Planta (2003) 217: 540–546
DOI 10.1007/s00425-003-1032-5

ORIGINAL ARTICLE

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Cytosolic potassium homeostasis revisited: ^{42}K-tracer analysis in *Hordeum vulgare* L. reveals set-point variations in [K^+]

Received: 23 January 2003 / Accepted: 15 March 2003 / Published online: 1 May 2003

Abstract Current models of potassium acquisition and cytochemical processes in plants assume that potassium concentrations in the cytosol ($[K^+]_{cyt}$) are maintained homeostatically at approximately 100 mM. Here, we use ^{42}K radiotracer data in the model plant species *Hordeum vulgare* L. (barley) to show that this assumption is incorrect. Our study reveals that $[K^+]_{cyt}$ in root cells of intact barley seedlings is held at a minimum of two physiological set points, coinciding with two fundamentally distinct modes of K^+ transport, each of which is characterized by a unique network of fluxes to and from the cytosol, and reflects variations in mechanisms and energetics of K^+ transport, cytosolic K^+ turnover, flux partitioning, and sensitivity to NH_4^+. Increased external potassium or ammonium concentrations caused a substantial drop in $[K^+]_{cyt}$, as well as a switch from a transport mode dominated by high-affinity, energy-dependent, influx to a mode dominated by channel-mediated fluxes in both directions across the plasma membrane. Our study provides the first subcellular demonstration of the flexibility, rather than strict homeostasis, of cellular K^+ maintenance, and of the dynamic interaction between plant membrane fluxes of the two major nutrient cations K^+ and NH_4^+.

Keywords Compartmental analysis · ^{42}K · *Hordeum* Ion transport · Homeostasis

Introduction

Potassium (K^+) availability in both agricultural and natural ecosystems frequently limits plant growth, development, and productivity (Asher and Ozanne 1967; Leigh 2001). K^+ is the most abundant cation in plant cells (Leigh and Wyn Jones 1984; Leigh 2001), and activates a wide range of biochemical reactions, including many that are central to primary metabolism and mRNA translation (Memon et al. 1985; Maathuis and Sanders 1996). K^+ is also a major osmotic solute, an important counterion to anionic pools in the cell (Leigh 2001), and its fluxes play a key role in stomatal and nastic movements and in membrane potential rectification (MacRobbie 1977; Leigh 2001). While tissue [K^+] can fluctuate with external K^+ supply, it has become widely accepted that the K^+ concentration in the cytosol of the plant cell ($[K^+]_{cyt}$) is maintained near 100 mM (Memon et al. 1985; Beilby and Blatt 1986; Maathuis and Sanders 1993; Walker et al. 1996; Leigh 2001), with high stringency, except under conditions of K^+ starvation (Vorobiev 1967; Leigh and Wyn Jones 1984; Memon et al. 1985; Maathuis and Sanders 1993, 1996; Walker et al. 1996; Leigh 2001). Why such a rigorous maintenance of $[K^+]_{cyt}$ should be necessary, however, is questionable, given that the [K^+] required for the in vitro activation of cytosolic enzymes is generally much lower than 100 mM (Memon et al. 1985), and that ionic and osmotic functions can be compensated for by other chemical components in the cell (Jeschke and Wolf 1988; Leigh and Wyn Jones 1984). Moreover, much of the evidence supporting strict $[K^+]_{cyt}$ homeostasis is problematic, as it frequently has involved the use of physiologically unrealistic culture or measurement conditions. The most recent, and apparently most solid, study supporting $[K^+]_{cyt}$ homeostasis has used potassium-selective microelectrodes (Walker et al. 1996), which yielded reasonably stable cytosolic K^+ activities of approximately 80 mM (or approx. 100 mM when expressed as a concentration). However, these results must be approached with caution, given the numerous difficulties associated with intracellular microelectrodes, including problems with invasiveness (Mertz and Higinbotham 1976; Beilby and Blatt 1986), with calibration appropriate to a cytosolic milieu of unknown composition

