Futile transmembrane NH_4^+ cycling: A cellular hypothesis to explain ammonium toxicity in plants

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Most higher plants develop severe toxicity symptoms when grown on ammonium (NH_4^+) as the sole nitrogen source. Recently, NH_4^+ toxicity has been implicated as a cause of forest decline and even species extinction. Although mechanisms underlying NH_4^+ toxicity have been extensively sought, the primary events conferring it at the cellular level are not understood. Using a high-precision positron tracing technique, we here present a cell-physiological characterization of NH_4^+ acquisition in two major cereals, barley (*Hordeum vulgare*), known to be susceptible to toxicity, and rice (*Oryza sativa*), known for its exceptional tolerance to even high levels of NH_4^+. We show that, at high external NH_4^+ concentration ($[NH_4^+]_o$), barley root cells experience a breakdown in the regulation of NH_4^+ influx, leading to the accumulation of excessive amounts of NH_4^+ in the cytosol. Measurements of NH_4^+ efflux, combined with a thermodynamic analysis of the transmembrane electrochemical potential for NH_4^+, reveal that, at elevated $[NH_4^+]_o$, barley cells engage a high-capacity NH_4^+-efflux system that supports outward NH_4^+ fluxes against a sizable gradient. Ammonium efflux is shown to constitute as much as 80% of primary influx, resulting in a never-before-documented futile cycling of nitrogen across the plasma membrane of root cells. This futile cycling carries a high energetic cost (we record a 40% increase in root respiration) that is independent of N metabolism and is accompanied by a decline in growth. In rice, by contrast, a cellular defense strategy has evolved that is characterized by an energetically neutral, near-Nernstian, equilibration of NH_4^+ at high $[NH_4^+]_o$. Thus our study has characterized the primary events in NH_4^+ nutrition at the cellular level that may constitute the fundamental cause of NH_4^+ toxicity in plants.

Plants can extract and use various forms of nitrogen (N) from soils, most importantly the inorganic ions ammonium (NH_4^+) and nitrate (NO_3^-). While one might expect NH_4^+ to be preferred by plants, as its assimilation requires less energy than that of NO_3^- (1), only a few species perform well when NH_4^+ is the only, or predominant, source of N (2). By contrast, most species develop toxicity symptoms when grown on moderate to high levels of NH_4^+ (1–6), whereas normal growth in these species is seen on NO_3^-. Ammonium toxicity is especially problematic in areas with intensive agriculture and cultivation of livestock, where high levels of NH_3 emission, and subsequent NH_4^+ deposition, are observed (6, 7). It is estimated that N deposition from atmospheric NH_3 can reach levels as high as 50 $kg \cdot ha^{-1} \cdot yr^{-1}$ in some parts of Europe, in extreme cases constituting 50–80% of total N deposited from all possible sources (6). As a result, N saturation in many natural ecosystems is exceeded by as much as 10-fold, and damage to forest and agricultural crops alike has been attributed directly to this phenomenon (8, 9). This is a problem of serious concern in both Europe and North America (6–10).

Several hypotheses have been advanced to explain why NH_4^+ is toxic to plants, but none is considered satisfactory (3, 5). As NH_4^+ uptake mechanisms are coupled to H^+ extrusion into the rooting medium and H^+ release is also associated with NH_4^+ incorporation into protein (6), it has been repeatedly suggested that root medium acidification (11) and/or intracellular pH disturbance (3, 12) may explain the observed symptoms. However, in many cases, toxicity is equally observed in pH-buffered media (13), and a recent study on pea (*Pisum sativum*), known to be ammonium sensitive, has discounted the occurrence of ammonium-induced cytosolic pH disturbance (12). Others have suggested that carbohydrate limitation may contribute to the toxicity syndrome, based on the finding that NH_4^+ *per se* is not translocated to the shoot in most plants (14), and, thus, all C skeletons for N assimilation must be provided in roots, causing local C deprivation (15). In some cases, external provision of α-ketoglutarate, a key carbon source for N assimilation, alleviated toxicity symptoms (4), but in other cases it failed to enhance NH_4^+ metabolism (16), suggesting that other factors may limit NH_4^+ assimilation. The hypothesis that NH_4^+ toxicity results from the uncoupling of photophosphorylation in chloroplasts (6) has long been shown to be incorrect, as even very large NH_4^+ concentrations do not affect this process in intact chloroplasts (17). A diminution of essential cations such as K^+, Mg^{2+}, and Ca^{2+} in tissues of plants grown under prolonged exposure to NH_4^+ has been widely documented (18–20), and while this may be a contributor to the toxicity syndrome, it is clearly a longer-term effect, resulting from nutrient imbalance.

