Plant Physiol. (1998) 116: 581–587

Effects of Hypoxia on $^{13}NH_4^+$ Fluxes in Rice Roots[1]

Kinetics and Compartmental Analysis

Herbert J. Kronzucker, Guy J.D. Kirk, M. Yaeesh Siddiqi, and Anthony D.M. Glass*

International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines (H.J.K., G.J.D.K.); and Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4 (H.J.K., M.Y.S., A.D.M.G.)

Techniques of compartmental (efflux) and kinetic influx analyses with the radiotracer $^{13}NH_4^+$ were used to examine the adaptation to hypoxia (15, 35, and 50% O_2 saturation) of root N uptake and metabolism in 3-week-old hydroponically grown rice (*Oryza sativa* L., cv IR72) seedlings. A time-dependence study of NH_4^+ influx into rice roots after onset of hypoxia (15% O_2) revealed an initial increase in the first 1 to 2.5 h after treatment imposition, followed by a decline to less than 50% of influx in control plants by 4 d. Efflux analyses conducted 0, 1, 3, and 5 d after the treatment confirmed this adaptation pattern of NH_4^+ uptake. Half-lives for NH_4^+ exchange with subcellular compartments, cytoplasmic NH_4^+ concentrations, and efflux (as percentage of influx) were unaffected by hypoxia. However, significant differences were observed in the relative amounts of N allocated to NH_4^+ assimilation and the vacuole versus translocation to the shoot. Kinetic experiments conducted at 100, 50, 35, and 15% O_2 saturation showed no significant change in the K_m value for NH_4^+ uptake with varying O_2 supply. However, V_{max} was 42% higher than controls at 50% O_2 saturation, unchanged at 35%, and 10% lower than controls at 15% O_2. The significance of these flux adaptations is discussed.

More than 70% of the world's rice (*Oryza sativa* L.) is produced in intensively cultivated, irrigated lowland systems in Asia (International Rice Research Institute, 1997). In these systems N is generally the main factor limiting the realization of yield potentials (Kropf et al., 1993; Cassman et al., 1997). As a consequence, large amounts of mineral N fertilizers are used. According to one estimate, 7×10^6 metric tons of N is applied each year to the 74×10^6 ha of irrigated rice in Asia (Cassman and Pingali, 1995). However, unless the application of N fertilizer is timed precisely to match plant demand (Cassman et al., 1998), less than 50% of fertilizer N is usually recovered by the crop, because of high rates of loss through ammonia volatilization and denitrification (Craswell and Vlek, 1979; Vlek and Byrnes, 1986; Cassman et al., 1993). Clearly, the capacity of the root system to capture N in competition with these processes is critical. Mathematical modeling of the uptake process (Kirk and Solivas, 1997) shows that, under typical field conditions and following the initial flush of available N after fertilization, N absorption from the soil is rate limiting.

In flooded lowland rice soils, where the bulk of the soil is hypoxic to anaerobic, the main form of plant-available N is NH_4^+ (Sasakawa and Yamamoto, 1978; Yu, 1985). This is in marked contrast to most (aerobic) agricultural soils, where NO_3^- is the predominant inorganic N species (Kronzucker et al., 1995b). There have been reports that NH_4^+ is the preferred N species taken up by rice (Bonner, 1946; Fried et al., 1965; Shen, 1969; Dijkshoorn and Ismunadji, 1972a, 1972b; Yoneyama and Kumazawa, 1974, 1975; Sasakawa and Yamamoto, 1978; Ancheng et al., 1993; Wang et al., 1993a, 1993b) and that NH_4^+ is superior to NO_3^- in terms of fertilizer efficiency (Craswell and Vlek, 1979). Information regarding NH_4^+ uptake capacity and affinity under hypoxic conditions is scarce, however (Sasakawa and Yamamoto, 1978; Youngdahl et al., 1982; Wang et al., 1993b). It is not known how intracellular compartmentation and metabolic processing of NH_4^+ are affected by lowered O_2 tensions (Wang et al., 1993a). With the goal of developing new rice varieties, which might be more efficient in N extraction from paddy soils (Kirk and Kronzucker, 1998), information concerning N uptake and metabolism under more realistic conditions is needed. In this paper we report a study of the adaptation of flux parameters for NH_4^+ to hypoxic growth conditions in roots of rice, using $^{13}NH_4^+$ as a tracer and combining techniques of kinetic flux and compartmental analyses.

MATERIALS AND METHODS

Plant Growth Conditions

Rice (*Oryza sativa* L., cv IR72) seeds were surface sterilized in 5% NaOCl for 10 min, then rinsed several times with deionized water, and left to soak in aerated, deionized water at 30°C in a water bath for 48 h. The partially

[1] This work was supported by funds from the New Frontier project grant to the International Rice Research Institute and by a National Sciences and Engineering Research Council of Canada grant to A.D.M.G.

* Corresponding author; e-mail aglass@unixg.ubc.ca; fax 1–604–822–6089.

