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RESEARCH PAPER

Non-reciprocal interactions between K^+ and Na^+ ions in barley (*Hordeum vulgare* L.)

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Abstract

The interaction of sodium and potassium ions in the context of the primary entry of Na^+ into plant cells, and the subsequent development of sodium toxicity, has been the subject of much recent attention. In the present study, the technique of compartmental analysis with the radiotracers $^{42}K^+$ and $^{24}Na^+$ was applied in intact seedlings of barley (*Hordeum vulgare* L.) to test the hypothesis that elevated levels of K^+ in the growth medium will reduce both rapid, futile Na^+ cycling at the plasma membrane, and Na^+ build-up in the cytosol of root cells, under saline conditions (100 mM NaCl). We reject this hypothesis, showing that, over a wide (400-fold) range of K^+ supply, K^+ neither reduces the primary fluxes of Na^+ at the root plasma membrane nor suppresses Na^+ accumulation in the cytosol. By contrast, 100 mM NaCl suppressed the cytosolic K^+ pool by 47–73%, and also substantially decreased low-affinity K^+ transport across the plasma membrane. We confirm that the cytosolic $[K^+]$:$[Na^+]$ ratio is a poor predictor of growth performance under saline conditions, while a good correlation is seen between growth and the tissue ratios of the two ions. The data provide insight into the mechanisms that mediate the toxic influx of sodium across the root plasma membrane under salinity stress, demonstrating that, in the glycophyte barley, K^+ and Na^+ are unlikely to share a common low-affinity pathway for entry into the plant cell.

Key words: Barley, compartmental analysis, cytosol, influx, efflux, potassium, radiotracers, salinity, salt stress, sodium.

Introduction

Increasing salinization of agricultural soils is one of the most challenging issues faced by modern agriculture. In excess of 30% of cultivated soils are affected by salinity (Epstein *et al*., 1980; Zhu *et al*., 1997; Zhu, 2001; Munns, 2005). Much of this salinization is attributable to the infiltration and accumulation of NaCl (Zhu, 2001; Munns, 2005), often resulting in soil Na^+ concentrations above 40 mM, and growth suppression in most crops (Munns, 2005). One of the key physiological processes disrupted by Na^+ supply in this toxic range is the maintenance of cellular and whole-plant potassium homeostasis (Rains and Epstein, 1967; Flowers and Läuchli, 1983; Watad *et al*., 1991; Gaxiola *et al*., 1992; Warne *et al*., 1996; Zhu *et al*., 1998; Santa-María and Epstein, 2001; Peng *et al*., 2004; Cakmak, 2005; Kader and Lindberg, 2005; Kronzucker *et al*., 2006; Takahashi *et al*., 2007). At the tissue level, the ratio of K^+ to Na^+ is considered an excellent indicator of plant tolerance to salinity; the higher the ratio, the higher the plant's tolerance (Flowers and Hajibagheri, 2001; Cakmak, 2005; Chen *et al*., 2007*b*; cf. Genc *et al*., 2007). As a result of this observation, selection or breeding cultivars that maintain high K^+:Na^+ ratios has emerged as an important strategy to counteract the detrimental effects of soil salinity (Deal *et al*., 1999; Santa-María and Epstein, 2001). A more precise proposal has been that a high K^+:Na^+ ratio specifically in the cytosolic compartment is critical to plant survival under sodium challenge, while a decrease in this ratio will predict the onset of salinity stress and growth decline (Hajibagheri *et al*., 1987, 1989; Maathuis and Amtmann, 1999; Flowers and Hajibagheri, 2001; Carden *et al*., 2003; Peng *et al*., 2004; Kader *et al*., 2006; James *et al*., 2006; Chen *et al*., 2007*a*; Davenport *et al*., 2007; Obata *et al*., 2007; Takahashi *et al*., 2007). This proposal has gained wide acceptance, even though cytosolic K^+:Na^+ ratios are, in fact, rarely measured.

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In a recent study in barley (*Hordeum vulgare* L.), it was shown that, at low to intermediate levels of external K^+ supply ($[K^+]_{ext}$=0.1–1.5 mM), and at varying salinity levels, the ratio did not in fact correlate with seedling growth in this major cereal (Kronzucker *et al*., 2006). On the contrary, no difference in growth was observed in the presence of a >5-fold variation in the cytosolic K^+:Na^+ ratio. The study further demonstrated that Na^+ suppressed K^+ influx across the plasma membrane to a similar extent at 0.1 and 1.5 mM $[K^+]_{ext}$, concentrations at which high-affinity transport systems for K^+ predominate (Epstein *et al*., 1963; Kochian and Lucas, 1982; Szczerba *et al*., 2006), while Na^+ influxes and cytosolic pools were unaffected by K^+. However, a significant suppression of the cytosolic K^+ pool by Na^+ was seen only at the higher $[K^+]_{ext}$, suggesting that different cellular responses may come into effect as high-affinity K^+ transport gives way to low-affinity transport (see Szczerba *et al*., 2006).

