

Sodium as nutrient and toxicant

Herbert J. Kronzucker · Devrim Coskun ·
Lasse M. Schulze · Jessie R. Wong · Dev T. Britto

Received: 27 March 2013 / Accepted: 31 May 2013 / Published online: 22 June 2013
© Springer Science+Business Media Dordrecht 2013

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,

Responsible Editor: Philippe Hinsinger.

H. J. Kronzucker (✉) · D. Coskun · L. M. Schulze ·
J. R. Wong · D. T. Britto
Department of Biological Sciences, University of Toronto,
1265 Military Trail, Toronto, ON, Canada M1C 1A4
e-mail: herbertk@utsc.utoronto.ca

D. Coskun
e-mail: devrim.coskun@mail.utoronto.ca

L. M. Schulze
e-mail: lasse.schulze@utoronto.ca

J. R. Wong
e-mail: jessierachel.wong@mail.utoronto.ca

D. T. Britto
e-mail: britto@utsc.utoronto.ca

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 Aron 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 venenum*" (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; Pessaraki 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 (<i>Hordeum vulgare</i>), <i>Atriplex nummularia</i> , <i>A. paludosa</i> , <i>A. quinii</i> , <i>A. semibaccata</i> , <i>A. inflata</i> , <i>A. leptocarpa</i> , <i>A. lindleyi</i> , <i>A. spongiosa</i> , <i>A. semilunalaris</i> , <i>A. hortensis</i> <i>Atriplex nummularia</i> and <i>A. inflata</i>	0.4 mM 0.1 and 0.6 mM	Total DW ^a Shoot FW ^b ; leaf chlorophyll, sugar, and starch concentration	Brownell 1968 Brownell and Jackman 1966
Japanese millet (<i>Echinochloa utilis</i>), Bermuda grass (<i>Cynodon dactylon</i>), Shortleaf spikeseed (<i>Kyllinga brevifolia</i>), Joseph's-coat (<i>Amaranthus</i> <i>tricolor</i>), <i>Kochia childsii</i> , Moss-rose (<i>Portulaca</i> <i>grandiflora</i>)	0.1 mM	Total DW	Brownell and Crossland 1972
<i>Kochia childsii</i> , Rhodes grass (<i>Chloris gayana</i>), <i>Amaranthus caudatus</i> L.	0.1 mM	Total FW	Johnston et al. 1988
Joseph's-coat (<i>Amaranthus tricolor</i> L.)	0.5 mM	Growth rate	Ohta et al. 1987
Maize (<i>Zea mays</i> L.), Barnyard grass (<i>Echinochloa</i> <i>crus-galli</i> L.), Hog millet (<i>Panicum miliaceum</i> L.), Kleingrass (<i>Panicum coloratum</i> L.), <i>Panicum</i> <i>dichotomiflorum</i> , <i>Panicum maximum</i> , Rhodes grass (<i>Chloris gayana</i>)	0.1 mM	Growth rate	Ohta et al. 1988
Joseph's-coat (<i>Amaranthus tricolor</i> L.)	0.5 mM	RGR ^c , nitrate reductase activity, O ₂ evolution, chlorophyll content	Ohta et al. 1989
Tomato (<i>Solanum lycopersicum</i> 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 NO₃⁻ 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 C₄ habit of photosynthesis. The paradigm that C₄ 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; Pessaraki and Marcum 2000; Pessaraki 2001; Subbarao et al. 2003). Important exceptions include the leading C₄ 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 C₄ 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 C₄ species *Amaranthus tricolor*, the C₃ 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 (<i>Atriplex vesicaria</i>)	0.04–1.2 mM	Total FW ^a , DW ^b	Brownell 1965
Bladder saltbush (<i>Atriplex vesicaria</i>)	0.1–1.2 mM	DW	Brownell and Wood 1957
Beet (<i>Beta vulgaris</i> L.)	1–32 mM	Shoot FW, DW; Root FW, DW	El-Sheikh et al. 1967
<i>Suaeda aegyptiaca</i>	5–500 mM	Shoot FW, DW; succulence	Eshel 1985
Cacao tree (<i>Theobroma cacao</i>)	0.25–2 mM	Photosynthetic rate, WUE ^c , mineral nutrition	Gattward et al. 2012
Beet (<i>Beta vulgaris</i> L.)	16 mM	Water content, total FW	Lawlor and Milford 1973
Barley (<i>Hordeum vulgare</i> L.)	8 mM	Total DW	Mullison and Mullison 1942
Beet (<i>Beta vulgaris</i> L.)	2–10 mM	Leaf area and DW	Nunes et al. 1984
<i>Atriplex portulacoides</i> L.	20–700 mM	RGR ^d	Redondo-Gómez et al. 2007
<i>Arthrocnemum macrostachyum</i>	171–510 mM	RGR	Redondo-Gómez et al. 2010
<i>Lolium perenne</i> L., Timothy (<i>Phleum pratense</i> L.)	1–13 mM	Nitrate reductase activity	Smith et al. 1980
Beet (<i>Beta vulgaris</i> L.)	3.75–4.90 mM	Leaf area and water content, total DW	Subbarao et al. 1999b
Beet (<i>Beta vulgaris</i> L.)	1.7–3.5 mM	Total DW	Truog et al. 1953
Ranger alfalfa (<i>Medicago sativa</i> L.)	4 mM	Total DW	Wallace et al. 1948
<i>Halogeton glomeratus</i>	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 C₄ metabolites, such as malate, aspartate, and PEP, decreased. In the C₃ species tomato, by contrast, none of these metabolites was influenced by Na⁺ (Johnston et al. 1988). Furthermore, in the C₄ 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 C₄ 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 C₄ species

Sodium is known to influence C₄ 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 CO₂-concentrating mechanism in C₄ 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 C₄ species engaging in the ‘MEP’ (methyl-erythritol-phosphate) pathway, pyruvate import into chloroplasts, across the envelope, is pivotal to the C₄ 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
<i>Eucalyptus grandis</i>	68 kg/ha	Tree height, basal area, biomass	Almeida et al. 2010
Beet (<i>Beta vulgaris</i> L.)	100–800 kg/ha	Sugar yield	Draycott and Bugg 1982
Beet (<i>Beta vulgaris</i> L.)	150 kg/ha	Sugar yield	Draycott and Durrant 1976
Beet (<i>Beta vulgaris</i> L.)	45–225 kg/ha	Sugar yield	Draycott et al. 1970
Beet (<i>Beta vulgaris</i> L.)	100 kg/ha	Sugar yield	Durrant et al. 1974
Beet (<i>Beta vulgaris</i> L.)	250 kg/ha	Water content, root DW ^a , sugar content	Durrant et al. 1978
Pangola grass (<i>Digitaria eriantha</i>)	87 and 176 kg/ha	DW	Gammon 1953
Beet (<i>Beta vulgaris</i> L.)	30–90 kg/ha	FW ^b , water content	Haneklaus et al. 1998
Beet (<i>Beta vulgaris</i> L.), Cabbage (<i>Brassica oleracea</i>), Swiss chard (<i>Beta vulgaris</i>)	560–1121 kg/ha	Yield (FW)	Harmer and Benne 1945
Celery (<i>Apium graveolens</i>), Beet (<i>Beta vulgaris</i> L.), Wheat (<i>Triticum aestivum</i> L.), Pea (<i>Pisum sativum</i> L.)	280–1120 kg/ha	Yield (FW)	Harmer et al. 1953
Cotton (<i>Gossypium</i> [species not specified])	129.92 or 163.52 kg/ha	Total FW	Lancaster et al. 1953
Beet (<i>Beta vulgaris</i> L.), Flax (<i>Linum usitatissimum</i> L.), Oat (<i>Avena sativa</i> L.)	49.28 and 98.56 kg/ha	Total DW	Larson and Pierre 1953
Oat (<i>Avena sativa</i> L.), Turnip (<i>Brassica rapa</i> var. <i>rapa</i>), Potato (<i>Solanum tuberosum</i> L.), Italian ryegrass (<i>Lolium multiflorum</i>)	100–800 kg/ha	Yield	Lehr 1950
Cotton (<i>Gossypium</i> [species not specified])	26.88, 53.76, and 80.64 kg/ha	Total FW	Marshall and Sturgis 1953
Beet (<i>Beta vulgaris</i> L.)	0.6 g/kg soil	Total DW, succulence	Milford et al. 1977
Beet (<i>Beta vulgaris</i> L.)	300 kg/ha	Total FW	Szulc et al. 2010
Beet (<i>Beta vulgaris</i> L.)	90–180 kg/ha	Yield	Tinker 1965

