Tansley review

Sodium transport in plants: a critical review

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Summary

Sodium (Na) toxicity is one of the most formidable challenges for crop production world-wide. Nevertheless, despite decades of intensive research, the pathways of Na^+ entry into the roots of plants under high salinity are still not definitively known. Here, we review critically the current paradigms in this field. In particular, we explore the evidence supporting the role of nonselective cation channels, potassium transporters, and transporters from the HKT family in primary sodium influx into plant roots, and their possible roles elsewhere. We furthermore discuss the evidence for the roles of transporters from the NHX and SOS families in intracellular Na^+ partitioning and removal from the cytosol of root cells. We also review the literature on the physiology of Na^+ fluxes and cytosolic Na^+ concentrations in roots and invite critical interpretation of seminal published data in these areas. The main focus of the review is Na^+ transport in glycophytes, but reference is made to literature on halophytes where it is essential to the analysis.

Abbreviations: AAG, amino-acid-gated; AKT, *Arabidopsis* K^+ transporter; AVP, *Arabidopsis* vacuolar pyrophosphatase; CCC, cation-chloride cotransporters; CNGC, cyclic-nucleotide-gated channel; DEPC, diethyl pyrocarbonate; DA, depolarization-activated; *esb*, enhanced suberin; GLR, glutamate receptor; HA, hyperpolarization-activated; HAK, high-affinity K^+ transporter; HKT, high-affinity K^+ transporter; KcsA, *Streptomyces* K^+ channel; K_i, inhibition constant; K_m, Michaelis constant; Kna, K^+/Na^+ discrimination locus; KT, K^+ transporter; KUP, K^+ uptake permease; LCT, low-affinity cation transporter; Nax, Na^+ exclusion; NHX, Na^+/H^+ exchanger; NSCC, nonselective cation channel; PTS, 8-hydroxy-1,3,6-pyrenetrisulphonic acid; QTL, quantitative trait loci; ROS, reactive oxygen species; SBFI, sodium-binding benzofuran isophthalate; SKC, shoot K^+ concentration; SOS, salt overly sensitive; TEA, tetraethyl ammonium; Trk, K^+ transporter; VI, voltage-insensitive.

I. Introduction

Soil salinity is a global environmental challenge, affecting crop production on over 800 million hectares, or a quarter to a third of all agricultural land on earth (Szabolcs, 1989; Rengasamy, 2010). The problem is particularly severe in irrigated areas (Flowers, 1999; Zhu, 2001), where as much as one-third of global food production takes place (Munns, 2002; Munns & Tester, 2008; Zhang *et al.*, 2010) and where infiltration of highly saline sea water (Flowers, 2004) is common. However, salinity is also increasing in dryland agriculture in many parts of the world (Wang *et al.*, 1993; Rengasamy, 2006). While saline soils contain numerous salts at elevated concentrations, NaCl typically dominates (Zhang *et al.*, 2010), and it is believed that the harmful effects of saline conditions on most species are principally brought about by a combination of osmotic stress and ionic stress exerted by the sodium component of NaCl (Blumwald, 2000; Hasegawa *et al.*, 2000; Munns & Tester, 2008). Only in the cases of some woody species, such as in the genera *Citrus* and *Vitis* (grapevine), does chloride appear to be the more toxic ion (White & Broadley, 2001). It is for this reason that decades of research activity have been dedicated to the characterization of Na^+ transport and distribution in plants, and in particular its first entry into plant roots. In recent years, this endeavour has been augmented by the search for molecular candidates for Na^+ transport, with some remarkable successes, but not without significant controversies. In this review, we will take a critical look at the main classes of transporters that have been identified, chiefly by means of electrophysiological and molecular techniques, and will discuss these achievements in the context of the whole plant and of plant cultivation in the field, to which significant discoveries must ultimately relate. We particularly focus on aspects where conclusions may have been drawn prematurely, and point out discrepancies that require further discussion or experimentation to achieve progress.

We shall show that the link between electrophysiological evidence of Na^+ transport via nonselective cation channels (NSCCs) in protoplasts and artificial bilayer systems on the one hand, and *in planta* 'toxic' Na^+ fluxes on the other, may have been accepted prematurely; that many published Na^+ flux values under saline conditions in plant roots are energetically difficult to explain, and may require a new interpretation; that participation in Na^+ uptake by transporters such as low-affinity cation transporter 1 (LCT1) and K^+ transporters from the KUP/HAK/KT and AKT families, and as yet poorly characterized 'back-up' systems of K^+ acquisition, cannot be discounted at this point; that evidence for the role of HKT2 transporters in primary Na^+ uptake under K^+ deprivation conditions is strong, as is evidence for the role of HKT1 transporters in controlling internal Na^+ distribution between the root and the shoot, while evidence for their roles in primary Na^+ uptake under saline conditions is limited; that evidence for the role of Salt Overly Sensitive 1 (SOS1) in Na^+ efflux back into the external medium is not as clear as frequently indicated, and its role in root–shoot Na^+ transfer is obscure; that evidence for the role of NHX in vacuolar Na^+ sequestration and subsequent rescue from Na^+ toxicity is strong, but important questions remain; and that a proper evaluation of the role of cytosolic Na^+, and, in particular, the cytosolic $Na^+ : K^+$ ratio, is hampered by a scarcity of direct measurements (these are summarized here) and its utility, as well as that of total-tissue Na^+ accumulation, as a predictor of sodium stress may not be as great as is often stated.

II. The role of nonselective cation channels in primary sodium influx – a solid consensus. How solid is the evidence?

1. The functional subclasses of NSCCs

Even though no definitive molecular candidates have thus far emerged, a strong consensus has developed in recent years, largely based on electrophysiological studies, that various classes of NSCCs catalyse primary influx of Na^+ under saline conditions. NSCCs are thoroughly characterized in

animals, and their functions are well understood in their cellular signaling, vascular endothelial function, Ca^{2+} influx in response to store depletion, and renal ion homeostasis (Kaupp & Seifert, 2002; Clapham, 2003; Firth *et al.*, 2007; Venkatachalam & Montell, 2007; Kauer & Gibson, 2009). In plants, several categories of NSCCs have also been identified, and these have been subdivided (Demidchik & Tester, 2002; Demidchik & Maathuis, 2007), according to their response to changes in membrane electrical potential, into the following major classes: (1) depolarization-activated NSCCs (DA-NSCCs), (2) hyperpolarization-activated NSCCS (HA-NSCCs), and (3) voltage-insensitive NSCCs (VI-NSCCs). Additional classification systems distinguish NSCCs by their reponsiveness to certain ligands and physical stimuli and include cyclic-nucleotide-gated NSCCs (CNGCs), amino-acid-gated NSCCs (AAG-NSCCs), and reactive-oxygen-species-activated NSCCs (ROS-NSCCs). These may well constitute representatives of subclasses (1) through (3), as may other minor types of NSCCs not discussed here (see Demidchik & Maathuis, 2007).

The first definitive demonstration, using patch-clamp approaches, of NSCC-type conductances in plants dates to 1989, when Stoeckel and Takeda reported constitutive cation fluxes across the plasma membranes of triploid endosperm cells in species from the genera *Haemanthus* and *Clivia* that displayed minimal selectivity for various alkali, and some earth alkali, ions, and could be activated following depolarizations of the membrane potential (Stoeckel & Takeda, 1989). Despite some constitutive activity, these types of NSCCs have thus been classified in category 1 above. DA-NSCC operation has since been confirmed in a large number of experimental systems, including leaf and root cell preparations from *Arabidopsis thaliana*, *Thlaspi arvense* and *T. caerulescens*, *Hordeum vulgare* and *Phaseolus vulgaris* (Cerana & Colombo, 1992; Spalding *et al.*, 1992; de Boer & Wegner, 1997; Pei *et al.*, 1998; Piñeros & Kochian, 2003; Zhang *et al.*, 2004). Their main function appears to be in conducting Ca^{2+} (White & Ridout, 1999; White *et al.*, 2000), although a role in catalyzing K^+ release from root cells under sudden imposition of saline conditions has also been proposed (Shabala *et al.*, 2006). By contrast, the role of DA-NSCCs in catalyzing primary Na^+ fluxes under salt stress conditions has been much less conclusively demonstrated. Nevertheless, in a major review on the topic (Demidchik & Maathuis, 2007), it was suggested that members of this depolarization-activated class of NSCCs may well be involved in this function. The proposal was based upon reference to a series of comparative electrophysiological studies conducted in *Arabidopsis thaliana* and its natural halophyte relative *Thellungiella halophila* (Volkov *et al.*, 2004; Volkov & Amtmann, 2006; Wang *et al.*, 2006); studies that, however, concluded that the predominant Na^+ conductances observed were *voltage-insensitive*, not depolarization-activated. A role for the subclass of depolarization-activated NSCCs in catalyzing significant Na^+ fluxes under saline conditions therefore remains purely speculative at this point.

NSCC category 2 (HA-NSCCs) can be excluded from further in-depth discussion in the context of primary Na^+ fluxes under salinity, as hyperpolarization of the plasma membrane, inherent to the gating properties of these channels (see e.g. Gelli & Blumwald, 1997; Hamilton *et al.*, 2000; Véry & Davies, 2000; Demidchik *et al.*, 2007), does not typically accompany the imposition of salinity, neither in short-term nor in long-term applications of Na^+ (Laurie *et al.*, 2002; Carden *et al.*, 2003; Shabala *et al.*, 2006; Volkov & Amtmann, 2006; Malagoli *et al.*, 2008).

2. VI-NSCCs: the current consensus

In contrast to the above categories, a substantial number of studies support a role for VI-NSCCs (category 3) in catalyzing Na^+ fluxes across the plasma membrane, in particular in roots (some reports have also focused on shoots: see e.g. Elzenga & van Volkenburgh, 1994; Véry *et al.*, 1998), and it is here where more extensive discussion is warranted. CNGCs, AAG-NSCCs and ROS-NSCCs may well represent subclasses of this type of NSCC (Demidchik & Maathuis, 2007). The earliest demonstration of VI-NSCCs was in wheat (*Triticum aestivum*; Moran *et al.*, 1984; see also: Tyerman *et al.*, 1997; Buschmann *et al.*, 2000; Davenport & Tester, 2000), followed by extensive work in rye (*Secale cereale*; White & Tester, 1992; White & Lemtiri-Chlieh, 1995; White & Ridout, 1995; White, 1996), maize (*Zea mays*; Roberts & Tester, 1997), barley (*Hordeum vulgare*; Amtmann *et al.*, 1997), *A. thaliana* (Maathuis & Sanders, 2001; Demidchik & Tester, 2002; Shabala *et al.*, 2006; Volkov & Amtmann, 2006), *Thellungiella halophila* (Volkov *et al.*, 2004; Volkov & Amtmann, 2006; Wang *et al.*, 2006), and *Capsicum annuum* (Murthy & Tester, 2006). Common features unite the observations in this large body of studies: VI-NSCCs are so named because their open probability is not significantly, or at best weakly, modulated by membrane potential, in contrast to the categories of NSCCs discussed above. Currents are constitutive and instantaneous (i.e. permanently present when ensemble averages, not individual channel traces, are examined), and they lack time-dependent activation (Tyerman *et al.*, 1997; Amtmann & Sanders, 1999; White, 1999b). VI-NSCCs have been shown, in classic current–voltage relationships, to conduct both inward and outward currents, and thus may constitute both influx and efflux pathways *in planta* (see e.g. Shabala *et al.*, 2006; Volkov & Amtmann, 2006). VI-NSCCs also exhibit several pharmacological characteristics that separate them from other classes of ion channels (see Demidchik *et al.*, 2002; Demidchik & Maathuis, 2007): they are not sensitive to the potassium channel inhibitors Cs^+ and tetra-ethyl-ammonium (TEA^+), are not affected by the alkali cations Li^+ and Na^+, the

sodium channel inhibitor tetrodotoxin (cf. Allen *et al.*, 1995) or the calcium channel inhibitors verapamil and nifedipine, but are greatly inhibited by the trivalent cations lanthanum (La^{3+}) and gadolinium (Gd^{3+}; it should be noted, however, that these two cations are very broad-spectrum; see e.g. Qu *et al.*, 2007). One class of VI-NSCCs can also be *partially* blocked by divalent cations, including Ba^{2+} and Zn^{2+}, as well as, especially importantly, Ca^{2+} and Mg^{2+}, while another class is not inhibited by these ions, but instead transports them (Demidchik & Maathuis, 2007). Some VI-NSCCs are also inhibited by the organic compound quinine (Demidchik & Tester, 2002), but this feature is not universal (White & Lemtiri-Chlieh, 1995; White & Broadley, 2000). Other treatments, including pH changes (stimulation by alkaline pH and inhibition by acidic pH) and application of the histidine modifier diethylpyrocarbonate (DEPC; strong inhibition), have also been shown to be effective in selected experimental systems, such as *A. thaliana* (Demidchik & Tester, 2002) and rye (White, 1999a), but have as yet not been tested widely. Within a given experimental system (e.g. *A. thaliana*), such responses, in addition to the more universally exhibited ones, provide valuable gauges for a critical comparative evaluation of physiological results obtained by different methods (for further discussion, see Section II.3 below).

