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ORIGINAL ARTICLE

OsAMT1.3 expression alters rice ammonium uptake kinetics and root morphology

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Abstract High-affinity ammonium transporters (AMT1) are responsible for ammonium (NH_4^+) acquisition and/or perception in the micromolar range, and their expressions can be differentially regulated by nitrogen (N) availability. The present study characterised the functions of the rice (*Oryza sativa*) *OsAMT1.3* transporter to understand its contribution to NH_4^+ acquisition and plant adaptation to environments with low N availability. Transgenic rice plants were obtained to study the activity of the *OsAMT1.3* promoter ($P_{OsAMT1.3}$:GFP:GUS) and the overexpression of the *OsAMT1.3* gene (UBIL:*OsAMT1.3*:3xHA) in plants. The *OsAMT1.3* promoter activity was induced strongly in the absence of N and occurred primarily in the zones of lateral root emission and root tips. Anatomical sections of the segment of root tips and the middle third showed a differential pattern of *OsAMT1.3* activity. Analysis of the *OsAMT1.1–1.3* transporter expression profiles indicated that overexpression of *OsAMT1.3* positively affected *OsAMT1.2* expression. When subjected to a low N supply, plants overexpressing *OsAMT1.3* showed lower K_M and C_{min} values. Additionally, these lines showed longer roots with a higher area, volume, and number of tips. The data suggested that *OsAMT1.3* is involved in the ability of rice plants to adapt to low NH_4^+ supplies.

Keywords *Oryza sativa* L. · Nitrogen · Ammonium transporter · qRT-PCR

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Introduction

Nitrogen (N) is an essential element for plants and is the most limiting element for crop productivity and cereal grain quality (Bu et al. 2011). The current high rice productivities became possible partly because of the intensive use of fertilisers, primarily N fertilisers. Approximately 110 million tons of N fertilisers are applied annually worldwide, at high cost (FAO 2012). Additionally, this excess application may be associated with severe environmental damage (Mulvaney et al. 2008).

Plants absorb N preferentially as ammonium (NH_4^+) and nitrate (NO_3^-). Under anaerobiosis, NH_4^+ is the main form of N available to plants (Funayama et al. 2013). However, excess NH_4^+ absorption can be toxic (Britto et al. 2001), and the absorption and metabolism of this nutrient is highly regulated in plants (Sonoda et al. 2003a, b).

Plants take up NH_4^+ through high-affinity (HATS) and low-affinity (LATS) transport systems, depending on the nutrient concentration in the external medium (Wang et al. 1993). Recent studies have characterised the high-affinity ammonium transporters (*AMT1*) because they act at low soil solution N concentrations and may be involved in NH_4^+ uptake efficiency in plants (Ranathunge et al. 2014; Lima et al. 2010; Gu et al. 2013).

Genome sequencing identified several ammonium transporters belonging to the *AMT1* family in various species, such as *Oryza sativa* (*OsAMT1.1–1.3*) (Suenaga et al. 2003; Sonoda et al. 2003a, b), *Lycopersicon esculentum* (*LeAMT1.1–1.3*) (von Wirén et al. 2000), *Arabidopsis thaliana* (*AtAMT1.1–1.5*, *AtAMT2*) (Kaiser et al. 2002; Sohlenkamp et al. 2002), and *Triticum aestivum* (*TaAMT1.1–1.3*) (Jahn et al. 2004) profiles. *OsAMT1.1* is an NH_4^+-responsive gene, expressed in the roots and shoots (Ranathunge et al. 2014), whereas the *OsAMT1.2* and *OsAMT1.3* genes are specifically expressed in the roots (Sonoda et al. 2003a, b). *OsAMT1.2* responds positively to resupply with increasing doses of ammonium, whereas *OsAMT1.3* shows increased expression at N concentrations below 0.15 mM and is repressed at higher concentrations (Gaur et al. 2012).

The *OsAMT1.2* and *OsAMT1.3* gene expression profiles in the roots suggest that both are key components of the uptake system. However, they play different roles in N utilisation. OsAMT1.3 appears to act as an N sensor, whereas OsAMT1.2 acts as an NH_4^+ transporter (assimilator) at low concentrations. The observation that *OsAMT1.3* was repressed not only in the presence of NH_4^+ but also in the presence of NO_3^- has led to the hypothesis that it may serve as an N sensor (Yao et al. 2008).

