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RESEARCH PAPER

NH_4^+-stimulated and -inhibited components of K^+ transport in rice (*Oryza sativa* L.)

Mark W. Szczerba*, Dev T. Britto, Shabana A. Ali, Konstantine D. Balkos and Herbert J. Kronzucker†

Department of Biological Sciences, University of Toronto, 1265 Military Trail, Toronto, Ontario, Canada M1C 1A4

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Abstract

The disruption of K^+ transport and accumulation is symptomatic of NH_4^+ toxicity in plants. In this study, the influence of K^+ supply (0.02–40 mM) and nitrogen source (10 mM NH_4^+ or NO_3^-) on root plasma membrane K^+ fluxes and cytosolic K^+ pools, plant growth, and whole-plant K^+ distribution in the NH_4^+-tolerant plant species rice (*Oryza sativa* L.) was examined. Using the radiotracer $^{42}K^+$, tissue mineral analysis, and growth data, it is shown that rice is affected by NH_4^+ toxicity under high-affinity K^+ transport conditions. Substantial recovery of growth was seen as $[K^+]_{ext}$ was increased from 0.02 mM to 0.1 mM, and, at 1.5 mM, growth was superior on NH_4^+. Growth recovery at these concentrations was accompanied by greater influx of K^+ into root cells, translocation of K^+ to the shoot, and tissue K^+. Elevating the K^+ supply also resulted in a significant reduction of NH_4^+ influx, as measured by ^{13}N radiotracing. In the low-affinity K^+ transport range, NH_4^+ stimulated K^+ influx relative to NO_3^- controls. It is concluded that rice, despite its well-known tolerance to NH_4^+, nevertheless displays considerable growth suppression and disruption of K^+ homeostasis under this N regime at low $[K^+]_{ext}$, but displays efficient recovery from NH_4^+ inhibition, and indeed a stimulation of K^+ acquisition, when $[K^+]_{ext}$ is increased in the presence of NH_4^+.

Key words: Ammonium toxicity, influx, ion transport, potassium, rice, translocation.

Introduction

Maintenance of potassium (K^+) homeostasis is critical to plant cell function. However, the uptake of K^+ and its distribution within the plant vary widely with environmental conditions. One of the chief factors influencing plant–potassium relations is the chemical speciation of inorganic nitrogen (N) in soil. In particular, ammonium (NH_4^+) has been shown to reduce the primary influx of K^+ from the external environment, and to suppress its accumulation in plant tissues (Kirkby and Mengel, 1967; Scherer *et al*., 1984; Vale *et al*., 1987, 1988; Van Beusichem *et al*., 1988; Engels and Marschner, 1993; Peuke and Jeschke, 1993; Wang *et al*., 1996; Gerendás *et al*., 1997; Santa-María *et al*., 2000; Bañuelos *et al*., 2002; Kronzucker *et al*., 2003). This is a key feature of NH_4^+ toxicity, which affects the majority of plant species when exposed to elevated soil concentrations of NH_4^+ (typically, when $[NH_4^+]$ >1 mM; Britto *et al*., 2001, 2002; Britto and Kronzucker, 2002). However, the NH_4^+-dependent inhibition of K^+ influx and accumulation can be alleviated by increasing the external K^+ concentration ($[K^+]_{ext}$; Cao *et al*., 1993; Spalding *et al*., 1999; Santa-María *et al*., 2000; Kronzucker *et al*., 2003; Szczerba *et al*., 2006*a*). The sensitivity of K^+ influx to NH_4^+ appears to depend on the mechanism of primary K^+ uptake that dominates at a given $[K^+]_{ext}$: at micromolar concentrations, K^+ uptake is mainly mediated by an NH_4^+-suppressible, high-affinity transport system (HATS), while at higher, millimolar $[K^+]_{ext}$, K^+ influx is mediated by an NH_4^+-resistant, low-affinity transport system (LATS) (Spalding *et al*., 1999; Santa-María *et al*., 2000; Kronzucker *et al*., 2003; Szczerba *et al*., 2006*a*). The precise mechanism by which NH_4^+ inhibits high-affinity K^+ influx has not been elucidated, although it has been suggested that NH_4^+ competitively inhibits K^+ transport at the protein level (Vale *et al*., 1987; Wang *et al*., 1996).

In ammonium-sensitive barley (*Hordeum vulgare* L.), NH_4^+ has been shown to disrupt not only the primary influx, but also the internal distribution, of K^+, at both

* Present address: Department of Plant Sciences, University of California, Davis, Davis, CA, USA
† To whom correspondence should be addressed. E-mail: herbertk@utsc.utoronto.ca

whole-plant and cellular levels. For example, Santa-María *et al*. (2000) and Kronzucker *et al*. (2003) found that NH_4^+ reduced K^+ translocation from root to shoot by 60–90%. At a subcellular level, radiotracer studies have shown that cytosolic $[K^+]$ is suppressed by high $[NH_4^+]_{ext}$ (Kronzucker *et al*., 2003; Szczerba *et al*., 2006*a*). The disruption of cytosolic K^+ homeostasis and the translocation of K^+ to the shoot are, most probably, related: while NH_4^+ is not transported in large amounts to the shoot (Kronzucker *et al*., 1998; Husted *et al*., 2000), its effect on cytosolic $[K^+]$ or upon K^+ translocation pathways in the root may play a critical role in NH_4^+ sensitivity by reducing the xylem loading of K^+ (Gaymard *et al*., 1998; Johansson *et al*., 2006; Liu *et al*., 2006).

