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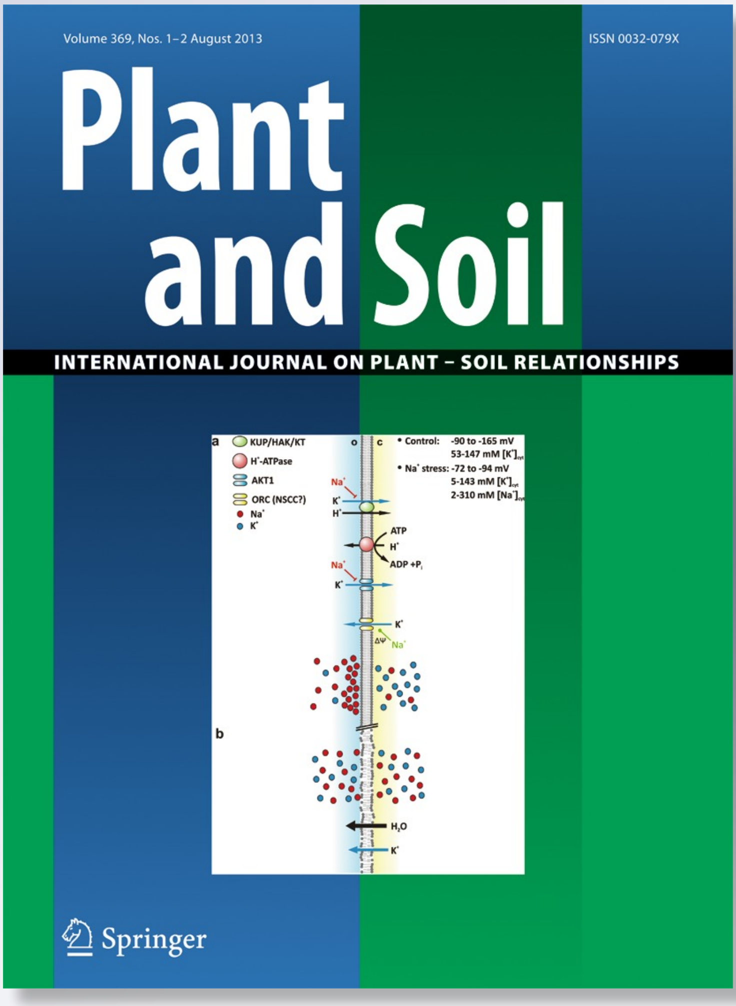

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MARSCHNER REVIEW

Sodium as nutrient and toxicant

**Herbert J. Kronzucker · Devrim Coskun ·
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Abstract

Background Sodium (Na^+) is one of the most intensely researched ions in plant biology and has attained a reputation for its toxic qualities. Following the principle of Theophrastus Bombastus von Hohenheim (Paracelsus), Na^+ is, however, beneficial to many species at lower levels of supply, and in some, such as certain C4 species, indeed essential.

Scope Here, we review the ion's divergent roles as a nutrient and toxicant, focusing on growth responses, membrane transport, stomatal function, and paradigms of ion accumulation and sequestration. We examine connections between the nutritional and toxic roles throughout, and place special emphasis on the relationship of Na^+ to plant potassium (K^+) relations and homeostasis.

Conclusions Our review investigates intriguing connections and disconnections between Na^+ nutrition and toxicity, and concludes that several leading paradigms in the field, such as on the roles of Na^+ influx and tissue accumulation or the cytosolic K^+/Na^+ ratio in the development of toxicity, are currently insufficiently substantiated and require a new, critical approach.

Keywords Salinity · Sodium · Plant nutrition · Sodium toxicity · Ion transport · Potassium

Introduction

Sodium is the sixth most abundant element in earth's crust, where it comprises some 2.8 % (Lutgens and Tarbuck 2003), and, after chloride, is the second most abundant solute in the oceans. In the latter, it typically prevails at concentrations near 470 mM (Harris 1996; Epstein and Bloom 2005), and these can be higher still in areas of high evaporation and limited rainfall, such as regions near 30° latitude. It is instructive to ponder the evolution of early life in this salt-dominated environment, and it, thus, comes as little surprise that marine organisms, from protists to animals, are highly salt-tolerant, and indeed require Na^+ for survival. Even in terrestrial animals, Na^+ has retained its role as an essential nutrient. In mammals, blood serum Na^+ concentrations are held high, by virtue of strict homeostatic mechanisms (see e.g. Grinstein and Rothstein 1986), near 135–145 mM, and even higher levels are routinely achieved in the renal system and in urine (Segen and Stauffer 1998). By contrast, the majority of land plants, when exposed to concentrations similar to those found in the mammalian blood stream, let alone that of the oceans, suffers moderate to severe toxicity symptoms. Such concentrations are, however,

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frequently found, and exceeded, in soils, and this occurs rarely as a uniform distribution but is associated with much spatial heterogeneity (Bazihizina et al. 2012). Only a few groups of terrestrial plants, the halophytes, have (re-)acquired the ability to tolerate, and indeed thrive on, high-Na^+ media, while others, however, appear to have the ability to utilize the Na^+ ion for several key cellular processes and can benefit from sodium's presence, as long as supply concentrations remain below osmotically challenging ones. Here, we will focus on the literature covering these beneficial effects in higher plants, with a special emphasis on the few mechanistic explanations that have emerged, and, in addition, on salient open questions in the otherwise more thoroughly researched area of sodium toxicity, in particular as they relate to, or emanate from, deliberations of the former. Special focus will also be brought to interactions between sodium and potassium, wherein much insight into mechanisms of growth and performance enhancement as well as impairment can be found.

Sodium as a nutrient

From beet to chocolate: the classic literature—Na^+ benefits are common

It has long been known that Na^+ can be of benefit to the growth of algae and cyanobacteria (Allen and Arnon 1955; Simonis and Urbach 1963; Brownell and Nicholas 1968), but, for higher plants, the reputation of the ion as a toxic one has held sway (Maathuis 2007; Munns and Tester 2008; Kronzucker and Britto 2011; Cheeseman 2013), and the vast majority of higher-plant literature on the ion has focused on this aspect, even though studies in a wide variety of species, including such important cultivated ones as tomato, potato, carrot, cacao, and cereals, have demonstrated the potential benefit of the ion for higher-plant growth as well (Wheeler and Adams 1905; Lehr 1941; Lehr and Wybenga 1955; Woolley 1957; Williams 1960; Brownell 1965; Brownell and Jackman 1966; Montasir et al. 1966; El-Sheikh et al. 1967; Hylton et al. 1967; Draycott and Durrant 1976; Galeev 1990; Takahashi and Maejima 1998; Gattward et al. 2012). It is important to emphasize that every substance has a threshold below which it is not toxic, in accordance with the "*sola dosis facit venemum*" (only the dose makes the poison) principle, famously attributed to Theophrastus Bombastus von Hohenheim (*Paracelsus*), but, for Na^+, beneficial effects are seen well into the range of concentrations that would be considered high for ordinary nutrient ions, such as NO_3^-, NH_4^+, or K^+, and, in the cases of halophytes, go far beyond that (Flowers and Colmer 2008). Many of the studies on beneficial effects have focused on the partial to near-complete replacement of potassium by sodium, typically in the concentration range of several millimolar, reporting either no negative, or indeed palpably positive, effects on plant growth and yield. In many of the cases, growth was particularly stimulated when K^+ supply was low, and plants suffered at least partial K^+ deprivation (Mullison and Mullison 1942; Wallace et al. 1948; Lehr 1950; Cope et al. 1953; Lancaster et al. 1953; Lehr 1953; Gammon 1953; Truog et al. 1953; Ulrich and Ohki 1956; Flowers and Läuchli 1983; Subbarao et al. 2001; Subbarao et al. 2003).

Tables 1, 2, and 3 summarize a substantial body of studies that have reported such beneficial effects, listing the species examined, the parameters reported, and the Na^+ concentrations at which effects were seen. Aside from growth (improved root and shoot biomass) and yield, results in early studies were often reported as readily visible changes, such as improvement in colour (i.e. greener leaves) and its maintenance in later growth stages, and, related to this, less pronounced manifestations of nutrient deficiency (chlorosis or necrosis), or greater "gloss" on leaves, suggesting an increase in cuticular wax formation (Harmer and Benne 1945; Brownell and Crossland 1972). Other reports have commented on the taste and texture of crops grown with additions of sodium (Harmer and Benne 1945; Truog 1953; Zhang and Blumwald 2001).

