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Feedback Regulation of the Ammonium Transporter Gene Family *AMT1* by Glutamine in Rice

Yutaka Sonoda [1, 6], Akira Ikeda [1, 2, 6], Satomi Saiki [3], Tomoyuki Yamaya [4, 5] and Junji Yamaguchi [1, 7]

[1] *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-ku N10-W8, Sapporo, 060-0810 Japan*
[2] *CREST, JST (Japan Science and Technology Agency)*
[3] *Division of Bioresources and Product Science, Graduate School of Agriculture, Hokkaido University, Kita-ku N9-W9, Sapporo, 060-8589 Japan*
[4] *Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, 981-8555 Japan*
[5] *Plant Science Center, RIKEN, 1-7-22 Suehiro, Tsurumi-ku, Yokohama, 230-0045 Japan*

The three members of the rice *OsAMT1* gene family of ammonium transporters show distinct expression patterns; constitutive and ammonium-promoted expression in shoots and roots for *OsAMT1;1*; root-specific and ammonium-inducible expression for *OsAMT1;2*; root-specific and nitrogen-repressible expression for *OsAMT1;3* [Sonoda et al. (2003), *Plant Cell Physiol.* 44: 726]. To clarify the feedback mechanisms, and to identify regulatory factors of the *OsAMT1* genes, the accumulation of the three mRNAs and its dependence on endogenous nitrogen compounds (as quantified by capillary electrophoresis) was studied. Ammonium application to roots following a period of nitrogen starvation induced accumulation of *OsAMT1;1* and *OsAMT1;2* mRNA, but a decrease of *OsAMT1;3* mRNA levels. The expression patterns of the three genes showed good correlation (positive in *OsAMT1;1* and *OsAMT1;2*, negative in *OsAMT1;3*) with the root tissue contents of glutamine but not of ammonium. The ammonium effects on *OsAMT1* expression were prevented by methionine sulfoximine, an inhibitor of glutamine synthetase. Moreover, glutamine had the same effect on transcriptional regulation of *OsAMT1* genes as ammonium, indicating that glutamine rather than ammonium controls the expression of ammonium transporter genes in rice. These results imply that rice possesses unique mechanisms of adaptation to variable nitrogen sources in the soil.

Keywords: Ammonium assimilation — Ammonium uptake — *AMT1* — Methionine sulfoximine — Root — *Oryza sativa*.

Abbreviations: AMT, ammonium transporter; CE, capillary electrophoresis; RT-PCR, reverse transcription-PCR; MSX, methionine sulfoximine.

Introduction

Inorganic nitrogen uptake into plant roots is under strict control in accordance with the nitrogen demand of the plant (Crawford and Glass 1998). Rice grown in paddy fields predominantly utilizes ammonium during most of the growing period, since ammonium is the major form of inorganic nitrogen in hypoxic and anaerobic soils (Sasakawa and Yamamoto 1978). However, excessive ammonium uptake into plants can lead to toxic effects (Britto et al. 2001, Kronzucker et al. 2001); ammonium uptake and metabolism therefore must be tightly regulated.

Ammonium uptake is predominantly mediated by ammonium transporters that have been isolated and partially characterized in several plant species, including *Arabidopsis thaliana* (*AtAMT1;1*, *AtAMT1;2*; *AtAMT1;3*, and *AtAMT2*; Gazzarrini et al. 1999, Sohlenkamp et al. 2002), *Lycopersicon esculentum* (*LeAMT1:1*, *LeAMT1;2*, and *LeAMT1:3*; Lauter et al. 1996, von Wirén et al. 2000) and *Lotus japonicus* (*LjAMT1;1*, *LjAMT2;1*; Salvemini et al. 2001, Simon-Rosin et al. 2003). Rice (*Oryza sativa* L.) possesses the *OsAMT2* gene family (Suenaga et al. 2003). In addition, three *OsAMT1* genes have been characterized by two groups independently (Sonoda et al. 2003, Kumar et al. 2003), and were designated *OsAMT1;1* (identical to *OsAMT1,1*), *OsAMT1;2* (identical to *OsAMT1,3*) and *OsAMT1;3* (identical to *OsAMT1,2*). We previously demonstrated (Sonoda et al. 2003) that the *AMT1* family of rice comprised of no more than these three genes, which showed distinct expression patterns. *OsAMT1;1* expression was constitutive in shoots and promoted by ammonium in roots; *OsAMT1;2* expression was root specific and ammonium inducible, while *OsAMT1;3* was also expressed specifically in roots but was repressed by nitrogen. These results implied feedback regulation, i.e. positive and negative control mechanisms for *AMT1* genes in rice.

