



BOTANICAL BRIEFING

K⁺ Nutrition and Na⁺ Toxicity: The Basis of Cellular K⁺/Na⁺ Ratios

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The capacity of plants to maintain a high cytosolic K⁺/Na⁺ ratio is likely to be one of the key determinants of plant salt tolerance. Important progress has been made in recent years regarding the identification and characterization of genes and transporters that contribute to the cytosolic K⁺/Na⁺ ratio. For K⁺ uptake, K⁺ efflux and K⁺ translocation to the shoot, genes have been isolated that encode K⁺ uptake and K⁺ release ion channels and K⁺ carriers that are coupled to either a H⁺ or Na⁺ gradient. Although the picture is less clear for the movement of Na⁺, one pathway, in the form of non-selective ion channels, is likely to play a role in Na⁺ uptake, whereas Na⁺ efflux and compartmentation are likely to be mediated by H⁺-coupled antiport. In addition, several proteins have been characterized that play prominent roles in the regulation of K⁺ and/or Na⁺ fluxes. In this Botanical Briefing we will discuss the functions and interactions of these genes and transporters in the broader context of K⁺ nutrition and Na⁺ toxicity.

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Key words: Salinity, K⁺/Na⁺ ratio, transporter, membrane.

INTRODUCTION

Plant growth depends on the supply of inorganic nutrients. Typically, plants are exposed to nutrient levels that vary widely in both time and space. To cope with such fluctuating environments plants have developed high levels of plasticity both at the individual level and within species and ecotypes. Nevertheless, extreme nutrient conditions will cause deficiency or toxicity to a varying extent for different plant species. This notion is clearly exemplified in the different degrees of salinity tolerance found in various plant species. Significant entry of Na⁺ will result in severe growth reduction or death in salt sensitive or glycophytic species, will lead to mild toxicity symptoms in salt tolerant species, but may benefit halophytes.

Salt tolerance is not exclusively correlated with adaptations to Na⁺ toxicity *per se* but also reflects adaptations to secondary effects of salinity such as water deficit and impaired nutrient acquisition (Flowers, Troke and Yeo, 1977; Greenway and Munns, 1980). The latter is particularly pertinent where the acquisition of K⁺ is concerned due to the physicochemical similarities between Na⁺ and K⁺. The capacity of plants to counteract salinity stress will strongly depend on the status of their K⁺ nutrition. Conversely, although most plants can cope (i.e. show no symptoms of either deficiency or toxicity) with external K⁺ concentrations ranging from low μM to tens of mM, the physiological 'window' of optimum K⁺ concentrations narrows in the presence of increasing amounts of Na⁺ (Marschner, 1995).

K⁺ is essential to all plant life, and in most terrestrial plants K⁺ is the major cationic inorganic nutrient. The cellular roles that K⁺ plays have been frequently reviewed

(e.g. Kochian and Lucas, 1988; Maathuis and Sanders, 1996) and can roughly be summarized as: (1) charge balancing in the cytoplasm, where K⁺ is the dominant counterion for the large excess of negative charge on proteins and nucleic acids; (2) activation of crucial enzymatic reactions such as occurring in the formation of pyruvate; and (3) a substantial contribution to the osmotic pressure of the vacuole and hence to cell turgor which endows non-lignified plant cells with structural rigidity. In contrast, Na⁺ is only essential for a number of C₄ species (for the translocation of pyruvate across the chloroplast envelope) where it functions as a micronutrient. In most other species Na⁺ does not act as a nutrient in the sense that it is strictly required for growth, but its addition to the growth medium may promote growth of many plants when the K⁺ supply is limited (Flowers and Läuchli, 1983) and in particular the growth of salt tolerant and halophytic plants by contributing to turgor formation.

Although the availability of Na⁺ as a 'cheap' osmoticum is generally beneficial, a large excess of Na⁺ ions over K⁺ is not, for several reasons. Firstly, the similar physicochemical structures of Na⁺ and K⁺ mean that Na⁺ competition at transport sites for K⁺ entry into the symplast may result in K⁺ deficiency. Secondly, cytoplasmic Na⁺ competes for K⁺ binding sites and hence inhibits metabolic processes that crucially depend on K⁺. Clearly, Na⁺ in the cytosol has to be restricted by limiting Na⁺ entry and/or operating an efficient system for Na⁺ efflux into the vacuole or the apoplast. Therefore, as has been pointed out by many authors (e.g. Yeo, 1998), one of the key elements in salinity tolerance is the capacity to maintain a high cytosolic K⁺/Na⁺ ratio.

In animal cells, K⁺/Na⁺ ratios of around 20 are maintained by strict regulation of cytosolic K⁺ around 100 mM and Na⁺ around 5 mM. Both ions are actively transported via the action of the K⁺-Na⁺ ATPase that moves K⁺ into the cell

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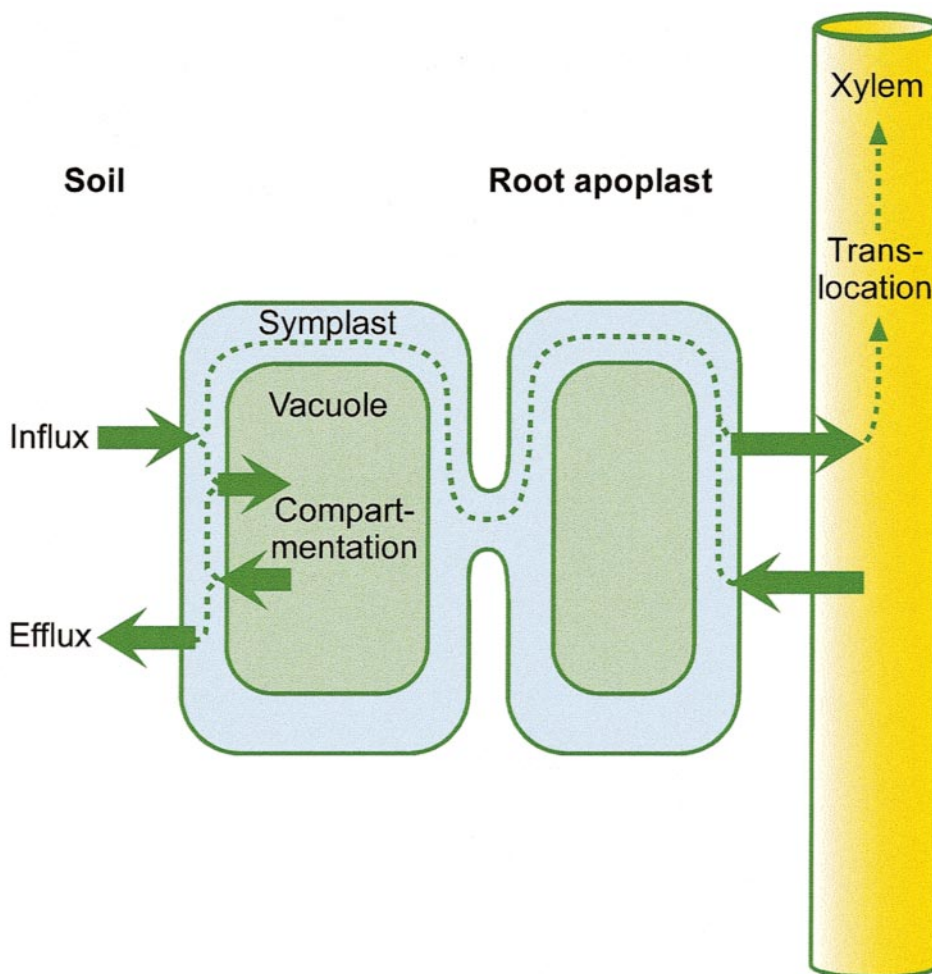