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(Coombs et al. 1994; Miller and Smith 1996), and with interferences from other intracellular ions and proteins that can produce severely erroneous readings with K^+-selective, and similar, electrodes (Coombs et al. 1994; Cuin et al. 1999). A less invasive experimental approach, using radioisotope methods to trace cellular K^+ fluxes, has also supported the idea of $[K^+]_{cyt}$ homeostasis, based on the concept that $[K^+]_{cyt}$ is an outcome of the multitude of fluxes introducing and removing K^+ to and from the cytosol. However, serious reservations must also be noted for these studies, in that they involved $^{86}Rb^+$ as an inappropriate K^+ analogue (Bange 1979; Memon et al. 1985), were limited to experiments with excised tissues (Pitman and Saddler 1967; Mertz and Higinbotham 1976; Macklon 1975), or, most importantly, did not investigate K^+ relations under varying external K^+ supply (Pitman and Saddler 1967; Macklon 1975; Bange 1979; Behl and Jeschke 1982; Hajibagheri et al. 1989). The present work, in root cells of intact barley seedlings, is the first cell-physiological study to use ^{42}K to provide non-invasive determinations of $[K^+]_{cyt}$ by examining the simultaneous operation of multidirectional K^+ fluxes in intact plants, over a wide, but physiologically realistic, range of external $[K^+]$.

Materials and methods

Plant culture

Seeds of barley (*Hordeum vulgare* L. cv. Klondike) were surface-sterilized for 15 min in 1% sodium hypochlorite and germinated under acid-washed sand for 3 days (Britto et al. 2001; Britto and Kronzucker 2001). Seedlings were then transferred to hydroponic tanks (8-l or 40-l capacity), for a growth period of 4 days, prior to experimentation, in controlled-environment chambers set at a 16 h/8 h light/dark cycle, 20 ± 2 °C temperature, 70% relative humidity, and approx. 250 μmol m^{-2} s^{-1} photon flux at plant level provided by fluorescent tubes with a spectral composition similar to sunlight (Vita-Lite Duro-Test, Fairfield, NJ, USA). Solutions in hydroponic tanks contained aerated 0.25-strength modified Johnson's solution, with NH_4^+ provided as $(NH_4)_2SO_4$ or NO_4^- as $Ca(NO_3)_2$, at pH 6–6.5 (Britto and Kronzucker 2001). Chemicals were purchased from VWR Canlab, Mississauga, ON, Canada. NH_4^+ or NO_3^- concentrations in solution were 0.1 mM or 10 mM. To maintain steady-state growth conditions, solutions were replenished daily. K^+ was provided as K_2SO_4 and KCl (the latter in a micronutrient concentration of 0.0125 mM), to give 0.0225, 0.1125, or 1.5 mM K^+. In total, 12 nutrient regimes were used (4 nitrogen conditions × 3 potassium conditions). The similarities in fluxes of potassium from cytosol to vacuole at the three potassium concentrations in the absence of ammonium (see Table 1) provide evidence that the plants used in our study were at a nutritional steady state, and that potassium was not limiting under these conditions (Asher and Ozanne 1967).

Radiotracer experiments

We used the radiotracer ^{42}K ($t_{1/2}$ = 12.36 h), provided by the McMaster Nuclear Reactor, Hamilton, Ontario, Canada, instead of the commonly used tracer $^{86}Rb^+$ (Memon et al. 1985; Vale et al. 1988; Hirsch et al. 1998; Spalding et al. 1999; Santa-Maria et al. 2000), to avoid elemental discrimination between Rb and K, which can be substantial (Jacoby et al. 1973; Santa-Maria et al. 2000). Labelling and desorption solutions used in these

Table 1 Component fluxes, and cytosolic concentrations, of K^+ in root cells of intact barley (*Hordeum vulgare*) seedlings, as a function of N and K treatment. Fluxes are expressed as μmol g^{-1} (fresh weight) h^{-1} and concentrations as mM, ± SE of 4–10 replicates. For details of calculations, see <crossref seeheading = "3" > Materials and methods </crossref >