^a Dry weight^b Fresh weight

explanation why only some C₄ 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 C₄ 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 C₄ 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

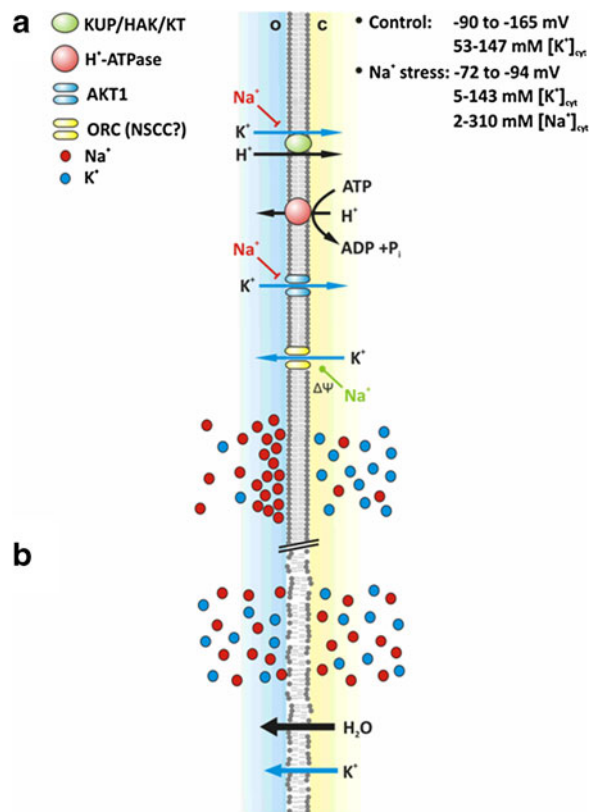


Fig. 1 Schematic illustration of the thermodynamics and mechanism of K^+ and Na^+ interactions at the plasma-membrane interface of plant roots. **a** Under non-osmotic challenges, Na^+ may inhibit (red) or stimulate (green) K^+ transport (blue arrows) (Santa-María et al. 1997; Thiel and Blatt 1991; Shabala et al. 2006). Cytosolic concentrations of K^+ and Na^+ ($[\text{K}^+]_{\text{cyt}}$ and $[\text{Na}^+]_{\text{cyt}}$, 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 disintegration (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

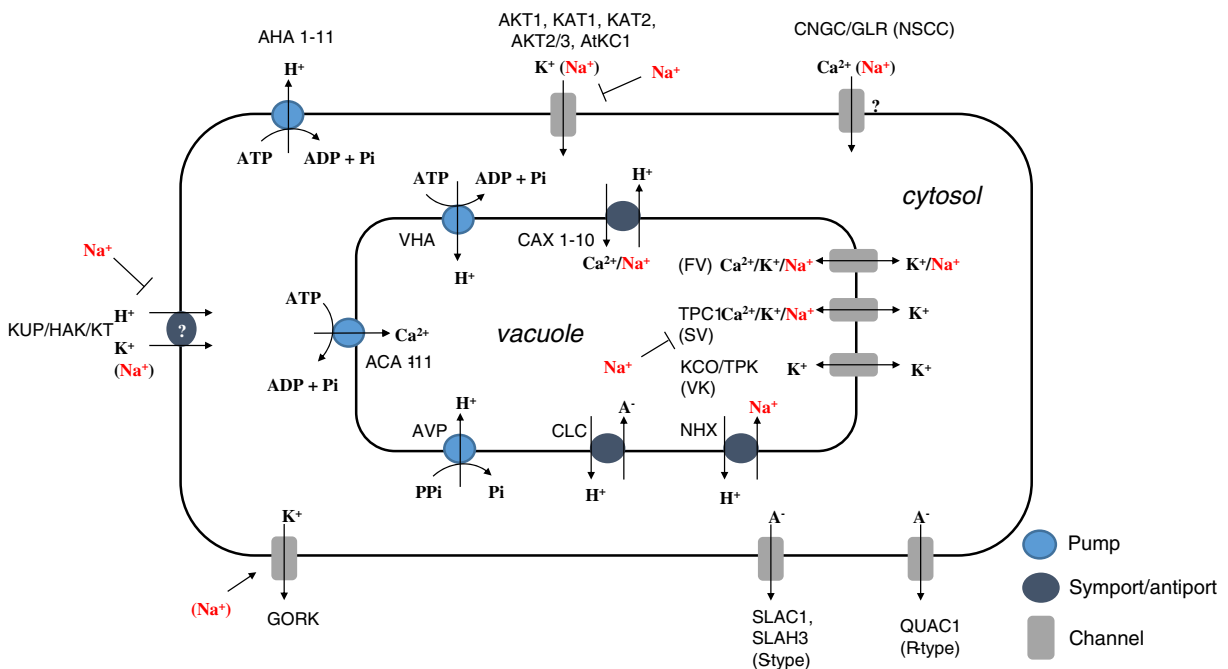


Fig. 2 Current model of ion transport in guard cells, highlighting Na^+ -specific mechanisms. K^+ influx channels (KAT1, KAT2, AKT1, AKT2/3, AtKC1; Pandey et al. 2007), integral to stomatal opening, can mediate Na^+ fluxes in some instances (Schroeder et al. 1987; Schroeder 1988; Obata et al. 2007), as can members of the KUP/HAK/KT family (Santa-Maria 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 $[\text{K}^+]$ ($[\text{K}^+]_{\text{cyt}}$) under saline conditions is well documented (Hajibagheri et al. 1987, 1988; Binzel et al. 1988; Schroepfel-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-Maria 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-Maria 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; Gollmack 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 $[K^+]_{\text{cyt}}$. Thus, a suppression of $[K^+]_{\text{cyt}}$ 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 disintegrality 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 $\text{Ca}^{2+}/\text{Na}^+$ 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 *AtAVPI*, 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 $[\text{Na}^+]_{\text{cyt}}$ 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 $[\text{Na}^+]_{\text{cyt}}$ (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 $[\text{Na}^+]$ 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 $[\text{Na}^+]$ 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 $[\text{Na}^+]$). 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 $300 \mu\text{mol g}^{-1}(\text{fresh wt}) \text{h}^{-1}$ is reported (at 200 mM external $[\text{Na}^+]$; 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 *extracellular* 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; Gollack 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 occurred *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.

Acknowledgments Funding for this review was generously provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chair (CRC) program.