Sonoda et al. (2003a, b) suggested that NH_4^+ transporters are regulated by the glutamine concentrations inside the roots and not by the NH_4^+ concentrations in the external solution (i.e., the control over the NH_4^+ uptake is internal). However, the regulation of the AMT1 family of transporters varies with the genotype and NH_4^+ availability (Gaur et al. 2012).

Plant varieties with increased N uptake efficiency in soils containing low N concentrations must be selected for sustainable agriculture (Glass et al. 2002). Therefore, the molecular and physiological responses of plants under low N concentrations should be well understood. Some researchers have suggested that the OsAMT1.3 transporter may function to signal the presence of N in the soil, in addition to its potential involvement as a transporter in the uptake of reduced NH_4^+ levels in the soil (Sonoda et al. 2003a, b; Gaur et al. 2012).

The present study aimed to evaluate the contribution of the OsAMT1.3 transporter in increasing the NH_4^+ uptake efficiency and to characterise its role in the mechanisms by which plants adapt to environments with low N availability.

Materials and methods

Plant material and growth conditions

Transgenic rice plants of the variety Nipponbare (*Oryza sativa* L. subsp. Japonica) were used in this study. The experiments were performed in a climatic chamber (light/dark cycle, 12/12 h; 28/26 °C; light intensity, 500 μmol m^{-2} s^{-1}; relative humidity, 70 %). The rice seeds were surface-sterilised with sodium hypochlorite (2 %) for 10 min and germinated in distilled water. Five days after germination (DAG), plants were transferred to Hoagland solution (Hoagland and Arnon 1950) with different N regimes. The solutions were changed every 3 days, and the pH was maintained at 5.8.

Gene constructs

Transgenic rice lines were generated by expressing *OsAMT1.3* under the control of the maize ubiquitin 1 promoter (UBIL:*OsAMT1.3*:3xHA) using the MultiSite Gateway cloning kit (Life Technologies, Carlsbad, CA, USA). The amplified fragment of *OsAMT1.3* was cloned into the molecular cloning site of the pH7m34GW vector, with the recombination sites necessary for cloning the Gateway vectors (Table S1). To generate the green fluorescent protein (GFP) and β-glucuronidase (GUS) *OsAMT1.3* construct, a 1500-bp fragment upstream of the translation initiation site was amplified from genomic DNA in two subsequent PCRs with hybrid primers (Table S2) and cloned into the molecular cloning site of the pGWFS7 vector (Karimi et al. 2002) to drive GFP expression and GUS activity. The resulting constructs were transformed into *Escherichia coli.*

Genetic transformation and in vitro development of transgenic plants

Rice plants were transformed according to the *Agrobacterium*-mediated transformation of embryogenic calli with the *OsAMT1.3* binary construct. The subsequent regeneration of transgenic lines and the separation of early events of independent stable transformations within the callus material have been described previously (Toki et al. 2006). Transformed calli were selected via hygromycin resistance conferred by a UBIL promoter-driven *hph* gene. Several T1 transformants were generated and confirmed by hygromycin resistance. The transgenic lines selected were grown in a greenhouse, and homozygous lines of the T3 generation were selected by segregation analysis for hygromycin resistance.

Localisation studies

Transgenic rice plants (L#4) were grown for 14 days in a Hoagland solution with two different N treatments: a constant N supply (2.0 mM NH_4^+) as a control or N deficiency. The roots were harvested, infiltrated with staining solution containing 5-bromo-4-chloro-3-indolyl-β-

D-glucuronide (X-gluc), and maintained at 37 °C for 3 h (Jefferson et al. 1987). Root segments (15 mm) from the tips of the roots and middle third were infiltrated with a 2.5 % glutaraldehyde solution in 0.01 M phosphate buffer. The samples were embedded in Leica® synthetic resin (hydroxyethyl methacrylate), according to the manufacturer's instructions, and sectioned using a Leica rotary microtome. The 2-µm sections were observed on an Axioplan light microscope (Zeiss) equipped with AxioVision software.

OsAMT1.3 promoter activity

Transgenic rice plants (L#4) were grown in a Hoagland solution with low N availability until 10 DAG (0.5 mM NO_3^-) to reduce *OsAMT1.3* expression after germination and reduce the interference of this dose in the subsequent treatments. The plants were then subjected to three different N regimes over 14 days: without N (control), 2.0 mM NO_3^-, and 2.0 mM NH_4^+. Roots were harvested at 0, 3, 7, and 14 days after treatment and stored at −80 °C for subsequent use.