It has been suggested that the cellular nitrogen status controls nitrogen and carbon metabolism as well as nitrogen absorption in roots (Stitt 1999, Stitt et al. 2002, Foyer et al. 2003). The endogenous level of glutamine is a useful index of cellular nitrogen status; it is tightly associated with the negative regulation of the genes for nitrate reductase and nitrate transporters (Hoff et al. 1994, Quesada et al. 1997, Krapp et al. 1998, Vidmar et al. 2000). A similar relationship was reported for the ammonium transporter gene, *AtAMT1;1* (Rawat et al. 1999). In addition, expression of *BnAMT1;2* in detached rape leaves was promoted by ammonium but repressed by glutamine

[6] Y.S. and A.I. contributed equally to this work.
[7] Corresponding author: E-mail, jjyama@sci.hokudai.ac.jp; Fax, +81-11-706-2737.

Table 1 Accumulation of nitrogen compounds in nitrogen-starved rice roots after ammonium application to the root medium

$(NH_4)_2SO_4$ treatment (h)	Nitrogen compound (μmol (g FW) $^{-1}$)				
	Ammonium	Glutamine	Glutamate	Aspartate	Asparagine
0	ND	ND	0.55±0.05	0.44±0.06	ND
0.25	1.31±0.11	1.60±0.16	0.39±0.03	0.25±0.02	ND
0.5	1.15±0.15	1.67±0.09	0.31±0.01	0.17±0.01	ND
1	1.67±0.15	4.39±0.53	0.50±0.08	0.35±0.05	ND
2	2.15±0.20	5.55±1.64	0.54±0.01	0.38±0.01	ND
4	1.67±0.10	6.08±1.32	0.69±0.07	0.55±0.02	0.70±0.11
8	2.33±0.49	2.75±0.21	0.74±0.06	0.36±0.03	1.31±0.16
24	ND	0.31±0.06	0.51±0.01	0.36±0.03	0.35±0.06
48	ND	ND	0.46±0.03	0.37±0.03	0.13±0.02

Rice seedlings were precultured hydroponically in nitrogen-free medium; 0.15 mM $(NH_4)_2SO_4$ were added at time 0. Ammonium and amino acid contents of root tissue were quantified by CE at the times indicated. ND, not detectable.

and glutamate (Pearson et al. 2002). Despite this information, the regulatory mechanisms of *AMT1* genes are still not entirely clear.

We describe feedback regulation in the *OsAMT1* gene family, and identify cytosolic glutamine as a promising candidate of the regulator. Glutamine in roots inhibits expression of *OsAMT1;3*, but also promotes expression of *OsAMT1;1* and *OsAMT1;2*. These findings hint at mechanisms by which rice might adapt to variable nitrogen sources in the soil solution.

Results

Relationship between ammonium assimilation and OsAMT1 expression

We previously reported short-term ammonium inducibility of *OsAMT1* genes in rice (Sonoda et al. 2003). To study long-term inducibility in roots, hydroponically grown rice was exposed to nitrogen deficiency for 3 weeks and then transferred to nutrient solution containing 0.15 mM $(NH_4)_2SO_4$. Ammonium in the nutrient solution decreased to about 3/4 after 4 h, to about 1/8 after 8 h, and was almost depleted after 24 h of the treatment (Fig. 1A). After 0, 2, 4, 8, 24, and 48 h of treatment, *OsAMT1* mRNA accumulation in the roots was examined by semi-quantitative RT-PCR (Fig. 1B). The *OsAMT1;1* transcript was transiently promoted after the addition of ammonium, but control levels were restored after 4 h. No *OsAMT1;2* transcript was detectable under nitrogen-starved conditions; ammonium induced expression that petered out after 24 h. In contrast, the *OsAMT1;3* transcript decreased substantially upon ammonium addition and recovered after 8 h.