Rice (*Oryza sativa* L.), the world's most important crop species, displays greater tolerance to NH_4^+ than other cereals (Sasakawa and Yamamoto, 1978). Given the pivotal role of K^+ nutrition in the development of NH_4^+ toxicity or tolerance, it was therefore important to investigate the degree to which rice plants may be able to resist NH_4^+-induced disruptions in primary K^+ acquisition, cellular K^+ homeostasis, and root-to-shoot K^+ translocation. These disruptions have been characterized in barley and other NH_4^+-sensitive plant species, but have only been examined in very limited detail in NH_4^+-tolerant plant species (Wang *et al*., 1996; Bañuelos *et al*., 2002). Here, compartmental analyses has been conducted using the radiotracer $^{42}K^+$ to evaluate K^+ transport and compartmentation in intact seedlings of NH_4^+-tolerant rice, examining plant performance at four levels of K^+ supply (0.02–40 mM, spanning the high- and low-affinity transport ranges), with either NH_4^+ or nitrate (NO_3^-) as the sole N source (10 mM). It was hypothesized that K^+ transport and distribution, at whole-plant and subcellular levels, would resist disruption by NH_4^+ provision, in ammonium-tolerant rice.

Materials and methods

Plant culture

Rice seeds (*O. sativa* L. cv. 'IR-72') were surface-sterilized for 10 min in 1% sodium hypochlorite, and germinated in water for 2 d prior to placement in 4.0 l vessels containing aerated, modified Johnson's solution (2 mM $MgSO_4$; 1 mM $CaCl_2$; 0.3 mM NaH_2PO_4; 0.1 mM Fe-EDTA; 20 μM H_3BO_3; 9 μM $MnCl_2$; 1.5 μM $CuSO_4$; 1.5 μM $ZnSO_4$; 0.5 μM Na_2MoO_4), pH 6–6.5, for an additional 19 d. The growth solutions were modified to provide four concentrations of potassium (as K_2SO_4), at 0.02, 0.1, 1.5, and 40 mM, and nitrogen (10 mM) as either $(NH_4)_2SO_4$ or $Ca(NO_3)_2$. Solutions were exchanged frequently to ensure that plants remained at a nutritional steady state, and to ensure that solution pH was maintained between 6 and 6.5. Solutions were exchanged on the following days (with the first 2 d spent in water for germination): 8, 12, 15, 17, 19, and 20. Plants were cultured in climate-controlled walk-in growth chambers under fluorescent lights, providing a tropical environment for the rice seedlings, with a day/night temperature cycle of 30 °C/20 °C, an irradiation of 425 μmol photons m^{-2} s^{-1} at plant height for 12 h d^{-1} (Sylvania Cool White, F96T12/CW/VHO), and a relative humidity of 70%. On day 19 (2 d prior to experimentation), seedlings were bundled together in groups of 3–5 at the stem base using a plastic collar, 0.5 cm in height. For ^{13}N experiments, rice seedlings were transferred to an experimental radiotracer facility that had similar irradiance and temperature to those of the growth chamber on day 20 (1 d prior to experimentation).

Steady-state influx, translocation, and pool size measurements

Plasma membrane fluxes, cytosolic pool sizes, and shoot translocation of K^+ were determined under steady-state conditions using compartmental analysis by tracer efflux (Lee and Clarkson, 1986; Siddiqi *et al*., 1991; Kronzucker *et al*., 1995, 2003; Szczerba *et al*., 2006*a*, *b*). Briefly, intact roots of seedlings were labelled for 60 min in a solution identical to the growth solution except that it contained the radiotracer $^{42}K^+$ ($t_{1/2}$=12.36 h, provided by McMaster University Nuclear Reactor, Hamilton, Ontario, Canada). Labelled seedlings were then attached to efflux funnels and eluted of radioactivity for 30 min, using a timed series [15 s (four times), 20 s (three times), 30 s (twice), 40 s (once), 50 s (once), 1 min (five times), 1.25 min (once), 1.5 min (once), 1.75 min (once), and 2 min (eight times); see Fig. 2] of non-radioactive desorption solutions (as 13 ml or 20 ml aliquots), identical to the growth solutions. All solutions were mixed using a fine stream of air bubbles. After elution, roots were detached from shoots and spun in a low-speed centrifuge for 30 s, and fresh weights were determined. Radioactivity from eluates, roots, and shoots was measured by gamma counting (Perkin-Elmer Wallac 1480 Wizard 3″, Turku, Finland, or Canberra-Packard, Quantum Cobra Series II, Model 5003).

Exponentially declining rates of $^{42}K^+$ release from roots over time were then analysed using linear regression (see Fig. 2). The function $\ln \phi_{co(t)}^* = \ln \phi_{co(i)}^* - kt$ [in which $\phi_{co(t)}^*$ is tracer efflux at elution time t, $\phi_{co(i)}^*$ is initial tracer efflux, and k, found from the slope of the changing tracer release rate, is the rate constant describing the exponential decline in tracer efflux] was used to resolve the kinetics of the slowest exchanging phase, which represents tracer exchange with the cytosolic compartment (Behl and Jeschke, 1981; Memon *et al*., 1985; Kronzucker *et al*., 2003). Chemical efflux, ϕ_{co}, was determined from $\phi_{co(i)}^*$, divided by the specific activity of the cytosol (S_c) at the end of the labelling period [this activity was determined using the exponential rise function $S_c = S_o (1 - e^{-kt})$, in which S_o is the specific activity of the external solution, t is labelling time, and k is as described above]. Net flux, ϕ_{net}, was found using total-plant $^{42}K^+$ retention after desorption. Influx, ϕ_{oc}, was calculated from the sum of ϕ_{net} and ϕ_{co}. Translocation of K^+ to the shoot was determined from tracer accumulation at the end of the loading period. Cytosolic $[K^+]$ ($[K^+]_{cyt}$) was determined using the flux turnover equation, $[K^+]_{cyt} = \Omega \times \phi_{oc}/k$, where Ω is a proportionality constant correcting for the cytosolic volume being ~5% of total tissue (Lee and Clarkson, 1986; Siddiqi *et al*., 1991). For ^{13}N experiments, compartmental analysis proceeded as described above, with the exception that seedlings were labelled for between 30 min and 60 min in a solution identical to the growth solution but containing the radiotracer ^{13}N ($t_{1/2}$=9.97 min; as $^{13}NH_4^+$) provided by the CAMH cyclotron facility (University of Toronto, Ontario, Canada).