	$[K^+]_o$ (mM)											
	0.0225				0.1125				1.5			
	$[NH_4^+]_o$ (mM)		$[NO_3^-]_o$ (mM)		$[NH_4^+]_o$(mM)		$[NO_3^-]_o$ (mM)		$[NH_4^+]_o$ (mM)		$[NO_3^-]_o$ (mM)	
	0.1	10	0.1	10	0.1	10	0.1	10	0.1	10	0.1	10
φ_{oc}	7.25 ± 0.45	2.55 ± 0.24	6.18 ± 0.79	8.38 ± 0.69	9.91 ± 0.78	3.54 ± 0.56	13.54 ± 0.71	11.45 ± 0.72	10.81 ± 1.49	11.45 ± 0.91	9.31 ± 1.04	9.14 ± 0.93
φ_{co}	1.07 ± 0.11	1.64 ± 0.06	1.18 ± 0.07	1.51 ± 0.31	3.03 ± 0.48	1.67 ± 0.27	3.84 ± 0.26	2.86 ± 0.21	3.74 ± 1.09	4.44 ± 0.41	3.50 ± 0.35	2.92 ± 0.36
φ_{net}	6.19 ± 0.42	0.91 ± 0.26	5.00 ± 0.86	6.87 ± 0.45	6.89 ± 0.52	1.96 ± 0.27	9.70 ± 0.50	8.59 ± 0.66	7.07 ± 0.98	7.01 ± 0.54	5.81 ± 0.69	6.22 ± 0.75
φ_{vac}	3.45 ± 0.38	0.65 ± 0.26	2.62 ± 0.79	2.90 ± 0.36	2.96 ± 0.40	1.59 ± 0.24	4.66 ± 0.38	3.52 ± 0.48	3.99 ± 0.72	4.01 ± 0.34	2.99 ± 0.50	3.61 ± 0.38
φ_{xylem}	2.74 ± 0.12	0.26 ± 0.01	2.38 ± 0.09	3.97 ± 0.49	3.92 ± 0.23	0.43 ± 0.09	5.04 ± 0.26	5.07 ± 0.36	3.08 ± 0.31	3.00 ± 0.31	2.82 ± 0.49	2.61 ± 0.42
$[K^+]_{cyt}$	102 ± 6	32 ± 2	98 ± 11	111 ± 18	125 ± 8	35 ± 5	131 ± 10	136 ± 15	75 ± 10	60 ± 1	42 ± 4	50 ± 6

experiments were chemically identical to the growth solutions described above; i.e., plants were at a nutritional steady state throughout the experimental period. Seedlings were then transferred from growth solutions to $^{42}K^+$ labelling solutions for 60 min (see Britto and Kronzucker 2001). This time period was long enough to substantially enrich the cytosolic compartments of root cells with radioisotope, but too short to label potentially interfering back-fluxes from the vacuole (Behl and Jeschke 1982; Jeschke 1982; Memon et al. 1985) and the phloem, the latter of which are known to be sizeable (White 1997; Peuke et al. 1998; Engels and Kirkby 2001). Labelled roots were attached to efflux funnels with clamped rubber spouts (Britto et al. 2001; Britto and Kronzucker 2001). Efflux was measured by desorbing radioactivity from the roots once per minute, for 28–30 min, into a series of 15-ml aliquots of non-labelled solution. Desorbed radioactivity was counted in a γ-counter (Canberra-Packard, Quantum Cobra Series II, Model 5003). Roots and shoots were separated after the final desorption step, surface water was removed from roots by low-speed centrifugation for 45 s, and the plant organs were weighed and counted for radioactivity. The changing rate of tracer efflux was plotted logarithmically over time (see Fig. 1), and linear regression was used to resolve the slow phase of efflux corresponding to the cytosolic compartment, based on phase-testing experiments and on previous studies (Memon et al. 1985; Britto et al. 2001; Britto and Kronzucker 2001). The coefficients of determination (r^2) for these regressed lines were typically >0.9. The slopes of the regression lines yielded kinetic (first-order) constants (k) for cytosolic $^{42}K^+$ exchange in the respective phases, which were used in subsequent calculations (see below). k constants, for convenience, are convertible to exchange half-times by the formula $t_{1/2}=(\ln 2)/k$. All fluxes are expressed in μmol K^+ g^{-1} (root FW) h^{-1}. Symbols used for fluxes, and basic calculation methods, are as follows:

