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RESEARCH ARTICLE

Ectomycorrhiza-mediated repression of the high-affinity ammonium importer gene *AmAMT2* in *Amanita muscaria*

Anita Willmann · Michael Weiß · Uwe Nehls

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Abstract A main function of ectomycorrhizas, a symbiosis between certain soil fungi and fine roots of woody plants, is the exchange of plant-derived carbohydrates for fungus-derived nutrients. As it is required in large amounts, nitrogen is of special interest. A gene (*AmAMT2*) coding for a putative fungal ammonium importer was identified in an EST project of functional *Amanita muscaria*/poplar ectomycorrhizas. Heterologous expression of the entire *AmAMT2* coding region in yeast revealed the corresponding protein to be a high-affinity ammonium importer. In axenically grown *Amanita* hyphae *AmAMT2* expression was strongly repressed by nitrogen, independent of whether the offered nitrogen source was transported by AmAMT2 or not. In functional ectomycorrhizas the *AmAMT2* transcript level was further decreased in both hyphal networks (sheath and Hartig net), while extraradical hyphae revealed strong gene expression. Together our data suggest that (1) *AmAMT2* expression is regulated by the endogenous nitrogen content of hyphae and (2) fungal hyphae in ectomycorrhizas are well supported with nitrogen even when the extraradical mycelium is nitrogen limited. As a consequence of *AmAMT2* repression in mycorrhizas, ammonium can be suggested as a potential nitrogen source delivered by fungal hyphae in symbiosis.

Keywords Ectomycorrhiza · Plant nitrogen nutrition · Ammonium · *Amanita muscaria* · Transport

Abbreviations
UTR Untranslated region

Introduction

Ectomycorrhizas are symbiotic associations of fine roots of woody plants with certain soil fungi (ascomycetes or basidiomycetes). Since the dominant trees of boreal and temperate forests (members of Fagaceae and Pinaceae) are obligate symbionts, ectomycorrhizas play a major role in forest ecosystems. In this type of symbiosis, mutual benefit is based on the exchange of plant-derived carbohydrates for nutrients supplied by the fungus (Marschner and Dell 1994; Smith and Read 1997).

Nitrogen is a major nutrient, since it is required in large amounts by plants and fungi. Due to the often acidic soil pH, rates of nitrogen mineralization (especially nitrification) of litter by bacterial activities are low in many forest ecosystems (Read 1991). Thus, the poorly mobile ammonium ion (Keeney 1980) together with organic nitrogen (e.g., amino acids or proteins) (Marschner and Dell 1994) predominate. It has been shown that under N deficiency hyphal ammonium acquisition can contribute up to 45% of total plant N uptake (Smith and Read 1997). In accordance with this, most ectomycorrhizal fungi grow better on ammonium

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A. Willmann · U. Nehls (✉)
Physiologische Ökologie der Pflanzen,
Eberhard Karls Universität, Auf der Morgenstelle 1,
72076 Tübingen, Germany
e-mail: uwe.nehls@uni-tuebingen.de

M. Weiß
Spezielle Botanik und Mykologie,
Eberhard Karls Universität, Auf der Morgenstelle 1,
72076 Tübingen, Germany

than on nitrate in pure culture (France and Reid 1984; Plassard et al. 1991; Finlay et al. 1992).

To date, three different ammonium importer genes (*HcAMT1*, *HcAMT2*, and *HcAMT3*) have been identified in the ectomycorrhizal basidiomycete *Hebeloma cylindrisporum* (Javelle et al. 2001, 2003). In fungal hyphae grown in axenic culture the expression of all three genes is induced by nitrogen starvation and suppressed by high nitrogen content in the medium. *HcAMT1* and *HcAMT2* turned out to be high-affinity ammonium importers with K_M values for ammonium of about 0.1 μM and lower, while HcAMT3 shows a much lower ammonium affinity (K_M value of about 12 μM). In contrast to *HcAMT3*, *HcAMT1,* and *HcAMT2* were able to restore pseudohyphal growth of a yeast mutant defective in all high-affinity ammonium importers. In agreement with this, *HcAMT1* and *HcAMT2* cluster together in the high-affinity ammonium transporter and sensor subfamily branch of ammonium importers in phylogenetic analysis, while *HcAMT3* cluster in the low affinity branch.

