Real Time Visualization of ^{13}N-Translocation in Rice under Different Environmental Conditions Using Positron Emitting Tracer Imaging System[1]

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The ammonium ion is an indispensable nitrogen source for crops, especially paddy rice (*Oryza sativa* L. cv Nipponbare). Until now, it has been impossible to measure ammonium uptake and nitrogen movement in plants in real time. Using the new technologies of PETIS (positron emitting tracer imaging system) and PMPS (positron multi-probe system), we were able to visualize the real time translocation of nitrogen and water in rice plants. We used positron-emitting ^{13}N-labeled ammonium ($^{13}NH_4^+$) and ^{15}O-water to monitor the movement. In plants cultured under normal conditions, $^{13}NH_4^+$ supplied to roots was taken up, and a ^{13}N signal was detected at the discrimination center, the basal part of the shoots, within 2 minutes. This rapid translocation of ^{13}N was almost completely inhibited by a glutamine synthetase inhibitor, methionine sulfoximine. In general, nitrogen deficiency enhanced ^{13}N translocation to the discrimination center. In the dark, ^{13}N translocation to the discrimination center was suppressed to 40% of control levels, whereas ^{15}O-water flow from the root to the discrimination center stopped completely in the dark. In abscisic acid-treated rice, ^{13}N translocation to the discrimination center was doubled, whereas translocation to leaves decreased to 40% of control levels. Pretreatment with NO_3^- for 36 hours increased ^{13}N translocation from the roots to the discrimination center to 5 times of control levels. These results suggest that ammonium assimilation (from the roots to the discrimination center) depends passively on water flow, but actively on NH_4^+-transporter(s) or glutamine synthetase(s).

More than 70% of the world's rice (*Oryza sativa*) is produced in intensively cultivated, irrigated lowland fields in Asia. In flooded lowland rice fields, the bulk of the soil is hypoxic or anaerobic, and the major form of nitrogen available to plants is NH_4^+. This is in marked contrast to most (aerobic) agricultural soils in which NO_3^- is the predominant inorganic nitrogen species. NH_4^+ is the preferred nitrogen species taken up by rice; it is superior to NO_3^- in terms of fertilizer efficiency in paddy fields (Yoshida, 1981).

Radioisotopes and stable isotopes are often used to study the uptake and translocation of nutrients in plants. Since nitrogen is the main nutrient of plants, many plant physiologists have used ^{15}N, which is a stable isotope, to elucidate the biochemical processes in rice root cells (Yoneyama and Kumazawa, 1974a; Arima and Kumazawa, 1975). They proved that the formation of the amide-nitrogen of Gln is the primary process in the fixation of ammonium absorbed from rice roots using $(^{15}NH_4)_2SO_4$ as the sole nitrogen source. Preparing samples for ^{15}N analysis is a very tedious process, and it is impossible to detect the excess percentage of ^{15}N in plants in real time. To overcome these shortcomings, ^{13}N has been adopted in plant physiology research (Glass et al., 1985; Ingemarsson et al., 1987). ^{13}N is a positron-emitting nuclide. When a positron decays, it emits two γ-rays in opposite directions. Several researchers have used $^{13}NH_4^+$ and $^{13}NO_3^-$ in plant nutrition research (Presland and McNaughton, 1986; Wang et al., 1993a, 1993b; Kronzucker et al., 1995a, 1995b, 1995c) and detected the positrons using a liquid scintillation counter. However, this is not real time analysis. Hamamatsu Photonics of Japan and the TIARA (Takasaki Ion Accelerators for Advanced Application) group recently developed a dynamic image measurement system called "PETIS" (Positron Emitting Tracer Imaging System). This system detects the γ-rays produced by positron-emitting nuclides with a scintillation camera and enables study of the movement of elements in plants in real time (Kume et al.,

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1997; Hayashi et al., 1998; Uchida et al., 1998; Matsunami et al., 1999; Nakanishi et al., 1999; Sato et al., 1999; Mori et al., 2000). In this study, we produced $^{13}NH_4^+$ and ^{15}O-water in a cyclotron and compared ^{13}N translocation with ^{15}O-water flow (mass flow) in rice supplied with $^{13}NH_4^+$ under different conditions in real time using PETIS and PMPS (positron multiprobe system).

RESULTS

Absorption and Translocation of $^{13}NH_4^+$ from Roots in Control Rice Plants

In control rice plants, $^{13}NH_4^+$ was absorbed from the roots and ^{13}N was translocated to all parts of the plant within 60 min (Fig. 1B). BAS images showed that the discrimination center (DC), the basal part of the shoots, was strongly labeled (Fig. 1, B and C). The youngest leaf was more strongly labeled than older leaves (Fig. 1B). PETIS detectors were focused on the DC (Fig. 1A), and real time ^{13}N translocation was monitored (Fig. 1, D–F). The image of the DC appeared 4 min after supplying $^{13}NH_4^+$ (Fig. 1D), and then images of the shoot appeared in the subsequent 3 to 4 min. The translocation curve made from the PETIS images revealed that the first ^{13}N arrived at the DC 2 min after the beginning of absorption (Fig. 1E), and the amount increased with time throughout the experiment (Fig. 1F).

When the PMPS detectors were focused on the DC (Fig. 2A) and on the part of the newest leaf (NL in Fig. 2A) that was 34.3 cm above the DC of control plants, radioactivity was detected in the DC and NL 2 and 6 min, respectively, after $^{13}NH_4^+$ supply to the roots (Fig. 2B). Therefore, the velocity of transport of ^{13}N to the newest leaf from the DC was 8.6 cm min^{-1} [=34.3 cm/(6 − 2 min)].

When $^{13}NH_4^+$ is absorbed from roots, the culture solution, and roots must be completely shielded with lead blocks to avoid direct irradiation of the PETIS camera or PMPS probes with positrons, otherwise, the high background radioactivity would affect the PETIS image or PMPS data. Therefore, it is impossible to observe the direct progression of $^{13}NH_4^+$ activity from outside to inside the roots using these methods.