In plants grown under identical conditions as those used for expression analysis, the contents of ammonium and various amino acids in roots were quantified by capillary electrophoresis (CE; Table 1 and Fig. 1C for ammonium and glutamine).

Table 2 Accumulation of nitrogen compounds in rice roots

Treatment	Nitrogen compound (μmol (g FW) $^{-1}$)				
	Ammonium	Glutamine	Glutamate	Aspartate	Asparagine
–N	ND	ND	0.55±0.05	0.44±0.06	ND
$+NH_4^+$	1.67±0.10	6.08±1.32	0.69±0.07	0.55±0.02	0.70±0.11
+MSX	1.15±0.15	ND	0.22±0.01	0.22±0.02	ND
$+NH_4^+$+MSX	2.39±0.37	ND	0.27±0.02	0.35±0.07	ND
+Gln	2.35±0.25	6.95±2.13	0.78±0.15	0.61±0.12	0.62±0.12

Rice seeds were precultured hydroponically in nitrogen-free medium for 3 weeks and then were transferred to the test solutions indicated (–N, nitrogen-free medium; $+NH_4^+$, nitrogen-free nutrient solution plus 0.15 mM $(NH_4)_2SO_4$; +MSX, nitrogen-free nutrient solution plus 1 mM MSX alone; $+NH_4^+$+MSX, nitrogen-free nutrient solution plus 0.15 mM $(NH_4)_2SO_4$ and 1 mM MSX; +Gln, nitrogen-free nutrient solution plus 5 mM glutamine). After 4 h, ammonium and amino acids in the root tissue were quantified by CE. ND, not detectable.