References

- Alekseev AM, Abdurakhmanov AA (1966) On the influence of ions of mineral nutrient salts on the state of the protoplasm of plant cells. *Fiziol Rast* 13:414–419
- Allen MB, Arnon DI (1955) Studies on nitrogen-fixing blue-green algae. I. growth and nitrogen fixation by *Anabaena cylindrica* Lemm. *Plant Physiol* 30:366–372
- Almeida JCR, Laclau JP, de Moraes Gonçalves JL, Ranger J, Saint-André L (2010) A positive growth response to NaCl applications in *Eucalyptus* plantations established on K-deficient soils. *For Ecol Manag* 259:1786–1795
- Amtmann A, Gradmann D (1994) Na⁺ transport in *Acetabularia* bypasses conductance of plasmalemma. *J Membr Biol* 139:117–125
- Amtmann A, Sanders D (1999) Mechanisms of Na⁺ uptake by plant cells. *Adv Bot Res* 29:75–112
- Amtmann A, Fischer M, Marsh EL, Stefanovic A, Sanders D, Schachtman DP (2001) The wheat cDNA LCT1 generates hypersensitivity to sodium in a salt-sensitive yeast strain. *Plant Physiol* 126:1061–1071
- Aoki N, Ohnishi J, Kanai R (1992) 2 different mechanisms for transport of pyruvate into mesophyll chloroplasts of C₄ plants—a comparative study. *Plant Cell Physiol* 33:805–809
- Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na⁺/H⁺ antiporter in *Arabidopsis*. *Science* 285:1256–1258
- Apse MP, Sottosanto JB, Blumwald E (2003) Vacuolar cation/H⁺ exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of AtNHX1, the *Arabidopsis* vacuolar Na⁺/H⁺ antiporter. *Plant J* 36:229–239
- Bañuelos MA, Garciadeblas B, Cubero B, Rodríguez-Navarro (2002) Inventory and functional characterization of the HAK potassium transporters of rice. *Plant Physiol* 130:784–795
- Barbier G, Chambannes J (1951) Accumulation du sodium dans les racines de plantes. *C R Acad Sci* 232:1372–1374
- Baxter I, Brazelton JN, Yu D, Huang YS, Lahner B, Yakubova E, Li Y, Bergelson J, Borevitz JO, Nordborg M, Vitek O, Salt DE (2010) A coastal cline in sodium accumulation in *Arabidopsis thaliana* is driven by natural variation of the sodium transporter AtHKT1;1. *Plos Genet* 6:1–8
- Bazihizina N, Barrett-Lennard EG, Colmer TD (2012) Plant growth and physiology under heterogeneous salinity. *Plant Soil* 354:1–19
- Benlloch M, Ojeda MA, Ramos J, Rodríguez-Navarro A (1994) Salt sensitivity and low discrimination between potassium and sodium in bean plants. *Plant Soil* 166:117–123
- Binzel HL, Hess FD, Bressan RA, Hasegawa PM (1988) Intracellular compartmentation of ions in salt adapted tobacco cell. *Plant Physiol* 86:607–614
- Blatt MR (1992) K⁺ channels of stomatal guard cells—characteristics of the inward rectifiers and its control by pH. *J Gen Physiol* 99:615–644
- Blumwald E, Poole RJ (1985) Na⁺/H⁺ antiporter in isolated tonoplast vesicles from storage tissue of *Beta vulgaris*. *Plant Physiol* 78:163–167
- Blumwald E, Aharon GS, Apse MP (2000) Sodium transport in plant cells. *Biochem Biophys Acta* 1465:140–151
- Botella MA, Martinez V, Pardines J, Cerdá A (1997) Salinity induced potassium deficiency in maize plants. *J Plant Physiol* 150:200–205
- Bowling DJF, Ansari AQ (1971) Evidence for a sodium influx pump in sunflower roots. *Planta* 98:323–329
- Bowling DJF, Ansari AQ (1972) Control of sodium transport in sunflower roots. *J Exp Bot* 23:241–246
- Box S, Schachtman DP (2000) The effect of low concentrations of sodium on potassium uptake and growth of wheat. *Aust J Plant Physiol* 27:175–182
- Britto DT, Kronzucker HJ (2001) Can unidirectional influx be measured in higher plants? A mathematical approach using parameters from efflux analysis. *New Phytol* 150:37–47

- Britto DT, Kronzucker HJ (2003) Ion fluxes and cytosolic pool sizes: examining fundamental relationships in transmembrane flux regulation. *Planta* 217:490–497
- Britto DT, Kronzucker HJ (2005) Nitrogen acquisition, PEP carboxylase, and cellular pH homeostasis: new views on old paradigms. *Plant Cell Environ* 28:1396–1409
- Britto DT, Kronzucker HJ (2006) Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. *Trends Plant Sci* 11:529–534
- Britto DT, Kronzucker HJ (2008) Cellular mechanisms of potassium transport in plants. *Physiol Plant* 133:637–650
- Britto DT, Kronzucker HJ (2009) Ussing's conundrum and the search for transport mechanisms in plants. *New Phytol* 183:243–246
- Britto DT, Kronzucker HJ (2011) Genes do not form channels. *Plant Soil* 346:15–17
- Britto DT, Kronzucker HJ (2012) Isotope techniques to study kinetics of Na⁺ and K⁺ transport under salinity conditions. *Meths Mol Biol* 913:389–398
- Britto DT, Ebrahimi-Ardebili S, Hamam AM, Coskun D, Kronzucker HJ (2010) ⁴²K analysis of sodium-induced potassium efflux in barley: mechanism and relevance to salt tolerance. *New Phytol* 186:373–384
- Brownell PF (1965) Sodium as an essential micronutrient element for a higher plant (*Atriplex vesicaria*). *Plant Physiol* 40:460–468
- Brownell PF (1968) Sodium as an essential micronutrient element for some higher plants. *Plant Soil* 28:161–164
- Brownell PF, Crossland CJ (1972) The requirement for sodium as a micronutrient by species having the C₄ dicarboxylic photosynthetic pathway. *Plant Physiol* 49:794–797
- Brownell PF, Jackman ME (1966) Changes during recovery from sodium deficiency in *Atriplex*. *Plant Physiol* 41:617–622
- Brownell PF, Nicholas DJD (1968) Some effects of sodium on nitrate assimilation and N₂ fixation in *Anabaena cylindrica*. *Plant Physiol* 42:915–921
- Brownell PF, Wood JG (1957) Sodium as an essential micronutrient element for *Atriplex vesicaria*, Heward. *Nature* 179:635–636
- Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J Plant Nutr Soil Sci* 168:521–530
- Carden DE, Walker DJ, Flowers TJ (1998) Root cell cytosolic ion differences between barley cultivars may contribute to salt tolerance. In: Abstracts of the 11th International Workshop on Plant Membrane Biology. The Society of Experimental Biology, 345
- Carden DE, Diamond D, Miller AJ (2001) An improved Na⁺-selective microelectrode for intracellular measurements in plant cells. *J Exp Bot* 52:1353–1359
- Carden DE, Walker DJ, Flowers TJ, Miller AJ (2003) Single-cell measurements of the contributions of cytosolic Na⁺ and K⁺ to salt tolerance. *Plant Physiol* 131:676–683
- Cheeseman JM (1982) Pump-leak sodium fluxes in low salt corn roots. *J Membr Biol* 70:157–164
- Cheeseman JM (2013) The integration of activity in saline environments: problems and perspectives. *Funct Plant Biol*. doi:10.1071/FP12285
- Chen H, An R, Tang J-H, Cui XH, Hao F-S, Chen J, Wang XC (2007) Over-expression of a vacuolar Na⁺/H⁺ antiporter gene improves salt tolerance in an upland rice. *Mol Breeding* 19:215–225
- Cope JT, Bradfield R, Peech M (1953) Effect of sodium fertilization on yield and cation content of some field crops. *Soil Sci* 76:65–79
- Corratgé-Faillie, Jabnoute M, Zimmermann S, Véry AA, Fizames C, Sentenac H (2010) Potassium and sodium transport in non-animal cells: the Trk/Ktr/HKT transporter family. *Cell Mol Life Sci* 67:2511–2532
- Coskun D, Britto DT, Jean YK, Kabir I, Tolay I, Torun AA, Kronzucker HJ (2013) K⁺ efflux and retention in response to NaCl stress do not predict salt tolerance in contrasting genotypes of rice (*Oryza sativa* L.). *PLoS One* 8:1–16
- Cram WJ (1969) Short term influx as a measure of influx across the plasmalemma. *Plant Physiol* 44:1013–1015
- Cramer GR (1992) Kinetics of maize leaf elongation II. Responses of a Na-excluding cultivar and a Na-including cultivar to varying Na/Ca salinities. *J Exp Bot* 43:857–864
- Cramer GR, Läubli A, Polito VS (1985) Displacement of Ca²⁺ by Na⁺ from the plasmalemma of root cells. *Plant Physiol* 79:207–211
- Cramer GR, Alberico GJ, Schmidt C (1994) Salt tolerance is not associated with the sodium accumulation of 2 maize hybrids. *Aust J Plant Physiol* 21:675–692
- Davenport RJ, Tester M (2000) A weakly voltage-dependent, nonselective cation channel mediates toxic sodium influx in wheat. *Plant Physiol* 122:823–834
- Desplanque B, Boudry P, Broomberg K, Saumitou-Laprade P, Cuguen J, Van Dijk H (1999) Genetic diversity and gene flow between wild, cultivated and weedy forms of *Beta vulgaris* L. (Chenopodiaceae), assessed by RFLP and microsatellite markers. *Theor Appl Genet* 98:1194–1201
- Ding L, Zhu JK (1997) Reduced Na⁺ uptake in the NaCl-hypersensitive *sos1* mutant of *Arabidopsis thaliana*. *Plant Physiol* 113:795–799
- Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. *Science* 280:69–77
- Draycott AP, Bugg SM (1982) Response by sugarbeet to various amounts and times of application of sodium chloride fertilizer in relation to soil type. *J Agric Sci* 98:579–592
- Draycott AP, Durrant MJ (1976) Response by sugar beet to potassium and sodium fertilizers, particularly in relation to soils containing little exchangeable potassium. *J Agric Sci* 87:105–112
- Draycott AP, Marsh JAP, Tinker PBH (1970) Sodium and potassium relationships in sugar beet. *J Agric Sci* 74:568–573
- Dreyer I, Uozumi N (2011) Potassium channels in plant cells. *FEBS J* 278:4293–4303
- Durrant MJ, Draycott AP, Boyd DA (1974) The response of sugar beet to potassium and sodium fertilizers. *J Agric Sci* 83:427–434
- Durrant MJ, Draycott AP, Milford GFJ (1978) Effect of sodium fertilizer on water status and yield of sugar beet. *Ann Appl Biol* 88:321–328
- El-Sheikh AM, Ulrich A, Broyer TC (1967) Sodium and rubidium as possible nutrients for sugar beet plants. *Plant Physiol* 42:1202–1208
- Epstein E (1961) Essential role of calcium in selective cation transport by plant cells. *Plant Physiol* 36:437–444

