13 Potassium Homeostasis in Salinized Plant Tissues

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13.1 Introduction

Potassium is an essential cation, comprising ~6% of a plant's dry weight and is involved in numerous functions such as osmo- and turgor regulation, charge balance, and control of stomata and organ movement. K^+ activates over 50 enzymes critical for numerous metabolic processes, including photosynthesis, oxidative metabolism and protein synthesis (Marschner 1995). Within the cytosol, K^+ neutralizes the soluble and insoluble macromolecular anions and stabilizes the pH at the level optimal for most enzymatic reactions (pH ~7.2). Thus, cytosolic K^+ homeostasis is crucial to optimal cell metabolism.

In contrast to K^+, Na^+ is not essential for plants (Marschner 1995). For the majority of crop species, Na^+ is toxic at mM concentrations in the cytosol. With cytosolic K^+ concentrations being around 150 mM (Leigh and Wyn Jones 1984; Leigh 2001) and cytosolic Na^+ in a lower mM range (Carden et al. 2003), the cytosolic K^+/Na^+ ratio is high, enabling many K^+-dependent metabolic processes to proceed (Rubio et al. 1995; Maathuis and Amtmann 1999). Under saline conditions, cytosolic Na^+ levels increase dramatically, estimates varying from 10 to 30 mM, up to 200 mM (Koyro and Stelzer 1988; Flowers and Hajibagheri 2001; Carden et al. 2003). At the same time, cytosolic K^+ content decreases dramatically. An almost 2-fold decrease in cytosolic K^+ activity was measured in salinized roots of barley (Carden et al. 2003), and cytosolic K^+ activity as low as 15 mM in epidermal leaf cells has been reported (Cuin et al. 2003). Thus the cytosolic K^+/Na^+ ratio falls dramatically under saline conditions, severely impairing cell metabolism (Maathuis and Amtmann 1999; Flowers and Hajibagheri 2001; Munns 2002). Not surprising, the ability to maintain a high cytosolic K^+/Na^+ ratio has often been cited as a key feature in plant salt tolerance (Gorham et al. 1990; Maathuis and Amtmann 1999; Tester and Davenport 2003; Chen et al. 2005).

Within the vacuole, K^+ mediates osmoregulation, and within specialized cells, stomatal movements and tropisms. Here the K^+ concentration is much more flexible and can be more readily replaced by other cations, including

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Na^+ (Leigh et al. 1986). However, the vacuolar PP-ase is critically dependent on K^+ for both hydrolytic activity and H^+ pumping (White et al. 1990). Thus, even in this organelle, maintenance of a minimal level of K^+ is vitally important for optimal plant performance. How is this achieved?

Molecular and ionic mechanisms of K^+ transport have been the subject of a large number of comprehensive reviews in recent years (Maathuis and Amtmann 1999; Maathuis and Sanders 1999; Tyerman and Skerrett 1999; Schachtman 2000; Mäser et al. 2001; Véry and Sentenac 2002, 2003; Shabala 2003) so are only briefly revised in our review. Many important questions, however, remain to be answered. It is not clear how the levels and ratios of K^+ to Na^+ are maintained within the plant, and why these ratios are different in cells within various plant tissues. It is also remains to be answered how plants distinguish between K^+ and Na^+, both at the root and cellular levels. This latter problem is not trivial, due to the similarity in ionic radius and ion hydration energies for K^+ and Na^+ (Hille 1992), factors which determine both the ion transport mode and the competition for enzyme binding sites within the cytosol. Despite a recent plethora of research (Apse et al. 1999, 2003; Hasegawa et al. 2000; Zhu 2000, 2003; Zhang and Blumwald 2001), we are still lacking full knowledge of the signal-transduction pathways involved in K^+ homeostasis and maintenance of the critical K^+/Na^+ ratios under salt stressed conditions.

This review addresses some of the above issues and summarizes molecular and electrophysiological evidence regarding mechanisms regulating K^+ homeostasis in salinized plant tissues. The main emphasis is made on the integration of K^+ transport mechanisms at various levels of plant structural organization.

13.2 Potassium acquisition and distribution in plants

Potassium enters the root symplast via the cell plasma membrane (PM). From there, it can travel through the symplast to the vascular tissues, where it is unloaded from the xylem parenchyma into xylem vessels for long-distance transport to leaves. K^+ is reabsorbed from the xylem into leaf cells. Being a highly mobile element (Marschner 1995), it can be easily loaded into the phloem for translocation to actively growing sink tissues (e.g. shoot and root apices) where it can be unloaded by way of symplasmic or apoplastic pathways. K^+ can also cross the tonoplast membrane for storage in vacuoles of both root and shoot cells. The integration and regulation of K^+ transport systems at different sites along the long-distance pathway allows the plant to direct the partitioning and circulation of K^+. Such an integrated system plays a central role in plant growth and development and in the allocation of mineral nutrients in response to changes in nutrient availability. This section

very briefly summarizes uptake and compartmentation of K^+ within a plant, at a physiological level.

13.2.1 Uptake at the root level

Net K^+ uptake at the root PM is classically viewed as the result of the operation of both active and passive transporters with different affinities for K^+ (systems *I* and *II*; Epstein et al. 1963). While the high-affinity K^+ uptake system *I* is strongly selective for K^+ over other alkali cations and shows increased gene expression or transport activity under K^+ starvation conditions, the low-affinity K^+ transport system *II* is less selective for K^+ over Na^+ and less influenced by changes in the K^+ status of the plant (Marschner 1995). Patch-clamp studies suggest that system *I* is an active transport mechanism (Maathuis and Sanders 1993), most likely via a K^+/H^+ symporter (Maathuis and Sanders 1994). The inward-rectifying K^+-selective (KIR) channels mediate uptake within the concentration range of system *II* (above 1 mM). These channels have been found in root cells of various species (White and Tester 1992; Gassmann and Schroeder 1994; Maathuis and Sanders 1995; Roberts and Tester 1995) and can mediate long-term K^+ influx into the cell (Schroeder et al. 1994; Schachtman et al. 1992; Gaymard et al. 1996). KIR channels are also found in root hairs (Gassmann and Schroeder 1994), suggesting their important role in K^+ acquisition beyond the root depletion zone.

13.2.2 Xylem loading

Once inside the root, K^+ is transported to the vascular tissues where it is unloaded from xylem parenchyma into xylem vessels for long-distance transport to leaves. Patch-clamp studies have demonstrated the presence of both anion and cation channels likely to be responsible for loading of solutes into the xylem for transport to the shoot (Maathuis et al. 1998; Köhler and Raschke 2000). The PM of cortical cells is dominated by a K^+ channel that favors K^+ influx into the cells, and thus uptake into the root, whereas the stelar cells are dominated by a K^+ channel favoring K^+ efflux into the apoplast, resulting in xylem loading (Roberts and Tester 1995). However, such clear-cut differences in channel activities are not seen in *Arabidopsis* cortical and stellar tissues (Maathuis et al. 1998). Some authors have argued against the role of outward K^+ channels in xylem sap K^+ loading (de Boer 1999), suggesting instead that K^+ secretion into the xylem occurs against the K^+ electrochemical gradient in a process mediated by active transport systems (Kochian and Lucas 1988; Moshelion et al. 2002). More likely, both types of K^+ transporters are involved. Experiments on SKOR, the *Arabidopsis* outward-rectifying Shaker channel, estimated that its activity contribute to about 50% of K^+ translocated towards

the shoot (Gaymard et al. 1998; Lacombe et al. 2000). The remainder might be attributed to some active transport system.

13.2.3 Potassium compartmentation at the tissue and whole-plant levels

Under normal growth conditions, K^+ is the most abundant cation in both the cytosol and the vacuole. The concentration to which K^+ accumulates is, however, different in root and leaf cells. K^+ activities in the leaf cell vacuoles were approximately 230 mM (Cuin et al. 2003) compared with 120 mM in the root cell vacuoles (Walker et al. 1996), while the cytosolic K^+ activities in root and leaf cells were comparable (Walker et al. 1996; Cuin et al. 2003).