FIG. 1. Key processes in plants that contribute to the establishment of cytoplasmic K^+/Na^+ ratios: (1) influx and efflux at the root/soil boundary; (2) translocation between root and shoot; (3) compartmentation e.g. into the vacuole.

and extrudes Na^+ . As in animal cells, cytosolic K^+ is under close homeostatic control in plant cells with 'set point' values of 100–150 mM (Walker, Leigh and Miller, 1996). Estimates of cytosolic levels of Na^+ in plant cells are based on X-ray analysis (Hajibagheri *et al.*, 1988) or the use of Na^+ -selective electrodes (Amtmann and Gradmann, 1994; Carden *et al.*, 1998) and range from low millimolar levels to around 100 mM. Therefore, a K^+/Na^+ ratio of around 1 provides a minimum value. Although lower than in animal cells, this value is many times higher than that found in saline external media. For instance, the K^+/Na^+ ratio of seawater is 0.02.

The K^+/Na^+ ratio that ultimately prevails in plant cells will depend on the concerted action of transport systems located at plasma and vacuolar membranes and probably involves K^+ selective, Na^+ selective and non-selective pathways. Key steps (Fig. 1) in the overall process can be identified at: (1) the soil/root-symplast interface where membranes of epidermal and cortical cells 'determine' which ion enters the symplast and to what extent; (2) the root/xylem interface in the form of xylem parenchyma cells where a second level of control can be exerted to adjust ion translocation to the shoot; and (3) partitioning, which may

occur at a cellular level, between cytoplasm and vacuole, and at a tissue level, e.g. recirculation of Na^+ and K^+ between old and young leaves. In addition, there is the possibility of Na^+ efflux into the root medium. However, in contrast to yeast (Rodriguez-Navarro, Quintero and Garcia-deblas, 1994) and some halophytic algae (Katz *et al.*, 1991), there are no observations that indicate the presence of a Na^+ efflux pump in higher plants, although there is circumstantial evidence for Na^+/H^+ antiport activity in some species (e.g. Wilson and Shannon, 1995).

Underlying all these processes is the activity of transport proteins in the membranes for which three main classes can be distinguished: (1) pumps: transporters fuelled 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^+ or 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' trans-membrane movement of H^+ , proceeding via a H^+ - K^+ symporter; (3) ion channels: proteins that catalyse the rapid 'downhill' dissipation of trans-membrane ionic gradients. Turnover rates are 10^6 – 10^8 per second and controlled via opening/closing (gating) of the channel. Channel gating is often under control of the membrane potential. The predominant type of channel found in plant membranes is K^+ selective: e.g. in guard cell membranes for the rapid release of K^+ during stomatal closure.

In this Botanical Briefing we will collate current data on the presence and regulation of individual membrane transporters for K^+ and/or Na^+ and their impact on the cytoplasmic K^+ / Na^+ ratio. We focus mainly on the soil/root interface since most knowledge regarding the molecular mechanisms of K^+ and, to a lesser extent, Na^+ transport deals with the uptake of K^+ and Na^+ into roots. In addition, since apoplastic loading of the xylem is low in most species (Yeo, 1998) the soil/root boundary ultimately determines the quantity and quality of the ionic species that enters the symplast.

CARRIERS INVOLVED IN K^+ AND Na^+ UPTAKE

High affinity potassium carriers

In most conditions K^+ uptake from micromolar external concentrations is 'uphill' and mediated by a carrier (but see below) with a high affinity for K^+ (10–50 μ M, Table 1). Two such mechanisms have been identified genetically in plant roots. Members of the plant *KUP-HAK* gene family (Fig. 2) were identified on the basis of homology to known genes from bacteria and fungi whereas *HKT1* was cloned by complementation of a yeast strain defective in K^+ -uptake with wheat root cDNA (Schachtman and Schroeder, 1994).

Members of the *KUP-HAK* gene family in plants were recently identified in barley (Santa-Maria *et al.*, 1997) and *Arabidopsis thaliana* (Quintero and Blatt, 1997; Fu and Luan, 1998; Kim *et al.*, 1998) by using RT-PCR. So far, at least two homologous genes have been found in barley (*HvHAK1* and 2) and four in *Arabidopsis* (*AtKT¹* or *AtKUP 1–4*). The Rb^+ and K^+ transport capacity of *HAK-KUP* gene products was analysed in yeast (Santa-Maria *et al.*, 1997; Fu and Luan, 1998), in *E. coli*, or after over-expression in *Arabidopsis* cell suspension cultures (Kim *et al.*, 1998). *KUP-HAK* transporters mediate K^+ and Rb^+ uptake with K_m values in the μ M range, similar to the values that are found for Rb^+ uptake in intact plants. The exact mechanism by which these transporters move K^+ is still under investigation but is believed to be via coupling to the H^+ gradient (Santa-Maria *et al.*, 1997). This would agree with an earlier conclusion, based on patch clamp studies, that high affinity K^+ transport in *Arabidopsis* root cells is H^+ -coupled (Maathuis and Sanders, 1994). *KUP-HAK* transporters are extremely selective for K^+ (K^+ / Na^+ ratio of 1000) and are competitively blocked by Na^+ when present in

mm concentrations (Fig. 2, Santa-Maria *et al.*, 1997). This clearly creates the potential for severe K^+ depletion when external K^+ / Na^+ ratios are low. Some studies report a low-affinity component for *AtKUP1*-mediated K^+ transport (Quintero and Blatt, 1997; Fu and Luan, 1998). This issue remains ambiguous however, since the dissection of indigenous background fluxes from those induced by the expressed transporter are difficult in the low affinity range. Expression patterns of *AtKUP1* differ in various reports and expression of at least one isoform (*AtKUP3*) is up-regulated upon K^+ -starvation (Kim *et al.*, 1998).

The *HKT1* gene product (Schachtman and Schroeder, 1994) shows weak homology to the TRK transporters of yeast as well as several genes from other fungi and bacteria involved in K^+ transport. So far, *HKT1* plant homologues have been found in rice (Golldack *et al.*, 1997), barley (Wang *et al.*, 1998) and *Arabidopsis* (Schachtman and Schroeder, 1994). Heterologous expression of *HKT1* in *Xenopus* oocytes allowed its functional characterization (Rubio, Gassmann and Schroeder, 1995): *HKT1*-mediated K^+ transport depends on the presence of Na^+ with two K^+ transported per one Na^+ . The K^+ binding site has a K_m of about 3 μ M and the Na^+ binding site a K_m of about 200 μ M. High affinity transport is highly selective for K^+ compared to Cs^+ and Li^+ . Rb^+ permeability in the high affinity range can be detected but is much smaller (approx. 1:15) than K^+ permeability. Although the expression of *HKT1* in wheat and barley is induced by K^+ -starvation (Wang *et al.*, 1998) the physiological relevance of the suggested high affinity K^+ transport function of *HKT1* remains controversial. Evidence from studies on various species, including wheat and *Arabidopsis*, supports the conclusion that the presence of external Na^+ is not essential for either plant growth or high-affinity K^+ transport (Maathuis *et al.*, 1996) and it was concluded that Na^+ -coupled K^+ transport has no or limited physiological relevance in terms of K^+ uptake.