Root samples were ground in liquid N_2; homogenised in three volumes of 50 mM Tris–HCl pH 8.0 buffer containing 1 mM EDTA, 1.5 % polyvinylpolypyrrolidone (PVPP), 10 mM dithiothreitol (DTT), 30 % glycerol, and 1 mM phenylmethylsulfonyl fluoride (PMSF); and then centrifuged at 14,000×*g* for 30 min. The supernatant was used to determine the enzyme activities. The protein concentration was determined according to Bradford (1976). The GUS activity was determined spectrophotometrically using the enzyme substrate *p*-nitrophenyl-β-D-glucuronide (PNPG), according to Aich et al. (2001). The activity was expressed as ΔOD_{405} in mg^{-1} protein h^{-1}.

OsAMT1.1–1.3 NH_4^+ transporter expression

Transgenic rice plants (L#2 and L#8), which overexpressed *OsAMT1.3*, and WT plants were grown in a Hoagland solution with 0.5 mM NO_3^-, to reduce the natural *OsAMT1.3* expression after germination. At 30 DAG, the plants were treated with a solution containing 0.5 mM NH_4^+. Plants were harvested at 2 and 6 h following treatment, and root samples were stored at −80 °C for subsequent use.

Total RNA was extracted according to GAO et al. (2001) in NTES buffer (0.2 M Tris–HCl pH 8.0, 25 mM EDTA, 0.3 M NaCl, 2 % SDS). The total RNA was quantified using a Qubit 2.0 fluorometer (Life Technologies), according to the manufacturer's instructions. The total RNA was treated with DNaseI (Life Technologies) and used for cDNA synthesis using a High-Capacity RNA-to-cDNA™ kit (Life Technologies) and oligo(dT) primers, according to the manufacturer's instructions. qRT-PCR was performed using the Power SYBR® Green PCR Master Mix kit and a StepOne real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). The PCR program consisted of 95 °C for 15 s and 60 °C for 60 s. Two qRT-PCR determinations were performed for each cDNA sample. The threshold cycle (C_t) values for each sample were normalised with the *O. sativa* elongation factor (eEF1-α) as a housekeeping gene. The relative quantity was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The primers designed by Duan et al. (2007) for the ammonium transporter genes (*OsAMT1.1*, *1.2*, and *1.3*) were used.

NH_4^+ uptake kinetics of rice lines overexpressing the *OsAMT1.3* gene

Transgenic rice plants (L#2 and L#8) were grown as previously described. At 27 DAG, the plants were submitted to N starvation for 72 h, followed by resupply with 0.2 mM NH_4^+. Samples of nutrient solution (1.0 mL) were collected at intervals of 30 min until N exhaustion. At the end of the experiment, shoots and roots were harvested (Table S3). The kinetic parameters, V_{max} and K_M, were measured by depletion of NH_4^+ in the uptake solution over time according to Claassen and Barber (1974). The ammonium content of the nutrient solution was determined according to Felker (1977). The integrated analysis of NH_4^+ uptake was calculated as $\alpha = V_{max}/K_M$ (Marschner 1995). In the final samples of nutrient solution, the concentration at which net uptake of ions ceases before the ions are completely depleted was measured (C_{min}) (Marschner 1995). Root parameters (root length, surface area, projected area, volume, and number of tips) were determined using the Winrhizo 4.1 software (Regent Instruments, Quebec, Canada).

Statistical analysis

A completely randomised experimental design was utilised, with four replicates in all experiments. Analysis of variance was performed by applying the *F* test, the averages were compared using a Scott–Knott test at $p \leq 0.05$, and the standard error was calculated.

Results

OsAMT1.3 tissue-specific localisation and quantification

To identify the sites of action of the OsAMT1.3 transporter and study its possible involvement in the adaptation of

plants to low N supplies, rice plants (L#4) were grown under high NH_4^+ supply or N starvation for 14 days. The roots were collected and infiltrated with a solution containing the β-glucuronidase (X-gluc) substrate (Fig. S1). Under treatment by 2.0 mM of NH_4^+, no promoter activity was observed (Fig. 1a), whereas under N starvation, an intense blue stain was observed (Fig. 1b). Two segments of roots (black boxes) were selected for further analyses: 15 mm from the tip and the middle third (Fig. 1c, d). In these segments, intense GUS and GFP activities were observed close to the epidermis, at the zones of lateral root emission and at the tips of the lateral roots (Fig. 1e–h). Histological sections of the selected segments are shown in Fig. 2a–f.