$^{13}NH_4^+$ Translocation from the Second Newest Leaf to the DC

To study $^{13}NH_4^+$ translocation from the leaf, $^{13}NH_4^+$ was supplied to the cut end of the second newest leaf (SNL) of a control plant (Fig. 3, two arrows). Only a few ^{13}N counts were detected at the

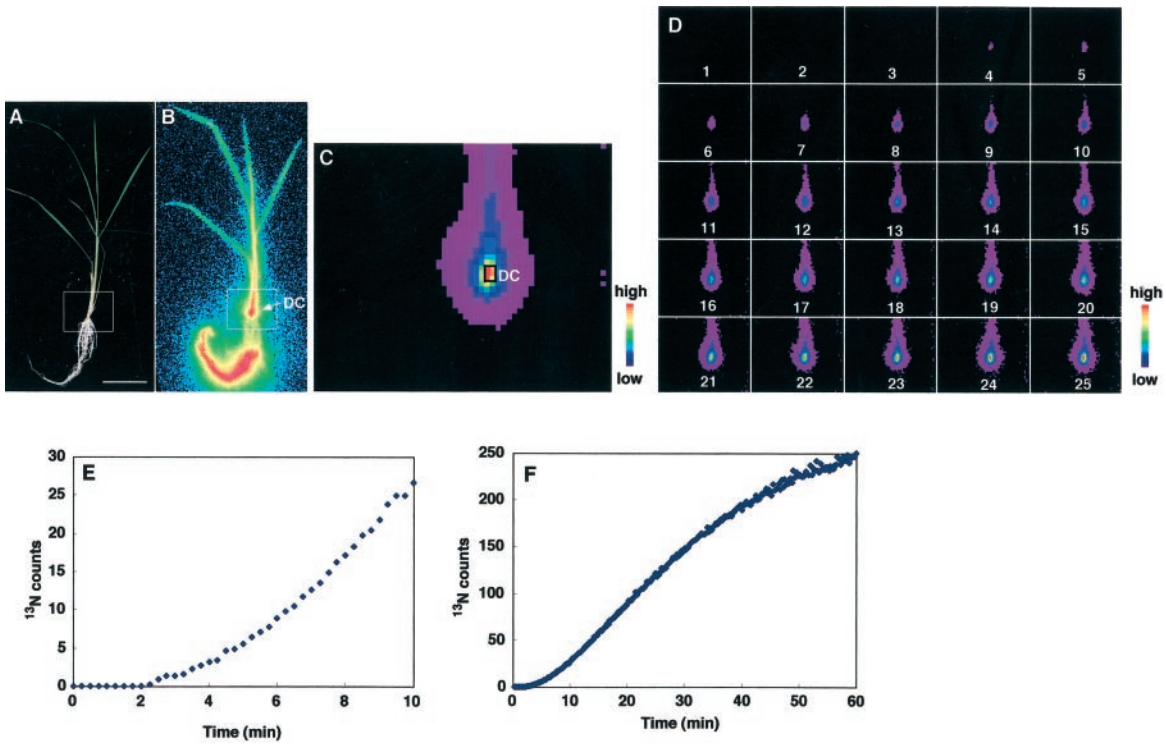

Figure 1. ^{13}N translocation from roots to the DC of control rice supplied with $^{13}NH_4^+$. A, Gross image of a control rice plant. B, Image of ^{13}N translocation using BAS-1500. Scale bar = 4 cm. C, Accumulative PETIS image after a 60-min analysis. D, The time course for the accumulation of radioactivity in the white squares in A and B was followed over time by PETIS. The images shown are for 1-min intervals and the data were scored every 15 s. E, Curve showing the accumulation of radioactivity in the DC after 10 (C) and 60 (F) min by PETIS.

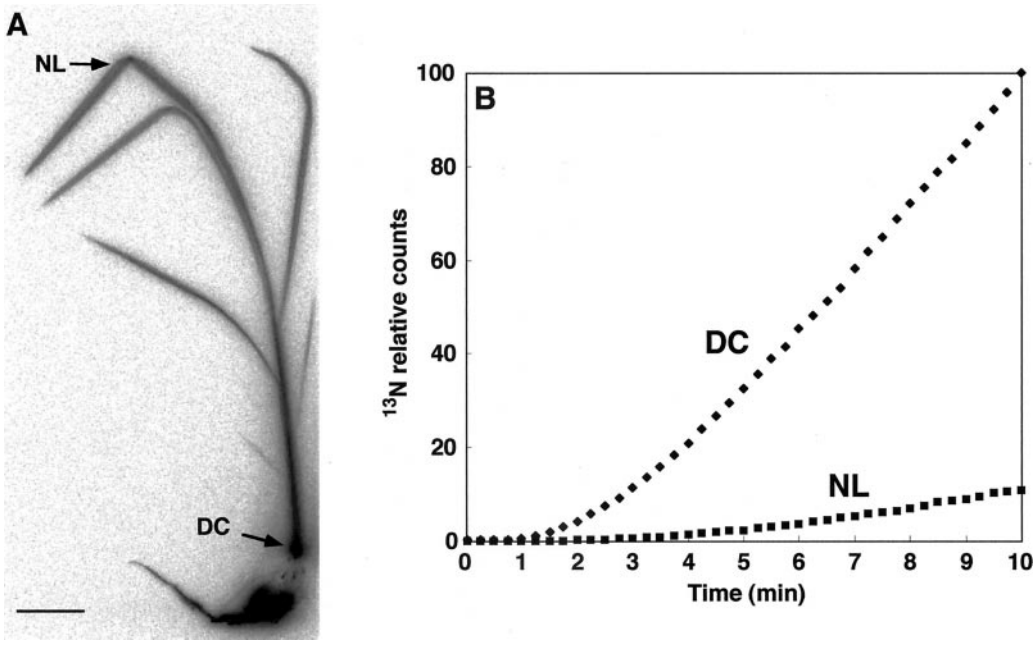

Figure 2. $^{13}NH_4^+$ translocation from roots to the DC of control rice. A, BAS image of ^{13}N. The arrows at the NL (newest leaf) and DC indicate where the radioactivity was traced with PMPS. B, Time course for radioactivity accumulation in the NL and DC.

DC (data not shown). The BAS image showed that a large amount of ^{13}N remained halfway along the leaf blade, and a very small amount of ^{13}N moved to the shoot (Fig. 3). $^{13}NH_4^+$ translocation from the cut leaf tip was also examined using 3-d nitrogen-deficient rice. Translocation was similar to that of controls (data not shown).

