root-to-shoot signaling, differential gene expression, hormones) and adaptation (morphological, anatomical, and ultrastructural) is presented in Fig. 3. Growth responses to salinity may be directly due to osmotic and ionic effects and indirectly due to photosynthesis or chemical and/or biochemical messengers. In this section, we attempt to understand how plant growth is affected by increasing salinity by integrating what is known at the levels from molecular and organelle to whole plant.

A. Osmotic Effects

Under saline conditions, low osmotic potentials of the soil solution induce water deficit in plant tissue. As a consequence, cell turgor pressure decreases. Since the growth of cells is correlated with turgor pressure in the growing tissues, decreased turgor is the major cause of inhibition of

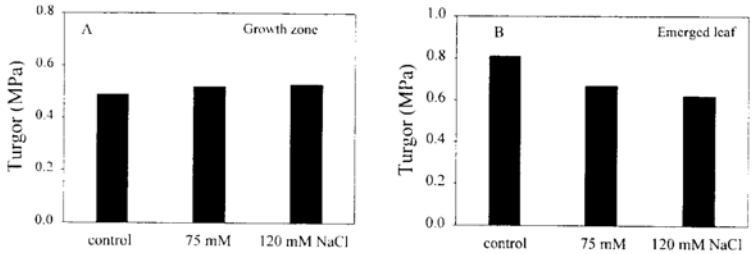

Figure 4 Epidermal cell turgor pressure of barley leaf grown under control conditions or in nutrient solution containing 75 and 120 mM NaCl: A, in the growth zone; B, in the emerged part. (Modified from Ref. 19.)

plant cell expansion under saline conditions (6). However, Munns (15) suggested that turgor is unlikely to play a role in the reduction of cell elongation in the growing leaf under saline conditions. There are only a few measurements of turgor in growing tissues of salt-affected plants. Thiel and associates (16) and Yeo and colleagues (17) using the pressure probe, found no detectable change in the turgor of elongating cells of plants grown in saline solution. According to the 1993 studies for wheat by Arif and Tomos (18) and 2002 studies for barley by Fricke and Peters (19), the turgor at the cell level measured with the pressure probe showed no clear relation to leaf elongation in the growth zone under saline conditions (Fig. 4A). That is, either turgor in elongating tissues is not affected by water deficit (e.g., Refs. 20 and 21) or, when it is, there is no correlation between the local elongating rate and the turgor of the cells (e.g., Refs. 22 and 23). In the emerged (air-exposed) leaf blade, i.e., maturity tissues, turgor was always higher than in actively growing cells and decreased significantly with increasing salt stress (Fig. 4B) (19).

Lack of change in turgor of the growing tissues under saline conditions may be due to osmotic adjustment, which helps to maintain the turgor in cells. Elongating cells adjusted osmotically to changes in external water potential by accumulating more solutes and by reducing the volume expansion (19, 24). Fricke and Peters (19) suggested that at high salinity levels, the largest increase in osmolality is achieved by reduced volume expansion rather than by increased solute deposition rates, a finding that can be explained in two ways: First, the total amount of solutes available for osmotic adjustment may have been limited, already reaching maximal deposition rates at moderate stress level. Sugar and other organic solutes contribute little to osmolality along the growth zone of NaCl-stressed wheat leaves (24). Therefore, the rate at which inorganic solutes

were supplied to the growth zone may have limited cell expansion. As a consequence, the rate of cell expansion had to slow to allow maintenance of the gradients of water potential between elongating cells and the xylem solution.

Second, a limitation in the rate at which solutes were taken up and deposited may have caused cells at high salinity (e.g., 120 mM NaCl) to grow most slowly. It is possible that expanding cells of plants exposed to high salinity levels were metabolically or energetically limited in their ability to accumulate solutes at rates as high as those of plants exposed to moderate salinity (25). If so, cells needed to expand and dilute solute contents at lower rates to maintain the gradients of water potential and water uptake.

B. Ionic Effect

Considerable attention has been focused on the hypothesis that Na^+ or Cl^- may be toxic to plants. In support of the hypothesis, the positive correlations between salt tolerance and Na^+ exclusion have been shown by Drew and Läuchli (26), Schubert and Läuchli (27), and Lazof and Bernstein (28). Accumulation of Na^+ and Cl^- in the leaves, through the transpiration flow, is generally a long-term process occurring in salt-stressed plants (29). High internal concentrations of Na^+ and Cl^- may provide toxic ions in the cellular compartment (6, 30, 31).

Plant growth is affected by the interactions of Na^+ or Cl^- and many mineral nutrients, causing imbalances in the nutrient availability, uptake, or distribution within plants and also increasing the plant's requirements for essential elements (6, 14, 32). For example, high concentrations of Na^+ in the external solution caused decreases in K^+ and Ca^{2+} concentrations in the tissues of many plant species (6, 32, 33). The decrease may be due to the antagonism between Na^+ and K^+ or Ca^{2+} at sites of uptake in roots, to the effect of Na^+ on the K^+ and Ca^{2+} transport into the xylem (32, 34, 35), or to indirect inhibition of the uptake process in other aspects, for example, hydrogen–adenosine triphosphatase (H^+-ATPase) activity (36, 37). Since Ca^{2+} is essential for maintaining selectivity and integrity of cell membranes (38, 39), the deficiency of Ca^{2+} could impair both the selectivity and the integrity of the membrane and then accelerate the passive accumulation of Na^+ in plant tissues.