In most studies on VI-NSCCs, clear demonstration of Na^+ conductance was provided. As suggested by their name, VI-NSCCs are, to a high degree, nonselective for cations, that is, similar permeation of a variety of cations can be observed when such tests are conducted. Nevertheless, ion preferences are still encountered, resulting in selectivity series. Many such series have been published, and, while generally similar, they vary in their detail. In a seminal study on *A. thaliana* (Demidchik & Tester, 2002), the series observed (cation permeabilities are listed relative to Na^+) was: K^+ (1.49) > NH_4^+ (1.24) > Rb^+ (1.15) > Cs^+ (1.10) > Na^+ (1.00) > Li^+ (0.73) > TEA^+ (0.47). In rye roots (White & Tester, 1992), the series was: K^+ (1.36) = Rb^+ (1.36) > Cs^+ (1.17) > Na^+ (1.00) > Li^+ (0.97) > TEA^+ (0.41). In wheat, NH_4^+ (2.06) > Rb^+ (1.38) > K^+ (1.23) > Cs^+ (1.18) > Na^+ (1.00) > Li^+ (0.83) > TEA^+ (0.20) was reported (Davenport & Tester, 2000). In other words, in these three benchmark studies (see also Tyerman *et al.*, 1997 and Volkov & Amtmann, 2006), the macronutrient potassium (and, where tested, also ammonium) was transported to a significantly greater extent than sodium, from equimolar concentrations (see also Zhang *et al.*, 2010). Thus, for this category of NSCCs, the cation selectivity series appear to follow a more consistent pattern than the frequently cited range of K^+ : Na^+ selectivity ratios for NSCCs of 0.3 to 3 (Demidchik *et al.*, 2002; Demidchik & Maathuis, 2007). The published selectivity series should provide an important gauge for determining the contribution of NSCCs to Na^+ conductance *in planta*.

Additional subclasses of NSCCs that have been the subject of some discussion in the context of Na^+ fluxes are cyclic nucleotide-gated and amino-acid- (in particular, glutamate-) gated NSCCs (CNGCs and AAG-NSCCs; see also Demidchik & Maathuis, 2007). Among these, CNGCs are perhaps the best studied. They are characterized by gating mediation involving the second messengers cAMP and cGMP, and their role in animal physiology is diverse and has been extensively investigated, in particular within the context of transduction of visual and olfactory stimuli and Ca^{2+} signalling (Kaupp & Seifert, 2002; Talke *et al.*, 2003; Gobert *et al.*, 2006; Takeuchi & Kurahashi, 2008). However, functional expression of plant CNGCs has proved difficult, and thus little functional consolidation has occurred to date, even though some 20 CNGCs have been found in the *A. thaliana* genome (Gobert *et al.*, 2006; Demidchik & Maathuis, 2007). However, in a few cases, expression in heterologous systems, including *Xenopus laevis* oocytes and yeast, has been successful (Leng *et al.*, 2002; Balagué *et al.*, 2003; Gobert *et al.*, 2006), and sensitivity to cAMP and cGMP has been observed, as well as sensitivity to Cs^+ (Balagué *et al.*, 2003) and Mg^{2+} (Leng *et al.*, 1999). Interestingly, *in planta* Na^+ fluxes, in glycophytes under toxic conditions, are typically reported to be insensitive to Cs^+ (see later discussion on fluxes; also see, however, Kader & Lindberg (2005) for work examining the protoplasts of rice; Wang *et al.* (2007) for work on the halophyte *Sueda maritima*, and Voigt *et al.* (2009) for Na^+ tissue content data in cowpea (*Vigna unguiculata*) – these studies present evidence of Cs^+ sensitivity of Na^+ uptake). Cesium sensitivity, and the voltage sensitivity seen in many CNGCs, reduce the likelihood of their significant involvement in catalyzing Na^+ fluxes in whole plants for extended periods of time (the roles of AtCNGC2, 4, 11 and 12 in response to pathogen attack, and the flow of Ca^{2+} under such conditions, are, by contrast, well documented; see e.g. Balagué *et al.*, 2003; Demidchik & Maathuis, 2007; Guo *et al.*, 2010). Two CNGCs from the *A. thaliana* genome, AtCNGC3 and AtCNGC10, have nevertheless been linked to primary K^+ and Na^+ fluxes in roots. In the case of the former (AtCNGC3), tissue expression analysis has localized the transporter to root epidermal and cortical cells, and a null mutation in the gene has been shown to reduce the net uptake rate of Na^+ during the initial (although not the later) stages of NaCl exposure, resulting in slightly enhanced growth on intermediate (40–80 mM) NaCl concentrations; the Na^+ content of mutant seedlings, however, was not different from that of the wild type following longer term treatments at high (80–120 mM) NaCl concentrations (Gobert *et al.*, 2006). The work may indicate a role for AtCNGC3 in Na^+ uptake in the early phases (the initial few hours) of salt stress. In the case of AtCNGC10, tissue expression studies have also localized the transporter to root tissues, and the gene was able to complement the reduced

K^+ uptake phenotype of the *A. thaliana akt1;1* mutant (see Hirsch *et al.*, 1998; Spalding *et al.*, 1999), establishing a possible role for the transporter in alkali ion fluxes in roots (Li *et al.*, 2005). Other more recent studies, however, have shown a greater role of AtCNGC3 in the transport of the earth alkali ions Ca^{2+} and Mg^{2+} (Guo *et al.*, 2010), although Na^+ transport may be involved indirectly (Guo *et al.*, 2008), while other CNGCs, such as AtCNGC2, are strongly selective for K^+ over Na^+ (Leng *et al.*, 2002). In support of an *in planta* involvement of CNGCs in Na^+ transport under toxic conditions, some studies have indeed reported a sensitivity of unidirectional or net fluxes of Na^+ to cyclic nucleotides (Maathuis & Sanders, 2001; Essah *et al.*, 2003; Rubio *et al.*, 2003; Maathuis, 2006; see, however, Section II.3). Additionally, the observation that salt-tolerant varieties of rice down-regulate OsCNGC1 to a greater extent than salt-sensitive varieties under saline conditions (Senadheera *et al.*, 2009) may also be taken as circumstantial evidence for an involvement of CNGCs in Na^+ influx. Based on these findings, therefore, the role of CNGCs in primary Na^+ fluxes cannot be dismissed at this point, and deserves careful further investigation, but the balance of the evidence does not currently favour a significant involvement (see also Zhang *et al.* (2010), who review conflicting information regarding whether CNGCs are blocked, or activated, by cyclic nucleotides).

Another subgrouping of ligand-sensitive NSCCs that may be involved in Na^+ transport is that of AAG-NSCCs, and, in particular, those gated by glutamate. Precedents for glutamate-activated NSCCs abound in the animal literature (Dingledine *et al.*, 1999; Traynelis *et al.*, 2010), but their role in plant physiology, and under conditions of sodium toxicity, is more obscure (Lam *et al.*, 1998; Davenport, 2002; Demidchik & Maathuis, 2007). At this time, convincing functional analyses of these channels are lacking, despite the fact that, as with CNGCs, some 20 AAG-NSCCs have been identified in the *A. thaliana* genome. The voltage insensitivity and instantaneous activation of currents, along with sensitivity to quinine and lanthanides in one study (Demidchik *et al.*, 2004), suggest that AAG-NSCCs may represent subclasses of VI-NSCCs. While some evidence from *Xenopus* oocytes indicates the possibility of Na^+ transport in at least some members of this family (AtGLR1;1, AtGLR 1;4 and AtGLR3;7; Roy *et al.*, 2008; Tapken & Hollmann, 2008), the preponderance of evidence currently supports a role for AAG-NSCCs in Ca^{2+} transport (Dennison & Spalding, 2000; Dubos *et al.*, 2003; Demidchik *et al.*, 2004) and signalling during development (Kim *et al.*, 2001; Turano *et al.*, 2002; Li *et al.*, 2006; Qi *et al.*, 2006; Walch-Liu *et al.*, 2006), rather than a role in primary Na^+ fluxes under saline conditions (cf. Essah *et al.*, 2003). Similarly, ROS-NSCCs (perhaps most NSCCs?) appear to be predominantly involved in Ca^{2+} transport (Demidchik & Maathuis, 2007).

3. Linking electrophysiological readings from protoplasts to fluxes in the whole plant: the challenge

It has to be strongly emphasized, and we will return to this critical point later, that essentially all demonstrations of the role of NSCCs, and in particular of VI-NSCCs, in catalyzing Na^+ fluxes have been achieved by patch-clamp analysis with isolated protoplasts or artificial lipid bilayers. By contrast, the connection between such measurements and Na^+ fluxes at the level of whole tissues and the whole plant is, in fact, much less secure (Malagoli *et al.*, 2008; Britto & Kronzucker, 2009; Zhang *et al.*, 2010), although the opposite conclusion is often stated (see e.g. Davenport, 2002; Munns & Tester, 2008). Several key studies have attempted to relate Na^+ currents measured by electrophysiology in protoplasts and artificial lipid bilayer systems to Na^+ fluxes and accumulation in intact plants and/or plant tissues. Once such set of comparative experiments was carried out in wheat (Davenport & Tester, 2000), and another in *A. thaliana* (Demidchik & Tester, 2002; Essah *et al.*, 2003). Both sets of studies employed $^{22}Na^+$-labelling of excised plants roots alongside electrophysiological examinations of protoplast and lipid bilayer preparations within a genotype. In the first of these studies, the authors showed that 'Na^+ influx through the NSC channel resembled $^{22}Na^+$ influx' (Davenport & Tester, 2000), and, indeed, concluded, even within the paper's title, that a 'nonselective cation channel mediates toxic sodium influx in wheat'.

This attribution was supported in large part by the partial sensitivity of both radiolabelled Na^+ fluxes and Na^+ currents to Ca^{2+}, Mg^{2+} and Gd^{3+}, and their insensitivity to other inhibitors, including those specific to potassium channels (TEA^+ and Cs^+; cf. Kader & Lindberg, 2005; Wang *et al.*, 2007; Zhang *et al.*, 2010). While Ca^{2+} sensitivity may indeed link NSCC operation well to the frequently (albeit not universally: see Yeo & Flowers, 1985; Schmidt *et al.*, 1993; Malagoli *et al.*, 2008) observed amelioration of Na^+ toxicity by Ca^{2+} in whole plants (LaHaye & Epstein, 1969; Greenway & Munns, 1980; Rengel, 1992; Epstein, 1998), it should be kept in mind that Ca^{2+} has a myriad of other effects on plants (Britto *et al.*, 2010; Zhang *et al.*, 2010) and thus can hardly be seen as specific, and that the similarly strong Mg^{2+} sensitivity documented for NSCC operation (Davenport & Tester, 2000; their Fig. 4) is not typically reflected in the Na^+ toxicity rescue of plants (LaHaye & Epstein, 1969). In addition, however, other issues deserve discussion. First, Ca^{2+} sensitivity, while exhibiting similar K_i values for electrical currents in bilayer preparations and tracer fluxes in roots (in the range of 610–650 μM; Davenport & Tester, 2000; see also White, 1999b; cf. Wang *et al.*, 2007; Malagoli *et al.*, 2008), was much more pronounced in single-channel preparations (> 50%) than it was in roots, where, at Ca^{2+} concentrations above 3 mM, *c.* 75% of the influx seen at the lowest [Ca^{2+}] was still observed, measuring in

Table 1 Selected plant Na^+ fluxes from the literature

Species	External [Na] (mM)	Flux (μmol g^{-1} FW h^{-1})	Calculated O_2 flux (μmol g^{-1} FW h^{-1})	Comments	References
Triglochin maritima	100	600	120	Compartmental analysis; 'influx to vacuole'	Jefferies (1973)
Triticum aestivum	150	125	25	2 min load; 7 min desorption	Wang *et al.* (2009a)
Puccinellia tenuiflora	150	87	17.4		
Zea mays	100	9.2–13.86 (with or without 1 mM Ca^{2+})	1.8–2.8	30 min load; 20 min desorption	Zidan *et al.* (1991)
	0.5	0.08	0.016	15 or 30 min load; 2 × 15 min desorption	Nocito *et al.* (2002)
Hordeum vulgare	100	80–110	16–22	Compartmental analysis	Kronzucker *et al.* (2006, 2008)
Arabidopsis thaliana	50	8.8	1.76	30 min load; 16 min desorption	Maathuis & Sanders (2001)
	50	5.05–7.77	1.0–1.6	5 min load; 20 min desorption	Elphick *et al.* (2001)
	200	300	60	2 min load; 2 × 2 min + 1 × 3 min desorption	Essah *et al.* (2003)
	50	104	21	As in Essah *et al.* (2003)	Møller *et al.* (2009)
	50	93–115	19–23	2 min load; 2 + 3 min desorption	Jha *et al.* (2010)
Spergularia maritima	90	600	120	Compartmental analysis (efflux)	Lazof & Cheeseman (1986)
Oryza sativa	50	*c.* 300 (root only; more when whole plant is considered)	60	Net fluxes (determined from tissue retention)	Senadheera *et al.* (2009)
	25	225	45	1 min load; 5 min desorption	Malagoli *et al.* (2008)
Triticum aestivum	100	17.0 (wild-type); 9.8 (line 271)	3.4; 1.96	30 min load; 2 × 8 min desorption	Laurie *et al.* (2002)
	100	145	29	w/verapamil; 5 min load plus 2 × 1 min ice-cold desorption	Davenport & Tester (2000)

Note the wide range of values presented, which reflect differences in species, tissues and protocols (e.g. applied concentrations and loading times).

excess of 70 μmol g^{-1} FW h^{-1}, a very high cationic flux indeed (see Britto & Kronzucker, 2009; and discussion of data in Table 1). Ascribing Ca^{2+} sensitivity of Na^+ influx in cereals exclusively to NSCCs (see also Davenport *et al.*, 1997) is further complicated by the recent demonstration of Ca^{2+} suppression of OsHKT2;1-mediated Na^+ transport in rice (Yao *et al.*, 2010), contrary to the earlier claim of Ca^{2+} insensitivity of HKT-mediated transport (Davenport & Tester, 2000; citing Schachtman *et al.*, 1997; see also other demonstrations of HKT-mediated Na^+ influx under toxicity in wheat, e.g. Laurie *et al.*, 2002 – to be discussed in Section V). Similarly, the Ca^{2+} sensitivity of other potential transport candidates, such as LCT1 (see Section III below), undermines the clear attribution of Ca^{2+}-sensitive fluxes to NSCCs. Moreover, it may be of paramount importance in this context that, as has been argued before (Schachtman & Liu, 1999; Amtmann *et al.*, 2001; Britto & Kronzucker, 2009), Ca^{2+} concentrations in saline soils are typically high (10 mM or more is not unusual: Schachtman & Liu, 1999; Garciadeblás *et al.*, 2003; Hirschi, 2004; Kronzucker *et al.*, 2008), and thus a Ca^{2+}-*insensitive* component(s) of Na^+ influx should, in fact, be of greater interest as a target for engineering salt tolerance. The at times nearly exclusive focus on NSCCs in the context of Na^+ acquisition under toxic, saline conditions is thus puzzling.