Effect of Met Sulfoximine (MSX)

The amount of ^{13}N in the DC in Met sulfoximine (MSX)-treated rice decreased drastically to 5% of control levels after 60 min of $^{13}NH_4^+$ supply (Fig. 4C). In the newest leaf, MSX also depressed ^{13}N translocation to 5% of control levels after 60 min (Fig. 4D). The BAS images showed that the ^{13}N signals of all the leaves and the DC in MSX-treated rice were much weaker than those of control rice (Fig. 4, A and B).

Effect of Nitrogen Deficiency

When $^{13}NH_4^+$ was supplied to the roots of nitrogen-deficient rice cultured under 500 μmol m^{-2} s^{-1}, the amount of ^{13}N in the DC increased to 5 times control levels after 60 min of $^{13}NH_4^+$ supply (Fig. 5A). However, it declined to 50% of control levels under pretreatment of stronger illumination (1,500 μmol m^{-2} s^{-1}; Fig. 5B).

Effect of the Dark

In the dark, the initial ^{13}N translocation from the roots to the DC occurred 5 min after $^{13}NH_4^+$ supply, 3 min later than in controls (Fig. 6A), and the radioactivity was reduced to 40% of control levels after 60 min of absorption (Fig. 6B).

Abscisic Acid Treatment

The amount of ^{13}N in the DC in abscisic acid (ABA)-treated rice was double that in control rice after 60 min of $^{13}NH_4^+$ supply (Fig. 7C), although there was no delay in the initial detection. However, ^{13}N translocation to the SNL in ABA-treated rice was depressed to 40% of control levels after 60 min (Fig. 7D). BAS images showed that ^{13}N signals in all the leaves of the ABA-treated rice were weaker than those of controls (Fig. 7, A and B).

Pretreatment with NO_3^-

^{13}N translocation to the DC in NO_3^- pretreated rice for 36, 24, and 12 h were 5, 3, and 2.3 times higher than in controls, respectively, after 60 min of $^{13}NH_4^+$ supply (Fig. 8).

Effect of Light on ^{15}O-Water Flow in Plants

In the dark (pretreatment for 30 min in dark conditions) ^{15}O-water flow from the roots to the DC stopped completely (Fig. 9, dark and light). After 60 min of illumination following 60 min of darkness, the ^{15}O-water flow rate from the roots to the DC recovered completely (Fig. 9, relight).

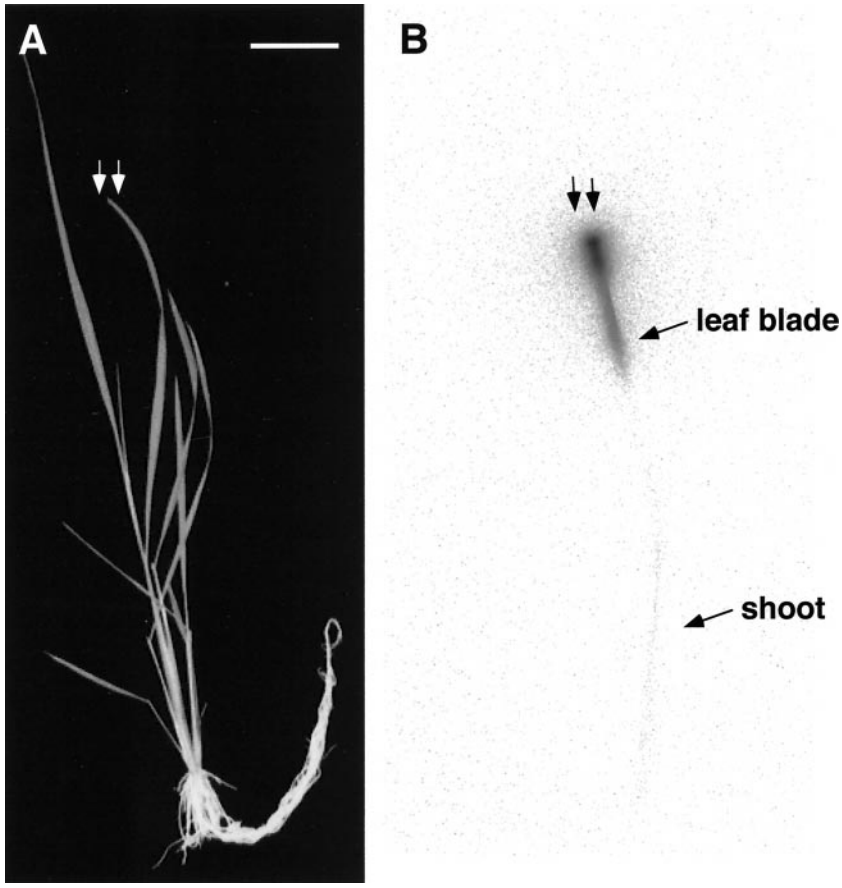

Figure 3. Poor translocation of $^{13}NH_4^+$ from the cut part of the SNL in a control plant to other parts of the plant. A, Gross image of a plant. B, Image of ^{13}N translocation using BAS-1500. $^{13}NH_4^+$ was absorbed from the SNL of a control plant (arrows) for 120 min. The rice plant was cultured in complete nutrient solution.

DISCUSSION

Normal Translocation Image of ^{13}N Supplied with $^{13}NH_4^+$ in Rice

In rice, $^{13}NH_4^+$ was absorbed by the roots and ^{13}N was translocated to all parts of the plant within 60 min. BAS images showed that ^{13}N-labeled younger leaves more strongly than older leaves, which indicates that younger leaves are stronger nitrogen sinks than older ones (Figs. 1B and 2A) (Yoneyama and Kumazawa, 1974b; Mori, 1998). In all cases, ^{13}N accumulated in the DC. The absorption curve made from the PETIS analysis revealed that ^{13}N reached the DC 2 min after $^{13}NH_4^+$ supply (Figs. 1E and 2B) and the tip of the newest leaf after 6 min (Fig. 2B). It took 4 min for ^{13}N to move from the DC to the tip of the newest leaf.