Abbreviations: $[NH_4^+]_{cyt}$, cytoplasmic NH_4^+ concentration; $[NH_4^+]_o$, NH_4^+ concentration in the external solution; ϕ, symbol for NH_4^+ flux (see "Materials and Methods" for subscripts denoting component fluxes); $t_{1/2}$, half-life of exchange.

germinated seeds were then placed onto plastic mesh mounted on Plexiglas discs (Rhom Co., Ltd., New York). The discs were transferred to 40-L hydroponic Plexiglas tanks (see below) located in walk-in controlled-environment growth chambers. The growth chambers were maintained at 30 ± 2°C and 70% RH and set to a 12-h day/12-h night photoperiod. A photon flux of approximately 500 μmol m^{-2} s^{-1} measured at plant level (with an LI-189 light meter and an LI-190SA quantum sensor, Li-Cor, Lincoln, NE) was provided by fluorescent lamps (215 W, 1500, F96T12/CW/VHO, Philips, Mahwah, NJ).

Nutrient Solutions

Rice seedlings were cultivated in hydroponic medium contained in 40-L Plexiglas tanks. Deionized, distilled water and reagent-grade chemicals were used in the preparation of all nutrient solutions. NH_4^+ was provided as the only source of N in the form of $(NH_4)_2SO_4$. Other 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), and $CuSO_4$ (1.5 μM). The complete solution was maintained from germination onward.

Nutrient solutions in tanks were continuously mixed via electric circulating pumps (circulator model IC-2, Brinkmann). Continuous infusion of the concentrated nutrient stock solution via peristaltic pumps (Technicon Proportioning Pump II, Technicon Instrument, Tarrytown, NY) allowed steady-state control of nutrient concentrations in the tanks. Solutions were checked daily for $[NH_4^+]$, measured using a Philips PU 8820 UV/VIS spectrophotometer, according to the method of Solorzano (1969); $[K^+]$, measured flame-photometrically (using an Instrumentation Laboratory Photometer, model 443, Lexington, MA); pH, measured with a microprocessor-based pocket-sized pH meter (pH Testr2 model 59000–20; Cole Parmer, Chicago, IL) and maintained at 6.5 ± 0.3 by addition of powdered $CaCO_3$; and $[O_2]$, measured using a biological O_2 monitor (YSI model 53, Yellow Springs Instruments, Yellow Springs, OH) equipped with an O_2 electrode (YSI 5331 Oxygen Probe, Yellow Springs Instruments). Nutrient solutions were degassed prior to filling of the tanks. O_2 concentrations of 7.5, 3.75, 2.6, and 1.1 μg mL^{-1} were maintained by infusion of N_2 gas (Praxair, Mississauga, Ontario, Canada) via aquarium stones placed at various solution depths (5, 10, and 15 cm; tanks were covered and the overall solution depth was 17 cm). The minimum O_2 concentration attainable with this method was 1.1. μg mL^{-1} (15% of saturation).

Measurement of Fluxes

The radiotracer ^{13}N (half-life = 9.96 min) was produced by the 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., 1995b). The irradiated solutions (approximately 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, 1995b, 1995c). A volume of 20 to 100 mL of $^{13}NH_4^+$-containing stock solution was prepared in a fumehood and was transferred to the controlled-environment chambers where experiments were carried out. All uptake solutions were premixed, and, in influx experiments, these 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) and contained NH_4^+ at the desired concentrations (Figs. 2 and 3). 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-L vessels for 5 min prior to immersion of the intact seedling roots in the labeled 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 of isotope loading, roots were dipped into nonlabeled solutions for 5 s to minimize carry-over of label by the root surface to the desorption solution. Roots were then desorbed in unlabeled solution, which was otherwise chemically identical, for 3 min to desorb $^{13}NH_4^+$ contained in the Donnan free space. The duration of these steps was based on the $t_{1/2}$ of NH_4^+ for the root surface, the Donnan free space, and the cytoplasm, as determined by efflux analysis (see below; Kronzucker et al., 1995c, 1995e). An exposure time of 10 min to $^{13}NH_4^+$ was chosen, since the contribution of tracer efflux from the cytoplasm can be expected to be negligible during this time (Kronzucker et al., 1995d; A.D.M. Glass, H.J. Kronzucker, and M.Y. Siddiqi, unpublished results).

Following desorption, seedling roots were excised from the shoots, the roots were spun in a low-speed centrifuge for 30 s to remove surface liquid, and the fresh weights of roots and shoots were determined. The plant organs were then introduced into 20-mL scintillation vials, and the radioactivities of roots and shoots were determined in a γ-counter (Minaxi δ, series Auto-γ 5000, Packard, Meriden, CT), measuring the 511-kV positron-electron annihilation radiation generated by recombination of ambient electrons and β^+ particles emitted from ^{13}N. Using the specific activity ($^{13}N/[^{13}N + {}^{14}N]$ [disintegrations per micromole]) of the loading solution and the total fresh root weight of each seedling, we calculated NH_4^+ fluxes and expressed the results in micromoles per gram fresh weight per hour.

