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GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*

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Abstract

We report the cloning and characterization of the first NH_4^+ transporter gene (*GintAMT1*) in an arbuscular mycorrhizal fungus. *GintAMT1* encodes a polypeptide of 479 amino acids sharing high sequence similarity with previously characterized NH_4^+ transporters from other fungi. Heterologous expression of *GintAMT1* in the yeast triple *mep* mutant complemented the defect of this strain to grow in the presence of less than 1 mM NH_4^+. As revealed by [^{14}C]methylammonium uptake experiments carried out in yeast, *GintAMT1* encodes a high-affinity NH_4^+ transporter. In mycelia developed in the presence of 0.9 mM NO_3^-, *GintAMT1* transcription was increased after the addition of 30 μM NH_4^+ but decreased after the addition of 3 mM NH_4^+. However, in mycelia grown in the presence of higher N concentrations, *GintAMT1* transcripts decreased after the addition of NH_4^+, irrespective of the concentration used. These data suggest that *GintAMT1* is involved in NH_4^+ uptake by the extraradical mycelia from the surrounding media when it is present at micromolar concentrations.

Keywords: Arbuscular mycorrhizal fungi; Nitrogen nutrition; Ammonium transport; Root organ culture

1. Introduction

The roots of most plant species are colonized by arbuscular mycorrhizal (AM) fungi to form mutually beneficial symbiotic associations called mycorrhizas. AM fungi, belonging to the fungal phylum Glomeromycota (Schüßler et al., 2001), are obligate biotrophs which depend entirely on the plant for their carbon supply (Bago and Bécard, 2002). The fungus in return provides the plant with essential mineral nutrients that are taken up from the soil by the extraradical mycelium and translocated to the plant (Smith and Read, 1997). Within the cortical cells of the root the fungus forms finely branched hyphal structures, the arbuscules, which are surrounded by a specialized plant membrane and which are believed to be the site of bi-directional nutrient transfer (Ferrol et al., 2002).

The main benefit of the AM association is an improved P status of the mycorrhizal plant, but there is increasing evidence that AM fungi also contribute to the N-budget of the plant. Different authors have shown the ability of the extraradical mycelium of AM fungi to deplete the inorganic ^{15}N, added as $^{15}NH_4^+$ to the soil of a root-free compartment (Frey and Schüepp, 1993; Johansen et al., 1992, 1993) and that AM extraradical hyphae were able to transport this N-isotope to a plant which was growing several centimetres away from the nitrogen source. The capability of AM extraradical hyphae to take up (Bago et al., 1996) and transport $^{15}NO_3^-$ to the host plant has also been reported (Tobar et al., 1994a,b). Much less is known about the uptake of organic nitrogen, but Hawkins et al. (2000) showed that AM hyphae are able to take up glycine and glutamic acid and transport nitrogen from these sources to the plant roots in a form that is presently unknown.

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Moreover, Hodge et al. (2001) reported that the AM symbiosis can both enhance decomposition of and increase nitrogen capture from complex organic material in soil.

Although AM fungi are able to take up both NO_3^- and NH_4^+, a clear preference for NH_4^+ has been demonstrated (Hawkins et al., 2000; Toussaint et al., 2004; Villegas et al., 1996), which is explained, at least in part, by the extra energy the fungus must expend in reducing NO_3^- to NH_4^+ before it can be incorporated into organic compounds (Marzluf, 1996). Ammonium is a ubiquitous intermediate in nitrogen metabolism and one of the major nutrients for plants and microorganisms. The whole process of NH_4^+ assimilation in the AM symbiosis involves NH_4^+ uptake and assimilation by the AM fungus and transfer to the plant. Several lines of evidence indicate that the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle is responsible for NH_4^+ assimilation in AM extraradical hyphae (Breuninger et al., 2004; Johansen et al., 1996), although the involvement of glutamate dehydrogenase has not been experimentally excluded. Recently, Govindarajulu et al. (2005) have shown that inorganic nitrogen taken up by the external mycelium is incorporated into amino acids, translocated from the extraradical to the intraradical mycelium as arginine, but transferred to the plant as NH_4^+.