In the present study, to examine further the proposed pivotal role of K^+ homeostasis in salinity stress and tolerance, the effects of K^+ supply across the low-affinity transport range of K^+ (up to 40 mM $[K^+]_{ext}$) upon the primary fluxes and cytosolic pools of Na^+, and, conversely, the effects of Na^+ upon K^+ fluxes and pools in this range were investigated. Such an examination was particularly necessary in the light of recent disagreements in the literature pertaining to (i) the size of cytosolic Na^+ pools (Flowers and Hajibagheri, 2001; Carden *et al*., 2003; James *et al*., 2006; Kronzucker *et al*., 2006), and (ii) the proposed, but as yet unresolved, roles of molecular candidates for toxic Na^+ influx into the plant (Tester and Davenport, 2003; Flowers, 2006; Wang *et al*., 2007). The primary candidates proposed are K^+-specific channels (Wang *et al*., 2007), non-selective cation channels (NSCCs; Demidchik *et al*., 2002), HKT-type transporters (Rodriguez-Navarro and Rubio, 2006), and the low-affinity cation transporter LCT (Amtmann *et al*., 2001). Indeed, were these candidates to catalyse toxicologically significant fluxes of sodium, they should be competitively influenced by the presence of potassium. For these reasons, a detailed study of the interactions between the two ions was carried out along a wide gradient of external K^+ supply.

Materials and methods

Plant growth

Seeds of barley (*Hordeum vulgare* L. cv. Klondike) were surface-sterilized by immersing seeds in 1.0% sodium hypochlorite for 10 min. Seeds were then washed under running tap water for 3 h, placed on discs of plastic mesh, and covered by 2 cm of moist sand. Germination proceeded for the following 3 d in a walk-in growth chamber equipped with fluorescent lights (Philips Econ-o-watt, F96T12, with an irradiation of 200 μmol photons m^{-2} s^{-1} at plant height, for 16 h d^{-1}), and having a day/night temperature cycle of 20 °C/15 °C, and relative humidity of ~70%.

Following germination, seedlings were transferred to opaque plastic 4 l hydroponic vessels, filled with modified quarter-strength Johnson's solution, consisting of: 5 mM $Ca(NO_3)_2$, 0.5 mM NaH_2PO_4, 0.25 mM $MgSO_4$, 0.2 mM $CaSO_4$, 0.125 μM Na_2MoO_4, 20 μM FeEDTA, 25 μM H_3BO_3, 2 μM $ZnSO_4$, 0.5 μM $MnSO_4$, and 0.5 μM $CuSO_4$. Control plants had no additional sodium, while salt-stressed plants were treated with 100 mM Na^+ (as NaCl). Potassium concentrations were adjusted to treatments of 1.5, 5, 10, 20, and 40 mM by addition of K_2SO_4. pH was adjusted to 6.3–6.5 by addition of NaOH. To prevent nutrient depletion, solutions were replaced after 2 d. Plants remained in hydroponic solutions for 4 d prior to experimentation. In select treatments (1.5 mM and 40 mM K^+, at low and high NaCl), plants were also grown for 2 weeks (11 d in solution; see Fig. 9B).

Flux analysis

Compartmental analysis by tracer efflux was conducted as described in detail elsewhere (Kronzucker *et al*., 1999, 2006; Britto *et al*., 2001, 2006). In brief, roots of intact seedlings were immersed for 1 h in a nutrient solution identical to the growth solution, except that it contained $^{24}Na^+$ or $^{42}K^+$ in addition to non-radioactive Na^+ or K^+. Roots were desorbed of radioactivity in tracer-free solutions for the monitoring of $^{24}Na^+$ or $^{42}K^+$ efflux, by periodic washing with a timed series of non-radioactive aliquots of nutrient solution (Kronzucker *et al*., 1999, 2006; Britto *et al*., 2001). The time course of aliquots was as follows: 15 s (4×), 20 s (3×), 30 s (2×), 40 s (1×), 50 s (1×), 1 min (5×), 1.25 min (1×), 1.5 min (1×), 1.75 min (1×), and 2 min (8×). Radioactivity was measured in aliquots and plant tissues by gamma counting, using a Canberra-Packard counter, Quantum Cobra Series II, Model 5003.

Unidirectional Na^+ or K^+ fluxes were determined using standard analyses (for further details, see Kronzucker *et al*., 1999, 2003, 2006; Britto *et al*., 2001):

(i) Efflux (ϕ_{co}) was calculated from the initial rate of $^{24}Na^+$ or $^{42}K^+$ release from the cytosol, divided by initial cytosolic specific activity (S_c) of $^{24}Na^+$ or $^{42}K^+$ in this compartment; S_c was estimated by using labelling time (t_L) in the external medium of known specific activity (S_o), and the kinetic constant k that describes the exponential rate of cytosolic tracer exchange, using the relationship $S_c=S_o(1-e^{-kt})$. This constant was determined from the slope of the cytosolic line (see Fig. 1).