Short-term non-steady-state influx measurements

To examine the effect of changing $[K^+]_{ext}$ on K^+ influx, unidirectional influx of K^+ under non-steady-state conditions was determined directly using short-term labelling with $^{42}K^+$ (see Britto

and Kronzucker, 2001). Seedlings grown at 0.1 mM $[K^+]_{ext}$ were pre-equilibrated for 5 min in growth solution, then immersed in labelling solution for another 5 min. This solution was identical to the growth solution, except that it contained $^{42}K^+$ for a final $[K^+]_{ext}$ between 0.1 mM and 5 mM. Plants were then transferred to a non-radioactive solution for 5 s to reduce tracer carryover to the desorption solution, and finally desorbed for 5 min in fresh nutrient solution. Influx of NH_4^+ was also determined directly, as described for $^{42}K^+$, but using short-term labelling (5 min) with ^{13}N. Seedlings were placed for 5 min in growth solution for equilibration, followed by immersion in labelling solution identical to the growth solution, but containing $^{13}NH_4^+$, for 5 min. Plants were then transferred to a non-radioactive solution for 5 s, and finally desorbed for 5 min in fresh nutrient solution, as described for $^{42}K^+$.

Tissue K^+ content

To measure tissue K^+ content, roots of rice seedlings were first desorbed for 5 min in 10 mM $CaSO_4$ to remove extracellular K^+. Roots and shoots were then separated and weighed. Tissue was oven dried for a minimum of 72 h at 80–85 °C, reweighed, pulverized, and digested with 30% HNO_3 for a minimum of 72 h. K^+ concentrations in tissue digests were determined using a single-channel flame photometer (Digital Flame Analyzer model 2655-00, Cole-Parmer, Anjou, Quebec, Canada).

Statistical analysis

Statistical analyses were conducted using either a paired-sample *t*-test or one-way analysis of variance (ANOVA), followed by *post hoc* multiple comparisons meeting the assumptions of the Dunnett's C exam (not assuming equal variances), with the statistical package SPSS (ver. 12).

Results

At the lowest external K^+ supply of 0.02 mM, growth of rice seedlings was suppressed by ~50% when nitrogen was supplied as NH_4^+, relative to NO_3^- controls (Table 1). Growth on NH_4^+ was also significantly lower at 0.1 mM $[K^+]_{ext}$, although to a much lesser extent (fresh weight was diminished by only 10%). At higher levels of K^+ supply, NH_4^+ either increased fresh weight (by nearly 50% at 1.5 mM $[K^+]_{ext}$), or had no significant effect relative to NO_3^- (at 40 mM). Maximal growth with NH_4^+ as sole N source was observed at 1.5 mM $[K^+]_{ext}$, rather than at the highest provision of 40 mM, at which suboptimal growth occurred.

Table 1. *Tissue fresh weight (root+shoot) for 3-week-old rice seedlings (shoot fresh weights are shown in parentheses)*

Error bars refer to ±SEM ($n \geqslant 5$ replicates). Asterisks indicate significantly higher means between N treatments for each K^+ condition examined, with $P < 0.05$.

$[K^+]_{ext}$	Plant fresh weight (mg)	
	NO_3^- treatment	NH_4^+ treatment
0.02	109±10* (55±6*)	52±4 (33±2)
0.1	300±7* (170±4)	267±5 (172±3)
1.5	251±27 (134±16)	367±31* (210±18*)
40	244±33 (128±17)	220±23 (130±15)

The growth trends shown in Table 1 were reflected in the K^+ content of roots and shoots (Fig. 1). At the lowest values of $[K^+]_{ext}$ (0.02 mM and 0.1 mM), tissue K^+ accumulation was strongly inhibited by NH_4^+ relative to NO_3^-, in both roots and shoots. At 1.5 mM and 40 mM $[K^+]_{ext}$, this relative inhibition was reversed in shoots, with NH_4^+-grown seedlings accumulating between 25% and 40% more K^+ than found in NO_3^--grown plants.

Compartmental analysis with the radiotracer $^{42}K^+$ was used to compare the influence of NH_4^+ and NO_3^- nutrition on subcellular K^+ fluxes and cytosolic K^+ compartmentation in the rice seedlings (Fig. 2). Unidirectional influx of K^+ across the plasma membrane of root cells generally increased with increasing $[K^+]_{ext}$, and a strong influence of N source on this flux was observed (Fig. 3). At the lowest values of $[K^+]_{ext}$ (0.02 mM and 0.1 mM), K^+ influx was significantly inhibited with NH_4^+ nutrition in rice, paralleling the inhibition of growth and K^+ accumulation in tissue. At 1.5 mM $[K^+]_{ext}$, no difference was seen in K^+ influx in seedlings grown with either NH_4^+ or NO_3^-, while, surprisingly, at the highest $[K^+]_{ext}$ value of 40 mM, influx was stimulated by NH_4^+ provision.

Figure 4 shows cytosolic concentrations of K^+ ($[K^+]_{cyt}$) for roots of rice seedlings, over the range of tested conditions. Again, a strong interaction between K and N nutrition was observed: at the same values of low $[K^+]_{ext}$ and high NH_4^+ that brought about growth inhibition, tissue K^+ suppression, and lower influx of K^+, there was a significant decline in $[K^+]_{cyt}$ in roots of rice seedlings. This trend was not seen at higher $[K^+]_{ext}$; on the contrary, at the highest $[K^+]_{ext}$, cytosolic K^+ pools of rice were larger under NH_4^+ nutrition. Interestingly, increasing $[K^+]_{ext}$ from the HATS range value of 0.1 mM to the