There is also a certain degree of heterogeneity between the vacuolar (but not cytosolic) K^+ content of different cell types in leaves under K^+-replete conditions (Cuin et al. 2003). Barley K^+ concentrations were only slightly lower in the mesophyll cells than the epidermal cells (Fricke et al. 1994; Cuin et al. 2003). Slight differences in vacuolar K^+ content between abaxial and adaxial epidermal cells were reported in *Lupinus* (Treeby and van Steveninck 1988) and *Sorghum* (Boursier and Läuchli 1989). However, the substantial heterogeneity in K^+ concentration between different cell types only became pronounced under K^+-limiting conditions, where concentrations were maintained in the mesophyll cells, but decreased in the epidermal cells (Fricke et al. 1994). Salinity is one such condition.

13.2.4 Intracellular K^+ compartmentation

The cytoplasmic K^+ level is strictly controlled (80–100 mM activity; Maathuis and Sanders 1994; Walker et al. 1996; Cuin et al. 2003), a homeostasis that is achieved by both the control of K^+ influx across the PM and by mobilizing K^+ from vacuolar reserves (Glass and Fernando 1992; Walker et al. 1996). Vacuolar K^+ content is not so strictly regulated and shows large fluctuations depending on K^+ supply (Leigh and Wyn Jones 1984; Walker et al. 1996). Under K^+-replete conditions, vacuolar K^+ is typically around 200–250 mM reaching 500 mM in open stomatal guard cells (MacRobbie 1998) but decreasing to 10 mM under K^+-deficient conditions (Walker et al. 1996). As the major role of K^+ in the vacuoles is in maintenance of cell turgor (required for cell extension and stomata opening), the osmotic functions of K^+ in the vacuole may replaceable to a varying degree by other cations (such as Na^+, Mg^{2+} and Ca^{2+}) or organic solutes (e.g. sugars). The concentration of K^+ in the apoplast is usually low (between 2 and 20 mM; Karley et al. 2000; Roelfsema and Hedrich 2002) with the exception of specialized cells or tissues such as stomata and pulvini, where it may transiently rise to 100 mM (Roelfsema and Hedrich 2002).

Potassium content is also high in chloroplasts, with 50–100 mM concentration range reported (Demmig and Gimmler 1983; Pier and Berkowitz 1987). In

addition to being an important stromal enzyme involved in leaf photochemistry, K^+ also plays a key role in charge balancing the massive light-driven transport of H^+ into the thylakoid lumen required for ATP synthesis (Pottosin and Schönknecht 1996). The extent to which intact chloroplasts are able to maintain a constant K^+ concentration, independently of changes in the external medium, is unknown. Also limited is our knowledge of K^+ transporters in this organelle. In addition to several types of cation-permeable channels (reviewed by Shabala 2003), there are also suggestions that various secondary active transport systems are present at the chloroplast envelope (Demming and Gimmler 1983; Wu and Berkowitz 1992). Recently, an apparently neutral $K^+(Na^+)/H^+$ antiporter has been characterized in the envelope membranes of *Arabidopsis* chloroplasts (Song et al. 2004). This exchanger was suggested to be located in the chloroplast envelope and is thought to function in the adjustment of pH in the cytosol thereby maintaining a high pH level in the chloroplast stroma. Much still remains to be described about K^+ homeostasis within this vitally important organelle.

13.2.5 Remobilization and recycling

After delivery to the leaf tissue, K^+ can be loaded into phloem cells for translocation to actively growing sink tissues (e.g. shoot and root apices), where it can be unloaded by way of symplasmic or apoplastic pathways.

Classical electrophysiological analysis shows that uptake by roots is tuned in response to shoot demand and K^+ recirculation via the phloem sap from shoots to roots is involved in this control. In this scheme, the rate of K^+ unloading from the root stele would act as a signal that would regulate, via as yet unidentified negative feedback mechanisms, K^+ uptake activity in root periphery cells (Kochian and Lucas 1988). This hypothesis has been supported by kinetic studies with rye (White 1997), and is likely to be significant in maintaining K^+ homeostasis, although the specific details on the underlying ionic mechanisms remain to be determined.

13.3 Ionic mechanisms of K^+ acquisition and transport in plants

13.3.1 General features of K^+ transporters in plants

Since the classical work of Epstein and coworkers (1963), many advanced electrophysiological and molecular techniques have become available, allowing considerable progress in the analysis of K^+ transport in plants at both the molecular and physiological level.

The recent completion of the *Arabidopsis* genome sequence has offered the opportunity to make an inventory of all the putative plant transporter

proteins (Ward 2001). A genome wide survey revealed seven major families of *Arabidopsis* cation transporters (75 genes in total) which mediate K^+ transport across plant membranes. These include (Mäser et al. 2001; Véry and Sentenac 2002, 2003; Shabala 2003): (i) Shaker-type K^+ channels (nine genes); (ii) two-pore K^+ channels (six genes); (iii) cyclic-nucleotide-gate channels (20 genes); (iv) putative K^+/H^+ antiporters (six genes); (v) KUP/HAK/KT transporters (13 genes); (vi) HKT transporters (one gene); (vii) glutamate receptors (20 genes). In addition, a low-affinity K^+-permeable transporter (LCT1) has been identified in wheat (Schachtman et al. 1997).

13.3.2 Basic features and control modes of potassium transporters

13.3.2.1 Shaker family of potassium channels

The Shaker family of K^+ channels comprises nine members in *Arabidopsis* (Mäser et al. 2001). They are related to animal K^+ channels initially cloned from *Drosophila* (thus, "Shakers"). Members of this family have also been identified in a number of other plant species (Véry and Sentenac 2003). Comparison of the functional properties of these channels in heterologous expression systems with channel activity recorded in planta suggests that they are active at the PM and mediate most K^+-selective voltage-gated currents that dominate the membrane K^+ conductance at hyper- and depolarized membrane potential (E_m) (Véry and Sentenac 2002). These channels are present in numerous cell types and operate at mM K^+ concentrations. They represent the best-characterized family of plant transporters at the molecular level.

Plant Shaker polypeptides typically display a short (about 60 amino acid) intracytoplasmic N-terminal domain, followed by a hydrophobic core composed of six transmembrane segments (S1–S6), the pore domain being inserted between S5 and S6, and a long intracytoplasmic region representing more than half the sequence (Véry and Sentenac 2003). The transmembrane segment 4 harbors positively charged amino acids and is expected to act as a voltage sensor. A highly conserved pore domain, carrying the hallmark GYGD/E motif of highly K^+ selective channels, is present between S5 and S6. The long C-terminal region harbors a putative cyclic nucleotide-binding domain and, in most Shaker channels, an ankyrin domain potentially involved in protein-protein interactions (Véry and Sentenac 2003).

