Minireview

Plants and sodium ions: keeping company with the enemy

Jose M Pardo and Francisco J Quintero

Address: Instituto de Recursos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, PO Box 1052, Sevilla 41080, Spain.

Correspondence: Jose M Pardo. E-mail: pardo@cica.es

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Abstract

Plants face a dilemma about sodium metabolism. Uptake of ubiquitous sodium ions is desirable as a way to build osmotic potential, absorb water and sustain turgor, but excess sodium ions may be toxic. Information from a number of plant species about the proteins involved in sodium-ion uptake helps to explain how plants manage to take in just the right amount.

Plants have a problem in dealing with cations. The potassium ion is the preferred inorganic cation of living cells, and plants are no exception to this rule; yet almost invariably the concentration of K^+ in the soil solution is lower than the cytosolic K^+ concentration (100-200 mM), meaning that plants must actively take up and concentrate K^+ using various types of ion transporter [1]. Because Na^+ is similar to K^+, and many K^+ transporters do not discriminate sufficiently between these cations, excess external Na^+ can not only impair K^+ acquisition but also lead to accumulation of Na^+ in plant cells, and as Na^+ is toxic to cells, this is undesirable.

In terms of their ability to tolerate saline (mainly NaCl) environments, plant species are categorized into two broadly defined groups. Glycophytes are salt-sensitive plants, including most cultivated species, that do not tolerate long exposure to even mild salinity. To avert Na^+ toxicity most glycophytes rely on restricting Na^+ intake, but because the cell's interior is electronegative relative to the extracellular space, and because cation transporters in cell membranes are somewhat permeable to Na^+, there is constant influx of Na^+ down this electrochemical gradient that cannot be completely prevented [2,3]. Moreover, the outcome of long-term inhibition of K^+ acquisition by competing Na^+ is chronic K^+ deficiency. Salt-tolerant plants, or halophytes (for example, the common ice plant *Mesembryanthemum crystallinum*), in contrast, implement the alternative strategy to cope with excess ions in the soil solution [3,4]. By coupling the uptake of ions by their roots with the compartmentation of ions into cellular vacuoles, halophytes effectively manage to convert potentially toxic ions into usable osmolytes, thereby accomplishing the greatest survival trick - turning a foe into a friend.

Most agriculturally important plants are glycophytes, so soil salinity represents a significant factor hindering crop yield in large areas of the world. Although the capacity for selective ion uptake and efficient vacuolar compartmentation have long been regarded as the basis for tolerance of salinity, and so have become desirable traits in crops, a lack of understanding about the molecular entities mediating Na^+ transport and how Na^+ acquisition is coordinated has impaired progress in obtaining salt-tolerant crops. Here, we summarize recent genetic and comparative studies in various plant species that have shed light on how Na^+ uptake and vacuolar compartmentation achieve ion homeostasis during episodes of salt stress. The roles of a number of classes of ion transporter have been clarified, and the pathways of transporter regulation are beginning to be identified.

Uptake of Na^+ and K^+

Pioneering studies conducted more than 30 years ago by Epstein and co-workers (see [5]) demonstrated that Na^+ competes with K^+ for uptake by plant roots, implying that K^+ transporters are also the gates for Na^+ entry. Although the first K^+ transporters were cloned a decade ago, however, the

identity of the major pathway(s) for Na^+ uptake has remained elusive. Transporters of the HAK-KT-KUP family (which includes the high-affinity K^+-uptake transporter HAK, K^+ transporter KT, and K^+-uptake transporter KUP) are highly selective for K^+ (K_m 10-50 μM) but can also transport Na^+, albeit with much lower affinity. HAK-KT-KUP proteins are thought to be K^+/H^+ symporters (moving both molecules in the same direction), permitting a concentration of K^+ against its electrochemical gradient, to ensure K^+ nutrition [1]. Another family of transporters, the HKT-TRK proteins (which includes the high-affinity K^+ transporter HKT and its homologs), shows mixed ion selectivity when expressed in heterologous systems. The wheat TaHKT1, for example, functions as a Na^+/K^+ symporter at micromolar Na^+ concentrations but as a Na^+ uniporter (transporting only this single type of ion) at millimolar Na^+ concentrations [6], whereas the *Arabidopsis* AtHKT1 transports only Na^+ [7]. Rice has both types of transporter: OsHKT1 is a Na^+ transporter like AtHKT1 but OsHKT2 behaves as a symporter or uniporter as does TaHKT1 [8]. Transcripts of the *OsHKT* genes accumulated under low K^+ concentrations and diminish in high external Na^+ [8]; together with the nature of the transporters, these data suggest that HKT proteins might mediate substantial Na^+ uptake [1,8].