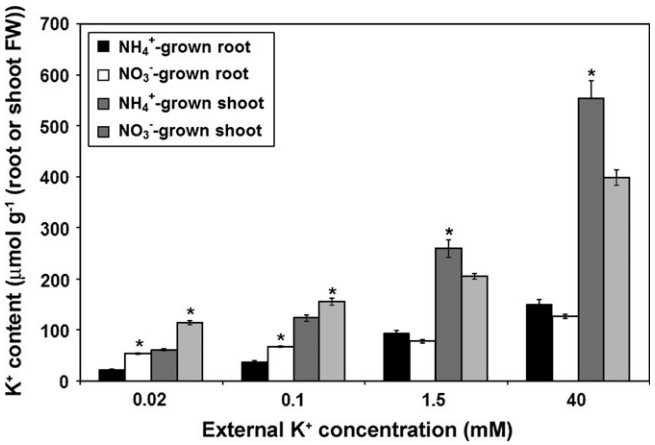

Fig. 1. K^+ tissue content of rice seedlings grown at four external K^+ concentrations (0.02, 0.1, 1.5, and 40 mM). Error bars refer to ±SEM of 6–18 replicates, with asterisks indicating significantly different means between N treatments (NO_3^- or NH_4^+) for each K^+ condition and plant organ (root or shoot) examined ($P < 0.05$).

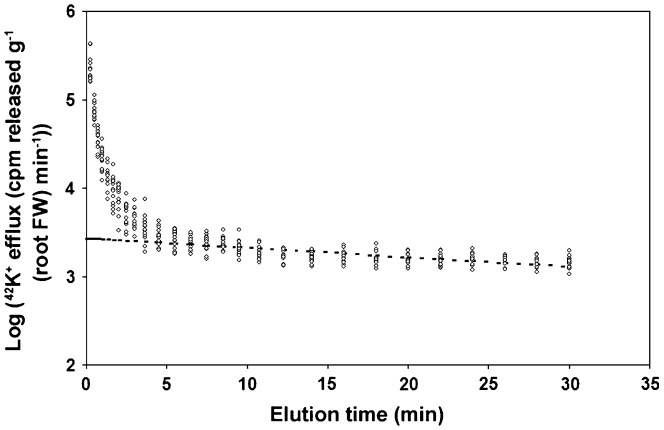

Fig. 2. $^{42}K^+$ efflux pattern in roots of rice seedlings grown at 0.1 mM K^+ and 10 mM NH_4^+. Shown is the entire data set (n=15) for this treatment, illustrating the reproducibility of the data. The dashed line represents averaged $^{42}K^+$ release from the cytosol. SEM for each point was within 10% of the mean.

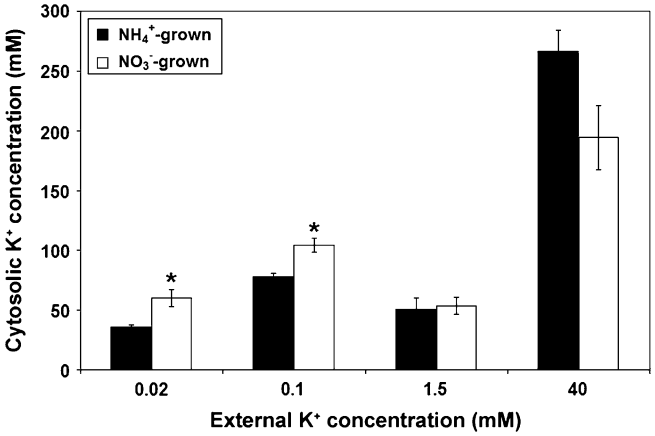

Fig. 4. Cytosolic K^+ concentrations (mM) as determined by compartmental analysis in roots of rice seedlings grown at 10 mM NO_3^- or NH_4^+, and at four external [K^+]. Error bars refer to ±SEM of 5–15 replicates, with asterisks indicating significantly different means between N treatments for each K^+ condition ($P < 0.05$).

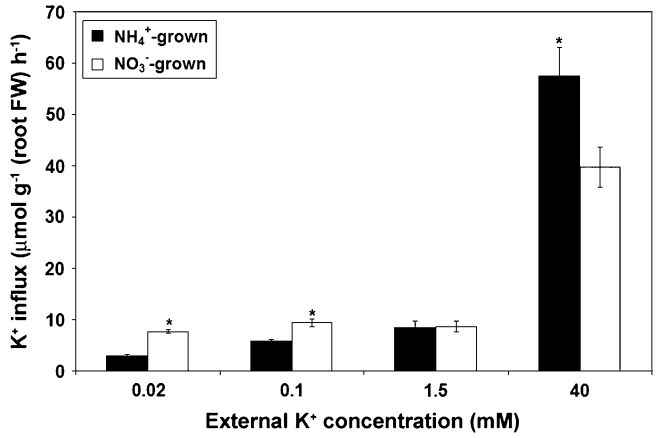

Fig. 3. Steady-state influx of K^+ in roots of rice at 10 mM NO_3^- or NH_4^+, and at four external [K^+]. Error bars refer to ±SEM of 5–15 replicates, with asterisks indicating significantly different means between N treatments for each K^+ condition ($P < 0.05$).

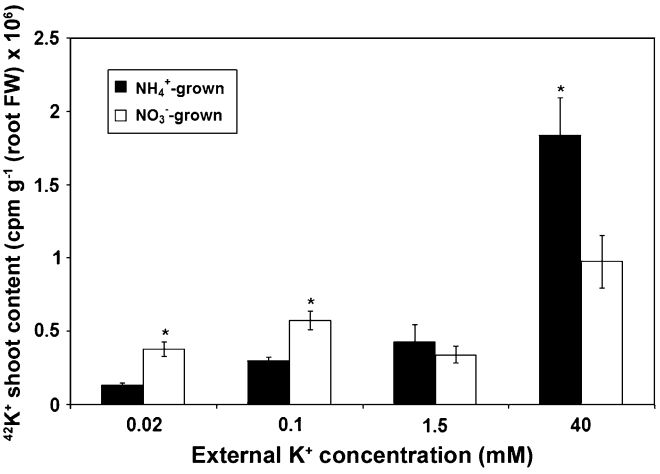

Fig. 5. Shoot accumulation of $^{42}K^+$ following labelling of rice seedlings grown at 10 mM NO_3^- or NH_4^+, and at four external [K^+]. Error bars refer to ±SEM of 5–15 replicates, with asterisks indicating significantly different means between N treatments for each K^+ condition ($P < 0.05$).