What these hypotheses have in common is that they are all contingent upon the permeation of NH_4^+ into the cell. Therefore, the study of transmembrane NH_4^+ fluxes is essential to the establishment of a proper context for the discussion of the issue of toxicity. Surprisingly, however, no study of the primary events of NH_4^+ acquisition, including the modes of entry, metabolism, and sequestration, has been undertaken in this context. Here we have used positron-emission tracing with the short-lived nitrogen isotope ^{13}N to characterize these primary events at the cellular level, in the model grass species rice (*Oryza sativa*) and barley (*Hordeum vulgare*).

Methods

Plant Culture and Radiotracer Experiments. Seedlings of barley (cv. CM-72) and of two major tropical-wetland varieties of rice (cv. IR-72 and cv. M-202) were cultivated hydroponically for 7 d and 21 d, respectively, in controlled-environment chambers. Hydroponic tanks contained aerated and N-free modified Johnson's solution (2, 14, 16). NH_4^+ was supplied as $(NH_4)_2SO_4$ at steady-state concentrations of 0.1 mM or 10 mM during growth and for radiolabeling experiments, which were conducted in the growth chambers. The short-lived radiotracer ^{13}N (half-life = 9.98 min) was used to trace NH_4^+ fluxes and to estimate cytosolic concen-

Abbreviation: $[NH_4^+]_o$, external NH_4^+ concentration.

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trations of NH_4^+ by compartmental analysis, as described previously (2, 16, 21). In brief, roots of intact seedlings were exposed to growth solution spiked with $^{13}NH_4^+$ for 1 h, to maximize the specific activity of tracer in the cytosolic compartment of root cells (21). Seedlings were then transferred to efflux funnels (16, 21), and tracer from the roots was eluted successively with 20-ml aliquots of nonlabeled solution for various washout periods. With $t = 0$ as the time of transfer from loading to washing solution and $t_{final} = 24$ min for the final elution, the time periods for the 25 successive washes were: 10 s (three times), 15 s (six times), 30 s (four times), 1 min (four times), and 2 min (eight times). γ-Ray emissions in the eluates were then measured (see Fig. 1). After the final elution, roots were excised from shoots, spin-dried for 30 s, and weighed, and the γ-ray emissions of roots and shoots were counted. Influx, efflux, net flux, and cytosolic concentrations of NH_4^+ were estimated from analysis of the wash-out kinetics as described in detail elsewhere (2, 16, 21). All fluxes are expressed in μmol per g (fresh weight) per h. Symbols, and basic calculation methods, for fluxes are as follows:

Φ_{co} = efflux from the cytosol, obtained from the rate of ^{13}N release from the cytosol at time 0.

Φ_{net} = net flux, obtained directly from the accumulation of ^{13}N in the plants at the end of the elution period.

Φ_{oc} = unidirectional influx, calculated from $\Phi_{net} + \Phi_{co}$.

Φ_{xylem} = xylem flux of ^{13}N, obtained directly from accumulation in the shoot, at the end of the elution period.

$\Phi_{met/vac}$ = combined flux to assimilation and vacuole, resulting from $\Phi_{net} - \Phi_{xylem}$.

Cytosolic NH_4^+ concentrations were calculated from the quotient of the rate of $^{13}NH_4^+$ release integrated over 5 times the half-life of cytosolic NH_4^+ exchange, and the ratio of efflux to all fluxes removing $^{13}NH_4^+$ from the cytosol, and were based on the assumption that the cytosol occupies 5% of cell volume (2, 16, 21). Experiments were repeated four to five times. Standard errors (SE) for fluxes were less than 20% of the means.