Among the species examined, the Chenopodiaceae, including the important crops spinach, beet, and sugar beet, have received particularly detailed attention, both in terms of physiological and field investigation (Larson and Pierre 1953; Lehr and Wybenga 1955; Tinker 1965; El-Sheikh et al. 1967; Draycott et al. 1970; Judel and Kuhn 1975; Draycott and Durrant 1976; Jeschke 1977; Milford et al. 1977; Durrant et al. 1978; Draycott and Bugg 1982; Flowers and Läuchli 1983; Nunes et al. 1984; Pessarakli and Tucker 1985; Peck et al. 1987; Magat and Goh 1990; Haneklaus et al. 1998; Subbarao et al. 1999a, b). Beneficial effects in this family are pronounced, and

Table 1 Survey of published studies that report Na^+ as a beneficial nutrient (micronutrient, ≤1 mM) for some plant species

Species	$[Na^+]_{external}$	Measure(s) improved by Na^+	Reference
Barley (*Hordeum vulgare*), *Atriplex nummularia*, *A. paludosa*, *A. quinii*, *A. semibaccata*, *A. inflata*, *A. leptocarpa*, *A. lindleyi*, *A. spongiosa*, *A. semilunalaris*, *A. hortensis*	0.4 mM	Total DW[a]	Brownell 1968
Atriplex nummularia and *A. inflata*	0.1 and 0.6 mM	Shoot FW[b]; leaf chlorophyll, sugar, and starch concentration	Brownell and Jackman 1966
Japanese millet (*Echinochloa utilis*), Bermuda grass (*Cynodon dactylon*), Shortleaf spikesedge (*Kyllinga brevifolia*), Joseph's-coat (*Amaranthus tricolor*), *Kochia childsii*, Moss-rose (*Portulaca grandiflora*)	0.1 mM	Total DW	Brownell and Crossland 1972
Kochia childsii, Rhodes grass (*Chloris gayana*), *Amaranthus caudatus* L.	0.1 mM	Total FW	Johnston et al. 1988
Joseph's-coat (*Amaranthus tricolor* L.)	0.5 mM	Growth rate	Ohta et al. 1987
Maize (*Zea mays* L.), Barnyard grass (*Echinochloa crus-galli* L.), Hog millet (*Panicum miliaceum* L.), Kleingrass (*Panicum coloratum* L.), *Panicum dichotomiflorum*, *Panicum maximum*, Rhodes grass (*Chloris gayana*)	0.1 mM	Growth rate	Ohta et al. 1988
Joseph's-coat (*Amaranthus tricolor* L.)	0.5 mM	RGR[c], nitrate reductase activity, O_2 evolution, chlorophyll content	Ohta et al. 1989
Tomato (*Solanum lycopersicum* L.)	1 mM	Total DW	Woolley 1957

[a] Dry weight

[b] Fresh weight

[c] Relative growth rate

this may well relate to their ecological habit as ruderal, r-strategic, species (Desplanque et al. 1999), capable of substantial growth rates and physiological flexibility in response to rapidly changing environments. The ability to, opportunistically, utilize the Na^+ ion when available, as an osmoticum to aid with rapid expansion growth (see later), may impart a significant competitive advantage in such potentially fast-growing species. Interestingly, many of the same species are also known to "luxury-consume" the NO_3^- ion when it is readily available, and accumulate it to high levels in particular in vacuoles, to the point that such levels are often considered a health hazard for human consumption, as, for instance, in spinach and beet (Phillips 1971; Stanford et al. 1977). The Chenopodiaceae are furthermore interesting in that they can benefit from Na^+ even when K^+ is present at significant concentrations alongside it in the growth medium (Marschner 1995; Subbarao et al. 2003), i.e. they do not require K^+ depletion before effects are seen. In other words, effects are not limited to those attributable to a "replacement" of K^+.

A further group for which much information is available, since early work focusing especially on the saltbush *Atriplex*, is that of species that engage in the C_4 habit of photosynthesis. The paradigm that C_4 species benefit particularly substantially, and indeed in many, albeit not all, cases require Na^+ as an essential nutrient, is now widely accepted (Harmer and Benne 1945; Barbier and Chambannes 1951; Brownell and Wood 1957; Brownell 1965; Alekseev and Abdurakhamanov 1966; Brownell and Jackman 1966; Brownell and Crossland 1972; Marschner 1995; Pessarakli and Marcum 2000; Pessarakli 2001; Subbarao et al. 2003). Important exceptions include the leading C_4 crops corn and sorghum, which have been shown not to benefit from Na^+ addition (Ohta et al. 1988; Ohnishi et al. 1990; Subbarao et al. 2003). In C_4 species, it is thought that Na^+ facilitates the conversion of pyruvate into phosphoenolpyruvate (PEP), which occurs in mesophyll cells, prior to the Calvin cycle (Johnston et al. 1988). Johnston et al. found that, under conditions of Na^+ deficiency in the C_4 species *Amaranthus tricolor*, the C_3 metabolites alanine and

Table 2 Survey of published studies that report Na^+ as a beneficial nutrient (macronutrient, >1 mM) for some plant species

Species	$[Na^+]_{external}$	Measure(s) improved by Na^+	Reference
Bladder saltbush (*Atriplex vesicaria*)	0.04–1.2 mM	Total FW[a], DW[b]	Brownell 1965
Bladder saltbush (*Atriplex vesicaria*)	0.1–1.2 mM	DW	Brownell and Wood 1957
Beet (*Beta vulgaris* L.)	1–32 mM	Shoot FW, DW; Root FW, DW	El-Sheikh et al. 1967
Suaeda aegyptiaca	5–500 mM	Shoot FW, DW; succulence	Eshel 1985
Cacao tree (*Theobroma cacao*)	0.25–2 mM	Photosynthetic rate, WUE[c], mineral nutrition	Gattward et al. 2012
Beet (*Beta vulgaris* L.)	16 mM	Water content, total FW	Lawlor and Milford 1973
Barley (*Hordeum vulgare* L.)	8 mM	Total DW	Mullison and Mullison 1942
Beet (*Beta vulgaris* L.)	2–10 mM	Leaf area and DW	Nunes et al. 1984
Atriplex portulacoides L.	20–700 mM	RGR[d]	Redondo-Gómez et al. 2007
Arthrocnemum macrostachyum	171–510 mM	RGR	Redondo-Gómez et al. 2010
Lolium perenne L., Timothy (*Phleum pratense* L.)	1–13 mM	Nitrate reductase activity	Smith et al. 1980
Beet (*Beta vulgaris* L.)	3.75–4.90 mM	Leaf area and water content, total DW	Subbarao et al. 1999b
Beet (*Beta vulgaris* L.)	1.7–3.5 mM	Total DW	Truog et al. 1953
Ranger alfalfa (*Medicago sativa* L.)	4 mM	Total DW	Wallace et al. 1948
Halogeton glomeratus	1–100 mM	Width of lateral branches; total DW	Williams 1960

[a] Fresh weight

[b] Dry weight

[c] Water-use efficiency

[d] Relative growth rate

pyruvate increased, whereas central C_4 metabolites, such as malate, aspartate, and PEP, decreased. In the C_3 species tomato, by contrast, none of these metabolites was influenced by Na^+ (Johnston et al. 1988). Furthermore, in the C_4 species *Atriplex tricolor* and *Kochia childsii*, when sodium-deficient, the activity of photosystem II (PS II), in mesophyll chloroplasts, was altered, whereas it remained unaffected in bundle-sheath chloroplasts (Johnston et al. 1989; Grof et al. 1989). Shoot nitrate assimilation is also confined to mesophyll cells in C_4 species (Moore and Black 1979; Vaughn and Campbell 1988). As Na^+ is known to enhance nitrate uptake in roots and nitrate assimilation in leaves, as shown in *Amaranthus tricolor* (Ohta et al. 1989), this may serve as one contributor to the beneficial effects seen.

Na^+-coupled pyruvate transport in chloroplasts: one solid role for Na^+ in C_4 species

Sodium is known to influence C_4 metabolism in several species in another significant way, by virtue of facilitation of pyruvate uptake into chloroplasts. This was first demonstrated physiologically in *Panicum miliaceum*, where pyruvate uptake into chloroplasts was found to be proportional to the concentrations of Na^+ supply, indeed following a 1:1 ratio for Na^+:pyruvate in the transport function (Ohnishi and Kanai 1987). This suggested the existence of a Na^+-dependent pyruvate cotransport system in chloroplasts, possibly driven by a light-stimulated, H^+-coupled, Na^+ efflux pump (Ohnishi et al. 1990; Furumoto et al. 2011). Pyruvate is central to the CO_2-concentrating mechanism in C_4 species, and it serves as a precursor to several major biochemical pathways in all plants, such as fatty acid synthesis and isoprenoid metabolism; furthermore, as the end product of glycolysis, it is positioned at a key intersection point of both primary and secondary metabolism (Schwender et al. 2004). In C_4 species engaging in the 'MEP' (methyl-erythritol-phosphate) pathway, pyruvate import into chloroplasts, across the envelope, is pivotal to the C_4 habit, and it is specifically here where Na^+-coupling is now established (Weber and von Caemmerer 2010). By contrast, at the functional level, the Na^+-coupled mechanism is not found in chloroplasts of corn, offering at least a partial

Table 3 Survey of published studies that report Na^+ as a beneficial nutrient for some plant species. Sodium concentrations in these studies were expressed as kg/ha or g/kg soil, but since field capacity of the soils were not specified, they cannot be compared directly with other studies