(ii) Net fluxes were determined from retention of tracer in root and shoot at the end of the desorption protocol, divided by S_o, while influx (ϕ_{oc}) was calculated from the sum of ϕ_{co} and the net flux.

(iii) Cytosolic concentrations of Na^+ ($[Na^+]_{cyt}$) or K^+ ($[K^+]_{cyt}$) were determined by integrating rates of radioactivity release from this compartment; in simplified form, this calculation is made using the equation $[Na^+ \text{ or } K^+]_{cyt}=\Omega\phi_{oc}/k$, where Ω is a proportionality constant accounting for the cytosolic compartment comprising 5% of tissue volume. Activity coefficients (γ) for cytosolic concentrations were estimated using the Debye–Hückel–Onsager equation, adapted for the monovalent cations Na^+ and K^+: $-\log\gamma=(0.5\sqrt{I})/(1+\sqrt{I})$ where I is ionic strength of the cytosol (assuming that the dominant cations are Na^+ and K^+, and that these are charge-balanced by monovalent anions; see Jander and Blasius, 1988).

Tissue K^+ and Na^+ content

Roots of barley seedlings were desorbed for 5 min in 10 mM $CaSO_4$ to remove extracellular K^+ and Na^+. Roots and shoots were then separated, weighed, and oven dried for a minimum of 72 h at 80–85 °C, then pulverized and digested with 30% HNO_3 for a minimum of 72 h. K^+ and Na^+ concentration was determined using a single-channel flame photometer (Digital Flame Analyzer model 2655-00; Cole-Parmer, Anjou, Québec).

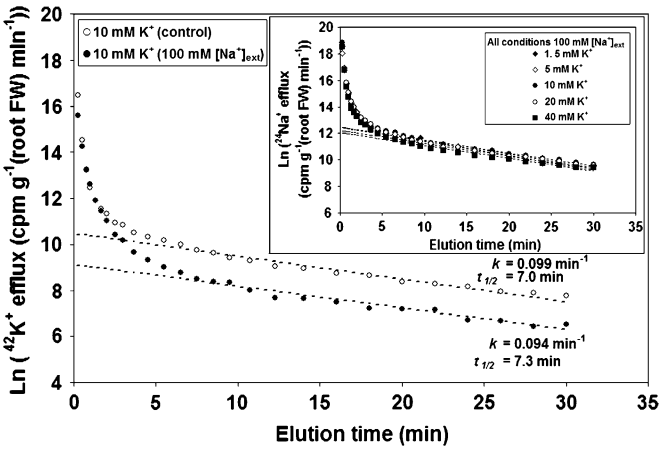

Fig. 1. Representative plots of $^{42}K^+$ and $^{24}Na^+$ (inset) efflux from roots of intact barley seedlings, under varying ionic conditions. Roots had been preloaded for 60 min in radioactive solution, then eluted of radioactivity in a timed series of non-radioactive growth-solution aliquots. Dashed lines represent the slowest exchanging compartment (cytosolic), with a minimum of 12 time points used for linear regression. Experiments were replicated between 4 and 14 times.

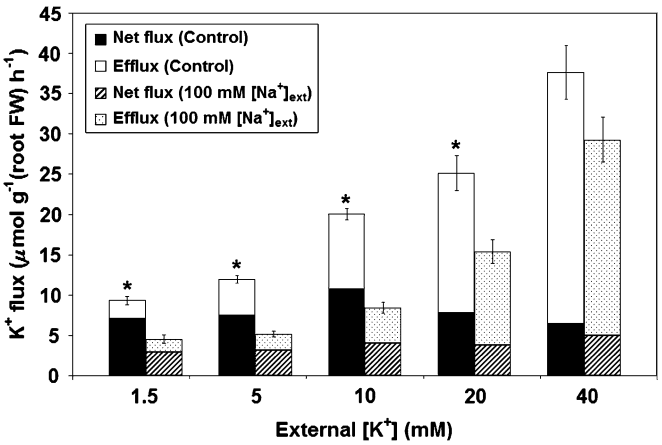

Fig. 2. Components of unidirectional K^+ influx in roots of intact barley seedlings, grown and monitored with or without 100 mM Na^+. Control values are drawn from Szczerba *et al*. (2006). Error bars refer to ±SEM of 4–14 replicates, with asterisks indicating significant differences in influx values within a given K^+ treatment ($P < 0.05$). Where differences in influx between Na^+ treatments were observed, significant differences in efflux and net flux were also found ($P < 0.05$).

Statistical analysis

Statistical analyses were conducted using one-way analysis of variance (ANOVA) with the statistical package SPSS (ver. 12).