The PETIS analysis showed that only negligible amounts of ^{13}N supplied to leaves were detected in the DC (data not shown). This was also confirmed by the BAS image after 120 min of NH_4^+ supply, which showed that a large amount of ^{13}N still remained halfway along the leaf blade, and that a very small amount of ^{13}N was translocated to the shoot of the leaf (Fig. 3). In our study $^{13}NH_4^+$ was supplied to the SNL, which was a sink for nitrogen flow. Rice Gln synthetase, GS2, is thought to assimilate any NH_4^+ evolved from photorespiration in the leaf (Redinbaugh and Campbell, 1993). Hence, $^{13}NH_4^+$ supplied to the leaf might be immediately assimilated by leaf GS2 and metabolized to other nitrogen compounds through the GS/GOGAT cycle (Sechley et al., 1992). This nitrogen does not leave the cells to be translocated because this leaf is a sink. No NH_4^+ was found in phloem sap collected from the young rice leaf (sink) using the insect laser technique (Hayashi and Chino, 1985). On the other hand, rice cytosolic GS1 functions in the bundle sheath cells in the senescence leaf blade, but little GS1 activity was detected in the sink leaf (Kamachi et al., 1992). This explains the Gln transport from the old (senescence) leaf to the young (sink) leaf (Mae et al., 1983). Therefore, in young rice leaves either $^{13}NH_4^+$ assimilation to Gln by GS1 and incorporation of the ^{13}N-labeled Gln into the phloem does not occur, or direct incorporation of $^{13}NH_4^+$ into the phloem is very weak (Fig. 3).

The DC, a Crucial Site for Material Transport in Graminaceous Plants

We recently reported the role of the DC in Fe and Met transport in barley using ^{59}Fe(III)-epihydroxymugineic acid and [^{11}C]Met, respectively (Mori, 1998; Nakanishi et al., 1999). When ^{59}Fe or [^{11}C]Met was supplied to roots or leaves, the DC was strongly labeled. ^{59}Fe and [^{11}C]Met subsequently

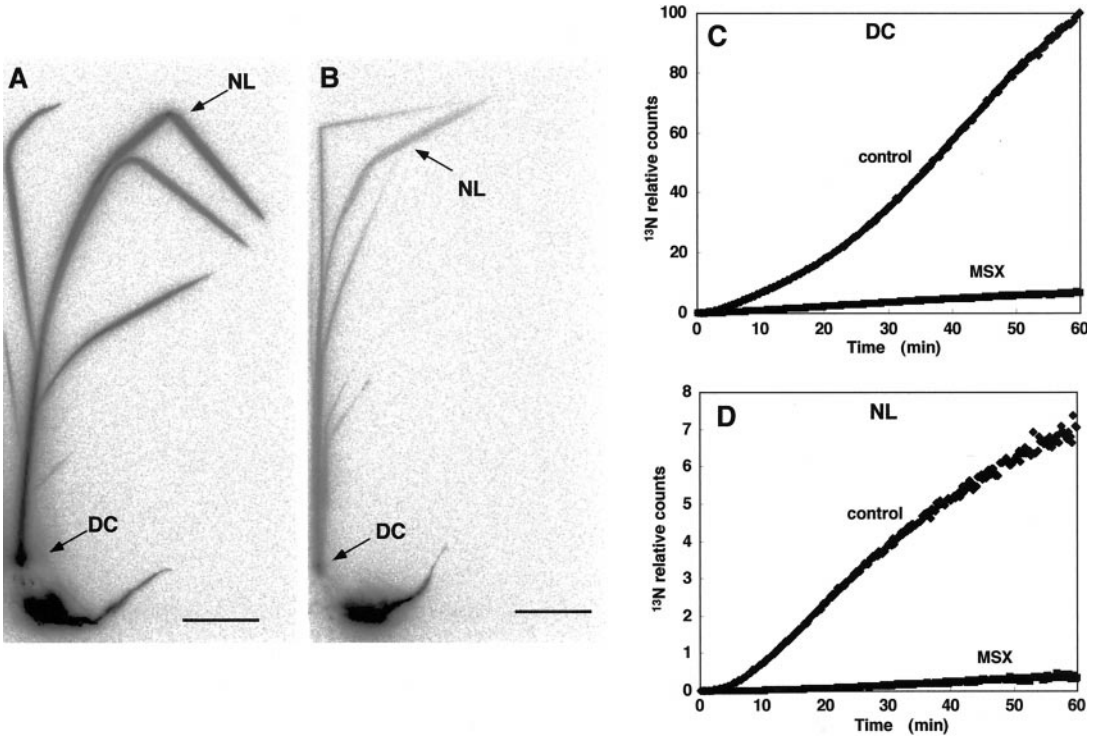

Figure 4. Severe suppression of $^{13}NH_4^+$ translocation from rice roots to the DC with MSX treatment. Image of the translocation of ^{13}N in control (A) and MSX-treated (B) rice plants using BAS-1500. Scale bar = 4 cm. Curves showing the accumulation of radioactivity in the DC (C) and newest leaf (D) using PMPS in two plants.

were distributed to other parts of the plant. Therefore, this part of the plant seems to play a crucial role in the translocation of minerals and metabolites in graminaceous plants and has been named the "discrimination center." In barley, [^{11}C]Met was translocated from the SNL tip to the DC at a velocity of 2 cm min^{-1}. In Fe-deficient barley, new chlorotic leaves were a strong Met sink, and leaf-to-leaf transfer through the DC occurred very rapidly (Nakanishi et al., 1999). ^{59}Fe-epihydroxymugineic acid supplied to cut barley leaves was also translocated through the DC to other new chlorotic leaves and to the root tips within 45 min as detected by radioautography (Mori, 1998). Photosynthetic $^{11}CO_2$ from an old leaf was translocated throughout the DC to the ears within 45 min at a velocity of 0.9 cm min^{-1} (Matsuhashi et al., 1998). Therefore, the translocation rate of the substrate depends on the substrate itself, the nutritional status of the plant, the plant's age, etc. Because the DC is also an important site controlling N translocation and partitioning as mentioned above, the structural characterization of the tissues involved using cytological methods should be considered in the future.