- Epstein E, Bloom AJ (2005) Inorganic components of plants. In: Mineral nutrition of plants: principles and perspectives, 2nd edn. Sinauer Associates, Inc., Massachusetts, pp 44–45
- Epstein E, Elzam OE, Rains DW (1963) Resolution of dual mechanisms of potassium absorption by barley roots. *Proc Natl Acad Sci U S A* 49:684–692
- Erdei L, Kuiper PJC (1979) Effect of salinity on growth, cation content, Na⁺ uptake and translocation in salt-sensitive and salt-tolerant *Plantago* species. *Physiol Plant* 47:95–99
- Eshel A (1985) Response of *Suaeda aegyptiaca* to KCl, NaCl, and Na₂SO₄ treatments. *Physiol Plant* 64:308–315
- Essah PA, Davenport R, Tester M (2003) Sodium influx and accumulation in *Arabidopsis*. *Plant Physiol* 133:307–318
- Evans HJ, Sorger GJ (1966) Role of mineral elements with emphasis on univalent cations. *Annu Rev Plant Physiol* 17:47–76
- Flowers TJ (1974) Salt tolerance in *Suaeda maritima* (L) Dum. A comparison of mitochondria isolated from green tissues of *Suaeda* and *Pisum*. *J Exp Bot* 101:101–110
- Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. *New Phytol* 179:945–963
- Flowers TJ, Hajibagheri MA (2001) Salinity tolerance in *Hordeum vulgare* ion concentrations in root cells of cultivars differing in salt tolerance. *Plant Soil* 231:1–9
- Flowers TJ, Läuchli A (1983) Sodium versus potassium: substitution and compartmentation. In: Läuchli A, Bielecki RL (eds) *Encyclopedia of plant physiology, new series, vol 15b, inorganic plant nutrition*. Springer, Berlin, pp 651–681
- Flowers TJ, Hajibagheri MA, Yeo AR (1991) Ion accumulation in the cell walls of rice plants growing under saline conditions—evidence for the Oertli hypothesis. *Plant Cell Environ* 14:319–325
- Fu HH, Luan S (1998) AtKUP1: a dual-affinity K⁺ transporter from *Arabidopsis*. *Plant Cell* 10:63–73
- Fuchs I, Stolzle S, Ivanshikina N, Hedrich R (2005) Rice K⁺ uptake channel OsAKT1 is sensitive to salt stress. *Planta* 221:212–221
- Furbank RT, von Caemmerer S, Sheehy J, Edwards G (2009) C₄ rice: a challenge for plant phenomics. *Funct Plant Biol* 36:845–856
- Furumoto T, Yamaguchi T, Ohshima-Ichie Y, Nakamura M, Tsuchida-Iwata Y, Shimamura M, Ohnishi J, Hata S, Gowik U, Westhoff P, Bräutigam A, Weber APM, Izui K (2011) A plastidial sodium-dependent pyruvate transporter. *Nature* 476:472–475
- Galamba N (2012) Mapping structural perturbations of water in ionic solutions. *J Phys Chem B* 116:5242–5250
- Galeev RR (1990) Application of sodium humate to potatoes. *Kartofel' I Ovoshchi* 2:12–13
- Gammon N (1953) Sodium and potassium requirements of pangola and other pasture grasses. *Soil Sci* 76:81–90
- Gassmann W, Rubio F, Schroeder JI (1996) Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1. *Plant J* 10:869–882
- Gattward JN, Almeida AAF, Souza JO, Gomes FP, Kronzucker HJ (2012) Sodium-potassium synergism in *Theobroma cacao*: stimulation of photosynthesis, water-use efficiency and mineral nutrition. *Physiol Plant* 146:350–362
- Gaxiola R, Delarrinoa IF, Villalba JM, Serrano R (1992) A novel and conserved salt-induced protein is an important determinant of salt tolerance in yeast. *EMBO J* 11:3157–3164
- Genc Y, McDonald GK, Tester M (2007) Reassessment of tissue Na⁺ concentration as a criterion for salinity tolerance in bread wheat. *Plant Cell Environ* 30:1486–1498
- Gierth M, Mäser P (2007) Potassium transporters in plants—involved in K⁺ acquisition, redistribution and homeostasis. *FEBS Lett* 581:2348–2356
- Golldack D, Quigley F, Michalowski CB, Kamasani UR, Bohnert HJ (2003) Salinity stress-tolerance and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently. *Plant Mol Biol* 51:71–81
- Greenway H, Munns R (1980) Mechanisms of salt tolerance in non-halophytes. *Annu Rev Plant Biol* 31:149–190
- Greenway H, Osmond CB (1972) Salt responses of enzymes from species differing in salt tolerance. *Plant Physiol* 49:256–259
- Grinstein S, Rothstein A (1986) Mechanisms of the regulation of the Na⁺/H⁺ exchanger. *J Membr Biol* 90:1–12
- Grof CPL, Johnston M, Brownell PF (1989) Effect of sodium nutrition on the ultrastructure of chloroplasts of C₄ plants. *Plant Physiol* 89:539–543
- Hajibagheri MA, Flowers TJ (2001) Salinity tolerance in *Hordeum vulgare*: ion concentrations in root cells of cultivars differing in salt tolerance. *Plant Soil* 231:1–9
- Hajibagheri MA, Harvey DMR, Flowers TJ (1987) Quantitative distribution within the root cells of salt-sensitive and salt-tolerant maize varieties. *New Phytol* 105:367–379
- Hajibagheri MA, Flowers TJ, Collins JC, Yeo AR (1988) A comparison of the methods of X-ray microanalysis, compartmental analysis and longitudinal ion profiles to estimate cytoplasmic ion concentrations in two maize varieties. *J Exp Bot* 39:279–290
- Hall JL, Flowers TJ (1973) Effect of salt on protein synthesis in halophyte *Suaeda maritima*. *Planta* 110:361–368
- Hampe T, Marschner H (1982) Effect of sodium on morphology, water relations and net photosynthesis in sugar beet leaves. *Z Pflanzenphysiol* 108:151–162
- Haneklaus S, Krudsen L, Schnug E (1998) Relationship between potassium and sodium in sugar beet. *Commun Soil Sci Plant Anal* 29:1793–1798
- Harmer PM, Benne EJ (1945) Sodium as a crop nutrient. *Soil Sci Soc Am J* 60:137–148
- Harmer PM, Benne EJ, Laughlin WM, Key C (1953) Factors affecting crop response to sodium applied as a common salt on Michigan muck soil. *Soil Sci* 76:1–17
- Haro R, Bañuelos MA, Senn MAE, Barrero-Gil J, Rodríguez-Navarro A (2005) HKT1 mediates sodium uniport in roots: pitfalls in the expression of HKT1 in yeast. *Plant Physiol* 139:1495–1506
- Haro R, Bañuelos MA, Rodríguez-Navarro A (2010) High-affinity sodium uptake in land plants. *Plant Cell Physiol* 51:68–79
- Harris L (1996) *Concepts in zoology*. Harper Collins, New York
- Hauser F, Horie T (2010) A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K⁺/Na⁺ ratio in leaves during salinity stress. *Plant Cell Environ* 33:552–565
- He T, Cramer GR (1993) Salt tolerance of rapid-cycling *Brassica* species in relation to potassium-sodium ratio and selectivity at the whole plant and callus levels. *J Plant Nutr* 16:1263–1277

