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Comparison of the Uptake of Nitrate and Ammonium by Rice Seedlings

INFLUENCES OF LIGHT, TEMPERATURE, OXYGEN CONCENTRATION, EXOGENOUS SUCROSE, AND METABOLIC INHIBITORS[1]

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ABSTRACT

The uptake of nitrate and ammonium by rice (*Oryza sativa*) seedlings was compared under various conditions. Nitrate uptake showed a 1-hour lag phase and then a rapid absorption phase, whereas ammonium uptake showed passive absorption during the first hour, then a shoulder of absorption, followed by a rapid metabolism-dependent absorption phase. Light did not affect the uptake of nitrate or ammonium. The uptake of nitrate and ammonium was markedly suppressed by removal of the endosperm. After removal of the endosperm, the uptake was restored by exogenous supply of 30 mM sucrose. No appreciable nitrate uptake was detected at temperatures below 15 C, whereas appreciable ammonium uptake occurred at 15 C, although lowered passive absorption and complete inhibition of the rapid metabolism-dependent absorption phase were observed at 5 C.

Nitrate uptake was decreased by aeration and also by bubbling the mixture with O_2. It was greatly depressed by bubbling the mixture with N_2. These results suggest that there is a low optimal O_2 concentration for nitrate uptake. In contrast, ammonium uptake was increased by aeration, not influenced by bubbling with O_2, and slightly decreased by bubbling the mixture with N_2.

Nitrate uptake was almost entirely inhibited by addition of cycloheximide, KCN, or arsenate. These compounds had scarcely any effect on the passive absorption of ammonium, but entirely depressed its successive metabolism-dependent absorption. Ammonium uptake occurred before nitrate uptake in solution containing both nitrate and ammonium.

There have been several reports on whether ammonium or nitrate is the better form of N for rice plants. Bonner (4) concluded from culture experiments that rice plants utilized ammonium in preference to nitrate, while Malavolta (13) later found that rice plants grew well in media containing nitrate as the sole N source.

Tang and Wu (24) first reported adaptive formation of nitrate reductase in 5-day-old rice seedlings. Shen (21) found NR[2] even in 3.5-day-old rice seedlings and showed that rice seedlings could assimilate nitrate from the beginning of germination. According to Oji and Izawa (16), the specific activity of NR in the stems and leaves of 21-day-old rice plants is higher than the activities in soybeans or wheats.

In East Asia young rice plants are grown in flooded fields and ammonium is the main form of N supplied in fertilizers. Nitrate has seldom been tested as a fertilizer for rice plants for the following reasons: (*a*) nitrate is scarcely adsorbed to soil particles and is readily washed away in flooded water; (*b*) nitrate is broken down to N_2 by denitrification under the reducing conditions existing in the soil under the water. Yamasaki and Seino (25) found that nitrate can be used in cultivation of rice plants, and that when it is used in rice nurseries, it increases the development of new roots after the seedlings are planted out in the paddy fields; it also has beneficial effects on growth and yield. Nitrate is also known to increase metabolic activity and cation absorption in rice roots (23, 25).

Comparative studies on the absorptions and assimilations of nitrate and ammonium have also been reported. Oji and Izawa (17) found that nitrate was converted to proteins at the same rate as ammonium ion in young rice seedlings, but that it was not so well absorbed or assimilated as ammonium. They (9) also reported that the absorption and assimilation of nitrate were significantly influenced by the level of respiratory substrates in the plants, the O_2 concentration around the roots, and the light intensity. According to Yoneyama and Kumazawa (26), the distribution of ^{15}N in the leaves of rice seedlings supplied with $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ changed with age, and the movement of the two forms of N to leaves in the dark was less than 1/10 of that in the light. Further systematic studies are needed on the utilization of nitrate by rice plants in comparison with that of ammonium. In the present work nitrate and ammonium absorption by rice seedlings were compared under various environmental conditions.