Efflux experiments were performed essentially as described elsewhere (Kronzucker et al., 1995b, 1995d, 1995e) under the same conditions as the influx experiments. Roots of intact rice seedlings were immersed for 45 to 60 min in 120-mL darkened plastic beakers containing the $^{13}NH_4^+$-labeled solution. Steady-state conditions, with respect to all nutrients as well as O_2 tensions, were maintained throughout growth, loading, and elution. A 60-min loading period was chosen on the basis of the $t_{1/2}$ for the cytoplasmic phase being approximately 14 min (see "Results and Dis-

cussion"). Therefore, 60 min of exposure to tracer ensured that the specific activity of the cytoplasm was approximately 95% of that in the loading solution (Kronzucker et al., 1995e). Following loading with $^{13}NH_4^+$, seedlings were transferred to "efflux funnels" (Wang et al., 1993a), and the roots were eluted with 20-mL aliquots of nonradioactive solution after varying intervals. These intervals ranged from 5 s to 2 min, over an experimental duration of 22 min. Eluates from a total of 25 intervals were collected separately, and the radioactivities of 20-mL samples from each eluate were determined (using a Minaxi δ counter, series Auto-γ 5000). After the final elution, roots and shoots were excised, introduced into scintillation vials and also counted for γ activity.

Data Analysis

All experiments were replicated three to five times. Each experimental treatment consisted of four replicates for influx experiments and two replicates for efflux experiments. Data from several experiments were pooled ($n \geq 6$ in efflux experiments; $n \geq 12$ in influx experiments) for calculations of means and SEs. These values were used for plotting time-dependence curves and uptake isotherms, as well as for calculating V_{max} and K_m values. The least-squares method by Cornish-Bowden and Wharton, based on the Michaelis-Menten equation, was used to obtain V_{max} and K_m estimates for the saturable high-affinity transport system isotherms (Kronzucker et al., 1995d, 1996). The calculation of $t_{1/2}$, fluxes and pool sizes in efflux analyses were as described in detail elsewhere (Kronzucker et al., 1995a, 1995b, 1995c, 1995e). All fluxes are expressed in micromoles of NH_4^+ per gram root fresh weight per hour.

Symbols used for fluxes are as follows: ϕ_{co}, efflux from the cytoplasm, obtained from the rate of ^{13}N release from the cytoplasm at time 0 divided by the specific activity of the loading solution; ϕ_{net}, net flux, obtained directly from the accumulation of ^{13}N in the plants at the end of the loading period; ϕ_{oc}, unidirectional influx, calculated from $\phi_{net} + \phi_{co}$; ϕ_{xylem}, flux of ^{13}N to the shoot, obtained directly from count accumulation in the shoot at the end of the elution period; and $\phi_{vac./ass.}$, combined fluxes to ammonium assimilation and to the vacuole, resulting from $\phi_{net} - \phi_{xylem}$.

RESULTS AND DISCUSSION

We found that NH_4^+ influx across the root plasmalemma responded within hours to the imposition of hypoxia (Fig. 1). Whereas in fully oxygenated plants NH_4^+ influx, measured at 100 μM $[NH_4^+]_o$, was 4.31 ($\pm$ 0.39) μmol g^{-1} h^{-1}, an increase in influx of approximately 35% was apparent after 1 to 2.5 h of hypoxia (15% O_2 saturation, i.e. approximately 1.1 μg mL^{-1}). Even though evolutionary adaptation strategies to deal with restricted O_2 supply in the rooting zone differ markedly between species and are poorly understood (Drew, 1990; Crawford, 1992), some rapid cellular responses appear to be universal. In particular, cytosolic acidification at the onset of hypoxia has been documented in several species, including rice (Roberts et al., 1985; Hoffman et al., 1986). This is believed to be due to lactic acid production preceding a switch to fermentative metabolism, as well as, in some cases, to proton leakage from the vacuole (Menegus et al., 1989, 1991). In species susceptible to damage from O_2 deprivation, this cytoplasmic acidosis is pronounced (as much as 0.8 pH unit) and not fully reversible. By contrast, in hypoxia-tolerant plants, it is of a relatively lesser magnitude ($\leq$ 0.4 pH unit in rice) and is followed by alkalinization of both the cytoplasm and the vacuole (Menegus et al., 1991). In fact, in rice cytosolic acidosis is complete after as little as 10 min of O_2 withdrawal and is sustained for no more than 4 h (Menegus et al., 1991), i.e. within an interval of time corresponding to our observed up-regulation of NH_4^+ influx into rice roots.