At higher ambient Na^+ concentrations, both *HKT1* binding sites are occupied by Na^+ . Hence, *HKT1* type systems may be relevant for Na^+ uptake rather than K^+ uptake. Interestingly, *HKT1* expression in rice is depressed during salt stress and a salt-sensitive rice variety was shown to maintain higher levels of *HKT1* expression than a salt-tolerant variety (Golldack *et al.*, 1997). It can be summarized that *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 other systems functioning in plants and may be limited to special cell types.

Low-affinity cation carriers

Together with *HKT1*, *LCT1* was cloned from wheat by complementation of yeast (Schachtman *et al.*, 1997). In contrast to *HKT1*, which restored yeast growth at micromolar K^+ concentrations, *LCT1* rescued the yeast cells only on media containing low millimolar concentrations of K^+ , indicating that *LCT1* may be involved in low affinity K^+ transport. The *LCT1* sequence does not show any homology to known proteins but *LCT1* transformed yeast cells show

¹ This nomenclature is no longer used; the *AtKT* genes are part of the *KUP/HAK* family.

TABLE 1. Gene products involved in carrier-mediated transport of K^+ and Na^+

Gene product	Species	Expression pattern	K_m	Putative localization	Putative function	Coupling	Reference
HvHAK	<i>H. vulgare</i>	Root	$K^+ = 27 \mu M$ $Rb^+ = 18 \mu M$	Plasma membrane	High-affinity uptake of K^+	K^+ - H^+ symport	Santa-Maria <i>et al.</i> , 1977
AtKUP1	<i>A. thaliana</i>	Stem, leaves and flowers Root and stem	$Rb^+ = 22 \mu M$ $Rb^+ = 44 \mu M/11 mM$	Plasma membrane	High-affinity K^+ uptake Dual affinity K^+ uptake	K^+ - H^+ symport	Kim <i>et al.</i> , 1998 Fu and Luan, 1998
HKT1	<i>T. aestivum</i>	Root cortex, leaves (around vascular tissue)	$K^+ = 3 \mu M$ $Rb^+ = 15 \mu M$ $Na^+ = 175 \mu M$ $Na^+ = 4-6 mM$	Plasma membrane	High-affinity K^+ uptake Low-affinity Na^+ uptake	K^+ - Na^+ symport Na^+ - Na^+ symport	Rubio <i>et al.</i> , 1995 Gassmann, Rubio and Schroeder, 1996
LCT1	<i>T. aestivum</i>	Root and leaves	mM for K^+ , Rb^+ and Na^+	Plasma membrane	Low-affinity K^+ , Na^+ and Ca^{2+} , uptake	Uniport?	Schachtman <i>et al.</i> , 1997
AtNHX	<i>A. thaliana</i>	Root, stem, leaves flowers		Tonoplast	Na^+ accumulation in the vacuole	Na^+ - H^+ antiport	Clemens <i>et al.</i> , 1998 Apse <i>et al.</i> , 1998

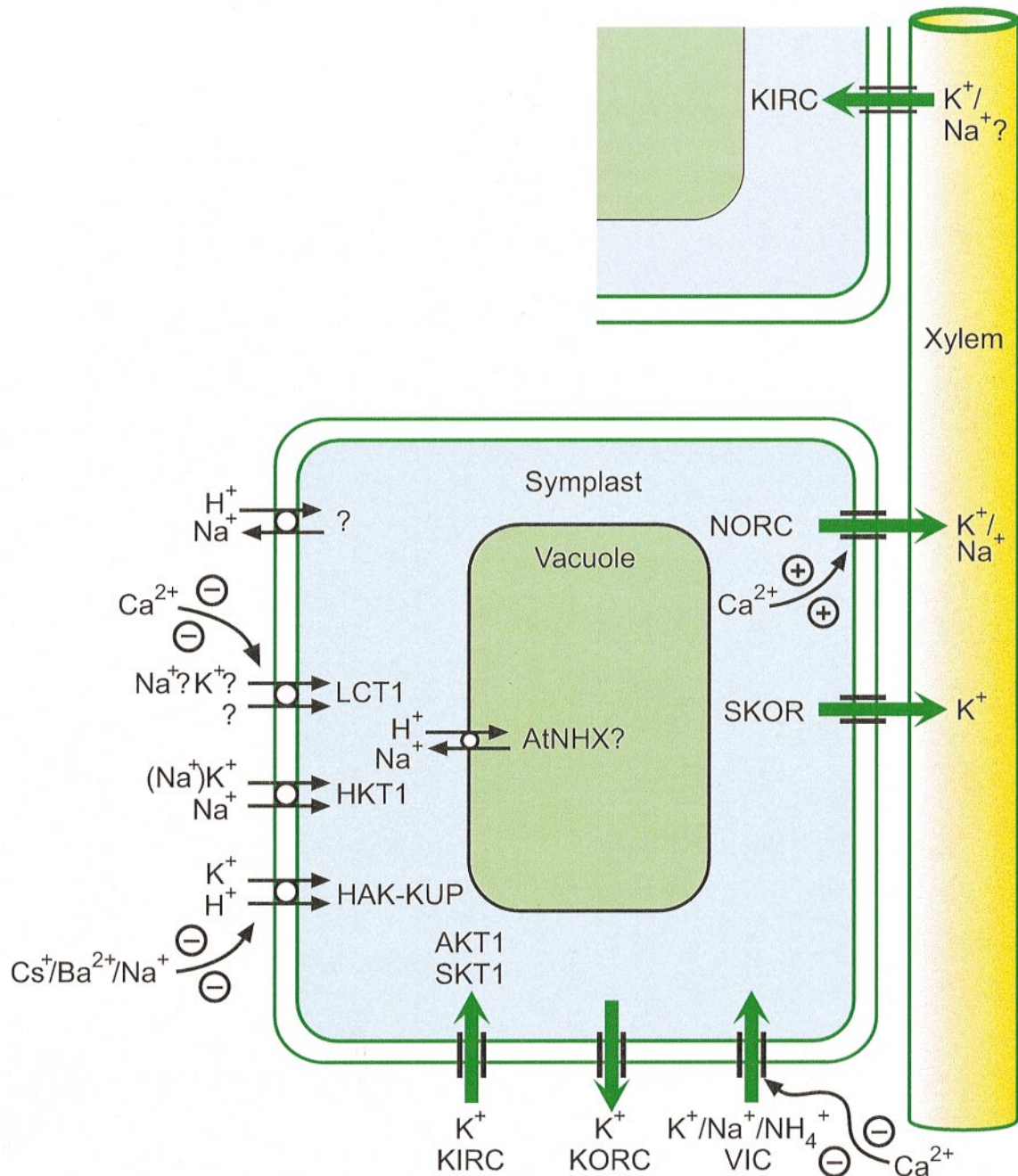


FIG. 2. Carrier and channel transport systems that are involved in influx, efflux, translocation and compartmentation of K^+ and Na^+ . Negative signs denote inhibition, positive signs denote activation. For details see text.

increased low-affinity uptake of Rb^+ as well as Na^+ , consistent with a non-selective pathway. Rb^+ uptake was strongly reduced by external Ca^{2+} , an ion that also permeates LCT1. Ca^{2+} inhibition of low-affinity Na^+ uptake in plants has been frequently reported and it is tempting to speculate that LCT1 may play a significant role as a pathway for Na^+ uptake in high-salt conditions. Complementation studies in a yeast strain with a deletion in *mid1*, a putative yeast Ca^{2+} channel subunit (Clemens *et al.*, 1998), suggest that LCT1 may also function in Ca^{2+} transport.