MATERIALS AND METHODS

Plants. Rice seeds (*Oryza sativa* var. Nihonbare) were germinated as described (19) for 2 days. Seedlings of nearly uniform size were selected and transplanted onto a Saran net floating on tap water in a pot. Plants were cultured in tap water under a schedule of 12-hr light and 12-hr dark in a phytotron for 9 days. The temperature was maintained at 30 C in the light and 25 C in the dark. Illuminance was 5,000 lux at the plant level.

Experiments on Nitrate and Ammonium Uptakes. After 9 days culture, the seedlings were removed and their roots were rinsed thoroughly with distilled H_2O. Groups of 20 seedlings were used for each treatment. The roots were blotted lightly with tissue paper and placed in an Erlenmeyer flask containing 60 ml of 0.5 mM $NaNO_3$ or 0.5 mM NH_4Cl. The flask was covered with aluminum foil to screen the roots from light. Absorption experiments were carried out in a phytotron with controlled lighting at 30 C. Illuminance was 5,000 lux at the level of the plants, except in the experiment on the effect of light.

Intact seedlings were used in most experiments. Shoots were cut

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[2] Abbreviation: NR: nitrate reductase.

off about 2 cm above the endosperm immediately before placing the seedlings in the culture solution (Figs. 1 and 2). The exuded xylem sap was removed with absorbent cotton so that it did not contaminate the culture solution. In some experiments the endosperm was also excised immediately before placing the root portions of the seedlings in the culture solution.

For the experiments of light illuminance, the appropriate light intensity was obtained by changing the distance of the light source from the plants. The dark condition was achieved by covering the box of plants with light-proof cloth. Under these conditions the increase of temperature was at most 3 C. In studies on the influence of temperature (see Fig. 3), plants were placed in rooms maintained at the respective temperatures.

In most experiments the solutions were not aerated, but for the results in Figure 4, air, O_2, or N_2 was bubbled through the solution during the culture period at a rate of 25 ml/min.

Samples of 1.0 ml of the solution were withdrawn at definite times after placing the roots in solution, except for the results in Figure 7, when samples of 0.5 ml were used to determine nitrate and ammonium. The culture solutions were not renewed, and so in all experiments the nitrate or ammonium concentration of the medium decreased progressively.

At the end of the experiment, the roots were thoroughly washed with running tap water and then with distilled H_2O, cut off just below the endosperm, blotted lightly, and weighed. All results are expressed per gram fresh weight of roots and the mean values of replicate experiments are shown.

Determinations of Nitrate and Ammonium. The nitrate content of the medium was usually determined by the phenol-disulfonic acid method (15). When sucrose was present in the ambient solution (see Fig. 2A), a nitrate ion electrode (model 92-07, Orion Research Inc.) was used because sucrose interferes with color development with phenol-disulfonate. The presence of ammonium ion also interfered with color development, and so when it was present 1 drop of 1 N NaOH was added to the solutions, and they were dried on a water bath before measuring nitrate by the phenol-disulfonic acid method (15).

The ammonium content of the ambient solution was estimated directly by Nessler's method (1). Ammonium in solution containing sucrose was determined by the microdiffusion method of Conway (1).

RESULTS AND DISCUSSION

Uptakes of Nitrate and Ammonium. The cumulative uptakes of nitrate and ammonium, and the tissue requirements of the two forms of N are shown in Figures 1A and 1B. There was a 1-hr lag phase in the uptake of nitrate in most experiments (*cf.* Figs. 1A and 4A). In contrast, ammonium uptake was rapid in the first hr (or sometimes less than 1 hr) and did not have a lag phase, but exhibited an absorption shoulder between 1 and 2 hr after the beginning of the experiment (*cf.* Fig. 3B). Figure 1C shows details of the initial ammonium uptake; considerable ammonium was taken up in the first 10 min. The surface of plant roots is negatively charged and this charge can be neutralized with H ions. Cations can be adsorbed from solution by exchange with H ions on the root surface (23). Ammonium exists as ammonium cation in the solution, and thus will be rapidly adsorbed by exchange with H ions. The initial rise in ammonium uptake was probably mainly due to adsorption of ammonium ions to the root surface, passive ion exchange, and diffusion of the ions into Donnan free spaces. The subsequent high rate of ammonium uptake, which continued for up to 1 hr (Fig. 1B), probably involved both above passive influx and metabolism-dependent uptake into the cells as discussed later.