Most plant Shaker-type K^+ channels identified so far have been successfully expressed and characterized in heterologous systems. Based on their voltage dependency, these channels can be grouped into three functional subfamilies: (i) inward, (ii) weakly-inward, and (iii) outward-rectifying (Véry and Sentenac 2003). Inward-rectifying channels are activated by membrane hyperpolarization from a threshold more negative than the K^+ equilibrium potential (E_k), and are mainly involved in K^+ uptake. Weak inward-rectifiers also are activated by membrane hyperpolarization, but

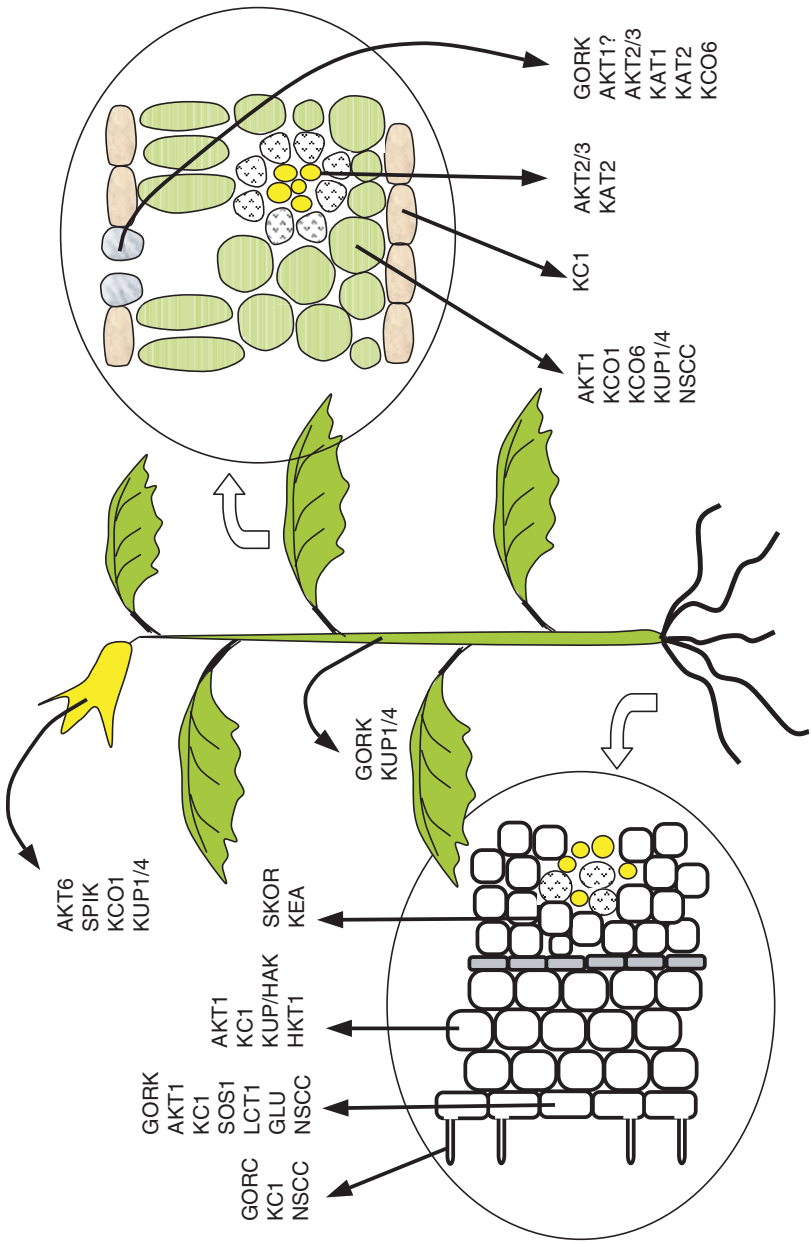

Fig. 13.1. Tissue-specific expression of K^+ transporters in plants. Most of the data comes from experiments with *Arabidopsis*, although results from other plant species are also included. With the exception of KCO channels, all other transporters shown are likely to be expressed at cell PM

never display null open probabilities within a physiological E_m range and are potentially able to mediate both K^+ uptake and release. Outward-rectifying channels activate at E_m more positive than E_k and are specialized in K^+ release (Véry and Sentenac 2003).

Physiological roles and functional expression of the channels from the Shaker family are diverse. AKT1 is a hyperpolarization-activated K^+ channel (Bertl et al. 1994) that is expressed in the roots (Lagarde et al. 1996) and plays a role in K^+ uptake, provided that the external K^+ concentration is in the mM range and the E_m is negative enough (Hirsch et al. 1998). SKOR is expressed in stelar tissue of the root and is thought to be involved in K^+ release into the xylem sap (Gaymard et al. 1998). KAT1 takes part in guard cell K^+ uptake, but is not essential for stomatal opening (Szyroki et al. 2001), probably because of inward-rectifier redundancy in guard cells (Pilot et al. 2001). SPIK is involved in K^+ uptake in pollen and is required for optimal pollen tube development and pollen competitive ability (Mouline et al. 2002). The roles of the other five Shakers are less well understood. Current data support the hypothesis that AKT2/3 is involved in long-distance K^+ transport via the phloem sap (Deeken et al. 2000; Lacombe et al. 2000). This channel has also been shown to be an important contributor, along with AKT1, to the mesophyll K^+ permeability (Dennison et al. 2001). Like KAT1, KAT2 is thought to play a role in K^+ influx into guard cells during stomatal opening (Pilot et al. 2001) and GORK to mediate K^+ release from these cells during stomatal closure (Ache et al. 2001; Hosy et al. 2003). GORK is also expressed in root hairs, where it could play a role in osmoregulation (Ivashikina et al. 2001). AtKC1 is expressed in root periphery cells (Ivashikina et al. 2001; Pilot et al. 2003) where it would be an integral component of functional K^+ uptake channels (Reintanz et al. 2002). Only localization data have been obtained for the remaining *Arabidopsis* Shaker channel, AKT6 (Lacombe et al. 2000), revealing expression in flowers.

13.3.2.2 "Two-pore" potassium channels

Two-pore K^+ channels display a hydrophobic core composed of either 4 TMS and 2 P domains (KCO-2P family) or 2 TMS and 1 P domain (KCO-1P family); none of the TMS acts as a voltage sensor. Their pore domains bear a high K^+ permeability hallmark motif. The channels have putative Ca^{2+}-binding sites in their cytosolic C-terminal region (Czempinski et al. 1999; Moshelion et al. 2002) and share some structural homologies with 4TMS-2P (leak-like) and 2TMS-1P (inward-rectifying) animal K^+ channels, respectively (Doupnik et al. 1995).

In *Arabidopsis*, the KCO-2P family has five members and the KCO-1P family, a single member (Czempinski et al. 2002). Only *KCO1* has been characterized, where it was shown to encode a K^+ selective outward-rectifying channel activated by cytosolic Ca^{2+} (Czempinski et al. 1999). KCO1 can also

be functionally distinguished from outward-rectifying Shaker channels by faster and non-sigmoidal kinetics of current activation and a higher single channel conductance. KCO1 is expressed throughout the plant (Czempinski et al. 2002). At the subcellular level, it has been localized at the tonoplast (Czempinski et al. 2002).

13.3.2.3 Cyclic nucleotide-gated (CNG) channels

Twenty members of CNG family were found in *Arabidopsis* (Köhler et al. 1999; Mäser et al. 2001), with CNG channel homologs identified in barley (Schuurink et al. 1998) and tobacco (Arazi et al. 1999). They share structural homologies with the animal cyclic nucleotide-gated channels (CNGCs) first identified in sensory cells. CNGCs are related to the Shaker family, but without the high K^+ selectivity hallmark motif in their P domains. As a result, they readily conduct both mono- and divalent cations (Véry and Sentenac 2002) and do not distinguish well between Na^+ and K^+ (Gamel and Torre 2000). Similar to their animal counterparts, CNG channels are gated by intracellular cGMP or cAMP (Maathuis and Sanders 2001), Ca^{2+} and calmodulin (Mäser et al. 2001; Véry and Sentenac 2002). Their physiological role is likely to be in cell signaling (Demidchik et al. 2002; Véry and Sentenac 2002).

13.3.2.4 K^+/H^+ antiporters

A family of cation/H^+ antiporters (CPA), comprising six putative K^+/H^+ antiporters, has been identified in *Arabidopsis* (Mäser et al. 2001). The latter systems (called KEA for *K*$^+$ *e*fflux *a*ntiporter) show substantial sequence similarities (up to 35% identity) with bacterial Ker (K^+ efflux) antiporters regulated by glutathione (Yao et al. 1997). Their tissue and subcellular localizations are unknown. Due to poor ion selectivity, other members of CPA family might also exchange H^+ for K^+, in addition to other ions. For instance, the AtNHX1 tonoplast located Na^+/H^+ exchanger (Apse et al. 1999) was shown to transport Na^+ and K^+ with equal affinity in reconstituted liposomes (Venema et al. 2002). Plant K^+/H^+ exchange activity is expected, at least at the tonoplast, to be an important mechanism in K^+ loading into the vacuole. AtNHX1 might be involved in osmoregulation and Na^+ detoxification of the cytoplasm, as well as in cytosolic pH regulation (Venema et al. 2002). It has also been suggested that K^+/H^+ exchangers might be at work at the PM, contributing to active K^+ secretion in the xylem sap (Kochian and Lucas 1988).