Cross sections obtained from the tips and from the middle third of the root exhibited different patterns of staining (Fig. 2). Cross section from the tips showed *OsAMT1.3* activity in the exodermis, sclerenchyma, cortex, and stele (Fig. 2a, b), while in cross and longitudinal sections from the middle third region, with longer lateral roots, a higher *OsAMT1.3* activity was observed in the sites of lateral root emission and at the exodermis (Fig. 2c–f; Fig. S2).

In addition to the histochemical assays (Figs. 1, 2), the in vitro GUS activity was also quantified under treatment with NH_4^+ or NO_3^- and under N deficiency for 14 days. High GUS activity was observed in plants (L#4) grown without N at 3 days following the treatment (Fig. 3). This high activity remained until 7 days and then decreased after 14 days. High *OsAMT1.3* activity was observed in all periods analysed under N deprivation. No changes were noted in the GUS activity in plants grown under a constant N supply. These results indicated that the OsAMT1.3 ammonium transporter is induced strongly by N deficiency and repressed under NO_3^- or NH_4^+ supply.

OsAMT1.3 positively affects *OsAMT1.2* expression

Rice plants overexpressing *OsAMT1.3* were obtained to examine the effect of its expression on the NH_4^+ uptake and root growth. L#2 and L#8 were selected for further study because they showed high *OsAMT1.3* expression levels. Rice lines showed increased levels of *OsAMT1.3* expression under constant N supply (Fig. 4a). In addition, O*sAMT1.2* expression showed the same pattern as *OsAMT1.3* (Fig. 4a, b; Fig. S3). The *OsAMT1.2* expression levels were higher at 2 h than at 6 h (Fig. 4c, d), and the lines did not present significant changes in their *OsAMT1.1* expression levels at 2 and 6 h (Fig. 4c).

Uptake kinetics and root parameters under low NH_4^+ supply and N deficient

The overexpression of the *OsAMT1.3* did not alter the V_{max} significantly in the rice lines; however, the K_M values were 26.4 and 52.4 % lower for L#2 and L#8, respectively, than for the WT (Table 1). The integrate analysis of NH_4^+ uptake using the α value indicated that the rice lines showed kinetic parameters more favourable to the uptake of NH_4^+ at low concentrations than the WT. Furthermore,