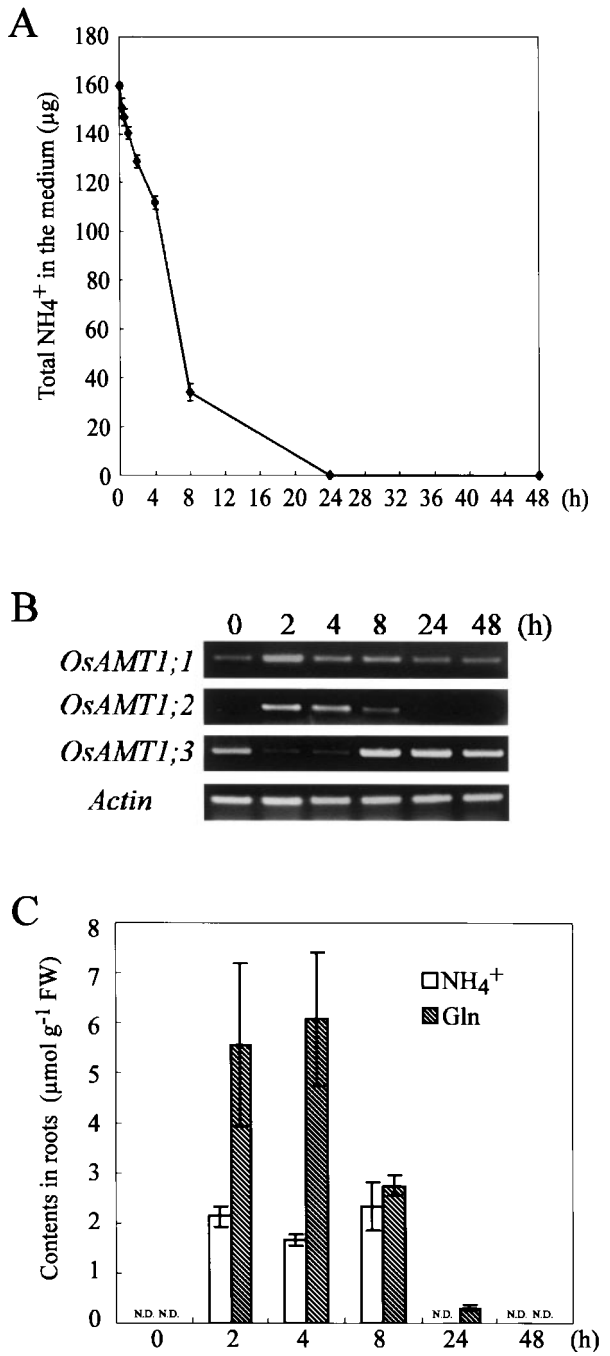

Fig. 1 Consumption of ammonium in the medium (A), ammonium-inducibility of *OsAMT1* mRNA accumulation (B), and contents of ammonium and glutamine in root tissues of rice plants (C). (A) Rice seeds were precultured hydroponically in a nitrogen-free medium before 0.15 mM $(NH_4)_2SO_4$ were added at time 0. Ammonium in the solution was quantified by CE at the times indicated. Bar indicates ± SE (n = 3). (B) Expression of *OsAMT1;1*, O*sAMT1;2*, and *OsAMT1;3* in roots determined by semi-quantitative RT-PCR at different times after the start of the ammonium treatment. As a control, *Actin* mRNA was also examined. (C) Levels of ammonium and glutamine in roots at different times after the start of the ammonium treatment, quantified by CE. Bars indicate ± SE (n = 3). ND, not detectable.

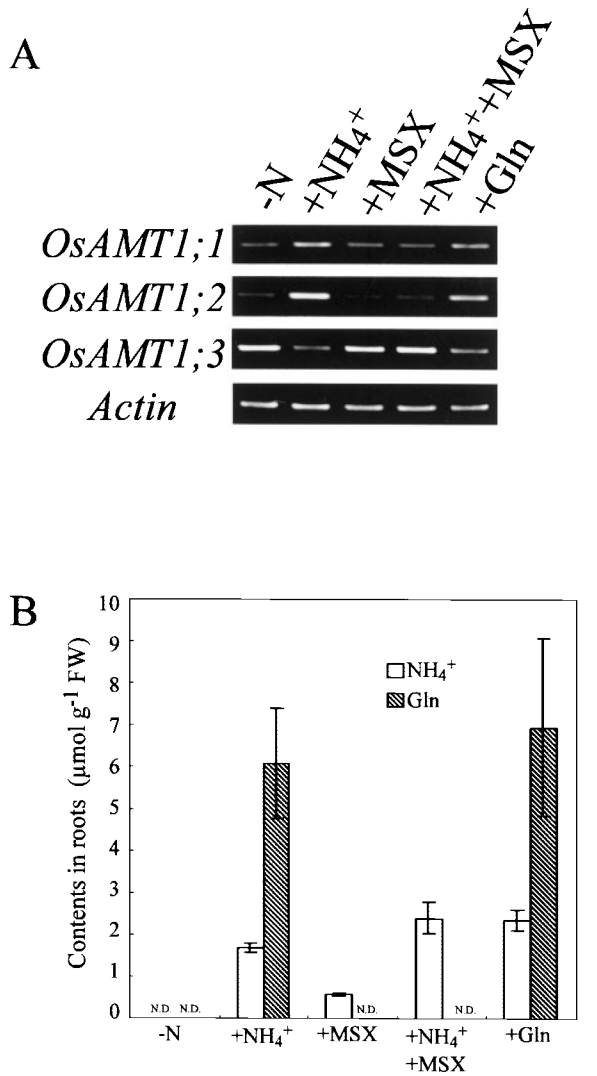

Fig. 2 Effects of ammonium, glutamine, and MSX on *OsAMT1* mRNA accumulation. (A) Rice seeds were precultured hydroponically in nitrogen-free medium and were then transferred to media containing either no nitrogen (–N), 0.15 mM $(NH_4)_2SO_4$ (+NH_4^+), 1 mM MSX (+MSX), 0.15 mM $(NH_4)_2SO_4$ plus 1 mM MSX (+NH_4^++MSX) or 5 mM glutamine (+Gln). After 4 h, mRNA accumulation of *OsAMT1;1*, *OsAMT1;2* and *OsAMT1;3* in the roots was determined by semi-quantitative RT-PCR. *Actin* mRNA was measured as a control. (B) Ammonium and glutamine contents of roots were quantified by CE in plants grown as described for (A). Bars indicate ± SE (n = 3). ND, not detectable.