13.3.2.5 KUP/HAK/KT transporters

This is a class of transporters which are homologous to the H^+-K^+ symporters first identified in *E. coli* (Schleyer and Bakker 1993) and *S. occidentalis* (Bañuelos et al. 1995). The plant homologues, called KUP (Fu and Luan 1998;

Kim et al. 1998), HAK (Santa-María et al. 1997; Rubio et al. 2000), or KT (Quintero and Blatt 1997), form a large family, with 13 members in *Arabidopsis* (Mäser et al. 2002) and at least 17 members in rice (Bañuelos et al. 2002). Little is known about the structure of these transporters. Hydrophobicity profiles suggest that they might possess 12 TMS and a long cytosolic loop between the second and third TMS (Kim et al. 1998; Rubio et al. 2000; Bañuelos et al. 2002).

Four groups of plant KUP/HAK/KT transporters can be distinguished on a phylogenetic tree (Rubio et al. 2000; Bañuelos et al. 2002); two of these have been characterized at the functional level (Rodríguez-Navarro 2000). While some transporters in group *I* are classified as high-affinity K^+ transporters, all members from group *II* operate in the low-affinity (mM) range. These are active transport systems, with poor discrimination between K^+, Rb^+ and Cs^+ (Rubio et al. 2000; Bañuelos et al. 2002) but reduced permeability to Na^+ and NH_4^+ (Fu and Luan 1998; Santa-María et al. 2000; Bañuelos et al. 2002). Their activity is inhibited by elevated Na^+ levels (Quintero and Blatt 1997; Kim et al. 1998) and alkaline pH (Garciadeblás et al. 2002), suggesting H^+-K^+ stoichiometry. These systems can mediate both influx and efflux of K^+ (Bañuelos et al. 2002; Garciadeblás et al. 2002).

Although both high- and low-affinity KUP/HAK/KT transporters are expressed in various plant organs/tissues, their subcellular localization is largely unknown. At least one of 17 members of OsHAK family in rice was targeted to the tonoplast (Bañuelos et al. 2002), while others were more likely to be located at the PM. Most of these transporters are expressed in roots (Santa-María et al. 1997; Kim et al. 1998; Rubio et al. 2000; Rigas et al. 2001) and are believed to mediate high-affinity K^+ uptake through the PM.

13.3.2.6 HKT transporters

Plant HKT transporters are related to the fungal Trk transporters and prokaryote KtrB and TrkH K^+ transporter subunits (Rodríguez-Navarro 2000). Sequence analysis suggests that these transporters evolved from bacterial 2TMS K^+ channels. They display a core structure with eight TMS and four P-forming domains, four repeats of 1TMS-1P-1TMS, with the four P loops lining a central P, and C-terminal cytosolic region (Durell et al. 1999; Kato et al. 2001).

Although HKT homologues have been isolated or detected in many species, including *Arabidopsis*, eucalyptus, rice, ice plant (*Mesembryanthemum crystallinum*) and poplar (Fairbairn et al. 2000; Uozumi et al. 2000; Horie et al. 2001), they do not constitute multigene families. There is only one member of this group in *Arabidopsis* (AtHKT1; Uozumi et al. 2000) and in diploid wheat (HKT1; Schachtman and Schroeder 1994). Eucalyptus and rice each have two HKT paralogs (Fairbairn et al. 2000; Horie et al. 2001). The only exception is japonica rice in which the genome shows the presence of up to nine OsHKT genes (Garciadeblás et al. 2003). All HKT transporters identified so far are expressed predominantly in roots.

Available information suggests that HKT homologues may operate in two transport modes. One has only a limited ability to transport K^+, while in the other mode, HKT transporters transport K^+ as readily as Na^+. For example, K^+ uptake was not observed when the *Arabidopsis* homolog AtHKT1 was expressed in either yeast or *Xenopus* oocytes, although high Na^+ uptake activity was detected (Uozumi et al. 2000). Wheat, TaHKT1 operates as a high affinity Na^+-K^+ symporter in the presence of low K^+ and Na^+ concentrations, and as a low-affinity Na^+-Na^+ (co)-transporter when the Na^+/K^+ ratio in the external solution is high (Rubio et al. 1995; Gassmann et al. 1996). In eucalyptus, two HKT1 homologs (EcHKT1 and EcHKT2) both show K^+ and Na^+ currents when expressed in *Xenopus* oocytes (Fairbairn et al. 2000). Experiments on rice suggested that the OsHKT1 isoform operated as a Na^+ transporter, while OsHKT2 displayed K^+-Na^+ symport activity (Horie et al. 2001).

Wheat HKT1 is expressed in the root cortex (Schachtman and Schroeder 1994) where it functions as a low-affinity Na^+ transport under low K^+:Na^+ ratios. K^+ starvation induces HKT1 expression in wheat and barley (Wang et al. 1998) as well as inward Na^+ currents in wheat root cortical cells (Buschmann et al. 2000). HKT1 plays a role in net Na^+ accumulation (Uozumi et al. 2000; Laurie et al. 2002; Mäser et al. 2002) and its decreased expression under salt stress often correlates with plant salt tolerance (Golldack et al. 1997). Direct evidence for the involvement of AtHKT1 in Na^+ uptake and salt sensitivity in *Arabidopsis* has emerged from a screen for suppressor mutations of the *Arabidopsis sos3* mutant (Liu and Zhu 1998). Disruption of AtHKT1 suppressed the *sodium-overly-sensitive* (*sos*) phenotype (Rus et al. 2001) and *sos3/athkt1* double mutant seedlings took up less Na^+ than either *sos3* or wild type (WT) plants (Rus et al. 2001). Not surprisingly, HKT1 has been proposed to be a determinant of salt sensitivity in plants (Rubio et al. 1995).

13.3.2.7 LCT1

LCT1 is low-affinity transporter found only in wheat. It is capable of mediating uptake of a wide range of monovalent cations, including K^+ and Na^+ (Schachtman et al. 1997; Amtmann et al. 2001) and is expressed in both roots and leaves (Schachtman et al. 1997). Expression of LCT1 in yeast caused Na^+ hypersensitivity (Amtmann et al. 2001), and LCT1 mediated Na^+ transport was inhibited by Ca^{2+} (Schachtman et al. 1997; Amtmann et al. 2001). LCT1 has no counterpart in *Arabidopsis* and shares no sequence homology with any other gene. Nonselective cation conductances have been described in vivo in wheat roots. The hypothesis of a role for LCT1 in this activity would, however, be highly speculative because poorly selective cation conductances have also been described in many species, including *Arabidopsis* (Demidchik et al. 2002).

13.3.2.8 Glutamate receptors

A family of polypeptides related to animal ionotropic glutamate receptors has been found in plants (Lam et al. 1998), with 20 members in *Arabidopsis* (Lacombe et al. 2001). Although the P domains of plant and animal receptors are quite distant, plant glutamate receptors might, like their animal counterparts, form cation channels permeable to K^+, Na^+ and/or Ca^{2+} (Lam et al. 1998; Nakanishi et al. 1990). Plant glutamate receptors are usually expressed in roots (Chiu et al. 2002). Their physiological role in plants remains unknown.