Species	$[Na^+]_{external}$	Measure(s) improved by Na^+	Reference
Eucalyptus grandis	68 kg/ha	Tree height, basal area, biomass	Almeida et al. 2010
Beet (*Beta vulgaris* L.)	100–800 kg/ha	Sugar yield	Draycott and Bugg 1982
Beet (*Beta vulgaris* L.)	150 kg/ha	Sugar yield	Draycott and Durrant 1976
Beet (*Beta vulgaris* L.)	45–225 kg/ha	Sugar yield	Draycott et al. 1970
Beet (*Beta vulgaris* L.)	100 kg/ha	Sugar yield	Durrant et al. 1974
Beet (*Beta vulgaris* L.)	250 kg/ha	Water content, root DW[a], sugar content	Durrant et al. 1978
Pangola grass (*Digitaria eriantha*)	87 and 176 kg/ha	DW	Gammon 1953
Beet (*Beta vulgaris* L.)	30–90 kg/ha	FW[b], water content	Haneklaus et al. 1998
Beet (*Beta vulgaris* L.), Cabbage (*Brassica oleracea*), Swiss chard (*Beta vulgaris*)	560–1121 kg/ha	Yield (FW)	Harmer and Benne 1945
Celery (*Apium graveolens*), Beet (*Beta vulgaris* L.), Wheat (*Triticum aestivum* L.), Pea (*Pisum sativum* L.)	280–1120 kg/ha	Yield (FW)	Harmer et al. 1953
Cotton (*Gossypium* [species not specified])	129.92 or 163.52 kg/ha	Total FW	Lancaster et al. 1953
Beet (*Beta vulgaris* L.), Flax (*Linum usitatissimum* L.), Oat (*Avena sativa* L.)	49.28 and 98.56 kg/ha	Total DW	Larson and Pierre 1953
Oat (*Avena sativa* L.), Turnip (*Brassica rapa* var. *rapa*), Potato (*Solanum tuberosum* L.), Italian ryegrass (*Lolium multiflorum*)	100–800 kg/ha	Yield	Lehr 1950
Cotton (*Gossypium* [species not specified])	26.88, 53.76, and 80.64 kg/ha	Total FW	Marshall and Sturgis 1953
Beet (*Beta vulgaris* L.)	0.6 g/kg soil	Total DW, succulence	Milford et al. 1977
Beet (*Beta vulgaris* L.)	300 kg/ha	Total FW	Szulc et al. 2010
Beet (*Beta vulgaris* L.)	90–180 kg/ha	Yield	Tinker 1965

[a] Dry weight

[b] Fresh weight

explanation why only some C_4 species rely on sodium as a nutrient (Ohnishi et al. 1990; Aoki et al. 1992; Weber and von Caemmerer 2010). Recently, in a benchmark study, Furumoto et al. (2011) characterized the Na^+-pyruvate cotransport mechanism at the molecular level, by identification of the *BASS2* gene in *Flaveria* and *Cleome*, and showing the protein's localization to the chloroplast envelope and the facilitation of pyruvate influx. They showed widespread distribution of the gene across various plant groups, but also its absence, at the protein expression level, in corn, in agreement with the earlier physiological observations, and in keeping with the great diversity known to exist among C_4 species (Weber and von Caemmerer 2010), and the multiple evolutionary origins of the trait (Langdale 2011; Sage et al. 2011; Williams et al. 2012). However, there now appears little doubt that the mechanism of Na^+-dependent pyruvate transport is central to the Na^+ requirement in many C_4 species. Na^+-coupled transport systems are very common in animals and marine protists, and one wonders how much flexibility might exist in other transport systems that are normally coupled to H^+ gradients (Britto and Kronzucker 2005), to utilize Na^+ gradients under special circumstances. This possibility is suggested, for instance, by the demonstration, in heterologous systems, such as *Xenopus* oocytes (albeit not necessarily *in planta*; Walker et al. 1996; Box and Schachtman 2000), that K^+ transporters of the HKT family, normally acting as uniporters or H^+-coupled mechanisms, can undergo Na^+ coupling (Rubio et al. 1995; Haro et al. 2005). Another intriguing suggestion is that fluxes of both phosphate and nitrate might be coupled to the steep electrochemical potential gradient for Na^+ in the marine angiosperm *Zostera marinus* (Rubio et al. 2005).

It is noteworthy to point out that the interest in understanding operation and optimization mechanisms, such as Na^+-pyruvate cotransport, in C_4 species is also of great importance as efforts are intensified to transfer the C_4 habit to major C_3 crops, such as rice (Furbank et al. 2009; Weber and von Caemmerer 2010).

HKT2—entry portal for Na^+ at low external concentrations, and prerequisite for the ion's benefits?

If Na^+ can be readily beneficial in so many plant species and, associated with this, accumulate to significant concentrations in plant organelles and organs to levels similar to those of K^+ (Gattward et al. 2012; Schulze et al. 2012), there must be efficient pathways for its entry across root plasma membranes. Interestingly, despite considerable effort, entry paths for Na^+ into roots have not as yet been successfully identified at the molecular level across taxonomic groups (Munns and Tester 2008; Zhang et al. 2010; Kronzucker and Britto 2011; Cheeseman 2013), while a strong body of evidence has shown, at least in grasses, that one family of genes, *HKT2* (formerly referred to as *HKT1*, but the latter designation is now reserved for a group of Na^+ transporters believed to be predominantly involved in intra-plant Na^+ transfer from root to shoot; Sunarpi et al. 2005; Møller et al. 2009), encodes transporters that can transport Na^+ at substantial rates across root plasma membranes, especially when K^+ is limiting (Horie et al. 2001, 2011; Laurie et al. 2002; Munns and Tester 2008; Hauser and Horie 2010). This is instructive, given that Na^+ benefits tend to be at their most pronounced when K^+ is in short supply, and, indeed, Na^+ can assume some of the functions of K^+. Also of interest, however, is that *HKT2* members appear to be absent in a great many plant species, including in the currently leading genetic model system *Arabidopsis thaliana* (Sunarpi et al. 2005; Munns and Tester 2008; Møller et al. 2009). *Arabidopsis* is a particularly interesting example in that it possesses only one gene from the entire *HKT* family, *AtHKT1*, and it has been suggested that this limited arsenal may be a common feature in dicotyledonous plants, while grasses typically have many members of both the *HKT1* and *HKT2* families. Haro et al. (2010), however, observed that, nevertheless, K^+-deprivation-enhanced Na^+ accumulation, and high-affinity Na^+ uptake, were observed in 16 randomly chosen crop species, including dicotyledonous ones (e.g. sunflower, onion, and alfalfa), and concluded that, therefore, the molecular picture for Na^+ uptake, even in the "high-affinity" range, is more complex than merely involving members from the HKT2 group of transporters (e.g. HKT1 might yet be found to engage in primary uptake, as suggested previously in *Arabidopsis*; Rus et al. 2001; Kronzucker and Britto 2011). Only in rice, and the Aveneae and Triticeae tribes of the Poaceae family, has high-affinity uptake been definitively associated with HKT transporters (Haro et al. 2010). Thus, HKT (in particular HKT2) transporters may be validly discussed as probable candidates for high-affinity Na^+ transport that may form a molecular foundation for Na^+ benefits in grasses, while the search for molecular candidates mediating high-affinity Na^+ uptake in other species must be considered ongoing.

In this context, it is interesting to speculate on the importance of Na^+/K^+ symport, which has been reported in several studies (Rubio et al. 1995; Spalding et al. 1999; Szczerba et al. 2008), as such a function could offer additional explanatory power for synergistic effects of co-provision of the two ions. However, only very limited (and misleading; see Haro et al. 2005) demonstrations of such a function outside heterologous expression systems, such as *Xenopus* oocytes and yeast cells, have thus far occurred (Rubio et al. 1995; Spalding et al. 1999; Haro et al. 2005). Other than these instances, little evidence for Na^+-coupled K^+ uptake exists in terrestrial plants (Maathuis et al. 1996; Rodríguez-Navarro and Rubio 2006; Corratgé-Faillie et al. 2010; Schulze et al. 2012), although it may play a significant role in aquatic angiosperms and algae (Maathuis et al. 1996). A far more common observation is that Na^+, at already modest (below-saline) concentrations, inhibits K^+-influx systems, both in the high- and low-affinity transport ranges for K^+ (Rains and Epstein 1967a, b, c; Cheeseman 1982; Jeschke 1982; Kochian et al. 1985; Benlloch et al. 1994; Schachtman and Schroeder 1994; Santa-María et al. 1997; Flowers and Hajibagheri 2001; Fuchs et al. 2005; Martínez-Cordero et al. 2005; Kronzucker et al. 2006, 2008; Nieves-Cordones et al. 2007; Wang et al. 2007), and can, additionally, stimulate K^+ efflux (Shabala et al. 2006; Britto et al. 2010; Coskun et al. 2013), thus depressing K^+-utilization efficiency in a two-pronged fashion.

Can Na^+ assume K^+ functions?