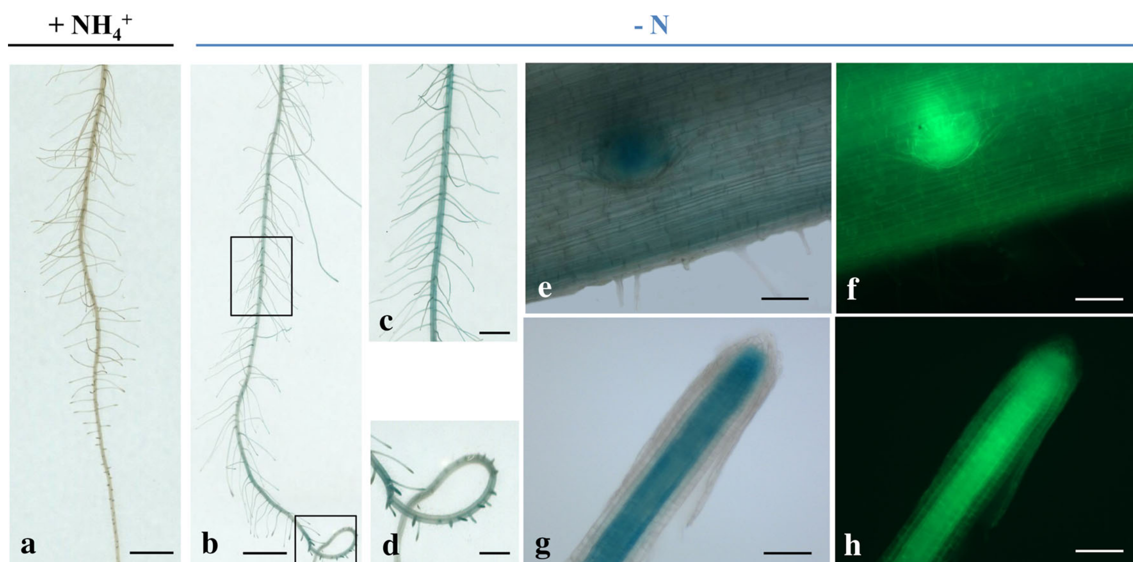

Fig. 1 Rice roots (L#4) grown with 2.0 mM NH_4^+ (**a**) and under N starvation (*scale bar* 10 mm) (**b**). *Black boxes* indicate the root segments selected for the anatomical sections. Middle third (**c**) and tip (**d**) (*scale bar* 2.0 mm). An intense blue stain (GUS) and fluorescence (GFP) were observed in the lateral root emission zone (*scale bar* 100 μm) (**e**, **f**) and in the tips (*scale bar* 400 μm) (**g**, **h**)

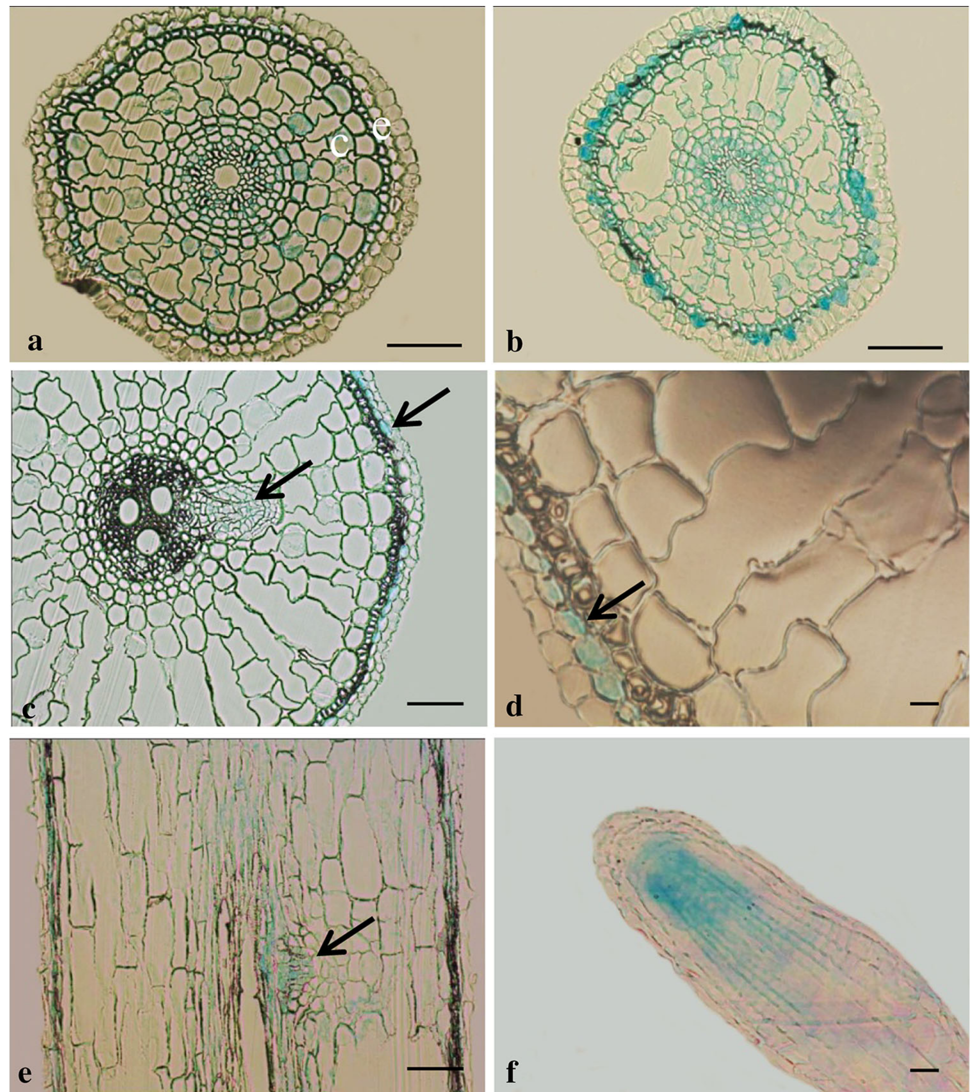

Fig. 2 Anatomical sections of rice roots (L#4) after 14 days without N. Blue staining (GUS) was observed at the segment of tips (**a**, **b**) (*scale bar* 100 μm) and in the middle third with lateral roots (**c**–**f**) (*scale bar* 50 μm). *Arrows* indicate the *OsAMT1.3* promoter activity zones. *c* cortex, *e* exodermis