Interestingly, in wheat, sensitivity to low concentrations of Gd^{3+}, a hallmark of many VI-NSCCs, was not observed (Demidchik & Tester, 2002; Demidchik *et al.*, 2002; Demidchik & Maathuis, 2007), and only at 1 mM Gd^{3+} were significant reductions in Na^+ flux evident (Davenport & Tester, 2000; unfortunately, only one flux value was provided in that study, in low-salt plants; sensitivity to La^{3+}, another key uniting feature of VI-NSCCs, was not tested). By contrast, in *A. thaliana*, strong Gd^{3+} sensitivity (complete inhibition could be achieved at 0.1 mM; this was similar for La^{3+}) was seen in electrophysiological

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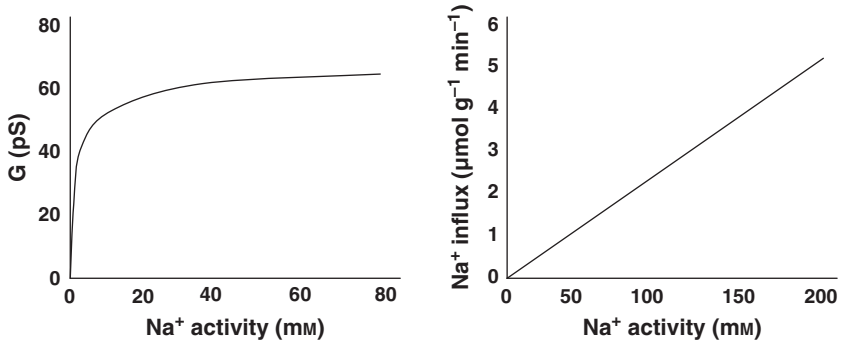

Fig. 1 Idealized comparison of Na^+ current measured electrophysiologically through nonselective cation channels (left; G, conductance through channel; redrawn from Davenport & Tester, 2000), and Na^+ influx into plant roots measured using radiotracing with $^{22}Na^+$ (redrawn from Essah *et al.*, 2003). Note the early saturability of the current (cf. White & Ridout, 1995), as compared with the continued linearity of the tracer flux.

characterizations of NSCC conductances (Demidchik & Tester, 2002), but none at all in corresponding tracer studies on plant roots of the same ecotypes (Essah *et al.*, 2003; in this study, La^{3+} actually produced a 34% *increase* in roots from the same genotype; their Table 4). Thus, the pharmacological agreement is actually far less compelling than is frequently stated.

More importantly, however, as illustrated in Fig. 1, a fundamental characteristic evident in electrophysiological trials, but not in root influx measurements, is the saturability of the Na^+ flux. In electrophysiological characterization, an NSCC proclaimed to mediate toxic Na^+ influx in wheat (Davenport & Tester, 2000) failed to produce any flux enhancement at Na^+ concentrations beyond 7–10 mM, that is, far below the toxicity threshold for the ion, with complete saturation being observed between 10 and 80 mM (using a simple Michaelis–Menten model, a K_m value of 1.2 mM was reported for this saturable pattern; see also the discussion by Amtmann *et al.* (2001) and that of White & Davenport (2002), who developed a permeation model for this NSCC). A similar, if slightly less pronounced, saturable pattern was reported in a follow-up study in *A. thaliana* (Demidchik & Tester, 2002; cf. White & Ridout, 1995, who did see a non-saturating increase in current with increasing external $[Na^+]$, in an NSCC from rye root plasma membranes; however, parallel tracer flux studies have not been conducted in this system). By stark contrast, Na^+ influx into roots produced a linearly increasing flux in both wheat and *A. thaliana* that showed no signs of abating even at 200 mM Na^+ (Fig 1; Davenport & Tester, 2000; Essah *et al.*, 2003). Interestingly, the influx measured at 200 mM external $[Na^+]$ in Essah *et al.* (2003) was *c.* 300 μmol g^{-1} (FW) h^{-1}, one of the highest purported trans-plasma-membrane cation fluxes ever reported in glycophytes (Table 1, and Britto & Kronzucker, 2009). Indeed, in the extensive study by Essah *et al.* (2003), all Na^+ fluxes, even some at rather low external Na^+ (see their Fig. 4, for values at 1 mM Na^+) were very high (e.g. a flux of over 210 μmol g^{-1} FW h^{-1} was reported at 1 mM). It is unclear whether translations of the magnitudes of currents in patch-clamp experiments (reported in pS or pA) into tissue fluxes (typically reported in μmol g^{-1} FW h^{-1}) can be achieved in principle, but what is clear, at this time, is that such correspondence has not yet been achieved in the case of NSCCs and their corresponding Na^+ fluxes at the tissue level.

We have previously shown (Malagoli *et al.*, 2008; Britto & Kronzucker, 2009), using established energetic models of transport (Poorter *et al.*, 1991; Scheurwater *et al.*, 1999; Kurimoto *et al.*, 2004; Britto & Kronzucker, 2006), that fluxes of the magnitudes reported in the above studies, and indeed many others (Table 1), are not explicable energetically, if they are to follow currently proposed mechanisms of Na^+ transport (Tester & Davenport, 2003; Apse & Blumwald, 2007; Malagoli *et al.*, 2008; Munns & Tester, 2008; Teakle & Tyerman, 2010). In Table 1, we summarize, using the currently established model of cation transport and its energization (Britto & Kronzucker, 2009), minimal respiratory oxygen fluxes required to energize the reported Na^+ fluxes. In halophytes, at 100 mM external Na^+ supply, unidirectional Na^+ fluxes as high as 600 μmol g^{-1} h^{-1} have been reported (Jefferies, 1973; Lazof & Cheeseman, 1986), corresponding to a respiratory O_2 flux of 120 μmol g^{-1} h^{-1}, with 50% of these values being attained in the glycophyte *A. thaliana* at 200 mM Na^+ (Essah *et al.*, 2003). No precedents for respiratory values of this magnitude can be found in the literature, and we previously showed (Malagoli *et al.*, 2008), in the IR29 variety of *Indica* rice, that the respiratory requirement for the measured Na^+ fluxes in that variety (as high as 225 μmol g^{-1} h^{-1} at 25 mM Na^+) exceeded measured total respiratory values by 100%. Thus, a critical look at many, although not necessarily all (see e.g. Laurie *et al.*, 2002), of the Na^+ fluxes summarized in Table 1 is essential, if one is to successfully interpret measured Na^+ fluxes and link them to plant performance. In addition, it would behoove experimenters to conduct respiratory analyses in their systems when exceptionally large fluxes are observed, as a partial test of the correct assignment of the measurements to a genuine plasma membrane flux. In this context, the need to distinguish between apoplastic and symplastic phases of uptake may be critically important (Yeo *et al.*, 1987; Kronzucker *et al.*, 1995, 1998; Britto & Kronzucker, 2001; see Section VIII). As we have also argued previously

(Malagoli *et al.*, 2008; Britto & Kronzucker, 2009), alternative explanations for such fluxes, or a revision of the accepted transport energization model, must be considered. These alternative possibilities may include tracer absorption by the plant root apoplast (see Yadav *et al.*, 1996; Gong *et al.*, 2006; Krishnamurthy *et al.*, 2009), or an as yet unsatisfactorily characterized means of flux coupling (Colmenero-Flores *et al.*, 2007), or vesicular transport (Peiter *et al.*, 2007).

Additional evidence, independent of tracer analysis, for the participation of NSCCs in Na^+ influx *in planta* comes from tissue analysis (Volkov & Amtmann, 2006), and use of Na^+-sensitive fluorescent dyes (Kader & Lindberg, 2005; Anil *et al.*, 2007). On the basis of insensitivity to the potassium-channel blockers Cs^+ and TEA^+ of both instantaneous Na^+ currents and tissue Na^+ accumulation, Volkov & Amtmann (2006) (see their Fig. 8) came to the conclusion that NSCCs are responsible for Na^+ fluxes in *T. halophila*. However, it should be pointed out that data sets for results obtained using fluorescing dyes are scant at this time, and relationships with tissue accumulation may be problematic in cases of long-term treatment with pharmacological inhibitors, as illustrated by the *increase* in Na^+ accumulation in plants treated with Cs^+ for 2 d in the aforementioned study, in disagreement with the premise that accumulation can directly reflect the pharmacological profile of the channels carrying instantaneous currents in patch-clamp experiments. In studies on protoplasts and suspension-culture cells using the sodium-sensitive dye SBFI, the appearance of Na^+ in the cytosol, upon sudden Na^+ exposure, was reduced by Ca^{2+} (Anil *et al.*, 2007) and some additional channel inhibitors (Zn^{2+} and La^{3+}; Kader & Lindberg, 2005) known to target NSCCs. However, it should be kept in mind that such pharmacological agents can give conflicting results (Balkos *et al.*, 2010), and assignment to specific mechanisms can be difficult.

We argue that, for a match-up between electrophysiology readings and excised tissue or whole- plant tracer studies to be achieved, several criteria must be met. First, responses to pharmacological treatments must match not just for a few, but for the majority of agents applied within a given genotype. They must produce changes in the *same* direction (inhibition vs enhancement) in both experimental approaches and, in particular, sensitivity to La^{3+} and Gd^{3+} at low concentrations should be observed as a gauge of NSCC involvement. Secondly, the kinetic response of currents and *in planta* fluxes must assume comparable shapes (saturable vs linear). Thirdly, fluxes measured *in planta* must be subjected to an energetic analysis, and, where excessive fluxes are seen, respiration data must be provided to test the proposed interpretation that fluxes in fact proceed across the plasma membrane. As such criteria are currently not met, assignments of electrophysiological Na^+ currents of the NSCC type to *in planta* Na^+ fluxes and vice versa must be viewed as preliminary.

III. Low-affinity cation transporter 1 – a forgotten link?

On account of some similarities with NSCCs, a brief discussion of LCT1, originally isolated from wheat (Schachtman *et al.*, 1997; Clemens *et al.*, 1998; Amtmann *et al.*, 2001), is useful. TaLCT1 from wheat has been shown to transport Na^+ when expressed heterologously in yeast cells (Schachtman *et al.*, 1997; Amtmann *et al.*, 2001), and a decrease in the intracellular K^+ : Na^+ ratio was shown to result from this expression (Amtmann *et al.*, 2001). Critically, TaLCT1 mediated Na^+ transport in yeast was sensitive to both K^+ and Ca^{2+} (Amtmann *et al.*, 2001); the latter feature is shared with NSCCs (see Section II.3). Notwithstanding these observations, the findings of Clemens *et al.* (1998) implicated TaLCT1 chiefly in the transport of Ca^{2+}, and possibly heavy metals, such as cadmium. TaLCT1 involvement in heavy metal transport has also been supported by Antosiewicz & Hennig (2004). In a recent review, Plett & Møller (2010) have argued that TaLCT1 does not, in and of itself, transport Na^+ but may enhance native transport systems already present in yeast membranes to acquire or enhance this function. This particular interpretation was not, however, suggested in the original studies (Amtmann *et al.*, 2001) to which the authors refer.