CHANNELS INVOLVED IN K^+ AND Na^+ UPTAKE

Three types of ion channel have been implicated in the transport of monovalent cations (Fig. 2). They can be distinguished by their ion selectivity and gating behaviour. The proportion of time that so-called KIRCs (K^+ inward rectifying channels) spend in the open state (expressed by their open probability) increases whenever the membrane voltage becomes more negative. And, since channel ac-

tivation usually occurs at voltages more negative than the equilibrium potential for K^+ , KIRCs only allow movement of K^+ into the cell. KORCs (K^+ outward rectifying channels) have opposite gating characteristics and thus favour K^+ efflux. The open probability of VICs (voltage independent channels) does not change with voltage. All three classes are capable, at least to some extent, of transporting K^+ and Na^+ .

KIRCs

Characterization of KIRCs in plant cell membranes is based on a vast amount of electrophysiological data (for review see Maathuis *et al.*, 1997). KIRCs were the first plant ion channels to be identified at a molecular level (Table 2; Anderson *et al.*, 1992; Sentenac *et al.*, 1992). In spite of mediating inward current, KIRCs show a high degree of homology to animal Shaker-channels which are exclusively outward rectifying. KIRCs have been found in most plant cell types and are involved in many physiological processes; in particular KIRCs in the plasma membrane of root epidermal and cortical cells have been shown to be the main pathway for low-affinity K^+ uptake by plants (Gassmann, Ward and Schroeder, 1993; Maathuis and Sanders, 1995). Most KIRCs are highly selective for K^+ over Na^+ as well as divalent cations but usually allow considerable passage of Rb^+ and NH_4^+ (for a detailed list of K^+/Na^+ permeability ratios of KIRCs see Amtmann and Sanders, 1999) and it is concluded that in general the contribution of KIRCs to low-affinity Na^+ uptake is minor.

With the molecular identification of KIRCs and other transport systems, their functional characterization becomes ever more urgent. *In planta*, the production of null mutants (knock-out mutants) where a specific gene is disrupted is an elegant approach. The isolation of akt1-1 (an *Arabidopsis* AKT1 null mutant; Hirsch *et al.*, 1998) has revealed that the simple division of carrier-mediated K^+ transport with a high affinity, and channel-mediated K^+ transport with a low affinity may be an oversimplification. Although AKT1 is believed to play a major role in root K^+ uptake (Lagarde *et al.*, 1996), akt1-1 showed diminished K^+ uptake from micromolar external concentrations but not from millimolar concentrations. These results indicate that AKT1 may play a role in high affinity K^+ uptake and that an alternative pathway for low affinity K^+ transport is available (e.g. via other KIRCs or VICs, see below). The akt1-1 null mutant showed similar salt sensitivity as the wildtype (Hirsch *et al.*,

1998) suggesting no or a minor role for AKT1 in Na^+ uptake.

KORCs

KORCs have been characterized by patch-clamp experiments in many different plant species and tissues (for a list of K^+/Na^+ permeability ratios of KORCs see Amtmann and Sanders, 1999). The main function of KORCs is assumed to be in stabilizing the membrane potential and/or providing a K^+ release pathway. Nevertheless it is worth considering KORCs with respect to cation uptake by plants. Although K^+ uptake via KORCs is unlikely, the driving force for Na^+ may be directed inward when these channels are open and Na^+ permeable KORCs could therefore provide a pathway for Na^+ uptake. However, quantitative modelling of KORC Na^+ -currents on the basis of typical features of KORC selectivity and gating (Amtmann and Sanders, 1999) as well as comparison of KORCs in salt-sensitive and tolerant wheat varieties (Schachtman, Tyerman and Terry, 1991) do not support this hypothesis.

VICs

VICs were first analysed in lipid bilayers containing plant plasma membrane fractions (White and Tester, 1992) and were subsequently observed in patch clamp experiments on protoplasts of various tissue origin (for review see Amtmann and Sanders, 1999) including wheat and maize roots. In general VICs are non-selective amongst monovalent and in some cases even divalent cations. Calculations of K^+ and Na^+ currents through an ensemble of KIRCs and VICs based on typical characteristics of both channel types leads to the conclusion that the contribution of VICs to K^+ influx is negligible since they are usually present in very small numbers compared to KIRCs. However, in conditions with high external Na^+/K^+ ratios such as a typical saline environment, VICs, if open, would allow massive influx of Na^+ into the cell over a wide range of voltages (Amtmann and Sanders, 1999).

Physiological evidence for a role of VICs in low affinity Na^+ uptake stems from the remarkable agreement between Ca^{2+} inhibition of Na^+ permeation through VICs, with the effect of Ca^{2+} on Na^+ influx into intact tissue (see below). Additional proposed roles for VICs include the stabilization of membrane potential and fast adaptation to osmotic stress. Also, it is speculated that this type of channel

TABLE 2. Genes encoding ion channels involved in K^+ transport

Gene	Species	Type	Expression	Inhibitors	Function	Reference
<i>AKT1</i>	<i>A. thaliana</i>	KIRC	Root cortex	$Cs^+/TEA/Ba^{2+}$	Low and high affinity K^+ uptake	Hirsch <i>et al.</i> , 1998
<i>AKT2</i>	<i>A. thaliana</i>	KIRC	Leaves	?		Cao <i>et al.</i> , 1995
<i>KAT1</i>	<i>A. thaliana</i>	KIRC	Guard cell	Cs^+/Ba^{2+}	Stomatal opening	Anderson <i>et al.</i> , 1992
<i>SKT1</i>	<i>S. tuberosum</i>	KIRC	Root Leaf epidermis	Cs^+	Root K^+ uptake	Zimmermann <i>et al.</i> , 1998
<i>KST1</i>	<i>S. tuberosum</i>	KIRC	Guard cells	Cs^+/Ba^{2+}	Stomatal opening	Müller-Röber <i>et al.</i> , 1995
<i>SKOR</i>	<i>A. thaliana</i>	KORC	Root pericycle	Ba^{2+}/TEA	Translocation to shoot	Gaymard <i>et al.</i> , 1998

participates in the uptake of NH_4^+ (Tyerman, Whitehead and Day, 1995; White, 1996).

CARRIERS INVOLVED IN Na^+ COMPARTMENTATION

Intracellular compartmentation of toxic solutes is a prerequisite for maintaining cellular integrity. Compartmentation of Na^+ in the vacuole avoids build up of cytoplasmic Na^+ (Fig. 2), raises the cytoplasmic K^+/Na^+ ratio and contributes to the vacuolar osmotic potential. In a number of species a Na^+/H^+ antiport is present in the tonoplast which allows accumulation of Na^+ in the vacuole by using the trans-tonoplast H^+ gradient as driving force [see Barkla and Pantoja (1996) for review]. Na^+/H^+ antiport exhibits K_m values in the mM region and is sometimes inhibited by amiloride. In *Arabidopsis* the genes for AtNHX1-3 were cloned and all show high homology with yeast and mammalian Na^+/H^+ antiporters (Apse *et al.*, 1998). Expression is observed in all tissues, although functional analysis of the AtNHX products have 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 (Barkla and Pantoja, 1996). Activity has only been reported for salt tolerant species such as red beet, sugar beet, barley and *Plantago maritima*, but appears absent in salt sensitive species such as *Plantago 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.