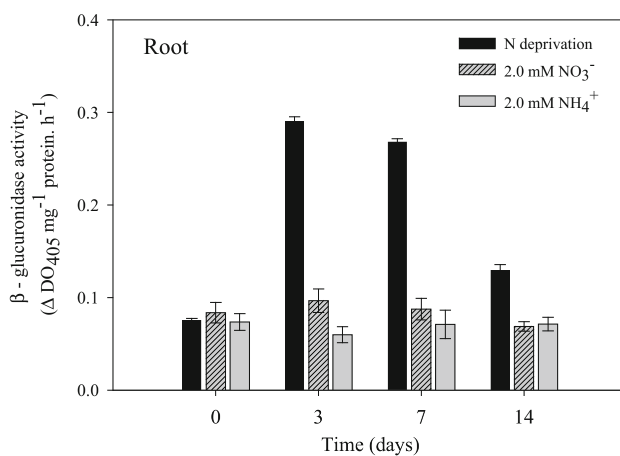

Fig. 3 *OsAMT1.3* activity in rice roots (L#4) under N starvation, 2.0 mM NO_3^- or 2.0 mM NH_4^+ supply. Roots were harvested at 0, 3, 7, and 14 days after treatment. Values represent averages ± SE ($n = 4$)

a significant reduction in C_{min} values of 4.2 and 17 % was observed for L#2 and L#8, respectively (Table 1). When plants were grown in N-deficient medium or 0.2 mM NH_4^+ for 14 days, several root parameters were modified in the rice lines. Greater root length, projection area, surface area, root volume, and number of tips were observed for L#2 and L#8 in both treatments (Table 2).

Despite the higher values observed for the root parameters in 0.2 mM NH_4^+, the differences between the lines and WT were more evident without N. L#2 and L#8 showed greater increases in the root length, approximately 32 and 48 %, respectively, without N, while with 0.2 mM NH_4^+, the increases were 12 and 20 % for L#2 and L#8, respectively. In addition, marked increases in the number of tips were observed for L#2 and L#8 (44 and 77 %, respectively) during N starvation, while under the treatment with 0.2 mM NH_4^+ a minor increase was observed: 4 and 7 %, for L#2 and L#8, respectively.

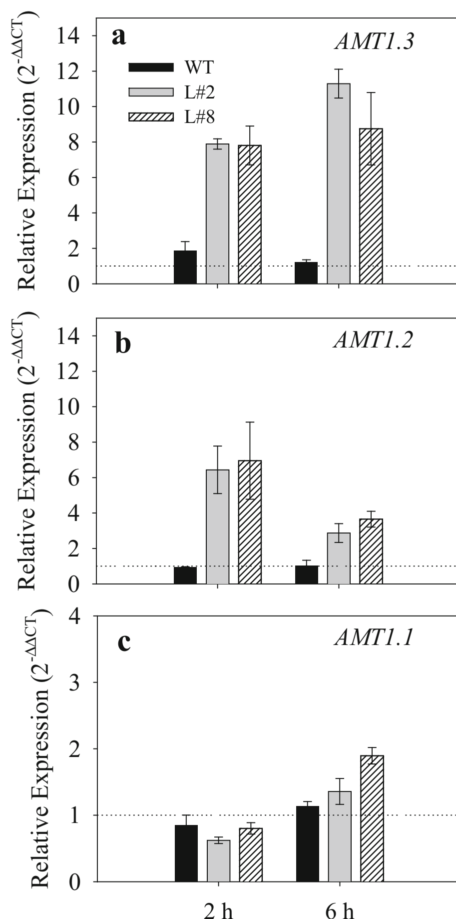

Fig. 4 Relative expression of three ammonium transporters (*OsAMT1.3*, *OsAMT1.2*, and *OsAMT1.1*) in the roots of wild type (WT), L#2, and L#8 rice under a constant 0.5 mM NO_3^-/NH_4^+ supply (**a**–**c**). WT plants at 2 h were used as the reference. Values are averages ± SE ($n = 3$)