Zhang *et al.* (2010) have argued that the involvement of LCT1 in primary Na^+ influx under saline conditions is not likely, because of its sensitivity to Ca^{2+}. Even though this sensitivity is less pronounced than the Ca^{2+} sensitivity of most NSCCs, soil Ca^{2+} concentrations are, nevertheless, high enough (several millimolar) in most saline soils (Schachtman & Liu, 1999; Garciadeblás *et al.*, 2003; Hirschi, 2004; Kronzucker *et al.*, 2008) to significantly suppress its activity, if present. More fundamentally, examination of the Ca^{2+} dependence of unidirectional Na^+ influx in a major glycophyte (rice; Malagoli *et al.*, 2008) and in a halophyte (*S. maritima*; Wang *et al.*, 2007) showed no significant alteration of influx as a function of imposed Ca^{2+} gradients, supporting, at least superficially, neither NSCC nor LCT1 operation. It has to be kept in mind, however, that many unidirectional flux analyses in the high salt range are problematic (see Section II.3), and thus proposed connections between such measurements and electrophysiological evidence in heterologous systems are currently not convincing. Moreover, several studies have documented a suppression of Na^+ accumulation at elevated external Ca^{2+} concentrations (Melgar *et al.*, 2006; Tuna *et al.*, 2007; Voigt *et al.*, 2009), leaving open possibilities for Ca^{2+}-sensitive influx pathways, even where direct influx measurements may not have detected such sensitivities. It is furthermore possible, as we argue later (see Sections IV and V) with respect to other transporter types, that even greatly suppressed activities of (e.g. Ca^{2+}-sensitive) transporters may nevertheless permit sufficient entry of Na^+ to account for 'toxic' build-up. Further investigation,

through critical refinement of concepts and methodology, will be essential to achieving progress.

IV. Are potassium transporters implicated in sodium influx?

Based on agreement between older kinetic models (Epstein *et al.*, 1963) and mutant analyses in *A. thaliana* (Gierth & Mäser, 2007), the current consensus is that, under nutritionally relevant conditions, some 80% of potassium acquisition by plants occurs through two major systems, KUP/HAK/KT and AKT. Respectively, they catalyse high- and low-affinity uptake (Gierth & Mäser, 2007; Britto & Kronzucker, 2008; Rubio *et al.*, 2008; Szczerba *et al.*, 2009), while as yet unidentified back-up systems provide additional K^+ acquisition capacity at higher external potassium concentrations (Hirsch *et al.*, 1998; Spalding *et al.*, 1999; Pyo *et al.*, 2010). The KUP/HAK/KT family has many gene members that are known to encode potassium transporters in roots, while the AKT family appears to be restricted to just one member implicated in root potassium acquisition, namely AKT1 (apart from a 'silent regulatory subunit' found in *A. thaliana*, AtKC1; Reintanz *et al.*, 2002; Pilot *et al.*, 2003; Britto & Kronzucker, 2008; Szczerba *et al.*, 2009). Both systems can be strongly inhibited by Na^+ (Fu & Luan, 1998; Senn *et al.*, 2001; Qi & Spalding, 2004; Kronzucker *et al.*, 2008; Britto *et al.*, 2010), but both have also been shown or proposed to be capable of transporting Na^+, in particular when Na^+ concentrations are high (Santa-María *et al.*, 1997; Amtmann & Sanders, 1999; Blumwald *et al.*, 2000; Golldack *et al.*, 2003; cf. Nieves-Cordones *et al.*, 2010), and thus must be discussed here.

1. The KUP/HAK/KT family

For the KUP/HAK/KT family of transporters, little inhibition of K^+ uptake is found at low concentrations of Na^+; indeed, at low substrate concentrations, or within the high-affinity range of transport, selectivities have been shown to be up to three orders of magnitude higher for K^+ than for Na^+ (Smith & Epstein, 1964; Santa-María *et al.*, 1997; Rubio *et al.*, 2000; Martínez-Cordero *et al.*, 2004, 2005). At high external [Na^+], however, Na^+ appears to inhibit HAK5 at both transcriptional and functional levels in several species (Nieves-Cordones *et al.*, 2008, 2010; Alemán *et al.*, 2009). In the halophyte *T. halophila*, this inhibition was less pronounced than in its glycophytic relative *A. thaliana* (Alemán *et al.*, 2009), and indeed the opposite response (a stimulation of K^+ influx by Na^+) was observed in the halophyte *Mesembryanthemum crystallinum* (Su *et al.*, 2002). During short-term (several-hour) exposure to high Na^+ concentrations, transcriptional up-regulation has also been shown in barley, for *HvHAK1* (Fulgenzi *et al.*, 2008), but was followed by an inhibition upon longer term Na^+ exposure. In the early phases of salt exposure, the authors also reported increased tissue sodium, but not potassium, concentrations, coincident with increased expression of the transporter (Fulgenzi *et al.*, 2008). This may well support a role for KUP/HAK/KT transporters in Na^+ uptake. In recent studies on reed (*Phragmites australis*) plants, Takahashi *et al.* (2007a,b) found expression of *PhaHAK5* to be more pronounced under salt stress in a salt-sensitive variant, and heterologous expression in yeast yielded Na^+ permeability, again suggesting that at least some members of the KUP/HAK/KT family may be involved in Na^+ accumulation under some conditions. In addition, HvHAK1 from barley has been shown to conduct both high-affinity K^+ and low-affinity Na^+ fluxes when expressed in *trk* double mutants of yeast (Santa-María *et al.*, 1997). However, the authors pointed out that relatively high endogenous cation conductances at high substrate concentrations in this experimental system (Ramos *et al.*, 1994) render such conclusions problematic. Of particular interest in this context is that low-affinity Na^+ fluxes in several systems have been shown to be strongly up-regulated by K^+ starvation (Pitman, 1967; Pitman *et al.*, 1968; Kochian *et al.*, 1985; Ding & Zhu, 1997; Buschmann *et al.*, 2000; Horie *et al.*, 2001, 2009), reminiscent of the behaviour of K^+ transporters of the KUP/HAK/KT family (Britto & Kronzucker, 2008; Szczerba *et al.*, 2009). However, Nieves-Cordones *et al.* (2010) recently reported no difference in Na^+ uptake between *athak5-3* T-DNA insertional mutant plants and wild type, based on tissue Na^+ accumulation under moderate Na^+ supply, which suggests a lack of involvement of KUP/HAK/KT transporters in Na^+ uptake *in planta*.

Given these contradictory results, to test for the involvement of KUP/HAK/KT transporters using nonmutant-based approaches, we propose that the very pronounced ammonium sensitivity of this transporter class (Smith & Epstein, 1964; Vale *et al.*, 1987, 1988; Santa-María *et al.*, 1997; Bañuelos *et al.*, 2002; Martínez-Cordero *et al.*, 2004, 2005; Nieves-Cordones *et al.*, 2007, 2010; Qi *et al.*, 2008) may be used gainfully. One would expect Na^+ fluxes to have similar sensitivity to NH_4^+ fluxes were the involvement of this class significant. It is noteworthy that, in side-by-side comparisons of plants grown on NO_3^- vs NH_4^+, sodium toxicity is typically more pronounced on the latter nitrogen source (Speer & Kaiser, 1994; Speer *et al.*, 1994; Abdolzadeh *et al.*, 2008; cf. Voigt *et al.*, 2009). If symplastic Na^+ accumulation is critical to the development of sodium toxicity, it may thus be taken to suggest that KUP/HAK/KT systems are not involved in primary Na^+ influx, unless the ionic stresses exerted by Na^+ and NH_4^+ aggravate each other in other ways.

2. The AKT family

As in the case of KUP/HAK/KT transporters, AKT1 has been shown to be capable of Na^+ transport (Santa-María

Table 2 Estimates of cytosolic/cytoplasmic Na^+ concentrations or activities obtained with a variety of techniques and plant systems

Method	Plant system	External $[Na^+]$ (mM)	Cytosolic/cytoplasmic Na^+ concentration or activity (mM)	References
Efflux analysis	*Suaeda maritima* leaf, root	340	150–165	Yeo (1981)
	Hordeum vulgare root	1–100	7–308	Kronzucker *et al.* (2006)
	Nicotiana tabacum cell suspension	428	54	Binzel *et al.* (1988)
	Atriplex nummularia root	3–50	61–198	Mills *et al.* (1985)
	Avena sativa root	3–50	8–39	Mills *et al.* (1985)
	Zea mays root	50	79–142	Hajibagheri *et al.* (1989)
	Zea mays root	25	9–11	Schubert & Läuchli (1990)
Fluorescence microscopy (SBFI)	*Oryza sativa* root protoplasts	5–100	2–24	Kader & Lindberg (2005)
	Oryza sativa callus cells	75–200	18 to > 80	Anil *et al.* (2007)
	Arabidopsis thaliana root	30–90	5–90	Halperin & Lynch (2003)
Na^+-selective microelectrodes	*Hordeum vulgare* root	200	≤ 0.1 to > 100	Carden *et al.* (2001)
	Hordeum vulgare root	200	2–28	Carden *et al.* (2003)
	Acetabularia acetabulum	460	60–295	Amtmann & Gradmann (1994)
X-ray microanalysis	*Hordeum vulgare* leaf	150–250	150–475	James *et al.* (2006b)
	Triticum turgidum leaf	150–250	100–400	James *et al.*, (2006b)
	Nicotiana tabacum cell suspension	428	96	Binzel *et al.* (1988)
	Hordeum vulgare root	0–200	2–350	Flowers & Hajibagheri (2001)
	Zea mays root	100	42–138	Hajibagheri *et al.* (1987)
	Zea mays leaf	200	100	Hajibagheri *et al.* (1987)
Subcellular fractionation[a]	*Suaeda maritima* leaf chloroplasts	340	437	Harvey & Flowers (1978)
	Spinacea oleracea leaf chloroplasts	0–100	19–60	Speer & Kaiser (1991)
	Pisum sativum leaf chloroplasts	0–100	26–43	Speer & Kaiser (1991)
	Spinacia oleracea leaf chloroplasts	0.002–200	96–165	Robinson *et al.* (1983)

[a]Chloroplastic $[Na^+]$ considered to be identical to the cytosolic concentration (Speer & Kaiser, 1991).
SBFI, sodium-binding benzofuran isopthalate.

et al., 1997; Amtmann & Sanders, 1999; Blumwald *et al.*, 2000; Golldack *et al.*, 2003; cf. Nieves-Cordones *et al.*, 2010). Nevertheless, inhibition of OsAKT1 transcription by Na^+ in rice has been shown (Fuchs *et al.*, 2005), and a specific mechanism of inhibition of channel conductance by Na^+ build-up to even modest levels (near 10 mM) on the cytosolic side of the channel has been inferred from electrophysiological analysis (Qi & Spalding, 2004). From the latter study, one may conclude that continued AKT1 function under salinity is unlikely, given even conservative estimates for cytosolic Na^+ under saline conditions (Carden *et al.*, 2003; Kader & Lindberg, 2005; Munns & Tester, 2008; also see Table 2, and our discussion of published cytosolic Na^+ concentrations in Section IX). By contrast, in a comparison of two rice cultivars differing in salt tolerance (Kader & Lindberg, 2005), cytosolic $[Na^+]$ in leaf protoplasts (measured with the fluorescent sodium-sensitive dye SBFI) was reduced by nearly 50% when the potassium-channels blockers Cs^+ and TEA^+, and the more generic channel blockers La^{3+}, Ba^{2+} and Zn^{2+}, were applied to the salt-sensitive variety (the salt-tolerant variety was not affected by Cs^+ and TEA^+). This provided support for the involvement of OsAKT1 in Na^+ uptake in the sensitive variety. Similarly, Voigt *et al.* (2009), in cowpea, found that the potassium channel inhibitors Cs^+ and TEA^+ could reduce tissue sodium concentrations. Work by Golldack *et al.* (2003), also contrasting two rice varieties differing in salt tolerance, found higher OsAKT1 expression levels in the sensitive variety IR29, whereas lower levels were found in response to Na^+ in the tolerant variety Pokkali. In the halophyte *S. maritima*, Wang *et al.* (2007) found evidence for the involvement of an AKT-type transporter in Na^+ acquisition at Na^+ supply under 150 (but not at 25) mM, based on the pharmacology of $^{22}Na^+$ fluxes (again, sensitivity to Cs^+ and TEA^+ was seen; in addition, the authors observed sensitivity to Ba^{2+}, which is often used, if not as commonly as Cs^+ and TEA^+, to gauge the involvement of AKT-type transporters; Garciadeblás *et al.*, 2003; Nieves-Cordones *et al.*, 2010). More recently, Nieves-Cordones *et al.* (2010), using the *A. thaliana atakt1-2* mutant (Rubio *et al.*, 2008), reported no difference between the wild type and the mutant in Na^+ uptake based on tissue accumulation of the ion, under moderate Na^+ supply. The authors, however, suggested a possible role for AtAKT1 in (Na^+-promoted) K^+ efflux, which is often seen to aggravate the inhibition of K^+ influx under saline conditions (Shabala *et al.*, 2006; Britto *et al.*, 2010). However, this contention would appear to be at odds with other reports that AtAKT1 does not catalyse K^+ efflux (Hirsch *et al.*, 1998; in their study, *atakt1* mutants showed no difference from wild type in the efflux direction). The possible involvement of AKT1 in primary Na^+ fluxes was also investigated in an elegant

electrophysiological study by Buschmann *et al.* (2000), who showed that, in whole-cell-configuration patch-clamp trials on wheat root cells, the characteristics of K^+ and Na^+ currents were fundamentally different from one another in at least three crucial respects: (1) currents measured from identical concentrations of the two ions were > 10-fold larger for K^+ than for Na^+; (2) Na^+ currents were Ca^{2+}-sensitive, while K^+ currents were not; and (3) the activation kinetics of the two types of current were very different – Na^+ currents were instantaneous/fast-activating, and K^+ currents were not. From this, the authors concluded that AKT1 does not mediate Na^+ fluxes in wheat.