- Hedrich R, Neher E (1987) Cytoplasmic calcium regulates voltage-dependent ion channels in plant vacuoles. *Nature* 329:833–836
- Horie T, Yoshida K, Nakayama H, Yamada K, Oiki S, Shinmyo A (2001) Two types of HKT transporters with different properties of Na⁺ and K⁺ transport in *Oryza sativa*. *Plant J* 27:129–138
- Horie T, Brodsky DE, Costa A, Kaneko T, Lo Schiavo F, Katsuhara M, Schroeder JI (2011) K⁺ transport by OsHKT2;4 transporter from rice (*Oryza sativa*) with atypical Na⁺ transport properties and competition in permeation of K⁺ over Mg²⁺ and Ca²⁺ ions. *Plant Physiol* 156:1493–1507
- Hosy E, Vavasseur A, Mouline K, Dreyer I, Gaymard F, Poree F, Boucherez J, Lebaudy A, Bouchez D, Véry AA, Simonneau T, Thibaud JB, Sentenac H (2003) The *Arabidopsis* outward K⁺ channel GORK is involved in regulation of stomatal movements and plant transpiration. *Proc Natl Acad Sci U S A* 100:5549–5554
- Humble GD, Hsiao TC (1969) Specific requirement of potassium for light-activated opening of stomata in epidermal strips. *Plant Physiol* 44:230–234
- Humble GD, Raschke K (1971) Stomatal opening quantitatively related to potassium transport—evidence from electron probe analysis. *Plant Physiol* 48:447–453
- Hylton LO, Ulrich A, Cornelius DR (1967) Potassium and sodium interrelations in growth and mineral content of Italian Ryegrass. *Agron J* 59:311–314
- Isayenkov S, Isner JC, Maathuis FJM (2010) Vacuolar ion channels: roles in plant nutrition and signalling. *FEBS Lett* 584:1982–1988
- Ivashikina N, Hedrich R (2005) K⁺ currents through SV-type vacuolar channels are sensitive to elevated luminal sodium levels. *Plant J* 41:606–614
- Jarvis RG, Mansfield TA (1980) Reduced stomatal response to light, carbon dioxide and abscisic acid in the presence of sodium ions. *Plant Cell Environ* 3:279–283
- Jennings DH (1976) The effects of sodium chloride on higher plants. *Biol Rev* 51:453–486
- Jeschke WD (1977) K⁺-Na⁺ selectivity in roots, localisation of selective fluxes and their regulation. In: Marre E, Ciferri O (eds) Regulation of cell membrane activities in plants. Elsevier, Amsterdam, pp 63–78
- Jeschke WD (1982) Shoot—dependent regulation of sodium and potassium fluxes in roots of whole barley seedlings. *J Exp Bot* 33:601–618
- Jha D, Shirley N, Tester M, Roy SJ (2010) Variation in salinity tolerance and shoot sodium accumulation in *Arabidopsis* ecotypes linked to differences in the natural expression levels of transporters involved in sodium transport. *Plant Cell Environ* 33:793–804
- Johnston M, Grof CPL, Brownell PF (1988) The effect of sodium nutrition on the pool sizes of intermediates of the C₄ photosynthetic pathway. *Aust J Plant Physiol* 15:749–760
- Johnston M, Grof CPL, Brownell PF (1989) Chlorophyll a/b ratios and photosystem activity of mesophyll and bundle sheath fractions from sodium-deficient C₄ plants. *Aust J Plant Physiol* 16:449–457
- Judel G, Kuhn H (1975) Über die Wirkung einer Natriumdüngung zu Zuckerrüben bei guter Versorgung mit Kalium in Gefäßversuchen. *Zucker* 28:68–71
- Kader MA, Lindberg S (2005) Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, *Oryza sativa* L. determined by the fluorescent dye SBFI. *J Exp Bot* 56:3149–3158
- Kim EJ, Kwak JM, Uozumi N, Schroeder JI (1998) *AtKUP1*: an *Arabidopsis* gene encoding high-affinity potassium transport activity. *Plant Cell* 10:51–62
- Kingsbury RW, Epstein E (1985) Salt sensitivity in wheat. A case for specific ion toxicity. *Plant Physiol* 80:651–654
- Kochian LV, Xin-Zhi J, Lucas WJ (1985) Potassium transport in corn roots. IV. characterization of the linear component. *Plant Physiol* 79:771–776
- Krishnamurthy P, Ranathunge K, Nayak S, Schreiber L, Mathew MK (2011) Root apoplastic barriers block Na⁺ transport to shoots in rice (*Oryza sativa* L.). *J Exp Bot* 62:4215–4228
- Kronzucker HJ, Britto DT (2011) Sodium transport in plants: a critical review. *New Phytol* 189:54–81
- Kronzucker HJ, Britto DT, Davenport RJ, Tester M (2001) Ammonium toxicity and the real cost of transport. *Trends Plant Sci* 6:335–337
- Kronzucker HJ, Szczerba MW, Moazami-Goudarzi M, Britto DT (2006) The cytosolic Na⁺:K⁺ ratio does not explain salinity-induced growth impairment in barley: a dual-tracer study using ⁴²K⁺ and ²⁴Na⁺. *Plant Cell Environ* 29:2228–2237
- Kronzucker HJ, Szczerba MW, Schulze LM, Britto DT (2008) Non-reciprocal interactions between K⁺ and Na⁺ ions in barley. *J Exp Bot* 59:2793–2801
- Lancaster JD, Andrews WB, Jones US (1953) Influence of sodium on yield and quality of cotton lint and seed. *Soil Sci* 76:29–40
- Langdale JA (2011) C₄ cycles: past, present, and future research on C₄ photosynthesis. *Plant Cell* 23:3879–3892
- Larson WE, Pierre WH (1953) Interaction of sodium and potassium on yield and cation composition of selected crops. *Soil Sci Soc Am J* 76:51–64
- Laurie S, Feeney KA, Maathuis FJM, Heard PJ, Brown SJ, Leigh RA (2002) A role for HKT1 in sodium uptake by wheat roots. *Plant J* 32:139–149
- Lawlor DW, Milford GFJ (1973) The effect of sodium on growth of water-stressed sugar-beet. *Ann Bot* 37:597–604
- Lebaudy A, Vavasseur A, Hosy E, Dreyer I, Leonhardt N, Thibaud JB, Véry AA, Simonneau T, Sentenac H (2008) Plant adaptation to fluctuating environment and biomass production are strongly dependent on guard cell potassium channels. *Proc Natl Acad Sci U S A* 105:5271–5276
- Lehr JJ (1941) The importance of sodium for plant nutrition I. *Soil Sci* 52:237–244
- Lehr JJ (1950) Importance of sodium for plant nutrition: v. responses of crops other than beet. *Soil Sci* 72:157–166
- Lehr JJ (1953) Sodium as a plant nutrient. *J Sci Food Agric* 4:460–471
- Lehr JJ, Wybenga JM (1955) Exploratory pot experiments on sensitiveness of different crops to sodium. *Plant Soil* 3:251–261
- Leigh RA, Wyn-Jones RG (1986) Cellular compartmentation in plant nutrition: the selective cytoplasm and the promiscuous vacuole. *Adv Plant Nutr* 2:249–279
- Lemtiri-Chlieh F, Berkowitz GA (2004) Cyclic adenosine monophosphate regulates calcium channels in the plasma membrane of *Arabidopsis* leaf guard and mesophyll cells. *J Biol Chem* 279:35306–35312
- Leonhardt N, Marin E, Vavasseur A, Forestier C (1997) Evidence for the existence of a sulfonylurea-receptor-