K^+ AND Na^+ TRANSLOCATION TO THE SHOOT

Growing plants with a shoot/root ratio > 1 will translocate most K^+ that enters the root symplast to the shoot. Yet, the majority of glycophytes tend to exclude Na^+ from the shoot by retaining it in the root and lower stem. The latter strategy is only successful at low to moderate external concentrations of Na^+ and relies on the selective release of Na^+ into the xylem and its resorption from the xylem stream. Several transport mechanisms that contribute to the translocation of K^+ and Na^+ and some aspects of their regulation have now been identified.

The *Arabidopsis* gene *SKOR* (Gaymard *et al.*, 1998) encodes a channel protein which is also a member of the Shaker family but displays gating characteristics that favour outward K^+ flux. *SKOR* has an important role in the translocation of K^+ to the shoot. GUS constructs with the *SKOR* promoter show high levels of expression in the root pericycle (Gaymard *et al.*, 1998). The *SKOR* expression pattern agrees with results from patch clamp experiments on maize roots which showed large K^+ outward currents in root stelar protoplasts as opposed to K^+ inward currents in cortical protoplasts (Roberts and Tester, 1995). Furthermore ABA, a plant hormone involved in drought-response of plants (Behl and Jeschke, 1981), reduces both outward

currents in maize stelar protoplasts (Roberts, 1998) and *SKOR* expression levels (Gaymard *et al.*, 1998). Transgenic *Arabidopsis* with knock-out mutations of *SKOR* display decreased shoot K^+ contents (Gaymard *et al.*, 1998) confirming its role in K^+ translocation to the shoot.

In addition to KORCs which are highly selective for K^+ over Na^+ (including KORCs in maize and barley stelar protoplasts and *SKOR*), a second type of outward-rectifying channel which does not discriminate between monovalent cations (NORC) has been found in patch clamp experiments on barley xylem parenchyma protoplasts (Wegner and Raschke, 1994; Wegner and De Boer, 1997). Opening of this channel requires μM concentrations of cytoplasmic Ca^{2+} and creates a potential passage for Na^+ release into the xylem. NORC remains to be identified at the gene level 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 this process may involve a NORC-mediated Na^+ release pathway into the stele.

A xylem parenchyma KIRC (Wegner and Raschke, 1994) with relatively low cation selectivity may also be implemented in Na^+ compartmentation (Fig. 2), since it potentially functions in basal parts of the xylem in Na^+ resorption (Maathuis *et al.*, 1997).

MODULATION OF K^+/Na^+ RATIO BY EXTERNAL Ca^{2+}

Application of external Ca^{2+} has long been known to ameliorate salinity stress symptoms in many species. Although this effect is probably based on several phenomena (Kinraide, 1998), a clear physiological basis is now available from the many studies on the impact of Ca^{2+} on ion channels. Several KIRCs are blocked by external Ca^{2+} but such blockage is usually very weak (e.g. Roberts and Tester, 1995). External Ca^{2+} has also been described to affect the open probability of KORCs in tobacco culture cells. A 100-fold increase in Ca^{2+} from 0.1 to 10 mM led to a three-fold reduction in KORC mediated current (Murata *et al.*, 1998).

A direct effect of Ca^{2+} on the movement of Na^+ is manifested in VICs: VIC-mediated Na^+ currents in wheat and maize root protoplasts were strongly reduced by external Ca^{2+} in the range of 0.01 to 1 mM. K_d s of Ca^{2+} inhibition in patch clamp experiments (around 300 μM ; Roberts and Tester, 1997; Tyerman *et al.*, 1997) agree perfectly with K_d s for Ca^{2+} inhibition of Na^+ flux measurements in intact roots (Zidan *et al.*, 1991; Davenport, Reid and Smith, 1997; Tyerman and Skerrett, 1999). Such findings not only implicate VICs as a major pathway for Na^+ uptake but provide an explanation of how increased external Ca^{2+} leads to lower Na^+ uptake and therefore helps to establish a high K^+/Na^+ ratio. Therefore, down-regulation of this channel type must be crucial for salt-tolerance.

Apart from its direct effects, external Ca^{2+} also interferes with signalling cascades that involve cytoplasmic Ca^{2+} . This was clearly demonstrated for the *Arabidopsis* salt over-sensitive (*sos3*) mutant where the salt sensitive phenotype was reverted by increased external Ca^{2+} (Liu and Zhu, 1997, see below).

TABLE 3. Genes products involved in K^+/Na^+ ratio

Gene product	Species	Action	Reference
SOS3	<i>A. thaliana</i>	Affects both K^+ and Na^+ movement, possibly via Ca^{2+} , calmodulin and calcineurin	Liu and Zhu, 1998
KNA1	<i>T. aestivum</i>	K^+ and Na^+ partitioning in leaves	Dubcovsky <i>et al.</i> , 1996
SAL1	<i>S. tuberosum</i>	Regulation of Na^+ and Li^+ fluxes, possibly via inositol phosphate signalling	Quintero <i>et al.</i> , 1996
HAL1	<i>S. cerevisiae</i>	Promotes Na^+ efflux and restricts K^+ efflux	Bordas <i>et al.</i> , 1997