At this time, the evidence for the involvement of K^+ transporters from both the KUP/HAK/KT and AKT families remains limited, but some credible connections have been made, and it is clear that this area deserves further investigation. It may well be that the involvement of these transporters is not universal, but is genotype-specific. It is furthermore important to keep in mind that the KUP/HAK/KT and AKT systems can catalyse some of the most sizable cation fluxes under normal nutritional conditions (note that many reports of excessive Na^+ fluxes may be in doubt – see Britto & Kronzucker, 2009; and Section II.3), so that, even as strongly inhibited systems, and when presented with high external [Na^+], their contribution to primary Na^+ influx is not implausible. In addition, it is now well established, from mutant analyses in *A. thaliana*, that back-up systems exist for K^+ acquisition at elevated substrate concentrations – systems that eliminate growth differences between wild-type plants and mutants defective in AtHAK5 and AtAKT1 function (Hirsch *et al.*, 1998; Spalding *et al.*, 1999; Broadley *et al.*, 2001; Pyo *et al.*, 2010). The identity of these back-up systems is currently unknown, and the potential for Na^+ flow through them cannot be discounted at this time. Future studies must examine the nature of these transporters with respect to their sensitivity to Na^+ as a toxicant, and their ability to transport it.

V. HKT: a saga of twists and turns – where do we stand?

1. A history of confusion

In a breakthrough study in 1994 (Schachtman & Schroeder, 1994), a purported high-affinity transporter for potassium was isolated from wheat, and originally named HKT1 (and now known as TaHKT2;1). This characterization was based upon transcripts isolated from plants grown under potassium-deprivation conditions, which induce high-affinity potassium transport (Britto & Kronzucker, 2008; Szczerba *et al.*, 2009). The transporter was then characterized functionally in heterologous yeast and (*Xenopus laevis*) oocyte systems, where it was shown to indeed transport K^+ with saturable, high-affinity characteristics (although *c.* 30% conductance for Na^+ was also seen, at low external concentrations, in agreement with the behaviour of high-affinity K^+ transporters identified in earlier kinetic studies: Epstein *et al.*, 1963; Rains & Epstein, 1967). Conclusions about the transporter's potential role in primary K^+ acquisition were additionally supported by *in situ* hybridization, localizing TaHKT2;1 throughout the cortical cells of wheat roots. Based on the pH dependence of its transport kinetics, TaHKT2;1 was furthermore thought to operate as a $K^+ : H^+$ symporter, with a 1 : 1 stoichiometry (Schachtman & Schroeder, 1994). However, an alternative explanation for this was later proposed, and its function was inferred to be a $Na^+ : K^+$ symporter, based upon the observation of enhanced cation currents in *Xenopus* oocytes when Na^+ and K^+ were provided together (Rubio *et al.*, 1995). In both *Xenopus* oocytes and yeast, TaHKT2;1 was also shown to function as a Na^+ uniporter at higher (millimolar) concentrations of Na^+ (Rubio *et al.*, 1995; Gassmann *et al.*, 1996). However, Maathuis *et al.* (1996) were unable to produce *in planta* evidence of $Na^+ : K^+$ symport function in wheat (see also Epstein *et al.*, 1963; Rubio *et al.*, 1996; Walker *et al.*, 1996), and there is now largely agreement that this member of the HKT family from wheat functions as a Na^+ uniporter (Uozumi *et al.*, 2000; Laurie *et al.*, 2002; Horie *et al.*, 2009; Corratgé-Faillie *et al.*, 2010). Claims of other functions have been attributed to heterologous expression systems which may possess either different protein translation initiation machineries and/or different membrane polarization states (Haro *et al.*, 2005; Huang *et al.*, 2008). More recent work (Yao *et al.*, 2010) has, however, produced better agreement among several heterologous systems (yeast, *Xenopus* oocytes, and bright yellow cells from *Nicotiana tabacum*) as well as *in planta* analyses, at least for OsHKT2;1 and OsHKT2;2 in rice, in essence reopening the debate. As a result of the early high-profile studies, and the ensuing debate surrounding them, HKT transporters are among the most intensively studied Na^+-permeable transporters in plants (Horie *et al.*, 2009). HKT functions have since been characterized in a number of experimental systems. The *A. thaliana* genome includes only one member of the family, AtHKT1;1, whereas multiple members of at least two subfamilies are found in monocot genomes (e.g. rice exhibits at least five members of the HKT1 subfamily and four members of the HKT2 subfamily, and even more are found in polyploid wheat; Golldack *et al.*, 2002; Garciadeblás *et al.*, 2003; Huang *et al.*, 2006, 2008; Byrt *et al.*, 2007; Horie *et al.*, 2009; Zhang *et al.*, 2010). Based on biophysical and phylogenetic considerations, two subfamilies (or classes) of HKT transporters are currently distinguished (Mäser *et al.*, 2002b; Platten *et al.*, 2006; Horie *et al.*, 2009; Corratgé-Faillie *et al.*, 2010). Class 1 transporters show a preference for Na^+ conductance over that of other cations and are characterized by a serine residue in the first of the four pore domains of the selectivity

filter (the others being occupied by glycine residues, for a motif of S-G-G-G), whereas most class 2 members are characterized by superior K^+ conductance and a glycine residue in the position occupied by serine in class 1 transporters (for a motif of G-G-G-G). However, there are notable exceptions, in particular HKT2;1 from cereals, in which the glycine has reverted to serine (Horie *et al.*, 2009; Corratgé-Faillie *et al.*, 2010). Moreover, the attractive simplicity of this broad classification system has been questioned by some workers (Haro *et al.*, 2010), based on the observation of substantial Na^+ transport capacity in G-G-G-G-motif HKT members (Schachtman & Schroeder, 1994; Haro *et al.*, 2005) and the widespread existence of high-affinity Na^+ uptake in species devoid of S-G-G-G-motif HKTs (Haro *et al.*, 2010). Interestingly, selectivity filters of HKTs bear close resemblance to that of potassium channels from the KcsA family, from which HKTs are believed to have evolved (Kato *et al.*, 2001; Mäser *et al.*, 2002b; Platten *et al.*, 2006). Lan *et al.* (2010) have recently shown a minimum of two conductance modes in one HKT member in rice (OsHKT2;4), one of which greatly resembles that of cation channels (in this case, transporting predominantly Ca^{2+}).

2. The HKT1 family

The best-characterized member of class-1 HKTs is AtAKT1;1 from *A. thaliana*. Its mediation of Na^+ transport is well established (Uozumi *et al.*, 2000; Mäser *et al.*, 2002a; Horie *et al.*, 2009; Jabnoune *et al.*, 2009; Møller *et al.*, 2009; Corratgé-Faillie *et al.*, 2010), although its main role is currently believed to be in regulating Na^+ distribution between root and shoot (Berthomieu *et al.*, 2003; Sunarpi *et al.*, 2005; Huang *et al.*, 2006; Rus *et al.*, 2006; Davenport *et al.*, 2007; Horie *et al.*, 2009; Møller *et al.*, 2009; Hauser & Horie, 2010), rather than in mediating primary Na^+ entry into roots. In disagreement with this, Rus *et al.* (2001) demonstrated that mutations in *AtHKT1*;1 (in a *sos3*-mutant background) led to lower total tissue Na^+ accumulation than in wild type, suggesting a potential role in Na^+ uptake by roots. Mäser *et al.* (2002a), however, found that T-DNA insertion mutants for the gene had identical total tissue Na^+ content, and only reduced content in roots (see also Berthomieu *et al.*, 2003; Gong *et al.*, 2004; Sunarpi *et al.*, 2005; Horie *et al.*, 2006); from this, its role was inferred to be in internal distribution, not primary uptake. This discrepancy between studies may require further careful experimental work, to rule out the involvement of the transporter in root sodium uptake definitively. It is of considerable significance that members of the HKT1 subfamily should have been successfully linked to quantitative trait loci (QTL) for Na^+ exclusion from shoots, in particular TmNax1 and TmNax2 in *Triticum monococcum* (Lindsay *et al.*, 2004; Huang *et al.*, 2006; James *et al.*, 2006a; Byrt *et al.*, 2007), TaKna1 in *Triticum aestivum* (Byrt *et al.*, 2007), and OsSKC1 in *Oryza sativa* (Ren *et al.*, 2005). The equivalent of AtHKT1;1 in the cereals rice and wheat appears to be HKT1;5 (Ren *et al.*, 2005; James *et al.*, 2006a; Byrt *et al.*, 2007; Huang *et al.*, 2008; Hauser & Horie, 2010), assuming similar functions in regulating root : shoot distribution in these species. While a model of phloem localization, and thus a role in rerouting shoot-absorbed Na^+ back to the roots, was briefly favoured in the case of AtHKT1;1 (Berthomieu *et al.*, 2003), evidence for its localization in xylem parenchyma and its involvement in Na^+ removal from the xylem, reducing Na^+ appearance in the shoot, now prevails (Sunarpi *et al.*, 2005; Huang *et al.*, 2006; Davenport *et al.*, 2007; Horie *et al.*, 2009). A recent demonstration of Na^+ exclusion from the shoot by virtue of its tissue-specific overexpression in xylem parenchyma cells of *A. thaliana* (Møller *et al.*, 2009) has received particular attention. Using the enhancement trap system developed by Haseloff (1999), the authors produced two lines with enhanced *AtHKT1;1* expression in mature stelar cells, which showed reduced shoot Na^+ accumulation and enhanced salt tolerance. However, closer analysis of the data invites some caveats: Na^+ accumulation in shoots actually correlated poorly with biomass in these lines (see also Jha *et al.* (2010) for this being more generally the case in *A. thaliana*), one of the lines (J2731) was barely affected by salinity, either in the wild-type background or following overexpression (i.e. it was very difficult to gauge the impact of overexpression on growth), and the second line (E2586) was afflicted by a major pleiotropic growth penalty on account of *AtHKT1;1* overexpression, even in the *absence* of Na^+ (a growth reduction as severe as that produced by salinity imposition in the wild type; results such as this render it desirable, in general, to compile transcriptomic data of important mutants vs wild types, to ensure that pleiotropies do not obscure interpretation of the data). Therefore, the promise of *HKT1* overexpression in the genetic engineering of salt tolerance has to be viewed with caution (see also Section IX on the relationship between shoot sodium accumulation and sodium toxicity). Nevertheless, the study does demonstrate that it is possible to reduce root–shoot transfer of Na^+ by cell-targeted overexpression of *AtHKT1;1*. Whether this negates any involvement of HKT transporters in primary Na^+ entry in *A. thaliana* remains to be demonstrated conclusively. While Møller *et al.* (2009) showed no significant differences in unidirectional influxes among any of the *hkt* or wild-type lines, the excessive nature of the measured Na^+ fluxes (Section II.3), and the likelihood of the need for their reinterpretation, must be considered.

3. The HKT2 family

In contrast to HKT1, members of the HKT2 class have been clearly shown to be involved in primary Na^+ uptake in roots. This is particularly so for OsHKT2;1 in *Japonica* rice,

where *oshkt2;1* mutant alleles have been shown to lead to greatly diminished Na^+ influx into roots (Horie *et al.*, 2007; note: our reanalysis of OsHKT2;1-mediated Na^+ fluxes leads us to suspect a calculation error in this work, and in related studies, of several orders of magnitude – otherwise, the results for OsHKT2;1 would be among the largest ion fluxes ever recorded in plants). In another study on rice, Golldack *et al.* (2002) provided evidence, based on electrophysiology traces and transcript responses to variable ion provision, for a more broad-spectrum transport function for alkali cations, including sodium. As in the case of KUP/HAK/KT transporters (see Section IV), both HKT1 and HKT2 transporters are greatly up-regulated by potassium deprivation (Wang *et al.*, 1998; Horie *et al.*, 2001; Garciadeblás *et al.*, 2003; Horie *et al.*, 2007, 2009; Yao *et al.*, 2010; cf. Haro *et al.*, 2010 for exceptions to this), and an attribution of potassium-starvation-enhanced Na^+ currents (Buschmann *et al.*, 2000) to HKT (in particular TaHKT2;1) has been made, albeit not by the original authors (Horie *et al.*, 2009). Indeed, it was potassium deprivation that initially led to the discovery, in wheat, of the first HKT transporter (Schachtman & Schroeder, 1994). It is now widely believed that HKT transporters, and, again, in particular HKT2;1, allow the partial functional replacement of potassium by sodium that is often observed under saline conditions that suppress potassium uptake (Mengel & Kirkby, 1982; Flowers & Läuchli, 1983; Rodríguez-Navarro, 2000; Subbarao *et al.*, 2003; Haro *et al.*, 2010). However, many, if not most, species appear to have additional, nonHKT systems in place that can assume this function (Haro *et al.*, 2010). When tested in heterologous systems, HKTs have been clearly shown to transport Na^+ in a variety of species, including *A. thaliana*, wheat, rice, *Eucalyptus camaldulensis* and *Mesembryanthemum crystallinum* (Rubio *et al.*, 1995, 1999; Gassmann *et al.*, 1996; Fairbairn *et al.*, 2000; Uozumi *et al.*, 2000; Horie *et al.*, 2001; Mäser *et al.*, 2002a; Garciadeblás *et al.*, 2003; Su *et al.*, 2003; Jabnoune *et al.*, 2009), and *in planta* demonstrations of OsHKT-mediated Na^+ transport in rice and wheat have also been successful (Laurie *et al.*, 2002; Horie *et al.*, 2009). Several reports, however, especially in rice, have suggested that HKTs (in particular OsHKT2;1) are down-regulated by elevated concentrations of sodium, in addition to the down-regulation by elevated potassium (Horie *et al.*, 2009). Indeed, a half-time of *c.* 1.5 h has been reported for OsHKT2;1 suppression by Na^+ (Horie *et al.*, 2007), and mRNA levels of at least three OsHKTs have been shown to be inhibited at Na^+ concentrations as low as 30 mM (Horie *et al.*, 2001). If the latter findings hold more universally (see also Fairbairn *et al.*, 2000; providing functional evidence for Na^+ sensitivity in EcHKTs from *Eucalyptus* in a heterologous system), this may preclude a significant role of HKTs in Na^+ acquisition under saline conditions, as has been concluded by several groups (Møller *et al.*, 2009). Nevertheless, Laurie and coworkers, in a particularly noteworthy study in wheat, found TaHKT2;1 to be active in plants grown in the presence of significant amounts of potassium, and saline provisions of Na^+, and showed a significant reduction in $^{22}Na^+$ influx in roots coincident with reductions in *TaHKT2;1* expression (Laurie *et al.*, 2002; see also Table 1). Wang *et al.* (2007), in the halophyte *S. maritima*, also concluded, based on the sensitivity of $^{22}Na^+$ influx to Ba^{2+} and its insensitivity to TEA^+ and Cs^+ (see also Fairbairn *et al.*, 2000; Liu *et al.*, 2001; Garciadeblás *et al.*, 2003), that an HKT-type transporter may be responsible for some of the Na^+ uptake observed in this species under moderately saline conditions. The latter observations caution against an all-out dismissal of HKT involvement in root sodium uptake under saline conditions, especially if, as with primary K^+ transporters (Section IV), the inherent flux capacities of these systems are high, and thus even greatly suppressed activities might yet suffice to catalyse Na^+ acquisition.