A high-affinity ammonium importer (TbAMT1, K_M value around 2 μM) was also characterized from the ectomycorrhizal ascomycete *Tuber borchii* (Montanini et al. 2002). As observed in *Hebeloma*, the *Tuber* gene was repressed by nitrogen and induced by nitrogen starvation. However, in contrast to *Hebeloma*, nitrogen starvation resulted only in a rather slow induction of gene expression. Furthermore, heterologous expression of *TbAMT1* could not restore pseudohyphal growth of a triple *mep* yeast mutant.

In addition to ectomycorrhizal fungi, a high-affinity ammonium importer was isolated from extraradical mycelia of the endomycorrhizal fungus *Glomus intraradices* (Lopez-Pedrosa et al. 2006). Like in other fungi, gene expression was strongly reduced at elevated ammonium concentrations.

While nitrogen uptake by soil-growing hyphae and its regulation by nitrogen starvation are well investigated, relatively little is known about the situation in ectomycorrhizas. In this contribution, we thus present gene expression analysis of a high-affinity ammonium importer isolated from the ectomycorrhizal fungus *Amanita muscaria* in functional ectomycorrhizas.

Materials and methods

Biological material

Amanita muscaria (L.: Fr.) Pers. strain CS83 was isolated from a fruiting body from Schönbuch, Germany (Schaeffer et al. 1995). Mycelia were grown in liquid culture or on Petri dishes for 2–24 days in modified *Melin Norkrans* (MMN; Marx 1969) medium in the presence of glucose (40 mM) as carbon source and various nitrogen sources and concentrations. Casein hydrolysate (N-Z amine HD, Sigma, St. Louis, MI, USA) was used as amino acid mixture. Fungal mycelium grown in liquid culture was collected by filtration with a Büchner funnel under suction, washed twice with deionized water, frozen in liquid nitrogen and stored at −80°C.

Populus tremula × *tremuloides* was used as plant partner for mycorrhiza formation under axenic conditions (Hampp et al. 1996) with MMN (Marx 1969) medium containing a reduced ammonium amount (final concentration 300 μM) as sole nitrogen source. Mycorrhized and non-mycorrhized fine roots were harvested, frozen in liquid nitrogen and stored at −80°C.

Heterologous expression of *AmAMT2* in yeast

A *Pst*I/*Xho*I digested cDNA fragment containing the entire *AmAMT2* coding region was inserted into the *Pst*I/*Xho*I digested yeast expression vector pDR196 (Rentsch et al. 1995). The *Saccharomyces cerevisiae* triple *mep* mutant 31019b (Marini et al. 1997) was transformed with the *AmAMT2* expression construct according to Gietz and Woods (2002). Uptake experiments of ^{14}C-labeled methylamine and competition experiments with NH_4^+ were performed according to Selle et al. (2005). Transformed yeast cells were grown in 0.67% yeast nitrogen base (without amino acids), 2% glucose, 4.3 mM proline at 30°C under agitation to an OD_{600} of 0.5. Cells were collected by centrifugation (10 min, 6,000 rpm, 15°C), washed once with cold distilled water and resuspended in 20 mM sodium-phosphate-buffer (pH 7) to an OD_{600} of 8. Aliquots (125 μl cell suspension) were centrifuged (5 min, 6,000 rpm, 20°C), resuspended in 125 μl 20 mM sodium-phosphate-buffer (pH 7), 2% glucose and incubated for 5 min at 30°C under agitation (750 rpm, Shutron thermoshaker, Reutlingen, Germany). Each sample was mixed with 125 μl 20 mM sodium-phosphate-buffer (pH 7) containing 2% glucose, different concentrations of non-labeled methylamine and 185 kBq ^{14}C-methylamine (Ge Healthcare Europe, Freiburg, Germany). Aliquots of 50 μl were taken at different times and cells were collected by filtration under suction using glass-fiber filters (GF/C; Whatman, Maidstone, UK). The filters were washed twice with ice-cold stop-solution (20 mM sodium-phosphate pH 7, 100 mM methylamine) and twice with ice-cold water and transferred to 6-ml scintillation vials containing 4 ml of scintillation cocktail (Ultima Gold; Packard,