Seedlings from which the endosperms had been removed (endospermless seedlings) showed low nitrate absorption after the lag phase and reduced the maximal nitrate absorption (Fig. 1A). Excision of the shoots (decapitation) did not affect the maximal nitrate absorption appreciably (Fig. 1A). Similarly, removal of the

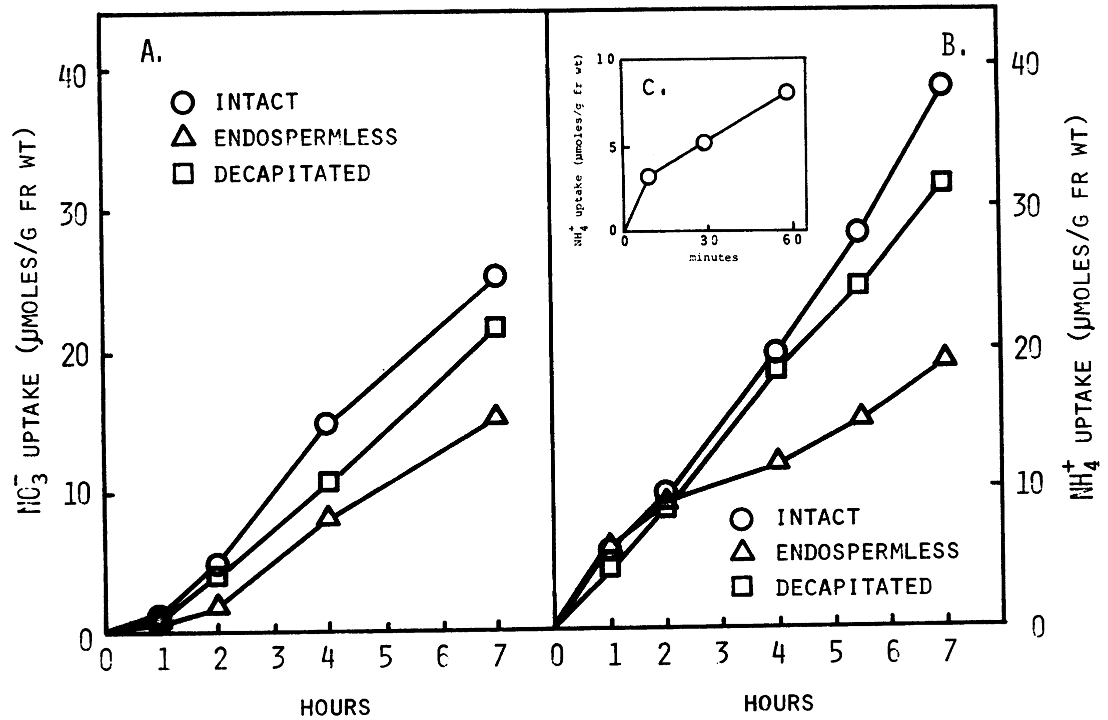

FIG. 1. Influence of tissue excision on nitrate and ammonium uptakes. Shoots or endosperms were excised immediately before placing seedlings in culture solution. Initial concentration of nitrate and ammonium solution was 0.5 mM (pH 6.5). Solutions were not renewed during experiment and not aerated. Temperature was 30 C throughout. Light illuminance was 5,000 lux at the level of plants. A: nitrate uptake; B: ammonium uptake. Ammonium uptake by intact seedlings in a short period is shown in inset (C).

endosperm markedly reduced the maximal ammonium uptake, whereas removal of the shoots had little effect (Fig. 1B). These results indicate that the uptakes of nitrate and ammonium both depend mainly on materials from the endosperm, probably for energy, but that they depend very little on the shoot. The small decrease in uptake on removal of the shoot (leaves) also suggests that transpiration from the leaves had no significant effect on the uptakes of nitrate and ammonium and that the products of photosynthesis in the shoot under these conditions did not provide sufficient energy for absorption.