13.3.2.9 Other transport systems

Non-selective cation channels. Non-selective cation channels (NSCC) are a large, heterogeneous group of channels. In addition to CNG channels and glutamate receptors, the group also includes a large number of other channels which show high selectivity for cations over anions, but low selectivity among monovalent cations under a wide range of ionic conditions (Demidchik et al. 2002) and usually have similar permeability to a wide range of monovalent cations. They show K^+:Na^+ selectivity ratios typically between 0.3 and 3, and make a key contribution to the uptake of Na^+ by plant cells (Tyerman et al. 1997; Demidchik et al. 2002; Tester and Davenport 2003). NSCC channels have been found at the PM, tonoplast and other endomembranes. Numerous methods of activation of these channels have been reported, and they are thought to function in low-affinity nutrient uptake (see Demidchik et al. 2002 for review). They are gated by diverse mechanisms including voltage, cyclic nucleotides, glutamate, reactive oxygen species and stretch.

CCC family. A few putative members of the cation chloride cotransporter family (CCC) have been found in plants (Véry and Sentenac 2003). In animal cells, the CCC family comprises K^+-Cl^-, Na^+-Cl^- and Na^+-K^+-$2Cl^-$ cotransporters (Gamba et al. 1993; Gillen et al. 1996; Isenring and Forbush 1997). Members of this family have important roles in cellular ionic and osmotic homeostasis in animal cells.

13.4 Specificity of salinity effect on K^+ homeostasis in plant tissues

13.4.1 K^+/Na^+ competition for uptake—channels and symporters

As Na^+ is not an essential element, it is no surprise that as yet, no specific Na^+-selective channels have been identified in higher plants. Due to their similar physicochemical structures, excessive Na^+ in the soil solution competes with binding sites in transport systems that mediate K^+ uptake resulting in K^+

deficiency (Niu et al. 1995; Hasegawa et al. 2000). The ionic mechanisms of pathways of Na^+ uptake into plant cells have been a subject of extensive electrophysiological studies (Maathuis and Sanders 1993, 1995; Gassmann and Schroeder 1994; Amtmann et al. 1997, 1999; Tyerman et al. 1997; Buschmann et al. 2000; Davenport and Tester 2000; Demidchik and Tester 2002). However, the task of attributing known ion currents to corresponding transporter genes appears to be particularly difficult for Na^+, since it is likely that several transporters contribute to Na^+.

Candidate genes for root Na^+ uptake are found in several K^+ transporter families. The high-affinity K^+ transporter (HKT1), low affinity cation transporter (LCT1) and nonselective cation channels (NSCCs) are the most likely specific transport systems that mediate Na^+ influx (Schachtman and Schroeder 1994; Schachtman et al. 1997; Amtmann and Sanders 1999; Davenport and Tester 2000; Amtmann et al. 2001). Genome wide analyses indicate that additional classes of Na^+ transporters are likely to exist and characterization of further complexities and interesting functions of Na^+ are on the horizon.

13.4.1.1 Inward-rectifying channels from the Shaker family

Under saline conditions, Na^+ would passively diffuse into the cell cytoplasm through Na^+-permeable PM channels. However, most inward-rectifying K^+ (KIR) channels from the Shaker family appear to be highly selective for K^+ over Na^+ (Amtmann and Sanders 1999) thus are unlikely to mediate significant net Na^+ influx into plant cells.

Despite this, there are reports in the literature of an adverse affect of Na^+ on the functioning of KIRs (e.g. AKT1). Sodium may have a direct effect on the AKT channel protein, an interaction that reduces the open probability or conductance of the channel (Qi and Spalding 2004). Alternatively, Na^+ impairs the activity of a positive regulator of AKT1 or interferes with the delivery of AKT1 channels to the membrane. Thus, KIRs functioning may be impaired by excessive Na^+, but it is not likely that these channels play any substantial role in Na^+ transport into the cell.

13.4.1.2 KUP/HAK/KT transporters

The KUP/HAK/KT family of K^+ transporters might mediate some low-affinity Na^+ influx at high Na^+ concentrations, although the full extent is not known. When expressed in yeast, HvHAK1 from barley mediated low-affinity Na^+ transport in addition to high-affinity K^+ uptake (Santa-María et al. 1997). Elevated Na^+ also inhibits K^+ transport through heterologously expressed AtKUP1 (Fu and Luan 1998) and AtHAK5 (Rubio et al. 2000). At the same time, the upregulation of *Mesembryanthemum crystallinum* McHAK1 and McHAK2 under both K^+ starvation and NaCl stress in roots and leaves has been reported (Su et al. 2002).

13.4.1.3 High affinity transporters—HKT1

Two different transport modes have been reported for HKT1: (i) a saturable high-affinity K^+-Na^+ symport and (ii) a low-affinity Na^+ transport, the latter operating at high external Na^+ concentrations when the transport of K^+ is blocked (Rubio et al. 1995; Gassmann et al. 1996).

The in planta function of AtHKT1 as an effector of Na^+ influx has been confirmed (Rus et al. 2001). T-DNA insertional and deletion mutants of AtHKT1 were identified in a screen for suppressors of NaCl sensitivity of the *sos3-1* mutant (Liu and Zhu 1997; Rus et al. 2001). Suppression of *sos3-1* NaCl sensitivity is related to reduced cellular accumulation of Na^+ and increased capacity to maintain internal K^+. Together, these results establish that AtHKT1 controls Na^+ influx into plants. It is likely that AtHKT1 is a Na^+ influx system but its function as a regulator of Na^+ and K^+ influx systems cannot be precluded. As the transcript is expressed predominantly in root cortical cells in wheat (Schachtman and Schroeder 1994), HKT1 most probably functions in the control of Na^+ loading into the xylem for export to the shoot (Uozumi et al. 2000; Rus et al. 2001). Through a combination of functional chimeric HKT analysis and sequence analyses, an amino acid was identified in HKT transporters that play an important role in determining the transport mode of HKT transporters (Mäser et al. 2002). This amino acid lies within the predicted "pore-loop" domain. The presence of a Gly residue resulted in K^+-Na^+ transport, whereas a Ser residue in this position caused more Na^+ selective transport (described above). Evidence is mounting that HKT1 systems are conserved in plant species and that these function in Na^+ transport (Rus et al. 2001; Golldack et al. 2002; Laurie et al. 2002; Garciadeblás et al. 2003).

Several reports have analyzed the physiological roles of HKT transporters in vivo. Laurie et al. (2002) found that transgenic wheat plants expressing an HKT1 antisense construct showed Na^+ tolerance under saline conditions with reduced Na^+ uptake activity and accumulation. However, Mäser et al. (2002) and Berthomieu et al. (2003) showed that loss-of-function mutations in the AtHKT1 gene lead to overaccumulation of Na^+ in shoots and rendered leaves Na^+ hypersensitive. Transgenic plants harboring an AtHKT1 promoter-GUS construct showed HKT1 expression in vascular tissues (Mäser et al. 2002; Berthomieu et al. 2003). Thus, a model was proposed in which AtHKT1 would facilitate recirculation of the Na^+ from the shoot to the root, thereby restricting its accumulation in the aerial part of the plant (Mäser et al. 2002; Berthomieu et al. 2003). These authors postulated that, in the shoot, HKT1 loads Na^+ into the phloem, which is then translocated to the root and removal of Na^+ from the root phloem occurs by efflux down the electrochemical gradient (Berthomieu et al. 2003). This model is support by Laurie et al. (2002), who showed that reduction of TaHKT1 expression in wheat resulted in a marked decrease in the root stele Na^+ content while poorly affecting the root epidermal and cortical contents.

13.4.1.4 LCT1

LCT1 is proposed to play a role in Na^+ uptake in wheat. When expressed in yeast, it functions as a non-selective cation permeable transporter, mediating both Na^+ and K^+ transport (Schachtman et al. 1997), and rendered yeast more salt sensitive (Amtmann et al. 2001). However, further analyses will be required to determine where LCT1 is targeted, as well as to quantify its contribution to the regulation of K^+ homeostasis in salinized plant tissues.

