manipulating cytosolic PP_i content can represent a cost-effective and environmentally responsible strategy to control tuber sprouting. Depending on which promoter is used, either delayed[13] or accelerated[14] sprouting can be achieved. However, to be used commercially, expression of the PPase gene during tuber development must be avoided.

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Ammonium toxicity and the real cost of transport

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Recently, it has been proposed that ammonium is toxic to barley because of the energetic cost of pumping ammonium that has leaked into root cells back into the soil. This does not occur in rice because high levels of ammonium reduce the potential difference across the plasma membrane of rice – whereas the potential difference in barley appears to be ammonium insensitive. These results highlight the potentially high costs of membrane transport, and thus the central importance of transport processes in plants.

Ammonium (NH_4^+) is one of the major nutrients for plants, and a ubiquitous intermediate in plant metabolism[1]. However, this ion is notorious for its toxic effects on many, if not most, plant species[2–4]. The reasons for this toxicity have been the subject of much speculation, and have included proton extrusion associated with NH_4^+ uptake, cytosolic pH disturbances, displacement of crucial cations such as K^+ and Mg^{2+}, shifts in plant carbohydrate status, and the uncoupling of photophosphorylation[4]. However, a comprehensive explanation of NH_4^+ toxicity has remained elusive. A recent paper by Dev Britto *et al.*[5] proposes a new hypothesis, that NH_4^+ toxicity is the result of the high energetic cost of pumping NH_4^+ back out of cells, after entering at unusually high rates in NH_4^+-sensitive species.

This proposal is based on a comparative study of ammonium fluxes and their energetics in an ammonium-tolerant and an ammonium-sensitive species (rice and barley, respectively). In this work, a short-lived radioactive isotope of nitrogen, ^{13}N, was used to label NH_4^+, enabling direct measures of influx and efflux of this nutrient into and out of roots of intact seedlings of rice and barley. Compartmental analyses of efflux kinetics were used to elucidate efflux across the plasma membrane; influx was calculated by the addition of efflux to measured net accumulation; cytosolic NH_4^+ concentrations were estimated using flux and specific activity data[6]. The authors did not include new direct measures of influx in the publication, but, importantly, these have been confirmed in later experiments (as well as, for rice, in previously published work[7]).

Although NH_4^+ influx at low concentrations of NH_4^+ ($[NH_4^+]_o = 0.1$ mM) in both tolerant and sensitive species was similar (at 5 to 6 μmol g^{-1} h^{-1}), influx increased at higher (although still physiologically reasonable) external NH_4^+ to a much greater extent in the NH_4^+-sensitive barley than in the NH_4^+-tolerant rice (65 versus 35 μmol g^{-1} h^{-1} at 10 mM $[NH_4^+]_o$). Concomitant with this large increase in influx was a disproportionately large increase in efflux in barley, with the NH_4^+ efflux being 76% that of the influx, compared with 'only' 53% in rice. (Under conditions of low NH_4^+ supply, only 14–28% of incoming NH_4^+ was returned to the outside of the root.) The authors used simple thermodynamic calculations to indicate that the efflux of this cation from barley roots must be active. So, how much energy must the plant be consuming to power such large effluxes?

Energetics of NH_4^+ efflux

For these calculations, both the free cytosolic NH_4^+ concentration and the plasma membrane electrical potential difference must be measured. Free cytosolic NH_4^+ concentration is notoriously difficult to ascertain – as discussed recently in several key papers[8,9]. Although there are clearly significant difficulties in using compartmental analysis to obtain accurate measures for compartmental concentrations in complex, heterogeneous tissues[6], they can provide an idea of the range of concentrations that might be found in subcellular compartments. Thus, although the values provided by Britto *et al.*[5] are

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Box 1. Calculation of the energy required to remove NH_4^+ from the barley cytosol

$$\Delta G(\phi_{co(NH_4^+)}) = -\left(zF\Delta\Psi - RT\ln\frac{[NH_4^+]_o}{[NH_4^+]_c}\right)$$

$$= -(+1)(96.5\,kJ\,mol^{-1}V^{-1})(-0.123V) + (0.00831\,kJ\,mol^{-1}K^{-1})(293K)\ln\frac{0.010\,M}{0.358\,M}$$

$$= 3.2\,kJ\,mol^{-1}$$

where: $\Delta G(\phi_{co(NH_4^+)})$ is the free energy change for ammonium efflux across the plasma membrane; z is the charge on the transported solute; F is the Faraday constant, relating to the energy required to move charge across a difference in electrical potential; $\Delta\Psi$ is the difference in electrical potential; R is the universal gas constant; T is absolute temperature.

higher than those reported using other methods[9], when combined with measures of membrane potential, they correspond, in rice, remarkably well to values predicted by the Nernst equation for passive NH_4^+ equilibration. In barley, they indicate that NH_4^+ efflux is an energy-demanding process. If cytosolic NH_4^+ concentrations are lower than calculated, then this energy demand in barley will be even greater, and there will be an energy demand in rice.