Groningen, The Netherlands). Radioactivity, incorporated into yeast cells, was measured in a liquid scintillation counter (WinSpectral; PerkinElmer, Boston, MA, USA). Inhibition studies were performed in sodium-phosphate-buffer (pH 7), 2% glucose containing 50 μM non-labeled methylamine, 185 kBq ^{14}C-methylamine (Ge Healthcare Europe) and an NH_4^+ concentration varying between 0.25 and 75 μM. K_M and K_I values were calculated using the Hyper-software (John Easterby's software, http://www.liv.ac.uk/~jse).

Quantitative RT-PCR

Total RNA was isolated according to Nehls et al. (1998). Aliquots of about 1 μg total RNA were treated with DNAse I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and used for first-strand cDNA synthesis in a total volume of 15 μl, containing 50 pmol oligo-d$(T)_{18}$-primer (Ge Healthcare Europe) and 200 U Superscript II RNAse H^- Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. After synthesis, 30 μl of 5 mM Tris/HCl, pH 8 were added and aliquots were stored at −80°C.

Quantitative RT-PCR was performed in a total volume of 20 μl using 10 μl Q-PCR-Master mix (containing Sybr green and fluorescein; ABgene, Hamburg, Germany), 0.5 μl cDNA, and 10 pmol of each primer in a MyiQ Real Time PCR system (BioRad, Hercules, CA, USA). PCR was always performed in duplicates together with the reference gene (see below). At least three different cDNA synthesis reactions of at least two different biological replicates were used for analysis.

Primers (the reverse primer was chosen to target the 3′-UTR of the genes): *AmAMT2*: 5′-TGACGCAGAAATGGGTG-3′; 5′-GCAATGTCGGAGTAAAGC-3′. The constitutively expressed *A. muscaria* gene *SCIV038* (Nehls et al. 1999b, 2001) (5′-CTCATCTGCTCTCGTGC-3′; 5′-CATAATAAGAACGGCGG-3′) was used as a reference.

Construction of the phylogenetic tree

The protein alignment was constructed from the deduced protein sequences of *AmAMT2* (*A. muscaria*) and all known *Candida albicans*, *Emericella nidulans*, *H. cylindrisporum*, *Phanerochaete chrysosporium*, *Neurospora crassa*, *T. borchii*, *S. cerevisiae*, *Schizzosaccharomyces pombe*, *Filobasidiella neoformans,* and *Ustilago maydis* ammonium transporters using MAFFT, version 5.734 (Katoh et al. 2002). Ambiguous alignment positions were excluded from the phylogenetic analysis. To estimate phylogenetic relationships, the alignment was analyzed using heuristic maximum-likelihood analyses as implemented in the PHYML (Guindon and Gascuel 2003) online version (Guindon et al. 2005), based on the WAG model of amino acid substitution (Whelan and Goldman 2001), additionally assuming a percentage of invariant sites and Γ-distributed substitution rates at the remaining sites (WAG + I + G; using a discrete approximation of the Γ distribution involving four rate categories) and starting from a BIONJ tree (Gascuel 1997). All model parameters were estimated using maximum likelihood. An unrooted phylogram was drawn using SplitsTree, version 4 (Huson and Bryant 2006). Branch support was inferred from 500 replicates of non-parametric bootstrapping (Felsenstein 1985).

Additionally, we performed a Bayesian Markov chain Monte Carlo (MCMC) analysis using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). We ran two independent MCMC analyses, each involving four incrementally heated chains over 1 million generations, using the WAG + I + G model of amino acid substitution and starting from random trees. Model parameters were not fixed but sampled during MCMC. Trees were sampled every 100 generations resulting in an overall sampling of 10,000 trees per run, from which the first 2,000 trees of each run were discarded (burn in). The remaining 8,000 trees sampled in each run were pooled and used to compute a majority rule consensus tree to get estimates for the posterior probabilities. Stationarity of the process was controlled using the Tracer software (Rambaut and Drummond 2003).