13.4.1.5 Non-selective cation channels

Physiological data implicate the involvement of non-selective cation channels (NSCCs) in Na^+ influx and these are considered to be the major route for Na^+ entry into plant cells (Tyerman et al. 1997; Amtmann and Sanders 1999; Tyerman and Skerrett 1999; Davenport and Tester 2000; Tyerman 2002). These channels have a similar permeability for K^+ and Na^+ (Amtmann and Sanders 1999). Na^+ influx currents through NSCC have been characterized electrophysiologically in root cortical cells of wheat (Tyerman et al. 1997; Davenport and Tester 2000), maize (Roberts and Tester 1997) and *Arabidopsis* (Demidchik and Tester 2002), as well as in barley suspension cells (Amtmann et al. 1997). The current amplitude of these channels was dependent on both the external Na^+ concentration and the external Ca^{2+} concentration. At a low Ca^{2+} concentration (40–100 μM), large increases were observed in the amplitude of the Na^+-dependent inward currents. At higher Ca^{2+} concentrations, Na^+ currents through NSCC were inhibited (Tyerman et al. 1997; Buschmann et al. 2000; Davenport and Tester 2000; Demidchik and Tester 2002), correlating with the reduction of Na^+ uptake by increased external Ca^{2+} concentration (LaHaye and Epstein 1969).

Calcium inhibition of NSCC conductance is not complete, so it is possible that these ion channels allow a substantial leak for Na^+ influx, particularly under high saline conditions (Davenport and Tester 2000; Demidchik and Tester 2002). This suggests that Ca^{2+}-insensitive Na^+ uptake pathways are probably also present and involved in Na^+ uptake. However, their full contribution to Na^+ uptake remains unknown.

13.4.2 Sodium/cation antiporters

Low cytosolic Na^+ concentration is attained by the operation of Na^+/H^+ antiporters located at both the PM (Shi et al. 2000) and the tonoplast (Apse et al. 1999). Electrochemical K^+ gradients generated by H^+-pumps at the PM (H^+-ATPase) and the tonoplast (H^+-ATPase, H^+-PPase) provide the energy used by the PM and tonoplast bound Na^+/H^+ antiporters to couple the passive movement of H^+ to the active movement of Na^+ out of the cell and into the

vacuole. The recent characterization of these systems has added considerably to our awareness of cytosolic Na^+ control. The identification and characterization of the yeast HAL1 gene which facilitates K^+/Na^+ selectivity and salt tolerance in yeast cells gives another dimension to our understanding of this issue. Consequently, our insight into Na^+ transport at both these membranes, and the control over K^+ and Na^+ homeostasis, has increased considerably, knowledge which is finally giving us the possibility of generating salt tolerant crops.

13.4.2.1 The SOS-signal transduction pathway

The SOS (for *S*alt-*O*verly-*S*ensitive) signal-transduction pathway is important in controlling ion homeostasis and salt tolerance in plants (Hasegawa et al. 2000; Sanders 2000; Zhu 2000, 2003). The current model for the SOS signal-transduction pathway is that high Na^+ induces a Ca^{2+} signal (Knight et al. 1997). A myristoylated Ca^{2+}-binding protein senses the salt-elicited Ca^{2+} signal and translates it to downstream responses (Liu and Zhu 1998; Ishitani et al. 2000). SOS3 interacts with, and activates SOS2, a serine/threonine protein kinase (Halfter et al. 2000; Liu et al. 2000). This SOS2/SOS3 complex regulates the expression level of SOS1, a salt effector gene encoding a PM Na^+/H^+ antiporter. The SOS1 Na^+/H^+ exchanger serves to extrude excess Na^+ from the cytosol and out of the cell, thereby maintaining a low cytosolic Na^+ concentration (Shi et al. 2000).

Activity of the SOS1 promoter has been found ubiquitously in virtually all tissues, but its greatest activity is found in root epidermal cells, particularly at the root tip and in cells bordering the vascular tissue (Shi et al. 2002). This suggests three major roles: (i) mediating Na^+ efflux from cytosol to the root medium, (ii) buying time for Na^+ storage in the vacuole by slowing down Na^+ accumulation in the cytoplasm, and (iii) controlling long-distance Na^+ transport between roots and shoots by loading Na^+ into and unloading Na^+ from the xylem and phloem (Zhu 2003). The role of SOS1 in long distance transport is important for coordination between transpirational Na^+ flow and the vacuolar sequestration of Na^+ in leaves. A higher concentration of Na^+ accumulates in shoots of *sos1* mutants than in WT, and transgenic plants overexpressing SOS1 showed improved salt tolerance and accumulated less Na^+ in the xylem transpiration stream as well as in the shoot (Shi et al. 2003).

Extrusion of excess Na^+ from the cell is a straightforward way to avoid Na^+ accumulation in the cytosol, so is widely employed by root epidermal cells, where SOS1 is preferentially expressed (Shi et al. 2002). This strategy would be problematic for most other types of cells, where extruded Na^+ will immediately become a problem for neighboring cells. This is especially important in leaves where, due to the small apoplasmic volume (~3%; Flowers and Yeo 1986), Na^+ extrusion via SOS1 would cause a rapid increase of apoplasmic Na^+ leading to cell dehydration, turgor loss and even death of leaf cells and tissues (Marschner 1995).

The problem may be overcome if SOS1 acts in concert with AtHKT1, which has been suggested to mediate Na^+ loading into the phloem in leaves and unloading in roots (Nublat et al. 2001; Berthomieu et al. 2003). These two mechanisms could remove Na^+ from the apoplast and symplast as long as their combined efficiency is greater than the rate of Na^+ delivery. According to this scenario, SOS1 Na^+/H^+ exchanger will remove Na^+ from the cell and AtHKT1 will load it into phloem for removal from the shoot. Earlier Rus et al. (2001) explained why the mutations in the AtHKT1 gene suppress the *sos3* mutant phenotype by this functional interaction between SOS1 and AtHKT1. The above model of functional interaction between AtHKT1 and AtNHX1 can also explain the otherwise puzzling result that transgenic plants overexpressing AtNHX1 accumulate about 30% more Na^+ in leaves than control plants (Apse et al. 1999). However, there are no reports of the functional expression or physiological characterization of the SOS-signaling pathway in leaves, and few on the functioning of the AtHKT1 gene and associated transporters. Therefore, the occurrence of this model in planta remains to be confirmed.

The SOS3-SOS2 kinase complex may also regulate Na^+ compartmentation by (i) activating NHX1 at the tonoplast, (ii) restricting Na^+ entry into the cytosol (by inhibiting the PM Na^+ transporter HKT1 activity; Zhu 2002), (iii) negatively controlling the expression of AtNHX family members (Yokoi et al. 2002), and (iv) controlling K^+ acquisition by the root (Wu et al. 1996). The latter is confirmed by the fact that under NaCl conditions, *sos1* mutant plants accumulate more Na^+ and less K^+ (Wu et al. 1996; Ding and Zhu 1997; Rus et al. 2001) and overexpression of SOS1 has been shown to result in increased Na^+ export from the cell and improved salt tolerance in transgenic *Arabidopsis* (Shi et al. 2002). Also, all *sos* mutants had a growth defect under K^+-limiting conditions (Zhu et al. 1998). Which specific K^+ transport system is targeted by SOS signaling pathway, remains a mystery. Patch-clamp experiments suggest that the extrusion of Na^+ from the cytoplasm by SOS1 protects the K^+ permeability of the membrane, and the AKT1 K^+ channel in particular, from inhibition by Na^+ (Qi and Spalding 2004). However, experiments in our laboratory showed no difference in NaCl-induced K^+ fluxes from roots of WT and *akt1 Arabidopsis* mutant (S. Shabala and L. Shabala, unpublished) questioning the AKT1 involvement. Also, NaCl-induced K^+ efflux from barley mesophyll was strongly inhibited by K^+ channel blocker TEA^+ (Shabala et al. 2005). The possible involvement of other K^+ transporters remains to be evaluated.