Figure 2 shows the effect of exogenous sucrose on endospermless seedlings. Added sucrose was more effective than substrate from intact endosperm on nitrate uptake (Fig. 2A). Similarly, ammonium absorption in endospermless seedlings was accelerated by addition of sucrose (Fig. 2B). These data suggest that energy-generating compounds, such as sucrose, are required for the uptake of both nitrate and ammonium and that there is little influx of N compounds into plants by passive influx. It is still unknown why exogenous sucrose was more effective than endogenous substrate(s) in the endosperm for nitrate uptake, and why removal of endosperm decreased NH_4^+ uptake so markedly.

Light Illumination. Both nitrate uptake and induction of NR have been reported to be promoted by light (3, 5) and ammonium uptake is also stimulated by light (26). The effects of light intensity on the uptake of nitrate and ammonium were measured. Nitrate uptake was scarcely affected by changing the illuminance from 2,000 to 7,000 lux, but it was reduced by 14 to 18% in the dark. Ammonium uptake was also hardly affected by changing the light intensity and was reduced by 7 to 8% in the dark.

Light had similar effects on nitrate and ammonium uptake in endospermless seedlings; namely, the uptakes of ammonium and nitrate under 7,000 lux were depressed by 27 and 15%, respectively, as placed in the dark.

The illuminance had scarcely any influence on either uptake. Thus, light is not a definitive factor in short term absorption in younger rice seedlings.

Temperature. The effects of temperature on nitrate and ammonium uptakes are shown in Figure 3. Nitrate uptake was about 20% less at 25 than at 30 C, and hardly any nitrate was absorbed at 15 or 5 C (Fig. 3A). Like nitrate uptake, ammonium uptake was about 15% less at 25 than at 30 C, but unlike nitrate uptake, there was appreciable absorption at 15 C (Fig. 3B). The absorption shoulder in ammonium uptake after the initial rise was prolonged up to 4 hr and the subsequent absorption phase appeared at 15 C. At 5 C, the initial rise of ammonium uptake was suppressed and the subsequent absorption phase was not observed (Fig. 3B). The decrease in ammonium in the ambient solution within 1 hr at 5 C was probably due to its adsorption on the root surface or passive influx, because afterwards there was no further decrease. Ion transport, which is closely linked to metabolic activity, is greatly influenced by temperature; the temperature coefficient (Q_{10}) of ion absorption is 2 or more in the range of about 10 to 30 C, as expected for a metabolic process (23). The present results strongly suggest that the uptakes of nitrate and ammonium at high temperature are closely associated with metabolism.

O_2 Concentration Around the Roots. The effects of bubbling air, O_2, or N_2 gas around the roots on the uptakes of nitrate and ammonium are shown in Figure 4. Aeration decreased nitrate uptake (Fig. 4A), but increased ammonium uptake (Fig. 4B).

Bubbling with O_2 decreased nitrate uptake to the same level as that observed on aeration. Contrary to expectation, bubbling with N_2 suppressed nitrate uptake about 75% (Fig. 4A). Ammonium uptake was not influenced at all by O_2, but was slightly reduced by N_2 bubbling, although less than nitrate uptake (Fig. 4B). The stirring of the solution by a magnetic stirrer had little effect on nitrate uptake.

Suppression of nitrate uptake by aeration has also been observed under different experimental conditions in rice seedlings (9). Yemm *et al.* (*cf.* 2) found that additions of ammonium, nitrate, and nitrite all promoted O_2 uptake in excised barley roots, but that the RQ was raised only by nitrate or nitrite. This suggests that NADH generated by various dehydrogenations is oxidized by NR as well as the respiratory chain. Nitrate can take the place of O_2 as a terminal electron acceptor (so-called nitrate respiration, *cf.* 2), and CO_2 output can proceed similarly in both nitrate and O_2 respiration (*cf.* 2).