For barley, using the cytosolic NH_4^+ concentration estimate ($[NH_4^+]_c$) of 358 mM and trans-plasma membrane potential ($\Delta\Psi$) of –123 mV from Britto *et al.*[5], one can calculate the free energy required to power the active efflux of ammonium (Box 1).

A realistic range of free energies released upon hydrolysis of ATP is 30 to 57 kJ mol^{-1} (Ref. 10), suggesting that, theoretically, 9.5 to 18 NH_4^+ ions could be pumped out of the cell per ATP hydrolysed. Britto *et al.* recorded a 41% increase in O_2 consumption, of 7.5 μmol g^{-1} FW h^{-1} in barley under high NH_4^+ conditions[5] (absolute rates were not reported in the original paper). This corresponds to an increase in ATP production of ~37.5 to 45.0 μmol g^{-1} FW h^{-1} [assuming a P/O ratio of 2.5 to 3.0 (Ref. 10)]. The accompanying increase in NH_4^+ efflux was 47.5 μmol g^{-1} FW h^{-1}, yielding an ATP:NH_4^+ stoichiometry approaching 1. Thus, the ATP turnover is 7 to 17 times in excess of the energy requirement for NH_4^+ efflux as outlined above. Clearly, this signifies either that the flux-coupling mechanism is highly inefficient (≤10%), or that increased energy from respiration is directed to processes other than NH_4^+ efflux.

Britto *et al.* provided evidence consistent with the first of these possibilities by showing that the increase in O_2 consumption was even seen when NH_4^+ transport was decoupled from subsequent events of N metabolism by use of the inhibitor methionine sulfoximine (MSX) (Ref. 5). It is also important to note that increasing NH_4^+ did not increase growth – in fact, growth was decreased in barley[5]. Such energetic inefficiency in transport processes is not uncommon, this being a consequence of particular ion movements being 'locked into' a particular mechanism, regardless of what the energy gradients happen to be at the time. In this case, the ATP:NH_4^+ stoichiometry is close to 1.0, much less than the theoretical 9.5 to 18.0 calculated above. Although the mechanism by which NH_4^+ efflux is coupled to cellular energetics is unknown, this stoichiometry is suggestive of either a direct NH_4^+-extruding ATPase that extrudes one NH_4^+ per ATP hydrolysed, or an H^+-linked antiporter where one NH_4^+ is extruded for the influx of one H^+ (which, in turn, was extruded at the expense of one ATP).

Crucially, increasing NH_4^+ around rice roots had no significant effect on root respiration, consistent with the above contention that NH_4^+ equilibrates passively in this species under high NH_4^+ conditions. Furthermore, the size of the increase in efflux was much lower in rice. The near-thermodynamic equilibrium of NH_4^+ across the plasma membrane of rice occurred mostly because the plasma membrane potential depolarized in response to elevated NH_4^+ (in contrast with barley, where membrane potential remained more negative). Thus, the main difference in the response of barley and rice to elevated NH_4^+, which explains their differential sensitivities to NH_4^+, is that tolerance was associated with depolarization of the cells under conditions of elevated NH_4^+. It is notable that the membrane potential in rice appears to follow closely the equilibrium potential for NH_4^+, whereas in barley the membrane potential is hyperpolarized negative of the equilibrium potentials for both NH_4^+ and K^+ (E_K = –100 mV, given that roots were growing in 1.5 mM K^+, and assuming a cytosolic K^+ activity of 80 mM)[11]. Given this observation, the issue of NH_4^+-induced depolarization, specifically in rice, can be addressed by asking, 'Why is the rice root plasma-membrane conductance dominated by NH_4^+?'