MSX Treatment

^{13}N translocation from the roots to the DC and to the newest leaf decreased drastically in MSX-treated rice (Fig. 4, C and D). BAS images also showed that ^{13}N translocation to the DC and to all leaves was suppressed by MSX treatment (Fig. 4, A and B). As we show in Figure 9, for ^{15}O-water, the time required for radiation to travel from the roots to the DC under light conditions is approximately 1 min. Because there is no physical barrier in the xylem flow, the barriers to radial transport are the main limiting factor for the passage of each mineral element from outside the root cells to the DC. These factors include cell membranes transporters (or channels), plasmodesmata, Casparian strip, and so forth. In case of MSX treated rice, ^{13}N-radioactivity appeared at the DC 10 min after supplying $^{13}NH_4^+$ under light conditions (MSX in Fig. 4C), whereas ^{13}N-radioactivity appeared after 2 min in the control rice (control in Fig. 4C or Fig. 1E). Therefore, a delay up to 8 min occurs in the process of radial transport. This strongly suggests that the conversion of $^{13}NH_4^+$ to ^{13}N-gluatamine by GS1 in the cytoplasm of root cells for xylem loading is the essential for process for $^{13}NH_4^+$ transport in rice.

Pretreatment with MSX is reported to completely inhibit Gln synthetase activity in rice roots (Kronzucker et al., 1998). Although data were not shown, it was also mentioned that the long distance translocation of $^{13}NH_4^+$ was markedly inhibited by MSX in rice (Kronzucker et al., 1998). In addition, the major nitrogen solute in the xylem of rice is Gln and not NH_4^+ (Fukumorita and Chino, 1982). Therefore, the ^{13}N translocation that we observed in control rice

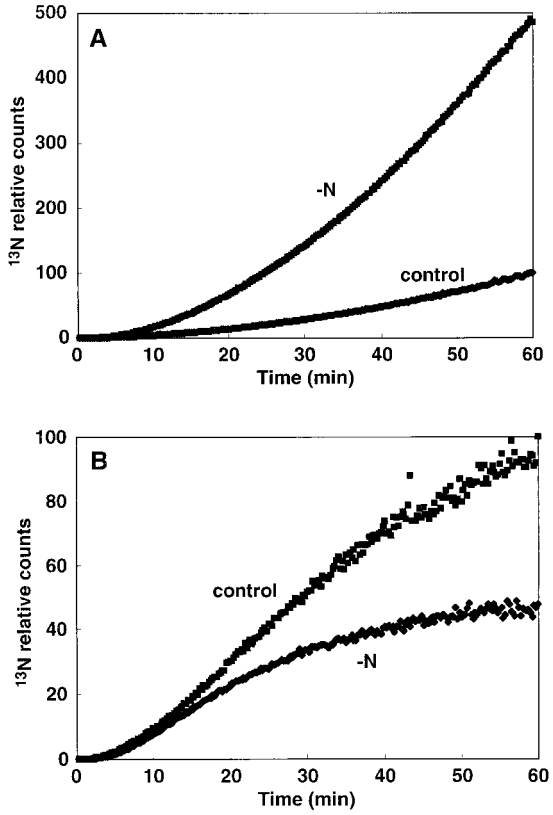

Figure 5. Enhanced or depressed translocation of $^{13}NH_4^+$ from rice roots to the DC of 3-d nitrogen deficiency pretreated rice under natural light. Time course study of the translocation of $^{13}NH_4^+$ to the DC from roots of rice cultured under light intensities of 500 μmol m^{-2} s^{-1} (A) and 1,500 μmol m^{-2} s^{-1} (B), traced by PETIS.

reflects the amount of ^{13}N-Gln synthesized by Gln synthetase (GS1) in roots after passage through an NH_4^+ transporter in the roots.

Nitrogen Deficiency Treatment

When $^{13}NH_4^+$ was supplied to the roots of 3-d nitrogen-deficient rice that had been cultured under 500 μmol m^{-2} s^{-1}, the rate of ^{13}N translocation to the DC was enhanced (Fig. 5A). It has been reported that nitrogen starvation increases $^{13}NH_4^+$ influx in the roots of rice (Wang et al., 1993b; Kronzucker et al., 1998), and that it induces one of the NH_4^+ transporter genes (*AtAMT1*) in the roots of Arabidopsis (Gazzarrini et al., 1999; Rawat et al., 1999) and tomato (*LeAMT1:1*) (von Wirén et al., 2000). Our results concur with these results. A rice NH_4^+ transporter gene is presumably induced by nitrogen deficiency; however, rice NH_4^+ transporter gene, OsAMT1–1, has not been characterized yet (von Wirén et al., 1997). In contrast, under natural light intensity (1,500 μmol m^{-2} s^{-1}), nitrogen deficiency depressed ^{13}N translocation to the DC (Fig. 5B). In this study, higher photosynthetic activity presumably caused severe nitrogen deficiency in the tops of plants very soon after the nitrogen deficiency treatment. This in turn resulted in the decreased translocation of an energy source (ATP) or carbon substrate (i.e. Suc, Glu, etc.) from the tops to the roots. These sequential processes might suppress ^{13}N translocation to the DC. Long distance nitrogen translocation is reported to be influenced by the availability of carbon skeletons (Kronzucker et al., 1998).

Effect of Darkness

In the dark, ^{13}N translocation to the DC decreased, but it was not completely stopped (Fig. 6, A and B). When translocation of ^{15}O-water in rice was traced by the PETIS method, the flow of ^{15}O-water from the roots to the DC was completely stopped by 30-min dark pretreatment (Fig. 9). Therefore, in rice, the dark may cause stomata closure, reducing the rate of flow of water in the xylem, resulting in low xylem loading. These sequential events might lead to the decrease in ^{13}N translocation from the DC to the top and cause the delay in the initial detection of ^{13}N from the roots at the DC. In tomato leaves, expression of the NH_4^+ transporters *LeAMT1;2* and *LeAMT1;3* showed reciprocal diurnal regulation with the highest transcription of *LeAMT1;3* in the dark and the highest levels of *LeAMT1;2* after the onset of illumination (von Wirén et al., 2000). In Arabidopsis, three NH_4^+ transporter genes (*AtAMT1;1*, *AtAMT1;2*, and *AtAMT1;3*) showed diurnal variation in expression. Of these, *AtAMT1;3* transcript levels peaked with ammonium uptake at the end of the light period, suggesting that *AtAMT1;3* links nitrogen assimilation and carbon provision in roots (Gazzarrini et al., 1999). Therefore, it is reasonable to assume that NH_4^+ absorption is not completely stopped in the dark, since some NH_4^+ transporter genes might be expressed diurnally, even if water flow in the xylem is stopped in the dark (Fig. 9). It is still unknown whether there is diurnally regulated expression of an NH_4^+-transporter in rice roots.

