2.34 Ammonium Uptake and Assimilation in Rice

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1. Introduction

More than 70% of the world's rice is produced in intensively cultivated, irrigated paddy fields. In flooded paddy fields, the bulk of the soil is hypoxic or anaerobic and the major form of nitrogen available to plants is ammonium. Rice plants evolutionally acquired the ability to be resistant to these conditions[1]. This is in marked contrast to most well-aerated agricultural soils in which nitrate is the predominant inorganic nitrogen species. Ammonium is the preferred nitrogen species taken up by rice and it is superior to nitrate in terms of fertilizer efficiency in paddy fields[2]. It has been demonstrated that influx of ammonium into rice roots gradually increased between $2x10^{-6}$ and $1x10^{-3}$ M external ammonium[3], indicating that rice plants may acclimate to the external ammonium supply by altering properties of their ammonium transport systems.

To study the regulation of ammonium uptake into rice roots, three ammonium transporter genes (*OsAMT1;1*, *1;2* and *1;3*; *Oryza sativa ammonium transporter*) were isolated and examined[4,5]. OsAMT1s belong to AMT1 family, containing 11 putative transmembrane-spanning domains. Southern blot analysis and screening of the rice genome database confirmed that with *OsAMT1;1-1;3* the complete AMT1 family of rice had been isolated. Heterologous expression of *OsAMT1s* in the yeast *Saccharomyces cerevisiae* mutant 31019b showed that all three OsAMT1s exhibit ammonium transport activity. Northern blot analysis showed a distinct expression in shoots and roots for *OsAMT1;1*, root-specific and ammonium-inducible expression for *OsAMT1;2*, and root-specific and nitrogen-derepressible expression for *OsAMT1;3*.

Real time monitoring of ammonium translocation in rice by PETIS (positron emitting tracer imaging system) demonstrated an active uptake and movement of ammonium[6]. Here we show the attempt of real time monitoring of ammonium translocation using rice for wild-type and transgenic plant constitutively expressing *OsAMT1;2* gene.

2. Experimental procedures

2.1 Transgenic Rice

The 35S promoter of cauliflower mosaic virus (CaMV) from pBI221 was connected with ORF of *OsAMT1;2* cDNA. Rice was transformed by the procedure described by Hiei et al.[7]. Transformants of rice were grown in greenhouse. Hygromycin resistance of rice seedlings was assayed and screened. Homozygous plants for the transgene were used in the experiments.

2.2 Plant Materials and Growth Conditions

Rice (*Oryza sativa* L) seeds were sterilized and then thoroughly rinsed in water. Seedlings were grown hydroponically first in tap water for a week, and then in nitrogen-free nutrient solution, which is described in the reference[5].

2.3 $^{13}NH_4^+$ Synthesis

The radiotracer ^{13}N (half-life = 9.96 min) was

produced in the cyclotron at TIARA by proton irradiation of water. This procedure was described in detail [6].

2.4 Ammonium translocation activity measurement by PETIS

To study $^{13}NH_4^+$ uptake and translocation from roots to whole plant, the roots of a single plant were placed in a 16 cm height glass test tube that contained 20 mL of culture solution with nitrogen source (0.015 mM $(NH_4)_2SO_4$). $^{13}NH_4^+$ (50 MBq, carrier-free in 6 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 analysis performed under ambient conditions described in detail[6]. For nitrogen-deficiency treatment, plants were transferred to culture solution without nitrogen source for 3 days.

2.5 Ammonium and amino acid determination by capillary electrophoresis

NH_4^+ and amino acids were grinding root tissues in 0.01 N HCl at 4°C. Further procedures are described in the reference[6].

3. Results and Discussion

We succeeded in production of the transgenic rice expressing *OsAMT1;2* ORF under constitutive promoter of CaMV 35S, designated as *OsAMT1;2* sense rice. Two transgenic lines were established, which called S1 and S2. Quantitative RT-PCR analysis revealed that transcript for *OsAMT1;2* is much accumulated in the S2 plant compared to that in the S1 (data not shown).