13.4.2.2 Tonoplast Na^+/H^+ antiporters

Compartmentation of Na^+ into the vacuole is important in the maintenance of lower cytosolic Na^+ concentrations, while maintaining a lower cellular osmotic potential. Significant progress has been made in deducing the genes and transporters responsible for Na^+ sequestration. Active transport across the tonoplast utilizes the electrochemical gradient generated by V-type H^+-ATPase and H^+-PPase. Vacuolar Na^+/H^+ antiporter activity was first measured in tonoplast

enriched membranes isolated from red beet storage tissue (Blumwald and Poole 1985), and has subsequently been measured in many plants (Apse et al. 1999; Blumwald et al. 2000 and references within). Salinity also upregulates the expression of a V-type H-ATPase (Golldack and Dietz 2001), and overexpression of the native vacuolar H^+-PPase gene (AVP1) increases salt tolerance in *Arabidopsis* (Gaxiola et al. 2001).

Cloning of AtNHX1 (Apse et al. 1999; Gaxiola et al. 1999) was followed by its functional complementation in yeast *nhx1* mutants (Gaxiola et al. 1999), measurement of its Na^+/H^+ exchange activity in vacuoles isolated from AtNHX1 overexpressing *Arabidopsis* (Apse et al. 1999) and in vacuolar vesicle membranes isolated from yeast expressing AtNHX1 (Darley et al. 2000). These studies clearly confirm the function of AtNHX1 as an Na^+/H^+ antiporter. Increased expression of AtNHX1 by transformation with AtNHX driven by a strong constitutive promoter, improves salinity tolerance in *Arabidopsis* (Apse et al. 1999), *Brassica napus* (Zhang et al. 2001) and tomato (Zhang and Blumwald 2001). These results show that an increased capacity for vacuolar Na^+ sequestration is important for salinity tolerance. Importantly, transgenic tomato and *Brassica* plants accumulated a high concentration of Na^+ in leaves, but not in fruit or seed, thus were highly tolerant of salt stress while at the same time maintaining the quality of fruit and oil (Zhang and Blumwald 2001; Zhang et al. 2001). This is the first real progress in the development of genetically modified salt tolerant crop species.

This increased capacity for Na^+ uptake by vacuoles by a NHX1 Na^+/H^+ antiport is found in many salt tolerant species, e.g. *Beta maritima*, *Atriplex gmelini* and *Mesembryanthemum crystallinum*. All show strong induction of both Na^+/H^+ antiporter expression and activity in response to NaCl treatment (Barkla et al. 1995; Hamada et al. 2001; Xia et al. 2002).

The AtNHX1 gene is also able to mediate K^+ transport in addition to Na^+/H^+ exchange (Zhang and Blumwald 2001; Venema et al. 2002; Apse et al. 2003). Similar K^+ transporter activity has been reported in rice, where overexpression of the tonoplast located OsNHX1 increased salt tolerance (Fukuda et al. 2004). A tomato tonoplast LeNHX2 antiporter maintained higher K^+ concentration in intracellular compartments under salt stress conditions (Venema et al. 2003) The AtCHX17 in *Arabidopsis* was reported to have a greater role in K^+ acquisition and homeostasis rather than in Na^+ transport (Cellier et al. 2004). This suggests that members of the NHX family may have different substrate specificities and play different roles in salt tolerance (Yokoi et al. 2002). Overall, the importance of NHX Na^+/H^+ antiporters at the tonoplast in the maintenance of K^+ and Na^+ homeostasis in plant cells is beyond doubt.

13.4.2.3 HAL genes

The yeast *HAL1* and *HAL3* genes are proposed to improve salt tolerance by increasing the cellular K^+/Na^+ ratio (Gaxiola et al. 1992; Serrano 1996; Rios et al. 1997). Transcription of HAL1 favors Na^+ extrusion and restricts K^+ efflux

through an unknown pathway (Bordas et al. 1997), effectively increasing the intracellular K^+/Na^+ ratio (Gaxiola et al. 1992).

In yeast, *HAL1* expression is induced by salt (Gaxiola et al. 1992) and its overexpression confers increased salt tolerance in transgenic *Saccharomyces cerevisiae* (Rios et al. 1997). Increased salt tolerance has also been reported in transgenic plants expressing the *HAL1* gene, including tomato (Gisbert et al. 2000; Rus et al. 2001) and melon (Bordas et al. 1997). Genes homologous to the yeast HAL genes could be present in higher plants and may be relevant to salt tolerance (Bordas et al. 1997). For example, Espinosa-Ruiz et al. (1999) isolated two *Arabidopsis* genes AtHAL3a and AtHAL3b which show homology with HAL3. Gain of AtHAL3a function *Arabidopsis* show increased growth rates and improved salt tolerance. Alterations in intracellular cation concentrations associated with changes in HAL3 expression indicated that HAL3 directly increased cytoplasmic K^+ concentration and decreased Na^+ concentrations (Espinosa-Ruiz et al. 1999).

13.4.3 Mitigating effect of calcium

An important determinant for plant salt tolerance that is particularly relevant to Na^+ and K^+ homeostasis is the sensitivity of many transport processes to Ca^{2+}. Increased Ca^{2+} supply has a protective effect on plants under salt stress (reviewed by Rengel 1992). Physiological effects of supplemental Ca^{2+} include (i) diminished membrane leakiness, (ii) improved PM structural integrity, (iii) improved K^+ status of the cell and (iv) reduced Na^+ accumulation in plants (Cramer et al. 1985, 1987; Rengel 1992; Bressan et al. 1998; Munns 2002).

At the transporter level, the traditional view is that elevated Ca^{2+} restricts Na^+ uptake via NSCC (Tyerman et al. 1997; Demidchik and Tester 2002; Tester and Davenport 2003). Other divalent cations may also control NSCC permeability for Na^+ (Elphick et al. 2001; Demidchik and Tester 2002). However, work in our laboratory suggested that NSCC blockage by elevated Ca^{2+} is not the only mechanism involved. MIFE experiments on root (Shabala et al. 2003) and leaf (Shabala 2000; Shabala et al. 2005) tissue of various species showed that both supplemental Ca^{2+} and other divalent cations (Mg^{2+}, Ba^{2+}, Zn^{2+}) reduce or prevent NaCl-induced K^+ efflux from the cell. Thus, our results suggest that, in addition to their known ability to block NSCC, divalent cations also control the activity or gating properties of PM K^+ transporters, assisting in maintaining an optimal K^+/Na^+ ratio. Results of pharmacological studies and patch-clamp experiments suggest that depolarization-activated outward-rectifying K^+ channels are involved (S. Shabala, V. Demidchik and J. Davies, unpublished data).

13.4.4 Ion compartmentation between roots and shoots

The regulation of Na^+ transport to the shoot is another feature that governs plant responses to salinity. The differences in the growth responses of salt

tolerant and salt sensitive species are often related to differences in the translocation of Na^+ to leaves (Marschner 1995). In salt tolerant species, increased salt supply leads to a large accumulation of Na^+ in the shoots where it is utilized in the vacuoles of leaf cells for osmotic adjustment (Flowers and Läuchli 1983), often replacing most of K^+ in the vacuole (Hawker et al. 1974). In more salt sensitive species, substitution of K^+ by Na^+ is much more limited. The higher salt tolerance of many species is often attributed to a more effective restriction on shoot directed transport of Na^+ (Tester and Davenport 2003). There is also some evidence of extensive recirculation of shoot Na^+ to the roots (Mäser et al. 2002; Berthomieu et al. 2003), although it has been suggested that Na^+ transport is largely unidirectional and results in progressive accumulation of Na^+ as the leaves age (Tester and Davenport 2003). As such, retranslocation of Na^+ from shoots to roots was found to contribute to low Na^+ contents in the shoots of beans (Matsushita and Matoh 1991) and clover (Winter 1982), but not barley (Munns et al. 1987).