However, much of the physiological research into salinity has concentrated on ionic effects (ionic toxicity and nutritional disturbance) in whole plants or in nongrowing tissues. The measurements in whole plants or nongrowing tissues do not allow estimation of the correlations between growth and direct causes. Leaf elongation in grasses, i.e., the growth zone, is restricted to a small region at the base of the blade enclosed by older

leaves (40). Although the growth zone is enclosed, grass leaves present a good opportunity to study leaf growth processes, because the growth zone is distinct and relatively simply organized (41). Elongation is largely unidirectional, and a cellular particle is displaced away from the leaf base as a result of the production of younger tissue and longitudinal growth. Since the growing tissues are most active in metabolism and strong sinks for nutrients, the leaf growth of grasses under control or stress conditions should be much more closely associated with metabolic and nutritional changes within the most actively growing tissues than within the whole plant or nongrowing tissues. By comparing the profiles of spatial distribution of leaf elongation of grasses with that of mineral elements, sugars, and water relations in growing tissues of grasses with and without salinity, it is possible to determine the causes of the direct effect of salinity on leaf elongation of grasses.

The results of Na^+ elemental analyses performed on the same scale as the growth analysis for the growing leaves of sorghum and wheat rule out sodium toxicity as a direct cause of growth inhibition (Fig. 5A) (42). Although Fig. 5A showed that Na^+ concentration was much higher at salt treatment than at control, the level of Na^+ concentration is far below the level of ionic toxicity in the leaf tissues (42). This finding supports suggestions (43) that the leaf growth of barley is not directly controlled by the local concentration of Na^+ in growing tissues. Furthermore, the pattern of spatial distribution of Na^+ in the leaf growth zone of wheat under saline conditions

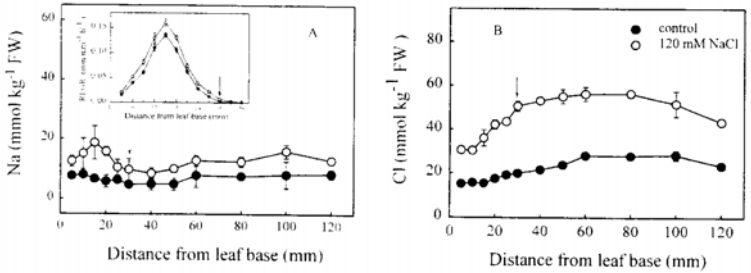

Figure 5 Sodium (A) and Cl^- (B) concentrations along the growth and maturity zones of wheat leaf 4 grown in soil with no added NaCl and 120 mM NaCl. Error bars ($n = 2$) represent standard errors and fit within the plot symbol if not otherwise shown. Arrow indicates the position of the end of the leaf growth zone. Inset illustrates the relative elemental growth rate (REGR) along the growth zone of wheat leaf 4 grown in soil with no added NaCl and 120 mM NaCl: FW, Fresh weight. (Modified from Ref. 42.)

was closely related to that of the relative elongation growth rate of wheat leaf along the leaf axis (Fig. 5A, inset), a relationship that is contrary to the behavior expected from growth inhibition. Along the leaf base there is a gradient in vacuolization. As cells elongate, during their displacement from the leaf base, larger vacuoles develop. It is unlikely that the degree of tissue vacuolization regulates Na^+ accumulation in the tissue, since the pattern of increased accumulation does not coincide with vacuole volume.

Hu and Schmidhalter (42) suggested that although Cl^- level in the growth zone of wheat under saline conditions was about four times higher than Na^+ level (Fig. 5b), it was also unlikely to be toxic. The maximal Cl^- content at the end of the elongating zone reached only (50–60 mmol kg^{-1} fresh weight [FW]) (Fig. 5b), and this concentration did not inhibit in vitro protein synthesis in a wheat germ system (44). In an earlier study by Hu and colleagues (9), Cl^- concentration in the mature leaves of wheat was about 10 times as high as that in growing leaves under similar conditions and had very little effect on the main stem grain yield. These results together suggest that the growing leaves may be able to regulate the Cl^- concentration to prevent an excessive accumulation of ions, because the expanding vacuoles in growing tissues would readily accommodate the salts and so prevent their buildup in the cytoplasm or the cell wall. Munns and coworkers (43, 45) found that Cl^- concentration in the growth zone of barley under saline conditions is unlikely to cause toxicity to leaf elongation as well. Chloride is very mobile, and plants tolerate it at high concentration (46). It preferentially accumulates in the old leaves and in the leaf sheath (6, 47).

For interaction of Na^+ or Cl^- and other nutrient elements, the direct elemental analyses in the growing leaves of wheat at 120 mM NaCl (42) showed that K^+ and Ca^{2+} concentrations were increased by salinity, especially in the elongation zone; this finding contrasted with findings of studies on growing leaves of sorghum (48) and on maize roots (49). This contrast was most likely due to the high Ca^{2+} content in the soil studied by Hu and Schmidhalter (42). Calcium enhanced the uptake of K^+ in pigeon pea (*Cajanus cajan* L. Huth), resulting in a higher concentration of K^+ in plants grown under saline conditions (50). Cramer and associates (51) reported higher concentrations of K^+ in the root tips of two corn cultivars in the presence of calcium. Similarly, higher external Ca^{2+} level may cause a higher Ca^{2+} concentration under saline conditions. Nevertheless, K^+ and Ca^{2+} contents in the leaf tissue are unlikely to limit leaf elongation of wheat under saline conditions. With these points in mind, however, the inconsistent data demonstrate that the effect of salinity on K^+ and Ca^{2+} in young tissue is different for various plant species and growth media.