Electrophysiological Measurements. Membrane potential differences were measured as described elsewhere (22). Intact barley or rice roots were held between steel pins and pieces of silicone tubing in a Plexiglas chamber affixed to a light microscope, and they were impaled with glass microelectrodes until a stable electrical potential difference between root and external solution was maintained for at least 2 min. Sixty-seven measurements in total were made on different plants for the 0.1 mM NH_4^+ condition in barley, and 20 for the 10 mM NH_4^+ condition. SE was <3% of the means. For rice, 17 measurements were undertaken at 0.1 mM external NH_4^+ concentration ($[NH_4^+]_o$), and 26 at 10 mM $[NH_4^+]_o$ (SE < 5% of the means).

Respiration Measurements. O_2 depletion was followed potentiometrically in intact roots of barley and rice with a Hansatech cuvette/electrode system attached to a chart recorder. Cuvettes were filled with air-saturated growth solutions. Calculations were based on slopes of steady O_2 depletion lines generated over 3–10 min, after which plants were weighed and discarded. The O_2 content of the cuvette was not reduced by more than 25% of the initial value. Measurements were repeated 20–25 times.

Relative Growth Rates. Individual barley plants were monitored over days 1, 3, and 7 after germination. Surface water was removed from roots before weighing, after which the plants were immediately returned to solution. Eight replicates were used for each of the two steady-state NH_4^+-growth concentrations.

Results and Discussion

In the present study, we assayed NH_4^+ transport properties in the root cells of two major cereal species known to differ markedly in their abilities to tolerate NH_4^+ as the sole N source. $^{13}NH_4^+$ efflux from roots of intact plants of the two species, prelabeled with this tracer, showed substantial differences in the rates of NH_4^+ extrusion from root cells to the external medium (Fig. 1). Interestingly, barley (*Hordeum vulgare*), which suffers from NH_4^+ toxicity (23), excretes NH_4^+ at a significantly higher rate than rice (*Oryza sativa*), a species whose tolerance to NH_4^+ is considered exceptional (24). Fig. 2, depicting bidirectional chemical fluxes of NH_4^+ across the plasma membranes of these two species, documents in both cases a large increase in total flux (equivalent to unidirectional influx) as the steady-state external NH_4^+ concentration is stepped up from a moderate level of 0.1 mM to a level of 10 mM, which represents the high end of NH_4^+ concentrations found in agricultural soils (1–11), and which is potentially toxic (24). Importantly, however, the increase in barley is much higher (12-fold) than in rice (7-fold). In fact, the magnitudes of the fluxes in barley are the highest ever reported for NH_4^+ in a plant root system, and strongly suggest the operation of channel-based NH_4^+-transport systems, whose existence has

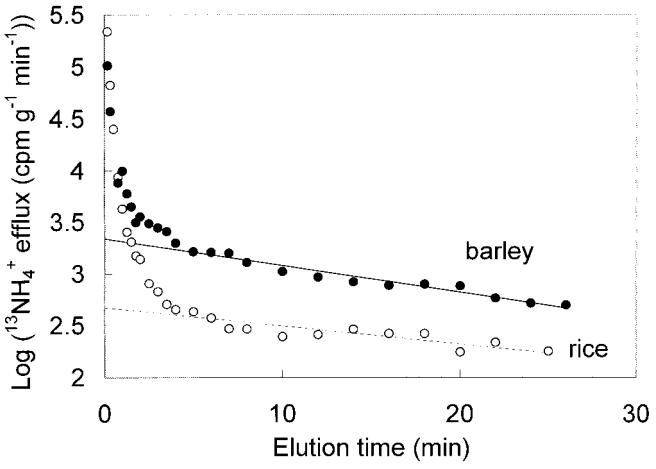

Fig. 1. Semilogarithmic plots of $^{13}NH_4^+$ efflux from the cytosolic compartments of barley and rice roots. Plants were prelabeled under steady-state conditions, with NH_4^+ provided externally at 10 mM. Plots have been corrected for specific activities of ^{13}N tracer (2, 21), allowing a direct comparison of initial efflux rates by inspection of the *y*-intercepts of the regression lines.

