NH₄⁺-stimulated and -inhibited components of K⁺ transport in rice (Oryza sativa L.)

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Abstract

The disruption of K⁺ transport and accumulation is symptomatic of NH₄⁺ toxicity in plants. In this study, the influence of K⁺ supply (0.02–40 mM) and nitrogen source (10 mM NH₄⁺ or NO₃⁻) on root plasma membrane K⁺ fluxes and cytosolic K⁺ pools, plant growth, and whole-plant K⁺ distribution in the NH₄⁺-tolerant plant species rice (Oryza sativa L.) was examined. Using the radiotracer ⁴²K⁺, tissue mineral analysis, and growth data, it is shown that rice is affected by NH₄⁺ toxicity under high-affinity K⁺ transport conditions. Substantial recovery of growth was seen as [K⁺]ₜₐₐₜ increased from 0.02 mM to 0.1 mM, and at 1.5 mM, growth was superior on NH₄⁺. 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 NH₄⁺ influx, as measured by ¹⁵N radiotracing. In the low-affinity K⁺ transport range, NH₄⁺ stimulated K⁺ influx relative to NO₃⁻ controls. It is concluded that rice, despite its well-known tolerance to NH₄⁺, nevertheless displays considerable growth suppression and disruption of K⁺ homeostasis under this N regime at low [K⁺]ₜₐₐₜ, but displays efficient recovery from NH₄⁺ inhibition, and indeed a stimulation of K⁺ acquisition, when [K⁺]ₜₐₐₜ is increased in the presence of NH₄⁺.

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 (NH₄⁺) 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 NH₄⁺ toxicity, which affects the majority of plant species when exposed to elevated soil concentrations of NH₄⁺ (typically, when [NH₄⁺] >1 mM; Britto et al., 2001, 2002; Britto and Kronzucker, 2002). However, the NH₄⁺-dependent inhibition of K⁺ influx and accumulation can be alleviated by increasing the external K⁺ concentration ([K⁺]ₜₑₓₜ; Cao et al., 1993; Spalding et al., 1999; Santa-María et al., 2000; Kronzucker et al., 2003; Szczerba et al., 2006a). The sensitivity of K⁺ influx to NH₄⁺ appears to depend on the mechanism of primary K⁺ uptake that dominates at a given [K⁺]ₜₑₓₜ: at micromolar concentrations, K⁺ uptake is mainly mediated by an NH₄⁺-suppressible, high-affinity transport system (HATS), while at higher, millimolar [K⁺]ₜₑₓₜ, K⁺ influx is mediated by an NH₄⁺-resistant, low-affinity transport system (LATS) (Spalding et al., 1999; Santa-María et al., 2000; Kronzucker et al., 2003; Szczerba et al., 2006a). The precise mechanism by which NH₄⁺ inhibits high-affinity K⁺ influx has not been elucidated, although it has been suggested that NH₄⁺ competitively inhibits K⁺ transport at the protein level (Vale et al., 1987; Wang et al., 1996).

In ammonium-sensitive barley (Hordeum vulgare L.), NH₄⁺ has been shown to disrupt not only the primary influx, but also the internal distribution, of K⁺, at both