Miscellaneous

Overlapping sequencing was performed using M13 universal and reverse primers (Stratagene, La Jolla, CA, USA) as well as gene specific primers (Invitrogen) and the *ABI PRISM*™ Dye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1 (Applied Biosystems, Foster City, CA, USA) on an automated sequencer ABI 3100 (Applied Biosystems).

For analysis of DNA and protein sequences, the program package GeneJockey II (Biosoft, Cambridge, UK) was used. The sequence data was compared to gene libraries using BlastX (NCBI, http://www.ncbi.nlm.nih.gov/blast; Altschul et al. 1997).

Ammonium ion concentration was measured using a Dionex (Sunnyvale, CA, USA) DX 120 ion chromatograph with a Dionex CS12A cation exchange column guarded with a CG12A guard column. A solution of methanesulfonic acid served as the eluent. Analyses were performed according to column specifications in

eluent recycle mode with an appropriate self-regenerating suppressor.

Results

cDNA and deduced protein sequence of *AmAMT2*

A cDNA clone (*AmAMT2*; EBI accession number AJ642592) coding for a putative high-affinity ammonium importer was obtained from an EST project (Nehls, unpublished) using fully developed *P. tremula* × *tremuloides*/*A. muscaria* ectomycorrhizas (Nehls et al. 2001) as a source. The deduced protein has a length of 470 amino acids with a calculated molecular mass of 50,955 Da. Using the algorithms TMHMM (Sonnhammer et al. 1998; Krogh et al. 2001) and TMMTOP (Hofmann and Stoffel 1993), 11 putative transmembrane helices with sufficient length to be considered potential membrane-spanning domains were predicted in AmAMT2 (data not shown).

To estimate the phylogenetic relationships of the deduced *AmAMT2* protein to other fungal ammonium importers, all available sequences of basidiomycetes (NCBI database; http://www.ncbi.nlm.gov) as well as those of *N. crassa*, *E. nidulans,* and *S. cerevisiae* were used (Fig. 1). AmAMT2 clusters together with its closest relatives HcAMT1 and HcAMT2 of *H. cylindrisporum* and PcAMT2 of *P. chrysosporium* (76, 74, and 75% identity, respectively) in subgroup I.

AmAMT2 encodes a high-affinity ammonium importer

To investigate whether *AmAMT2* encodes a functional ammonium importer, the entire coding region of the cDNA was cloned into the yeast expression vector pDR196 (Rentsch et al. 1995). The *S. cerevisiae* triple *mep* mutant 31019b (Marini et al. 1997) was transformed with the *AmAMT2* expression construct. Only transformants expressing the *AmAMT2* cDNA in sense orientation were able to grow on agar plates containing 0.5 mM ammonium as sole nitrogen source.

Because ammonium uptake conferred by AmAMT2 could not be determined directly in yeast, the kinetic properties of the transporter were investigated by uptake experiments using radioactively labeled methylamine (Fig. 2a). A K_M value of 5 μM (±1.5 μM) was determined for this artificial substrate. Inhibition of methylamine uptake by ammonium addition revealed a K_I of about 0.5 μM, indicating that *AmAMT2* encodes a high-affinity ammonium importer (Fig. 2b).