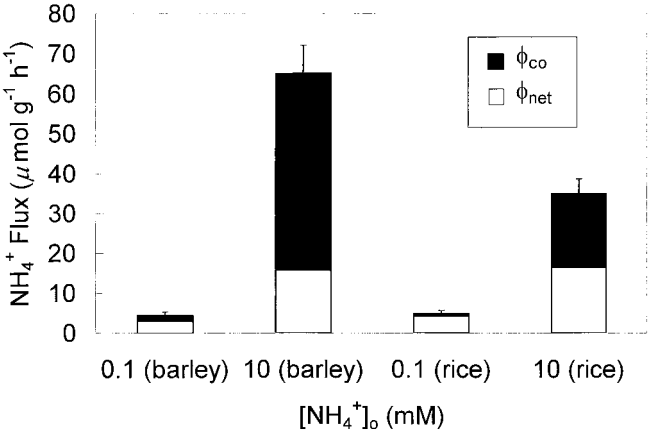

Fig. 2. Comparison of steady-state bidirectional plasma-membrane NH_4^+ fluxes in barley and rice roots at 0.1 mM and 10 mM $[NH_4^+]_o$. Column height represents total influx from the external medium (Φ_{oc}), while the filled areas depict the portion of influx returned to the environment by efflux transport (Φ_{co}). The net flux (Φ_{net}) is the difference between these two fluxes. Vertical bars indicate standard errors of influx means.

Table 1. Thermodynamic analysis of cytosolic NH_4^+ pool sizes in barley and rice

Plant	$[NH_4^+]_o$, mM	Influx, μmol·g^{-1}·h^{-1}	Efflux, μmol·g^{-1}·h^{-1}	Flux ratio	$\Delta\Psi$, mV	$[NH_4^+]_c$, mM	
						Predicted	Measured
Barley	0.1	5.46	1.53	0.28	−121	12	28
	10	65.1	49.4	0.76	−123	1,320	358
Rice	0.1	4.85	0.67	0.14	−132	15.6	33.4
		5.97 (ref. 34)	1.17 (ref. 34)	0.19	−122 (ref. 22)	10.7	20.6 (ref. 34)
	1	10.51 (ref. 34)	3.09 (ref. 34)	0.29	−88 (ref. 22)	28.78	38.1 (ref. 34)
	10	35.0	18.6	0.53	−87.9	289	232
					−82 (ref. 22)	233	ND

The Nernst equation {$\Psi_N = RT/(nF)\ \ln([NH_4^+]_o/[NH_4^+]_c)$, where Ψ_N = the Nernst potential; R = 8.3144 J·K^{-1}·mol^{-1}; F = 96,485 C·mol^{-1}; n = 1; and T = 293.15 K (for barley) or 303.15 K (for rice)} was used to predict equilibrium cytosolic concentrations ($[NH_4^+]_c$) on the basis of external concentration, membrane potential ($\Delta\Psi$), and an ambient temperature of 20°C for barley and 30°C for rice. Cytosolic concentrations were measured by using compartmental analysis (see text). Additional membrane potential readings and estimates of cytosolic NH_4^+ concentrations for rice (cv. M-202) are included on the basis of analysis of previously published work (22, 34). ND, not determined.

been demonstrated by several recent studies (25, 26). As the steady-state net fluxes are almost identical in the two species, a substantially larger fraction of incoming nitrogen is subsequently excreted in the ammonium-sensitive species. This pronounced excretion of N is inextricably linked to an apparent inability of barley to exclude NH_4^+ at the more primary, intake, step. Our preliminary analyses on other species known to suffer NH_4^+ toxicity, including wheat, tomato, and the tree species Douglas-fir and trembling aspen (27), suggest that this phenomenon is by no means isolated, but may well occur universally among susceptible higher plants.