Ammonium and glutamine were undetectable under nitrogen starvation, but accumulated 15 min after the addition of ammonium (Table 1), while *OsAMT1;2* mRNA also became detectable at the same time (data not shown in this study, but see Fig. 5A in Sonoda et al. (2003)); they decreased to below the detection level again after 8 h (Table 1, Fig. 1C). The correspondence of the decrease in ammonium in the nutrient solution (Fig. 1A) and the transient peaks of endogenous ammonium and

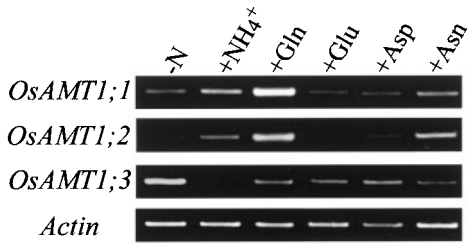

Fig. 3 Amino acid-inducibility of *OsAMT* mRNA accumulation. Rice seeds were precultured hydroponically in nitrogen-free medium before transfer to media containing either no nitrogen (–N), 0.15 mM $(NH_4)_2SO_4$ ($+NH_4^+$), 5 mM glutamine (+Gln), 5 mM glutamate (+Glu), 5 mM aspartate (+Asp) or 5 mM asparagine (+Asn). After 4 h, mRNA accumulation of *OsAMT1;1*, *OsAMT1;2*, and *OsAMT1;3* in the roots was determined by semi-quantitative RT-PCR. *Actin* mRNA was quantified as a control.

glutamine (Fig. 1C) suggest that the plants efficiently took up ammonium from the solution and converted it into organic nitrogen compounds. Asparagine became detectable in the roots 4 h after addition of ammonium and then decreased again after 8 h, thus showing a qualitatively similar development as glutamine did. On the other hand, glutamate and aspartate levels showed no clear dependence on ammonium supply (Table 1). Taken together, the expression patterns of the *OsAMT1* genes showed good correlation with the cellular glutamine level but less so with the concentrations of other amino acids and the cellular ammonium contents.

Effects of the glutamine synthetase inhibitor MSX on OsAMT1 expression

To clarify whether *OsAMT1s* expression is dependent on ammonium or glutamine, we applied methionine sulfoximine (MSX), an inhibitor of glutamine synthetase (GS). GS functions in the glutamine synthetase/glutamine-2-oxoglutarate aminotransferase cycle (GS/GOGAT cycle) to generate glutamine from ammonium and glutamate (Lam et al. 1996). MSX blocks the action of GS and prevents glutamine synthesis.

Ammonium application following nitrogen starvation increased mRNA levels of *OsAMT1;1* and *OsAMT1;2*, and decreased the level of *OsAMT1;3* mRNA (Fig. 2A). These ammonium responses did not occur when MSX was applied together with ammonium, and MSX alone showed no effect (Fig. 2A). Glutamine mimicked the ammonium effect (Fig. 2A), suggesting that this amino acid rather than ammonium itself controlled the expression of *OsAMT1* genes. To evaluate this hypothesis, we examined endogenous levels of nitrogen compounds in roots by CE (Fig. 2B, Table 2). Ammonium as well as glutamine accumulated in roots following application of ammonium or glutamine to the root medium. However, application of MSX in addition to ammonium resulted in increased ammonium levels, while glutamine remained below the detection threshold. These results confirmed the hypothesis that the transcription of *OsAMT1* genes is regulated by glutamine.

Effects of various amino acids on OsAMT1 expression

Ammonium showed positive effects on the expression of *OsAMT1;1* and *OsAMT1;2*, and a negative one on *OsAMT1;3*. These effects were mimicked not only by glutamine but also by asparagines (Fig. 3). On the other hand, glutamate and aspartate were ineffective with respect to *OsAMT1;1* and *OsAMT1;2*. However, the negative effect on *OsAMT1;3* expression observed after application of ammonium or glutamine also occurred when glutamate and aspartate were added (Fig. 3). This implies that differences exist in the regulatory mechanisms of *OsAMT1;3* on one hand, and *OsAMT1;1* and *OsAMT1;2* on the other. Tissue levels of glutamate and aspartate were similar in all treatments, while asparagine resembled glutamine by increasing under both the ammonium and the glutamine treatment (Table 2).