Another feature of HKT2 transporters, their Ca^{2+} sensitivity (Fairbairn *et al.*, 2000; Horie *et al.*, 2009; Yao *et al.*, 2010; cf. Davenport & Tester, 2000), may also be examined in this light. It is well known that elevated soil Ca^{2+} concentrations can often alleviate salt stress symptoms in crops (LaHaye & Epstein, 1969; Greenway & Munns, 1980; Rengel, 1992; Epstein, 1998), and it is interesting that HKT2, like VI-NSCCs and LCT1 (Sections II and III), are greatly suppressed by Ca^{2+}. On account of this feature, the case for involvement of HKT2 in primary Na^+ influx under salinity conditions is both weakened and strengthened; weakened because soil Ca^{2+} concentrations in saline soils are usually quite high (Schachtman & Liu, 1999; Garciadeblás *et al.*, 2003; Hirschi, 2004; Kronzucker *et al.*, 2008; Zhang *et al.*, 2010), and strengthened because, if such suppressions are not complete, residual transport capacity might be sufficient to lead to Na^+ build-up in tissues. It is also interesting that at least one HKT2 transporter (OsHKT2;4) was recently shown to indeed be capable of transporting substantial quantities of Ca^{2+} (when activated by hyperpolarization), while displaying some pharmacological properties reminiscent of nonselective cation channels (such as sensitivity to La^{3+}, Gd^{3+} and Ba^{2+}; Lan *et al.*, 2010). Other workers have more directly suggested that HKT2 transporters are in fact a type of nonselective cation channel (Horie *et al.*, 2009). These are interesting suggestions, and require further examination. In this context, it is also important to point out that, in general, neat distinctions between 'transporters' and 'channels' are difficult to maintain, and dual-mode behaviour, at least at the level of electrophysiological investigation and in heterologous systems, has emerged in very many cases (Gassmann *et al.*, 1996; Fu & Luan, 1998; Kim *et al.*, 1998; Miller, 2006, 2010; Conde *et al.*, 2010; Lan *et al.*, 2010), which further complicates the picture.

VI. SOS: an ambiguous tale

The SOS1 phenotype was first identified in *A. thaliana* by means of a root-bending assay based on salt stress (Wu *et al.*, 1996), which also yielded SOS2 and SOS3 phenotypes (Zhu *et al.*, 1998), and, more recently, SOS4 and SOS5 (Shi *et al.*, 2002). Curiously, of these five proteins, AtSOS1 may be the best studied, but still has a poorly defined function (Oh *et al.*, 2010). In *A. thaliana*, AtSOS2 and AtSOS3 are essential components of a stress signalling pathway: AtSOS3, a calcineurin-like, myristoylated Ca^{2+}-binding protein, responds to an unknown primary signal (presumably a change in intra- or extracellular sodium), via changes in cytosolic Ca^{2+}, and activates AtSOS2, a serine/threonine kinase which in turn activates AtSOS1, probably via phosphorylation (Qiu *et al.*, 2002; Quintero *et al.*, 2002). How the second activation step occurs is not fully understood, but it probably involves the phosphorylation and complexing of at least one additional protein, an AtSOS3-like calcium-binding protein (SCaBP8), in addition to the phosphorylation of AtSOS1 itself (Lin *et al.*, 2009). The AtSOS2/3 signalling complex has been suggested to be involved in the regulation of other pathways and proteins that are related to salt stress, including ABA synthesis and the transporters AtNHX1, AtAKT1 and AtHKT1 (Zhu, 2002, 2003; Qiu *et al.*, 2004). AtSOS4 is involved in pyridoxal phosphate (vitamin B6) synthesis and root hair development (Shi & Zhu, 2002), while AtSOS5 is probably a cell-surface proteoglycan essential for cell expansion and for normal root growth under saline conditions (Shi *et al.*, 2003a).

Although identified using a salt-stress protocol, AtSOS1 was initially suggested to be primarily involved in high-affinity K^+ transport (Wu *et al.*, 1996). This suggestion was not unreasonable, given the close connection between salt stress and K^+ homeostasis, and is consistent with several experimental observations.These include the drastically reduced K^+ uptake at external [K^+] below 100 μM in *sos1* mutants of *A. thaliana*, even in the absence of Na^+ stress, and the abnormal growth of *atsos* mutants in general below 20 mM external [K^+] (Wu *et al.*, 1996; Ding & Zhu, 1997). In addition, there is a correlation between the salt tolerance of *atsos1*, *atsos2* and *atsos3* mutants and their K^+ (but not Na^+) tissue contents (Zhu *et al.*, 1998). Subsequent work based on sequence homologies with bacterial and fungal genes suggested that *AtSOS1* encodes a strict Na^+ / H^+ antiporter at the plasma membrane (Shi *et al.*, 2000), which was later confirmed by an 80% reduction in electroneutral Na+/H+ exchange capacity in purified plasma membrane vesicles from *sos1* mutant plants, relative to wild type (Qiu *et al.*, 2002). Nevertheless, clear links between AtSOS1 activity and K^+ nutrition exist, even if they are difficult to explain. One fruitful line of inquiry may come from the investigation of how *athkt1* mutations suppress the salt sensitivity and the low-K^+ phenotype of *A. thaliana sos* mutants (Rus *et al.*, 2004). Alternatively, Qi & Spalding (2004) suggested that K^+ and Na^+ fluxes might be tied together via AtSOS1, based on the impairment of AtAKT1-mediated K^+ influx as a result of increased intracellular Na^+, a proposed outcome of AtSOS1 malfunction. However, this explanation appears incomplete, given the finding by Ding & Zhu (1997) that *atsos1* plants are impaired in high-affinity K^+ uptake, independent of saline conditions. Other cellular functions of AtSOS1 have been suggested, including Ca^{2+} and H^+ homeostasis (Shabala *et al.*, 2005; Guo *et al.*, 2009; Oh *et al.*, 2010), oxidative and osmotic stress tolerances (Zhu *et al.*, 1998; Katiyar-Agarwal *et al.*, 2006; Chung *et al.*, 2008), vacuolar morphology and membrane trafficking (Oh *et al.*, 2010) and, possibly, signal transduction (Chung *et al.*, 2008). It must be considered, however, that some of these functions may be pleiotropisms; recent work has indicated that a large number of changes in the expression of other genes are brought about by *atsos1* mutations, even in the absence of salt stress (Oh *et al.*, 2010).

At the whole-plant level, the specific role of AtSOS1 remains uncertain. It has been variously proposed to: promote Na^+ efflux from roots into the external medium (Elphick *et al.*, 2001); facilitate Na^+ retrieval from, and delivery to, the xylem (under high and medium salt stress, respectively; Shi *et al.*, 2002); and maintain a low-sodium zone at the root meristem and elongation zone (Oh *et al.*, 2009). In addition to affecting sodium distribution, it appears to be involved in K^+ acquisition under low-K^+ conditions.

Some of the problems in assigning an unambiguous role to SOS1 come from ambiguous data from localization, mutant and cross-species studies. While AtSOS1 has been found in all vegetative tissues of *A. thaliana* (Ward *et al.*, 2003) it appears to be more specifically enriched in the root tip epidermis (Shi *et al.*, 2002), suggesting that the meristem requires special protection, particularly given the lack of vacuolation and, therefore, the lack of expression of the tonoplast Na+/H+ antiporter AtNHX1 (see Section VII below) in these cells (Shi *et al.*, 2002). Thus, root tip cells may require a mechanism distinct from AtNHX1 to restrict Na^+ concentrations in the cytosol (Oh *et al.*, 2010). AtSOS1 is also enriched in root parenchyma cells lining the vasculature, consistent with a proposed role in Na^+ partitioning between root and shoot (Shi *et al.*, 2002). However, these enriched areas of SOS1 expression have not been well confirmed in functional assays, and indeed use of vibrating microelectrodes has shown that SOS1 activity can be found throughout the length of the root (Shabala *et al.*, 2005). Moreover, the presence of AtSOS1 in xylem parenchyma, and the thermodynamic gradient powering Na+/H+ exchange (Munns & Tester, 2008), suggest that the more significant role of AtSOS1 is to direct sodium towards the leaves, a function that, however, does not seem reasonable

for a transporter associated with salt tolerance, especially given the greater sensitivity to sodium typically found in leaf tissue of glycophytes (Tester & Davenport, 2003). Nevertheless, the xylem-loading role of AtSOS1 is consistent with the observation that, under mild salt stress (25 mM NaCl), *sos1* mutants of *A. thaliana* accumulated less Na^+ in shoots (Shi *et al.*, 2002). By contrast, this role is contradicted by evidence showing that, under both low (25 mM) and high (100 mM) NaCl stress, tomato (*Solanum lycopersicum*) plants expressing low SlSOS1 activity have much more sodium in the leaves (though not in the stem) than wild type (Olías *et al.*, 2009). Moreover, at 100 mM NaCl, *A. thaliana sos1* mutants accumulated more Na^+ in shoots (Shi *et al.*, 2002), while overexpressors accumulated less (Shi *et al.*, 2003b). Shi *et al.* (2002) suggested a scenario in which the direction of the Na+/H+ antiport switches as the sodium status of the plant changes, and thus AtSOS1 could serve to retrieve Na^+ from the xylem under high sodium stress. Reversal of the direction of a flux can certainly occur depending on the experimental situation, at least in simple cellular systems (Bañuelos *et al.*, 2002), but the thermodynamic analysis by Munns & Tester (2008) indicates that the thermodynamic conditions likely to prevail *in planta* are unlikely to favour the proposed function for SOS1 in taking Na^+ up from the xylem, while extruding protons. Thus, the role of SOS1 in long-distance Na^+ transport remains ambiguous.

SOS1 is more commonly considered to drive the expulsion of sodium from the plant, but evidence for this role is likewise problematic. For instance, in *A. thaliana*, *sos1* mutants were shown to have slightly *higher* sodium efflux relative to wild type, and reduced sodium content, despite their much greater sensitivity to salt stress (Ding & Zhu, 1997). By contrast, the *sos3* mutant of *A. thaliana* did show a substantial reduction in Na^+ efflux (Elphick *et al.*, 2001). A recent study of four ecotypes of *A. thaliana* revealed an inverse correlation between *AtSOS1* expression and plant sodium content, supporting its role in efflux from the plant (Jha *et al.*, 2010). However, a comparative study between *A. thaliana* and its salt-tolerant relative *T. halophila* (synonymous to *T. salsuginea*), showed that, while the latter had 8–10 times higher expression levels of SOS1 (Oh *et al.*, 2009), it nevertheless had less Na^+ efflux than did *A. thaliana* (Wang *et al.*, 2006). In another recent study, transgenic *A. thaliana* lines constitutively overexpressing AtSOS1 did not substantially alter plant Na^+ accumulation (Yang *et al.*, 2009). Thus, multiple strands of apparently contradictory evidence obscure the details of the undeniable role of SOS1 in plant salt tolerance.