LATS range value of 1.5 mM resulted in a lowering of $[K^+]_{cyt}$ under steady-state conditions, regardless of the N source.

Figure 5 illustrates the effect of N source on $^{42}K^+$ transport to the shoot in rice seedlings. Rice seedlings showed suppression of $^{42}K^+$ translocation at the lowest $[K^+]_{ext}$ values (0.02 mM and 0.1 mM), with a maximum 65% reduction at the lowest K^+ condition. At higher $[K^+]_{ext}$ (1.5 mM and 40 mM), NH_4^+-grown rice displayed substantially (as much as 90%) greater translocation of $^{42}K^+$, compared with NO_3^- controls.

Figure 6 shows the influx of NH_4^+ into intact rice seedlings determined by short-term (5 min) labelling using $^{13}NH_4^+$. Maximal NH_4^+ influx was found when $[K^+]_{ext}$ was low (0.02 mM or 0.1 mM), ranging between 61 μmol g^{-1} h^{-1} and 86 μmol g^{-1} h^{-1}. Elevating $[K^+]_{ext}$ into the LATS concentration range for K^+ significantly reduced NH_4^+ influx, by >60% of the maximum NH_4^+ influx determined under K^+ HATS conditions. Compartmental analysis conducted using $^{13}NH_4^+$ showed similar trends, with elevated K^+ supply drastically reducing NH_4^+ influx (Fig. 6, inset). In addition, when seedlings were grown under a K^+ LATS, rather than a K^+ HATS condition (5 mM versus 0.02 mM $[K^+]_{ext}$), NH_4^+ efflux was reduced to a greater extent than influx, resulting in a decrease of the efflux:influx ratio from ~90% to <70%.

Figure 7 shows the influx of K^+ into rice seedlings, as determined by short-term (5 min) accumulation of $^{42}K^+$. Non-steady-state influx experiments, in which seedlings grown at low $[K^+]_{ext}$ were transiently exposed to elevated

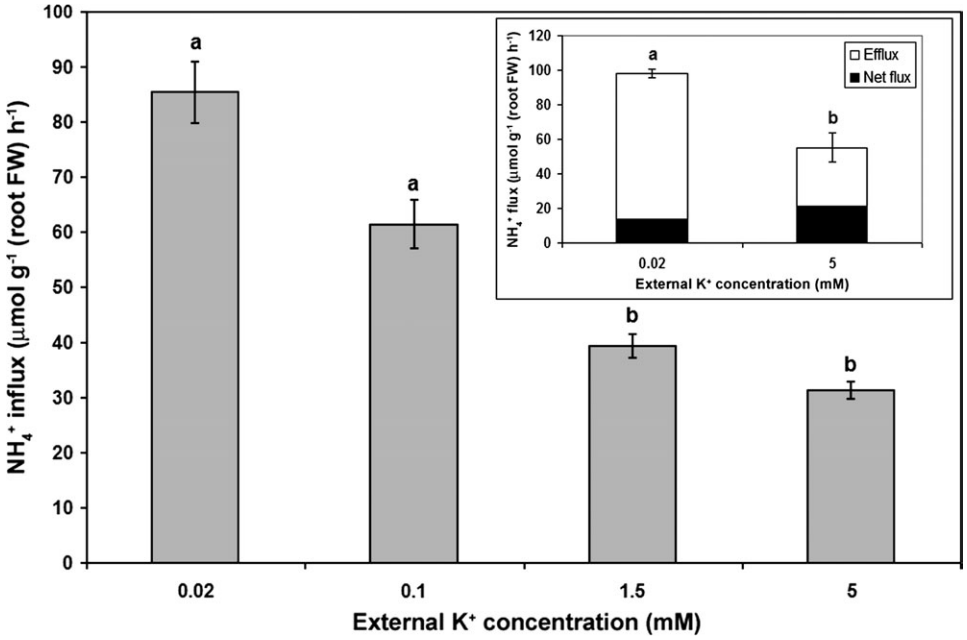

Fig. 6. Effect of $[K^+]_{ext}$ on NH_4^+ influx, directly measured using short-term (5 min) labelling with ^{13}N. Rice seedlings were grown and tested under steady-state conditions, at 10 mM NH_4^+ and four external $[K^+]$. Error bars refer to ±SEM of seven replicates. Different letters refer to significantly different means ($P < 0.05$). Inset: steady-state component fluxes of NH_4^+ in roots of rice grown at 10 mM NH_4^+ and external K^+ concentrations representing K^+ HATS (0.02 mM $[K^+]_{ext}$) and LATS (5 mM $[K^+]_{ext}$). Bars are divided into net flux (filled segments) and efflux (open segments), which together comprise the influx term. Error bars refer to ±SEM of three replicates. Different letters refer to significantly different influx means ($P < 0.05$).

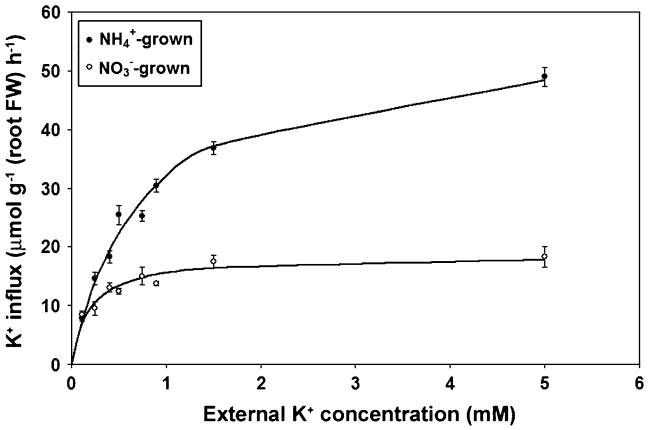

Fig. 7. Effect of changing external $[K^+]$ on K^+ influx, measured directly using short-term labelling. Rice seedlings were grown at 0.1 mM $[K^+]_{ext}$, and either 10 mM $[NO_3^-]_{ext}$ (open circles) or 10 mM $[NH_4^+]_{ext}$ (filled circles), and labelled in solutions spanning 0.1–5 mM $[K^+]_{ext}$ for 5 min. Error bars refer to ±SEM of 4–10 replicates.