Genetic evidence supporting a significant role for HKT proteins in Na^+ uptake has been provided recently by Rus *et al.* [9]. The *Arabidopsis SOS3* (salt overly-sensitive 3) gene product is a Ca^{2+}-binding protein, deficiency in which elicits Na^+ sensitivity and an inability to grow at low external K^+ concentrations [10]. Theoretically, hypersensitivity of *sos3* mutant plants to NaCl could arise either from increased Na^+ entry or from reduced K^+ uptake. Searching for mutations in other genes that suppressed the salt-sensitive phenotype of *sos3* mutants, Rus *et al.* [9] isolated two independent loss-of-function mutants in *AtHKT1*, the only gene of the *HKT-TRK* family in *Arabidopsis*. The *hkt1* mutation dramatically reduced the net Na^+ content of *hkt1 sos3* double-mutant plants under a saline regime, to levels even lower than those of wild- type plants. Interestingly, the *hkt1* mutation also suppressed, instead of exacerbating, the low-K^+ phenotype of *sos3* plants. In fact, the K^+ content of *hkt1 sos3* double-mutant plants was higher than that of wild-type plants. Together, these results indicate that AtHKT1 is not a relevant K^+-uptake system and provide evidence of substantial Na^+ influx through AtHKT1. Because AtHKT1 is preferentially expressed in roots, it may mediate physiological Na^+ uptake in conditions of poor K^+ availability, in which Na^+ could partially substitute for K^+, for instance as osmoticum (a solute contributing to osmotic pressure) in the vacuole [1].

The recent study by Rus *et al.* [9] leaves open the question of whether or not the cation selectivity of AtHKT1 is regulated by a SOS3-dependent signaling pathway. Fungal TRK K^+ transport proteins, which are structurally related to HKTs, modulate their Na^+/K^+ selectivity according to the ionic environment and the K^+ status of the cell [1]. Because the *hkt1* mutation was not segregated from the *sos3* mutant background to assess the phenotype of a single *hkt1* mutant [9], it is unclear whether AtHKT1 is an unconditional Na^+ transporter or if its Na^+/K^+ selectivity is distorted in the *sos3* mutant background, allowing unrestricted Na^+ entry. Matters are further complicated by the likelihood that SOS3 regulates Na^+ efflux [11]. Thus, it remains possible that Na^+ uptake through AtHKT1 becomes detrimental to the plant only when other relevant Na^+ fluxes are compromised by the *sos3* mutation (Figure 1).

Roles of calcium ions and cyclic nucleotides

An interesting determination made by Rus *et al.* [9] is that the capacity of the *hkt1* mutation to suppress the Na^+ sensitivity of *sos3* mutant plants disappeared in medium with low concentrations of Ca^{2+} (0.15 mM). This suggests the operation of an alternative Na^+-influx system, different from AtHKT1, that is hampered by high Ca^{2+} (2 mM) but becomes the prevalent pathway for Na^+ entry at low external Ca^{2+} concentrations. Electrophysiological studies have confirmed the presence of non-selective voltage-independent cation channels (known as NSCCs or VICs) in *Arabidopsis* root cells [2,12,13]. These channels lack significant selectivity between monovalent cations, their affinity for K^+ being only slightly higher than their Na^+ affinity (P_K/P_{Na} = 1.49), and they are strongly blocked by external Ca^{2+} at low concentrations, with half-maximal inhibition at 0.1 mM. The probability of the NSCC/VIC channel being open decreased 10-15-fold with a 10-fold increase in external Ca^{2+} concentration [13]. On the basis of similarities between NSCC/VIC transport behavior and Na^+ uptake by intact roots, NSCC/VICs are thought to contribute substantially to total Na^+ influx in saline conditions [2,12,13].