- φ_{co} = efflux from the cytosol, obtained from the rate of $^{42}K^+$ release from the cytosol at time zero (i.e., the y-intercept of the regressed cytosolic line), divided by the specific activity of the cytosolic compartment (S_c) at the end of ^{42}K labelling. This is calculated by the equation $S_c=S_o(1-e^{-kt})$, where S_o is the specific activity of the external solution (Walker and Pitman 1976).
- φ_{net} = net flux, obtained from the accumulation of $^{42}K^+$ in the plants at the end of the elution period. This quantity is corrected for counts that would have been released had efflux continued for a longer period, i.e. by subtracting, from the accumulated counts, the result of the integration of the equation $\varphi^*_{co(t)}=\varphi^*_{co(t=0)}e^{-kt}$ (i.e., the exponential form of the cytosolic regression line, where $\varphi^*_{co(t)}$ is the tracer efflux at time t, and $\varphi^*_{co(t=0)}$ is the initial tracer efflux), from the end of elution to $t=\infty$. This difference is divided by S_o and by the duration of $^{42}K^+$-labelling.
- φ_{oc} = unidirectional influx, calculated from $\varphi_{net}+\varphi_{co}$.
- φ_{vac} = flux to the vacuole, resulting from $\varphi_{net}-\varphi_{xylem}$.
- φ_{xylem} = flux to the xylem, calculated according to the following rationale: Radioactivity (cpm) recovered in shoots accumulates over the course of the experiment as a result of two oppositely changing isotopic fluxes: I—the flux from a rising specific activity in the xylem-labelling (cytosolic) pool during the labelling period; and II—the flux from a declining specific activity in this pool during the elution period. Mathematically, these isotopic fluxes can be expressed in the following terms:

$$\mathrm{I}: \phi_{xylem}* = \phi_{xylem}S_o(1-e^{-kt})$$

$$\mathrm{II}: \phi_{xylem}* = \phi_{xylem}S_o(1-e^{-kt(L)})e^{-kt}$$

where φ^*_{xylem} is the radioisotopic flux from the cytosol to the xylem, t is time, and $t(L)$ is labelling time. The sum of these terms, when integrated, respectively, over $t(L)$ and over elution time $t(E)$, is equal to the total tracer remaining in the shoot, and this relationship can be used to solve for φ_{xylem} in the following way:

$$\begin{aligned}\text{cpm remaining } g^{-1} &= \int_0^{t(L)} \phi_{xylem}S_o(1-e^{-kt})dt \\ &+ \int_0^{t(E)} \phi_{xylem}S_o(1-e^{-kt(L)})e^{-kt}dt \\ &= \phi_{xylem}S_o\left\{\left(\int_0^{t(L)}dt - \int_0^{t(L)}e^{-kt}dt\right) + (1-e^{-kt(L)})\int_0^{t(E)}e^{-kt}dt\right\} \\ &= \phi_{xylem}S_o\left\{\left(t+\frac{1}{k}e^{-kt}\right)\Big|_0^{t(L)} - \frac{1-e^{-kt(L)}}{k}e^{-kt}\Big|_0^{t(E)}\right\}\end{aligned}$$

Evaluating the integral and solving for φ_{xylem} gives the equation used in the present study:

$$\phi_{xylem} = \frac{\text{cpm remaining } g^{-1}}{t(L)S_o - \frac{S_o}{k}(1-e^{-kt(L)})(e^{-kt(E)})}$$

Cytosolic concentrations of K^+ were derived from unidirectional influxes and cytosolic kinetic exchange constants, according to the flux-turnover equation $[K^+]=\Omega\varphi_{oc}/k$ (Britto and Kronzucker 2001), where Ω is a proportionality constant accounting for the cytosolic compartment comprising 5% of tissue volume (Britto and Kronzucker 2001). Experiments were repeated 4–10 times. Standard errors for flux and compartmentation parameters (half-times, fluxes, pool sizes) were within 15% of the means.