Barnal and colleagues (52) proposed that the relatively greater uptake of Cl^- than of Na^+ in salt-stressed plants may also be responsible

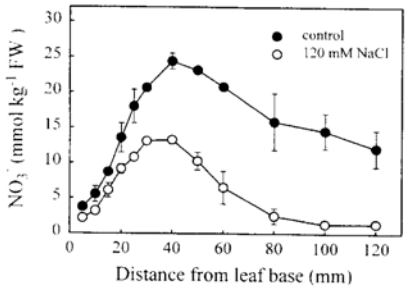

Figure 6 Nitrate concentration along the growth and maturity zones of wheat leaf 4 grown in soil with no added NaCl and 120 mM NaCl. Error bars ($n = 2$) represent standard errors and fit within the plot symbol if not otherwise shown. Arrow indicates the position of the end of the leaf growth zone. FW, Fresh weight. (Modified from Ref. 42.)

for the growth reduction by depressing the uptake of other anions such as NO_3^-. Although the effect of salinity on NO_3^- in the whole plant or mature tissues is well described, much less is known about the effect of salinity on NO_3^- in growing tissues. The lower supply of NO_3^- to growing leaves may be limiting leaf elongation of wheat by salinity (Fig. 6) (42). The difference in NO_3^- concentration between the two treatments increased along the leaf axis. The decrease in NO_3^- concentration with distance beyond the elongation zone may be due to the greater reduction of NO_3^- in the exposed part of the leaf, since light stimulates the NO_3^- reduction, and/or due to the decreased NO_3^- uptake by salinity. However, Cram (53) showed that net NO_3^- influx was reduced by a high Cl^- concentration in root tissue, such that if the abundance of NaCl increased, the concentration of NO_3^- decreased.

There are very few data on effects of salinity on micronutrients in growing leaves of grasses. Hu and coworkers (54) reported that salinity affected the distribution pattern of Fe concentration on the FW basis, whereas it did not affect those of Zn and Mn. Therefore, the decreased leaf growth is probably not due to the causes of toxicity or deficiency of these micronutrients in the growing leaves of wheat.

C. Osmotic and Ion Effects on a Time Scale: Biphasic Model

The two-phase model for the inhibition of growth by salinity was proposed by Munns (15). The first phase of growth reduction in this model is due to

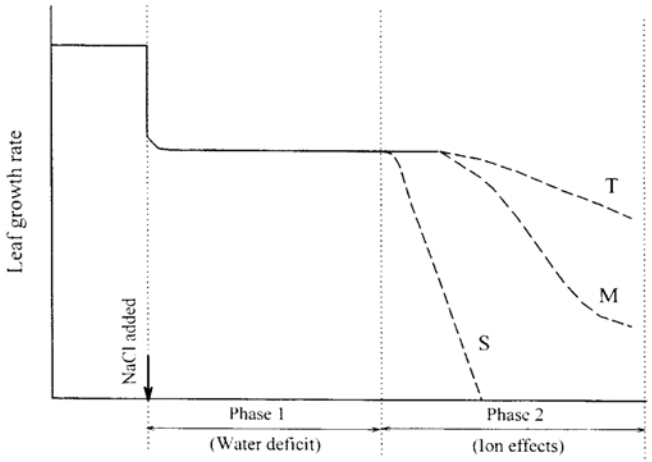

Figure 7 The two-phase model for salt inhibition of plant growth. T, Tolerant crop; M, moderately tolerant crop; S, sensitive crop to salinity. (Adapted from Ref. 15.)

the osmotic effect of soil salinity (Fig. 7). Early in phase 1 of growth reduction, turgor pressure has an influence on growth, but the majority of phase 1 growth inhibition is maintained and regulated by hormonal signals from roots (time scale here is from hours to weeks). The rate of leaf expansion can similarly be influenced by other osmotica (17, 55, 56), so this effect is extremely unlikely to be salt-specific. Thus, phase 1 of growth reduction depends on salt outside the plant rather than salt in tissues.

During extended periods, i.e., the second phase, salt begins to accumulate in older leaves, and salt injury becomes apparent. The rate of growth reductions in phase 2 depends on the rate of leaf turnover. If a few of these older leaves die and many new leaves are still produced, then the rate of phase 2 growth inhibition is minimized. However, if the number of older leaves affected approaches the rate of new leaf production, then there are changes in the flow of assimilates or hormonal balance, leading to a more rapid phase 2 growth inhibition.

The hypothetical two-phase growth response is illustrated in Fig. 7 by comparing closely related genotypes differing in salt tolerance. The differences in growth response to salinity among varieties of a crop should only be evident during the second phase of inhibition, as was found for wheat, barley, and corn varieties (57, 58). However, in some reports,

variation in salinity responses among genotypes of a species did not correspond to differential phase 2 effects (59–61).

The biphasic model of growth inhibition by salinity describes glycophyte responses to salinity well. However, growth responses to salinity in halophytes do not follow the same model. Halophytes are able to avoid the development of phase 2 growth inhibition by a number of physiological, morphological, anatomical, and behavioral mechanisms.

D. Photosynthesis

At low or moderate soil salinity, decreased growth is primarily associated with a reduction in photosynthetic area, rather than a reduction in photosynthesis per unit leaf area (15). At high salinity, however, leaf photosynthesis can be reduced by lowered stomatal conductance as a result of water imbalance (62) or by a change in the ionic relations of the chloroplasts (63). In addition, the transport of photosynthates in the phloem may be inhibited (64).

