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Dynamic Interactions between Root NH_4^+ Influx and Long-Distance N Translocation in Rice: Insights into Feedback Processes

Herbert J. Kronzucker [1, 2, 6], Jan K. Schjoerring [3], Yair Erner [4], Guy J.D. Kirk [2], M. Yaeesh Siddiqi [5] and Anthony D.M. Glass [5]

[1] *Department of Plant Sciences, University of Western Ontario, London, ON, Canada N6A 5B7*
[2] *International Rice Research Institute (IRRI), P.O. Box 933, 1099 Manila, Philippines*
[3] *Plant Nutrition Laboratory, Royal Veterinary and Agricultural University, DK-1871 FC, Copenhagen, Denmark*
[4] *Department of Citriculture, ARO The Volcani Center, Bet-Dagan 50250, Israel*
[5] *Department of Botany, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4*

Ammonium influx into roots and N translocation to the shoots were measured in 3-week-old hydroponically grown rice seedlings (*Oryza sativa* L., cv. IR72) under conditions of N deprivation and NH_4^+ resupply, using $^{13}NH_4^+$ as a tracer. Root NH_4^+ influx was repressed in plants continuously supplied with NH_4^+ (at 0.1 mM), but a high proportion of absorbed N (20 to 30%) was translocated to the shoot in the form of N assimilates during the 13-min loading and desorption periods. Interruption of exogenous NH_4^+ supply for periods of 1 to 3 d caused NH_4^+ influx to be de-repressed. This same treatment caused N translocation to the shoot to decline rapidly, until, by 24 h, less than 5% of the absorbed ^{13}N was translocated to the shoot, illustrating a clear priority of root over shoot N demand under conditions of N deprivation. Upon resupplying 1 mM NH_4^+, root NH_4^+ influx responded in a distinct four-phase pattern, exhibiting periods in which NH_4^+ influx was first enhanced and subsequently reduced. Notably, a 25 to 40% increase in root influx, peaking at ~2 h following re-exposure was correlated with a 4- to 5-fold enhancement in shoot translocation and a repression of root GS activity. The transient increase of NH_4^+ influx was also observed in seedlings continuously supplied with NO_3^- and subsequently transferred to NH_4^+. Extended exposure to NH_4^+ caused root NH_4^+ influx to decrease progressively, while shoot translocation was restored to ~30% of incoming NH_4^+. The nature of the feedback control of NH_4^+ influx as well as the question of its inducibility are discussed.

Key words: Ammonium — Influx — Nitrogen — Nitrogen stress — N-13 — Rice.

It has been widely documented that higher plants increase their root capacity to absorb nitrogen when subjected to N deprivation for prolonged periods of time. This enhancement of uptake is observed for both NO_3^- (Humphries 1951, Jackson et al. 1976, Clement et al. 1979, MacKown et al. 1981, Breteler and Nissen 1982, Talouitze et al. 1984, Lee and Rudge 1986, Ingemarsson et al. 1987, Oscarson et al. 1987, Teyker et al. 1988, Siddiqi et al. 1989, Jackson and Volk 1992) and NH_4^+ (Tromp 1962, Lycklama 1963, Minotti et al. 1969, Ivanko and Ingversen 1971, Jackson et al. 1976, Lee and Rudge 1986, Morgan and Jackson 1988a, b, Jackson and Volk 1992, Wang et al. 1993b). Evidently, negative-feedback mechanisms are in place which down-regulate N uptake when N supply is sufficient, while de-repression of plasmamembrane N transport occurs as N is withdrawn during starvation. There has been much debate in the literature as to whether NO_3^- and NH_4^+ themselves or rather downstream products of N assimilation, such as glutamine and other amino acids, are the more potent negative-feedback agents in this regulation of membrane transport of N (Lee and Rudge 1986, Morgan and Jackson 1988b, Jackson and Volk 1992, King et al. 1993, Wang et al. 1993a, Kronzucker et al. 1995b, e).

In the case of NO_3^-, the response of root uptake to N starvation is complicated by a concurrent de-induction of the inducible high-affinity transport system ('IHATS'; Siddiqi et al. 1989, King et al. 1992) for NO_3^- influx. Just as the presence of NO_3^- as a signal is essential for the full expression (induction) of NO_3^- influx (MacKown and McClure 1988, Tischner et al. 1993), its withdrawal results in a rather rapid decay (within hours) of the IHATS (Siddiqi et al. 1989). Several workers have claimed that a similar process of induction and de-induction is involved in regulating NH_4^+ influx (Goyal and Huffaker 1986, Morgan and Jackson 1988a, Jackson and Volk 1992, Mäck and Tischner 1994), but the evidence is much less conclusive than in the case of NO_3^-.