Results and discussion

We grew barley seedlings under 12 nutritional conditions, by varying both potassium and nitrogen provision rates, and nitrogen source (NO_3^- vs. NH_4^+). The external K^+ concentrations were chosen to reflect the operational ranges of the two known kinetically and energetically distinct influx patterns for K^+ (Epstein et al. 1963; Vale et al. 1988; Leigh 2001), that of saturable, high-affinity, influx (0.0225 mM and 0.1125 mM) on the one hand, and linear, low-affinity, influx (1.5 mM) on the other. Such a distinction is critical to the question of $[K^+]_{cyt}$ homeostasis, because, as external $[K^+]$ increases, the change in influx mechanism, from one of active (high-affinity) transport, to one of passive, channel-mediated (low-affinity), transport is most likely to reveal changes, if any, in K^+ distribution across the plasma membrane. Variations in nitrogen source in these experiments were important firstly because we had hypothesized that one reason why NH_4^+ is toxic to many plant species is that unrestricted accumulation of this ion displaces cytosolic potassium from its presumed homeostatic set point (Britto et al. 2001; Kronzucker et al. 2001; Britto and Kronzucker 2002), and, secondly, because application of high NH_4^+ deactivates the major component of high-affinity K^+ influx, but has little effect on low-affinity K^+ influx (Hirsch et al. 1998; Spalding et al. 1999; Santa-Maria et al. 2000; Vale et al. 1988). This treatment can thus be used to reveal a special flux condition in which low-affinity ion channels may be mediating fluxes from dilute external K^+ solutions (Hirsch et al. 1998), and one in which cytosolic potassium concentrations must play a key thermodynamic role.

Figure 1 shows outward time-dependent $^{42}K^+$ currents from roots of radiolabelled barley plants under the 12 growth conditions. Differences in half-times for

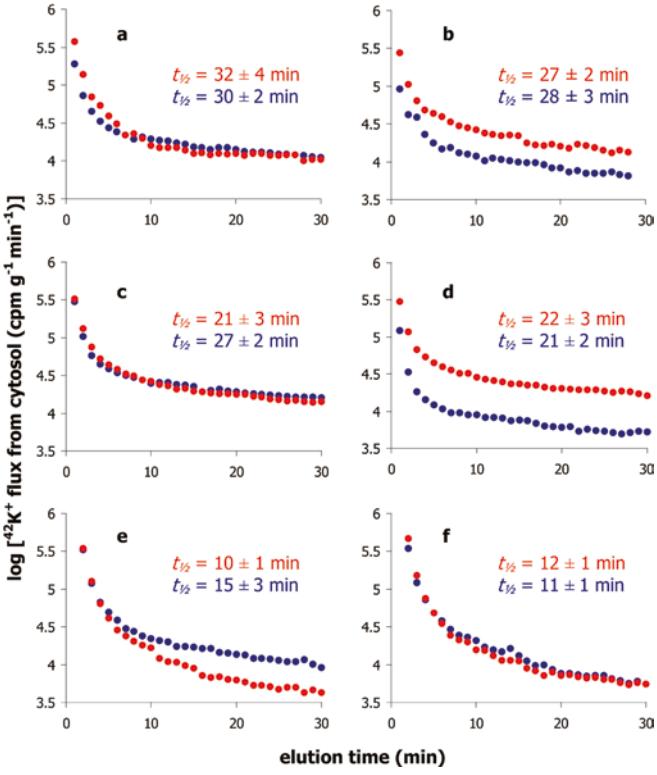

Fig. 1a–f $^{42}K^+$ efflux from radiolabelled root cells of intact barley (*Hordeum vulgare*) seedlings in each of 12 nutritional treatments, averaged over 4–10 runs per treatment. *Red symbols* indicate NO_3^- treatment; *blue symbols* indicate NH_4^+ treatment. Plots at *left* (**a, c, e**) indicate low-N (0.1 mM) treatment; those at *right* (**b, d, f**) indicate high-N (10 mM) treatment. Potassium treatments are arranged by rows: **a, b** 0.0225 mM; **c, d** 0.1125 mM; **e, f** 1.5 mM. Exchange half-times for cytosolic K^+ are given for each condition (±SE). The plots were normalized for variations in specific tracer activity (to an arbitrary value of 200,000 cpm/μmol) and root mass between experiments. Changing tracer efflux into the external solution was divided by the ratio of efflux to influx, such that plotted rates indicate total tracer flux from the cytosol, which equals total tracer influx under the steady-state conditions used here (see Kronzucker et al. 2000). This mathematical treatment is used so that regression of the slow phases of the normalized efflux traces yields *y*-intercepts that permit direct comparisons in $[K^+]_{cyt}$ among treatments with similar slopes or half-times ($t_{1/2}$), given that $[K^+]_{cyt}$ is proportional to the product $t_{1/2}\varphi_{oc}$ (see, e.g., Walker and Pitman 1976, Britto and Kronzucker 2001; also see Materials and methods)