Knowledge of the kinetic constants of NH_4^+ exchange for the cytosolic component in the experimental root system, and of the tracer content in this pool at the onset of the elution protocol, allowed us to compare the concentrations of NH_4^+ on either side of the plasma membrane (2), and hence to investigate the energetics of fluxes across this membrane. Our analysis shows that, at 10 mM $[NH_4^+]_o$, the cytosolic concentrations of NH_4^+ in both barley and rice are of a level higher than ever reported in plant cells (Table 1), and indeed are irreconcilable with the widely held dogma that no appreciable quantities of free NH_4^+ can exist in the cytosol (1, 5, 6). Nevertheless, a thermodynamic analysis, using measurements of electrical potential ($\Delta\Psi$) across the plasma membrane, reveals that, in barley, the cytosolic levels of NH_4^+ at 10 mM $[NH_4^+]_o$ are substantially lower than predicted by the Nernst equation, which applies when NH_4^+ is passively distributed across a highly permeable membrane (Table 1). Rice, by contrast, maintains a lower internal concentration than barley under this condition, but because of the relatively depolarized state of its plasma membrane under high NH_4^+ provision (22), the transmembrane distribution ratio of NH_4^+ very closely approximates that predicted by Nernstian analysis (Table 1). This lowering of membrane polarization with increasing NH_4^+ provision, which is not followed by a restoration of that polarization in the steady state, is confirmed by previously published work on rice (ref. 22; see Table 1) and the aquatic NH_4^+-specialist plant *Lemna gibba* (28). Table 1 shows that, in rice, a depolarized membrane potential is already achieved at 1 mM $[NH_4^+]_o$, a more modest NH_4^+ concentration. The reduction of $\Delta\Psi$ with increasing $[NH_4^+]_o$ apparent in rice has the important biophysical consequence of lowering the ceiling for NH_4^+ accumulation in the cytosol, thus eliminating the gradient against which efflux transporters must work to remove excess cytosolic NH_4^+. The failure of barley to down-regulate $\Delta\Psi$, on the other hand, sustains a gradient for very large inward NH_4^+ fluxes, which we in fact observe (Fig. 2). Homeostatic restoration of $\Delta\Psi$ to preset values has been previously reported for barley exposed to various concentrations of NO_3^- (29), the second major N source used by plants. Under NH_4^+ nutrition, an undiminished $\Delta\Psi$ in barley yields a high potential for NH_4^+ accumulation, and a significantly larger demand on the efflux process (Table 1). Our data show that, whereas the net flux of NH_4^+ is nearly identical in both species at 10 mM, the efflux process in barley mediates a 2- to 3-fold higher removal of NH_4^+ from the cytosol, effectively reducing a Nernstian NH_4^+ concentration of 1.32 M by the equivalent of 962 mM (Table 1).

Such a process must carry a substantial energetic burden, and this prediction was consistent with measurements of oxygen consumption in roots of intact barley plants under the steady-state conditions of NH_4^+ supply corresponding to those of flux measurements. As indicated in Fig. 3, upon transition from low steady-state NH_4^+ provision to high, respiration in barley increased by a startling 41%, whereas no significant difference was found in rice between growth conditions (not shown). To isolate, in barley, the flux processes at the plasma membrane level from other potentially energy-requiring pathways of NH_4^+ processing, we applied methionine sulfoximine (MSX), a compound known to completely block NH_4^+ assimilation in the species under investigation (14, 16, 30). Importantly, MSX did not diminish respiratory activity associated with elevated external NH_4^+ (Fig. 3), emphasizing that the respiratory stimulation was attributable to processes upstream of NH_4^+ metabolism—i.e., residing at the level of membrane transport—and independent of activities of glutamine synthetase and other processes related to N metabolism. Other work on barley has shown that the proportion of

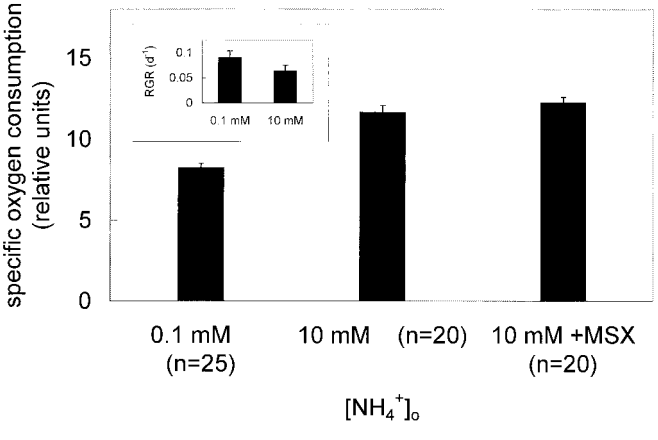

Fig. 3. Respiration rates of intact barley roots at 0.1 mM and 10 mM $[NH_4^+]_o$. In one experiment, 1 mM methionine sulfoximine (MSX) was applied to block NH_4^+ metabolism (see text). Relative growth rates under the two conditions are shown in the *Inset*. Rice experienced no significant difference in respiration under the two NH_4^+ regimes (see text).