In the present study, we have used the short-lived radiotracer ^{13}N to examine the responses of root-plasmalemma NH_4^+ influx to NH_4^+ deprivation and subsequent NH_4^+ resupply in rice, within a time frame of a few hours to several days. We have also monitored the concomitant development of N translocation to the shoot. Our aim was to characterize the dynamics of NH_4^+ flux regulation, including the question of inducibility of NH_4^+ influx, within

Abbreviations: GS, glutamine synthetase; MSO, methionine sulphoximine; $[NH_4^+]_{cyt}$, cytoplasmic ammonium concentration; $[NH_4^+]_o$, NH_4^+ concentration in the external solution.

[6] Corresponding author; e-mail kronzuck@julian.uwo.ca; fax 1-519-661-3935.

the root system of rice and the whole-plant regulation of N transport between root and shoot.

Materials and Methods

Plant growth conditions—Rice seeds (*Oryza sativa* L., cv. IR72) were surface-sterilized by exposure to 5% NaOCl for 10 min, rinsed several times with deionized water, and incubated in aerated deionized water for 48 h in a waterbath (set at 30°C). Subsequently, the seeds were placed on plastic mesh mounted onto Plexiglas discs. The discs were transferred to Plexiglas hydroponic tanks (volume: 40 literes; see below for composition of nutrient solutions) which were located in walk-in controlled-environment growth chambers. Temperatures in the growth chambers were maintained at 30/20°C, RH at 70%, and photoperiod was 12 h/12 h. Photon flux was ~500 μmol m^{-2} s^{-1}, as measured at the level of shoot tips; light was provided by fluorescent lamps (Philips 1500, F96T12/CW/VHO, 215 W), and photon flux was measured using an LI-189 light meter equipped with an LI-190SA quantum sensor (Li-Cor, Lincoln, NE).

Nutrient solutions—Rice seedlings were cultivated for a period of three weeks in nutrient solution contained in 40-liters hydroponic tanks (see above). Deionized-distilled water and reagent-grade chemicals were used in the preparation of all nutrient solutions. Nutrient salts added were as follows: K_2SO_4 (1 mM), $MgSO_4$ (2 mM), $CaCl_2$ (1 mM), NaH_2PO_4 (300 μM), Fe-EDTA (100 μM), $MnCl_2$ (9 μM), $(Na)_6Mo_7O_{24}$ (25 μM), H_3BO_3 (20 μM), $ZnSO_4$ (1.5 μM), $CuSO_4$ (1.5 μM). Ammonium was added in the form of $(NH_4)_2SO_4$, at 0.1 mM for the duration of growth, and at 1 mM in "re-exposure" experiments (following −N treatment for 2 to 4 d), as indicated in Results and Discussion. Uptake was always measured at 0.1 mM $[NH_4^+]_o$ in order to determine high-affinity NH_4^+ influx. When NO_3^- was used as a pretreament agent in NH_4^+-flux experiments, plants were exposed to 0.1 mM NO_3^- (added in the form of $Ca(NO_3)_2$) for 7 d prior to flux determinations. The complete solution was maintained throughout the growth period. Nutrient solutions in tanks were continuously mixed by means of electric circulating pumps (Circulator model IC-2, Brinkmann Instruments Canada Ltd., Rexdale, Ontario). Steady-state control of nutrient concentrations was ensured by continuous and controlled infusion of a concentrated nutrient stock solution through peristaltic pumps (Technicon Proportioning Pump II, Technicon Instrument Corp., Tarrytown, NY). Solutions were checked daily for $[NH_4^+]$, measured according to the method of Solorzano (1969), using a Philips PU 8820 UV/VIS spectrophotometer; $[NO_3^-]$, measured spectrophotometrically according to Cawse (1967); $[K^+]$, measured by flame photometry (using an Instrumentation Laboratory Photometer, model 443, Lexington, MA); and pH, measured with a microprocessor-based pocket-size pH meter (pH Testr2 model 59000-20; Cole Parmer, Chicago, IL) and maintained at 6.5±0.3 by addition of powdered $CaCO_3$.