cytosolic K^+ exchange, as determined from the rates of first-order decline in the slow phase of tracer efflux (visible in the tails of each plot starting at 5–10 min after the onset of elution), indicate a significantly faster turnover of cytosolic K^+ at the external concentration representative of low-affinity influx (Fig. 1e, f), as compared to the turnover at the two concentrations representative of high-affinity influx (Fig. 1a–d). This indicates that the well-established differences in high- and low-affinity influx systems are only one aspect of a more comprehensive shift in cellular behavior from one transport mode to another, a shift which has important implications for $[K^+]_{cyt}$ (see below). While this effect was independent of N nutrition, high NH_4^+ concentrations in the external medium caused a dramatic and consistent suppression of the $^{42}K^+$ current in the high-affinity, but not the low-affinity, K^+ influx range, further distinguishing the two transport modes. Differences in the $^{42}K^+$ current reflected a suppression of all measured cytosolic K^+ fluxes under high-NH_4^+ and low- to medium-K^+ conditions (Table 1). By contrast, the measured K^+ fluxes at high external $[K^+]$ were very similar in value to fluxes at low external $[K^+]$, when not suppressed by ammonium. This broad flux optimum is not surprising, given that plants tend to have broad growth optima with respect to K^+ supply (Asher and Ozanne 1967), although it conceals the differences in cytosolic K^+ turnover seen in Fig. 1.

Cytosolic $[K^+]$ was determined by analysis of flux and turnover data (Fig. 2). At low external $[K^+]$, estimates of $[K^+]_{cyt}$ agreed well with values previously reported in the literature (Pitman and Saddler 1967; Vorobiev 1967; Macklon 1975; Memon et al. 1985; Beilby and Blatt 1986; Maathuis and Sanders 1993; Walker et al. 1996), the notable exception being that the high NH_4^+ treatment had a profound effect on $[K^+]_{cyt}$, suppressing it 3- to 4-fold. While it has been firmly established that NH_4^+ can cause a decline in total tissue K^+, the present work is the first demonstration that NH_4^+ alters the magnitude specifically of $[K^+]_{cyt}$. This finding would offer a compelling explanation of the toxic effect of NH_4^+, as hypothesized above, were the tight control of $[K^+]_{cyt}$ indeed essential to cell function. However, further experiments showed that, at high external $[K^+]$, $[K^+]_{cyt}$ also fell dramatically (40–50%) below the approx. 100 mM set point seen at low and intermediate external $[K^+]$. Because this decline was independent of N source, and occurred under potassium-replete, non-stressed, conditions, deviations from a presumed $[K^+]_{cyt}$ homeostasis cannot by themselves be

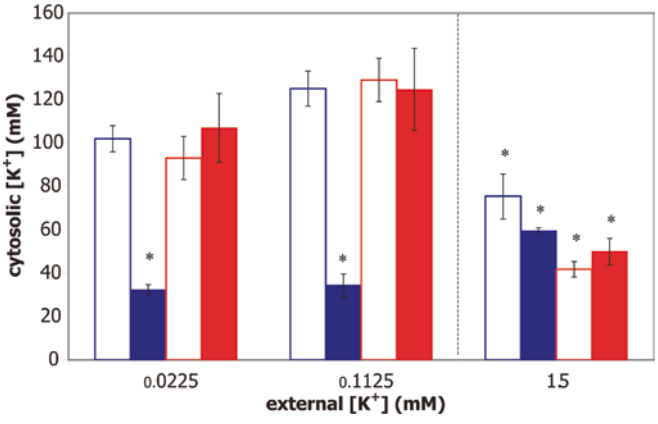

Fig. 2 Cytosolic K^+ concentrations ($[K^+]_{cyt}$) in root cells of intact barley seedlings as a function of N and K treatment. *Blue bars* indicate NH_4^+ treatments; *red bars* indicate NO_3^- treatments. *Open bars* indicate low-N, and *closed bars* indicate high-N treatment (see Fig. 1). Error bars indicate ± SE of 4–10 replicates. *Asterisks* indicate means that differ from the 0.1125 mM K^+, 0.1 mM NO_3^- used as a standard of comparison (Student's *t*-test, $P > 0.99$). The *dashed line* indicates a distinction between external K^+ concentrations pertaining to the two modes of K^+ transport

implicated as a mechanism of NH_4^+ toxicity, although it is still possible that NH_4^+ pushes $[K^+]_{cyt}$ below a critical threshold level at which damage to the cell may occur. While this possibility was not addressed in this study, our data reveal a more fundamental phenomenon, that a universal set point for $[K^+]_{cyt}$ does not occur, and that $[K^+]_{cyt}$ heterostasis is fully compatible with normal plant function.