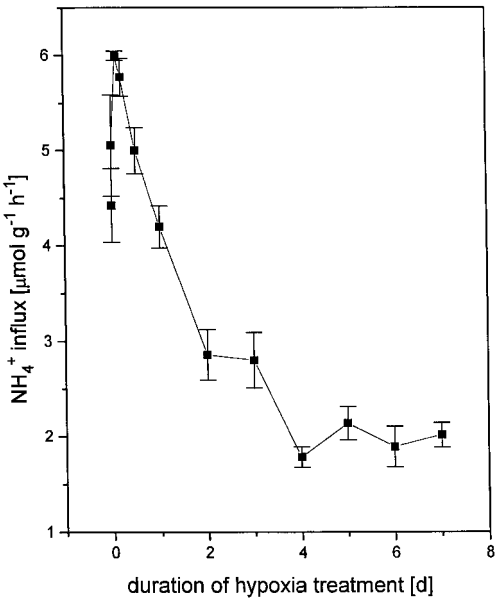

Figure 1. NH_4^+ influx into roots of intact cv IR-72 rice seedlings as a function of increasing time of O_2 deprivation. Seedlings were cultivated hydroponically at 100 μM $[NH_4^+]_o$ for 3 weeks. Plants were exposed for varying periods to 15% O_2 (growth and control at 100%). Data are means $\pm$ SE ($n \geq 12$).

Several reports suggest that in hypoxia-tolerant plants, cytosolic alkalinization following the initial acidosis appears to involve metabolic H^+ consumption through glutamate and Arg decarboxylation, leading to the formation of either γ-aminobutyric acid or polyamines such as putrescine, respectively (Reggiani et al., 1989, 1990, 1993; Reggiani, 1994; Aurisano et al., 1995). Polyamines in turn have been shown to stimulate plasmalemma H^+-ATPase activity (Reggiani et al., 1992). Thus, the observed higher N acquisition rates may be consistent with the N requirements associated with pH regulation during the first hours under conditions of O_2 restriction. Also, higher NH_4^+ influx might meet the needs of an apparently generally increased N metabolism under O_2-restriction conditions (Reggiani et al., 1988, 1989). Increased N acquisition might be operating in parallel to the documented N remobilization by degradation of storage proteins (Reggiani et al., 1988). Ultimately, i.e. under prolonged O_2 stress, such up-

regulation responses of N uptake must be compromised by restrictions in ATP supply (Reggiani et al., 1985).

In our study, following the initial increase, NH_4^+ influx declined to an apparent steady-state value of approximately 2 μmol g^{-1} h^{-1} by 4 d. To ensure steady-state conditions in subsequent kinetic experiments, 7 d of hypoxia pretreatment was therefore used prior to labeling with ^{13}N.

From the kinetics of NH_4^+ influx at different O_2 tensions (Figs. 2 and 3), it is evident that rice can maintain substantial influx of N even at 15% O_2. V_{max} for fully oxygenated plants was 5.22 μmol g^{-1} h^{-1} ($\pm$0.48), with a K_m of 31.78 μM ($\pm$11.8). K_m values did not change significantly with varying O_2 supply; V_{max} was unchanged at 35%, approximately 10% lower at 15% and 42% higher at 50% saturation. The increase of NH_4^+ influx observed at 50% O_2 is interesting and appears to be another manifestation of increased N demand under O_2 stress, realized in up-regulated N uptake. Apparently, below 35% O_2 rice roots are no longer able to up-regulate NH_4^+ influx. However, N acquisition rates remain considerable. The maintenance of appreciable N uptake rates in deoxygenated hydroponic systems has been reported previously for Japonica rice (Sasakawa and Yamamoto, 1978).

Compartmental analysis (efflux analysis) was used in the present study to examine in greater detail the adaptation of component fluxes and subcellular compartmentation of NH_4^+ to hypoxia over a period of 5 d. Experiments were conducted at 100 μM $[NH_4^+]_o$ and with hypoxia treatment

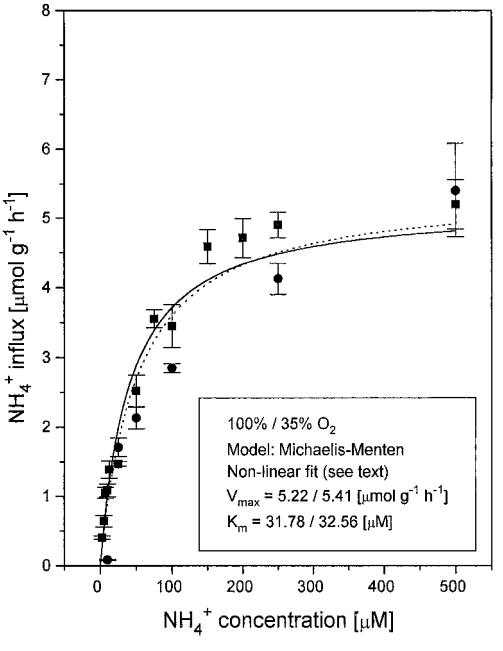

Figure 2. Steady-state NH_4^+ influx into roots of intact cv IR-72 rice seedlings as a function of $[NH_4^+]_o$ in the high-affinity transport range (2.5–500 μM) at 100 and 35% O_2. ■ (solid line for isotherm fit), Control plants at 100% O_2 provision; and ● (dotted line for isotherm fit), plants exposed to 35% O_2 for 7 d prior to the influx determinations. Kinetic analysis according to Cornish-Bowden and Wharton (see text) was used for the derivation of V_{max} and K_m values. Data are means $\pm$ SE ($n \geq 12$).