Measurement of fluxes—The radiotracer ^{13}N (half-life=9.96 min) was produced by the 'TRIUMF' (Tri-University Meson Facility) cyclotron at the University of British Columbia (Vancouver, Canada) by proton irradiation of water. This procedure produced mostly $^{13}NO_3^-$ with high radiochemical purity (Kronzucker et al. 1995a). The irradiated solutions (~700–740 MBq) were supplied in sealed 20-ml glass vials. Procedures for the removal of radiocontaminants and conversion of $^{13}NO_3^-$ to $^{13}NH_4^+$ using Devarda's alloy were as described in detail elsewhere (Kronzucker et al. 1995a, b, c). A volume of 100 ml of $^{13}NH_4^+$-containing "stock" solution was prepared in a fumehood and was transferred into the controlled-environment chambers where experiments were carried out. All uptake solutions were premixed and were contained in individual 500-ml plastic vessels behind lead shielding. The chemical composition of the uptake solution was identical to the growth solution in the hydroponic tanks (see above), but contained NH_4^+ at 0.1 mM. Tracer was then added by syringe to the individual uptake vessels. At the start of influx experiments, rice seedlings were transferred from the hydroponic growth tanks to prewash solutions in 1-liter vessels for 5 min prior to immersion of the intact seedling roots in the labelled uptake solutions. This protocol minimized perturbation and allowed the roots to equilibrate to the exact solution temperature and to the solution composition used during influx. The roots were then exposed to tracer for 10 min. Immediately following the 10-min isotope loading, roots were dipped into non-labelled solutions for 5 s to minimize carry-over of label by the root surface to the desorption solution. Roots were then desorbed for 3 min in unlabelled solution, which was otherwise chemically identical to influx solution, to desorb $^{13}NH_4^+$ contained in the *Donnan free space*. The duration of these steps was based on the half-lives of exchange of NH_4^+ for the root surface, the *Donnan free space*, and the cytoplasm as determined by efflux analysis (see below as well as Kronzucker et al. 1995c, e). An exposure time of 10 min was chosen for $^{13}NH_4^+$ influx, since the contribution of tracer efflux from the cytoplasm can be expected to be negligible during this time (Wang et al. 1993a, Kronzucker et al. 1995e, 1996). Following desorption, seedling roots were excised from the shoots, the roots spun in a low-speed centrifuge for 30 s to remove surface liquid, and the fresh weights of roots and shoots determined. The plant organs were then introduced into 20-ml scintillation vials, and the radioactivities of roots and shoots were determined in a Packard γ-counter (Minaxi δ, Auto-γ 5000 Series), measuring the 511-keV positron-electron annihilation radiation generated by recombination of ambient electrons and β^+ particles emitted from ^{13}N. Using the specific activity ($^{13}N/(^{13}N+^{14}N)$ [dpm μmol^{-1}]) of the loading solution and the total fresh root weight of each seedling, NH_4^+ fluxes were calculated and expressed in μmol (g FW)$^{-1}$ h^{-1}. All experiments were replicated at least four to five times. Each experimental treatment consisted of four replicates. Representative experiments are shown ($n \geq 4$).

Glutamine synthetase assay—Determination of GS activity essentially followed the biosynthetic reaction assay by Lea et al. (1990). Roots were pulverized in liquid nitrogen and incubated for 10 min at 4°C in a buffer containing 50 mM Tris-Cl, 1 mM EDTA, 2 mM dithiothreitol (DTT), 10 mM $MgSO_4$, 5 mM glutamate, 10% v/v ethanediol, and 0.1% insoluble polyvinylpyrrolidone (PVP); buffer pH was 7.8. The ratio of plant material to extraction buffer was 1 : 5. Following centrifugation (30,000 × g at 4°C for 20 min), the activity of GS was measured in an assay buffer consisting of 100 mM Tris-HCl at pH 7.8, 5 mM NH_2OH, 50 mM $MgSO_4$, 50 mM glutamate, and 20 mM ATP; 0.25 ml of the assay buffer were preincubated at 30°C, followed by addition of 0.2 ml supernatant. The reaction was allowed to proceed for 30 min, as a linear time course was observed for at least 45 min. The assay was terminated by the addition of 0.7 ml of $FeCl_3$ reagent (2.5% w/v $FeCl_3$, 5% w/v trichloroacetic acid in 1.5 M HCl). Controls were performed under identical conditions, except that ATP was absent. The resulting precipitate was spun down at 10,000 × g for 5 min, and the absorbance of the supernatant was measured at 540 nm. A standard curve was made using glutamyl hydroxamate.