Despite its reputation as a toxic ion in the plant biology realm, from a physico-chemical perspective, Na^+ does not particularly stand out—it shares its basic chemical properties, and reactivities, with most of its alkali-metal (principal-quantum-number-one) peers, and, despite some differences in both hydrated and non-hydrated radii, is, in fact, quite similar to K^+. It certainly does not possess inherent qualities that flag it as especially toxic in relation to biological matrices and processes, unlike, for instance, strongly Lewis-acidic metals, such as transition metals and heavy metals. Indeed, on account of its physico-chemical similarity to K^+, a role for Na^+ as a generic, "benign" osmoticum in plant vacuoles is especially plausible and reasonable. However, differences in chaotropic and lyotropic properties of the two ions in terms of their effects on water and molecular structure, while controversial (Mancinelli et al. 2007; Galamba 2012), may yet emerge as important to toxicity manifestations in intracellular compartments (Cheeseman 2013), and as a foundation for the maintenance of a high cytosolic K^+/Na^+ ratio, although this has not yet been investigated stringently. A good number of the studies listed in Tables 1, 2, and 3 supports this notion. For instance, in beet, a replacement of 95 % of the plant's leaf-tissue K^+ by Na^+ resulted in no measurable negative impact on osmotic potential (Subbarao et al. 1999b). It has, thus, been concluded that a near-complete replacement of K^+ by Na^+ in its osmotic function is possible (Shabala and Mackay 2011a, b; Gattward et al. 2012). For other, non-osmotic functions of K^+, replacement by Na^+ may, however, not be as easily achieved. From the perspective of biochemical functions, such as in the cytoplasm, poorly characterized as it remains (Cheeseman 2013), there is believed to be a rather strict requirement for K^+, and a strong maintenance of its concentration (Britto and Kronzucker 2008); indeed, K^+ is considered essential for protein synthesis (Hall and Flowers 1973; Wyn Jones et al. 1979) and oxidative phosphorylation (Flowers 1974), both of which are equally inhibited by Na^+ in glycophytes and halophytes (Greenway and Osmond 1972). More generally, K^+ is considered essential for the functioning of 50–60 enzymes (Leigh and Wyn-Jones 1986). However, it has also been shown that, at the enzyme level, Na^+ can assume some of the roles of K^+ at least in several prominent instances, as, e.g., in the case of sucrose synthase (Nitsos and Evans 1969). The latter authors showed a requirement of some 50 mM K^+ for the enzyme's optimal function, and found equimolar concentrations of Na^+ to be only ~20 % effective, but not inhibitory. Similarly, NH_4^+, Rb^+, and Cs^+ were able to substitute for K^+ at ~80 % effectiveness. Thus, while not as effective as K^+, it is not inconceivable that Na^+, at modest cytosolic levels, such as those reported in some studies even under saline conditions (Carden et al. 2003), may aid enzyme function rather than inhibit it, as long as cytosolic K^+ concentration concurrently remains at a reasonable level (Kronzucker et al. 2008; and see Fig. 1). Thus, if high-uptake situations can be maintained for Na^+, in particular under low

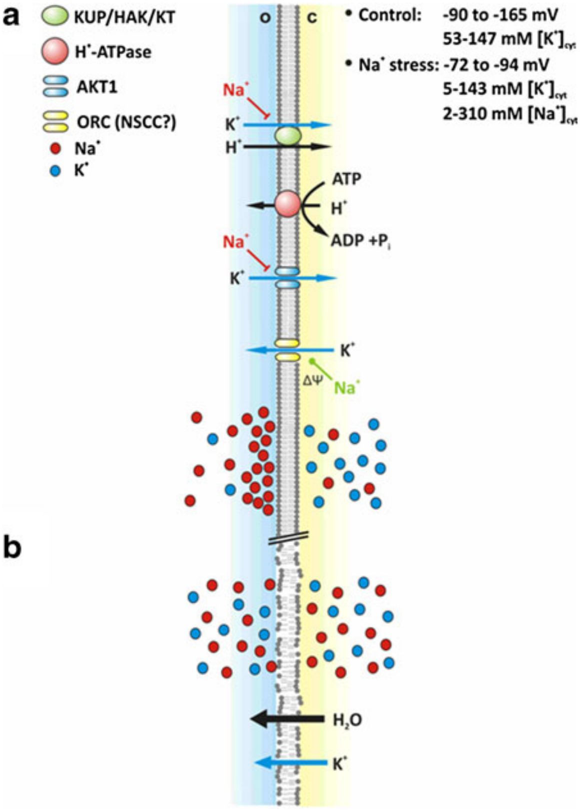

Fig. 1 Schematic illustration of the thermodynamics and mechanism of K^+ and Na^+ interactions at the plasma-membrane interface of plant roots. **a** Under non-osmotic challenges, Na^+ may inhibit (*red*) or stimulate (*green*) K^+ transport (*blue arrows*) (Santa-María et al. 1997; Thiel and Blatt 1991; Shabala et al. 2006). Cytsolic concentrations of K^+ and Na^+ ($[K^+]_{cyt}$ and $[Na^+]_{cyt}$, respectively) and resting membrane potentials can alter with long-term Na^+ stress (Hajibagheri et al. 1988; Flowers and Hajibagheri 2001; Carden et al. 2003; Kronzucker et al. 2006). **b** Sudden salinity stress can result in membrane disintegrity (osmotic shock) and the release of cellular contents, including K^+ and water (Nassery 1975; Cramer et al. 1985; Britto et al. 2010; Coskun et al. 2013)

K^+ provision (but not complete deprivation; Evans and Sorger 1966), or when K^+ uptake is partially inhibited, including by Na^+ itself (see above, and Figs. 1 and 2), Na^+ may well "rise to the challenge" of filling in for K^+ in more than one of its key functions. In this context, it is also informative that several transport proteins, previously thought to be highly ion-specific, such as KUP/HAK/KT, AKT and NHX proteins, have been shown to engage in the transport of either K^+ and Na^+ under variable circumstances (Zhang and Blumwald 2001; Venema et al. 2002; Kronzucker and Britto 2011).

Can Na^+ replace K^+ in stomatal guard cells? The mechanistic implications are not trivial

One specific subset of K^+ functions where Na^+ has received particular attention is the replacement of K^+ in stomatal guard cells (Humble and Hsiao 1969; Pallaghy 1968; Terry and Ulrich 1973; Jarvis and Mansfield 1980; Hampe and Marschner 1982; Véry et al. 1998; for review, see Robinson et al. 1997; Roelfsema and Hedrich 2005). This is to be regarded as a special case of replacement of K^+ in its osmotic role, affecting especially the vacuoles of guard cells. This stipulation is particularly interesting, given that highly K^+-specific component mechanisms have been identified for both stomatal opening and closing, and large rates of ion flow must be established to facilitate prompt, and meaningful, stomatal response (see Fig. 2; Humble and Raschke 1971; Leonhardt et al. 1997; Schroeder et al. 1984, 1987; Schroeder 1988). Indeed, while some electrophysiological studies have shown that high Na^+ flux activity, in a domain similar to what is normally seen for K^+, can be observed in guard cells (Zhao et al. 2011; cf. Schroeder et al. 1987; Schroeder 1988), current molecular-mechanistic models for stomatal function leave little room for