Results and discussion

Figure 1 shows representative plots of $^{42}K^+$ (main plot) and $^{24}Na^+$ (inset) release from roots of intact barley seedlings, previously labelled with respective tracers for 60 min. Steady-state tracer efflux curves of this type were analysed under five external potassium conditions (1.5, 5, 10, 20, and 40 mM) in control (1 mM NaCl) or salt-treated (100 mM NaCl) plants, to determine unidirectional fluxes of Na^+ and K^+ across the plasma membrane of root cells, kinetic constants for cytosolic exchange of the two ions, and estimates of the ions' cytosolic activities. In Fig. 1, the main plot shows the potent reduction, by elevated Na^+ provision, of the efflux of K^+ from barley roots (note that the *y*-axis is logarithmic), a phenomenon paralleled in reductions in the influx and net flux of K^+ (see Fig. 2). By contrast, the inset of this figure shows the lack of a reciprocal effect: a 27-fold difference in K^+ supply had no influence on the efflux of Na^+ under saline conditions.

Figure 2 shows the steady-state unidirectional and net fluxes of K^+ into barley roots, obtained using compartmental tracer analysis under the 10 conditions examined. Over the range of K^+ supply, the influx of potassium in salt-treated plants was lower than in controls (by 20–60%), significantly so in all cases except for 40 mM $[K^+]_{ext}$ ($P < 0.05$). Work by Kochian *et al*. (1985) showed that application of 3 mM Na^+ resulted in a 50% suppression of K^+ influx in maize, but this effect was limited to the low-affinity transport range for K^+, and was only seen in 'low-salt' plants. This result contrasts with the present study and with previous work in barley, which showed the suppression, by Na^+, of K^+ influx at the high-affinity supply provision of 0.1 mM (Kronzucker *et al*., 2006), as well as in the low-affinity range in the 'high-salt' plants (grown at up to 40 mM K^+); two possible explanations for this disagreement include the use of a much higher Na^+ provision (100 mM), and the examination of plants grown and tested under steady-state nutritional conditions. Consistent with the present work, effects of Na^+ on K^+ uptake have also been observed in both high- and low-affinity transport ranges in a wide range of other studies (Rains and Epstein, 1967; Santa-María *et al*., 1997; Fu and Luan, 1998; Rubio *et al*., 2000, 2003; Martínez-Cordero *et al*., 2005).

Net fluxes and unidirectional effluxes of K^+ were also reduced under elevated NaCl, significantly so in all cases except for the 40 mM K^+ treatment (Fig. 2). The reduction of K^+ efflux with high NaCl provision (Figs 1, 2) may appear, on the surface, to contradict the finding that Na^+ stimulated a net efflux of K^+ from roots of several plant species (see, for instance, Chen *et al*., 2005; Cuin and Shabala, 2007), but it must be pointed out that such demonstrations of Na^+-stimulated K^+ efflux involve short-term changes following NaCl shock, whereas the present study involves measurements made under steady-state conditions.

Influx and efflux of Na^+ at 100 mM $[Na^+]_{ext}$ were high under all K^+ conditions (Fig. 3), and indicated substantial futile cycling of the ion at the plasma membrane. Rapid unidirectional influxes of Na^+ in glycophytes under salinity conditions have been observed by others (Essah *et al*., 2003; Wang *et al*., 2006; Davenport *et al*.,

2007; Horie *et al*. 2007); in particular, the influx values reported here are in excellent agreement with short-term $^{22}Na^+$ labelling data in a recent study by Chen *et al*. (2007*a*) that examined Na^+ influx in four cultivars of barley. The high ratios of efflux to influx seen consistently throughout the present treatments are also supported by previous studies (Cheeseman, 1982; Mills *et al*., 1985; Essah *et al*., 2003). In sharp contrast to the suppression of unidirectional and net K^+ fluxes by Na^+, a reciprocal influence was not seen. At 100 mM $[Na^+]_{ext}$, the 27-fold variation in K^+ supply resulted in no significant differences in influx, efflux, or net flux of sodium. Previous work on the interactions between these ions in the high-affinity K^+ transport range (Kronzucker *et al*., 2006) also showed no significant differences in Na^+ between 0.1 mM and 1.5 mM external $[K^+]$. The two studies together therefore show that a 400-fold range of K^+ provision fails to modify Na^+ fluxes, while K^+ fluxes across this range are generally suppressed by elevated sodium. This lack of reciprocity was further confirmed by experiments conducted in 2-week-old seedlings of barley, indicating that the pattern was not limited to one developmental stage (data not shown). This conclusion, however, was not drawn in a classic study, also with barley, by Rains and Epstein (1967): a strong reciprocal suppression of the flux of one ion (K^+ or Na^+) by the co-presence of the other was observed in the low-affinity 'mechanism 2 of alkali cation transport'. Again, however, it is important to note that the present study differed from that of Rains and Epstein (1967) in that the fluxes in the present study were measured under steady-state nutritional supply conditions, which may be more relevant to plant performance in the field than perturbational conditions might be. In a recent study, Wang *et al*. (2007) also showed that K^+ supply had no effect on Na^+ uptake in the halophyte *Suaeda maritima*, but only when the external $[Na^+]$ was below 75 mM. Above that concentration, a suppression of Na^+ fluxes was found, indicating, in *Suaeda*, the involvement of AKT-type potassium channels in Na^+ influx under some conditions.