NSCC/VICs appear to be widespread among plants but their physiological function and molecular identity have remained obscure. Recently, Maathuis and Sanders [12] have shown that some NSCC/VIC currents are rapidly and reversibly deactivated by cyclic nucleotides (cAMP and cGMP) on the cytosolic side of root cell membranes, suggesting a direct interaction of cyclic nucleotides with the channel. There are 20 candidate genes in the *Arabidopsis* genome that encode putative cyclic-nucleotide-gated channels (CNGCs), having hallmark cyclic-nucleotide- and calmodulin-binding domains [14]. In keeping with the idea that a nucleotide-sensitive NSCC/VIC forms a major pathway for Na^+ influx, supplemental cAMP and cGMP were found to improve the tolerance of *Arabidopsis* seedlings to NaCl but not to equivalent concentrations (equiosmolar amounts) of sorbitol [12]. Salt tolerance induced by cyclic nucleotides correlated with less accumulation of Na^+ after 5 days exposure to NaCl and reduced uptake of radioactively labeled $^{22}Na^+$ in short-term experiments [12]. It appears that only a subtype of NSCC/VICs are CNGCs, however, because nucleotide sensitivity was found

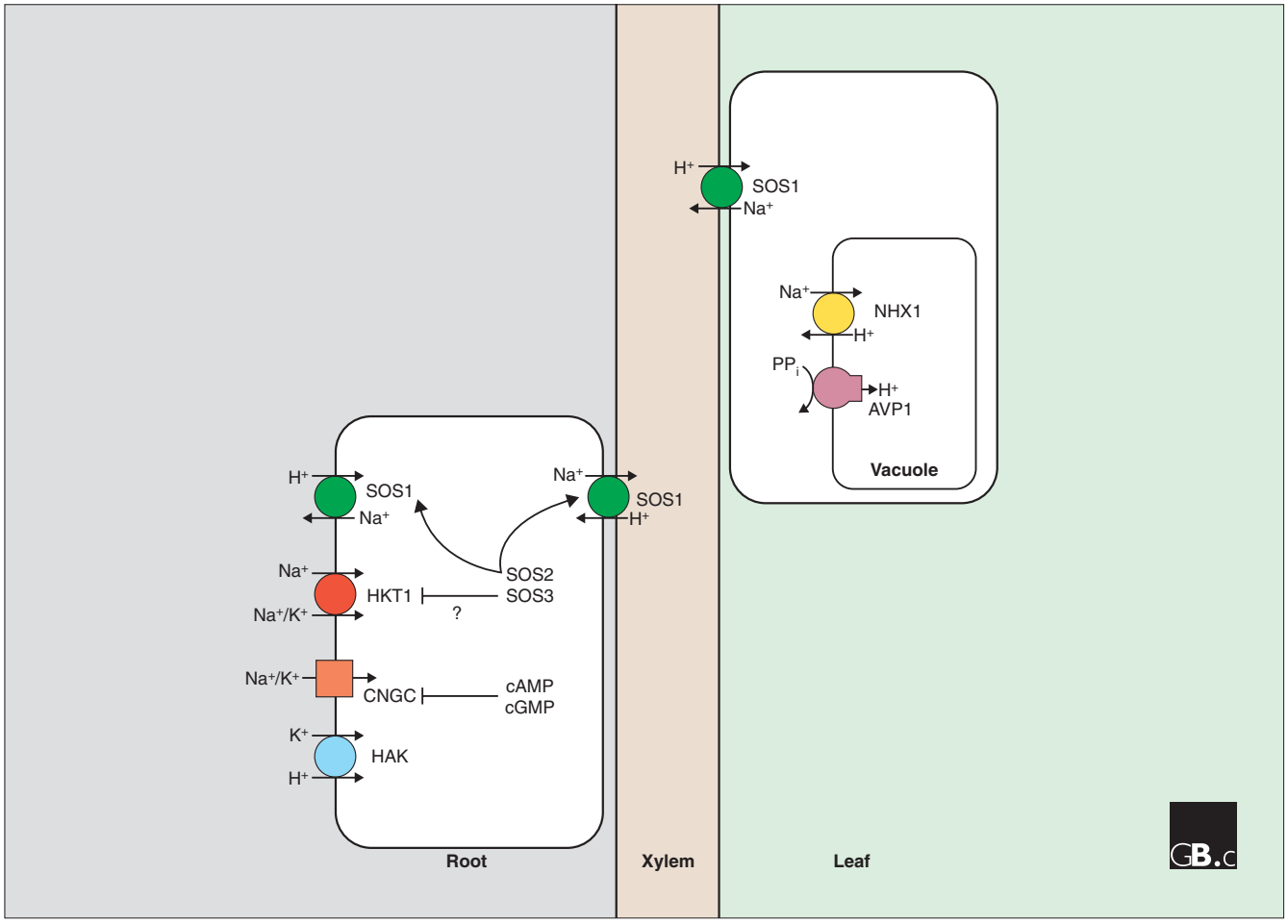

Figure 1
Model of Na^+ fluxes in plant cells. Sodium ions enter root cells through HKT proteins and non-selective voltage-independent cation channels, some of which (labeled CNGC) are inactivated by cyclic nucleotides (cAMP and cGMP). Although there is no direct experimental evidence for this suggestion, the transport activity or ion selectivity of HKT proteins could be regulated by a process dependent on the Ca^{2+} sensor SOS3 to prevent excessive Na^+ uptake. SOS3 associated with the protein kinase SOS2 positively regulates the activity of the plasma membrane Na^+/H^+ antiporter SOS1, which in turn mediates Na^+ extrusion and possibly long-distance Na^+ transport from roots to shoots [11,21]. HAK is a K^+/H^+ symporter that can transport Na^+ at low affinity. Cytoplasmic Na^+ is compartmentalized into vacuoles within cells by the tonoplast (vacuolar membrane) Na^+/H^+ antiporter NHX1, dissipating the H^+ gradient generated by the V-ATPase (not shown) and the pyrophosphatase AVP1 (which hydrolyzes pyrophosphate, PP_i).

in only a fraction of cells exhibiting NSCC/VIC currents [12]. Moreover, the *Arabidopsis* polypepetide CNGC2, which has homology to animal ion channels, is activated by cyclic nucleotides, rather than inactivated, and displays different transport properties from NSCC/VICs [15]. A mutant screen for improved tolerance to Na^+ at low external Ca^{2+}, perhaps in the *sos3* mutant background, could confirm whether or not NSCC/VICs contribute to the Ca^{2+}-sensitive pathway for Na^+ influx unmasked by the *hkt1* mutation, and could establish whether these channels belong to the CNGC family [9].

Ion compartmentation