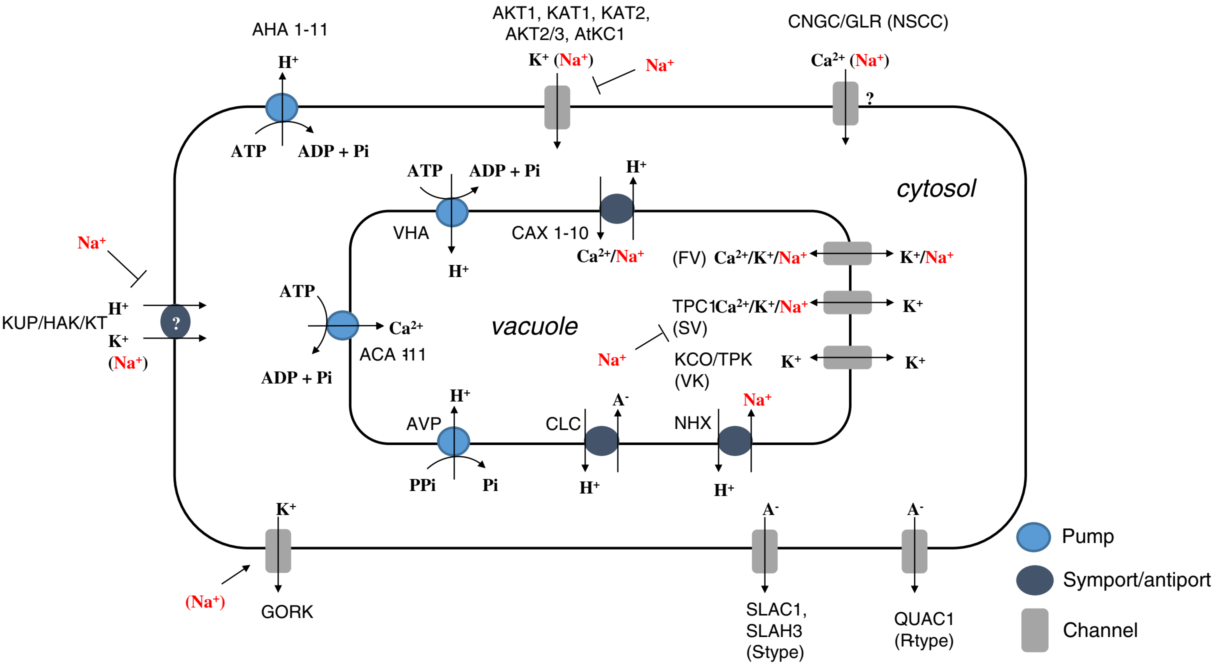

Fig. 2 Current model of ion transport in guard cells, highlighting Na^+-specific mechanisms. K^+ influx channels (KAT1, KAT2, AKT1, AKT2/3, AtKC1; Pandey et al. 2007), integral to stomatal opening, can mediate Na^+ fluxes in some instances (Schroeder et al. 1987; Schroeder 1988; Obata et al. 2007), as can members of the KUP/HAK/KT family (Santa-María et al. 1997; Takahashi et al. 2007), although their involvement in guard cell functioning is currently speculative. Moreover, both classes of transporters can be directly inhibited by Na^+ (Fu and Luan 1998; Thiel and Blatt 1991). Non-selective cation channels (NSCCs) can also mediate Na^+ fluxes (Zhao et al. 2011), although their molecular/genetic characterization is currently unknown. At the tonoplast, Na^+ sequestration is linked to cation exchangers (CAX and NHX; Zhao et al. 2008; Apse et al. 2003) and vacuolar channels (SV and FV; Ivashikina and Hedrich 2005; Isayenkov et al. 2010). SV channels are also blocked by luminal Na^+ (Ivashikina and Hedrich 2005). K^+ efflux via channels (GORK; Hosy et al. 2003), critical to stomatal closing, can be stimulated by Na^+ indirectly, via membrane depolarization (Shabala et al. 2006)

Na^+, and, to postulate its involvement, necessitates that one step outside the box of established thought.

As Fig. 2 illustrates, the entry of K^+ into guard cells, to facilitate stomatal opening, requires the engagement of potassium channels from the Shaker family (Schroeder et al. 1984, 1987; Schroeder 1988; Thiel et al. 1992; for review, see Schroeder et al. 2001; Pandey et al. 2007). The Nobel-Prize-winning work on Shaker channels has furthermore provided a model, based on X-ray crystallography, of K^+ binding on the outside of the channel that specifically disallows the binding of Na^+—according to the model, the ions are bound in their "naked", dehydrated state, and the difference in ionic radii makes it impossible for the smaller Na^+ ion to bind successfully to the oxygen-enriched "corners" of the channel (Doyle et al. 1998; Dreyer and Uozumi 2011). This model is supported by electrophysiological studies on guard cell protoplasts that demonstrate channel conductances with high (more than an order of magnitude larger) K^+ selectivity over Na^+ (Schroeder et al. 1984, 1987; Schroeder 1988; Blatt 1992; Müller-Röber et al. 1995; Nakamura et al. 1995; Véry et al. 1995; see also Véry et al. 1998; Lebaudy et al. 2008). However, given the multiplicity of inward-rectifying K^+ channels being expressed in guard cells (Szyroki et al. 2001), and that some of these have been demonstrated to be capable of mediating Na^+ fluxes, albeit mostly in other cell types (Golldack et al. 2003; Obata et al. 2007; Wang et al. 2007), routes for high-capacity Na^+ entry might exist under some conditions that are not currently accounted for in stomatal guard cell models (Fig. 2). Other such possible routes include non-selective cation channels (NSCCs), such as the cyclic nucleotide gated (CNGC) and/or glutamate receptor (GLR) channels (Lemtiri-Chlieh and Berkowitz 2004; Meyerhoff et al. 2005; Wolf et al. 2005; Zhao et al. 2011; see also Véry et al. 1998; Tyerman and Skerrett 1999). It is also possible that members of the KUP/HAK/KT family, generally attributed to primary K^+ uptake in roots (Gierth and Mäser 2007), might contribute, as these have been shown to be capable of mediating low-affinity Na^+ fluxes in roots under special circumstances (Santa-María et al. 1997; Takahashi et al. 2007; see also Mäser et al. 2002), and are also expressed in shoots (Kim et al. 1998; Rubio et al. 2000; Bañuelos et al. 2002; Su et al. 2002). However, a specific demonstration in guard cell opening and closing has not been made in any study of which we are aware. At the tonoplast, vacuolar sequestration of Na^+ has been attributed to the function of Na^+/H^+ exchangers (NHX; Blumwald and Poole 1985; Apse et al. 1999, 2003) and both slow-vacuolar (SV) (Hedrich and Neher 1987; Schönknecht et al. 2002; Ivashikina and Hedrich 2005) and fast-vacuolar (FV) channels (Isayenkov et al. 2010). It has further been suggested that members of the cation exchanger (CAX) family could transport Na^+ (Luo et al. 2005; see also Zhao et al. 2008), but, again, none of these candidates is a part of current models of guard cell function. No leading reviews or textbook treatises on stomatal guard cell function have incorporated these flexibilities and, instead, they make the case for very specific, and central, engagement of K^+ channels/transporters as integrated into a tightly orchestrated cascade of events, involving hormone binding, Ca^{2+} fluxes, and membrane potential activation of K^+ fluxes (Schroeder et al. 2001). This, when combined with established molecular channel models that predict strict exclusion of Na^+ in particular for Shaker channels (Doyle et al. 1998; Dreyer and Uozumi 2011; cf. Obata et al. 2007; Zhao et al. 2011), poses profoundly interesting questions both about physiological plasticities and the applicability of strict molecular-mechanistic paradigms.

In addition to mediating Na^+ fluxes, several guard cell transporters are affected, either directly or indirectly, and usually negatively, by sodium's presence, particularly at higher concentrations (see below). Lastly, K^+ efflux via outward-rectifying K^+ channels (e.g. GORK; Hosy et al. 2003) has been shown to be stimulated by sodium's depolarization effects at the plasma membrane, which could have adverse effects on cytosolic K^+ homeostasis (Shabala et al. 2006), and might, in turn, be expected to affect stomatal guard cell function negatively.

It is instructive that, in some cases, authors have reported superior stomatal performance in plants cultivated on high Na^+/K^+ ratios than on K^+ alone (Marschner 1995), and improved water-use efficiency (Gattward et al. 2012), suggesting that Na^+ may not only fill in for K^+ in the specific context of stomatal guard cell function, but may indeed be capable of outperforming it. We believe, in the light of the established model for stomatal function shown in Fig. 2, this warrants detailed attention at the mechanistic level, with potential for intriguing, and indeed paradigm-shifting, insights into membrane transporter function. Of further interest is the relationship between such transport activities and the tendency of some halophytes to reduce stomatal aperture in response to Na^+ supply, while some non-halophytes do the opposite (Robinson et al. 1997; Véry et al. 1998).

Sodium toxicity

The potassium target

A large number of studies has shown the disruption of both cellular and whole-plant potassium homeostasis under sodium stress (Rains and Epstein 1967a, b, c; Flowers and Läuchli 1983; Watad et al. 1991; Gaxiola et al. 1992; Warne et al. 1996; Zhu et al. 1998; Santa-Maria and Epstein 2001; Peng et al. 2004; Cakmak 2005; Kader and Lindberg 2005; Kronzucker et al. 2006; Takahashi et al. 2007; Kronzucker et al. 2008; Britto et al. 2010; Coskun et al. 2013, cf. Seemann and Critchley 1985). Potassium homeostasis is critical for proper cell function, and one might ask, applying the "*lex parsimoniae*" principle of William of Ockham, whether its disruption by Na^+ may perhaps be sufficient to explain a large part of Na^+ toxicity. It is common in complex toxicological syndromes to invoke a large number of causes, or confound causes and their effects. Manifold as the effects of Na^+ on critical processes such as photosynthesis, transpiration, production of reactive oxygen species and, ultimately, growth and yield, are (Bazihizina et al. 2012; Cheeseman 2013), many, if not most, of these must be considered as downstream effects rather than primary causes of toxicity. There is no doubt that Na^+, when appearing suddenly at high concentrations, in "shock" scenarios (see discussion in Cheeseman 2013), carries osmotic consequences that disrupt, typically temporarily, the membrane integrity of roots (Britto et al. 2010; Coskun et al. 2013), or also those of shoots, such as shown in rice (Flowers et al. 1991), preceding, or perhaps coinciding with, more "ion-specific" effects (see later discussions). In addition to this well-recognized osmotic effect, a second parsimonious explanation may lie in the disruption of potassium homeostasis—one that may reasonably and fruitfully supplant alternative, to date ill-substantiated (Kronzucker and Britto 2011), hypotheses, such as those of "toxic" Na^+ fluxes or cytosolic K^+/Na^+ ratios (Maathuis and Amtmann 1999; Davenport and Tester 2000; Yao et al. 2010; see below). The decline in cytosolic $[K^+]$ ($[K^+]_{cyt}$) under saline conditions is well documented (Hajibagheri et al. 1987, 1988; Binzel et al. 1988; Schroeppel-Meier and Kaiser 1988; Speer and Kaiser 1991; Hajibagheri and Flowers 2001; Carden et al. 2003; Kronzucker et al. 2006; see also Fig. 1) and is attributable to sodium's effects on K^+ transport (Szczerba et al. 2008; Kronzucker and Britto 2011). Na^+ has been shown to suppress K^+ influx in both its high- and low-affinity ranges, particularly at millimolar concentrations (Cheeseman 1982; Jeschke 1982; Schachtman and Schroeder 1994; Rubio et al. 1995; Gassmann et al. 1996; Maathuis et al. 1996; Santa-María et al. 1997; Martínez-Cordero et al. 2005; Kronzucker et al. 2006, 2008; Nieves-Cordones et al. 2007). Some studies have reported only weak Na^+ effects (Epstein 1961; Epstein et al. 1963; see also Seemann and Critchley 1985), or even stimulations of K^+ influx by Na^+ (Rubio et al. 1995; Spalding et al. 1999), but such studies are in the minority. As pointed out earlier in this review, and powerfully by others (Walker et al. 1996), some conflicting data on this front most probably arise from differences in experimental approaches, in particular between heterologous expression systems, excised roots, and intact plants (Haro et al. 2010). Overall, there is now broad consensus that the predominant effect of Na^+ co-presence in K^+-containing media is one of affecting K^+ homeostasis negatively (Kronzucker et al. 2008).