In the present study, the steady-state, non-reciprocal effect of one ion on the fluxes of the other is borne out in the tissue accumulation patterns for K^+ and Na^+ (Figs 4, 5). For potassium, there was a general reduction of tissue content with salt treatment, particularly in the shoot, where tissue K^+ levels were seen to decline by as much as 50% (Fig. 4). The contrasting situation for sodium is seen in Fig. 5; as with Na^+ fluxes, the accumulation of this ion was not changed over the range of applied external $[K^+]$, either in the root or in the shoot.

On a finer scale of analysis, a steady-state, non-reciprocal interaction was also seen in the effects of ion supply on the activities of Na^+ and K^+ in the cytosolic compartment of root cells ($a_{Na+,cyt}$ and $a_{K+,cyt}$; Figs 6, 7), as estimated by compartmental analysis. Under salinity conditions, $a_{Na+,cyt}$ proved to be resistant to variations in K^+ provision, showing no significant changes across the entire range of external K^+ supply (Fig. 7). By comparison, 100 mM sodium provision resulted in severe drops in $a_{K+,cyt}$, relative to low-sodium controls (Fig. 6), under all levels of K^+ supply. This effect was somewhat alleviated with increasing K^+ provision, but, even at the highest $[K^+]_{ext}$, the pool size of K^+ was reduced by nearly one-half. Again, this pattern of no reciprocal effect on cytosolic pool sizes of Na^+ and K^+ was confirmed in 2-week-old barley seedlings (not shown).

Other workers, using techniques of X-ray microanalysis, radiotracer analysis, subcellular fractionation, and ion-selective microelectrodes, have reported very similar values for cytosolic activities of sodium (Harvey and Flowers, 1978; Yeo, 1981; Mills *et al*., 1985; Amtmann

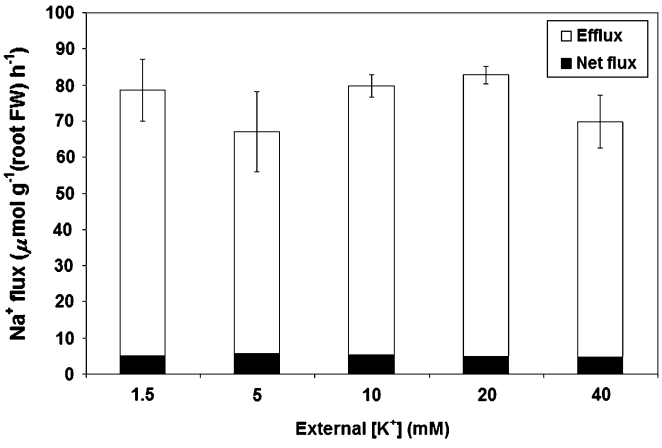

Fig. 3. Components of unidirectional Na^+ influx in roots of intact barley seedlings, grown and monitored at 100 mM Na^+ and at varying external K^+ provision. Error bars refer to ±SEM of four to seven replicates. No significant differences between treatments were observed.

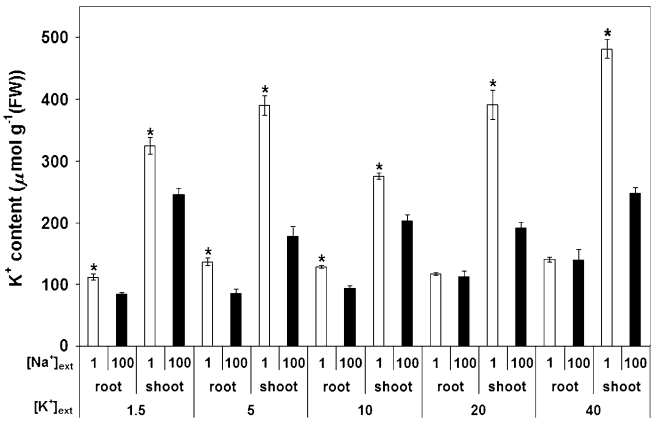

Fig. 4. Tissue content of K^+ in roots and shoots of barley plants, grown with or without 100 mM Na^+, and under varying external K^+ provision. Control (1 mM Na^+) values are drawn from Szczerba *et al*. (2006). Error bars refer to ±SEM of four to eight replicates. Asterisks denote significant differences within a given K^+ treatment and plant organ ($P < 0.05$).