It is essential to point out that analyses of the kind presented here provide information about the intact plant, rather than a homogeneous cell preparation, and hence report averages of cytosolic potassium concentrations for the entire root. While our approach, then, lacks the special precision of single-cell sampling, it provides relevance at the organismal level. Interestingly, in the case of potassium, Leigh (2001) has emphasized the relative uniformity in K^+ relations among cell types in the root, lending credence to an intrapolation of integrated tissue data to single-cell behavior. Moreover, even if there were substantial heterogeneity in K^+ fluxes and compartmentation among different cell types, our data would then imply even more substantial heterostasis in some cells, as these would have to differentially absorb the variations observed, or a concerted shift among all cells that would conserve the ratio of $[K^+]_{cyt}$ among all cells. Either way, the conclusion that cytosolic K^+ concentrations are not fixed at a single homeostatic set point is inescapable.

An analysis of the component fluxes governing K^+ distribution across the plasma membrane provides important insight into the mechanistic underpinnings, and implications, of $[K^+]_{cyt}$ heterostasis. Table 1 and Fig. 1 show that the two conditions under which $[K^+]_{cyt}$ is reduced (i.e., the high-NH_4^+/low-K^+ treatments, and the high-K^+ treatments) have some superficial differences, but may be interpreted as being caused by deeper similarities in K^+ transport mechanisms. In the case of high-NH_4^+/low-K^+, the primary cause of the depression in $[K^+]_{cyt}$ is a severe reduction in influx by high NH_4^+ (Table 1), in excellent agreement with recent demonstrations that high-affinity K^+ influx is mediated by NH_4^+-sensitive HAK (KUP/HAK/KT/POT—see Mäser et al. 2001) transporters in both barley and in *Arabidopsis thaliana* (Spalding et al. 1999; Santa-Maria et al. 2000; Mäser et al. 2001), as well as with more traditional physiological measurements of K^+ influx in the presence of NH_4^+ (Vale et al. 1988). By contrast, K^+ influx in the low-affinity range was NH_4^+-insensitive, and its similarity in magnitude to high-affinity influx (Table 1) into a K^+ pool of diminished size must result in a more rapid turnover of that pool, which is precisely what is observed in Fig. 1. Low-affinity influx is believed to be primarily mediated by Shaker-type (AKT) channels, one of which was demonstrated by Hirsch et al. (1998) to be NH_4^+-insensitive in *A. thaliana*, to the extent that it was able to mediate K^+ influx from external $[K^+]$ as low as 0.01 mM when high-affinity K^+ influx was suppressed by high external $[NH_4^+]$. In that study, it was pointed out that such a flux could only occur under the relatively rare condition in which membrane potentials were highly hyperpolarized, i.e. negative of −200 mV, a situation which was found in approx. 20% of cells, mostly in the apical region (Hirsch et al. 1998). Importantly, as in barley (Cheeseman and Hanson 1979; Britto et al. 2001; Kronzucker et al. 2001), membrane potentials in *Arabidopsis* are sensitive to external $[K^+]$, but insensitive to external $[NH_4^+]$ (Hirsch et al. 1998; Spalding et al. 1999), but in their thermodynamic evaluation, Hirsch et al. had assumed that $[K^+]_{cyt}$ would remain unaltered under high external $[NH_4^+]$. Our results, showing that NH_4^+ suppresses $[K^+]_{cyt}$ by as much as 4-fold, indicate that channel-mediated influx under conditions of very low external $[K^+]$ is even more feasible than first proposed by Hirsch et al. At high external $[K^+]$, Nernst potentials calculated from our $[K^+]_{cyt}$ values are very similar to numerous published values of membrane electrical potential differences ($\Delta\Psi$) in barley (generally between −80 and −100 mV; see Pitman and Saddler 1967; Mertz and Higinbotham 1976; Cheeseman and Hanson 1979; Walker et al. 1996), suggesting that, in the low-affinity influx range, both K^+ influx and K^+ efflux across the plasma membrane are passive, resulting in a thermodynamic K^+ equilibrium. By contrast, reported membrane potentials at the two lower external $[K^+]$ are in most cases significantly more positive than predicted on the basis of $[K^+]_{cyt}$, indicating active transport. Again, the exceptions are seen in the high NH_4^+ treatments, which appear to mimic the high K^+ condition both in terms of suppressed $[K^+]_{cyt}$ and in terms of the likelihood of passive transport. That the absolute rates of influx under these two latter conditions are much less than those under high external $[K^+]$ can be explained simply by the concentration dependence of low-affinity influx.