(between 0.1 mM and 5 mM) $[K^+]_{ext}$, showed that K^+ influx increased significantly with increased substrate, regardless of N condition. However, K^+ influx was the highest in NH_4^+-grown seedlings following the change in $[K^+]_{ext}$, with K^+ influx increasing by 5–6.5 times, as compared with NO_3^--grown seedlings, in which influx only doubled.

Discussion

NH_4^+ toxicity affects many, if not most, plant species, although the mechanisms by which this occurs are still poorly understood (see review by Britto and Kronzucker, 2002). However, a common feature of NH_4^+ toxicity in plant systems is the suppression of tissue cation content, particularly that of potassium (Kirkby and Mengel, 1967; Kirkby, 1968; Van Beusichem *et al*., 1988; Engels and Marschner, 1993; Gerendás *et al*., 1997; Santa-María *et al*., 2000). K^+ homeostasis is also implicated as a central factor in resistance to sodium toxicity (Benlloch *et al*., 1994; Cuin and Shabala, 2005), and may thus play a broad role in ion stress tolerance. To understand better the role of K^+ in NH_4^+ toxicity and tolerance, the influence of nitrogen source and K^+ supply on plant growth and K^+ uptake, accumulation, cytosolic pools, and root-to-shoot translocation, in rice, an ammonium-tolerant plant species, was examined. An NH_4^+ concentration of 10 mM was used to induce toxicity under conditions that still fall within the range found in fertilized agricultural soils (Britto and Kronzucker, 2002), and the K^+ concentrations were chosen to represent the high- and low-affinity transport system ranges, as well as to reflect soil concentrations (Reisenauer, 1966; Hawkesford and Miller, 2004). The one exception to this was the 40 mM K^+ treatment, which was used to test the possible limits to which elevated K^+ supply can relieve NH_4^+ stress.

Rice has been traditionally considered to be an ammonium specialist (Wang *et al*., 1993), partly because the low oxygen environment found in rice paddy yields NH_4^+, rather than NO_3^-, as the dominant nitrogen source (Shen, 1969; Arth *et al*., 1998). On the other hand, it has been shown that rice seedlings are able to take up NO_3^- at higher rates than NH_4^+ (Kronzucker *et al*., 2000). In support of the claim that rice may not be an NH_4^+ specialist under all conditions, the present study shows that, at low concentrations of K^+ (0.02 mM or 0.1 mM), NH_4^+ nutrition suppresses growth (Table 1), and reduces K^+ accumulation (Fig. 1) and influx (Fig. 3), relative to NO_3^- controls. Similarly, Bañuelos and co-workers (2002) found that NH_4^+ suppressed K^+ uptake in excised rice roots at low $[K^+]_{ext}$. In the present study, the effects observed at low $[K^+]_{ext}$ were relieved when $[K^+]_{ext}$ was raised to 1.5 mM and higher, indicating that NH_4^+ tolerance in rice depends upon a substantial K^+ supply. Increasing $[K^+]_{ext}$ also reduced the amount of NH_4^+ futile cycling, with significant reductions in NH_4^+ efflux, influx, and the ratio of the two (Fig. 6). A comparison of all growth conditions shows that the maximal biomass achieved was found not with NO_3^- but with NH_4^+, and when K^+ supply was moderately high (1.5 mM). This indicates that rice indeed prefers this N source as long as K^+ conditions are optimized (Table 1).

Despite reduced growth with low $[K^+]_{ext}$, rice seedlings were not as severely affected by NH_4^+ as was previously shown for seedlings of barley (Kronzucker *et al*., 2003; Szczerba *et al*., 2006*a*), considered to be an NH_4^+-sensitive species. Although growth in both species was reduced by ~50% at the lowest $[K^+]_{ext}$ (0.02 mM) with NH_4^+ as the N source, the influx, cytosolic pool size and tissue content of K^+ were reduced by 80–90% in barley, but only by ~60% in rice. Moreover, increasing $[K^+]_{ext}$ from 0.02 mM to 0.1 mM resulted in marked improvements in rice grown with NH_4^+: growth was suppressed only by 10%, and influx, $[K^+]_{cyt}$, and tissue K^+ content only by 20–40%, as compared with NO_3^--grown seedlings. In contrast, barley seedlings still showed a substantial (30%) growth depression, and an even greater (60–90%) suppression of influx, $[K^+]_{cyt}$, and K^+ tissue content at this external $[K^+]$. These differences illustrate that, despite displaying some sensitivity to NH_4^+, K^+ homeostasis in rice shows more effective recovery from NH_4^+ toxicity than barley. This difference may be attributable to three possible effects. First, the high-affinity K^+ transport mechanism may be more resistant to NH_4^+ in rice, perhaps due to greater binding affinity for K^+, thus providing greater relief from competitive inhibition with NH_4^+ (Vale *et al*., 1987; Wang *et al*., 1996). Secondly, NH_4^+-resistant K^+ transport via channels may occur at a lower external concentration of K^+ in rice. It has been shown by Spalding *et al*. (1999) in *Arabidopsis* that 55–63% of K^+ permeability in the HATS range can be mediated by AKT1, the channel believed to be the dominant mediator of low-affinity K^+ transport (Gierth and Mäser, 2007). This contribution may perhaps be even higher in rice, particularly under conditions with NH_4^+, as has been suggested by Rodríguez-Navarro and Rubio (2006). On the other hand, it has been shown that membrane potentials in rice are typically much less negative than those in *Arabidopsis*, particularly when grown with NH_4^+, which causes permanent membrane depolarization in rice (Wang *et al*., 1994; Britto *et al*., 2001). Thirdly, NH_4^+ may promote gene expression of high-affinity K^+ transporters in rice, as has been found with *LeHAK5* in tomato plants (Nieves-Cordones *et al*., 2007). Conversely, NH_4^+ may reduce expression of HAK/KUP/KT transporters in rice, as has been found in *Arabidopsis* and pepper plants (Martínez-Cordero *et al*., 2005; Qi *et al*., 2008); however, NH_4^+ may be less effective in this capacity in rice than in barley.