- like protein in plants: modulation of stomatal movements and guard cell potassium channels by sulfonylureas and potassium channel openers. *Proc Natl Acad Sci U S A* 94:14156–14161
- Luo GZ, Wang HW, Huang J, Tian AG, Wang YJ, Zhang JS, Chen SY (2005) A putative plasma membrane cation/proton antiporter from soybean confers salt tolerance in *Arabidopsis*. *Plant Mol Biol* 59:809–820
- Lutgens FK, Tarbuck EJ (2003) *Foundations of earth science*. Pearson, New Jersey
- Lynch J, Läuchli A (1984) Potassium transport in salt-stressed barley roots. *Planta* 161:295–301
- Maathuis FJM (2007) Monovalent cation transporters; establishing a link between bioinformatics and physiology. *Plant Soil* 301:1–15
- Maathuis FJM, Amtmann A (1999) K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Ann Bot* 84:123–133
- Maathuis FJM, Verlin D, Smith FA, Sanders D, Fernandez JA, Walker NA (1996) The physiological relevance of Na^+ -coupled K^+ -transport. *Plant Physiol* 112:1609–1616
- MacRobbie EAC (1971) Fluxes and compartmentation in plant cells. *Annu Rev Plant Physiol* 22:75–96
- Magat SS, Goh KM (1990) Effect of chloride on fertilizers on ionic composition and cation-anion balance and ratio of fodder beet (*Beta vulgaris* L.) grown under field conditions. *New Zealand J Agric Res* 33:29–40
- Malagoli P, Britto DT, Schulze LM, Kronzucker HJ (2008) Futile Na^+ cycling at the root plasma membrane in rice (*Oryza sativa* L.)—kinetics, energetics, and relation to salinity tolerance. *J Exp Bot* 59:4109–4117
- Mancinelli R, Botti A, Bruni F, Ricci MA, Soper AK (2007) Hydration of sodium, potassium, and chloride ions in solution and the concept of structure maker/breaker. *J Phys Chem B* 111:13570–13577
- Marschner H (1995) *Mineral nutrition of higher plants*. Academic, London
- Marshall JG, Sturgis MB (1953) Effects of sodium fertilizers on yield of cotton. *Soil Sci* 76:75–80
- Martínez-Cordero MA, Martínez V, Rubio F (2005) High-affinity K^+ uptake in pepper plants. *Plant Mol Biol* 56:413–421
- Mäser P, Gierth M, Schroeder JI (2002) Molecular mechanisms of potassium and sodium uptake in plants. *Plant Soil* 247:43–54
- Meyerhoff O, Müller K, Roelfsema M, Latz A, Lacombe B, Hedrich R, Dietrich P, Becker D (2005) AtGLR3.4, a glutamate receptor channel-like gene is sensitive to touch and cold. *Planta* 222:418–427
- Mian A, Oomen RJFJ, Isayenkov S, Sentenac H, Maathuis FJM, Véry AA (2011) Over-expression of an Na^+ and K^+ -permeable HKT transporter in barley improves salt tolerance. *Plant J* 68:468–479
- Milford GFJ, Cormack WF, Durrant MJ (1977) Effects of sodium chloride on water status and growth of sugar beet. *J Exp Bot* 28:1380–1388
- Møller IS, Tester M (2007) Salinity tolerance of *Arabidopsis*: a good model for cereals? *Trends Plant Sci* 12:534–540
- Møller IS, Gilliam M, Jha D, Mayo GM, Roy SJ, Coates JC, Haseloff J, Tester M (2009) Shoot Na^+ exclusion and increase salinity tolerance engineered by cell type-specific alteration of Na^+ transport in *Arabidopsis*. *Plant Cell* 21:2163–2178
- Montasir AH, Sharoubeem HH, Sidrak GH (1966) Partial substitution of sodium for potassium in water cultures. *Plant Soil* 25:181–194
- Moore R, Black CC (1979) Nitrogen assimilation pathways in leaf mesophyll and bundle sheath cells of C4 photosynthetic plants formulated from comparative studies with *Digitaria sanguinalis* (L.) Scop. *Plant Physiol* 64:309–313
- Müller-Röber B, Ellenberg J, Provart N, Willmitzer L, Bush H, Becker D, Dietrich P, Hoth S, Hedrich R (1995) Cloning and electrophysiological analysis of KST1, an inward rectifying K^+ channel expressed in potato guard cells. *EMBO J* 14:2409–2416
- Mullison WR, Mullison E (1942) Growth responses of barley seedlings in relation to potassium and sodium nutrition. *Plant Physiol* 17:632–644
- Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environ* 25:239–250
- Munns R, James RA (2003) Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil* 253:201–218
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Munns R, Schachtman DP, Condon AG (1995) The significance of a two-phase growth response to salinity in wheat and barley. *Aust J Plant Physiol* 22:561–569
- Munns R, James RA, Lauchli A (2005) Increasing salt tolerance in monocotyledonous plants, with emphasis on wheat. *Comp Biochem Phys A* 141:S337
- Nakamura RL, McKendree WL, Hirsch RE, Sedbrook JC, Gaber RF, Sussman MR (1995) Expression of an *Arabidopsis* potassium channel in guard cells. *Plant Physiol* 109:371–374
- Nassery H (1975) The effect of salt and osmotic stress on the retention of potassium by excised barley and bean roots. *New Phytol* 75:63–67
- Nassery H (1979) Salt-induced loss of potassium from plant roots. *New Phytol* 83:23–27
- Nieves-Cordones M, Martínez-Cordero MA, Martínez V, Rubio F (2007) An NH_4^+ -sensitive component dominates high affinity K^+ uptake in tomato plants. *Plant Sci* 172:273–280
- Nitsos RE, Evans HJ (1969) Effects of univalent cations on activity of particulate starch synthetase. *Plant Physiol* 44:1260–1266
- Nocito FF, Sacchi GA, Cocucci M (2002) Membrane depolarization induces K^+ efflux from subapical maize root segments. *New Phytol* 154:45–51
- Nunes MA, Dias MA, Correia M, Oliveira MM (1984) Further studies on growth and osmoregulation of sugar beet leaves under low salinity conditions. *J Exp Bot* 35:322–331
- Obata T, Kitamoto HK, Nakamura A, Fukuda A, Tanaka Y (2007) Rice shaker potassium channel OsKAT1 confers tolerance to salinity stress on year and rice cells. *Plant Physiol* 144:1978–1985
- Ohnishi J, Kanai R (1987) Na^+ -induced uptake of pyruvate into mesophyll chloroplasts of a C4 plant, *Panicum miliaceum*. *FEBS Lett* 219:347–350
- Ohnishi J, Flügge UI, Heldt HW, Kanai R (1990) Involvement of Na^+ in active uptake of pyruvate in mesophyll chloroplasts of some C4 plants. *Plant Physiol* 94:950–959
- Ohta D, Matoh T, Takahashi E (1987) Early responses of sodium-deficient *Amaranthus tricolor* L. plants to sodium application. *Plant Physiol* 84:112–117