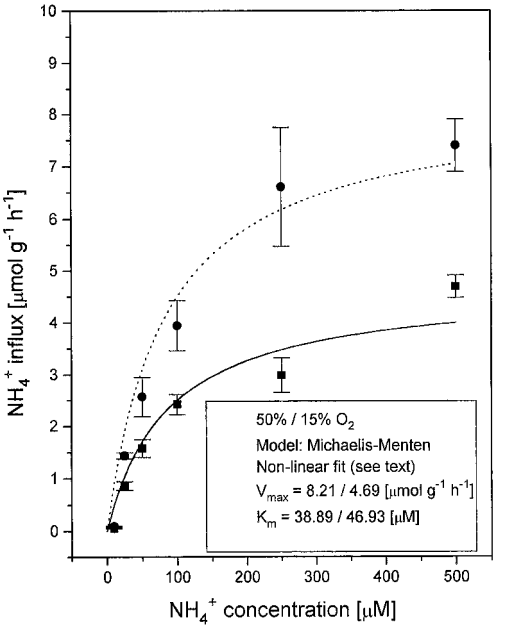

Figure 3. Steady-state NH_4^+ influx into roots of intact cv IR-72 rice seedlings as a function of $[NH_4^+]_o$ in the high-affinity transport range (2.5–500 μM) at 50 and 15% O_2. ● (dotted line for isotherm fit), Plants exposed to 50% O_2; and ■ (solid line for isotherm fit), plants exposed to 15% O_2. See Figure 2.

at 15% O_2 imposed for 0, 1, 3, and 5 d. Efflux analysis requires that plants be at a quasi-steady state with respect to ambient conditions and that the plant's physiological status should not change during the experimental probing. Thus, we could not examine the effects of very short times of exposure to lowered O_2 tensions via efflux analysis (Kronzucker et al., 1995a). Semilogarithmic plots of the rate of $^{13}NH_4^+$ release from cv IR-72 roots versus time of elution showed three distinct phases of ^{13}N efflux (Wang et al., 1993a; Kronzucker et al., 1995c, 1995e). All three efflux phases could be described adequately by first-order kinetics ($r^2 \geq 0.95$). Eluates representative of each efflux phase were passed through cation-exchange resins (analytical grade AG 50 W-X 8 cation-exchange resin, 200–400 mesh, Na^+ form, Bio-Rad; Kronzucker et al., 1995c), and it was confirmed that $\geq$99.2% of the ^{13}N was positively charged. Since the concentration of positively charged amino acids in 3-week-old rice roots is typically less than 5% of total amino acid concentrations (Yoneyama and Kumazawa, 1974; Wang et al., 1993a) and the metabolic pool of assimilation products can be expected to be labeled more slowly than the cytoplasmic NH_4^+ pool (Macklon et al., 1990), the contribution of the N species other than $^{13}NH_4^+$ to the pool of effluxing ^{13}N was considered negligible.

Based on previous $^{13}NH_4^+$-efflux studies in which extensive compartment identification tests were carried out (Kronzucker et al., 1995e), the three phases of NH_4^+ exchange in the present study could be interpreted as a surface film of NH_4^+ adhering to the roots (including the water free space), the Donnan free space, and the cytoplasm. This is in keeping with previously published tentative compartment assignments in similar ^{13}N studies

(Kronzucker et al., 1995e, and refs. therein). For the three compartments in the present study $t_{1/2}$ values were approximately 2 to 3 s, 30 s, and 14 min, respectively. These estimates are very close to those reported by Wang et al. (1993a) for rice, except for $t_{1/2}$ of the cytoplasmic phase, which was significantly longer in our study (14 as opposed to 7 min). However, 10 to 14 min for NH_4^+ was also found in other species, such as spruce (Kronzucker et al., 1995c, 1995e), other tree species, Arabidopsis, and barley (*Hordeum vulgare* L.) (A.D.M. Glass, H.J. Kronzucker, X.-J. Min, and M.Y. Siddiqi, unpublished results).

Under all conditions efflux analysis revealed high cytoplasmic NH_4^+ concentrations, in the range from 15 to 20 mM (at 100 μM $[NH_4^+]_o$). This has also been reported by Wang et al. (1993a) for rice and is similar to results obtained in spruce, which is known to be better adapted to NH_4^+ uptake than NO_3^- (Kronzucker et al., 1997). The high cytoplasmic NH_4^+ levels raise interesting questions with respect to NH_4^+ toxicity (Givan, 1979). It has long been assumed that high intracellular NH_4^+ concentrations are incompatible with physiological functioning for various reasons (Magalhaes and Fernandes, 1995), especially in species such as barley, wheat, pea, or tomato, which show pronounced symptoms of NH_4^+ toxicity when grown on NH_4^+ as the sole N source (Kronzucker et al., 1995c; Magalhaes and Fernandes, 1995; Bligny et al., 1997). It is unclear why NH_4^+ is not toxic in rice and spruce. There are also implications pertaining to some traditional assumptions regarding substrate limitation for enzymic NH_4^+-processing reactions alternative to Gln synthetase, in particular glutamate dehydrogenase and Asn synthase (Cedar and Schwartz, 1969a, 1969b; Oaks and Ross, 1984). Our results suggest that genetic engineering, through overexpression of genes that code for such enzymes in rice, might well be a useful technological approach to increasing N-utilization capacity by enhancing metabolic processing of the freely available NH_4^+.