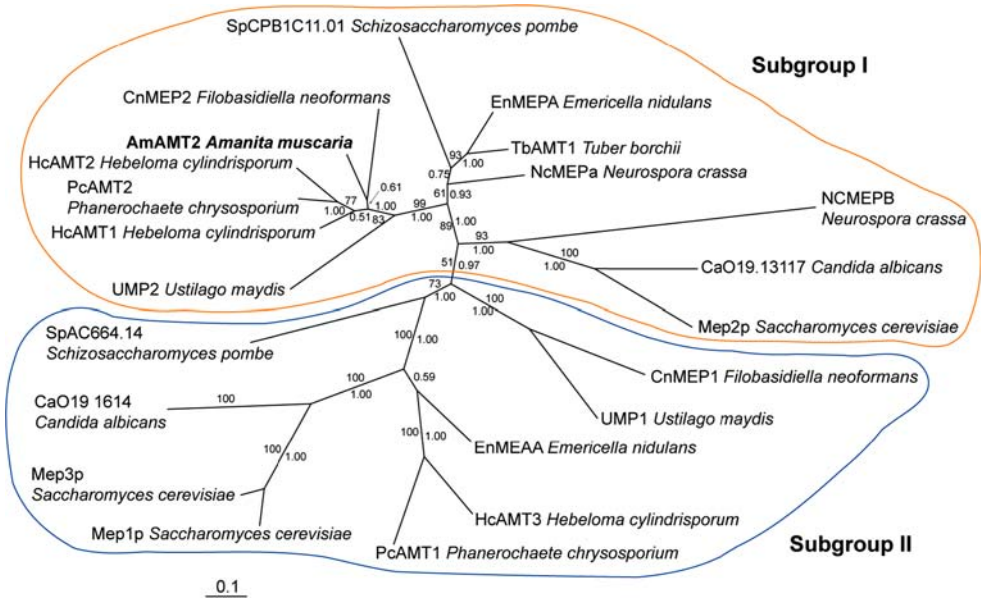

Fig. 1 Phylogenetic relationships between the deduced protein sequences of AmAMT2 (*Amanita muscaria*) and all known *Candida albicans*, *Emericella nidulans*, *Hebeloma cylindrisporum*, *Neurospora crassa*, *Phanerochaete chrysosporium*, *Saccharomyces cerevisiae*, *Tuber borchii*, *Ustilago maydis* ammonium transporters. The unrooted tree was derived with heuristic maximum likelihood (*ML*) analyses using the WAG amino acid substitution model, additionally assuming a portion of invariable sites and Γ-distributed substitution rates at the remaining sites (WAG + I + G). Ambiguously aligned regions were excluded from the phylogenetic analysis. Branch support values were calculated from 500 replicates of non-parametric ML bootstrap analysis (percentages) and from Bayesian Markov chain Monte Carlo analysis, also using the WAG + I + G substitution model (decimal fractions). Values below 50% or 0.5 are not shown. Branch lengths are scaled in terms of expected numbers of amino acid substitutions per site