Figure 1 summarizes the key events that are expected to lead to compromised cytosolic K^+ homeostasis in typical root cells. Na^+ can directly inhibit high-affinity K^+ transporters of the KUP/HAK/KT family (Santa-María et al. 1997; Quintero and Blatt 1997; Fu and Luan 1998; Senn et al. 2001) and Shaker-type K^+ channels (Thiel and Blatt 1991; Qi and Spalding 2004; Fuchs et al. 2005; Wang et al. 2007). Moreover, transcript abundances of both KUP/HAK/KT transporters (Su et al. 2002; Nieves-Cordones et al. 2007) and Shaker-type K^+ channels (Su et al. 2001; Golldack et al. 2003; Pilot et al. 2003) can be affected negatively by NaCl. In most cases, transport inhibition is believed to be mediated by Na^+ binding to the outside of carriers and channels (Szczerba et al. 2008), although, in the case of some Shaker-type channels, a mechanism has also been proposed whereby small concentrations of cytosolic Na^+ (near 10 mM) can effect inhibition from the inside of the cell (Qi and Spalding 2004); such concentrations are considered easily attained (Carden et al. 2003; Kronzucker and Britto 2011). In addition, as pointed out previously, K^+ efflux via outward-rectifying channels has been shown to be stimulated by Na^+-induced depolarization of the plasma membrane (Shabala et al. 2006; Fig. 1a), which could contribute to the decline in $[K^+]_{cyt}$. Thus, a suppression of $[K^+]_{cyt}$ is one of the clear consequences of sodium's actions (perhaps not necessitating the invoking of the miraculous powers of a "cytosolic K^+/Na^+ ratio"), as is a depolarization of the plasma-membrane potential, both instantaneously, upon first exposure to Na^+ (Shabala et al. 2003, 2006; Mian et al. 2011; cf. Bowling and Ansari 1971, 1972; Cheeseman 1982; Nocito et al. 2002), and in the longer term (Malagoli et al. 2008) (Fig. 1a). Upon sudden, "shock", applications of higher concentrations of Na^+, membrane disintegrity due to osmotic shock and ionic displacement (particularly of Ca^{2+}), result in the release of cellular contents, including K^+ and water (Nassery 1975, 1979; Lynch and Läuchli 1984; Cramer et al. 1985; Britto et al. 2010; Coskun et al. 2013; Fig. 1b), offering an alternative, or additional, explanation of enhanced K^+ release under Na^+ exposure (Britto et al. 2010; Coskun et al. 2013). Combined, the above effects of impaired K^+ influx and enhanced efflux are expected to result in blockage of K^+ translocation to the shoot (Erdei and Kuiper 1979; Botella et al. 1997; Kronzucker et al. 2006; Munns and Tester 2008), with obvious consequences for downstream events such as photosynthesis and stomatal function.

Sodium accumulation: what is better—more or less?

Many studies (e.g. Schubert and Läuchli 1990; Cramer 1992; Schachtman and Munns 1992; Davenport and Tester 2000; Munns and Tester 2008; Møller and Tester 2007; Møller et al. 2009) have suggested that the excessive accumulation of Na^+, particularly in shoot tissue, lies at the heart of its toxicity. Indeed, this notion has driven much investigation into the identification of transport systems that either catalyze primary Na^+ influx into the root (Davenport and Tester 2000; Essah et al. 2003), or its distribution within the plant (Møller et al. 2009), with the tantalizing prospect of conferring salt tolerance by genetically modifying the influx or localization of Na^+. However, as we shall now discuss, evidence is mounting that the assumption that sodium accumulation must be causally linked with its toxicity is not always a safe one.

In the case of wheat (*Triticum* spp.), clearly negative correlations have been observed between Na^+ accumulation and growth (e.g. Schachtman and Munns 1992; Munns and James 2003). More recent work, however, has shown that there is considerable deviation from this simple picture. For example, Rajendran et al. (2009), who examined salt stress and accumulation in 12 varieties of *Triticum monococcum*, found that two of the four most salt tolerant varieties accumulated among the highest amounts of Na^+ in the fourth leaf (Rajendran et al. 2009); this contrasted starkly with the other two most tolerant varieties, which had the lowest Na^+ levels. Thus, within the most salt tolerant tertile for a single species of wheat, there was a three- to seven-fold variation in sodium content, indicating that the relationship between accumulation and tolerance is by no means straightforward. Similarly, in a recent large survey of bread wheat (*Triticum aestivum*) genotypes, there was no clear relationship between tissue Na^+ exclusion and salinity tolerance (Genc et al. 2007). Although it has been suggested that this was due to shoot Na^+ accumulation being below the toxicity threshold (Møller and Tester 2007), these levels were nevertheless far from negligible (100–300 mg kg^{-1}), and, more importantly, did reduce shoot dry matter, on average, by one-third compared to controls, and reduced total biomass and yield by 50 %. In the case of corn, increased influx and shoot accumulation of Na^+ has been linked to its toxicity in some studies (Schubert and Läuchli 1990; Cramer 1992), while Cramer et al. (1994) later showed that Na^+ accumulation in the shoots of two varieties of hybrid corn did not reflect their relative salt tolerances, concluding that salinity-induced growth depression was primarily due to osmotic stress. Perhaps the most surprising study of this nature in the realm of cereal grasses (Yeo et al. 1990) was conducted in rice, a particularly salt-sensitive species. In this survey of 150 rice genotypes, shoot sodium concentrations accounted for only a small degree of variation in survival under saline conditions. This is surprising, given that one of the leading causes of Na^+ toxicity in rice is thought to be apoplastic bypass flow to the shoot (Flowers et al. 1991).

Because of the substantial work that has been done at both ecophysiological and molecular levels in *Arabidopsis thaliana*, it is instructive to consider the question of how Na^+ accumulation, particularly in the shoot, affects Na^+ tolerance in this model species. In a pioneering study on Na^+ transport and accumulation in *A. thaliana*, Essah et al. (2003) showed that, although *A. thaliana* plants grown under 60-fold differences in external $[Ca^{2+}]$ showed 6-fold differences in per-plant accumulation of Na^+ (and 4-fold differences in Na^+ accumulation per gram root), there were no differences in biomass between them. More recently, screening of 12 *A. thaliana* accessions yielded two coastal populations with reduced *AtHKT1* expression that apparently resulted in increased shoot Na^+ and, surprisingly, greater salt tolerance (Rus et al. 2006). In a follow-up study (Baxter et al. 2010), many more such salt-tolerant accessions displaying this "weak allele" at the *AtHKT1;1* locus were discovered, often in populations growing in close proximity to coastal areas. Another recent survey, comparing four ecotypes of *A. thaliana*, indicated no inverse relationship between shoot Na^+ concentration and Na^+ tolerance (Jha et al. 2010).

Studies on *A. thaliana* mutations which confer salt tolerance or hypersensitivity also cast doubt on the concept of elevated tissue Na^+ being detrimental under salinity stress. For instance, *Arabidopsis* plants overexpressing the vacuolar Na^+/H^+ antiporter AtNHX1 were able to thrive on NaCl concentrations as high as 200 mM, exhibiting far superior growth than the wild type while containing about 30 % more Na^+ (Apse et al. 1999). Similarly, overexpression of *OsNHX1* in upland rice resulted in growth significantly higher than that of wild type, while at the same time displaying both increases in shoot Na^+ and decreases in root and shoot K^+ (Chen et al. 2007). Overexpression of the *HvHKT2;1* gene (whose product is implicated in sodium influx—see above) in young barley plants resulted in improved growth despite increased Na^+ uptake, translocation, and shoot accumulation (Mian et al. 2011). Conversely, disruption of the *sos1* gene, which putatively encodes an efflux transporter for Na^+ (but which, importantly, is also implicated in xylem loading for root-to-shoot Na^+ transfer), has been shown to result in *Arabidopsis* plants that are over 20 times more salt-sensitive than wild type, while accumulating as little as half the amount of tissue Na^+ (Ding and Zhu 1997).