total respiration assigned to processes associated with NH_4^+ uptake and assimilation constitutes as much as 14% of the plant's total respiratory expenditure at 0.1 mM external NH_4^+ provision (31). Most of this expenditure appears to be due to plasma-membrane transport activity, hence we conclude that the 41% increase in total root respiration observed in the present study for barley at 10 mM NH_4^+ can be attributed directly to the approximately 30-fold increase in NH_4^+ efflux from the cytosol under this condition. Work with the NH_4^+ analogue methylammonium shows that a respiratory increase is also observed in barley (32), as well as other species (33), on addition of this nonmetabolized compound. In the absence of metabolic sinks, it becomes clear that membrane processes *per se* can impose a substantial energetic burden on the plant root system. Indeed, in barley the increase in respiration at 10 mM external NH_4^+ is not correlated with an increase in growth-related energy demands; on the contrary, it accompanies a depression in relative growth rate at the whole plant level (Fig. 3 *Inset*) not seen in ammonium-tolerant species such as rice (2, 14, 21, 22, 34, 35). It is intriguing that in rice, where no respiratory increase was found with increasing NH_4^+ provision (data not shown), a thermodynamic equilibrium is achieved across the plasma membrane (Table 1), suggesting that passive inwardly and outwardly directed NH_4^+ channel activities may mediate NH_4^+ distribution across this membrane without energetic cost. These findings underscore the cellular adaptations rice has evolved to use NH_4^+ as an N source (34, 35), which allow it to thrive in flooded soil environments where hypoxic to anoxic conditions typically render NH_4^+ the only major N source available to plant growth (35).

We propose that the inability of barley, and that of other species susceptible to ammonium toxicity, to exclude NH_4^+ by regulation of plasma-membrane influx systems constitutes a fundamental breakdown in plant cell function and must precede any intracellular toxicity-associated events such as cation displacement or carbohydrate depletion (see above). The operation of the energy-intensive NH_4^+ extrusion mechanism we describe appears to be central to the ammonium toxicity syndrome, and is similar in principle to mechanisms evolved by bacteria and carcinomas to actively excrete cytotoxins such as antibiotics and chemotherapeutic agents (36, 37). The ecological significance of this syndrome is substantial, not only because soil nitrogen profiles profoundly determine the spatial and temporal dynamics of ecosystems (2, 38), but especially in the light of recent interventions by humans in the functioning of the global nitrogen cycle (5–7, 39).