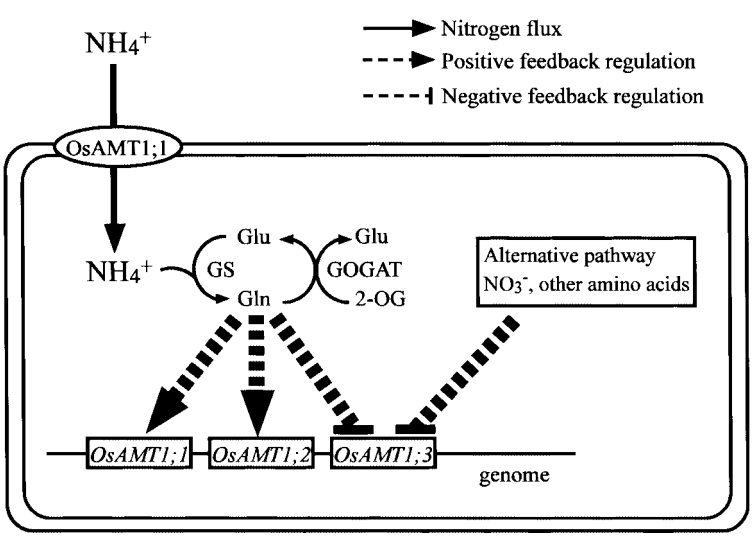

Fig. 4 Hypothetical model of the regulation of *OsAMT1* genes in rice roots. *OsAMT1* gene expression is positively feedback-regulated (dotted arrow) and negatively feedback-regulated (dotted T line) by cytosolic glutamine (Gln). Nitrogen fluxes are shown as solid arrows. See text for details.

Discussion

Expression patterns and physiological roles of OsAMT1 genes

Previously, we had suggested that the three genes of the rice ammonium transporter gene family *OsAMT1* were regulated by ammonium, since the expression of the genes had been found to be modulated by ammonium (Sonoda et al. 2003). However, in the present study we show that the expression of *OsAMT1* genes does not depend on endogenous ammonium levels but on endogenous glutamine. The putative regulatory pathways are summarized in Fig. 4. The product of *OsAMT1;1* is likely to be responsible for basic ammonium uptake into the roots, because this gene is constitutively expressed. Ammonium is rapidly converted into glutamine by the GS/GOGAT cycle (Lam et al. 1996). Increased glutamine levels cause an additional promotion of *OsAMT1;1*, strongly induce *OsAMT1;2*, and suppress *OsAMT1;3* (Fig. 4). In fact, accumulation of *OsAMT1;2* mRNA was detectable 30 min after ammonium application to nitrogen-starved rice plants (Sonoda et al. 2003), and its level remained high as long as endogenous glutamine concentrations were increased (Fig. 1B, C). Therefore it seems likely that OsAMT1;2 mainly functions in ammonium uptake from ammonium-enriched soils. On the other hand, a low glutamine status favors the expression of *OsAMT1;3*, which showed maximal mRNA accumulation when endogenous glutamine was not detectable (Fig. 1, 2). Thus, OsAMT1;3 seems to support OsAMT1;1 in taking up ammonium when ammonium availability in the soil is low.

Positive feedback regulation of OsAMT1;1 and OsAMT1;2 by glutamine

Glutamine is the main transportable form of organic nitrogen and a nitrogen storing compound in plants (Lam et al. 1996). It is a key element not only in primary nitrogen metabolism but also in nitrogen recycling in the processes of photorespiration and leaf senescence (Lam et al. 1996).

In this communication we demonstrated that cytosolic glutamine may act as a feedback regulator of the *OsAMT1* gene family. Glutamine suppressed the expression of the *OsAMT1;3*, the same down-regulation of the *AMT1* genes was previously reported in *Arabidopsis* (Rawat et al. 1999). We showed in this study that glutamine also promoted the expression of *OsAMT1;1* and *OsAMT1;2*. Previously, three *AtAMT1* genes (*AtAMT1;1–1;3*) have been characterized in detail but no positive regulation by ammonium and/or glutamine were detected (Gazzarrini et al. 1999, Rawat et al. 1999). In tomato, *LeAMT1;2* expression was enhanced by ammonium application, but it was suppressed by glutamine (Becker et al. 2002), representing a regulatory pattern that we did not detect in any of the *OsAMT1* genes. Previously, glutamine had been considered to be a negative regulatory factor of nitrogen uptake, because (1) ammonium uptake was reduced by concomitant glutamine application in barley and wheat roots (Causin and Barneix 1993, Lee et al. 1992), and (2) expression of ammonium and nitrate transporter genes was suppressed by amino acids including glutamine in *Arabidopsis* and barley (Quesada et al. 1997, Krapp et al. 1998, Rawat et al. 1999, Vidmar et al. 2000). The results presented in this study provide evidence for a hitherto unknown role of glutamine as a positive regulator of ammonium transporter genes in rice.