In most organisms, ammonium uptake is mediated by members of the NH_4^+ transporter family (AMT/MEP). The first of the genes involved in NH_4^+ transport were cloned from yeast (Marini et al., 1994) and *Arabidopsis* (Ninnemann et al., 1994). Since then, these genes have been found in a variety of bacteria (Thomas et al., 2000), fungi (Javelle et al., 2001, 2003b; Montanini et al., 2002) and homologues have been found in humans (Marini et al., 2000). Aside from their role in NH_4^+ uptake, NH_4^+ transporters can also act as NH_4^+ sensors. In yeast, the high-affinity transporter MEP2 has been considered to act as the sensor for low NH_4^+ availability and evidence suggests that it is associated with the signal transduction cascades leading to filamentous growth (Lorenz and Heitman, 1998). The objective of the present work was to characterize an NH_4^+ transporter of *Glomus intraradices* in order to get some insights into the mechanisms of nitrogen acquisition and sensing in AM fungi.

2. Materials and methods

2.1. Arbuscular mycorrhizal monoxenic cultures and N treatments

Arbuscular mycorrhizal monoxenic cultures were established as described by St-Arnaud et al. (1996). Briefly, clone DC2 of carrot (*Daucus carota* L.) Ri-T DNA transformed roots were cultured with the AM fungus *G. intraradices* Smith & Schenck (DAOM 197198, Biosystematic Research Center, Ottawa, Canada) in two-compartment Petri dishes. Cultures were initiated in one compartment ("root compartment") of each plate, which contained M medium (Chabot et al., 1992). Fungal hyphae, but not roots, were allowed to grow over to the second compartment ("hyphal compartment"), which contained M medium without sucrose and modified to provide different NO_3^- concentrations (0.9, 1.8, or 3.6 mM). Ca^{2+} and K^+ losses, resulting from the reduction of NO_3^- salts, were compensated by the addition of the corresponding Cl^- and SO_4^{2-} salts, respectively. Plates were incubated in the dark at 24 °C until the transition from absorptive to sporulative phase was initiated (approximately 2–3 months from the establishment of the cultures). At this stage, 0, 30 μM, or 3 mM $(NH_4)_2SO_4$ pulses were applied to the hyphal compartment of the plates of the different NO_3^- treatments by placing 500 μL of stock solutions in droplets dispersed throughout the compartment, allowing diffusion to distribute the added solution evenly. The time of $(NH_4)_2SO_4$ addition was referred as time 0 and mycelia were harvested 24 and 48 h after the pulse.

2.2. Isolation of the GintAMT1 cDNA

The 3′ end of *GintAMT1* was obtained by chance while trying to isolate the 3′ end of a different *G. intraradices* gene by rapid amplification of cDNA ends (RACE). The 5′ end of *GintAMT1* was obtained by RACE by using the SMART RACE cDNA Amplification kit (Clontech, Palo Alto, CA, USA) and the *GintAMT1* specific primer AMT1 (5′-CTAAGAGCAGCAGCACCAGAAGAAA TG-3′) following the manufacturer's instructions. 5′ RACE reactions were performed using 1 μg total RNA extracted from *G. intraradices* extraradical mycelium grown in MC plates at the standard nitrate concentration (3.6 mM). PCR products were subcloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced. The full-length clone was obtained by ligating the 3′ and 5′ fragments in a *Sac*II/*Mef*I-digested pGEM-T Easy vector.

2.3. Sequence analyses

Nucleotide sequences were determined by *Taq* polymerase cycle sequencing and using an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Computer database comparisons were performed using BLAST algorithm (Altschul et al., 1990) and computer translation by using the Translate tool from EXPASY Molecular Biology Server. Amino acid sequence comparisons were made with the BESTFIT program of the Genetics Computer Group (Madison, WI, USA), and multiple sequence alignments of translated gene sequences were carried out with the program CLUSTALW (version 1.5; Thompson et al., 1994). Genetic distances were estimated by using the Kimura's two-parameter method employed by PHYLIP (Felstein, 1993). The tree was displayed with the help of the TREEVIEW program (Page, 1996). Transmembrane domains of GintAMT1 were predicted by using SOSUI (Hirokawa et al., 1998).

2.4. Functional complementation assays

The full-length of *GintAMT1* was cloned into the *Not*I site of the yeast expression vector pFL61 (Minet et al., 1992).