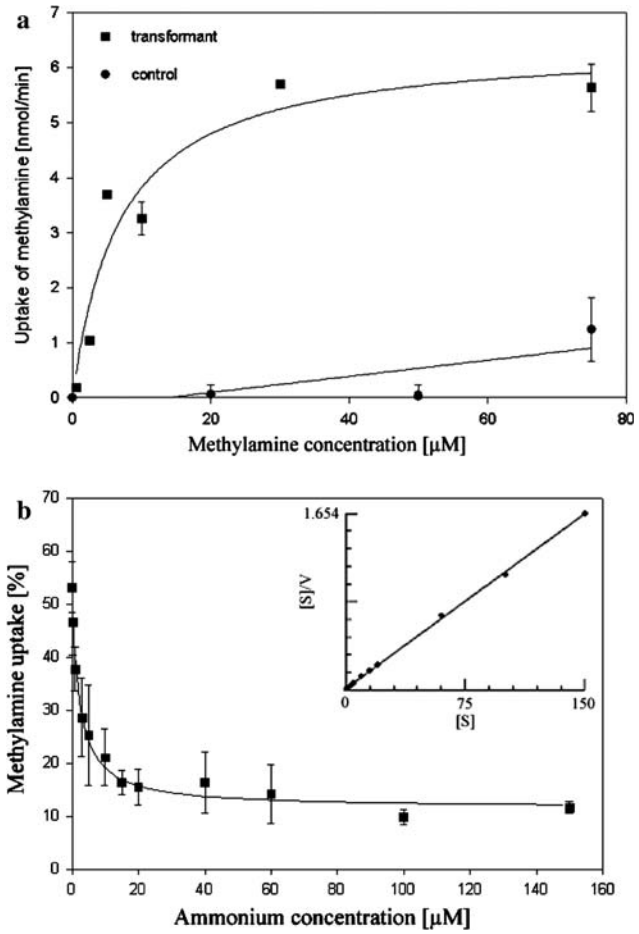

Fig. 2 Functional analysis of AmAMT2 by heterologous expression in yeast. The entire coding region of *AmAMT2* was expressed in a yeast mutant defective in high-affinity ammonium importer. Yeast cells were transformed either with the expression vector alone or the vector containing *AmAMT2*. **a** Yeast cells were incubated with increasing methylamine concentrations (1–100 µM) containing the same specific activity (40 kBq ^{14}C-methylamine/µmol). The rate of methylamine uptake (nmol/min) was calculated from the radioactivity incorporated by yeast cells expressing *AmAMT2* (*filled square*) or containing only the empty expression vector (*filled circle*) and used to calculate the K_M value. **b** Yeast cells expressing *AmAMT2* (*filled square*) were incubated with 20 µM methylamine and increasing concentrations (0.025–150 µM) of ammonium sulphate. Methylamine uptake (%) was measured and used to calculate the K_I value for ammonium from the Hanes plot (*inset*)

AmAMT2 expression is regulated by fungal nitrogen nutrition and ectomycorrhiza formation

AmAMT2 is highly expressed in the absence of a nitrogen source (Fig. 3). Since nitrate cannot be utilized by *A. muscaria* (Abuzinadah and Read 1988), *AmAMT2* transcript levels were comparably high in the presence of nitrate and in the absence of any nitrogen source. The presence of an easily metabolizable nitrogen source inhibits *AmAMT2* expression by a factor of about four (Fig. 3), independent of whether this nitrogen source is transported by AmAMT2 (ammonium) or not (amino acid mixture).

Inhibition of *AmAMT2* expression was dependent on the external nitrogen concentration. Increasing ammonium concentrations resulted in decreased *AmAMT2* transcript levels, reaching a minimum at about 500 µM ammonium (Fig. 4).

Compared to hyphae grown at optimal nitrogen nutrition (2 mM ammonium), ectomycorrhiza formation was accompanied by a further 40% reduction of *AmAMT2* expression ($P = 0.001$; Fig. 4). Since the ammonium concentration in the growth medium was below the detection limit of about 20 µM (data not shown), extraradical hyphae (obtained from the same agar plates as mycorrhizas) revealed a maximal *AmAMT2* expression at the time when mycorrhizas were harvested (Fig. 4).

Fully developed ectomycorrhizas are composed of two fungal networks with different functions in symbiosis. Mycorrhizas were thus dissected into fungal sheath and remaining Hartig net according to Nehls et al. (2001), and gene expression was analyzed separately for both networks by quantitative RT-PCR (Fig. 5). *AmAMT2* transcript levels were about the same in hyphae of the fungal sheath and the Hartig net.

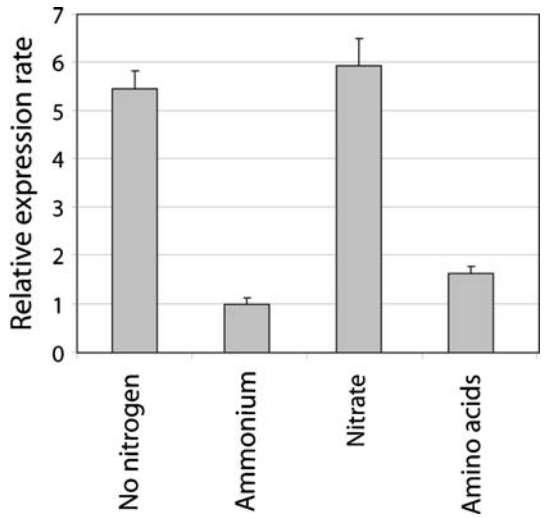

Fig. 3 Impact of the nitrogen source on *AmAMT2* expression. *A. muscaria* mycelia were pre-cultivated in liquid culture in absence of any nitrogen source for 1 week. After medium exchange and addition of different nitrogen sources (each at final concentration of 2 mM; amino acids were supplied as casein hydrolysate), mycelia were cultivated for additional 2 days (with a change of the respective growth medium twice a day). Total RNA was isolated, aliquots of about 1 µg were DNAse treated and used for first-strand cDNA synthesis. Expression analysis was performed by quantitative RT-PCR using specific primers for *AmAMT2* and the constitutively expressed *A. muscaria* gene SCIV038 (Nehls et al. 2001a) as calibration standard