Surprisingly, however, at the highest $[K^+]_{ext}$ (40 mM), a growth decline was observed in rice seedlings, regardless of N source, even though K^+ influx and tissue accumulation, cytosolic $[K^+]$, and $^{42}K^+$ translocation were all maximized. In previous work, a similar decline was found in NH_4^+-grown barley seedlings when $[K^+]_{ext}$ was increased from 1.5 mM to 40 mM (Szczerba *et al*., 2008). These reductions in growth under the extreme K^+ condition may in part be a consequence of the energetic drain on root cells catalysing substantial futile cycling of both K^+ and NH_4^+ under high nutrient supply (Britto *et al*., 2001, 2002; Britto and Kronzucker, 2006; Szczerba *et al*., 2006*a*).

It is remarkable that the steady-state acquisition of K^+ at 40 mM in rice should be substantially (~40%) higher under NH_4^+ nutrition than under NO_3^-, particularly when both NH_4^+ and K^+ can have a depolarizing effect on the plasma membrane in this species, thus reducing the driving force for K^+ entry into the cell (Wang *et al*., 1994; Britto *et al*., 2001; Kronzucker *et al*., 2001). A stimulation of low-affinity K^+ influx by NH_4^+ was also seen in measurements of K^+ influx following brief exposure (5 min) of seedlings grown at 0.1 mM $[K^+]_{ext}$ to higher K^+ concentrations (Fig. 7). This shows that NH_4^+-grown plants have significantly enhanced K^+ influx under non-steady-state conditions, relative to NO_3^- controls. Indeed, at the highest $[K^+]_{ext}$ tested in this experiment, the influx of K^+ was more than double that of seedlings grown with NO_3^- (Fig. 7). Under such non-steady-state conditions as shown in Fig. 7, NH_4^+ appears to 'prime' K^+ influx, allowing the plant to capitalize upon a transient flush of K^+ in the dynamic soil environment. Such a priming mechanism may be the result of K^+ utilizing NH_4^+ transporters, as has been suggested by a recent investigation in barley (Szczerba *et al*., 2008). As was found in rice (Fig. 6), a reduction in NH_4^+ influx was observed following elevation of $[K^+]_{ext}$ under non-steady-state and steady-state conditions. NH_4^+ transport has been

shown to follow a pattern of uptake similar to K^+, with a high-affinity system at micromolar $[NH_4^+]_{ext}$, and a low-affinity one at millimolar concentrations (Kronzucker *et al*., 1996), but a peculiar aspect of low-affinity NH_4^+ transport is that it is not down-regulated by high plant N status, but, on the contrary, is substantially increased (Wang *et al*., 1993; Rawat *et al*., 1999; Min *et al*., 2000; Cerezo *et al*., 2001). It has been suggested that this increase is due to the induction, or enhancement, of low-affinity NH_4^+ transport by NH_4^+ itself (Cerezo *et al*., 2001). Therefore, it is possible that under high $[NH_4^+]_{ext}$, K^+ utilizes an induced NH_4^+ transporter to enter the plant cell, if K^+ is present at a sufficiently high concentration, thus accounting for the increased K^+ flux under K^+ LATS conditions. The existence of common pathways for the two ions is substantiated by numerous indications that NH_4^+ flux can occur via K^+ transporters (Scherer *et al*., 1984; Vale *et al*., 1987; Wang *et al*., 1996; White, 1996; Nielsen and Schjoerring, 1998), a phenomenon that has also been postulated for some components of Na^+ influx (e.g. Kader and Lindberg, 2005).

It should be pointed out, however, that the effect shown in Fig. 7, when seedlings were transferred from a condition of 0.1 mM $[K^+]_{ext}$ to higher K^+ concentrations, was only temporary. At the steady state, K^+ influx parity between NH_4^+ and NO_3^- growth conditions was achieved at 1.5 mM $[K^+]_{ext}$, signalling a longer term down-regulation of NH_4^+-related component(s) of K^+ acquisition. The enhancement of K^+ influx by NH_4^+ seen at the 40 mM steady-state condition may also be the result of longer-term adaptations, a view supported by others who have found that NH_4^+ can enhance K^+ uptake in plant species when K^+ is supplied under nutrient-replete conditions (Daliparthy *et al*., 1994, and references therein).

A broad correlation was seen between unidirectional K^+ influx (Fig. 3) and cytosolic $[K^+]$ (Fig. 4) in root cells. Accordingly, a number of different set points for $[K^+]_{cyt}$ were observed as the flux increased, confirming a previous conclusion that the homeostatic control of cytosolic K^+ pools is not as rigid as generally thought (Kronzucker *et al*., 2003, 2006; Szczerba *et al*., 2006*a*). A particularly striking observation was seen at 1.5 mM $[K^+]_{ext}$, in plants growing with either N source: at this K^+ concentration, a dip in $[K^+]_{cyt}$ was seen relative to the 0.1 mM or 40 mM levels of $[K^+]_{ext}$. This pattern has been observed before for nitrate-grown barley (Kronzucker *et al*., 2003, 2006; Szczerba *et al*., 2006*a*), and it receives strong confirmation in the present study by being visible in a second species, and under two nitrogen regimes. The reasons for this decline are not clear, but may be associated with the switch between a condition dominated by high-affinity K^+ transport to one dominated by a low-affinity system (Britto and Kronzucker, 2006).