Using the thermodynamic considerations outlined above, it emerges that, under most physiological circumstances, all outwardly directed transmembrane K^+ fluxes from the cell, including efflux into the external medium, and the more specialized flux into the xylem stream (φ_{xylem}), are likely to be mediated by channels. These may include the recently identified KCO channels (Mäser et al. 2001) in the case of efflux, and SKOR channels (Gaymard et al. 1998) in the case of the xylem flux. It was of further interest to examine responses of these fluxes to high external NH_4^+, because the latter leads to even higher cytosolic NH_4^+ concentrations (Britto et al. 2001; Kronzucker et al. 2001), which could in principle compete with K^+ for KCO- or SKOR-mediated transport (Kronzucker et al. 2001; Britto and Kronzucker 2002). On the other hand, it has also been proposed that the well-documented suppression of net K^+ uptake by high NH_4^+ is attributable mainly to enhanced K^+ efflux (Vale et al. 1988). The data in Table 1 support the first of these two ideas, showing that fluxes from the cell mirror the differential sensitivities of high- and low-affinity K^+ influx to high NH_4^+, with one exception, that of efflux under the lowest external $[K^+]$. In that case, the suppression of net flux in the high NH_4^+ treatment is attributable almost exclusively to a

suppression of influx. Efflux is not diminished, but rather a minimum transport velocity (V_{min}) appears to exist, which operates even in the presence of high NH_4^+. Nevertheless, the xylem flux in this case is profoundly diminished, offering a plausible new explanation for ammonium toxicity, which is often reported with lower, but not higher, potassium supply (Britto and Kronzucker 2002). Importantly, neither flux from the cell is a simple function of $[K^+]_{cyt}$; note that the lowest efflux is observed in a 100 mM $[K^+]_{cyt}$ condition, while the highest is observed in a 60 mM $[K^+]_{cyt}$ condition (Fig. 2, Table 1). However, $[K^+]_{cyt}$ correlated moderately ($r^2 = 0.67$) with φ_{xylem}, as did the net flux into the plant ($r^2 = 0.69$). This indicates that a system regulating root/shoot K^+ flux partitioning may respond to both the cytosolic K^+ pool size and to net K^+ acquisition, allowing the plant to repress shoot fluxes under conditions of K^+ stress.

Our results document, for the first time, both a pathological (NH_4^+-induced) and a non-pathological (K^+-dependent) heterostasis in the magnitude of the cytosolic K^+ pool, which parallels an inherent plasticity in cellular K^+ fluxes. The suppression of $[K^+]_{cyt}$ seen under conditions of high NH_4^+, and low to intermediate K^+, cannot in itself explain NH_4^+ toxicity, although the extent of that suppression, or the suppression of shoot fluxes of K^+, are likely to be key factors. The discovery of $[K^+]_{cyt}$ heterostasis and the new information we present on K^+ fluxes provide a whole-systems context for the interpretation of molecular information currently emerging about both K^+ and N transport mechanisms. Our study clarifies essential elements in the energetics of K^+ transport, and identifies differential physiological sensitivities of key cellular transport processes, which will help guide the design of experiments aimed at the functional characterization of transporter proteins in their native settings.

Acknowledgments We thank A.D.M. Glass, N.P.A. Huner, G.S. Khush, M.Y. Siddiqi, and G.J.D. Kirk for insightful discussion of the manuscript, M. Butler at the McMaster University Nuclear Research Reactor for developing the ^{42}K radioisotope production protocol used here, and the University of Western Ontario for providing research space. This work was supported by grants from the National Science and Engineering Research Council of Canada and the Canada Foundation for Innovation.

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