ABA Effect

ABA is a plant hormone that affects stomata closure. Therefore, we predicted that ^{13}N movement would be reduced by lower water flow in the xylem, as occurs in the dark. In fact, ABA decreased ^{13}N transport to the SNL (Fig. 7D); the BAS images also showed that ^{13}N translocation to leaves was depressed in ABA-treated rice (Fig. 7, A and B). Unexpectedly, however, with ABA treatment the ^{13}N accumulation observed at the DC was greater than in control rice (Fig. 7C). This was quite different from the results of the dark treatment, suggesting that ABA not only closes the stomata, but also stimulates ammonium assimilation. Treatment with ABA (1 or 10 mM) is reported to increase the activity of Gln synthetase in maize roots and shoots (Sengar and Srivastava, 1995). No direct or indirect effects of ABA

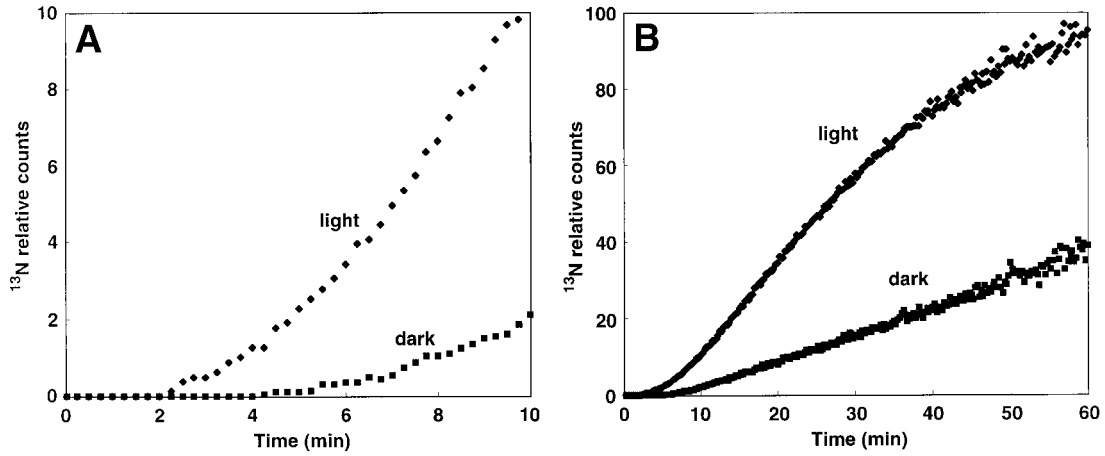

Figure 6. Suppression of the translocation of $^{13}NH_4^+$ from the roots to the DC of rice in the dark. Time course study of the translocation of ^{13}N into the DC from the roots. Accumulation of radioactivity within 10 (A) and 60 (B) min.

on NH_4^+ transporters have been reported. Presumably, enhanced assimilation of $^{13}NH_4^+$ to Gln by ABA is the major reason for the enhanced NH_4^+ translocation to the DC from the roots.

NO_3^- Effect

NO_3^- pretreatment for 36, 24, and 12 h enhanced ^{13}N translocation to the DC (Fig. 8). In radish (Ota and Yamamoto, 1989), Arabidopsis (Gazzarrini et al., 1999), and rice (Kronzucker et al., 1998, 1999) simultaneous application of nitrate and ammonium enhanced NH_4^+ assimilation and translocation to shoots. Our result also strongly suggests that nitrate regulates ammonium assimilation by rice roots, perhaps via enhanced expression of either NH_4^+-

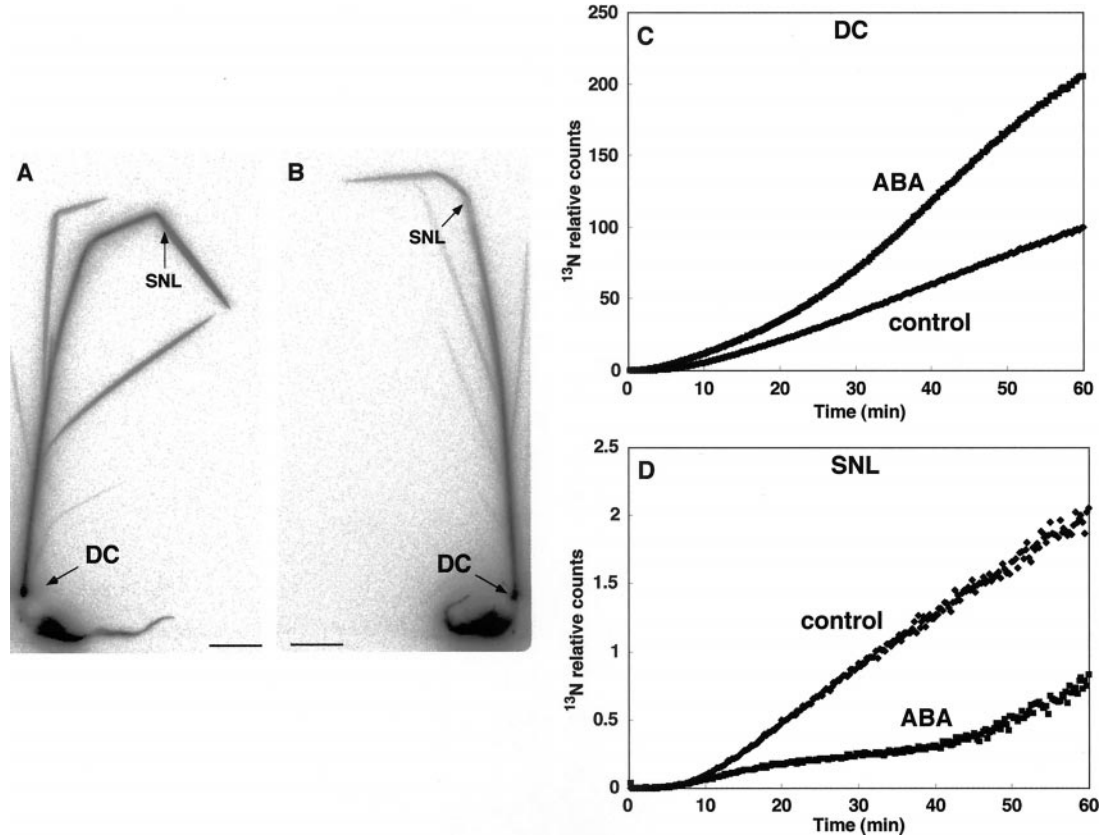

Figure 7. $^{13}NH_4^+$ translocation from roots to the DC and to the SNL in rice with ABA treatment. Image of the translocation of ^{13}N in control (A) and ABA-treated (B) rice plants using BAS-1500. The accumulation of radioactivity in the DC (C) and the SNL (D) over 60 min.

transporter or Gln synthetase, GS1, genes (Li et al., 1993; Cren and Hirel, 1999).