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1. Reisenauer, H. M. (1978) in *Nitrogen in the Environment*, eds. Nielsen, D. R. & Macdonald, J. G. (Academic, New York), pp. 157–189.
2. Kronzucker, H. J., Siddiqi, M. Y. & Glass, A. D. M. (1997) *Nature (London)* **385,** 59–61.
3. Gerendas, J., Zhu, Z., Bendixen, R., Ratcliffe, R. G. & Sattelmacher, B. (1997) *Z. Pflanzenernähr. Bodenkd.* **160,** 239–251.
4. Magalhaes, J. R., Huber, D. M. & Tsai, C. Y. (1992) *Plant Sci.* **85,** 135–141.
5. De Graaf, M. C. C., Bobbink, R., Roelofs, J. G. M. & Verbeek, P. J. M. (1998) *Plant Ecol.* **135,** 185–196.
6. Pearson, J. & Stewart, G. R. (1993) *New Phytol.* **125,** 283–305.
7. Fangmeier, A., Hadwiger-Fangmeier, A., Van der Eerden, L. & Jäger, H.-J. (1994) *Environ. Poll.* **86,** 43–82.
8. Van Breeman, N., Burrough, P. A., Velthorst, E. J., van Dobben, H. F., de Wit, T., Ridder, T. B. & Reijnders, H. F. R. (1982) *Nature (London)* **299,** 548–550.
9. Van Breeman, N. & Van Dijk, H. F. G. (1988) *Environ. Poll.* **54,** 249–274.
10. Padgett, P. E., Allen, E. B., Bytnerowicz, A. & Minich, R. A. (1999) *Environ. Poll.* **104,** 179–187.
11. Barker, A. V., Volk, R. J. & Jackson, W. A. (1966) *Plant Physiol.* **41,** 1193–1199.
12. Bligny, R., Gout, E., Kaiser, W., Heber, U., Walker, D. & Douce, R. (1997) *Biochim. Biophys. Acta* **1320,** 142–152.
13. Blacquière, T., Voortman, E. & Stulen, I. (1998) *Plant Soil* **106,** 23–34.
14. Kronzucker, H. J., Schjoerring, J. K., Erner, Y., Kirk, G. J. D., Siddiqi, M. Y. & Glass, A. D. M. (1998) *Plant Cell Physiol.* **39,** 1287–1293.
15. Lewis, O. A. M, James, D. M. & Hewitt, E. J. (1982) *Ann. Bot.* **49,** 39–49.
16. Kronzucker, H. J., Siddiqi, M. Y. & Glass, A. D. M. (1995) *Plant Physiol.* **109,** 481–490.
17. Heber, U. (1984) *in Advances in Photosynthesis Research*, ed. Sybesma, C. (Nijhoff/Junk, The Hague, The Netherlands), pp. 381–389.
18. Holldampf, B. & Barker, A. V. (1993) *Commun. Soil Sci. Plant Anal.* **24,** 1945–1957.
19. Cao, W. & Tibbits, T. W. (1993) *J. Plant Nutr.* **16,** 1691–1704.
20. Speer, M. & Kaiser, W. M. (1994) *Plant Cell Environ.* **17,** 1223–1231.
21. Kronzucker, H. J., Glass, A. D. M., Siddiqi, M. Y. & Kirk, G. J. D. (2000) *New Phytol.* **145,** 471–476.
22. Wang, M. Y., Glass, A. D. M., Shaff, J. E. & Kochian, L. V. (1994) *Plant Physiol.* **104,** 899–906.
23. Lewis, O. A. M., Soares, M. I. M. & Lips, S. H. (1986) in *Fundamental, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants,* Developments in Plant and Soil Sciences, eds. Lambers, H., Neeteson, J. J. & Stulen, I. (Nijhoff, Dordrecht, The Netherlands), Vol. 19, pp. 295–300.
24. Magalhaes, J. R. & Huber, D. M. (1989) *Fert. Res.* **21,** 1–6.
25. Tyerman, S. D., Whitehead, L. F. & Day, D. A. (1995) *Nature (London)* **378,** 629–632.
26. Nielsen, K. H. & Schjoerring, J. K. (1998) *Plant Physiol.* **118,** 1361–1368.
27. Min, X. J., Siddiqi, M. Y., Guy, R. D., Glass, A. D. M. & Kronzucker, H. J. (1999) *Plant Cell Environ.* **22,** 821–830.
28. Ullrich, W. R., Larsson, M., Larsson, C.-M., Lesch, S. & Novacky, A. (1984) *Physiol. Plant.* **61,** 369–376.
29. Glass, A. D. M., Shaff, J. & Kochian, L. V. (1992) *Plant Physiol.* **99,** 456–463.
30. Wieneke, J. & Roeb, G. W. (1998) *Z. Pflanzenernähr. Bodenkd.* **161,** 1–7.
31. Bloom, A. J., Sukrapanna, S. S. & Warner, R. L. (1992) *Plant Physiol.* **99,** 1294–1301.
32. Rigano, C., Di Martino Rigano, V., Vona, V., Carfagna, S., Carillo, P. & Esposito, S. (1996) *New Phytol.* **132,** 375–382.
33. Larson, T. R. & Rees, T. A. V. (1994) *Planta* **195,** 195–200.
34. Wang, M. Y., Siddiqi, M. Y., Ruth, T. J. & Glass, A. D. M. (1993) *Plant Physiol.* **103,** 1249–1258.
35. Kronzucker, H. J., Kirk, G. J. D., Siddiqi, M. Y. & Glass, A. D. M. (1998) *Plant. Physiol.* **116,** 581–587.
36. McMurry, L., Petrucci, R. E., Jr., & Levy, S. B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3974–3977.
37. Nikaido, H. (1994) *Science* **264,** 382–388.
38. Tilman, D. (1986) *Ecology* **67,** 555–563.
39. Vitousek, P. M. (1994) *Ecology* **75,** 1861–1876.