Nitrate assimilation will proceed slower when more NADH is oxidized by respiration than by nitrate reduction. During short term absorption of low concentrations of nitrate under the present conditions, there was no significant induction of NR (19), and thus the depression of absorption by aeration could hardly be due to competition between respiratory enzymes and NR for NADH.

Nitrate uptake was low in O_2-enriched solution, and it was decreased further by bubbling the solution with N_2 to remove O_2 (N_2 bubbling). These results suggest that a narrow range of O_2

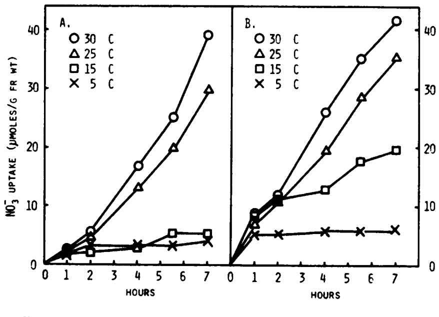

FIG. 3. Influences of temperature on nitrate and ammonium uptakes. Other conditions except temperature were as in Figure 1. A: nitrate uptake; B: ammonium uptake.

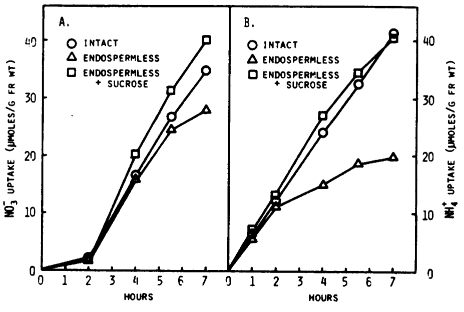

FIG. 2. Effects of exogenous sucrose on nitrate and ammonium uptake in endospermless seedlings. Sucrose was added to solution at a concentration of 30 mM. Other conditions were as in Figure 1. A: nitrate uptake; B: ammonium uptake.

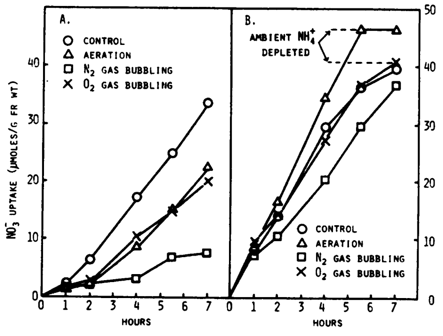

FIG. 4. Influences of bubbling air, O_2, or N_2 gas through solution on nitrate and ammonium uptakes. Air, O_2, or N_2 gas was bubbled through solution during culture period at a rate of 25 ml/min. Other conditions were as in Figure 1. A: nitrate uptake; B: ammonium uptake.

concentration lower than that in air is suitable for nitrate uptake in rice seedlings.

Metabolic Inhibitors. Cycloheximide (2 μg ml^{-1}) as added to the culture solution inhibited nitrate uptake almost completely (Fig. 5A), and suppressed ammonium uptake moderately (Fig. 5B). It suppressed the rapid influx of nitrate or ammonium in the first hr and the difference between the control and cycloheximide treatment groups indicates that it prevented metabolism-dependent absorption.

Cycloheximide, an inhibitor of protein synthesis, has been found to suppress salt uptake (6). Jackson *et al.* (10) found that inhibitors of RNA and protein synthesis reduced the maximal development of nitrate uptake and also induction of NR in the tips of corn roots. They suggested that nitrate uptake may depend upon continuous protein synthesis. There may be a specific transport protein associated with nitrate uptake. It is uncertain whether a similar hypothesis could apply to ammonium uptake.