Reductions in photosynthesis due to nonstomatal factors may be caused by toxic ions. Correlations have been observed in a number of species, including bean (65), cotton (66), citrus (67), grapevine (68), and rice (69). Evidence in support of this hyphothesis is found in strong negative correlations between ions and photosynthetic activity, in which Na^+ has been implicated primarily in crop species such as rice (69) and wheat (70), and Cl^- in woody perennials such as citrus (71) and grapevine (68, 72). Fig. 8 (73) shows negative relationships between both Na^+ and Cl^- accumulation and photosynthetic rate. Ion concentrations can be detrimental to the integrity of the cell and affect photosynthetic processes directly through membrane damage or enzyme inhibition, if the vacuole can no longer sequester incoming ions. For example, Seemann and Critchley (65) found that high Cl^- concentrations (250–300 mM) in the chloroplast of phaseolus correlated with the efficiency of ribulose bisphosphate carboxylase oxygenase (RUBISCO). In that study, similar Cl^- concentrations were found in both cytoplasm and chloroplasts and vacuole, indicating a breakdown in vacuolar compartmentation.

In contrast to these observations, different types of experiments have found poor correlations between ion accumulation and photosynthetic rates. For example, Tattini and associates (74) observed a full recovery of net photosynthetic rate in olive relieved of a 200-mM NaCl stress, with leaf Na^+ contents remaining high during relief. Furthermore, Rawson and associates (75) found different relationships between gas exchange and ion concentrations for different leaves and for different salinities.

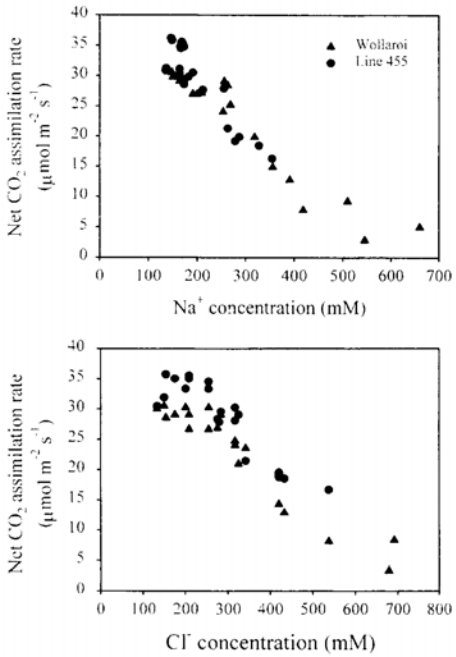

Figure 8 Relationship between net CO_2 assimilation rate and ion content (Na^+ and Cl^-) in leaf 3 of two wheat varieties (Wollaroi and Line 455) grown in 150 mM NaCl. Each data point represents measurements from an individual leaf. (Modified from Ref. 73.)

E. Molecules Involved in Cell Wall Hardening

The cell elongation of growing leaves is related not only to the turgor, but also to cell wall extensibility and turgor threshold (76). The rate of leaf elongation is regulated or controlled by alterations in any of three parameters: cell wall extensibility, turgor pressure, and yield threshold (76). Thus, the possible causes for the reduction in the longitudinal elongation of leaves under saline conditions may be either decreases in the cell wall extensibility or increases in yield threshold. Under saline conditions, decreases in the cell wall extensibility of maize leaves (32, 77) and increases in the yield threshold of maize leaves (32) may be responsible for the reduction in leaf elongation. However, there is a need for direct

measurements of cell wall extensibility and turgor threshold in the growth zones of grass leaves.

Apoplastic pH is considered to play an important role in cell wall loosening and tissue growth. Several environmental conditions that affect growth were shown to alter apoplast acidification. For example, growth inhibition by water stress is accompanied by an increase in apoplastic pH and a decrease in acidification rate (78, 79). However, Neves-Piestun and Bernstein (80) reported that salinity-induced inhibition of leaf elongation in maize is not mediated by changes in cell wall acidification capacity in growing tissues of leaves.

In 1993 experiments highlighted the biochemical regulation of cell wall extensibility as a key process in controlling growth in plants and led to the identification of a number of proteins that are potentially involved in this process (81). Xyloglucan endotransglycosylase (XET) has been proposed to be involved in the control of cell wall relaxation (81); it catalyses the transglycosylation of xyloglucan, the major hemicellulose polymer that mediates the cross-linking of cellulose microfibrils in the cell wall (82, 83). Significant correlations between high levels of XET activity and tissue elongation have been described [84–86]. Expansins are a family of cell proteins proposed to play a key role in the regulation of tissue elongation, as well as cell wall differentiation (87). However, a study of in vitro wall analysis (88) showed the lack of effect of moderate salinity on apoplastic protein concentrations and enzyme assays in the growth zone of maize leaves in a short-term experiment.

A candidate gene approach was taken in an attempt to identify genes whose expression pattern might function as a marker of tissue elongation and leaf growth of grasses. Reidy and colleagues (89) reported that a detailed analysis of the spatial expression of α- and β-expansin genes along the leaf elongation zone of *Festuca pratensis* showed no correlation between an expression pattern of these genes and leaf elongation. In another study, however, Reidy and coworkers (90) found that XET-related gene *FpXET1* is a potential marker for leaf elongation in the growth zone of *Festuca pratensis*. However, it is unclear how salinity affects α- and β-expansin genes in growing tissues.

F. Cell Elongation and Division Regulated by Signaling and Genes

Because of the important roles of several hormones in regulating cell elongation, it would not be surprising if stress inhibits cell expansion by changing the concentration of growth-promoting hormones such as abscisic acid (ABA), auxin, cytokinin (CK), gibberellin (GA), and brassinolides (91).