Efflux analysis essentially confirmed results from our time-dependence influx study (Table I). Estimates from compartmental analysis for unidirectional influx were 3.97 μmol g^{-1} h^{-1} for fully oxygenated controls, 4.02 after 1 d of hypoxia, 2.78 after 3 d, and 2.12 after 5 d. Efflux, as a percentage of influx, was approximately 15% to 25% and as such was not significantly different between treatments. Significant differences were seen, however, in the amounts of N allocated to NH_4^+ assimilation and the vacuole ($\phi_{vac./ass.}$) and to the shoot (ϕ_{xylem}). $\phi_{vac./ass.}$ was 52% of incoming N in controls and approximately 45, 34.5, and 62% after 1, 3, and 5 d, respectively, whereas ϕ_{xylem} was approximately 25, 31, 50, and 22% of incoming N, respectively. These observed shifts in the allocation pattern of N may reflect a redirection of N metabolism during adaptation to hypoxia. Significant changes in amino acid profiles in rice under hypoxic/anaerobic conditions have been documented by Reggiani et al. ([1988]; also see above). These workers also found a substantial accumulation of polyamines and speculated that these compounds play a critical role in triggering shoot elongation beyond the flooded zone (Reggiani et al., 1989). The changes we observed in ^{13}N transfer between the root and shoot might indicate the transfer of such N compounds. The exact role of these N shifts in the context of adaptation to hypoxia is unknown.

CONCLUSIONS

(a) The capacity for NH_4^+ acquisition in rice seedlings in the vegetative stage remains high, even at very low O_2 concentrations (approximately 1 μg mL^{-1}). Both up- and down-regulation of NH_4^+ influx were observed as rice seedlings adapted to hypoxic conditions. These involve only changes in V_{max} for NH_4^+ influx, whereas uptake affinity for NH_4^+ (i.e. K_m) is unchanged. (b) An up-regulatory response in NH_4^+ uptake in the initial phases (first few hours) of hypoxia appears to occur in response to cytoplasmic acidosis in rice. It is speculated that additional N is supplied through plasma membrane influx to satisfy the requirements for pH restoration, as related to the production of N compounds, such as polyamines or γ-aminobutyric acid. (c) Reproducible changes in N allocation between different compartments inside root cells and the shoot occur in response to hypoxia. (d) $[NH_4^+]_{cyt}$ under hypoxic as well as fully aerated conditions are high and at a given external concentration appear to be maintained within a defined range (15–20 mM at 0.1 mM $[NH_4^+]_o$). At the cellular level such a high $[NH_4^+]_{cyt}$ illustrates the unique ability of rice plants to tolerate NH_4^+ as the sole N source, and they point to the possibility of engineering transgenic rice plants with higher N-utilization capacity by overexpressing genes coding for NH_4^+-assimilation enzymes, such as Asn synthetase or glutamate dehydrogenase. This is presently being pursued at the International Rice Research Institute.

Table I. *Component fluxes of NH_4^+ as estimated from compartmental analysis (for derivation of flux parameters and symbols, see text)*

Rice seedlings were grown under steady-state nutritional conditions for 3 weeks prior to conducting efflux experiments. For each flux component, the respective percentage of influx is indicated in parentheses. Data are means ± SE ($n \geq 6$).

Pretreatment O_2	NH_4^+ Fluxes				
	ϕ_{oc}	ϕ_{co}	ϕ_{net}	$\phi_{vac./ass.}$	ϕ_{xylem}
	μmol g^{-1} h^{-1}				
100% (control)	3.97 ± 0.42	0.95 ± 0.17 (23)	3.02 ± 0.36 (77)	2.04 ± 0.13 (52)	0.97 ± 0.07 (25)
15% (1 d)	4.02 ± 0.37	0.96 ± 0.11 (24)	3.06 ± 0.33 (76)	1.81 ± 0.04 (45)	1.25 ± 0.17 (31)
15% (3 d)	2.78 ± 0.41	0.43 ± 0.02 (16)	2.35 ± 0.38 (84)	0.96 ± 0.09 (34)	1.39 ± 0.08 (50)
15% (5 d)	2.12 ± 0.19	0.35 ± 0.02 (17)	1.77 ± 0.14 (83)	1.31 ± 0.07 (62)	0.46 ± 0.11 (21)

ACKNOWLEDGMENTS

For provision of ^{13}N we wish to thank M. Adam, T. Hurtado, and T. Ruth at the particle acceleration facility Tri-university Meson Facility at the University of British Columbia. Our thanks also to D.T. Britto, Y. Erner, X.-J. Min, J.K. Schjoerring, and D. Zhuo for essential assistance with ^{13}N experiments.