Summarizing results, NH_4^+ assimilation from the roots to the DC in rice depends passively on water flow, and actively on NH_4^+ transporter(s) or Gln synthetase(s) activity in the roots. Some of these genes may be regulated by NO_3^-, nitrogen deficiency, ABA, or diurnally. Cloning of these genes in rice is awaited.

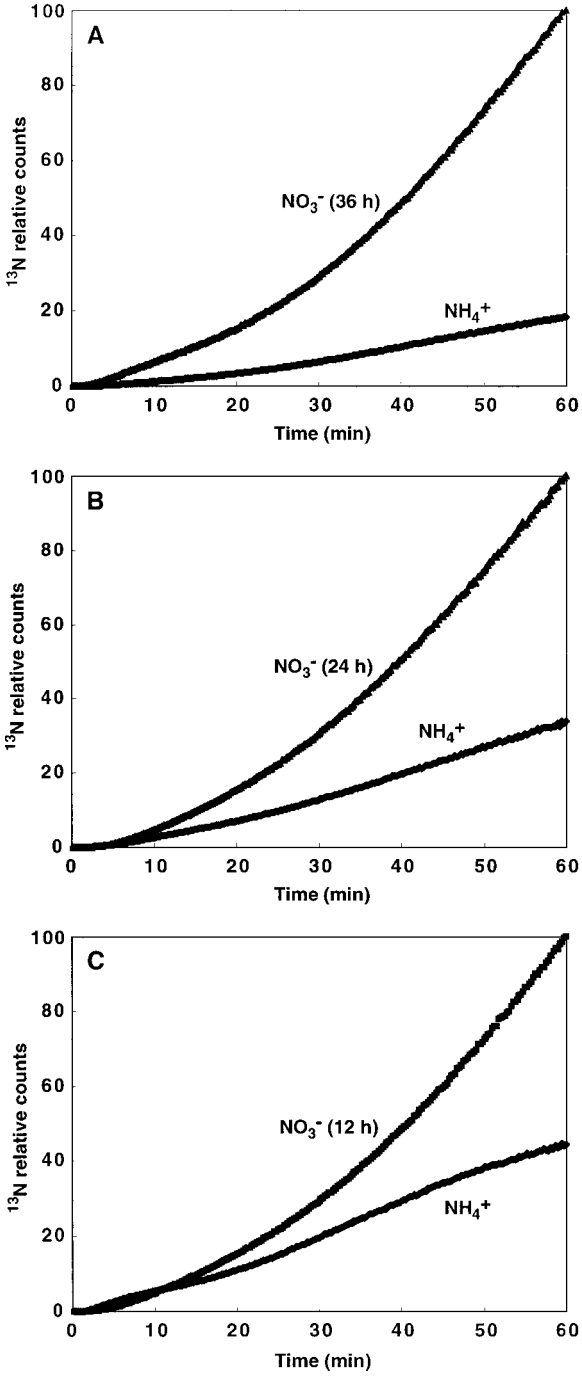

Figure 8. Enhancement of $^{13}NH_4^+$ translocation from roots to the DC of rice with NO_3^- pretreatment for 36, 24, and 12 h.

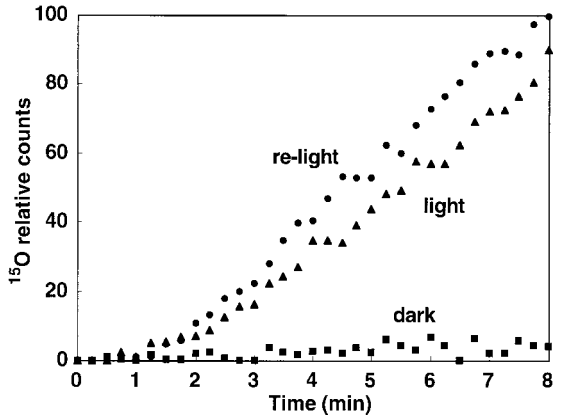

Figure 9. Water flow from the roots to the DC. Conditions were changed sequentially from light → dark → light. In the dark, water flow was stopped completely.

For a time course study, using a scintillation counter to monitor ^{13}N requires the preparation of many plants of the same age (Presland and McNaughton, 1986) as to stop the enzyme reactions plants must be killed at each sampling time. In contrast, PETIS enables visualization of the movement of labeled substances in a single intact plant body in real time, reproducibly. TIARA now produces nine positron-emitting nuclides for biological studies: ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{22}Na, ^{48}V, ^{52}Mn, ^{52}Fe, and ^{62}Zn. Many transporter genes for heavy metal ions (Mori, 1999; Guerinot, 2000), potassium (Schachtman, 2000), sugars (Lemoine, 2000), phosphate (Raghothama, 2000), sulfate (Saito, 2000), and amino acids (Fischer et al., 1998; Ortiz-Lopez et al., 2000) recently have been isolated, and various transgenic plants harboring such genes will be developed in the future. Positron-emitting nuclide studies of such transgenic plants using the PETIS method will provide novel dynamic knowledge about the movement of nutrients and metabolites in plants in real time under various nutrient and environmental stresses. In other words, it will be easy to visualize where in a plant body some transporter gene is not functioning by using a transgenic plant that is defective in the transporter gene and vice versa.

MATERIALS AND METHODS

$^{13}NH_4^+$ Synthesis

The radiotracer ^{13}N (half-life = 9.96 min) was produced in the cyclotron (Sumitomo Cypris-HM, Japan) at Hamamatsu Photonics (Hamamatsu, Japan) by proton irradiation of water. This procedure produces mostly $^{13}NO_3^-$ with high radiochemical purity (Kronzucker et al., 1995a). The irradiated solutions were supplied in sealed 20-mL glass vials. The procedures used to remove the radiocontaminants and convert $^{13}NO_3^-$ to $^{13}NH_4^+$ using Devard's alloy have been described in detail elsewhere (Kronzucker et al., 1995a, 1995b, 1995c).