While perhaps surprising given the emphasis on reduced Na^+ uptake and translocation being a key to improved salt tolerance, the above studies in *Arabidopsis thaliana* are consistent with others involving members of the Brassicaceae. Indeed, the salt-including habit of many species in this family has been documented as early as 1896 (von Marilaun 1896). In more recent times, He and Cramer (1993) investigated K^+/Na^+ ratios in shoot tissue of six *Brassica* species, near relatives of *Arabidopsis*, and found that they bore no relation to salt tolerance, while Porcelli et al. (1995) showed that yields and shoot growth of *Brassica napus* plants were not correlated with tissue K^+/Na^+ or Ca^{2+}/Na^+ ratios.

In cases where improvements in growth under salinity are accompanied by increased shoot Na^+ content, the concept of "tissue tolerance" is often proposed. Tissue tolerance is currently interpreted as involving effective sequestration of Na^+ into vacuoles, via transporters such as NHX1, where it will not harm cytosolic functions (Apse et al. 1999; Munns and Tester 2008; Møller et al. 2009; Jha et al. 2010). In support of this idea, Jha et al. (2010) have shown that there is a positive relationship between salt tolerance and expression levels of *AtAVP1*, which encodes an H^+-pumping pyrophosphatase that is likely to be critical in the vacuolar sequestration of Na^+. In contrast to such studies, however, the survey of rice cultivars by Yeo et al. (1990; see above) indicated that there was no correlation between shoot Na^+ and tissue tolerance; moreover, tissue tolerance was negatively correlated with both plant survival and plant vigour. In any case, even when increased shoot Na^+ and increased salt tolerance occur together, the term "tissue tolerance" appears somewhat weak. Such plants not only appear to "tolerate" higher levels of Na^+ in their tissues, but increased sequestration may in fact be a net benefit in such instances, possibly conferring greater osmotic capacity upon cells having vacuoles enriched in sodium. In this respect, beneficial Na^+-including processes in glycophytes under salt stress may operate as they do under benign conditions or in halophytes (see Section I). A more startling speculation that arises from this analysis is that engineering plants to reduce their uptake of Na^+ and/or its translocation to the shoot might not be an effective solution to the problem of salt stress; indeed, the reverse could be true in many instances. Until these accumulation issues are better resolved, the roles of Na^+ transporters in salt tolerance and toxicity will not be adequately understood. What is clear at this point is that salinity stress and tolerance is a complex, multi-faceted trait, and cannot be predicted by simple indicators such as Na^+ accumulation (Yeo et al. 1990; Møller and Tester 2007; Rajendran et al. 2009).

Osmotic and ionic effects: what is the difference?

Osmotic stresses due to the presence of high salt concentrations in both the rooting medium and plant cell walls have long been known to be the chief initial cause of sodium toxicity (Jennings 1976; Greenway and Munns 1980; Munns and Tester 2008). Severe reductions in extracellular water potentials due to high salt loads can produce rapid dehydration and consequent damage of cells, and the similarities between salt and drought stresses are remarkable (Munns 2002). In the case of rice, most, if not all, salt-induced damage in the leaves might be due to osmotic stresses caused by Na^+ buildup in the leaf apoplast (the "Oertli hypothesis"; Flowers et al. 1991; Krishnamurthy et al. 2011). Less clear, however, are the mechanisms underlying the secondary, "ion-specific" aspects of salt stress (Munns et al. 1995). Studies investigating these aspects often compare plant responses to salt provision against isosmotic provisions of non-ionic compounds such as polyethylene glycol, but these cannot provide a distinction, for example, between ion-specific effects of Na^+ and Cl^- or K^+, or between NaCl and other salts. Instead, chemically distinct, but isosmotic, salt treatments must be used (Greenway and Munns 1980; Kingsbury and Epstein 1985; Tavakkoli et al. 2010). Another caveat of such work is that, since these effects occur over a longer time scale than osmotic effects, short-term experiments are not appropriate (Munns et al. 2005; Tavakkoli et al. 2010).

While most studies of this nature do reveal non-osmotic consequences of excessive salt accumulation, their results can vary widely among plant systems; for instance, Na^+ has been found to inhibit photosynthetic processes in cereals such as rice and wheat, while Cl^- appears to be the more important ion in fava bean, and woody perennials such as citrus and grapevine (Tavakkoli et al. 2010). In addition, it remains difficult to distinguish between ion-specific and strictly osmotic effects (Greenway and Munns 1980; Yadav et al. 2011). Moreover, little is known about the deleterious action of hyperaccumulating ions *in vivo*, particularly within the cell. By contrast, the harmful effects of Na^+ on the transport and accumulation of nutrient ions, particularly K^+, is becoming very clear (see above), as reinforced by the recent finding that the Na^+-specific effects in salinity-treated bean plants were impaired K^+ and Ca^{2+} nutrition, and stomatal regulation (Tavakkoli et al. 2010).

One reason why Na^+-specific toxicity mechanisms within the cell are poorly understood is that there is little certainty regarding intracellular Na^+ concentrations, particularly in the cytosol. While the maintenance of a high K^+/Na^+ activity ratio in the cytosol of plant root cells is frequently described in the literature as being a critical determinant of plant performance under salinity stress, direct evidence supporting this contention is, by comparison, extremely rare. While there is a strong consensus from independent lines of evidence that the cytosolic levels of K^+ in plant cells are typically around 100 mM (Walker et al. 1996; Britto and Kronzucker 2008), measurements of cytosolic Na^+ differ dramatically from one another, depending on the methods used (Kronzucker and Britto 2011). This can be illustrated by comparing two studies examining the same pair of barley cultivars, one using X-ray microanalysis (Flowers and Hajibagheri 2001), the other using intracellular Na^+-specific electrodes (Carden et al. 2003); estimates from the two studies disagree from 4- to 90-fold (although it should be noted that different exposure times to elevated NaCl were used). Despite lack of consensus on this critical parameter, cytosolic K^+/Na^+ ratios are frequently invoked as central to sodium toxicity. For instance, in what is perhaps the most highly influential review on this subject (cited over 300 times), Maathuis and Amtmann (1999) provide only three references to support the claim that "cytosolic levels of Na^+ in plant cells range from low millimolar levels to around 100 mM". One of these references is to a conference abstract (Carden et al. 1998), one to a study using the giant alga *Acetabularia* (Amtmann and Gradmann 1994, which reports values for $[Na^+]_{cyt}$ of up to 300 mM), and the third reports estimates of cytosolic K^+ and Cl^- using multiple methods, but does not contain any data on Na^+ (Hajibagheri et al. 1988). Despite the rather glaring lack of convincing measurements of cytosolic sodium, however, this and other similarly data-poor reviews continue to be cited in support of its presumed toxicological role.

Nevertheless, the issue of cytosolic Na^+ accumulation may yet prove important to explaining the causes of sodium toxicity. New experimental evidence supporting this connection might soon be achieved thanks to recent improvements in intracellular Na^+-specific microelectrode technology. While not without pitfalls of their own (Carden et al. 2001), measurements with such electrodes are more direct and, perhaps, less afflicted by assumptions and technical problems than those using older methods such as compartmental analysis by tracer efflux (CATE) or X-ray microanalysis. X-ray microanalysis studies must contend with extraneous contributors to relevant spectral signals, low count rates, specimen instability, preparative artifacts, and determinations of cytosolic water content (Roomans 1988; Carden et al. 2003). CATE is often afflicted by the appearance of large tracer fluxes from poorly understood compartments, which, if erroneously identified as cytosolic, can yield extremely large artificial values of $[Na^+]_{cyt}$ (Britto and Kronzucker 2012). Indeed, reported unidirectional Na^+ fluxes across the plasma membrane, as measured with CATE or with short-term tracer uptake experiments, appear at times to be too high to be energetically feasible (Britto and Kronzucker 2009). When coupled with even the small cytosolic exchange half-times for Na^+ found in the literature (Essah et al. 2003), these values can, with CATE, result in cytosolic $[Na^+]$ values an order of magnitude higher than those reported using electrodes (Kronzucker et al. 2006; see below). Perhaps because of uncertainties with these and other methods, ion-specific microelectrode measurements of cytosolic Na^+ accumulation have become the new standard (Munns and Tester 2008). Surprisingly, however, given that cytosolic $[Na^+]$ is considered to be a critical factor in Na^+ toxicity, very few studies have measured it using microelectrode technology (Carden et al. 2003; Rubio et al. 2005); clearly, much more progress needs to be made in this area.