Discussion

In this study, transgenic rice plants (L#4) were submitted to N deprivation or 2.0 mM NH_4^+ for 14 days to identify whether the promoter activity is regulated by N and the sites of action of OsAMT1.3. The *OsAMT1.3* activity was restricted to the roots under N deficiency (Fig. 1), supporting the data found by Sonoda et al. (2003a, b) and Yao et al. (2008). The plants subjected to N deprivation showed higher *OsAMT1.3* promoter activity, mainly in the root emission zone and roots tips (Fig. 1e–h). Furthermore, root sections at the segment from the tips and middle third exhibited different patterns of *OsAMT1.3* promoter activity (Fig. 2). In the tips, the *OsAMT1.3* activity was uniformly distributed at the exodermis, sclerenchyma, cortex, and stele (Fig. 2a, b), while in the middle third segment, it was observed only at the cortex and exodermis (Fig. 2c–f). The ammonium taken up by the AMTs is readily assimilated by cytosolic GS1 and NADH-GOGAT isozymes in the surface cell layers of the roots, epidermis, and exodermis (Hirose et al. 1997; Ishiyama et al. 1998). The *OsAMT1.3* promoter activity observed mainly in the exodermis (Fig. 2c, d) is related to the sites of primary ammonium assimilation, indicating a role of the encoded protein in the ammonium uptake. The *OsAMT1.3* promoter activity at root emission zones also suggested a role in signalling events that result in lateral root emission under N deficiency (Fig. 2e, f; Fig. S2). Yao et al. (2008) observed that *OsAMT1.3* was expressed preferentially at the apex of the lateral and seminal roots. These data support the idea that OsAMT1.3 acts as a sensor for nutrients present in the soil, changing the plant metabolism through the activation of signal transduction pathways (Gojon et al. 2011).

In *A. thaliana* with a quadruple knockout of ammonium transporter genes (*amt1.1*, *amt1.2*, *amt1.3*, and *amt2.1*), no lateral root formation was observed, and the lateral root formation decreased significantly in a *atamt1.3* mutant under conditions of low N, supplied as NH_4^+ and NO_3^- (Lima et al. 2010). These authors suggested that *AtAMT1.3* might be involved in triggering lateral root formation in *Arabidopsis*. The AtAMT1.3 ammonium transporter does not show high sequence similarity with *OsAMT1.3* (Li et al. 2009); however, *OsAMT1.3* could also act as a signal for the emission of lateral roots under N deficiency in rice (Fig. 2c–f).

The *OsAMT1.3* activity in plants grown in nutrient solution without N was higher at 3 and 7 days after treatment. When these plants were transferred to solutions containing N as NH_4^+ or NO_3^- ions, no changes were observed in the *OsAMT1.3* promoter activity (Fig. 3). This result is consistent with the data of Sonoda et al. (2003a, b), where rice plants submitted to N starvation showed upregulation of *OsAMT1.3* expression, while in plants submitted to N supply as NH_4^+ or NO_3^-, the opposite

Table 1 NH_4^+ uptake kinetic parameters (V_{max}, K_M, α, and C_{min}) for wild type (WT), L#2, and L#8 plants under resupply with 0.2 mM NH_4^+

Lines	V_{max} (μmol g^{-1} h^{-1})	K_M (μmol L^{-1})	α (V_{max}/K_M) (L g^{-1} h^{-1})	C_{min} (μmol L^{-1})
WT	9.28a	24.52a	0.38c	26.24a
L#2	7.68b	18.04b	0.48b	25.13a
L#8	9.39a	11.68c	0.82a	21.76b

Averages followed by the same letter within the same column do not differ significantly according to the Scott–Knott test at $p \leq 0.05$

Table 2 Root parameters for wild type (WT), L#2, and L#8 plants grown in a nutrient solution without N or with 0.2 mM NH_4^+ for 14 days

Rice plants	Treat.	Length (cm)	Proj. area (cm^2)	Surf. area (cm^2)	Root vol. (cm^3)	Tips
WT	Without N	366.2b	99.7b	313.3b	19.8b	184.1b
L#2		485.2a	136.5a	411.4a	27.8a	265.9a
L#8		543.9a	133.4a	419.2a	25.9a	327.0a
WT	0.2 mM NH_4^+	614.2b	154.5b	499.5b	35.8b	432.3b
L#2		691.3a	205.8a	648.7a	48.8a	450.2a
L#8		739.7a	206.5a	667.8a	45.5a	466.6a