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whole-plant and cellular levels. For example, Santa-María et al. (2000) and Kronzucker et al. (2003) found that NH₄⁺ 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 [NH₄⁺]_{ext} (Kronzucker et al., 2003; Szczersa et al., 2006a). The disruption of cytosolic K⁺ homeostasis and the translocation of K⁺ to the shoot are, most probably, related: while NH₄⁺ 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 NH₄⁺ sensitivity by reducing the xylem loading of K⁺ (Gyamart 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 NH₄⁺ than other cereals (Sasakawa and Yamamoto, 1978). Given the pivotal role of K⁺ nutrition in the development of NH₄⁺ toxicity or tolerance, it was therefore important to investigate the degree to which rice plants may be able to resist NH₄⁺-induced disruptions in primary K⁺ acquisition, cellular K⁺ homeostasis, and root-to-shoot K⁺ translocation. These disruptions have been characterized in barley and other NH₄⁺-sensitive plant species, but have only been examined in very limited detail in NH₄⁺-tolerant plant species (Wang et al., 1996; Bañuelos et al., 2002). Here, compartmental analyses has been conducted using the radiotracer ⁴²K⁺ to evaluate K⁺ transport and compartmentation in intact seedlings of NH₄⁺-tolerant rice, examining plant performance at four levels of K⁺ supply and distribution, at whole-plant and subcellular levels, would resist disruption by NH₄⁺ 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 MgSO₄; 1 mM CaCl₂; 0.3 mM NaH₂PO₄; 0.1 mM Fe-EDTA; 20 μM H₂BO₃; 9 μM MnCl₂; 1.5 μM CuSO₄; 1.5 μM ZnSO₄; 0.5 μM Na₂MoO₄), pH 6–6.5, for an additional 19 d. The growth solutions were modified to provide four concentrations of potassium (as K₂SO₄), at 0.02, 0.1, 1.5, and 40 mM, and nitrogen (10 mM) as either (NH₄)₂SO₄ or Ca(NO₃)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 ¹⁴N 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; Szczersa et al., 2005a, 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 ⁴²K⁺ (t½=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 Wallace 1480 Wizard 3”., Turku, Finland, or Canberra-Packard, Quantum Cobra Series II, Model 5003).

Exponentially declining rates of ⁴²K⁺ release from roots over time were then analysed using linear regression (see Fig. 2). The function ln φ_{co(0)^i} = ln φ_{co(0)^{i}*} − kt [in which φ_{co(0)^{i}*} is tracer efflux at elution time t, φ_{co(0)^*} 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 (Bebh and Jeschke, 1981; Memon et al., 1985; Kronzucker et al., 2003).

Chemical efflux, φ_{co}, was determined from φ_{co(i)*}, divided by the specific activity of the cytosol (S₀) at the end of the labelling period [this activity was determined using the exponential rise function S₀=S_{₀}^c (1 − e^{-kt}), in which S₀ is the specific activity of the external solution, t is labelling time, and k is as described above], Net flux, φ_{co}, was found using total-plant ⁴²K⁺ 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}=Ω×φ_{co}/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 ¹⁴N 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 ¹³N (t½=9.97 min; as ¹⁴NH₃) 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 ⁴²K⁺ (see Britto
and Kronzucker, 2001). Seedlings grown at 0.1 mM [K\textsuperscript{+}]\textsubscript{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 \textsuperscript{42}K\textsuperscript{+} for a final [K\textsuperscript{+}]\textsubscript{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\textsubscript{4}\textsuperscript{+} was also determined directly, as described for \textsuperscript{42}K\textsuperscript{+}, but using short-term labelling (5 min) with \textsuperscript{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 \textsuperscript{13}NH\textsubscript{4}\textsuperscript{+}, 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 \textsuperscript{42}K\textsuperscript{+}.

**Tissue K\textsuperscript{+} content**

To measure tissue K\textsuperscript{+} content, roots of rice seedlings were first desorbed for 5 min in 10 mM CaSO\textsubscript{4} to remove extracellular K\textsuperscript{+}. 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\textsubscript{3} for a minimum of 72 h. K\textsuperscript{+} 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\textsuperscript{+} supply of 0.02 mM, growth of rice seedlings was suppressed by ~50% when nitrogen was supplied as NH\textsubscript{4}\textsuperscript{+} relative to NO\textsubscript{3}\textsuperscript{-} controls (Table 1). Growth on NH\textsubscript{4}\textsuperscript{+} was also significantly lower at 0.1 mM [K\textsuperscript{+}]\textsubscript{ext}, although to a much lesser extent (fresh weight was diminished by only 10%). At higher levels of K\textsuperscript{+} supply, NH\textsubscript{4}\textsuperscript{+} either increased fresh weight (by nearly 50% at 1.5 mM [K\textsuperscript{+}]\textsubscript{ext}), or had no significant effect relative to NO\textsubscript{3}\textsuperscript{-} (at 40 mM). Maximal growth with NH\textsubscript{4}\textsuperscript{+} as sole N source was observed at 1.5 mM [K\textsuperscript{+}]\textsubscript{ext}, rather than at the highest provision of 40 mM, at which suboptimal growth occurred.