Pathways of NH_4^+ influx

To address this, the identity of the specific transporters involved in NH_4^+ influx must be clarified. It is clear from Britto *et al.*[5] that toxicity-related NH_4^+ influxes occur at high levels of external NH_4^+, where low-affinity influx transporters are primarily responsible for NH_4^+ uptake[7]. Indeed, the activity of high-affinity NH_4^+ influx transporters, active at NH_4^+ concentrations below 1 mM, is dramatically reduced by an increase in NH_4^+ supply[1]. By contrast, the activity of the low-affinity transporters is much higher at higher NH_4^+ concentrations and is not downregulated by the substrate[7]. Transporters responsible for toxic NH_4^+ uptake are therefore likely to be constitutively expressed, low-affinity, high-capacity, channel-type transporters.

Although the inventory of such transporters is incomplete, some tentative assignments can be made based on existing information. Some potassium channel proteins (e.g. KAT1, expressed in guard cells) have been shown to transport NH_4^+ at relatively high rates[12], although root AKT1-type inwardly-rectifying K^+ (KIR) channels have been found to be relatively impermeable to NH_4^+ (Ref. 13). Root outwardly-rectifying K^+ (KOR) channels could possibly also mediate small inward fluxes of NH_4^+ (Ref. 14).

By contrast, nonselective cation (NSC) channels, known to transport a range of monovalent cations, have been found to transport NH_4^+ at higher rates than K^+ in rye and wheat root plasma membranes[15,16]. Although investigations

into the physiological roles and molecular biology of NSC channels in plants are in their early stages, the high and apparently constitutive activities, and low selectivities, of these channels strongly suggest that they are major contributors to the NH_4^+ flux system observed in Britto *et al.*[5]. Furthermore, their apparent lack of feedback regulation can give rise to the futile cycling (i.e. the movement in to, then out from, the cell, with no net accumulation) observed in the case of both NH_4^+ and Na^+ (Ref. 17).

A possibility following from the above discussion is that the plasma membrane of the root cells of rice is dominated by a conductance of NH_4^+ through NSC channels, whereas, in barley, the membrane potential is controlled by the action of other transporters. But how is this reconciled with a lower rate of NH_4^+ influx into rice roots? We propose that membrane conductance is dominated by NH_4^+-permeable NSC channels because other membrane conductances (notably via K^+-selective channels, and H^+-ATPase activity) are lower, rather than because NSC channel activity is higher. This needs to be tested using electrophysiological techniques. Under conditions of high levels of external NH_4^+, and the high levels of cytosolic NH_4^+ measured by Britto *et al.*[5], NSC channels would conduct mainly NH_4^+, and maintain the membrane potential close to $E_{NH_4^+}$.

In barley, membrane potential is maintained negative of the equilibrium potential for NH_4^+ probably because of a higher membrane activity of H^+-ATPases, ironically leading to a situation where NH_4^+ influx is elevated and the energy required for active NH_4^+ extrusion can give rise to toxity symptoms. The NSC channels thought to be responsible for NH_4^+ influx might function beneficially in barley as a mechanism for exploiting short-term abundance of NH_4^+, but are dysfunctional at high levels of NH_4^+ (such as would more likely be found in soils in which rice grows).

The paper by Britto *et al.*[5] provides one of the few insights into the real cost of solute transport in terms of cellular energy balance. It is remarkable that the cost of transport of just one ionic species can have such a significant effect on the total plant energy balance. As such, this paper by Britto *et al.*[5] advances our understanding of the mechanism of NH_4^+ tolerance, and also gives insight into the central importance of transport processes in the life of a cell and an intact plant.

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Techniques & Applications

Using complex plant pedigrees to map valuable genes

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Statistical methods pioneered by human and animal geneticists use marker and pedigree information to detect quantitative trait loci within complex pedigrees. These methods, adapted to plants, promise to expand the range of data useful for identifying the genetic factors influencing plant growth, development and evolutionary responses, and to increase the relevance and cost effectiveness of quantitative trait loci mapping in applied contexts.

With the advent of molecular markers, geneticists have developed new analytical methods to identify the quantitative trait loci (QTL) that affect traits showing continuous variation. These methods have sought to answer basic questions concerning QTL (e.g. number, mode of action, effect magnitude), and to map QTL, facilitating their manipulation for breeding purposes. In plants, QTL mapping experiments generally use populations derived from single crosses of inbred lines[1,2]. This situation contrasts with that of mapping in domestic animals and humans, which use extended pedigrees founded by multiple individuals[3]. The general ease of creating inbred lines and of

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