Abscisic acid inhibits leaf expansion (92–95). Several reports have suggested that a signal from the roots communicates with the expanding leaves and growing tissues of the shoots (29), and this may be a similar process to water stress. Experiments within a longer period suggest that ABA levels may regulate cell expansion during salt stress. The increase of ABA concentration in plants by moderate salt stress (96) and water stress (93, 95) is correlated with reduced leaf expansion.

The 2002 study by Cramer and Quarrie (97) showed that ABA concentrations in the leaf growth zone of maize were highly correlated with the inhibition of leaf elongation rate for all four genotypes (Fig. 9). Their results suggest that ABA concentration in the growth zone of leaf is a good predictor of leaf elongation response to salinity.

The characteristics of the leaf growth zone of grasses are similar to those of the root growth zone. The studies by Saab and associates (98, 99) dealt with the effects of ABA content in growing tissues of roots on elongation in the root growth zone at low water potential. After measuring ABA, water content, and elongation of millimeter segments of roots under water stress conditions, Saab and colleagues (98) concluded that a gradient of responsiveness to ABA developed in the cells of the growth zone. The ability of ABA to protect cell expansion of the growth zone, e.g., in the root

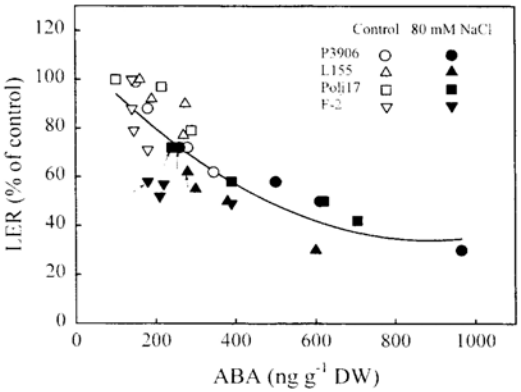

Figure 9 The relation between leaf elongation rate (LER) and the ABA concentration of the growth zone of the third leaf of four genotypes (P3906, L155, Polj 17, and F-2). The ABA concentrations were increased and LER decreased after additions of ABA to control and salt-stressed plants for 23 h. Solid symbols represent the values for 80 mM NaCl-treated plants. Arrows point to values of salt-stressed plants without ABA added. DW, Dry weight. (Modified from Ref. 97.)

at low water potential, decreased with distance from the tip. At low water potential, ABA became more inhibiting to cell expansion with increasing distance from the tip of the root (98). However, it is still not clear how salinity affects ABA content in the leaf growth zone of grasses and how ABA content is directly related to the cell elongation in the growing tissues of grass leaves.

The final size of a leaf is not only determined by the cell size; it is also contributed to by the number of cells. Munns and Termaat (29) reported that cell numbers in grass leaves were significantly reduced by salinity. The zone of cell division in grass leaves is located at the leaf base. For tall fescue, epidermal cell division is restricted to the basal 1.5 to 2 mm, and the division zone of mesophyll cells is extended to 5 to 10 mm above the leaf base (100, 101). Cell division is probably controlled by signaling and candidate genes. However, the connection between stress signaling and control of cell division needs to be better understood (100). A potentially important link between stress and cell division was revealed by induction of a cyclin-dependent protein kinase inhibitor (ICK1) in *Arabidopsis* species by ABA (102). Cell division by reduction of the activities of cyclin-dependent protein kinases that help to drive the cell cycle (91). Salt stress may inhibit cell division by causing the accumulation of ABA, which, in turn, induces ICK1. Furthermore, salinity interferes cell cycle regulatory genes such as *CDC2aAt* and Arath;*CycB1;1* and Arath; *CyA2;1* in *Arabidopsis thaliana* (103).

There is evidence that the activity of the protein kinase $p34^{cdc2}$, a product of the *cdc*2 gene, is involved in the progression of cell cycle in plants. The $p34^{cdc2}$ kinase activity is necessary to start S and M phases of the cell cycle (104, 105). The $p34^{cdc2}$ kinase activity and final cell number are deceased in transgenic plants overexpressing a dominant negative mutant of the $p34^{cdc2}$ kinase (106) and in leaves of wheat plants in water deficit (107). Granier and coworkers (108) showed that the pattern of the spatial distribution of $p34^{cdc2}$ kinase activity on a per cell basis at the cell division of maize leaves is linked to that of cell division rate. There was a linear relationship between the $p34^{cdc2}$ kinase activity and cell division rate of a growing maize leaf under water deficit and contrasting temperature conditions.

III. MECHANISMS OF SALT TOLERANCE: SALT UPTAKE, TRANSPORT, AND COMPARTMENTATION

In principle, salt tolerance can be achieved by salt inclusion or salt exclusion. However, inclusion or exclusion of salt is relative. Excluder plants show a

much lower salt uptake in comparison with includers. In halophytes, high salt tolerance is mainly based on the inclusion of salts and use of salt to lower the osmotic potential for turgor maintenance in aerial plant parts, which facilitates water uptake and transport and lowers the metabolic cost for the production of osmolytes, or Na^+ in plants can be used for the replacement of K^+ in various metabolic functions. Therefore, adaptation by salt inclusion requires high tissue tolerance to Na^+ and Cl^- or avoidance of high tissue concentrations. In some halophytes, salt can be excreted through salt glands and bladders.