Results and Discussion

Withdrawal of NH_4^+ from growth solution for periods of up to 3 d resulted in a progressive increase in NH_4^+ influx across the root plasmalemma in rice. Plants under steady-state provision of 100 μM NH_4^+ exhibited an influx of 4.2 ± 0.12 μmol (g FW)$^{-1}$ h^{-1} (measured at the same concentration), while plants starved for NH_4^+ for 24 h showed an influx of 6.3 ± 0.32 μmol g^{-1} h^{-1}. No further enhancement of influx was observed after an additional 1 to 2 d of NH_4^+ withdrawal (Fig. 1). In N-sufficient plants, 20 to 30% of incoming ^{13}N was translocated to the shoot within the 13 min (10 min loading plus 3 min desorption) of isotope exposure in our experiments (Fig. 1, 2); this percentage of N translocation to the shoot dropped rapidly as NH_4^+ was withdrawn (significantly even within the first hour) and reached a new steady-state level around 5% of incoming N after 24 h of N withdrawal and onwards (Fig. 2). This relative shutdown of shoot N translocation indicates a priority of root over shoot demand for N under starvation conditions. As

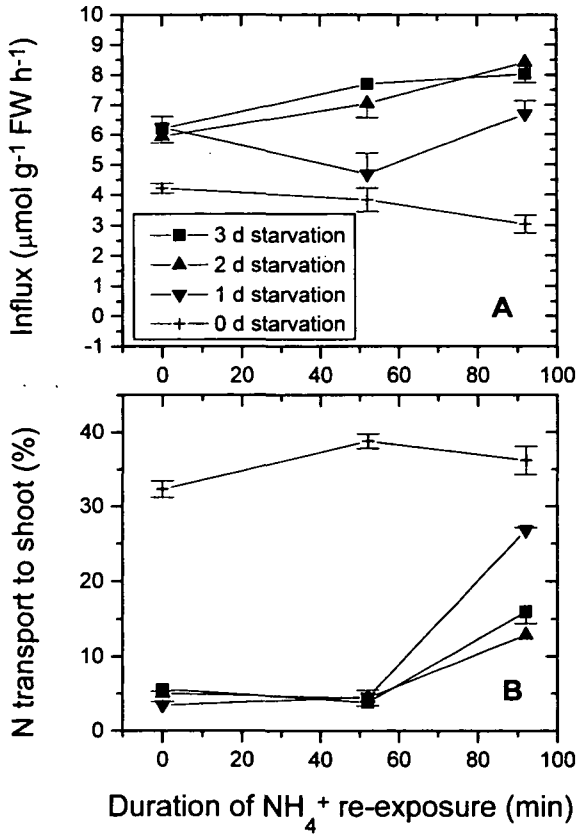

Fig. 1 NH_4^+ influx into roots (A) and N translocation to the shoot (B) in intact rice seedlings. Plants, previously grown at 0.1 mM $[NH_4^+]_o$, were deprived of N for 0 to 3 d and then re-exposed to 1 mM NH_4^+ for varying periods up to 90 min prior to flux determinations. Fluxes were measured at 0.1 mM $[NH_4^+]_o$. Data are means ± SE ($n \geq 4$).