Negative and multiple feedback regulation of OsAMT1;3

Expression of *OsAMT1;3* was suppressed by all amino acids tested (Fig. 3), indicating that *OsAMT1;3* expression responds to the nitrogen status of the tissue rather than to the concentration of a single compound. *OsAMT1;3* seems also to be regulated by nitrate (as indicated in Fig. 4), since levels of *OsAMT1;3* mRNA decreased after application of nitrate (Sonoda et al. 2003). The dose effects of applied nitrate and amino acids on the expression of the *OsAMT1* genes, however, are likely to be depend on their permeability and pool sizes of root cells. Indeed 6–8 h treatment with nitrate affects increase in endogenous levels for ammonium, glutamine, glutamate, aspartate and asparagine in maize seedlings (Sivasankar et al. 1997) and barley roots (Vidmar et al. 2000) and the application of amino acid also affects the contents for the other amino acids (Vidmar et al. 2000). Therefore, further careful experiments will be needed.

AtAMT1;1, which resembles *OsAMT1;3* in its expression pattern, seems to be regulated by sugars as well as by nitrogen sources (Kaiser et al. 2002). Bacteria such as *E. coli* sense the concentration of glutamine as an index of cytosolic nitrogen status, and 2-oxoglutarate as a measure of carbon status to regulate nitrogen metabolism (Arcondéguy et al. 2001). The *E. coli* PII protein is believed to be a regulatory factor for nitrogen metabolism (Arcondéguy et al. 2001), and is involved in the control of the activity of ammonium transporters (Coutts et al. 2002). GLB1, an *Arabidopsis* PII protein homolog, has been suggested to regulate glutamine utilization (Hsieh et al. 1998, Smith et al. 2003). Future studies will have to examine the relationship between *OsAMT1* genes, especially *OsAMT1;3*, and the rice PII homolog with regard to the sensing of the cytosolic glutamine status. However, preliminary studies showed that 2-oxoglutarate does not affect the expression of the *OsAMT1* genes (data not shown).

Unique regulatory mechanisms in the ammonium uptake system in rice

Why does rice differ from other species concerning glutamine effects on nitrogen uptake in roots? Rice must be expected to have developed adaptations to environments in which toxic levels of ammonium prevail, as they are found in hypoxic, flooded soils. Ammonium taken up into the roots must be promptly assimilated by GS/GOGAT cycle because of its toxicity (Lam et al. 1996). Indeed GS1 and NADH-dependent GOGAT (NADH-GOGAT) is localized in epidermis and exodermis in rice roots (Ishiyama et al. 1998, Ishiyama et al. 2003). In addition, glutamine also promoted the expression of

NADH-GOGAT (Hirose et al. 1997). In rice, glutamine and asparagine, which carry ammonium-derived nitrogen, are transported to growing organs through the vascular system, while nitrate is translocated in the xylem. In fact, phloem sap of rice contains as much as 0.1 M free amino acids including glutamine and asparagine (Hayashi and Chino 1990). In most other plant species, the majority of the nitrogen allocated to the stem and leaves is translocated as nitrate, which is thought to be harmless to cells (Crawford and Glass 1998, Williams and Miller 2001). Thus, higher concentrations of glutamine would be expected in rice as compared to plants which mainly transport nitrogen as nitrate. Consequently, in plants such as tomato and *Arabidopsis*, cytosolic nitrate would seem a useful indicator of environmental nitrogen availability, while glutamine could represent an index of in planta nitrogen status. In contrast, plants mainly utilizing ammonium such as rice, cannot use intracellular levels of the primary nitrogen source as a measure of nitrogen availability, due to its toxicity. Thus, early intermediates of nitrogen metabolism such as glutamine have to serve as indicators of both the environmental and the cytosolic nitrogen status.