The growth trends shown in Table 1 were reflected in the K\textsuperscript{+} content of roots and shoots (Fig. 1). At the lowest values of [K\textsuperscript{+}]\textsubscript{ext} (0.02 mM and 0.1 mM), tissue K\textsuperscript{+} accumulation was strongly inhibited by NH\textsubscript{4}\textsuperscript{+} relative to NO\textsubscript{3}\textsuperscript{-}, in both roots and shoots. At 1.5 mM and 40 mM [K\textsuperscript{+}]\textsubscript{ext}, this relative inhibition was reversed in shoots, with NH\textsubscript{4}\textsuperscript{+}-grown seedlings accumulating between 25% and 40% more K\textsuperscript{+} than found in NO\textsubscript{3}\textsuperscript{-}-grown plants.

Compartmental analysis with the radiotracer \textsuperscript{42}K\textsuperscript{+} was used to compare the influence of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} nutrition on subcellular K\textsuperscript{+} fluxes and cytosolic K\textsuperscript{+} compartmentation in the rice seedlings (Fig. 2). Unidirectional influx of K\textsuperscript{+} across the plasma membrane of root cells generally increased with increasing [K\textsuperscript{+}]\textsubscript{ext}, and a strong influence of N source on this flux was observed (Fig. 3). At the lowest values of [K\textsuperscript{+}]\textsubscript{ext} (0.02 mM and 0.1 mM), K\textsuperscript{+} influx was significantly inhibited with NH\textsubscript{4}\textsuperscript{+} nutrition in rice, paralleling the inhibition of growth and K\textsuperscript{+} accumulation in tissue. At 1.5 mM [K\textsuperscript{+}]\textsubscript{ext}, no difference was seen in K\textsuperscript{+} influx in seedlings grown with either NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{-}, while, surprisingly, at the highest [K\textsuperscript{+}]\textsubscript{ext} value of 40 mM, influx was stimulated by NH\textsubscript{4}\textsuperscript{+} provision.

Figure 4 shows cytosolic concentrations of K\textsuperscript{+} ([K\textsuperscript{+}]\textsubscript{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\textsuperscript{+}]\textsubscript{ext} and high NH\textsubscript{4}\textsuperscript{+} that brought about growth inhibition, tissue K\textsuperscript{+} suppression, and lower influx of K\textsuperscript{+}, there was a significant decline in [K\textsuperscript{+}]\textsubscript{cyt} in roots of rice seedlings. This trend was not seen at higher [K\textsuperscript{+}]\textsubscript{ext}, on the contrary, at the highest [K\textsuperscript{+}]\textsubscript{ext}, cytosolic K\textsuperscript{+} pools of rice were larger under NH\textsubscript{4}\textsuperscript{+} nutrition. Interestingly, increasing [K\textsuperscript{+}]\textsubscript{ext} from the HATS range value of 0.1 mM to 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)**

<table>
<thead>
<tr>
<th>[K\textsuperscript{+}]\textsubscript{ext}</th>
<th>Plant fresh weight (mg)</th>
<th>NH\textsubscript{4}\textsuperscript{+} treatment</th>
<th>NO\textsubscript{3}\textsuperscript{-} treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>109±10* (55±6*)</td>
<td>52±4 (33±2)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>300±7* (170±4)</td>
<td>267±5 (172±3)</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>251±27 (134±16)</td>
<td>367±21* (210±18*)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>244±33 (128±17)</td>
<td>220±23 (130±15)</td>
<td></td>
</tr>
</tbody>
</table>