An unusual, possibly unique, feature of *AtSOS1* is that not only is its transcript up-regulated under salt stress, but the stability of the transcript itself is maintained in the presence of NaCl (Shi *et al.*, 2000; Ward *et al.*, 2003). This has been demonstrated by use of a 35S promoter driving the constitutive transcription of *AtSOS1*; even under these conditions, the transcript was only stable in the presence of salt. The very long hydrophilic C-terminus of AtSOS1, which occupies some 60% of the coding region (Katiyar-Agarwal *et al.*, 2006), appears to be essential to salt stabilization; this may occur via direct interactions with Na^+, in a manner analogous to the sensing of glucose, in yeast, by glucose transporters (Zhu, 2002). More recent evidence has indicated that the Na^+-induced stability of AtSOS1 mRNA is mediated by reactive oxygen species (ROS) (Chung *et al.*, 2008).

VII. Vacuolar storage via NHX: some lingering questions

Debates as to actual concentrations of sodium (and its deleterious effects) in the cytosol (see Section IX) aside, the sequestration of Na^+ in the central vacuole appears to be important to salt tolerance in plants. The use of Na^+ as a 'cheap osmoticum' is well established (Lehr, 1953; Marschner *et al.*, 1981) and its vacuolar sequestration, to this end, may be as important as the reduction of cytosolic Na^+. This may be particularly true under conditions where K^+ uptake is limited as a result of low soil K^+ and/or high Na^+ concentrations (see Sections IV and V). The *A. thaliana* genome project has led to the identification of a gene encoding a putative tonoplast Na+/H+ exchanger homologous to the Na+/H+ antiport system at the prevacuolar compartment of *Saccharomyces cerevisiae* (Gaxiola *et al.*, 1999). This gene, *AtNHX1*, expressed in root, leaf and floral tissues, and localized to the tonoplast membrane (in most cases; see Rodríguez-Rosales *et al.*, 2008), was shown to confer salt tolerance when overexpressed in *A. thaliana* (Apse *et al.*, 1999). Interestingly, the resultant tolerance was also associated with a greater plant Na^+ content relative to wild type, a condition that has since been reported in rice, tomato and barley (Apse & Blumwald, 2007; Liu *et al.*, 2010; see Section IX).

NHX overexpression (endogenous or transgenic) has been shown to confer salt tolerance in a wide range of plant species (cf. Yang *et al.*, 2009), including tomato (Zhang & Blumwald, 2001), *Brassica napus* (Zhang *et al.*, 2001), rice (Ohta *et al.*, 2002), maize (Yin *et al.*, 2004), wheat (Xue *et al.*, 2004), cotton (*Gossypium hirsutum*; He *et al.*, 2005), tobacco (*Nicotiana tabacum*; Lu *et al.*, 2005) and sugar beet (*Beta vulgaris*; Liu *et al.*, 2008), in addition to yeast (Aharon *et al.*, 2003). This impressive list, however, highlights a number of unanswered questions. Does the increased sequestration of Na^+ into the vacuole have consequences for primary uptake of Na^+ into the plant? How does the sequestration system prevent energy-dissipating leakage of Na^+ back into the cytosol, via nonselective cation channels in the tonoplast (see Tester & Davenport, 2003)? Does the cytosolic Na^+ concentration in fact drop in the cytosol in salt-tolerant, NHX-overexpressing plants, and is this the primary means by which tolerance is conferred? This crucial parameter has never been measured in this

context (see Section IX), and without such information, the thermodynamics and energetic cost of NHX1 activity cannot be properly evaluated. Finally, how important to survival is the increased vacuolar osmolyte (Na^+) concentration by comparison to a simultaneously reduced cytosolic Na^+ activity, under high salinity?

While NHX transporters tend to be up-regulated in response to salt stress and may be regulated by the SOS pathway (Qiu *et al.*, 2004), their strong constitutive expression suggests that they have functions other than vacuolar sequestration of sodium (Hanana *et al.*, 2009). These functions may include a role in plant development (Apse *et al.*, 2003; Hanana *et al.*, 2007); in vesicle trafficking and protein targeting (Sottosanto *et al.*, 2007); in the transport of monovalent cations besides Na^+, such as Li^+, Rb^+ and, in particular, K^+ (Wu *et al.*, 2005), all of which have been shown to be substrates for NHX antiport with protons; and a role in pH homeostasis. Interestingly, with regard to the last possibility, the NHX1 protein in morning glory (*Ipomea nil*) appears to be involved in the pH control of flower colour; an insertional mutation in *InNHX1* resulted in the partial inhibition of vacuolar alkalanization, and inhibited change in floral colour (Fukada-Tanaka *et al.*, 2000).

The roles of NHX in pH homeostasis, and in Na^+ sequestration, are inextricably linked to the activity of proton pumps in the tonoplast; simultaneous overexpression of NHX and the vacuolar pyrophosphatase AVP has led to enhanced salt tolerance in rice (Zhao *et al.*, 2006) and *A. thaliana* (Brini *et al.*, 2007). While the up-regulation of NHX in response to salt stress is well documented, that of vacuolar proton pumps is ambiguous. In wheat, for instance, one study examining the expression of the pyrophosphatase showed that at least one isoform is salt-inducible (Wang *et al.*, 2009b), while another study showed little change in response to salt stress (Brini *et al.*, 2005). In a study on cucumber (*Cucumis sativus*), vacuolar pyrophosphatase activity was inhibited by salt via putative post-translational regulation, whereas the vacuolar H^+ ATPase was stimulated by NaCl, at least in the short term (Kabala & Klobus, 2008). Nevertheless, in a recent study of *A. thaliana* ecotypes (Jha *et al.*, 2010), the up-regulation of *AtNHX1* and *AtAVP1* was positively correlated in response to salt stress in both roots and shoots.

Interestingly, a recent study (Liu *et al.*, 2010) showed that overexpression in *A. thaliana* of *NHX* genes from four plant species, and from yeast, resulted in salt tolerance, higher photosynthetic activity, more negative water potential, more Na^+ and K^+ accumulation, and more ROS scavenging in all five transformed plant types under salt stress. How many of these effects are the direct result of *NHX* overexpression, and how many are pleiotropic, remains to be determined.

The NHX story, in sum, is by and large a successful one in terms of its promise for the engineering of salt tolerance in plants (cf. Yang *et al.*, 2009). Nevertheless, the full understanding of its function will require further investigations into the manifold cellular and developmental consequences (pleiotropies?) of its expression, the thermodynamics of its mechanism, and its cross-talk with other transporters and cellular functions.

VIII. Other pathways – the apoplast and possibilities of symport with chloride

In addition to flow of Na^+ across cellular membranes to facilitate entry into the root, and consequent infiltration of the shoot, it has long been known that, at least in some species, interruptions in the endodermis can also lead to unimpeded entry of Na^+ into the xylem stream via the cell wall, a process referred to as 'apoplastic bypass' (Yeo & Flowers, 1985; Yeo *et al.*, 1987; Yadav *et al.*, 1996; Yeo, 1999; Faiyue *et al.*, 2010a,b; Fig. 2). This is particularly pronounced in many cultivars of rice (Garcia *et al.*, 1997;

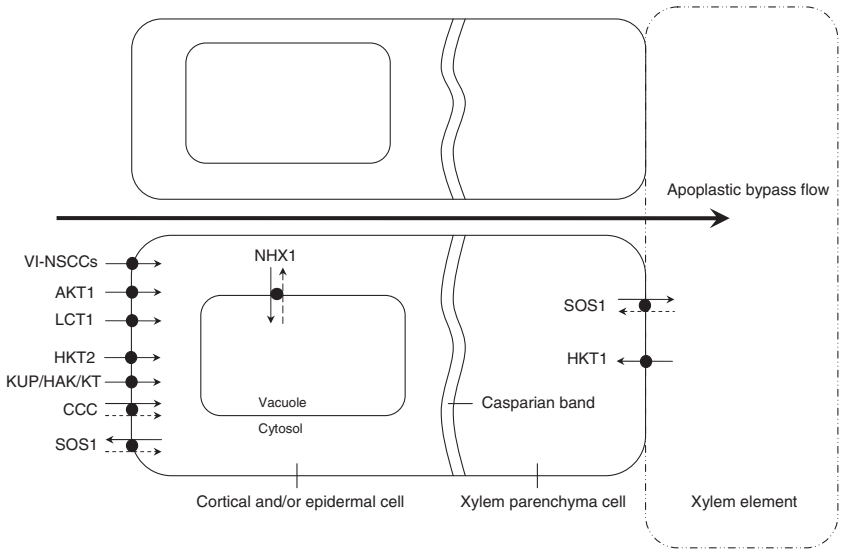

Fig. 2 Diagram of the most likely candidates for Na^+ transporters in plant root cells. Solid arrows indicate the direction of Na^+ flux, while dashed arrows indicate the direction of flux of protons (in the case of NHX1 or SOS1) or accompanying ions (in the case of cation–chloride cotransporters (CCCs)). LCT, low-affinity cation transporter; VI-NSCC, voltage-insensitive nonselective cation channel.

Malagoli *et al.*, 2008; Krishnamurthy *et al.*, 2009), where the apoplast is considered to be a major port of Na^+ entry into roots and shoots, but apoplastic bypass is also known to occur in maize, pea (*Pisum sativum*), squash (*Cucurbita pepo*) and bean (*Vicia faba*) (Peterson *et al.*, 1981), as well as barley, *Salicornia virginica* and *Spartina alterniflora* (Peterson *et al.*, 1986). Peculiarly, however, apoplastic bypass in the model system *A. thaliana*, on which most of the recent genetic and mechanistic ideas regarding Na^+ transport have been based, has not been investigated using standard methods (e.g. tracing using radiolanthanum, or the fluorescent apoplastic dye PTS, neither of which crosses the plasma membrane). In fact, we have found only one study in the literature that addresses this issue in *A. thaliana* (Essah *et al.*, 2003), but even this study only involved the use of a crude boiling technique. It may be crucial to conduct proper bypass investigations in this plant species, given its exceptional prominence in ion transport physiology.

Using the apoplastic dye PTS, Flowers and co-workers (Yadav *et al.*, 1996; Yeo, 1999) showed that rice plants displaying high Na^+ accumulation in shoots also had high rates of apoplastic flow, and that apoplastically delivered quantities of Na^+ could be sufficient to desiccate leaf tissue through osmotic stress in this species (Flowers *et al.*, 1991), in agreement with earlier proposals by Oertli (1968). The authors measured, using X-ray microanalysis, up to 600 mM free Na^+ in the apoplast of rice leaves when external Na^+ supply was at 50 mM for 7 d, enough to cause osmotic damage as proposed by the Oertli hypothesis. Similarly, Speer & Kaiser (1991), using measurements of apoplastic washing fluid, found 90 mM Na^+ in the apoplast of salt-sensitive pea at 100 mM Na^+ provided externally for 14 d. In the salt-tolerant comparator spinach (*Spinacia oleracea*), by contrast, only 7 mM apoplastic Na^+ was found, again supportive of the Oertli hypothesis (Speer & Kaiser, 1991). In a study in corn (*Zea mays*) and cotton, however, Mühling & Läuchli (2002) concluded that apoplastic bypass, while clearly present, was insufficient in these two species to produce sufficient extracellular build-up of Na^+ to produce osmotic damage in leaves; the authors found Na^+ in apoplastic washing fluid to be limited to 10–30 mM, at 150 mM Na^+ externally, in both short- and long-term applications. Thus, observations on the role of apoplastic accumulation and resulting osmotic damage are contradictory, may be species-specific (being especially pronounced in rice), and may require more thorough investigation under variable growth conditions, and in particular with respect to the duration of external Na^+ supply and the protocol of Na^+ addition, which has either been raised gradually, in smaller concentration steps, or more suddenly in the contrasting studies (Mühling & Läuchli, 2002). In the case of rice (a particularly salt-sensitive species), more recent investigations have strengthened the case for apoplastic bypass. Anatomical and histochemical analyses have documented pronounced interruptions in the endodermal layers of suberin, in particular in zones where lateral roots emerge (Ranathunge *et al.*, 2004, 2005). However, such proposals about the exact site of Na^+ entry into the apoplast have even more recently been questioned by use of rice mutants deficient in lateral root development (Faiyue *et al.*, 2010a,b). While apoplastic water flow (measured using the apoplastic dye PTS) in the latter studies was, in fact, increased in these mutants (as well as following chemical treatments reducing lateral root formation), shoot Na^+ accumulation was nevertheless reduced by 20–23%. Whether this may indicate an uncoupling of water flow from Na^+ flow in the apoplast, or limitations in the efficacy of the large molecule PTS to trace water or Na^+ movement, is unclear. Nevertheless, the authors concluded that the site of Na^+ entry is more likely to be via the tips of lateral (and, presumably, seminal) roots rather than through the zones where laterals emerge and interrupt the endodermis. A recent, detailed correlation analysis of suberin deposition, leaf Na^+ accumulation and biomass in rice (Krishnamurthy *et al.*, 2009) strongly supports a link between the integrity of the endodermis (and possibly also the exodermis, where present; Peterson *et al.*, 1993; Kotula *et al.*, 2009), apoplastic flow (the authors found a negative correlation with suberin deposition) and biomass (positive correlation with suberin deposition) under salinity challenge. Such correlations are in good agreement with the observation that in many halophytes the Casparian strip is up to three times thicker than in glycophytes (Poljakoff-Mayber, 1975; Peng *et al.*, 2004), and that such layers can be plastic and thicken under salinity stress in some species (Reinhardt & Rost, 1995). Surprisingly, however, an analysis of a suberin overexpressor mutant of *A. thaliana*, *esb1*, showed *increased* rather than decreased Na^+ infiltration into the shoot (Baxter *et al.*, 2009). The latter conclusion clearly awaits more thorough characterization of the particular mutant in question, however, in particular as external Na^+ was not raised into the saline range, and possible pleiotropies must also be considered. Also interesting to us is the observation that, in *T. halophila,* Na^+ movement to the shoot increases dramatically following disruptions in tissue integrity in the roots, as measured using propidium iodide staining (Oh *et al.*, 2009). This study supports the notion of increased apoplastic Na^+ entry into the shoot under saline conditions, and also indicates a time sequence, suggesting that tissue damage in roots may have to precede increases in apoplastic Na^+ transfer to the shoot.