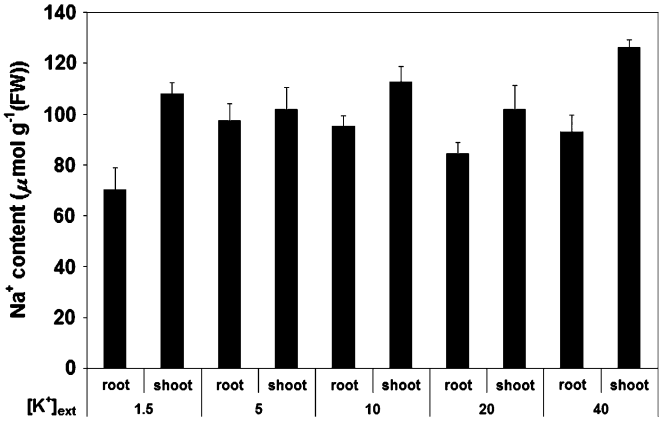

Fig. 5. Tissue content of Na^+ in roots and shoots of barley plants, grown at 100 mM Na^+, and under varying external K^+ provision. Error bars refer to ±SEM of 8–12 replicates. No significant differences between treatments were observed.

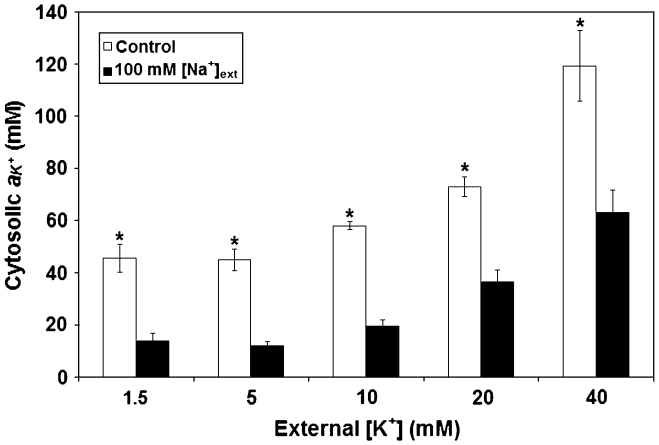

Fig. 6. Cytosolic K^+ activity in roots of barley seedlings grown under varying K^+ provision, with or without 100 mM Na^+. Error bars refer to ±SEM of 4–14 replicates. Asterisks denote significant differences within a given K^+ treatment ($P < 0.05$).

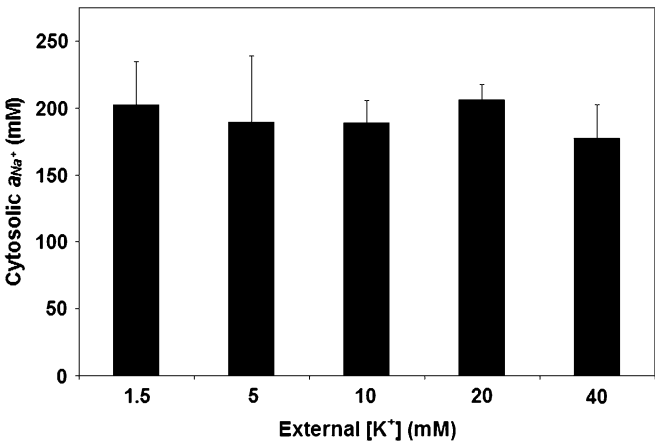

Fig. 7. Cytosolic Na^+ activity in roots of barley seedlings grown under 100 mM Na^+ and varying K^+ provision. Error bars refer to ±SEM of four to seven replicates. No significant differences between treatments were observed.

and Gradmann, 1994; Flowers and Hajibagheri, 2001; James *et al*., 2006) and potassium (Pitman and Saddler, 1967; Vorobiev, 1967; Macklon, 1975; Memon *et al*., 1985; Beilby and Blatt, 1986; Maathuis and Sanders, 1993; Walker *et al*., 1996). There have also been reports of suppressed cytosolic K^+ pool by Na^+ (Jeschke and Stelter, 1976; Harvey *et al*., 1981; Mills *et al*., 1985; Hajibagheri *et al*., 1987, 1988, 1989; Flowers and Hajibagheri, 2001; Carden *et al*., 2003; Chen *et al*., 2007*a*). A similar suppression of $a_{K+,cyt}$ has been observed to result from high ammonium (NH_4^+) provision in the same model system (Kronzucker *et al*., 2003), but, unlike the effect of Na^+, this effect was only seen in the high-affinity K^+ transport range (0.1 mM $[K^+]_{ext}$). By contrast, suppression of $a_{K+,cyt}$ by high Na^+ was not observed at this level of K^+ supply in previous work (Kronzucker *et al*., 2006), suggesting that K^+ homeostasis is more resilient to salt stress in the high-affinity range.