Error bars refer to ±SEM (n ≥ 5 replicates). Asterisks indicate significantly higher means between N treatments for each K\textsuperscript{+} condition examined, with \( P < 0.05 \).
LATS range value of 1.5 mM resulted in a lowering of [K\(^+\)]\(_{\text{cyt}}\) under steady-state conditions, regardless of the N source. Figure 5 illustrates the effect of N source on 42K\(^+\) transport to the shoot in rice seedlings. Rice seedlings showed suppression of 42K\(^+\) translocation at the lowest [K\(^+\)]\(_{\text{ext}}\) values (0.02 mM and 0.1 mM), with a maximum 65% reduction at the lowest K\(^+\) condition. At higher [K\(^+\)]\(_{\text{ext}}\) (1.5 mM and 40 mM), NH\(_4\)^+—grown rice displayed substantially (as much as 90%) greater translocation of 42K\(^+\), 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 13NH\(_4\)^+. Maximal NH\(_4\)^+ influx was found when [K\(^+\)]\(_{\text{ext}}\) was low (0.02 mM or 0.1 mM), ranging between 61 \(\mu\)mol g\(^{-1}\) h\(^{-1}\) and 86 \(\mu\)mol g\(^{-1}\) h\(^{-1}\). Elevating [K\(^+\)]\(_{\text{ext}}\) 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. Compartmenal analysis conducted using 13NH\(_4\)^+ 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\(^+\)]\(_{\text{ext}}\)), 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 42K\(^+\). Non-steady-state influx experiments, in which seedlings grown at low [K\(^+\)]\(_{\text{ext}}\) were transiently exposed to elevated
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 NH4+-grown seedlings following the change in [K+]ext, with K+ influx increasing by 5–6.5 times, as compared with NO3-grown seedlings, in which influx only doubled.

**Fig. 6.** Effect of [K+]ext on NH4+ influx, directly measured using short-term (5 min) labelling with 13N. Rice seedlings were grown and tested under steady-state conditions, at 10 mM NH4+ 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 NH4+ in roots of rice grown at 10 mM NH4+ 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 [NO3]ext (open circles) or 10 mM [NH4]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.

**Discussion**

NH4+ 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 NH4+ 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 NH4+ 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 NH4+ 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 NH4+ 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, Banuelos 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., 2006a), 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 showing similar 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 42K$^+$ 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., 2006a).

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; Rayat 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 Schoerring, 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., 2006a). 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., 2006a), 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 transport to the shoot (Fig. 8b; $R^2=0.82$) and $^{42}K^+$ transport to the shoot (Fig. 8b; $R^2=0.94$).
translocation in rice, by reducing both \( \text{NH}_4^+ \) influx (Fig. 6) and \([\text{NH}_4^+]_{\text{cyt}}\), as was also demonstrated recently in barley (Szczera et al., 2008). In that study, increasing \([\text{K}^+]_{\text{ext}}\) from a HATS-mediated to LATS-mediated transport condition, reduced \( \text{NH}_4^+ \) influx by >60% and \([\text{NH}_4^+]_{\text{cyt}}\) 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 \( \text{K}^+ \) supply leads to a reduced driving force for passive \( \text{NH}_4^+ \) entry into the cell.

The hypothesis that \( \text{K}^+ \) acquisition and homeostasis in rice is resistant to \( \text{NH}_4^+ \) nutrition was only partially borne out. Indeed, as with most other plant species, some disruption of growth, and of \( \text{K}^+ \) acquisition and distribution, was seen under low \( \text{K}^+ \) (reflective of high-affinity \( \text{K}^+ \) transport conditions). However, at 1.5 mM \([\text{K}^+]_{\text{ext}}\) growth was markedly greater under \( \text{NH}_4^+ \) nutrition, and \( \text{NH}_4^+ \)-stimulated \( \text{K}^+ \) acquisition at elevated \([\text{K}^+]_{\text{ext}}\), resulting in increased \( \text{K}^+ \) transport into root cells, tissue \( \text{K}^+ \), and \( ^{34} \text{K}^+ \) translocation to the shoot. Importantly, these apparent advantages translate into superior growth at the moderate LATS concentration of 1.5 mM \([\text{K}^+]_{\text{ext}}\). At 40 mM, in contrast, increased \( \text{K}^+ \) acquisition was associated with a growth depression, which may be attributable to the combined energy demands of futile \( \text{NH}_4^+ \) and \( \text{K}^+ \) cycling at the root plasma membrane, as demonstrated elsewhere for the two nutrient ions (Britto et al., 2001, 2002; Szczera et al., 2006a, 2008). The efficient recovery from \( \text{NH}_4^+ \) toxicity, and superior growth of rice with \( \text{NH}_4^+ \), under moderate \( \text{K}^+ \) 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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