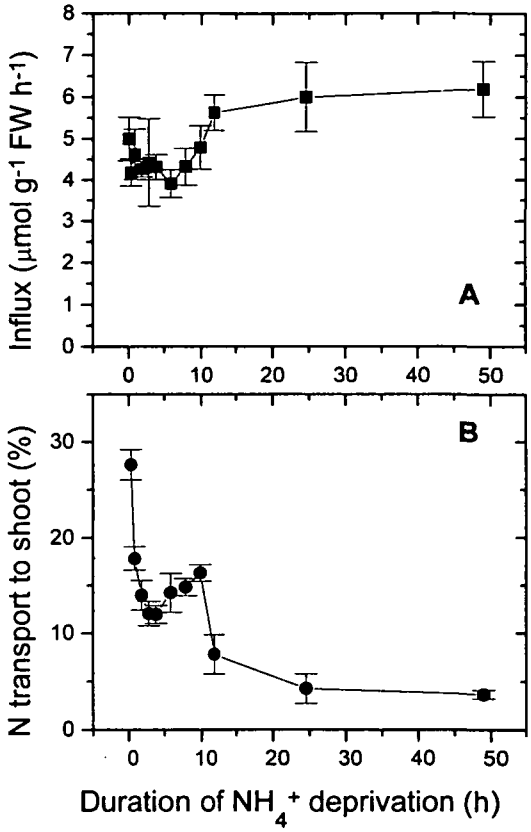

Fig. 2 NH_4^+ influx into roots (A) and N translocation to the shoot (B) in intact rice seedlings. Plants, previously grown at 0.1 mM $[NH_4^+]_o$, were deprived of N for increasing times up to 50 h. Fluxes were measured at 0.1 mM $[NH_4^+]_o$. Data are means ± SE ($n \geq 4$).

NH_4^+ was resupplied to N-deprived plants, an equally rapid recovery of N translocation to the shoot was observed (Fig. 1, 3). This recovery was essentially complete within 6 h of NH_4^+ resupply at 1 mM (Fig. 3). When N translocation to the shoot is expressed in absolute terms (cpm g^{-1} h^{-1}) rather than in percent of root NH_4^+ influx, shoot translocation patterns in N-supplied and N-deprived plants (cf. Fig. 1) were practically identical (not shown). ^{13}N translocation to the shoot must reflect the specific activities of the N solutes (NH_4^+ as well as amino acids) available for translocation at the xylem-loading step; unfortunately, values for those specific activities rise according to transport and metabolic reactions during the loading process and are unknown under the conditions of our experiments. In our plants, pretreatment with the GS inhibitor MSO (provided at 1 mM for 6 h) essentially eliminated long-distance ^{13}N transport (not shown); thus, amino acids appear to be the sole N substances translocated to the xylem in rice under the given conditions of external NH_4^+ supply (see also Wang et al. 1993a). As the cytoplasmic pool size of amino

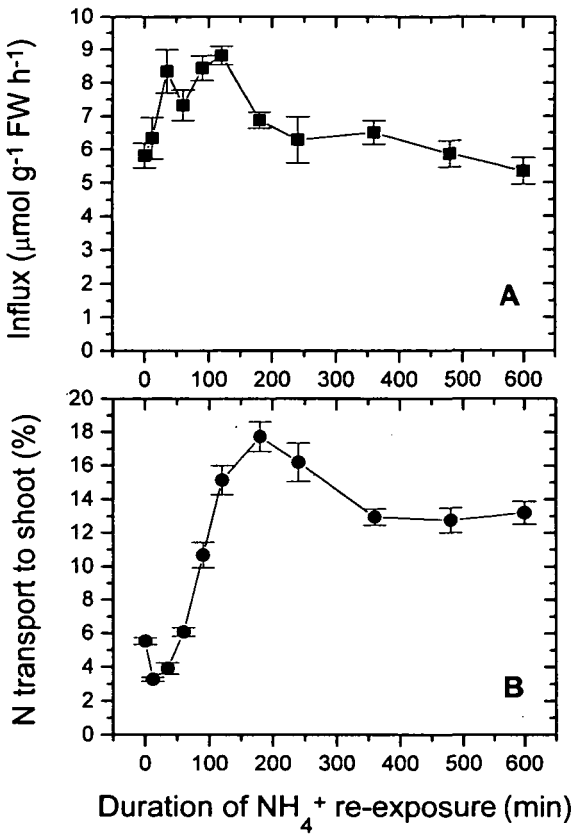

Fig. 3 NH_4^+ influx into roots (A) and N translocation to the shoot (B) in intact rice seedlings. Plants were deprived of N for 2 d and then re-exposed to 1 mM NH_4^+ for increasing times up to 10 h prior to flux determinations. Fluxes were measured at 0.1 mM $[NH_4^+]_o$. Data are means±SE ($n \geq 4$).

acids can be expected to be lower and the specific activity of that pool consequently to be higher in N-deprived compared to N-sufficient plants following identical exposure times to tracer, the close correspondence between treatments in absolute tracer translocation values further accentuates the priority of root over shoot demand for N under starvation conditions. Interestingly, in absolute terms, the shoot translocation pattern in response to NH_4^+ resupply as shown in Fig. 3, i.e. up-regulation at 2 h followed by down-regulation, was essentially the same in all N treatments, including N-sufficient plants. Evidently, N sink strength exerted by the shoot increases, although transiently, after N is resupplied. Given that NH_4^+ per se is not translocated to the shoot in rice, long-distance N translocation can be expected to be influenced by the availability of carbon skeletons (in particular α-ketoglutarate; see Kronzucker et al. 1995e) and thus depend upon photosynthetic activity. As this could potentially modify the allocation pattern of N to root and shoot, it is important to point out that the rice seedlings in our study were cultivated in controlled-environment rooms which allowed a photon flux of ~500 μmol $m^{-2} s^{-1}$ to be provided continuously for 12-h periods in each light/dark cycle; thus, carbon limitation was unlikely a factor in the experiments presented here.