The variability of the cytosolic K^+ pool in response to both salinity and external K^+ supply, and, by contrast, the uniformity of the Na^+ pool with changes in external K^+, together result in substantial (greater than 4-fold) changes in the ratio of the two cytosolic pools (Fig. 8). This K^+:Na^+ ratio peaked at 40 mM $[K^+]_{ext}$, and was minimized at 5 mM $[K^+]_{ext}$, but did not correlate with the growth of the experimental plants (Fig. 9A; also seen in 2-week-old plants, Fig. 9B). As with previous work (Kronzucker *et al*., 2006), this lack of correlation calls into question the view that the cytosolic K^+:Na^+ ratio is a critical determinant of plant growth in response to salinity stress (see Introduction). On the other hand, the ratio of K^+ to Na^+ on the coarser, tissue level, is rather uniform among salinity-treated plants (Fig. 8), as is the growth of plants under these conditions, indicating that this measure is indeed more of a more accurate predictor of plant performance under salt stress (see Introduction). This conclusion is supported by previously observed correlations between salinity tolerance and the tissue K^+:Na^+ ratio in other cultivars of barley (Flowers and Hajibagheri, 2001; Chen *et al*., 2007*b*). Interestingly, however, a recent study by Genc *et al*. (2007) showed that even this measure did not correlate with growth in 21 cultivars of bread wheat. The authors explained this as possibly reflecting alternative strategies of salt tolerance in different cereal species.

In summary, neither Na^+ fluxes nor cytosolic Na^+ pools responded to K^+ over a wide range of K^+ supply [400-fold, when results from a previous work (Kronzucker *et al*., 2006) are included], and elevated K^+ provision was unable to rescue barley plants from the primary toxic entry of Na^+, contrary to the rescue from NH_4^+ toxicity that is effected by increasing external provision of K^+ (Britto and Kronzucker, 2002; Kronzucker *et al*., 2003; Szczerba *et al*., 2006). The non-reciprocal nature of potassium–sodium interactions in barley provides insight into the

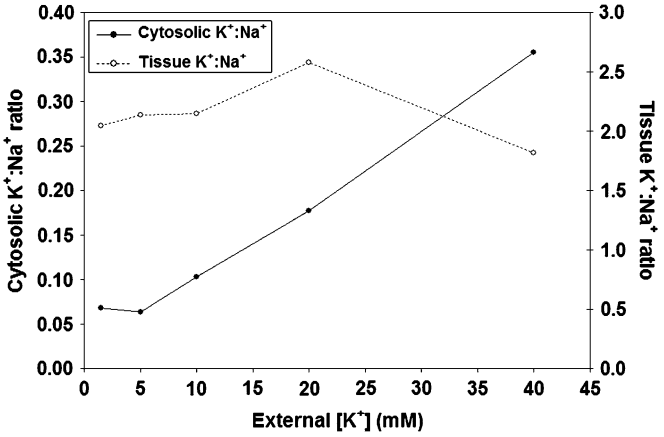

Fig. 8. Cytosolic and tissue K^+:Na^+ ratios in roots of intact barley seedlings, grown at 100 mM Na^+ and varying K^+ provision.

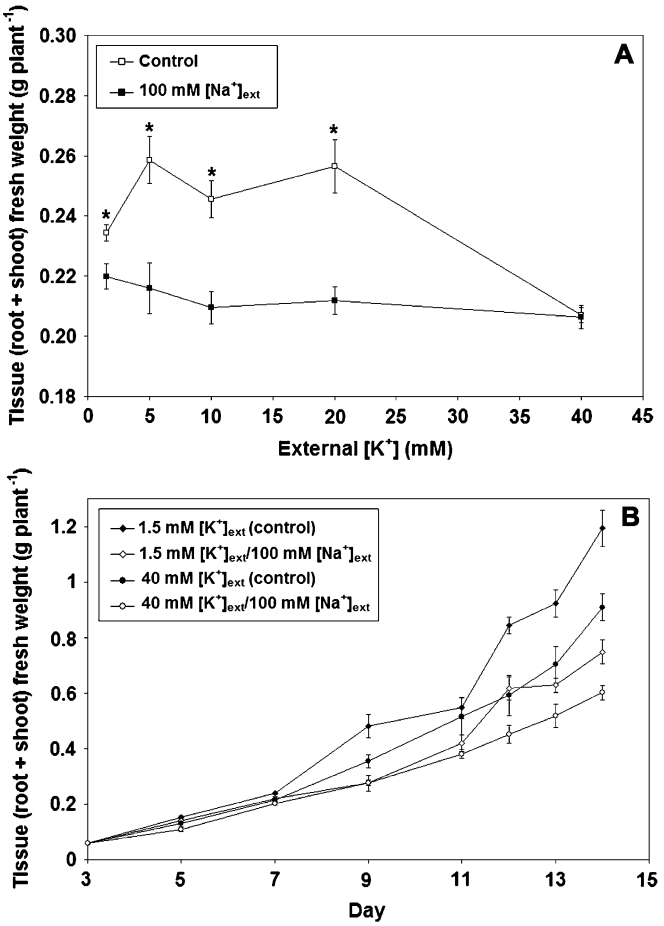

Fig. 9. (A) Fresh weights of barley seedlings (roots+shoots), grown and monitored with or without 100 mM Na^+ and varying levels of K^+ supply. Error bars refer to ±SEM of 12–72 replicates. Asterisks denote significant differences within a given K^+ treatment ($P < 0.05$). (B) Fresh weights of barley seedlings (roots+shoots), grown and monitored with or without 100 mM Na^+ and two different $[K^+]_{ext}$ (1.5 mM and 40 mM) for 2 weeks. Error bars refer to ±SEM of 5–82 replicates.