Treat. Treatment, *proj. area* projected area, *surf. area* surface area, *root vol.* root volume

Averages followed by the same letter within the same column do not differ significantly according to the Scott–Knott test at $p \leq 0.05$

behaviour was observed: downregulation of *OsAMT1.3* expression. Gaur et al. (2012) reported that the repression of *OsAMT1.3* through an increase in N might not be a universal mechanism, but may depend on the genotype and on the N level required by a given genotype. These authors observed repression of *OsAMT1.3* with increasing NH_4^+ concentrations in the solution, up to 1.0 mM, for the rice cultivar Kalanamak 3119, whereas the cultivar Pusa Basmati showed the opposite behaviour, increasing the *OsAMT1.3* expression under higher NH_4^+ levels. The Nipponbare rice variety used in our study requires low N supply and also showed high *OsAMT1.3* expression under N deficiency (Fig. 3). *OsAMT1.3* expression is believed to be useful as a biomarker to determine the optimal N supply for rice varieties adapted to low and high N environments. These differences in induction of the high-affinity *AMT* genes might be attributed to differences in N perception and signalling (Gaur et al. 2012).

Thus, overexpression of members of the AMT1 family might be useful to improve N uptake from soils with low NH_4^+ concentrations (Ranathunge et al. 2014). However, some members of the AMT1 family may not be directly involved in the acquisition of NH_4^+ from the external solution, acting instead as sensors of the intracellular NH_4^+ status (Hoque et al. 2006). Thus, we developed rice plants overexpressing *OsAMT1.3* to identify whether the gene product is involved in ammonium uptake or signalling.

The relative expression of the high-affinity ammonium transporter genes (*OsAMT1.1–1.3*) was performed at 2 and 6 h with a constant N supply (Fig. 4). L#2 and L#8 showed high *OsAMT1.3* expression (Fig. 4a). In addition, a strong, positive correlation was observed between the *OsAMT1.3* and *OsAMT1.2* expressions at 2 and 6 h (Fig. 4a, b; Fig. S3). No significant change was observed in the expression of the *OsAMT1.1*. These data support the hypothesis that the overexpression of *OsAMT1.3* could alter the expression of other members involved in high-affinity ammonium transport, such as *OsAMT1.2*. At 6 h, a decrease in the expression of *OsAMT1.2* was observed, possibly indicating negative feedback regulation by glutamine (Sonoda et al. 2003a, b).

Rice lines overexpressing *OsAMT1.3* showed lower K_M and C_{min} values than WT plants when supplied with 0.2 mM NH_4^+ (Table 1). This result suggested that *OsAMT1.3* overexpression, associated with a higher *OsAMT1.2* expression (Fig. 4), resulted in increased uptake efficiency by these plants, considering that *OsAMT1.2* is involved in NH_4^+ uptake from soil solutions (at concentrations <200 nM) and in the retrieval of NH_4^+ in the vascular system (Sonoda et al. 2003a, b). The AMT1 family might show different K_M values at low concentrations of NH_4^+, as observed in Arabidopsis and maize plants (Gazzarrini et al. 1999 and Gu et al. 2013). This suggested that the higher expression of *OsAMT1.3* and *OsAMT1.2* changed the K_M of the NH_4^+ high-affinity transport system, resulting in increased uptake efficiency.

The combination of low K_M and C_{min} values associated with increased root growth is a desirable characteristic in crop plants because it equates to increased N uptake efficiency (Barber 1995). In addition to improved kinetic parameters, the rice lines showed longer roots with more tips, indicating that *OsAMT1.3* expression might contribute to an increase in lateral root emission under N deficiency or low N supply (Table 2). A greater difference was observed for root length and number of tips during N starvation, indicating a contribution of the natural *OsAMT1.3* expression to the root parameters.

Our results showed that overexpression of *OsAMT1.3* was associated with the natural expression of *OsAMT1.2*, and promoted the uptake of NH_4^+ at low concentrations and changes to the root morphology of the lines. However, further studies should be carried out to isolate the role of the *OsAMT1.3* using knockout plants.

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