While N translocation to the shoot has not been previously examined alongside root NH_4^+ influx, general increases in NH_4^+ uptake capacity in response to N deprivation are well documented (see Introduction), and have been observed even in cases where growth rate was already measurably restricted due to N starvation (Lee and Rudge 1986, Morgan and Jackson 1988a). For rice, Wang et al. (1993b) have presented kinetic data for NH_4^+ influx under three different conditions of N supply. In that study, V_{max} was found to increase from 3.4±2 μmol $g^{-1} h^{-1}$ in plants previously grown in 1 mM $[NH_4^+]_o$ to 8.2±7 μmol $g^{-1} h^{-1}$ for 100 μM-grown plants and to 12.8±0.2 μmol $g^{-1} h^{-1}$ for 2 μM-grown plants. Our observed flux values are similar in magnitude to these values. Also, in the same rice variety as was used in the present study, our group has previously recorded similar values for steady-state NH_4^+ influx using in-

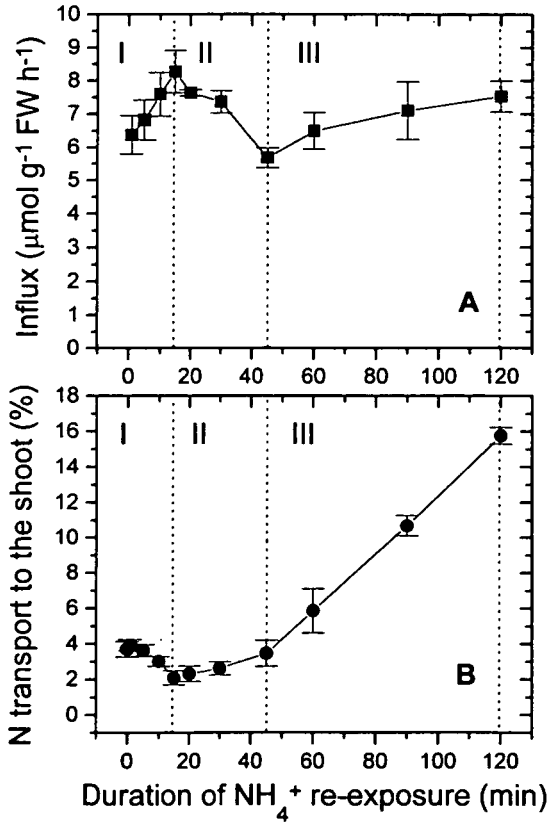

Fig. 4 NH_4^+ influx into roots (A) and N translocation to the shoot (B) in intact rice seedlings. Plants were deprived of N for 2 d and then re-exposed to 1 mM NH_4^+ for varying times up to 2 h. Fluxes were measured at 0.1 mM $[NH_4^+]_o$. Distinct phases of flux adaptation are indicated by Roman numerals. Data are means± SE ($n \geq 4$).

flux measurements as well as compartmental analysis (Kronzucker et al. 1998).