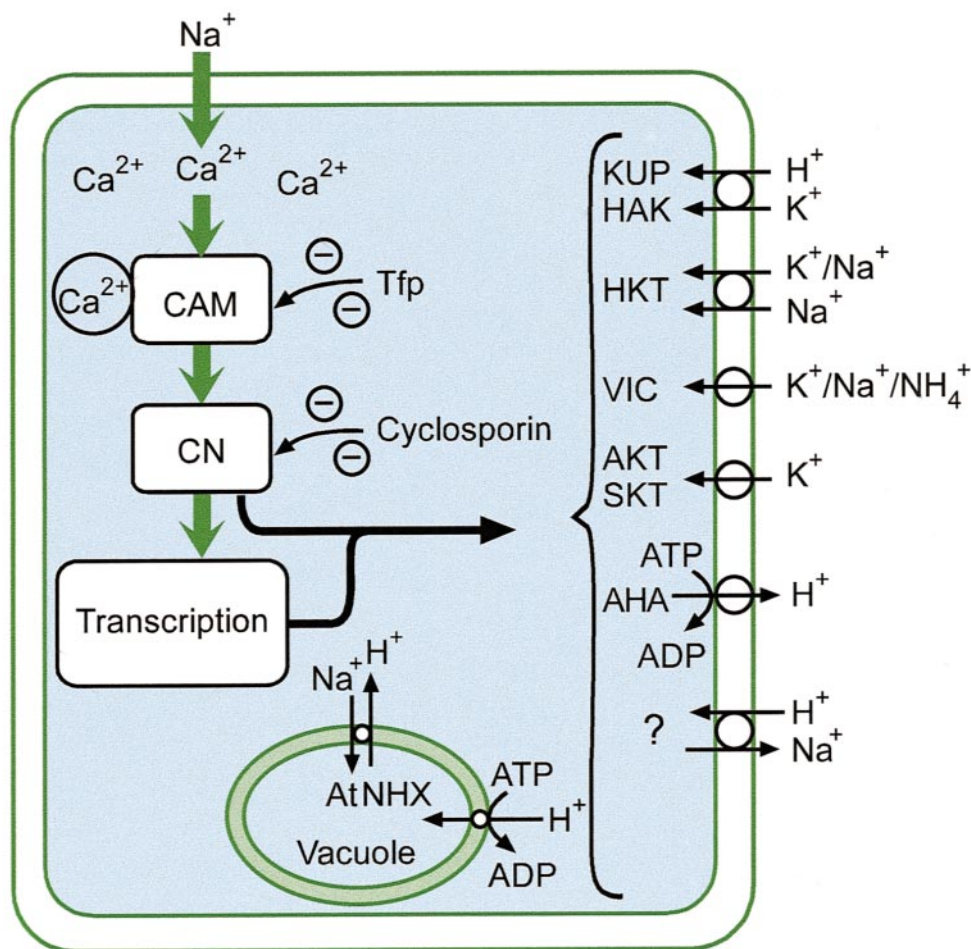


FIG. 3. Putative signalling pathway that impacts on transport of K^+ and Na^+ . Na^+ stress initiates a rise in cytoplasmic Ca^{2+} which in turn activates calmodulin (CAM). Subsequent binding of calmodulin and Ca^{2+} to calcineurin (CN, a Ca^{2+} /calmodulin dependent phosphatase) leads to modulation of transcription factors and/or direct modulation of K^+ and Na^+ transport systems. Negative signs denote inhibition. Tfp, Trifluoperazine.

GENES THAT AFFECT K^+/Na^+ RATIOS

Plant membranes contain a large number of transporters that contribute to the movement of K^+ and Na^+ . The relative magnitudes of influx, efflux and translocation ultimately determine the steady state levels of both ions (Fig. 1). Little is known about the regulation of these transport systems and how cells sense cytoplasmic ion levels. For K^+ , it has been proposed that part of the regulation stems from allosteric feedback that ensures down-regulation of K^+ uptake when cytoplasmic K^+ is

abundant (Glass and Fernando, 1992), but split root experiments have shown that long distance signals must also play a role (Marschner, 1995). Whatever the exact process is, since plants strictly control their cytoplasmic K^+ , this almost certainly implies the presence of a K^+ sensing mechanism. On the other hand 'Na⁺ sensing' may be limited to general perception of ionic/drought stress and/or K^+ deficiency. Nevertheless, several genes have now been identified in yeast and plants that appear to be involved in ionic homeostasis by modulating the movement of K^+ and Na^+ (Table 3).

As a second messenger, Ca^{2+} is involved in signalling cascades that modify transport activities. Studies on yeast cells showed Ca^{2+} - and calmodulin-based signalling pathways that affect uptake ratios of K^+ and Na^+ . One pathway of Na^+ stress perception in yeast mediates a raise in cytoplasmic Ca^{2+} , subsequently leading to activation of the phosphatase calcineurin which in turn modifies the action of both Na^+ efflux and K^+ influx systems (Bressan, Hasegawa and Pardo, 1998). Calcineurin activates ENA^1 pumps that are responsible for Na^+ efflux. At the same time, the main K^+ uptake system TRK1 is switched from a rather non-selective mode (low K^+/Na^+ selectivity) to a K^+ -selective mode. Both processes tend to reduce the cytoplasmic Na^+ load and increase the K^+/Na^+ ratio.

An analogous signalling cascade appears to be present in plants (Fig. 3). From a NaCl based mutant screen in *Arabidopsis*, *SOS3*, a locus involved in hypersensitivity to Na^+ and Li^+ , was identified (Liu and Zhu, 1997). In addition to salt over-sensitivity, *sos3* plants are unable to grow on low K^+ . Both components of the phenotype, Na^+ over-sensitivity and reduced high affinity K^+ transport, were partly suppressed by increased (> 3 mM) levels of external Ca^{2+} . The nature of the link between external Ca^{2+} and *SOS3* was only revealed after the gene was sequenced: *SOS3* codes for a protein that shows 27–31% sequence identity with the B subunit of the Ca^{2+} dependent calcineurin and a neuronal Ca^{2+} sensor (Liu and Zhu, 1998). As in yeast, cytosolic Ca^{2+} in plants is increased in response to salinity shock (Knight, Trewavas and Knight, 1997) and, similar to the pathway in yeast (Mendoza *et al.*, 1994), a calcineurin-like phosphatase in plants may impact on the K^+/Na^+ ratio. This could occur directly by modifying transport activities or indirectly via the activation of transport specific transcription factors.

Increasing additional support for the signalling pathway described above is emerging: transformation of tobacco with a constitutively activated yeast calcineurin conferred salt tolerance to several transgenic lines (Pardo *et al.*, 1998). Also, growth experiments with wildtype *Arabidopsis* plants exposed to various antagonists of the proposed signalling cascade show that the calcineurin inhibitor cyclosporin and the calmodulin antagonist trifluoperazine have little effect on plant growth in the absence of Na^+ but greatly reduce growth in the presence of Na^+ (C. Elphick and F. Maathuis unpubl. res.).

Other plant and yeast genes have been isolated that alter the cellular K^+/Na^+ ratio and impact on salt tolerance. In yeast, expression of *HAL1* is induced by salt and causes a rise in cellular K^+ and a decrease in Na^+ (Gaxiola *et al.*, 1992). Transcription of *HAL1* induces *ENA1* expression in a calcineurin independent way and hence favours Na^+ extrusion. *HAL1* also restricts K^+ efflux through a hitherto unknown pathway (Bordas *et al.*, 1997). The combined effects of *HAL1* effectively increase the intracellular K^+/Na^+ ratio from 1 to 4 (Gaxiola *et al.*, 1992). The *HAL1* gene is conserved in plants, and transformation of melon with

HAL1 produced a higher degree of salt tolerance in transgenic than in untransformed cultured shoots.

The yeast gene *HAL2* encodes a salt-sensitive enzyme in yeast sulphur metabolism and homologous genes have been identified in *Arabidopsis* (*SAL1*) and rice (Quintero, Garcia-deblas and Rodriguez-Navarro, 1996). Although the exact role of *SAL* type genes remains to be elucidated, it is speculated that the product of *SAL1* is involved in a signalling pathway that affects Na^+ fluxes.

In hexaploid wheat, more K^+ and less Na^+ is accumulated in young expanding leaves compared to tetraploid wheat (Dubcovsky *et al.*, 1996). The resulting higher leaf K^+/Na^+ ratio in the hexaploid variety is reflected in its greater salt tolerance. This trait was found to be controlled by a single locus *KNA1*. Although it is not known what *KNA* codes for, it does not resemble any transport protein. However, *KNA* might encode a regulatory protein that affects resorption of K^+ and/or Na^+ from the leaf xylem, the recycling of either ion through the leaf phloem, or both processes.

The overall picture that emerges from reports on genes involved in K^+/Na^+ homeostasis suggests that genes such as *SOS*, *KNA* and *SAL* are involved in modulation of K^+ and/or Na^+ transporter activities. Modulation of transport activity may occur directly if genes are part of a signalling cascade that, for instance, affects transporter phosphorylation status via phosphatases and kinases. This is exemplified by the phosphoinositide and phosphatase activities of *SAL* and *SOS* genes, respectively. Alternatively, gene-products of *SOS*, *KNA* and *SAL* may be, or act on, transcription factors that affect expression levels of specific transporters as is the case in yeast where salt stress stimulates over expression of the Na^+ extrusion pump.