Received September 9, 1997; accepted November 4, 1997.
Copyright Clearance Center: 0032–0889/98/116/0581/07.

LITERATURE CITED

Ancheng L, Jianming X, Xiaoe Y (1993) Effect of nitrogen (NH_4NO_3) supply on absorption of ammonium and nitrate by conventional and hybrid rice during reproductive growth. *In* NJ Barrow, ed, Plant Nutrition—From Genetic Engineering to Field Practice. Kluwer Academic Publishers, Wageningen, The Netherlands, pp 537–540

Aurisano N, Bertani A, Reggiani R (1995) Anaerobic accumulation of 4-aminobutyrate in rice seedlings; causes and significance. Phytochemistry **38:** 1147–1150

Bligny R, Gout E, Kaiser W, Heber U, Walker D, Douce R (1997) pH regulation in acid-stressed leaves of pea plants grown in the presence of nitrate or ammonium salts: studies involving ^{31}P-NMR spectroscopy and chlorophyll fluorescence. Biochim Biophys Acta **1320:** 142–152

Bonner J (1946) The role of organic matter, especially manure, in the nutrition of rice. Bot Gaz **107:** 267–278

Cassman KG, Kropff MJ, Gaunt J, Peng S (1993) Nitrogen use efficiency of rice reconsidered: what are the key constraints? Plant Soil **155/156:** 359–362

Cassman KG, Peng S, Olk DC, Ladha JK, Reichardt W, Dobermann A, Singh U (1998) Opportunities for increased nitrogen use efficiency from improved resource management in irrigated rice systems. Field Crops Res (in press)

Cassman KG, Puigali PL (1995) Intensification of irrigated rice systems: learning from the past to meet future challenges. Geo J **35:** 299–305

Cedar H, Schwartz JH (1969a) The asparagine synthetase of *Escherichia coli*. I. Biosynthetic role of the enzyme, purification, and characterization of the reaction products. J Biol Chem **244:** 4112–4121

Cedar H, Schwartz JH (1969b) The asparagine synthetase of *Escherichia coli*. II. Studies on mechanism. J Biol Chem **244:** 4122–4127

Craswell ET, Vlek PLG (1979) Fate of fertilizer nitrogen applied to wetland rice. *In* Nitrogen and Rice. International Rice Research Institute, Los Banos, Philippines, pp 174–192

Crawford RMM (1992) Oxygen availability as an ecological limit to plant distribution. Adv Ecol Res **23:** 93–185

Dijkshoorn W, Ismunadji M (1972a) Nitrogen nutrition of rice plants measured by growth and nutrient content in pot experiments. 2. Uptake of ammonium and nitrate from waterlogged soil. Neth J Agric Sci **20:** 44–57

Dijkshoorn W, Ismunadji M (1972b) Nitrogen nutrition of rice plants measured by growth and nutrient content in pot experiments. 3. Changes during growth. Neth J Agric Sci **20:** 133–144

Drew MC (1990) Sensing soil oxygen. Plant Cell Environ **13:** 681–693

Fried MF, Zsoldos F, Vose PB, Shatokhin IL (1965) Characterizing the NO_3^- and NH_4^+ uptake process of rice roots by use of ^{15}N-labelled NH_4NO_3. Physiol Plant **18:** 313–320

Givan CV (1979) Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry **18:** 375–382

Hoffman NE, Bent AF, Hanson AD (1986) Induction of lactate dehydrogenase isozymes by oxygen deficit in barley root tissue. Plant Physiol **82:** 658–663

International Rice Research Institute (1997) Rice Almanac, Ed 2. International Rice Research Institute, Manila, Philippines

Kirk GJD, Kronzucker HJ (1998) Roots and N acquisition. *In* TM Thiyagarajan, HFM ten Berge, eds, Rice—Nitrogen Relations. Kluwer Academic Publishers, Wageningen, The Netherlands (in press)

Kirk GJD, Solivas JL (1997) On the extent to which root properties and transport through the soil limit nitrogen uptake by lowland rice. Eur J Soil Sci (in press)

Kronzucker HJ, Glass ADM, Siddiqi MY (1995a) Nitrate induction in spruce: an approach using compartmental analysis. Planta **196:** 683–690

Kronzucker HJ, Siddiqi MY, Glass ADM (1995b) Compartmentation and flux characteristics of nitrate in spruce. Planta **196:** 674–682

Kronzucker HJ, Siddiqi MY, Glass ADM (1995c) Compartmentation and flux characteristics of ammonium in spruce. Planta **196:** 691–698

Kronzucker HJ, Siddiqi MY, Glass ADM (1995d) Kinetics of NO_3^- influx in spruce. Plant Physiol **109:** 319–326