It is intriguing that the existence of the apoplastic bypass route should be accepted readily for one species (rice), but entirely dismissed for others (Essah *et al.*, 2003; Plett & Møller, 2010). We consider it more reasonable for this to be a matter of degree. We further suggest, based upon a critical appraisal of published Na^+ influx values (see earlier discussion in Table 1), that, indeed, a substantial portion of reported short-term Na^+ fluxes may represent entry, and

exchange, of Na^+, by an exact route yet to be elucidated, into, and with, the root apoplast, rather than the symplast. It is of further interest in this context that the extent of apoplastic barrier development can vary substantially with growth conditions and that, in particular, hydroponic growth conditions, where roots are relatively unsupported, can lead to increased apoplastic bypass (Kotula *et al.*, 2009). Most plants subjected to Na^+ flux measurements (Table 1) have been grown hydroponically, perhaps explaining the high magnitude of many of the observed fluxes and the high degree of futile Na^+ cycling. Clearly, this important issue will require further examination.

Another potential pathway for Na^+ entry that has received relatively little attention, but should not be discounted at this time, is that of cation–anion symporters, in particular those that simultaneously, and electroneutrally, transport Na^+ (or K^+) along with Cl^-, known as the cation–chloride cotransporters (CCCs) (Haas & Forbush, 1998; Colmenero-Flores *et al.*, 2007; Zhang *et al.*, 2010). Given the typical co-presence of Na^+ and Cl^- at high concentrations in saline soils, this is a particularly attractive possibility. In animal cells, CCCs are well known to play critical roles in osmoregulation (Gamba *et al.*, 1993; Hoffmann & Dunham, 1995; Gillen *et al.*, 1996; Haas & Forbush, 1998), and their presence in plants has been known for some time. Harling *et al.* (1997) demonstrated an important role of CCCs in auxin-independent cell division control. More recently, a member of the CCC family in *A. thaliana*, AtCCC, was characterized in *Xenopus* oocytes, following microinjection of mRNA, and the authors observed simultaneously increased $^{22}Na^+$ (or $^{86}Rb^+$) and $^{36}Cl^-$ uptake which was furthermore sensitive to the sulfamyl loop diuretic bumetanide (Colmenero-Flores *et al.*, 2007). The latter pharmaceutical is used widely in gauging the participation of CCC-type transporters in animals (Blaesse *et al.*, 2009), and has a resultant therapeutic use as a highly effective diuretic in humans (reduced tissue water retention via blockage of CCCs, resulting in reduced cellular osmotic competence; Hebert *et al.*, 1996). Similarly, Zhang and coworkers (Zhang *et al.*, 2010), in *S. maritima*, observed that 100 μM bumetanide reduced tissue Na^+ accumulation in the saline range of 150–200 mM Na^+ by > 50%, lending support to the possible involvement of CCC-type transporters in primary Na^+ influx under saline conditions in this halophyte. Clearly, the possibility of more widespread CCC involvement in catalysing electroneutral Na^+ entry into plant roots deserves more attention in the future, and we have included this transporter class as a potential player in Na^+ entry in Fig. 2.

IX. 'Toxic' Na^+ fluxes, Na^+ 'homeostasis', and the question of cytosolic Na^+

Sodium exclusion, in particular sodium exclusion from the shoot, is frequently cited as one of the chief mechanisms by which salinity tolerance can be achieved (Munns, 2002; Tester & Davenport, 2003; Colmer *et al.*, 2006; Munns & Tester, 2008; Plett & Møller, 2010; Zhang *et al.*, 2010). Central to this, apart from how much Na^+ accumulates in total tissue and the relationship of this with biomass, are the issues of the rates of Na^+ intake and the resultant Na^+ concentrations in both extracellular and intracellular matrices. One of the most commonly referred to variables in this context is that of the cytosolic Na^+ concentration, and the ratio of cytosolic Na^+ concentration to cytosolic K^+ concentration (Maathuis & Amtmann, 1999). The attention paid to cytosolic Na^+ is, in part, predicated upon the notion of the direct toxic effects of the ion on enzymes, and the corresponding attention paid to cytosolic K^+ is justified by the well-established role of that ion in the activation of enzymes resident in the cytosol, or compartments that directly communicate with it and have a similar chemical composition. Indeed, it is well known that homeostatic control of K^+ in the cytosol near 70–100 mM is essential to the function of over 50 enzymes (Walker *et al.*, 1996; Leigh, 2001; Britto & Kronzucker, 2008; Szczerba *et al.*, 2009). A decrease in cytosolic $[K^+]$ under saline conditions has been documented using several methods (Hajibagheri *et al.*, 1987, 1998, 1988; Binzel *et al.*, 1988; Schröppel-Meier & Kaiser, 1988; Speer & Kaiser, 1991; Carden *et al.*, 2003; Kronzucker *et al.*, 2006, 2008), and is the result of inhibitory effects of Na^+ on both high- and low-affinity K^+ transporters (see Section IV), coupled to a stimulation of K^+ efflux (Shabala *et al.*, 2006; Britto *et al.*, 2010). Under saline conditions, cytosolic K^+ may fall to approximately one half to a third of the cytosolic K^+ concentration under healthy, nonsaline conditions; that is, to values near 30–40 mM (Carden *et al.*, 2003; Shabala *et al.*, 2006; Kronzucker *et al.*, 2008). Indeed, given the widespread observation of disruption of K^+ homeostasis by Na^+, it is an interesting question whether, rather than focusing on the ratio between Na^+ and K^+, a more parsimonious approach relating only K^+ concentrations to biomass under saline conditions may be more fruitful (that tissue Na^+ will concomitantly rise, at least somewhat, with external increases in Na^+ supply seems trite, and not particularly informative). This approach may also be more judicious, considering that, in contrast to the situation with K^+, the issue of how much Na^+ accumulates in the cytosolic compartment under saline conditions is controversial. Several seminal reviews have summarized the evidence to conclude that cytosolic concentrations of Na^+ probably do not exceed 30 mM (Tester & Davenport, 2003; Munns & Tester, 2008 – their Fig. 3a), and that 'maintenance of low concentrations of Na^+ within the cytoplasm of cells is of the utmost importance to the survival of plants in saline environments' (Plett & Møller, 2010). It has also often been stated more specifically that a critical threshold for cytosolic Na^+ lies near 100 mM (Munns & Tester, 2008), and that Na^+ concentrations above this value

would lead to toxicity for most enzymes, although for some the threshold may be lower (Greenway & Osmond, 1972; Flowers & Dalmond, 1992). Interestingly, enzymes from salt-tolerant genotypes are perhaps not significantly more tolerant of Na^+ than their counterparts in salt-sensitive genotypes (Greenway & Osmond, 1972), and, if maximal Na^+ concentrations in the cytosol of plant cells indeed are near 30 mM (Tester & Davenport, 2003; Munns & Tester, 2008), discussions of direct toxic effects of Na^+ would become moot. However, we have rarely seen this point raised. Table 2 shows that measured concentrations of cytosolic Na^+, arrived at by at least five different methods, in fact vary greatly, with consensus values under saline conditions perhaps closer to 50–200 mM (see also discussions in Binzel *et al.*, 1988; Maathuis & Amtmann, 1999; Flowers & Hajibagheri, 2001; Shabala *et al.*, 2006). It would thus in our view not be scientifically defensible to summarize the literature as having produced a consensus, at this time, that cytosolic values do not exceed 30 mM (Tester & Davenport, 2003; Munns & Tester, 2008). Rather, the latter conclusion appears to be principally based on only one study using ion-selective microelectrodes in barley (Carden *et al.*, 2003), while another method in the same genotypes, in fact, produced significantly larger values (Flowers & Hajibagheri, 2001).

The great range of reported cytosolic Na^+ concentrations, and the variability seen even when only one method is used (Carden *et al.*, 2003; a method not without significant problems of its own – see Carden *et al.*, 2001), call into question the use of the term 'Na^+ homeostasis' that has become common parlance in the salt tolerance literature (Blumwald, 2000; Adler *et al.*, 2010). To us, the term 'homeostasis' implies maintenance of a physiological condition within narrow limits, which is brought about by an intricate, cybernetic regulatory network ensuring that deflections from set points are quickly rectified. Such a condition clearly does not apply to Na^+ concentrations in plants, in particular given the toxic scenario that typically accompanies the variable accumulation levels of the ion, cytosolically and elsewhere. We therefore propose to abandon the use of this term, and replace it with more straightforward references to tissue sodium content. Similarly, we also discourage the use of the term 'toxic sodium flux' (Davenport & Tester, 2000) as unhelpful – unless an ion flux *per se* can be shown to be, in and of itself, toxic, for instance on account of a substantial energetic burden it may carry (Britto *et al.*, 2001), such a term can only be misleading. The term additionally loses meaning if resultant cytosolic and/or tissue Na^+ concentrations do not correlate well with salt tolerance.

The relationship between tissue accumulation of Na^+, particularly in the shoot, and salt sensitivity or tolerance does not appear to be straightforward either. Older paradigms often equated high shoot Na^+ concentrations with salt sensitivity and biomass decline (Tester & Davenport, 2003), and recent breakthroughs in cell-specific overexpression of *AtHKT1;1* to facilitate relative exclusion of Na^+ from shoots has been, accordingly, hailed as a major step towards engineering salt tolerance (Møller *et al.*, 2009). However, in many cases, including in *A. thaliana*, where the breakthrough was achieved, correlations between shoot Na^+ concentration and biomass under saline conditions are actually not strong (Jha *et al.*, 2010), an issue also evident in the aforementioned study (Møller *et al.*, 2009). More pertinent to agronomic concerns, an extensive study in bread wheat (Genc *et al.*, 2007) also failed to establish a correlation between salt tolerance and tissue Na^+ exclusion, or the potassium:sodium ratio on a total-tissue basis. It is of obvious related interest that, in many halophytes, high concentrations of tissue Na^+, including in shoots, have evolved as an adaptive strategy to confer osmotic competence (Cheeseman, 1988; Flowers *et al.*, 2010). Furthermore, the successful engineering of salt tolerance by overexpression of NHX transporters in the vacuolar membranes of several species (Blumwald, 2000; Yamaguchi & Blumwald, 2005; Apse & Blumwald, 2007; Adler *et al.*, 2010) has in many cases led to elevated tissue Na^+ concentrations (see Section VII), in part emulating the strategy of halophytes. The solidity of the relationship between tissue Na^+ exclusion and salt tolerance is, therefore, questionable and, consequently, so may be the significance (let alone universality) of approaches that will only minimize transfer of Na^+ to shoots as a promising path to the engineering of salt tolerance.

X. Concluding remarks

In his 1986 review, J. M. Cheeseman stated that 'it is unclear how many different types of transporters must actually be involved' in Na^+ transport. Some two and a half decades of intensive and novel research later, transport physiologists continue to be beset by this lack of clarity (Zhang *et al.*, 2010). In some ways, the complexity of sodium transport in plants appears to exceed that of most other ions, resulting in models of influx, efflux, sequestration, long-distance transport and recirculation whose complexity seems disproportionate to the extremely limited value of Na^+ as a provisional plant nutrient, and, perhaps, at odds with its toxic nature.

We must question why there has simultaneously been so much apparent progress in this field since Cheeseman's review, while at the same time the fundamental mechanistic principles of sodium transport in plants remain obscure. Certainly, the great variety of strategies by which plants cope with saline environments (Flowers *et al.*, 2010) suggests that universal principles are unlikely to be found. Such an impasse may be unavoidable; however, as scientists, we may also be amiss in our efforts to understand this important phenomenon, a situation that is, by contrast to nature's complexities, not irredeemable. For one thing, statements

are frequently put forward as basic facts, even when the evidence for them is quite lacking; a case in point is the cytosolic Na^+ : K^+ ratio, which may indeed be a key factor in sodium toxicity and tolerance, but has been measured only in exceedingly rare cases (see Section IX). Another major example in which an idea with weak experimental support is put forward as the scientific consensus is the idea that 'toxic Na^+ fluxes' are mediated by nonselective cation channels. As we have shown here (Section II), the links between electrophysiological analyses and macroscopic flux studies that have been used to promote this idea are very weak, and therefore NSCCs should not be put forward as the definitive means of Na^+ uptake by plants at this time.

We must therefore remain, for the time being, in a state of unknowing, however uncomfortable this may be, and be skeptical about conclusions that have perhaps been reached too hastily. This also means that we should less easily dismiss alternative possibilities underlying Na^+ transport and toxicity, including the numerous transport proteins discussed here, as well as the sobering realization that many of the ostensible plasma membrane fluxes measured *in planta* may have large artifactual components associated with them, as a result of the likely presence of apoplastic bypass.

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