A detailed time-resolution of the response of N-deprived rice plants to NH_4^+ resupply revealed a complex pattern, with NH_4^+ influx and N translocation to the shoot displaying distinct phases of both up- and down-regulation. Fig. 3 and 4 show that the response to resupply in both root and shoot could be subdivided into four distinct phases. When 1 mM NH_4^+ was first resupplied to N-deprived plants after 1 to 3 d without N, root NH_4^+ influx increased by as much as 35% within the first 20 min of resupply (phase I). This was followed by a decline of influx to values slightly below the initial influx within the first hour (phase II). Shoot translocation of N during this time, measured as % of influx translocated to the shoot, was a mirror image of the root response. A decline in NH_4^+ uptake following resupply, within a similar time frame as our phase II response, has been reported by Morgan and Jackson (1988a) and Jackson and Volk (1992) for maize, oat and wheat. In those studies, the decline was clearly correlated with an increasing tissue NH_4^+ concentration as well as an increase in net NH_4^+ efflux from the cytoplasm. In agreement with the above authors, we believe that NH_4^+ may act as a positive feedback agent, initially stimulating its own transport (phase I). When a certain intracellular threshold of accumulation is achieved, negative feedback ensues, as evidenced by a decline in influx and an accelerated NH_4^+ efflux (phase II). Wang et al. (1993a) have previously shown that cytoplasmic levels of NH_4^+ vary from less than 4 mM in N-insufficient rice seedlings to almost 40 mM in seedlings grown at 1 mM $[NH_4^+]_o$, while efflux increases from 11 to 29% of incoming NH_4^+ over this same range of $[NH_4^+]_o$. Interestingly, in our study, root GS activity decreased below that of N-deprived plants during phase I and II (Fig. 5). We attribute this temporary decline in GS to end-product inhibition of GS (see Garcia Fernández et al. 1995) by rapidly accumulating glutamine; we have previously demonstrated such a rise in root glutamine levels in rice within the time frame in question (Glass et al. 1997), which is confirmed by studies on other organisms (Amâncio and Santos 1992, Lee and Lewis 1994, see also Kronzucker et al. 1996). Ultimately, over several hours of NH_4^+ resupply, this decline in GS activity was offset by an apparent inductive effect on GS (Fig. 5), presumably involving transcriptional events (see also below).

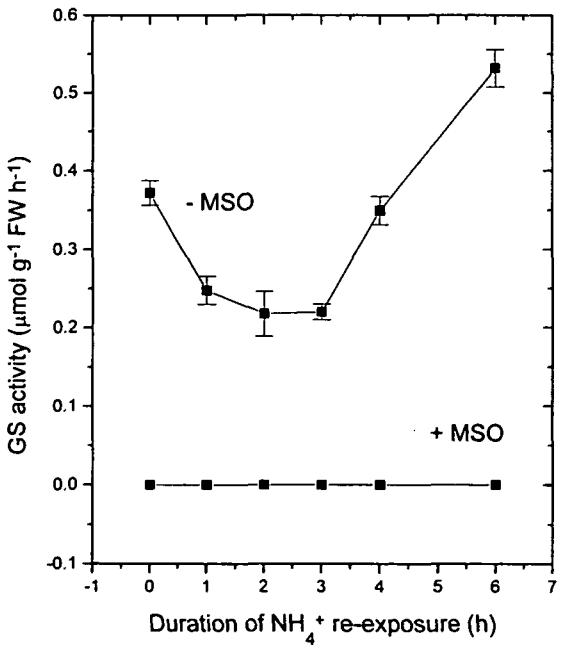

Fig. 5 In-vitro glutamine synthetase (GS) activities in roots of rice seedlings. Plants were deprived of N for 2 d and then re-exposed to 1 mM NH_4^+ for varying times up to 6 h prior to enzyme assay. Methionine sulphoximine (MSO) pretreatment was applied for 6 h at 1 mM. Data are means ± SE ($n \geq 4$).

A second rise in NH_4^+ influx, resulting in a second influx peak (phase III), was observed after approximately 2 h of re-exposure (Fig. 1, 3). The increase was 30 to 40% with respect to initial fluxes in 2-d and 3-d starved plants (see Fig. 3 for 2-d starved seedlings) and coincided with an increase of shoot translocation (both in absolute terms and percentage). Thus, root and shoot responses appear to be synchronized in this phase. The same root-localized accumulation of glutamine which led to an initial decline in GS activity may be necessary for the induction of the N translocation system. There have been several reports of transient up-regulation of NH_4^+ uptake in the first hours following NH_4^+ resupply in N-deprived cereals (Goyal and Huffaker 1986, Morgan and Jackson 1988a, Mäck and Tischner 1994). In analogy to the well-documented induction of NO_3^- transport by NO_3^- (see Kronzucker et al. 1995b, for refs.), an induction of NH_4^+ influx by NH_4^+ has been suggested for this phase (Goyal and Huffaker 1986, Mäck and Tischner 1994). We, however, disagree with the notion of inducibility of NH_4^+ transport, for several reasons. Although the up-regulatory response at 2 h in our study was also observed when plants were grown on 0.1 mM NO_3^- for 7 d prior to exposure to 1 mM NH_4^+ (data not shown), which may suggest an NH_4^+-specific response, the transient increase in NH_4^+ influx was very small in magnitude (~30% of initial influx prior to N resupply). By contrast, NO_3^- influx, for which NO_3^--specific induction has been clearly demonstrated (MacKown and McClure 1988, Tischner et al. 1993), can be enhanced up to 30-fold as NO_3^- is resupplied to NO_3^--deprived plants (Siddiqi et al. 1989). In a separate study, we found an approximately 10-fold induction of NO_3^- influx in rice upon provision of NO_3^- (Kronzucker et al. unpublished results). To our knowledge, the largest reported up-regulation of NH_4^+ uptake, based upon NH_4^+ depletion experiments, was less than 3.5-fold (Goyal and Huffaker 1986), interpreted by the authors as induction of transport. However, as $[NH_4^+]_o$