CONCLUSIONS

The capacity of plants to maintain a high cytosolic K^+/Na^+ ratio is likely to be one of the key determinants of plant salt tolerance. The overall K^+/Na^+ ratio is heritable in species such as wheat but not in others such as rice (Garcia *et al.*, 1997), and probably involves the contribution of different genes in different species. The steady state levels of both ions will be a function of many transport steps involved in the uptake, efflux, translocation and compartmentation. The influx of K^+ at the root boundary has now, to a large extent, been characterized for both high and low affinity uptake. Far less clear is how Na^+ enters the root symplast, although there is accumulating evidence that non-selective VICs play a substantial role. As a generalization, it can be concluded that K^+ uptake at the root/soil boundary is via highly K^+ selective pathways whereas Na^+ , at least in part, appears to move through less selective systems which in some cases are blocked by Ca^{2+} .

In addition, we now partially understand the underlying processes and genes that are involved in K^+ translocation to the shoot and compartmentation of Na^+ in the vacuole. Further identification and functional characterization of genes such as *SOS*, *KNA* and *SAL* will increasingly show us how plants modulate K^+ and Na^+ transport activities in order to establish ionic homeostasis.

¹ Efflux of Na^+ : these are ATP-fuelled pumps that extrude Na^+ in yeast.

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LITERATURE CITED

- Amtmann A, Gradmann D. 1994. Na^+ transport in *Acetabularia* bypasses conductance of plasmalemma. *Journal of Membrane Biology* **139**: 117–125.
- Amtmann A, Sanders D. 1999. Mechanisms of Na^+ uptake by plant cells. *Advances in Botanical Research* **29**: 76–112.
- Anderson JA, Huprikar SS, Kochian LV, Lucas WJ, Gaber RF. 1992. Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences (USA)* **89**: 3736–3740.
- Apse MP, Aharon GS, Snedden WA *et al.* 1998. Cloning and characterisation of plant sodium/proton antiports. In: *Abstracts of the 11th International Workshop on Plant Membrane Biology*. The Society of Experimental Biology, 84.
- Barkla BJ, Pantoja O. 1996. Physiology of ion transport across the tonoplast of higher plants. *Annual Review of Plant Physiology and Molecular Biology* **47**: 159–184.
- Behl R, Jeschke JD. 1981. Influence of abscisic acid on unidirectional fluxes and intracellular compartmentation of K^+ and Na^+ in excised barley root segments. *Physiologia Plantarum* **53**: 95–100.
- Bordas M, Montesinos C, Dabauza M, Salvador A, Roig LA, Serrano R, Moreno V. 1997. Transfer of the yeast salt tolerance gene *hal1* to *Cucumis melo* 1 cultivars and *in vitro* evaluation of salt tolerance. *Transgenic Research* **6**: 41–50.
- Bressan RA, Hasegawa PM, Pardo JM. 1998. Plants use calcium to resolve salt stress. *Trends in Plant Science* **3**: 411–412.
- Cao Y, Ward JM, Kelly WB, Ichida AM, Gaber RF, Anderson JA, Uozumi N, Schroeder JI. 1995. Multiple genes, tissue specificity, and expression-dependent modulation contribute to the functional diversity of potassium channels in *Arabidopsis thaliana*. *Plant Physiology* **109**: 1093–1106.
- Carden DE, Walker DJ, Flowers TJ *et al.* 1998. Root cell cytosolic ion differences between barley cultivars may contribute to salt tolerance. In: *Abstracts of the 11th International Workshop on Plant Membrane Biology*. The Society of Experimental Biology, 345.
- Clemens S, Antosiewicz DM, Ward JM, Schachtman DP. 1998. The plant cDNA *LCT1* mediates the uptake of calcium and cadmium in yeast. *Proceedings of the National Academy of Sciences USA* **95**: 12043–12048.
- Davenport RJ, Reid RJ, Smith FA. 1997. Sodium-calcium interactions in two wheat species differing in salinity tolerance. *Physiologia Plantarum* **99**: 323–327.
- Dubcovsky J, Maria GS, Epstein E, Luo MC, Dvorak J. 1996. Mapping of the K^+/Na^+ discrimination locus *knal* in wheat. *Theoretical and Applied Genetics* **92**: 448–454.
- Flowers TJ, Läuchli A. 1983. Sodium versus potassium: substitution and compartmentation. In: Läuchli A, Pirson A, eds. *Encyclopedia of plant physiology* 158. *Inorganic plant nutrition*. Berlin: Springer, 651–681.
- Flowers TJ, Troke PF, Yeo AR. 1977. The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology and Molecular Biology* **28**: 89–121.
- Fu H, Luan S. 1998. AtKUP1: A dual affinity K^+ transporter from *Arabidopsis*. *Plant Cell* **10**: 63–67.
- Garcia A, Rizzo RJ, Bartos SL, Senadhira D, Flowers TJ, Yeo AR. 1997. Sodium and potassium transport to the xylem are inherited independently in rice, and the mechanism of sodium:potassium selectivity differs between rice and wheat. *Plant, Cell and Environment* **20**: 1167–1174.
- Gassmann W, Rubio F, Schroeder JI. 1996. Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1. *Plant Journal* **10**: 869–882.
- Gassmann W, Ward JM, Schroeder JI. 1993. Physiological role of inward rectifying K^+ channels. *Plant Cell* **5**: 1491–1493.
- Gaxiola R, Larrinoa IF, Villalba JM, Serrano R. 1992. A novel and conserved salt-induced protein is an important determinant of salt tolerance in yeast. *EMBO Journal* **11**: 3157–3164.
- Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Bouchez J, Michaux-Ferriere M, Thibaud J, Sentenac H. 1998. Identification and disruption of a plant Shaker-like outward channel involved in K^+ release into the xylem sap. *Cell* **94**: 647–655.
- Glass ADM, Fernando M. 1992. Homeostatic processes for the maintenance of the K^+ content of plant cells: a model. *Israel Journal of Botany* **41**: 145–166.
- Gollmack D, Kamasani UR, Quigley F, Bennett J, Bohnert HJ. 1997. Salt stress-dependent expression of a HKT1-type high affinity potassium transporter in rice. *Plant Physiology* **114**: S529.
- Greenway H, Munns R. 1980. Mechanisms of salt tolerance in non-halophytes. *Annual Review of Plant Physiology and Molecular Biology* **31**: 149–190.
- Hajibagheri MA, Flowers TJ, Collins JC, Yeo AR. 1988. A comparison of the methods of X-ray microanalysis, compartmental analysis and longitudinal ion profiles to estimate cytoplasmic ion concentrations in two maize varieties. *Journal of Experimental Botany* **39**: 279–290.
- Hirsch RE, Lewis BD, Spalding EP, Sussman MR. 1998. A role for the AKT1 potassium channel in plant nutrition. *Science* **280**: 918–921.
- Katz A, Bentall M, Degani H, Avron M. 1991. *In vivo* pH regulation by a Na^+/H^+ antiporter in the halotolerant alga *Dunaliella salina*. *Plant Physiology* **96**: 110–115.
- Kim EJ, Kwak JM, Uozumi N, Schroeder JI. 1998. AtKUP1: An *Arabidopsis* gene encoding high-affinity potassium transport activity. *Plant Cell* **10**: 51–62.
- Kinraide TB. 1998. Three mechanisms for the calcium alleviation of mineral toxicities. *Plant Physiology* **118**: 513–520.
- Knight H, Trevas AJ, Knight MR. 1997. Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. *Plant Journal* **12**: 1067–1078.
- Kochian LV, Lucas WJ. 1988. Potassium transport in roots. *Advances in Botanical Research* **15**: 93–178.
- Lagarde D, Basset M, Lepetit M, Conegero G, Gaymard F, Astruc S, Grignon C. 1996. Tissue-specific expression of *Arabidopsis AKT1* gene is consistent with a role in K^+ nutrition. *Plant Journal* **9**: 195–203.
- Liu J, Zhu JK. 1997. An *Arabidopsis* mutant that requires increased calcium for potassium nutrition and salt tolerance. *Proceedings of the National Academy of Sciences USA* **94**: 14960–14971.
- Liu J, Zhu JK. 1998. A calcium sensor homolog required for plant salt tolerance. *Science* **280**: 1943–1945.
- Maathuis FJM, Sanders D. 1994. Mechanism of high affinity potassium uptake in roots of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA* **91**: 9272–9276.
- Maathuis FJM, Sanders D. 1995. Contrasting roles in ion transport of two K^+ -channel types in root cells of *Arabidopsis thaliana*. *Planta* **197**: 456–464.
- Maathuis FJM, Sanders D. 1996. Mechanisms of potassium absorption by higher plant roots. *Physiologia Plantarum* **96**: 158–168.
- Maathuis FJM, Ichida AM, Sanders D, Schroeder JI. 1997. Roles of higher plant K^+ channels. *Plant Physiology* **114**: 1141–1149.
- Maathuis FJM, Verlin D, Smith FA, Sanders D, Fernandez JA, Walker NA. 1996. The physiological relevance of Na^+ -coupled K^+ -transport. *Plant Physiology* **112**: 1609–1616.
- Marschner H. 1995. *Mineral nutrition of higher plants*. 2nd edn. London: Academic Press.
- Mendoza I, Rubio F, Rodriguez-Navarro A, Pardo JM. 1994. The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. *Journal of Biochemistry* **269**: 8792–8796.
- Murata Y, Yoshihashi M, Obi I, Kakutani T. 1998. Ca^{2+} regulation of outward rectifying K^+ channel in the plasma membrane of tobacco cultured cells in suspension: a role of the K^+ channel in mitigation of salt-stress effects by external Ca^{2+} . *Plant Cell Physiology* **39**: 1039–1044.
- Müller-Röber B, Ellenberg J, Provart N, Willmitzer L, Busch H, Becker D, Dietrich P, Hoth S, Hedrich R. 1995. Cloning and electrophysiological analysis of KST1, an inward rectifying K^+ channel expressed in potato guard cells. *EMBO Journal* **14**: 2409–2416.