The importance of Na^+ and K^+ compartmentation at the whole-plant level was highlighted in a recent study on two closely related species, contrasting in their salt tolerance. *Thellungiella halophila* (salt cress) is closely related to *Arabidopsis* (90–95% identity at the cDNA level; Bressan et al. 2001). Volkov et al. (2003) showed that under saline conditions *T. halophila* had a much better ability to retain or even increase shoot elemental K^+ content compared with *Arabidopsis*. The observed differences in K^+ accumulation were larger in roots than in shoots. At the same time, the differences in Na^+ accumulation (higher in *Arabidopsis*) were more pronounced in shoots than in roots. This suggests that control of Na^+ loading into xylem is particularly strong in *T. halophila* (Volkov et al. 2003).

The control of shoot Na^+ will undoubtedly affect the K^+/Na^+ ratio in leaf cells. For example, the *sas1* mutant of *Arabidopsis* shows a deficiency in the control of radial transport of Na^+ (Nublat et al. 2001). This led to a 5.5-fold higher concentration of Na^+ in the xylem and a severe overaccumulation of Na^+ in the shoot, corresponding with increased sensitivity to NaCl. Although Na^+ was accumulated preferentially over K^+ in a similar manner for *sas1* and WT, the greater amounts of Na^+ in the *sas1* mutants resulted in a much higher Na^+/K^+ ratio than in the WT. Overaccumulation of Na^+ was only in shoots, not in roots, which suggested that *sas1* mutation impaired Na^+ long-distance transport from roots to shoots. This emphasizes the importance of xylem loading in salinity tolerance. In wheat, a K^+/Na^+ discrimination factor limiting Na^+ translocation to the shoot for the benefit of K^+ loading operates at the xylem uptake step (Gorham et al. 1990). In soybean, Na^+ is removed from the xylem vessels and exchanged for K^+ at the xylem parenchyma (Läuchli 1976; Lacan and Durand 1996).

Another component regulating Na^+ xylem content is SOS1. In *sos1* mutants, the Na^+ concentration in the xylem sap is higher than in the WT, suggesting that SOS1 controls Na^+ loading into, and/or retrieval from the xylem (Zhu 2003). SOS1 is expressed around the vacuolar tissue, consistent with it function in xylem Na^+ concentration (Shi et al. 2002).

13.4.5 Compartmentation at the tissue level

Salt stress is known to have different effects on the K^+/Na^+ ratio in various plant tissues in both roots and leaves (Fricke 2004). In leaves, epidermal cells accumulate a greater amount of Na^+ than the mesophyll cells and the latter show a greater ability to maintain a high K^+ levels (Fricke et al. 1996; Cuin et al. 2003). Volkov et al. (2003) found different trends in the distribution between the epidermis and the bulk tissue of *T. halophila* and *Arabidopsis*. Salt stress decreased epidermal K^+ concentrations dramatically, but bulk K^+ increased in *T. halophila* while decreasing in both epidermal and bulk K^+ after salt treatment in *Arabidopsis* (Volkov et al. 2003). This is consistent with the important role of the tissue-specific compartmentation of Na^+ and K^+ for plant salt tolerance and suggests that some mechanisms are in place within the mesophyll, but not the epidermis, to ameliorate ionic changes and protect and maintain the photosynthetic activity of the mesophyll cells. This is consistent with recent studies in our laboratory showing that improving K^+/Na^+ ratios by externally applied divalent cations enables normal leaf photochemistry in plant grown even under high (100 mM) salinity conditions (Shabala et al. 2005).

The ionic mechanisms underlying the above difference in Na^+ and K^+ compartmentation between epidermis and mesophyll remain to be revealed. One explanation could be that differences in gene expression account for the differences in ion compartmentation under saline conditions. For example, in fully expanded leaves, under unsalinized conditions, the K^+ channel genes AtKC1 and AKT1 are expressed in hydrathodes and stipules (Lagarde et al. 1996; Pilot et al. 2003). However, upon the imposition of salt stress, the expression pattern of AtKC1 broadens out to the leaf epidermis (Pilot et al. 2003) where both AKT1 and AKT2 are also expressed (Dennison et al. 2001), indicating reprogramming of K^+ channel gene expression in leaves (Pilot et al. 2003). The strong increase in expression in leaves upon salt stress could underlie changes in the compartmentalization of Na^+ and K^+ ions between the different tissues. As described above, in barley, the leaf epidermis may act as a "storage compartment" for Na^+, thus protecting the mesophyll cells, at least for a period, from Na^+ toxicity, allowing it to maintain higher concentrations of K^+ (Dietz et al. 1992; Fricke et al. 1996). It might be speculated that the leaf epidermis in *Arabidopsis* could play a similar role, requiring high AtKC1 expression levels for as yet unidentified reasons.

However, Karley et al. (2000) reported similar types of ion-selective channels and membrane transporters catalyzing the transport of K^+ and Na^+ in epidermal and mesophyll cells from barley. They suggest that the presence or absence of ion transporters cannot explain cell type specific differences in K^+/Na^+ ratios. More likely, altered permeability or gating properties of these transporters may be the key to understanding salt tolerance. The difference in salt tolerance between *T. halophila* and *Arabidopsis* was attributed to much higher selectivity for K^+ over Na^+ of both inward- and outward-rectifying K^+ channels between these species (Volkov et al. 2003). This highlights the

importance of a plant's ability to retain K^+ as a key feature of salt tolerance and is consistent with our recent findings that the magnitude of NaCl-induced K^+ efflux from plant roots correlates with salt tolerance in barley (Chen et al. 2005).

13.5 Conclusions and future perspectives

Knowledge regarding K^+ transport and the effects of salinity on K^+ homeostasis has increased considerably in recent years. New awareness concerning Na^+ compartmentalization into the vacuole opens up exciting prospects for developing salt tolerant crops. The sequencing of the *Arabidopsis* genome has led to the identification of a plethora of K^+ transporters, some of which have already been characterized electrophysiologically. Microarray experiments are starting to indicate the genes induced by salt stress. The more recent sequencing of the rice genome, which is likely to be followed by other crops, will add to our knowledge of K^+ transporters and the effects of salinity on these, as these putative genes are characterized.

However, there is still a very long way to go to gain a full extent of knowledge about the mechanisms of salt tolerance in plants. Only a small numbers of genes responsible for K^+ or Na^+ transport are characterized physiologically. Moreover, the majority of these results are through experiments in heterologous systems. Thus, in planta studies on ionic mechanisms regulating K^+ homeostasis under saline conditions are needed. Also, we have just scratched the surface of the signaling mechanisms that mediate the salt stress regulation of the expression and activities of ion transporters. Other questions such as the involvement of compatible solutes in ion homeostasis and the interaction of salt stress with other abiotic stress such as drought, high temperatures, light intensity, pollution etc also remain a grey area.

Much research on salinity tolerance has recently focused on *Arabidopsis*. Being methodologically convenient, this species is rather "non-typical" from the point of view of plant physiologists. As a result, direct extrapolation of findings from *Arabidopsis* to other species is not always possible. Now that we appear to have most of the "basics" concerning *Arabidopsis* salt tolerance mechanisms, it is time that the salt tolerance mechanisms other species are tackled at the same level of scrutiny.

Another area that is severely lacking information and is of particular concern is the regulation of K^+ and Na^+ at the leaf level, most studies on K^+ transport under saline conditions have been attributed to root tissues. Being central to plant photosynthesis, leaf mesophyll cells are almost crying to be studied in this context!

Finally, with all the excitement of a magic of molecular techniques, we should not forget that plants are more than a combination of genes. Therefore, the whole-plant perspective should be also kept in mind when

doing in-depth studies on expression and control modes of some specific transporters mediating K^+ homeostasis in plants under saline conditions.

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