^{15}O Water Synthesis

^{15}O was produced by the $^{14}N(d, n)^{15}O$ reaction in a nitrogen gas target. The target gas contained 0.5% (v/v) oxygen as carrier and was kept in continuous flow at rates of 500 mL min^{-1} and a pressure of 3 kg cm^{-2}. The gas in the target chamber was irradiated with 10 MeV deuteron beams at a current of 15 μA using the Sumitomo Cypris-HM cyclotron, and then transferred into an automated radio-synthesizing system supplied by Sumitomo Heavy Industries Ltd. The system purifies $^{15}O_2$ using an Ascarite column to remove ^{15}O-CO_2 from the ^{15}O-labeled gases. Then, $^{15}O_2$ is converted into ^{15}O-water in the form of vapor by the platinum-catalyzed reaction of $^{15}O_2$ with hydrogen at 150°C. ^{15}O-water is finally recovered from the vapor by passage of the vapor through distilled water. Almost 3 GBq of ^{15}O-water could be produced from a 4-min irradiation.

Plant Materials and Growth Conditions

Rice (*Oryza sativa* L. cv Nipponbare) seeds were germinated at room temperature on paper towels soaked with distilled water. After germination, plantlets were transferred to a plastic net floating on tap water, pH 5.5, in a greenhouse under natural light. After 3 weeks, plants were transferred to nutrient solution consisting of 1 mM $(NH_4)_2SO_4$, 0.3 mM NaH_2PO_4, 0.7 mM K_2SO_4, 2.0 mM $CaCl_2$, 0.5 mM $MgSO_4$, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.2 μM $CuSO_4$, 0.5 μM $ZnSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, and 0.1 mM Fe-EDTA. The nutrient solution was changed every week.

The $^{13}NH_4^+$ absorption experiments were carried out when the plants had one to three tillers and there were four to six leaves on the main shoots. (a) For nitrogen-deficiency treatment, plants were cultured under a light intensity of 500 μmol m^{-2} s^{-1} in a growth chamber under artificial light, or under 1,500 μmol m^{-2} s^{-1} in a greenhouse under natural light, and then transferred to culture solution without NH_4^+ for 3 d. In all cases, plants grown with complete nutrient solution under the same light conditions were used as controls. (b) For NO_3^- pretreatment, NH_4^+-fed plants were cultured for 36 h with NO_3^- as the sole nitrogen source in nutrient solution consisting of 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.1 mM KCl, 0.1 mM KH_2PO_4, 0.5 mM $MgSO_4$, 0.1 mM Fe(III)-EDTA, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.2 μM $CuSO_4$, 0.5 μM $ZnSO_4$, and 0.01 μM $(NH_4)_6Mo_7O_{24}$.

Absorption and Translocation of $^{13}NH_4^+$ in Plants

To study $^{13}NH_4^+$ absorption from roots, the roots of a single plant were placed in a polyethylene bag that contained 15 mL of culture solution without NH_4^+. To maintain geometry, the plant and bag were placed between two acrylic boards centered between the PETIS detectors. $^{13}NH_4^+$ (100–500 MBq, carrier-free in 1 mL) was added to the culture solution after synthesis with gentle aeration for immediate mixing. The light intensity was 500 μmol m^{-2} s^{-1} unless otherwise described. The PETIS detectors (the detection area was 50 × 60 mm) were focused on the DC at the basal part of the shoot (Nakanishi et al., 1999) or on the leaves. The γ-rays emitted from decaying positrons from ^{13}N were counted over time using the coincident method with the paired detectors. The data were automatically corrected using 9.96 min as the half-life of ^{13}N. After a 60-min trace analysis, the plant was removed from the polyethylene bag and the roots were gently washed for 1 min in 100 mL of 5× complete culture solution containing NH_4^+. Then the plant was placed inside the cassette of a BAS-imaging plate for 10 to 20 min. This is very sensitive to positrons and produced a clear radioautograph using the BAS-1500 Imaging System (Fuji Photo Film, Tokyo). In some cases, part of a leaf or the DC was placed between two PMPS probes to directly count the paired γ-rays from decaying positrons in the tissues using the coincidence method (Uchida et al., 1998). The spatial position of PMPS probe should be such that it escapes direct irradiation by $^{13}NH_4^+$ solution. For this reason the longest leaf (the newest leaf or the SNL) was selected as detection point of ^{13}N translocation from roots.

To study $^{13}NH_4^+$ absorption from the leaf, we also used the SNL (not the newest leaf), because this leaf was the longest one. The leaf was cut at the tip in distilled water to avoid the intrusion of air into the exposed leaf tissues. The cut end of the leaf was dipped in 3 mL of culture solution (5× culture solution without NH_4^+) in the vial and $^{13}NH_4^+$ (1 GBq, carrier-free in 1 mL) was added. This vial was shielded with lead blocks to protect the probes of the PETIS camera from direct irradiation positrons from the $^{13}NH_4^+$ solution.

Dark (2-h pretreatment), nitrogen deficient (3-d pretreatment), and 1 mM MSX (30-min pretreatment), NO_3^- (36-, 24-, and 12-h pretreatment), and 0.1 mM ABA (30 min pretreatment) treatments were used to evaluate their effects on $^{13}NH_4^+$ assimilation/translocation.

^{15}O Water Flow Experiments

^{15}O-water (1 mL = 0.5 GBq) was supplied to 15 mL of 5× culture solution. The PETIS detectors were focused on the DC and the time course followed under illumination (500 μmol m^{-2} s^{-1}) for 15 min. After 60 min in darkness, additional ^{15}O-water (0.5 GBq) was supplied and the DC was monitored for 30 min in darkness. Then, after an additional 60 min of illumination, more ^{15}O-water was supplied and the DC was monitored for 15 min with illumination (500 μmol m^{-2} s^{-1}). The data were automatically corrected using 2.04 min as the half-life of ^{15}O.

In all $^{13}NH_4^+$ and ^{15}O-water experiments, each experiment was repeated at least three times to confirm the reproducibility of the results.

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