To evaluate the ability of uptake and assimilation of ammonium in the *OsAMT1;2* sense rice, quantitation of ammonium and glutamine in roots after ammonium treatment was examined (Fig. 1). Level of ammonium in

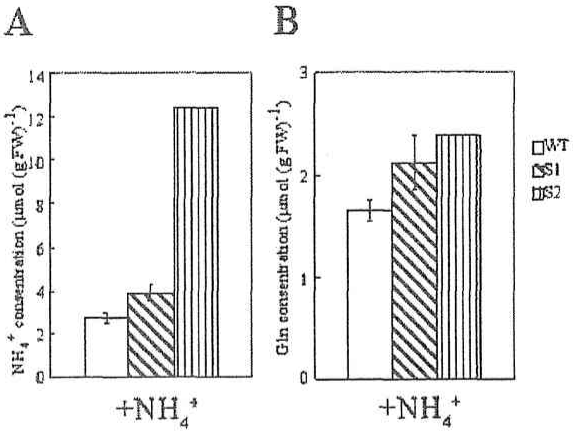

Fig. 1 Levels of ammonium and glutamine in roots quantified by capillary electrophoresis after ammonium treatment. Nitrogen-deprived plants (wild-type, WT; S1 and S2) was treated with 0.15 mM $(NH_4)_2SO_4$ for 4 hr and subjected to the quantitation.

roots of the S1 and S2 plants was approximately 1.4-fold and 4.5-fold higher than that in the wild-type (Fig. 1A), while glutamine was also accumulated in 1.4-fold in both S1 and S2 plants (Fig. 1B), indicating that Overexpression of ammonium transporter gene leads to efficient uptake of ammonium in rice plant.

To confirm the ability of the *OsAMT1;2* sense rice having efficient uptake of ammonium, shoot translocation of $^{13}NH_4^+$ from roots was performed by the PETIS analysis (Fig. 2). Nitrogen-deprived S1 and wild-type plants were transferred to 0.015 mM $(NH_4)_2SO_4$ for 30 min and then subjected to the radioactive $^{13}NH_4^+$. At 45 min after the radioactive ammonium treatment, ^{13}N signal was detected at the basement of leaf sheath in the wild-type (Fig. 2B and 2C), whereas the signal was detectable in most parts of the S1 plant (Fig. 2E and 2F). Accumulation of the signal at the leaf sheath was remarkably enhanced in the S1 compared to that in the wild-type (Fig. 2G). These results indicate that over expression of the *OsAMT1;2* results in enhanced ability for uptake and translocation of ammonium from roots.

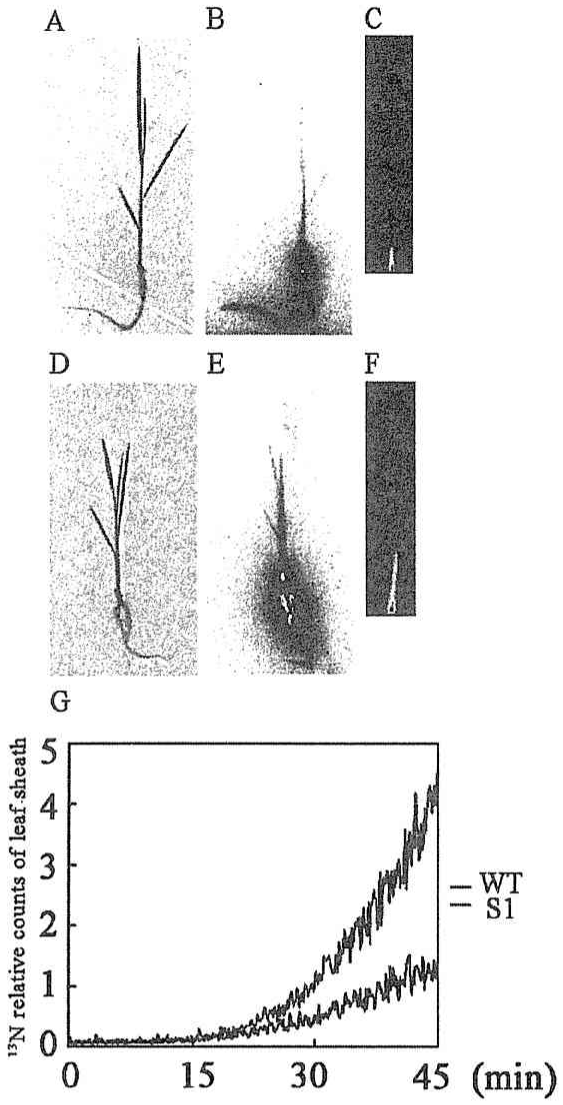

Fig. 2 Uptake and translocation of $^{13}NH_4^+$ from roots to shoot (A to F) and time-course study of translocation of $^{13}NH_4^+$ into leaf sheath (G) in *OsAMT1;2* sense rice. A to C, wild-type; D to F, S1 plant. A and D, photo; B and E, radioactive imaging by BAS1500; C and F PETIS ^{13}N-imaging.

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 studies will be needed to evaluate the idea.

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