Kronzucker HJ, Siddiqi MY, Glass ADM (1995e) Analysis of $^{13}NH_4^+$-efflux in spruce roots. A test case for compartment identification in efflux analysis. Plant Physiol **109:** 481–490

Kronzucker HJ, Siddiqi MY, Glass ADM (1996) Kinetics of NH_4^+ influx in spruce. Plant Physiol **110:** 773–779

Kronzucker HJ, Siddiqi MY, Glass ADM (1997) Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature **385:** 59–61

Kropf MJ, Cassman KG, Van Laar HH, Peng S (1993) Nitrogen and yield potential of irrigated rice. *In* NJ Barrow, ed, Plant Nutrition—From Genetic Engineering to Field Practice. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 533–536

Macklon AES, Ron MM, Sim A (1990) Cortical cell fluxes of ammonium and nitrate in excised root segments of *Allium cepa* L; studies using ^{15}N. J Exp Bot **41:** 359–370

Magalhaes JR, Fernandes MS (1995) Nitrate versus ammonium nutrition and plant growth. *In* HS Srivastava, RP Singh, eds, Nitrogen Nutrition in Higher Plants. Associated Publishers, New Delhi, India, pp 131–143

Menegus F, Cattaruzza L, Chersi A, Fronza G (1989) Differences in the anaerobic lactate-succinate production and in the changes of cell sap pH for plants with high and low resistance to anoxia. Plant Physiol **90:** 29–32

Menegus F, Cattaruzza L, Mattana M, Beffagna N, Ragg E (1991) Response to anoxia in rice and wheat seedlings. Plant Physiol **95:** 760–767

Oaks A, Ross DW (1984) Asparagine synthetase in *Zea mays*. Can J Bot **62:** 68–73

Reggiani R (1994) Purification and synthesis under anaerobic conditions of rice arginine decarboxylase. Plant Cell Physiol **35:** 1245–1249

Reggiani R, Aurisano N, Mattana M, Bertani A (1993) Effect of potassium ions on polyamine levels in wheat seedlings under anoxia. J Plant Physiol **142:** 94–98

Reggiani R, Brambilla I, Bertani A (1985) Effect of exogenous nitrate on anaerobic metabolism in excised rice roots. II. Fermentative activity and adenylic energy charge. J Exp Bot **36:** 1698–1704

Reggiani R, Cantu CA, Brambilla I, Bertani A (1988) Accumulation and interconversion of amino acids in rice roots under anoxia. Plant Cell Physiol **29:** 981–987

Reggiani R, Giussani P, Bertani A (1990) Relationship between the accumulation of putrescine and the tolerance to oxygen-deficient stress in Gramineae seedlings. Plant Cell Physiol **31:** 489–494

Reggiani R, Hochkoeppler A, Bertani A (1989) Polyamines and anaerobic elongation of rice coleoptile. Plant Cell Physiol **30:** 893–898

Reggiani R, Zaina S, Bertani A (1992) Plasmalemma ATPase in rice coleoptiles: stimulation by putrescine and polyamines. Phytochemistry **31:** 417–420

Roberts JKM, Andrade FH, Anderson IC (1985) Further evidence that cytoplasmic acidosis is a determinant of flooding intolerance in plants. Plant Physiol **77:** 492–494

Sasakawa H, Yamamoto Y (1978) Comparison of the uptake of nitrate and ammonium by rice seedlings. Influences of light, temperature, oxygen concentration, exogenous sucrose, and metabolic inhibitors. Plant Physiol **62:** 665–669

Shen TC (1969) Induction of nitrate reductase and the preferential assimilation of ammonium in germinating rice seedlings. Plant Physiol **44:** 1650–1655

Solorzano L (1969) Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol Oceanogr **14:** 799–801

Vlek PLG, Byrnes BH (1986) The efficacy and loss of fertilizer N in lowland rice. Fert Res **9:** 131–147

Wang MY, Siddiqi MY, Ruth TJ, Glass ADM (1993a) Ammonium uptake by rice roots. I. Fluxes and subcellular distribution of $^{13}NH_4^+$. Plant Physiol **103:** 1249–1258

Wang MY, Siddiqi MY, Ruth TJ, Glass ADM (1993b) Ammonium uptake by rice roots. II. Kinetics of $^{13}NH_4^+$ influx across the plasmalemma. Plant Physiol **103:** 1259–1267

Yoneyama T, Kumazawa K (1974) A kinetic study of the assimilation of ^{15}N-labeled ammonium in rice seedling roots. Plant Cell Physiol **15:** 655–661

Yoneyama T, Kumazawa K (1975) A kinetic study of the assimilation of ^{15}N-labeled nitrate in rice seedling roots. Plant Cell Physiol **16:** 21–26

Youngdahl LJ, Pacheco R, Street JJ, Vlek PLG (1982) The kinetics of ammonium and nitrate: uptake by young rice plants. Plant Soil **69:** 225–232

Yu T-R (1985) Soil and plants. *In* T-R Yu, ed, Physical Chemistry of Paddy Soils. Science Press, Beijing, China, pp 197–217