In glycophytes, which comprise most crop species, exclusion is the predominant strategy; i.e., there is generally an inverse relationship between salt uptake and salt tolerance. Glycophytes restrict the uptake of toxic ions by roots from soils and the movement of toxic ions to the shoot by attempting to control influx into root xylem by root cells and show a selectivity of K^+ over Na^+ by roots and a preferential loading of K^+ rather than of Na^+ into xylem in order to maintain the high ratio of K^+/Na^+ in plants (Fig. 10). Toxic ions can be further transported into the vacuole of cells away from cytoplasm through intracellular compartmentation. Cytoplastic concentration of Na^+ is regulated by sequestering Na^+ from

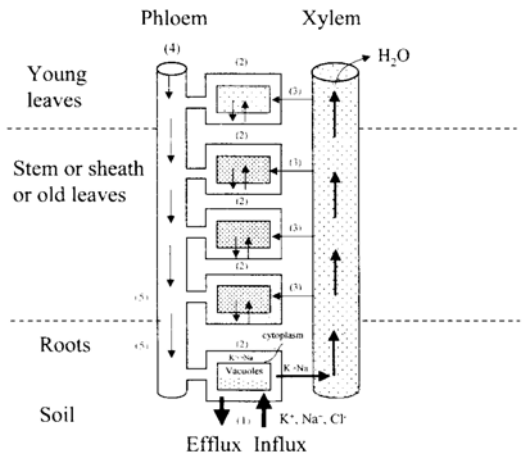

Figure 10 Model indicating the key processes of salt transport of salt-tolerant plants: (1), Influx and efflux of salt at root–soil boundary (selectivity, $K^+ \gg Na^+$); (2), compartmentation of salt into the vacuole; (3), removal of salt from the xylem; (4), translocation of salt between shoot and root; (5), Na^+ retained in the upper part of the root system and in the lower part of the shoot (e.g., stem or sheath).

the cytoplasm into the vacuole or across the plasma membrane by sodium and proton exchanger (antiports). The capacity to compartmentalize Na^+ into the vacuole via antiports is dependent on the activity of H^+-ATPase and perhaps the vacuolar H^+-pyrophosphatase, which establishes the H^+ gradient that energizes the transport of Na^+ against the electrochemical gradient (109). The importance of these various strategies in the overall salt tolerance may vary among plant varieties and severity of salt stress. Tolerant plants can also remove salt from the xylem in the roots to the stem, petiole, or leaf sheaths. In many species, Na^+ is sequestered in the upper part of the root system and the lower part of the shoot such as stem, leaf sheath, or old leaves, indicating an exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems and petioles. However, there is little retranslocation of Na^+ or Cl^- in the phloem, particularly in the more tolerant species. This limited retranslocation ensures that salt is not exported to growing tissues of the shoot. Salt-tolerant crop species are able to maintain steep concentration gradients of Na^+ and Cl^- between old and young leaves by restricting the import into the young leaves or apex, for example, in wheat (110) and in maize (111). Excluders adapt to saline conditions by prevention of internal water deficit by enhanced synthesis of organic solutes (e.g., sugars).

Therefore, understanding of the mechanisms of salt tolerance of plants at the molecular level should consider the genes and proteins that may control or regulate salt uptake, compartmentation, translocation, and distribution in plants.

A. Genes and Proteins Involved in Salt Uptake and Transport

There is no specific Na^+ transporter. Possible mechanisms for Na^+ entry into roots include permeation of Na^+ through K^+ and Ca^{2+} transporters, use of Na^+ transport to energize K^+ uptake, and Na^+ selective uptake. Candidate genes for root Na^+ uptake are found in several K^+ transporter families: (a) *HKT* transporters, (b) *KUP/HAK/KT* transporters, (c) cyclic-nucleotide-gated channels, and (d) *LCT1*. Na^+ can be effluxed from the cytoplasm through Na^+/H^+ antiporters, driven by the pH gradient across the plasmalemma (112, 113). These transport processes all work together to control the rate of net uptake of Na^+ by a cell.

The tolerance of most crop species (i.e., glycophytes) requires a high selectivity of K^+ over Na^+ at the root–soil boundary and compartmentation of Na^+ into the vacuole. The accumulation of K^+ by plant root symplast imposes a substantial energetic cost and requires specialized transport systems (114). The processes of selectivity can be distinguished according to

the activity of proteins (i.e., turnover rate) in the membranes by the three main classes (reviewed by Maathuis and Amtmann [115]):

1. Pumps

Transporter fueled by metabolic energy and able to transport substrates against an electrochemical gradient:turnover rates are low, around 10^2 per second. A prime example is the ubiquitous H^+-ATPase. No pumps have been identified in higher plants that directly transport K^+ and Na^+.

2. Carriers

Transport proteins that undergo specific conformational changes during substrate transport: they generally function in transport of substrates against a gradient are energized via coupling to an electrochemical gradient, and have turnovers of 10^2–10^3 per second. In plants, "uphill" (high-affinity) accumulation of K^+ is energized through coupling to the "downhill" transmembrane movement of H^+, proceeding via a H^+-K^+ symporter. Genes as carriers involved in K^+ and Na^+ uptake are presented in Table 2. *KUP-HAK* transporters are extremely selective for K^+ and are competitively blocked by Na^+ when present in millimolar concentrations (Fig. 11) (115, 116). This system clearly creates the potential for severe K^+ depletion when external K^+/Na^+ ratios are low. *HKT1* represents a putative pathway for high-affinity K^+ uptake and low-affinity Na^+ uptake, but its role in uptake of both ions is probably minor compared to that of other systems functioning in plants and may be limited to special cell types (115). In contrast to *HKT1*, *LCT1* may be involved in low affinity K^+ transport.