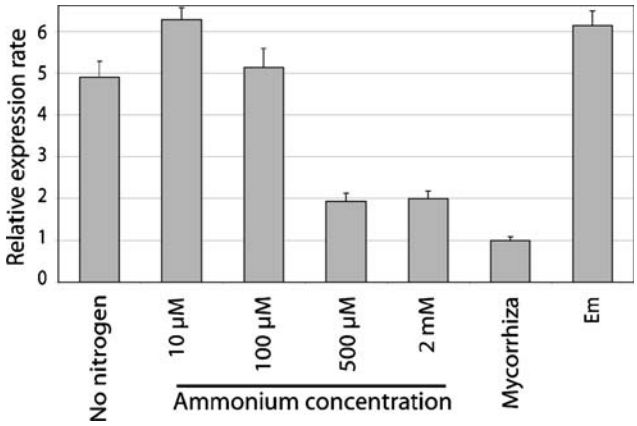

Fig. 4 Impact of the external ammonium concentration and ectomycorrhiza formation on the expression of *AmAMT2*. *A. muscaria* mycelia were pre-cultivated in liquid culture in absence of any nitrogen source for 1 week. After medium exchange and addition of different ammonium concentrations mycelia were cultivated for additional 2 days (with a change of the respective growth medium twice a day). Ectomycorrhizas as well as non-mycorrhizal extraradical mycelium (*Em*) were obtained from three-month-old *P. tremula* × *tremuloides* plants incubated with *A. muscaria* in a Petri dish system. Expression analysis was performed by quantitative RT-PCR

Discussion

In fungi, two different subgroups of ammonium importers can be distinguished (Javelle et al. 2003). According to phylogenetic analysis AmAMT2 clearly belongs to subgroup I. Members of this subgroup are indicated as high-affinity ammonium importers revealing apparent K_M values for ammonium between 0.1 and 2 µM (Fig. 1). This could be confirmed by functional characterization of AmAMT2.

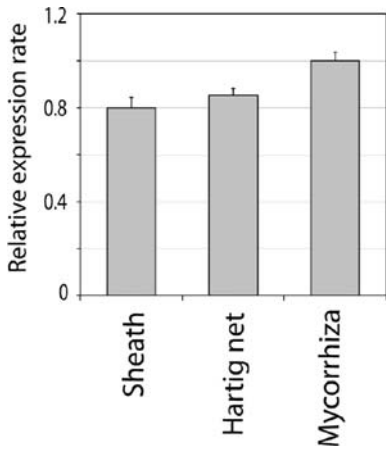

Fig. 5 Expression pattern of *AmAMT2* in dissected ectomycorrhizas. Three-month-old *P. tremula* × *tremuloides*/*A. muscaria* ectomycorrhizas were dissected into fungal sheath and Hartig net according to Nehls et al. (2001a). Expression analysis was performed by quantitative RT-PCR

Interestingly, all basidiomycotic members of subgroup I, but not those of subgroup II cluster together in a separated branch. Since ammonium importer genes contain highly conserved regions and gene fragments could thus be easily amplified by PCR, ammonium importer genes from subgroup I could be a useful tool for phylogenetic analysis of fungi.