Ion compartmentation is one successful strategy used by plant cells to cope with saline stress, because it couples growth by cell expansion, mainly due to an enlarging vacuole, with ion sequestration and detoxification from the cytosol. Accrued ions provide energetically cheap osmoticum that helps balance the plant's osmotic potential with that of the hypertonic soil solution, thereby alleviating the water deficit that is inherent to plants in a saline environment. The Na^+/H^+ antiporters (which transport the two ions in opposite directions) at the tonoplast (vacuolar membrane), mediate the transport of Na^+ into the vacuoles, with the energy for this coming from the electrochemical gradient of protons generated by the vacuolar H^+-ATPase and H^+-pyrophosphatase [4].

Gaxiola *et al.* [16] have reported inducing increased salt- and drought-tolerance in *Arabidopsis* plants by moderate overexpression of the endogenous *Arabidopsis* vacuolar H^+-pyrophosphatase, AVP1. The stress tolerance of plants

exposed to salinity was correlated with more negative solute potential (that is, greater concentration of total osmolytes), which permitted better water retention by desiccating plants, and with larger pools of Na^+ and K^+ (a 20-40% increase in concentration). Presumably, Na^+ accumulation occurs because of a greater H^+ gradient and enhanced Na^+/H^+ exchange across the tonoplast, although the activity of the Na^+/H^+ antiporter in vesicles of this membrane was not experimentally determined. Accumulation of K^+ could also result from the activity of the vacuolar antiporters, one of which, NHX1 (Na^+/H^+ exchanger 1), has been shown to transport Na^+ and K^+ [17]. Consistent with this interpretation, overexpression of the vacuolar cation antiporter NHX1 also increased the salt tolerance of *Arabidopsis*, tomato and canola plants [18-20]. Vacuoles isolated from leaves of transgenic *Arabidopsis* sustained higher Na^+/H^+ exchange rates, which directed greater Na^+ accumulation into aerial tissues than in wild-type plants. Drought tolerance of NHX1 transgenics was not examined, however, leaving the unanswered question of whether stress tolerance imparted by AVP1 can be explained solely by NHX1 upregulation.

It would be informative to compare AVP1 and NHX1 plants side by side and to test whether plants overexpressing both proteins are more stress-tolerant than either of the single-gene transgenics. Similarly, given that plants engineered to amass more organic solutes, such as proline or polyalcohols, are also stress-tolerant [3], super-plants combining a greater capacity for the accumulation of ions in the vacuole and for accumulation of compatible solutes in the cytosol hold great promise for substantially improved fitness in adverse environments. By looking in a variety of plant species, genomic tools promise to assist in furthering our understanding of the molecular basis for salt-tolerance, and our ability to engineer it, in the future.

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