3. Ion Channels

Proteins that catalyze the rapid downhill dissipation of transmembrane ionic gradients:turnover rates are 10^6–10^8 per second and controlled via opening and closing (gating) of the channel. Channel gating is often under control of the membrane potential. Three types of ion channel, i.e., K^+ inward rectifying channels (KIRCs), K^+ outward rectifying channels (KORCs), and voltage-independent channels (VICs), have been implicated in the transport of monovalent cations (Fig. 11). They can be distinguished by their ion selectivity and gating behavior. The proportion of time that so-called KIRCs spend in the open state (expressed by their open probability) increases whenever the membrane voltage becomes more negative. And, since channel activation usually occurs at voltage more negative than the equilibrium potential for K^+, KIRCs allow movement of K^+ only into the cell. K^+ outward rectifying channels (KORCs) have opposite gating

Table 2 Gene Products Involved in Carrier-Mediated Transport of K^+ and Na^+

Gene product	Species	Expression pattern	Putative localization	Putative function	Coupling
HvHAK	*Hordeum vulgare*	Root	Plasma membrane	High-affinity uptake of K^+	K^+-H^+ symport
AtKUP1	*Arabidopsis thaliana*	Stem, leaves, and flowers Root and stem	Plasma membrane	High-affinity K^+ uptake Dual-affinity K^+ uptake	K^+-H^+ symport
HKT1	*Triticum aestivum*	Root cortex Leaves (around vascular tissue)	Plasma membrane	High-affinity K^+ uptake	K^+-Na^+ symport
LCT1	*Triticum aestivum*	Root and leaves	Plasma membrane	Low-affinity Na^+ uptake Low-affinity K^+, Na^+, and Ca^{2+} uptake	Na^+-Na^+ symport Uniport?
AtNHX	*Arabidopsis thaliana*	Root, stem, leaves, flowers	Tonoplast	Na^+ accumulation in vacuole	Na^+-H^+ antiport

Source: Adapted from Ref. 115.

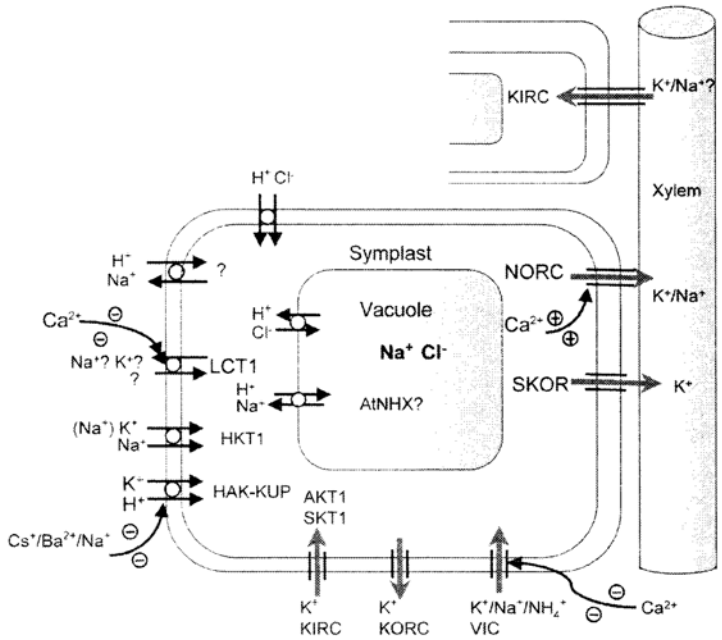

Figure 11 Carrier and channel transport systems that are involved in influx, efflux, translocation, and compartmentation of K^+, Na^+, and Cl^-. Minus signs, Inhibition, Pws signs, activation. (Modified from Ref. 115.)

characteristics and thus favor K^+ efflux. The open probability of VICs does not change with voltage: all three classes are capable, at least to some extent, of transporting K^+ and Na^+. Genes involved in K^+ transport are shown in Table 3.

As a result of the negative plasma membrane potential, the electrochemical potential gradient for Cl^- is uphill into the cell under nonsaline conditions. Uptake can be achieved by means of an active mechanism (117). There is indirect evidence that anion channels do indeed open to allow Cl^- efflux under salinity. Salt stress–induced enhancement of Cl^- permeability was noted by Yamaguchita and associates (118). Boursier and Läuchli (119) reported about the extrusion of Cl^- from the roots of sorghum plants. At very high salinity, the electrochemical potential for Cl^- has been estimated possibly to be reversed, allowing passive Cl^- influx into the cells (120, 121).

Table 3 Genes Encoding Ion Channels Involved in K^+ Transport[a]

Gene	Species	Type	Expression	Inhibitors	Function
AKT1	*Arabidopsis thaliana*	KIRC	Root cortex	Cs^+/TEA/Ba^{2+}	Low- and high-affinity K^+ uptake
AKT2	*Arabidopsis thaliana*	KIRC	Leaves	?	
KAT1	*Arabidopsis thaliana*	KIRC	Guard cell	Cs^+/Ba^{2+}	Stomatal opening
SKT1	*Solanum tuberosum*	KIRC	Root Leaf epidermis	Cs^+	Root K^+ uptake
KST1	*Solanum tuberosum*	KIRC	Guard cells	Cs^+/Ba^{2+}	Stomatal opening
SKOR	*Arabidopsis thaliana*	KORC	Root pericycle	Ba^{2+}/TEA	Translocation to shoot

[a]KIRC, K^+ inward rectifying channel; KORC, K^+ outward rectifying channel.
Source: Adapted from Ref. 115.