Based on protein sequence and kinetic properties, the *A. muscaria* ammonium importer AmAMT2 is highly related to HcAMT1 and HcAMT2 from *H. cylindrisporum* (Javelle et al. 2001, 2003). In both organisms ammonium transporter gene expression is maximal in the absence of nitrogen, while the transcript levels are strongly reduced in the presence of a readily metabolizable nitrogen source. However, in contrast to both *Hebeloma* genes, the expression of *AmAMT2* was still detectable in *A. muscaria* hyphae that were well supported with nitrogen. This expression pattern is comparable to that of high-affinity amino acid transporters in both organisms (Nehls et al. 1999a; Wipf et al. 2002). Like what is known about ammonium importers, gene expression of amino acid transporters is repressed in the presence of a good nitrogen source and this effect is again more drastic in *Hebeloma*, indicating that nitrogen dependent gene repression is (at the transcript level) less strict in *A. muscaria*.

However, the amount of plasma membrane proteins is often regulated by posttranslational mechanisms in addition to transcriptional control (Wendell and Bisson 1994; Stanbrough and Magasanik 1995). Thus, further investigations at the protein level are necessary to evaluate whether posttranscriptional regulation is more pronounced in *A. muscaria* compared to other ectomycorrhizal model fungi.

Ammonium importers present in the plasma membrane could have at least two different functions: (a) uptake of nitrogen for fungal nutrition and (b) prevention of ammonium leakage (Marini et al. 1997; Javelle et al. 2003). Both aspects are important for the function of soil growing ectomycorrhizal hyphae (for reviews, see Javelle et al. 2004; Nehls 2004), since nitrogen is usually rather limited in forest soils. Part of the nitrogen, which is taken up by soil growing hyphae, is later exported at the plant/fungus interface to meet the demand of the host plant. Here, nitrogen retrieval by fungal hyphae would interfere with their function in nitrogen export. The repression of ammonium importer gene expression, as observed for *AmAMT2* in functional ectomycorrhizas, could thus indicate a reduced ammonium retrieval rate by fungal hyphae in symbiosis. In consequence, a net ammonium efflux would be the result as shown for yeast *mep* mutants (Marini et al. 1997). Elevated

ammonium concentration in the growth medium as the reason for a suppressed *AmAMT2* expression in mycorrhizas can be ruled out. At the time point of ectomycorrhizal harvest, the ammonium concentration in the growth medium was below 20 μM, a concentration where *AmAMT2* expression was observed to be at a maximum in extraradical hyphae.

Ammonium as a fungal nitrogen source delivered towards the plant would also explain the symbiosis-regulated strong increase in ammonium uptake capacity of poplar fine roots in ectomycorrhizas (Selle et al. 2005). In addition, strong evidence exists that ammonium is exported by intraradical fungal hyphae in arbuscular mycorrhizal symbiosis as well (Govindarajulu et al. 2005; Jin et al. 2005).

Fully developed ectomycorrhizas are composed of two functionally different fungal networks (Kottke and Oberwinkler 1986; Jordy et al. 1998; Nehls et al. 2001). Hyphae of the fungal sheath isolate the infected fine root from the surrounding soil and nitrogen export is supposed to occur through hyphae of the Hartig net (Smith and Read 1997). To investigate hyphae of both ectomycorrhizal fungal networks independently of each other, we dissected functional *A. muscaria* ectomycorrhizas. However, in contrast to sugar-regulated *A. muscaria* genes (Nehls et al. 2001), the expression of the high-affinity ammonium (*AmAMT2*) importer gene turned out to be comparable in both fungal networks. This indicates that hyphae of the fungal sheath and the Hartig net are well supported with nitrogen by soil growing hyphae and that no further transcriptional suppression of *AmAMT2* expression occurs at the plant/fungus interface.

As shown for asco- and basidio-mycotic fungi, the existence of several ammonium importers in *A. muscaria* can be supposed. However, with respect to nitrogen nutrition, expression of high-affinity ammonium importer genes (*AmAMT2*, *A. muscaria*; *HcAMT1,* and *HcAMT2, H. cylindrisporum*) of subgroup I is regulated in the same manner, making it rather likely that other genes of the high-affinity ammonium importer gene family are also repressed in ectomycorrhizas.

In summary, together with the strong increase in plant ammonium uptake capacity (Selle et al. 2005) our data indicate that ammonium might be a potential nitrogen source delivered by fungal hyphae in ectomycorrhizal symbiosis. However, further investigations at the level of the whole ammonium importer gene family have to be performed in future.

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