- Ohta D, Matsui J, Matoh T, Takahashi E (1988) Sodium requirement of monocotyledonous C_4 plants for growth and nitrate reductase activity. *Plant Cell Physiol* 29:1429–1432
- Ohta D, Yasuoka S, Matoh T, Takahashi E (1989) Sodium stimulates growth of *Amaranthus tricolor* L. plants through enhanced nitrate assimilation. *Plant Physiol* 89:1102–1105
- Pallaghy CK (1968) Electrophysiological studies in guard cells of tobacco. *Planta* 80:147–153
- Pandey S, Zhang W, Assmann SA (2007) Roles of ion channels and transporters in guard cell signal transduction. *FEBS Lett* 581:2325–2336
- Peck NH, van Buren JP, MacDonald GE, Hemmat H, Becker RF (1987) Table beet plant and canned root responses to Na, K, and Cl from soils and from applications of NaCl and KCl. *J Am Soc Hort Sci* 112:188–194
- Peng YH, Zhu YF, Mao YQ, Wang SM, Su WA, Tang ZC (2004) Alkali grass resists salt stress through high $[K^+]$ and an endodermis barrier to Na^+ . *J Exp Bot* 55:939–949
- Pessaraki M (2001) Handbook of plant and crop stress. *Biol Plant* 44:304–304
- Pessaraki M, Marcum KB (2000) Growth responses and Nitrogen-15 absorption of *Distichlis* under sodium chloride stress. ASA-CSSA-SSSA Annual Meetings, Minneapolis, Minnesota
- Pessaraki M, Tucker TC (1985) Uptake of Nitrogen-15 by cotton under salt stress. *Soil Sci Soc Am J* 49:149–152
- Phillips WEJ (1971) Naturally occurring nitrate and nitrite in foods in relation to infant methaemoglobinaemia. *Food Cosmet Toxicol* 9:219–228
- Pilot G, Gaymard F, Mouline K, Cherel I, Sentenac H (2003) Regulated expression of *Arabidopsis* Shaker K^+ channel genes involved in K^+ uptake and distribution in the plant. *Plant Mol Biol* 51:773–787
- Porcelli CA, Boem FHG, Lavado RS (1995) The K/Na and Ca/Na ratios and rapeseed yield, under soil salinity or sodicity. *Plant Soil* 175:251–255
- Qi Z, Spalding EP (2004) Protection of plasma membrane K^+ transport by the salt overly sensitive1 Na^+ - H^+ antiporter during salinity stress. *Plant Physiol* 136:2548–2555
- Quintero FJ, Blatt MR (1997) A new family of K^+ transporters from *Arabidopsis* that are conserved across phyla. *FEBS Lett* 415:206–211
- Rains DW, Epstein E (1965) Transport of sodium in plant tissue. *Science* 148:1161
- Rains DW, Epstein E (1967a) Sodium absorption by barley roots—role of dual mechanisms of alkali cation transport. *Plant Physiol* 42:314–318
- Rains DW, Epstein E (1967b) Preferential absorption of potassium by leaf tissue of mangrove *Avicennia marina*—an aspect of halophytic competence in coping with salt. *Aust J Biol Sci* 20:847–857
- Rains DW, Epstein E (1967c) Sodium absorption by barley roots—its mediation by mechanism 2 of alkali cation transport. *Plant Physiol* 42:319–323
- Rajendran K, Tester M, Roy SJ (2009) Quantifying the three main components of salinity tolerance in cereals. *Plant Cell Environ* 32:237–249
- Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos EM, Luque T, Figueroa ME (2007) Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. *Ann Bot* 100:555–563
- Redondo-Gómez S, Mateos-Naranjo E, Figueroa ME, Davy AJ (2010) Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*. *Plant Biol* 12:79–87
- Robinson MF, Véry AA, Sanders D, Mansfield TA (1997) How can stomata contribute to salt tolerance? *Ann Bot* 80:387–393
- Rodríguez-Navarro A, Rubio F (2006) High-affinity potassium and sodium transport systems in plants. *J Exp Bot* 57:1149–1160
- Roelfsema MRG, Hedrich R (2005) In the light of stomatal opening: new insights into ‘the Watergate’. *New Phytol* 167:665–691
- Roomans GD (1988) Quantitative X-Ray microanalysis of biological specimens. *J Electr Micr Tech* 9:19–43
- Rubio F, Gassmann W, Schroeder JI (1995) Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* 270:1660–1663
- Rubio F, Santa-Maria GE, Rodríguez-Navarro A (2000) Cloning of *Arabidopsis* and barley cDNAs encoding HAK potassium transporters in root and shoot cells. *Physiol Plant* 109:34–43
- Rubio L, Linares-Rueda A, García-Sánchez MJ, Fernández JA (2005) Physiological evidence for a sodium-dependent high-affinity phosphate and nitrate transport at the plasma membrane of leaf and root cells of *Zostera marina* L. *J Exp Bot* 56:613–622
- Rus A, Yokoi S, Sharkhuu A, Reddy M, Lee BH, Matsumoto TK, Koiwa H, Zhu JK, Bressan RA, Hasegawa PM (2001) AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots. *Proc Natl Acad Sci U S A* 98:14150–14155
- Rus A, Baxter I, Muthukumar B, Gustin J, Lahner B, Yakubova Y, Salt DE (2006) Natural variants of AtHKT1 enhance Na^+ accumulation in two wild populations of *Arabidopsis*. *Plas Genet* 2:1964–1973
- Sage RF, Christin PA, Edwards EJ (2011) The C_4 plant lineages of planet Earth. *J Exp Bot* 62:3155–3169
- Santa-Maria GE, Epstein E (2001) Potassium/sodium selectivity in wheat and the amphiploid cross wheat X *Lophopyrum elongatum*. *Plant Sci* 160:523–534
- Santa-Maria GE, Rubio F, Dubcovsky J, Rodríguez-Navarro A (1997) The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. *Plant Cell* 9:2281–2289
- Schachtman DP, Munns R (1992) Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. *Funct Plant Biol* 19:331–340
- Schachtman DP, Schroeder JI (1994) Structure and transport mechanism of a high-affinity potassium uptake transporter from higher-plants. *Nature* 370:655–658
- Schachtman DP, Kumar R, Schroeder JI, Marsh EL (1997) Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. *Proc Natl Acad Sci USA* 94:11079–11084
- Schönknecht G, Spoormaker P, Steinmeyer R, Brüggeman L, Ache P, Dutta R, Reintanz B, Godde M, Hedrich R, Palme K (2002) KCO1 is a component of the slow-vacuolar (SV) ion channel. *FEBS Lett* 511:28–32
- Schroeder JI (1988) K^+ transport properties of K^+ channels in the plasma membrane of *Vicia faba* guard cells. *J Gen Physiol* 92:667–683

- Schroeder JI, Hedrich R, Fernandez JM (1984) Potassium-selective single channels in guard cell protoplasts of *Vicia faba*. *Nature* 312:361–362
- Schroeder JI, Raschke K, Neher E (1987) Voltage dependence of K^+ channels in guard-cell protoplasts. *Proc Natl Acad Sci U S A* 84:4108–4112
- Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001) Guard cell signal transduction. *Annu Rev Plant Physiol Mol Biol* 52:627–658
- Schroepel-Meier G, Kaiser WM (1988) Ion homeostasis in chloroplasts under salinity and mineral deficiency. *Plant Physiol* 87:822–827
- Schubert S, Luchli A (1990) Sodium exclusion mechanisms at the root surface of two maize cultivars. *Plant Soil* 123:205–209
- Schulze LM, Britto DT, Li M, Kronzucker HJ (2012) A pharmacological analysis of high-affinity sodium transport in barley (*Hordeum vulgare* L.): a $^{24}Na^+/^{42}K^+$ study. *J Exp Bot* 63:2479–2489
- Schwender J, Goffman F, Ohlrogge JB, Shachar-Hill Y (2004) Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. *Nature* 432:779–782
- Seemann JR, Critchley C (1985) Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of salt-sensitive species, *Phaseolus vulgaris* L. *Planta* 2:151–162
- Segen JC, Stauffer J (1998) The patient's guide to medical tests. Checkmark books, New York
- Senn ME, Rubio F, Bauelos MA, Rodriguez-Navarro (2001) Comparative functional features of plant potassium HvHAK1 and HvHAK2 transporters. *J Biol Chem* 276:44563–44569
- Shabala S, Mackay AS (2011a) Ion transport in halophytes. In: Kader J, Delseny M (eds) *Advances in botanical research*. Academic, Massachusetts, pp 151–199
- Shabala S, Mackay AS (2011b) Ion transport in halophytes. *Adv Bot Res* 57:151–199
- Shabala S, Shabala L, van Volkenburgh E (2003) Effect of calcium on root development and root ion fluxes in salinised barley seedlings. *Funct Plant Biol* 30:507–514
- Shabala S, Demidchik V, Shabala L, Cuin TA, Smith SJ, Miller AJ, Davies JM, Newman IA (2006) Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from *Arabidopsis* root and leaf cells by controlling plasma membrane K^+ -permeable channels. *Plant Physiol* 141:1653–1665
- Shi HZ, Ishitami M, Kim CS, Zhu JK (2000) The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ antiporter. *Proc Natl Acad Sci U S A* 97:6896–6901
- Simonis W, Urbach W (1963) ber eine Wirkung von Natriumionen auf die Phosphataufnahme und die lichtabhangige Phosphorylierung von *Ankistrodesmus braunii*. *Arch Mikrobiol* 46:265–286
- Smith GS, Middleton KR, Edmonds AS (1980) Sodium nutrition of pasture plants. II. effects of sodium chloride on growth, chemical composition and reduction of nitrate nitrogen. *New Phytol* 84:613–622
- Spalding EP, Hirsch RE, Lewis DR, Qi Z, Sussman MR, Lew BD (1999) Potassium uptake supporting plant growth in the absence of AKT1 channel activity—inhibition by ammonium and stimulation by sodium. *J Gen Physiol* 113:909–918
- Speer M, Kaiser WM (1991) Ion relations of sympylastic and apoplatic space in leaves from *Spinacia oleracea* L. and *Pisum sativum* L. under salinity. *Plant Physiol* 97:990–997
- Stanford G, Carter JN, Westermann DT, Meisinger JJ (1977) Residual nitrate and mineralizable soil nitrogen in relation to nitrogen uptake by irrigated sugar beets. *Agron J* 69:303–308
- Su H, Golldack D, Katsuhara M, Zhao CS, Bohnert HJ (2001) Expression and stress-dependent induction of potassium channel transcripts in the common ice plant. *Plant Physiol* 125:604–614
- Su H, Golldack D, Zhao C, Bohnert HJ (2002) The expression of HAK-type K^+ transporters is regulated in response to salinity stress in common ice plant. *Plant Physiol* 129:1482–1493
- Subbarao GV, Mackowiak C, Wheeler RM (1999a) Recycling of Na in advanced life support: strategies based on crop production systems. *Life Support Biosph Sci* 6:153–160
- Subbarao GV, Wheeler RM, Stutte GW, Levine LH (1999b) How far can sodium substitute for potassium in red beet? *J Plant Nutr* 22:1745–1761
- Subbarao GV, Wheeler RM, Levine LH, Stutte GW (2001) Glycine betaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J Plant Physiol* 158:767–776
- Subbarao GV, Ito O, Berry WL, Wheeler RM (2003) Sodium—a functional plant nutrient. *Crit Rev Plant Sci* 22:391–416
- Sunarpi, Horie T, Motoda J, Kubo M, Yang H, Yoda K, Horie R, Chan WY, Leung HY, Hattori K, Konomi M, Osumi M, Yamagami M, Schroeder JI, Uozumi N (2005) Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na^+ unloading from xylem vessels into xylem parenchyma cells. *Plant J* 44:928–938
- Szczerba MW, Britto DT, Ali SA, Balkos KD, Kronzucker HJ (2008) NH_4^+ -stimulated and -inhibited components of K^+ transport in rice (*Oryza sativa* L.). *J Exp Bot* 59:3415–3423
- Szulc PM, Kobierski M, Kubicki K, Keutgen N (2010) The potential of sodium fertilization for sugarbeet production. *Zuckerindustrie* 135:721–724
- Szyroki A, Ivashikina N, Dietrich P, Roelfsema MRG, Ache P, Reintanz B, Deeken R, Godde M, Felle H, Steinmeyer R, Palme K, Hedrich R (2001) KAT1 is not essential for stomatal opening. *Proc Natl Acad Sci U S A* 98:2917–2921
- Takahashi E, Maejima K (1998) Comparative research on sodium as a beneficial element for crop plants. *Mem Fac Agr Kinki Univ* 31:57–72
- Takahashi R, Nishio T, Ichizen N, Takano T (2007) High-affinity K^+ transporter *PhaHAK5* is expressed only in salt-sensitive reed plants and shows Na^+ permeability under NaCl stress. *Plant Cell Rep* 26:1673–1679
- Tavakkoli E, Rengasamy P, McDonald GK (2010) High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J Exp Bot* 61:4449–4459
- Terry N, Ulrich A (1973) Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet under conditions of low sodium supply. *Plant Physiol* 51:1099–1101
- Thiel G, Blatt MR (1991) The mechanism of ion permeation through K^+ channels of stomatal guard cells—voltage dependent block by Na^+ . *J Plant Physiol* 138:326–334
- Thiel G, MacRobbie EAC, Blatt MR (1992) Membrane transport in stomatal guard cells: the importance of voltage control. *J Membrane Biol* 126:1–18