The effect of pretreatment with chloramphenicol (chloromycetin powder, 100 μg ml^{-1}) for 2 hr before immersing rice seedlings into nitrate solution was studied. Chloramphenicol reduced nitrate uptake by 25 to 30%.

Chloramphenicol, a powerful inhibitor of protein synthesis on 70S ribosomes and also a producer of uncoupled respiration, is reported to induce NR in corn seedlings (20), rice plants (19, 22), and detached cucumber cotyledons (12). Nitrate uptake was reduced in rice seedlings that had been treated with chloramphenicol.

The respiratory inhibitors, KCN and arsenate, almost completely inhibited nitrate uptake (Fig. 6A). They had no effect on ammonium uptake in the first hr, but suppressed subsequent uptake (Fig. 6B).

Uptake of Nitrate and Ammonium from Solutions Containing $NaNO_3$ and NH_4Cl, or NH_4NO_3. Groups of 20 plants were transferred to 60-ml volumes of solution containing 30 μmol of $NaNO_3$ and 30 μmol of NH_4Cl, or 30 μmol of NH_4NO_3. Ammonium uptake was much faster than nitrate uptake, and almost all of the ammonium ion was absorbed within the experimental period (Fig. 7).

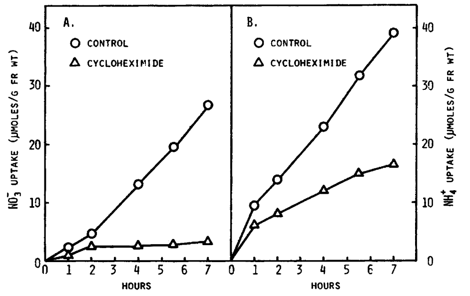

FIG. 5. Influences of cycloheximide on nitrate and ammonium uptakes. Cycloheximide was added to solution at a concentration of 2 μg/ml at beginning of experiment. Other conditions were as in Figure 1. A: nitrate uptake; B: ammonium uptake.

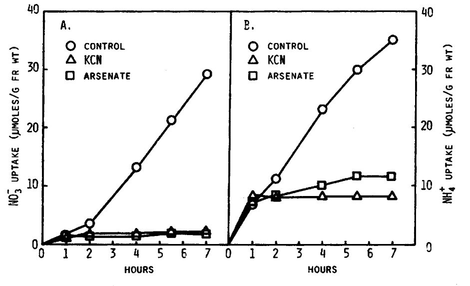

FIG. 6. Influences of respiratory inhibitors on nitrate and ammonium uptakes. KCN or arsenate was added to solution at a final concentration of 1 mM at beginning of experiment. Other conditions were as in Figure 1. A: nitrate uptake; B: ammonium uptake.

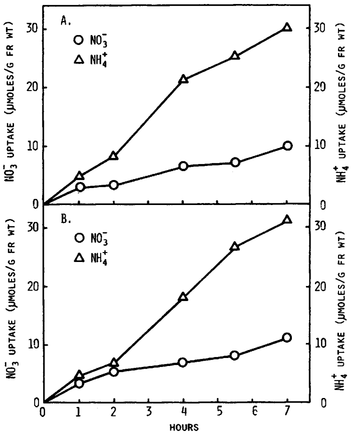

FIG. 7. Absorptions of nitrate and ammonium from solution containing both ions. Each concentration of nitrate and ammonium ion was 0.5 mM. Other conditions were as in Figure 1. A: solution containing $NaNO_3$ and NH_4Cl; B: solution containing NH_4NO_3 only.

Nitrate uptake is inhibited by ammonium in various plants (11, 14, 21) and *Penicillium* (8). Under our conditions ammonium uptake was much faster than nitrate uptake in solution containing both forms of N (Fig. 7), indicating that ammonium disturbed nitrate uptake. Ammonium also represses the formation of NR in *Lemna minor* L. (18) and apple seedlings (7). Studies are now in progress on the relation between nitrate uptake and induction of NR in the presence of ammonium.

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