B. Genes and Proteins Involved in Na^+ and Cl^- Compartmentation

Compartmentation of Na^+ in the vacuole prevents building up of high cytoplasmic Na^+ (Fig. 11), which raises the cytoplasmic K^+/Na^+ ratio and contributes to the vacuolar osmotic potential. This is one of the strategies for increasing the salt tolerance of plants. In a number of species a Na^+/H^+ antiport present in the tonoplast allows accumulation of Na^+ in the vacuole by using the transtonoplast H^+ gradient as driving force (115). In *Arabidopsis* sp. the genes for AtNHX1-3 were cloned and all show high homology with yeast and mammalian Na^+/H^+ antiporter (Fig. 11). Expression is observed in all tissues, although functional analysis of the AtNHX products has not yet been carried out and physiological role as well as membrane location are yet to be established. In general, tonoplast Na^+/H^+ antiport activity is induced by growth in NaCl (122). Activity has been reported only for salt-tolerant species such as red beet, sugar beet, barley, and *Plantago* sp. maritime but appears absent in salt-sensitive species such as *Plantago* sp. media. The functioning of Na^+/H^+ antiport is therefore likely to be important in halotolerance, and it is unclear whether glycophytes contain alternative Na^+ accumulating mechanisms or rely solely on passive Na^+ distribution over the tonoplast.

Na^+ compartmentation in the vacuole require energy-dependent transport, and an immediate effect of NaCl is vacuolar alkalization [123–125]. Na^+/H^+ antiporter activity has been associated with tonoplast vesicles (123, 126), and this association is presumed to be at least partially

responsible for the alkalization. In 1999 plant deoxyribonucleic acids (cDNAs) encoding NHE-like proteins that can functionally complement a yeast mutant defiant for endomembrane Na^+/H^+ transporter, NHX1 (127), were isolated. Overexpression of an NHE-like antiporter substantially enhanced salt tolerance of *Arabidopsis* sp., confirming the function of the antiporter in Na^+ compartmentation.

Tonoplast Cl^- transport determinants are predicted to be a channel or a carrier that couples Cl^- influx to the H^+ gradient (Fig. 11). A +50-mV (inside positive) tonoplast membrane potential would be sufficient to facilitate an almost 10-fold concentration Cl^- in the vacuole based on electrophoretic flux through an anion-permeable channel (128). Secondary active transport (H^+ anion antiporter) has also been proposed (129).

Compartmentation of salt in the halophytes depends on regulation of permeability rather than structural proteins. In other words, the differences in the regulatory pathway such as at perception, signaling, or signal transduction rather than structural genes for transport processes with different properties may be the targets for understanding and manipulation in the future (130).

C. Molecules Associated with Salt Translocation to the Shoot

The glycophytes tend to exclude Na^+ and Cl^- from the growing tissues of shoots by retaining them in the upper part of roots and lower stem, or leaf sheath, or old leaves. This strategy is only successful at low to moderate external salt concentrations and relies on the selective release of Na^+ into the xylem and its resorption from the xylem stream. Several mechanisms that contribute to the translocation of K^+ and Na^+ and some aspects of their regulation have now been identified (reviewed by Maathuis and Amtmann [115]).

The *Arabidopsis* sp. gene *SKOR* (131) encodes a channel protein that is also a member of the Shaker family but displays gating characteristics that favor outward K^+ flux. The *SKOR* gene has an important role in the translocation of K^+ to the shoot (Fig. 11). In addition to KORCs, which are highly selective for K^+ over Na^+ (including KORCs in maize and barley stellar protoplasts and *SKOR*), a second type of outward-rectifying channel that does not discriminate between monovalent cations (non-selective outward rectifying channels) has been found in patch clamp experiments on barley xylem parenchyma protoplasts (132, 133). Opening of this channel requires micromolar concentrations of cytoplasmic Ca^{2+} and creates a potential passage for Na^+ release into the xylem. At the gene level NORC remains to be identified and its exact role is not yet clear. However, data

suggest a regulatory role for cytoplasmic Ca^{2+} in Na^+ compartmentation between root and shoot, and the process may involve a NORC-mediated Na^+ release pathway into the stele.

A xylem parenchyma KIRC (132) with relatively low cation selectivity may also be implemented in Na^+ compartmentation (Fig. 11), since it potentially functions in basal parts of the xylem in Na^+ resorption.

The salt overly sensitive 1 (SOS1) protein, which is responsible for Na^+ loading/unloading into xylem, has recently been identified (134). The protein encodes a putative plasma membrane Na^+/H^+ antiporter. Loss-of-function mutations in SOS1 confer salt hypersensitivity and SOS1 mutants also cannot grow well under low K^+ conditions (135). Indirect evidence suggesting that SOS1 may function in Na^+ unloading is twofold: first, SOS1 is mainly expressed in the pericycle cells surrounding the xylem vessels and also in the veins. This expression pattern in the root is reminiscent of that of SKOC1 (131), which functions in leading K^+ into the xylem. Second, it was found that when plants were supplied with NaCl, Na^+ in the xylem sap of SOS1 mutant plants is higher than that in the wild-type plants. This finding suggests that SOS1 may actually prevent Na^+ from entering the xylem vessel. Interestingly, *SOS1* gene was also expressed in root tips. It is thus likely that SOS1 may have additional functions other than regulating long-distance transport of salts. As the root tip cells are not well developed and are deficient in prototype vacuoles, this expression pattern of SOS1 is consistent with the idea that SOS1 is localized on the plasma membrane, as suggested by its sequence characteristics (133). It is interesting that as the root cells differentiate, the expression of SOS1 becomes restricted to specific cell files, suggesting that positional information is involved in the regulation of SOS1 expression.

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