- Tinker PBH (1965) The effects of nitrogen, potassium and sodium fertilizers on sugar beet. *J Agric Sci* 65:207–212
- Truog E, Berger KC, Attoe OJ (1953) Response of nine economic plants to fertilization with sodium. *Soil Sci Soc Am J* 76:41–50
- Tyerman SD, Skerrett IM (1999) Root ion channels and salinity. *Sci Hortic-Amsterdam* 78:175–235
- Ulrich A, Ohki K (1956) Chlorine, bromine and sodium as nutrients for sugar beet plants. *Plant Physiol* 31:171–181
- Vaughn KC, Campbell WH (1988) Immunogold localization of nitrate reductase in maize leaves. *Plant Physiol* 88:1354–1357
- Venema K, Quintero FJ, Pardo JM, Donaire JP (2002) The *Arabidopsis* Na⁺/H⁺ exchanger AtNHX1 catalyzes low affinity Na⁺ and K⁺ transport in reconstituted liposomes. *J Biol Chem* 277:2413–2418
- Véry AA, Gaymard F, Bosseux C, Sentenac H, Thibaud JB (1995) Expression of a cloned plant K⁺ channel in *Xenopus* oocytes: analysis of macroscopic currents. *Plant J* 7:321–332
- Véry AA, Robinson MF, Mansfield TA, Sander D (1998) Guard cell cation channels are involved in Na⁺-induced stomatal closure in a halophyte. *Plant J* 14:509–521
- von Marilaun AK (1896) The natural history of plants. Blackie & Son Ltd, London
- Walker NA, Sanders D, Maathuis FJM (1996) High-affinity potassium uptake in plants. *Science* 273:977–978
- Wallace A, Toth SJ, Bear FE (1948) Influence of sodium on growth and composition of ranger alfalfa. *Soil Sci* 65:477–486
- Wang SM, Zhang JL, Flowers TJ (2007) Low-affinity Na⁺ uptake in the halophyte *Suaeda maritima*. *Plant Physiol* 145:559–571
- Wang CM, Zhang JL, Liu XS, Li Z, Wu GQ, Cai JY, Flowers TJ, Wang SM (2009) *Puccinellia tenuiflora* maintains a low Na⁺ level under salinity by limiting unidirectional Na⁺ influx resulting in a high selectivity for K⁺ over Na⁺. *Plant Cell Environ* 32:486–496
- Warne TR, Hickok LG, Kinraide TB, Vogelien DL (1996) High salinity tolerance in the stl2 mutation of *Ceratopteris richardii* is associated with enhanced K⁺ influx and loss. *Plant Cell Environ* 19:24–32
- Watad AEA, Reuveni M, Bressan RA, Hasegawa PM (1991) Enhanced net K⁺ uptake capacity of NaCl-adapted cells. *Plant Physiol* 95:1265–1269
- Weber APM, von Caemmerer S (2010) Plastid transport and metabolism of C₃ and C₄ plants—comparative analysis and possible biotechnological exploitation. *Curr Opin Plant Biol* 13:257–265
- Wetson AM, Flowers TJ (2010) The effect of saline hypoxia on growth and ion uptake in *Suaeda maritima*. *Funct Plant Biol* 38:1024–1025
- Wheeler HJ, Adams GE (1905) Concerning the agricultural value of sodium salts. Rhode Island Agricultural Experiment Station, Kingston
- Williams MC (1960) Effect of sodium and potassium salts on growth and oxalate content of Halogeton. *Plant Physiol* 35:500–509
- Williams BP, Aubry S, Hibberd JM (2012) Molecular evolution of genes recruited into C₄ photosynthesis. *Trends Plant Sci* 17:213–220
- Wolf T, Guinot DR, Hedrich R, Dietrich P, Marten I (2005) Nucleotides and Mg²⁺ ions differentially regulate K⁺ channels and non-selective cation channels present in cells forming the stomatal complex. *Plant Cell Physiol* 46:1682–1689
- Woolley JT (1957) Sodium and silicon as nutrients for the tomato plant. *Plant Physiol* 1:317–321
- Wyn Jones RG, Brady CJ, Speirs J (1979) Ionic and osmotic relations in plant cells. In: Laidman DL, Wyn Jones RG (eds) Recent advances in the biochemistry of cereals. Academic Press, New York, New York, pp 63–103
- Yadav S, Irfan M, Ahmad A, Hayat S (2011) Causes of salinity and plant manifestations to salt stress: a review. *J Environ Biol* 32:667–685
- Yao X, Horie T, Xue S, Leung H-Y, Katsuhara M, Brodsky DE, Wu Y, Schroeder JI (2010) Differential sodium and potassium transport selectivities of the rice OsHKT2;1 and OsHKT2;2 transporters in plant cells. *Plant Physiol* 152:341–355
- Yeo AR, Yeo ME, Flowers SA, Flowers TJ (1990) (1990) Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theor Appl Genet* 79:377–384
- Zhang HX, Blumwald E (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature* 19:765–768
- Zhang JL, Flowers TJ, Wang SM (2010) Mechanisms of sodium uptake by roots of higher plants. *Plant Soil* 326:45–60
- Zhao J, Barkla BJ, Marshall J, Pittman JK, Hirschi KD (2008) The *Arabidopsis cax3* mutants display altered salt tolerance, pH sensitivity and reduced plasma membrane H⁺-ATPase activity. *Planta* 227:659–669
- Zhao X, Wang YJ, Wang YL, Wang XL, Zhang X (2011) Extracellular Ca²⁺ alleviates NaCl-induced stomatal opening through a pathway involving H₂O₂-blocked Na⁺ influx in *Vicia* guard cells. *J Plant Physiol* 168:903–910
- Zhu JK, Liu J, Xiong L (1998) Genetic analysis of salt tolerance in *Arabidopsis*: evidence for a critical role of potassium nutrition. *Plant Cell* 10:1181–1191