We focused on the initial steps of ammonium uptake and translocation in roots in this study. However, the ammonium translocation would be also regulated by the nitrogen status via a consequence of internal nitrogen cycling and remobilization. Further experiments will be needed to evaluate the idea.

Materials and Methods

Plant material and growth conditions

Rice (*O. sativa* L. cv Nipponbare) seeds were sterilized in 1% (v/v) NaClO for 20 min and then thoroughly rinsed in sterile distilled water. Seedlings were grown hydroponically first in tap water for a week, and then in nitrogen-free nutrient solution (7 μM Na_2HPO_4, 16 μM KCl, 7 μM $CaCl_2{\cdot}2H_2O$, 15 μM $MgCl_2{\cdot}6H_2O$, 36 μM $FeSO_4{\cdot}7H_2O$, 9 μM Mn $FeSO_4{\cdot}4$ H_2O, 45 μM H_3BO_4, 3 μM $ZnSO_4{\cdot}7H_2O$, 0.2 μM $CuSO_4{\cdot}7H_2O$, 0.05 μM $Na_2MoO_4{\cdot}2H_2O$) for 2 weeks. Plants were then transferred in batches of three to one of the test media (nitrogen-free nutrient solution; nitrogen-free nutrient solution plus 0.15 mM $(NH_4)_2SO_4$; nitrogen-free nutrient solution plus 1 mM MSX alone; nitrogen-free nutrient solution plus 0.15 mM $(NH_4)_2SO_4$ and 1 mM MSX; nitrogen-free nutrient solution plus 5 mM of one of the amino acids indicated in Fig. 3). Plants were grown under continuous light, at 60% relative humidity and 30°C.

RNA extraction and semi-quantitative RT-PCR

Total RNA was extracted by the guanidine thiocyanate method. One to three μl RNA (0.6 μg RNA) were used as a template for the first strand cDNA synthesis, which was performed using ReverTraAce-α-® (TOYOBO, Osaka, Japan) in a reaction volume of 20 μl containing 1× RT buffer, 1 mM dNTPs, 0.5 μM oligo-dT primer, and 0.5 U RNase inhibitor (Promega, Tokyo, Japan). Aliquots of 1.5 μl of the produced cDNA served as a template for *OsAMT* cDNA synthesis. PCR amplification was performed in 25 cycles using either the AmpliTaq Gold™ polymerase (Applied Biosystems, Tokyo, Japan; for *OsAMT1;1*, *OsAMT1;2*, and *Actin*) or the *Takara Ex-Taq*™ polymerase (for *OsAMT1;3*). We used the primer pairs 5′-AAGAAGCTCGGCCTGCTCCGC and 5′-TGTGCAAAAGAAAATTAAACC (*OsAMT1;1*), 5′-AACAAGCTGGGCTTGCTGCGC and 5′-ACTATCTTTTTCTTCCTATTA (*OsAMT1;2*), 5′-GCACATCGTGCAGATCCTGG and 5′-CTGATACAAACAGGACACGTC (*OsAMT1;3*). For *Actin* (*RAc1*; Mcelroy et al. 1990) amplification the primers 5′-CTTCATAGGAATGGAAGCTGCGGGTA and 5′-CGACCACCTTGATCTTCATGCTGCTA were used. Amplified fragments were electrophoresed on 1.2% (w/v) agarose gels and visualized by ethidium bromide staining. In the previous study (Sonoda et al. 2003), we carefully evaluated the conditions for the semi-quantitative RT-PCR to quantify accurate amounts of the *OsAMT1* mRNAs.

NH_4^+ and amino acid determination by capillary electrophoresis

NH_4^+ and amino acids were extracted by grinding root tissues in 0.01 M HCl at 4°C. After centrifugation at 20,400×*g* for 5 min at 4°C and filtration of the supernatant through a Ultrafer®-MC Centrifugal Filter Unit (Millipore Corporation, Bedford, U.S.A.) at 4,400×*g* at 4°C, NH_4^+ and amino acids were respectively determined in the filtrate with a HP^{3D} Capillary Electrophoresis System (Hewlett-Packard, Waldbronn, Germany) as described by François et al. (1995), Soga and Ross (1999) and Soga and Imaizumi (2001) with minor modifications.

Acknowledgments

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