- Pardo JM, Reddy MP, Yang S *et al.* 1998. Stress signalling through Ca^{2+} /calmodulin-dependent protein phosphatase calcineurin mediates salt adaptation in plants. *Proceedings of the National Academy of Sciences USA* **95**: 9681–9686.
- Quintero FJ, Blatt MR. 1997. A new family of K^+ transporters from *Arabidopsis* that are conserved across phyla. *FEBS* **415**: 206–211.
- Quintero FJ, Garcıadeblas B, Rodrıguez-Navarro A. 1996. The *SAL1* gene of *Arabidopsis*, encoding an enzyme with 3'(2'), 5'-bisphosphate nucleotidase and inositol 1-phosphatase activities, increases salt tolerance in yeast. *Plant Cell* **8**: 529–537.
- Roberts SK. 1998. Regulation of K^+ channels in maize roots by water stress and abscisic acid. *Plant Physiology* **116**: 145–153.
- Roberts SK, Tester M. 1995. Inward and outward K^+ -selective currents in the plasma membrane of protoplasts from maize root cortex and stele. *Plant Journal* **8**: 811–825.
- Roberts SK, Tester M. 1997. A patch clamp study of Na^+ transport in maize roots. *Journal of Experimental Botany* **48**: 431–440.
- Rodrıguez-Navarro A, Quintero FJ, Garcıadeblas B. 1994. Na^+ -ATPases and Na^+ / H^+ antiporters in fungi. *Biochimica et Biophysica Acta* **1187**: 203.
- Rubio F, Gassmann W, Schroeder JI. 1995. Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* **270**: 1660–1663.
- Santa-Maria G, Rubio F, Dubcovsky J, Rodrıguez-Navarro A. 1997. The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. *Plant Cell* **9**: 2281–2289.
- Schachtman DP, Schroeder JI. 1994. Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. *Nature* **370**: 655–658.
- Schachtman DP, Tyerman SD, Terry BR. 1991. The K^+ / Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. *Plant Physiology* **97**: 598–605.
- Schachtman DP, Kumar R, Schroeder JI, Marsch EL. 1997. Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. *Proceedings of the National Academy of Sciences USA* **94**: 11079–11084.
- Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon JM, Gaymard F, Grignon C. 1992. Cloning and expression in yeast of a plant potassium ion transport system. *Science* **256**: 663–665.
- Tyerman SD, Skerrett IM. 1999. Root ion channels and salinity. *Scientiae Horticulturae* **78**: 175–235.
- Tyerman SD, Whitehead LF, Day DA. 1995. A channel-like transporter for NH_4^+ on the symbiotic interface of N_2 -fixing plants. *Nature* **378**: 629–632.
- Tyerman SD, Skerrett M, Garrill A, Findlay GP, Leigh RA. 1997. Pathways for the permeation of Na^+ and Cl^- into protoplasts derived from the cortex of wheat roots. *Journal of Experimental Botany* **48**: 459–480.
- Walker DJ, Leigh RA, Miller AJ. 1996. Potassium homeostasis in vacuolate plant cells. *Proceedings of the National Academy of Sciences USA* **93**: 10510–10514.
- Wang TB, Gassmann W, Rubio F, Schroeder JI, Glass ADM. 1998. Rapid upregulation of *HKT1*, a high-affinity potassium transporter gene, in roots of barley and wheat following withdrawal of potassium. *Plant Physiology* **118**: 651–659.
- Wegner LH, De Boer AH. 1997. Properties of two outward-rectifying channels in root xylem parenchyma cells suggest a role in K^+ homeostasis and long-distance signaling. *Plant Physiology* **115**: 1707–1719.
- Wegner LH, Raschke K. 1994. Ion channels in the xylem parenchyma of barley roots. *Plant Physiology* **105**: 799–813.
- White PJ. 1996. The permeation of ammonium through a voltage-independent K^+ channel in the plasma membrane of rye roots. *Journal of Membrane Biology* **152**: 89–99.
- White PJ, Tester MA. 1992. Potassium channels from the plasma membrane of rye roots characterized following incorporation into planar lipid bilayers. *Planta* **186**: 188–202.
- Wilson C, Shannon MC. 1995. Salt-induced Na^+ / H^+ antiport in root plasma-membrane of a glycophytic and halophytic species of tomato. *Plant Science* **107**: 147–157.
- Yeo AR. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. *Journal of Experimental Botany* **49**: 915–929.
- Zidan I, Jacoby B, Ravina I, Neumann PM. 1991. Sodium does not compete with calcium in saturating plasma membrane sites regulating ^{22}Na influx in salinized maize roots. *Plant Physiology* **96**: 331–334.
- Zimmermann S, Talke I, Ehrhardt T, Nast G, Müller-Röber B. 1998. Characterization of SKT1, an inwardly rectifying potassium channel from potato, by heterologous expression in insect cells. *Plant Physiology* **116**: 879–890.