was allowed to deplete to undetectable values over the same time period, the latter results are complicated by a concomitant up-regulatory adaptation to N-deprived conditions (see earlier). The (smaller) peak values recorded by Jackson and Volk (1992) and Mäck and Tischner (1994) are based on a similar depletion approach and are likewise affected by the problem of flux adaptation during measurement, although to a lesser extent, as solutions were replaced periodically (Jackson and Volk 1992). In general, it is difficult to rationalize an inductive, i.e. signalling, role for NH_4^+ with respect to its own transport, as tissue NH_4^+ levels, unlike those of NO_3^- (cf. Kronzucker et al. 1995b), will always be appreciably above zero due to amino acid metabolism and protein breakdown, even if NH_4^+ is withdrawn entirely from the external solution (cf. Wang et al. 1993a) or if plants are grown on NO_3^-. In addition, distinct constitutive and inducible high-affinity transport systems for NO_3^- have been demonstrated kinetically (see Kronzucker et al. 1995b, d), whereas no convincing evidence exists to suggest the presence of corresponding high-affinity NH_4^+ transport systems. We furthermore question the adaptive significance, and hence the evolution, of an induction process whose end result is a lowered rather than an increased rate of uptake; steady-state NH_4^+ influx after several days of NH_4^+ resupply was lower than initial influx in N-starved plants (see below), notwithstanding the transient increase. By contrast, in the case of NO_3^- induction, steady-state NO_3^- influx is substantially larger than constitutive influx in NO_3^--deprived plants.

With continued NH_4^+ exposure in our study, influxes progressively declined in all treatments (phase IV) until, by 6 h of re-exposure, values were either close to (0-d and 3-d starved plants) or slightly below the initial influx values (2-d starved plants; ~70% of control; data not shown). All plants reached steady-state (repressed) fluxes below initial values by 10 h of re-exposure (see Fig. 3). This repression of NH_4^+ influx in N-supplied versus N-deprived plants is well established and has also been observed in other cereal species within 5 to 10 h of NH_4^+ resupply (Goyal and Huffaker 1986, Morgan and Jackson 1988b, Jackson and Volk 1992, Mäck and Tischner 1994). Most authors have attributed the NH_4^+ influx repression at the root plasma membrane to negative feedback by amino acids, especially glutamine, as well as by NH_4^+ itself; both N agents may act at either the protein and/or transcriptional levels (Kronzucker et al. 1996, Glass et al. 1997). The exact coordination of pre- and post-translational feedback remains to be resolved.

Conclusions

(1) The present results demonstrate the dynamic nature of the interplay between root and shoot N demands and the apparent priority of root demand when the exogenous NH_4^+ supply is discontinued.

(2) Root NH_4^+ influx exhibited four distinct phases of alternating positive and negative feedback. While only brief and marginal enhancement was detected in root uptake, N translocation to the shoot was increased substantially following re-exposure to NH_4^+ after a period of N deprivation. Virtually identical root and shoot responses were observed in plants pretreated with NO_3^-.

(3) A transient increase in root NH_4^+ influx and N translocation to the shoot occurred simultaneously at 2 h following re-exposure to NH_4^+. Even though this increase in NH_4^+ influx was also seen in plants previously grown on NO_3^-, existing evidence does not support the notion of inducibility of NH_4^+ transport by NH_4^+ as suggested by others. Rather, our data suggest that the transient increase in root NH_4^+ influx represents a "synchronization" response of root uptake with shoot N translocation as the latter is up-regulated following NH_4^+ resupply.

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