The study by Rubio et al. (2005) is particularly striking as it was conducted in the marine angiosperm *Zostera marina*, and found only 10.7 mM cytosolic Na^+ in an artificial seawater medium (500 mM $[Na^+]$). Less of an extreme gradient was found by Carden et al. (2003) in barley seedlings exposed to 200 mM NaCl, but here too the cytosolic Na^+ activity value was low, maximally, 29 mM. Interestingly, these are levels at which little enzyme inhibition occurs (Greenway and Osmond 1972; Munns and Tester 2008), so mechanisms of Na^+-specific toxicity may need to be sought elsewhere. Of additional importance is the conundrum that these low values, relative to CATE and other methods, suggest that either unidirectional Na^+ fluxes reported across the membrane using tracers (Britto and Kronzucker 2009), or exchange half-times for cytosolic Na^+ pools (Essah et al. 2003), have been substantially overestimated. Another way to look at this problem is to predict cytosolic pool sizes on the basis of unidirectional influx across the plasma membrane and exchange half-times for the cytosol (Cram 1969; MacRobbie 1971; Britto and Kronzucker 2001, 2003). If we use results from Essah et al. (2003) in an example, in which an exchange half-time of 5 min is endorsed (Cheeseman 1982), and a unidirectional influx of as high as 300μmol g^{-1}(fresh wt) h^{-1} is reported (at 200 mM external $[Na^+]$; see their Fig. 2), a cytosolic concentration of Na^+ is predicted to be about 700 mM, more than two orders of magnitude higher than some reported microelectrode measurements at the same, or higher, external Na^+ concentrations (Carden et al. 2003; Rubio et al. 2005).

A novel aspect of ion-specific effects of Na^+ can be seen in work using *extra*cellular ion-selective microelectrodes (e.g. Shabala et al. 2006). Here, a sudden provision of moderate to high NaCl has been shown to elicit a short-term K^+ efflux from the cell. However, radiotracer and tissue experiments have shown that, under higher NaCl, there is a much more pronounced K^+-loss effect over the long term, which has been attributed to osmotic, rather than ionic, Na^+ specific stresses (Britto et al. 2010; see above).

The role of membrane transporters

Several recent reviews have summarized the postulated mechanisms of Na^+ transport into, and out of, plant cells (Munns and Tester 2008; Zhang et al. 2010; Kronzucker and Britto 2011; Cheeseman 2013), and, yet, with only few exceptions, no consensus has emerged as to the actual relative *in planta* roles of the various components, and no molecular candidate has been convincingly identified for Na^+ influx under toxic conditions. The strongest agreement in the literature currently is that certain subclasses of non-selective cation channels (NSCCs), the voltage-insensitive VI-NSCCs, participate critically, and most of the evidence for this derives from electrophysiological studies, where evidence appears incontrovertible that NSCCs can conduct Na^+ currents (see summary in Kronzucker and Britto 2011). However, as we have previously pointed out, the relationship of currents obtained from such electrophysiological studies, mostly conducted in patch-clamp configurations in membrane patches and naked protoplasts, to Na^+ fluxes and accumulation at the whole-plant level has, by no means, been established, and many questions remain (Kronzucker and Britto 2011). Indeed, *in planta* fluxes in excess of 100 micromoles per gram (fresh weight) per hour have been repeatedly reported in root systems (Essah et al. 2003; Malagoli et al. 2008; Møller et al. 2009; Wang et al. 2009; Wetson and Flowers 2010), and one can show, using established models of cation transport and energization (Kronzucker et al. 2001; Britto and Kronzucker 2006), that ion fluxes of this magnitude, were they to indeed proceed across plasma membranes, would be associated with a respiratory energy cost vastly in excess of the entire respiratory budget of the plant (Malagoli et al. 2008; Britto and Kronzucker 2009; Kronzucker and Britto 2011).

Other transporters that have received attention as potential candidates for Na^+ influx into (in particular) roots cells *in planta* are the low-affinity cation transporter LCT1 (Schachtman et al. 1997; Amtmann et al. 2001), the high- and low-affinity (and, at times, dual-affinity) K^+ transporters from the KUP/HAK/KT and AKT families (Santa-María et al. 1997; Amtmann and Sanders 1999; Blumwald et al. 2000; Golldack et al. 2003; Zhang et al. 2010), and members from the HKT family. Those from the HKT1 subfamily are believed to operate mostly in regulating root-to-shoot Na^+ translocation (Sunarpi et al. 2005; Møller et al. 2009), while those from the HKT2 subfamily have been implicated in primary Na^+ influx at least at lower Na^+ concentrations and in grasses (Horie et al. 2001; Laurie et al. 2002; Munns and Tester 2008; Hauser and Horie 2010; Horie et al. 2011; see also Schulze et al. 2012). In addition, two Na^+/H^+ antiport systems have been identified, one of which, SOS1, is believed to be responsible predominantly for Na^+ efflux at the plasma membrane (Shi et al. 2000), the other, NHX1, for Na^+ sequestration into the vacuole (Apse et al. 1999).

An interesting conundrum arises for modern biotechnologically oriented approaches, not only because the primary influx transporters are not yet known, but because it is, in fact, not clear whether more or less Na^+ entry should be a desirable goal. As previous sections of this review show, many species benefit from inclusion rather than exclusion of Na^+, including, or perhaps especially, under saline external conditions, and the Na^+ ion's inherent toxicity is, from a chemical perspective, to be regarded largely as a myth. Thus, were engineering efforts successful to produce plant lines with silenced or downregulated influx mechanisms, and/or overexpressed plasma-membrane efflux systems, the result may, in many genotypes, be less tolerance, not more. Only experiments can show what may occur, and one eagerly awaits these. However, fundamental current hypotheses in terms of the preference of Na^+ inclusion versus exclusion might be too simplistic. Additionally, as touched upon in an earlier section of this review, with respect to the role of membrane transporters in the development of salt toxicity, the application of William of Ockham's "*lex parsimoniae*" might guide a closer look at, for instance, sodium's established effects on other critical membrane transport systems, such as those mediating potassium influx and efflux, and, related to this, osmotic effects, before implicating additional paradigms such as those of "toxic sodium fluxes" or "cytosolic sodium toxicity" (Kronzucker and Britto 2011; Cheeseman 2013).

The supersession of organismal physiology: evolution or mistake?

One final word pertains to a recent trend in the plant sciences that may have, in our view, contributed to a lack of progress in several critical areas within the sodium field, that is the near-solitary focus on molecular approaches to answer outstanding research questions. The study of function has, by contrast, recently only received marginal attention, and, more frequently than not, has not occured *in planta intacta*, but instead in excised systems (e.g. Essah et al. 2003) or heterologous expression systems (e.g. Rubio et al. 1995), such as *Xenopus* oocytes, yeast, or *Escherichia coli.* Some of the caveats relating to this have been discussed by others (e.g. Haro et al. 2010), but have received insufficient hearing overall. Further, in some prominent cases, gene and function have indeed been treated as synonymous, even linguistically (Britto and Kronzucker 2011). A related issue is a lack of remembrance with regard to work conducted in the 1950s, 60s, 70s, and 80s (and even earlier), prior to the onset of the molecular age, which is in evidence with increasing frequency. Indeed, it is not unusual for older discoveries to be re-discovered, while much available context is missed by insufficient awareness of the older literature. Examples of this include the large body of literature on Na^+ as a nutrient (reviewed here), Emanuel Epstein's early work on the mechanisms of Na^+ transport (Rains and Epstein 1965, 1967a, b, c; by contrast, in the related area of K^+ research, Epstein's early kinetic studies form a pillar of the field), and the literature on K^+ loss under Na^+ stress (see Britto et al. 2010). In addition, a readiness to accept dogma without, in many cases, sufficient measurement, has, in our view, and that of others (Cheeseman 2013), afflicted the sodium field to an unusual extent. Two examples include the role of the "cytosolic K^+/Na^+ ratio", for which few actual measurements exist and which is yet referred to in numerous publications in the field, and, secondly, the involvement of non-selective cation channels (NSCCs) in mediating futile Na^+ cycling under salinity conditions, which has, in reality, not as yet been demonstrated conclusively in intact plant systems (Kronzucker and Britto 2011). It would be unwise to endorse the sidelining of stringent physiological examination and critical analysis in the above areas. We suggest that the sodium research field would benefit from greater awareness of, and dialogue between, both historical and recent discoveries and divergent approaches that have been, and yet should be, brought to bear on the field's critical outstanding questions.

Similarly, discoveries in the genetics and physiology of Na^+ nutrition and toxicity should continually be related to the performance of plants in ecological and agro-ecological settings. The productivity of natural and man-made biological systems will increasingly be subjected to potential salinity hazards, and the knowledge of how Na^+ cycles through and interacts with plants, in both beneficial and deleterious ways, is critical to the understanding and management of these systems. Insights from the above discussions may thus guide future selection and breeding approaches in novel ways, by favouring Na^+ inclusion traits in species where this may be of benefit (e.g. *Hordeum vulgare*, or members of the Chenopodiaceae and Brassicaceae), rather than simply attempting to silence them. The paucity of success in producing salt-tolerant cultivars for many crops may in part relate to a lack of thorough, and critical, examination of leading paradigms about Na^+ exclusion and cytosolic toxicity; such a critical examination is indispensable if advances are to successfully transfer from scientific concept and the lab bench to the agricultural field.

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