mediation of primary Na^+ entry into plant roots under toxicity-inducing conditions, an unresolved issue under much current debate (Schachtman and Liu, 1999; Golldack *et al*., 2003; Tester and Davenport, 2003; Qi and Spalding, 2004; Fuchs *et al*., 2005; Kader and Lindberg, 2005; Flowers, 2006; Davenport *et al*., 2007; Obata *et al*., 2007; Takahashi *et al*., 2007; Wang *et al*., 2007).

The present results suggest that several ion transporters favoured as candidates in the recent literature should likely be discounted, at least in barley. These include, on the one hand, K^+ channels, such as less selective members of the AKT family of transporters, and, on the other, NSCCs and the low-affinity cation transporter LCT, which have been shown to allow the passage of a variety of cations under certain conditions (Amtmann and Sanders, 1999; Maathuis and Amtmann, 1999; Uozumi *et al*., 2000; Amtmann *et al*., 2001; Rus *et al*., 2001; Kader and Lindberg, 2005; Kader *et al*., 2006; Wang *et al*., 2007). Were members of these families of transporters, or of high-affinity K^+ transporters (Takahashi *et al*., 2007), critically involved in toxic Na^+ influx, increasing external K^+ concentrations over an extensive range as employed in the present study would be expected to strongly reduce Na^+ influx by virtue of ion competition effects. While background levels of Ca^{2+} were high in the present study (5 mM), and it is recognized that this may have minimized to some extent Na^+ influx contribution from NSCCs and LCT transporters that are known to be Ca^{2+}-sensitive (see Rains and Epstein, 1967; Schachtman and Liu, 1999; Amtmann *et al*., 2001; Essah *et al*., 2003; Wang *et al*., 2006; Davenport *et al*., 2007; Wang *et al*., 2007), it should be pointed out that Ca^{2+} levels in soils are also typically in excess of 1 mM (Reisenauer, 1966; Hawkesford and Miller, 2004). Thus, if a major contribution from NSCCs or LCT cannot be realized or rationalized under such conditions, the contribution from these transporters may also not be particularly relevant in the field (Schachtman and Liu, 1999; for a similar conclusion, also see Wang *et al*., 2007). Two recent studies have also suggested that K^+-specific Shaker-type channels are unlikely candidates for Na^+ influx; in one case, a knockout mutation of *AKT1* in *Arabidopsis thaliana* resulted in reduced growth under Na^+ stress, showing that toxic entry of Na^+ can still proceed in the absence of AKT1 (Qi and Spalding, 2004). In the other case, overexpression of *KAT1* in yeast cells resulted in lowered Na^+ accumulation (Obata *et al*., 2007). Another group of transporters whose role in primary Na^+ influx has been rigorously discussed is the HKT family. It has been proposed that the most well-studied member of this group, HKT1;1 (Rubio *et al*., 1995) is a major determinant of sodium influx in *Arabidopsis* (Rus *et al*., 2001), but other reports suggest that its function is in long-distance recirculation of Na^+ (Berthomieu *et al*., 2003; Davenport *et al*., 2007). The strong suppression of HKT2-mediated Na^+ influx by even

low amounts of external K^+ in grasses (Laurie *et al*., 2002; Horie *et al*., 2007) suggests that this transporter is not likely to play a significant role in Na^+ uptake under normal soil conditions or in the present study.

However, other transporter types may emerge as mediators of toxic Na^+ entry into the plant (Horie *et al*., 2001; Golldack *et al*., 2002, 2003; Garciadeblás *et al*., 2003; Haro *et al*., 2005; Ren *et al*., 2005; Kader *et al*., 2006). In particular, the involvement of sodium-specific influx transporters remains a possibility, even though such systems have not yet been identified through *Arabidopsis* genomic analysis (see Hua *et al*., 2003). Another possibility may involve the coupled transport of Na^+ with ions such as chloride. A transporter of this kind has recently been identified in *Arabidopsis* (Colmenero-Flores *et al*., 2007).

Clearly, however, a definitive answer has not been forthcoming, and the search for the primary Na^+ influx transporter mediating toxic plasma-membrane influx continues (Flowers, 2006). The present study indicates that, in barley, this transporter (or transport system) appears to be K^+-independent, suggesting that attempts to improve salt tolerance in this species, via increased selectivity of K^+ uptake pathways, are unlikely to be effective.

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