A high correlation was found in rice between root $[K^+]_{cyt}$ (Fig. 4) and both shoot K^+ content (Fig. 8a; R^2=0.82) and $^{42}K^+$ transport to the shoot (Fig. 8b; R^2=0.94). This suggests that the cytosolic concentration of K^+ in the root is an important driver of long-distance K^+ transport. A similar conclusion was derived for barley seedlings, also grown under low K^+ and high N nutrient conditions, with NH_4^+ suppressing $[K^+]_{cyt}$ by 70%, and shoot transport of K^+ by 90% (Kronzucker *et al*., 2003). Root-to-shoot K^+ translocation is thought to be mediated (in *Arabidopsis*) at least in part by one outwardly rectifying, Shaker-type channel, designated as SKOR (Gaymard *et al*., 1998; Mäser *et al*., 2001). The findings suggest that NH_4^+ may act directly on shoot K^+ transporters, such as SKOR, or may disrupt K^+ translocation to the shoot by reducing the driving force for shoot transport by reducing $[K^+]_{cyt}$ (Liu *et al*., 2006). Such effects may be reduced in rice, unlike in barley, as rice has been shown to maintain lower $[NH_4^+]_{cyt}$ than found under identical conditions in barley (Britto *et al*., 2001). Moreover, elevating $[K^+]_{ext}$ may mitigate the effects of NH_4^+ upon K^+ shoot

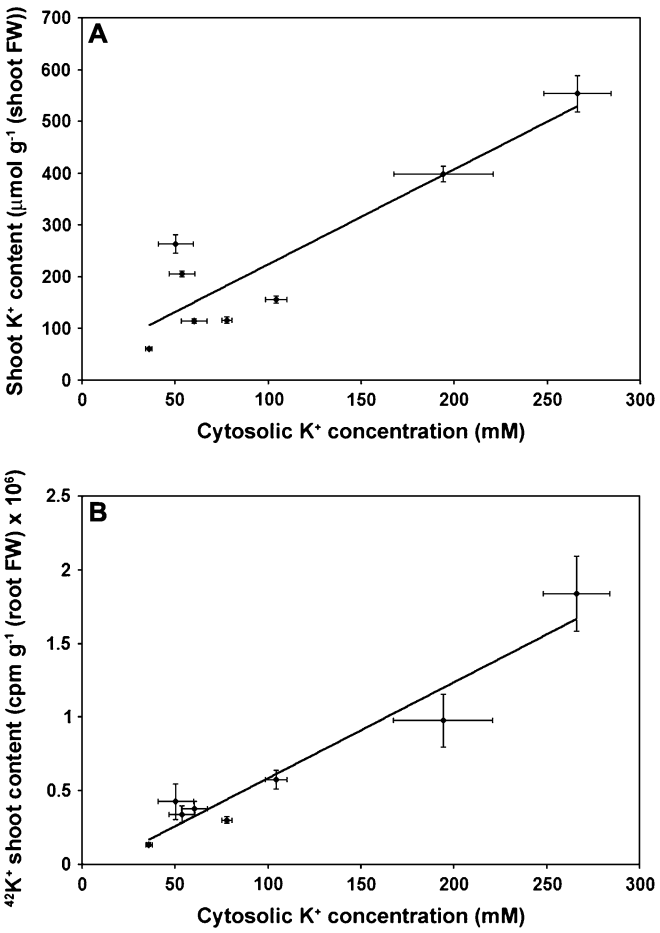

Fig. 8. Relationship between (A) shoot K^+ content and the root cytosolic K^+ concentration, and (B) shoot $^{42}K^+$ content and root cytosolic K^+ concentration in rice seedlings. Regression equations are: (A) y=1.839x + 39.513, with R^2=0.82, and (B) y=5565.4x + 75.846, with R^2=0.94.

translocation in rice, by reducing both NH_4^+ influx (Fig. 6) and $[NH_4^+]_{cyt}$, as was also demonstrated recently in barley (Szczerba *et al*., 2008). In that study, increasing $[K^+]_{ext}$, from a HATS-mediated to LATS-mediated transport condition, reduced NH_4^+ influx by >60% and $[NH_4^+]_{cyt}$ by 3–4 times. There, as well as in the present study, it is possible that the plasma membrane depolarization typically brought about by increased K^+ supply leads to a reduced driving force for passive NH_4^+ entry into the cell.

The hypothesis that K^+ acquisition and homeostasis in rice is resistant to NH_4^+ nutrition was only partially borne out. Indeed, as with most other plant species, some disruption of growth, and of K^+ acquisition and distribution, was seen under low K^+ (reflective of high-affinity K^+ transport conditions). However, at 1.5 mM $[K^+]_{ext}$, growth was markedly greater under NH_4^+ nutrition, and NH_4^+ stimulated K^+ acquisition at elevated $[K^+]_{ext}$, resulting in increased K^+ transport into root cells, tissue K^+, and $^{42}K^+$ translocation to the shoot. Importantly, these apparent advantages translate into superior growth at the moderate LATS concentration of 1.5 mM $[K^+]_{ext}$. At 40 mM, in contrast, increased K^+ acquisition was associated with a growth depression, which may be attributable to the combined energy demands of futile NH_4^+ and K^+ cycling at the root plasma membrane, as demonstrated elsewhere for the two nutrient ions (Britto *et al*., 2001, 2002; Szczerba *et al*., 2006*a*, 2008). The efficient recovery from NH_4^+ toxicity, and superior growth of rice with NH_4^+, under moderate K^+ conditions, demonstrate the close association of these two ions in the context of optimal plant growth, and may offer a focal point for the bioengineering of ammonium tolerance into sensitive crop genotypes.

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