Introduction

The arbuscular mycorrhizal (AM) symbiosis which occurs between fungi belonging to the Glomeromycota (Schuessler *et al.* 2001) and more than 80% of all plants species (Smith and Read 2008) is one of the most widespread terrestrial symbioses. The characteristic structures of AMs are the eponymous arbuscules: they are intracellular, highly branched hyphae where the majority of nutrient exchanges between the two partners is supposed to occur. Because AM fungi are obligate biotrophs, they depend on the carbohydrates supplied by the plant roots (Bago *et al.* 2003), whereas the plant improves its phosphorus (P) and nitrogen (N) nutrition via the fungal partner (Govindarajulu *et al.* 2005, Smith and Read 2008).

The uptake of inorganic phosphate (Pi) is the key physiological process by which AM fungi improve plant growth (Bucher 2007). AM fungi indeed express Pi transporters which acquire Pi from the soil and deliver it to the host plant (Harrison and van Buuren 1995). On the other hand, the plant acquires the Pi from the interfacial apoplast via own mycorrhiza specific Pi transporters.

Besides phosphorus, nitrogen is the other macronutrient for which mycorrhizal fungi play an important role. In soils nitrogen is found in two major forms, organic and inorganic compounds. The organic compounds are simple molecules, as urea, amino acids (AA), amines and peptides, or complex ones as proteins, whereas inorganic nitrogen compounds are mainly represented by nitrate (NO_3^-) and ammonium (NH_4^+). Uptake of organic and inorganic N-sources from the soil via specialised transporters has been described for ectomycorrhizal (ECM) and AM fungi (Gobert and Plassard 2008). Recent studies on AMs provide evidence that the subsequent N-transfer from the fungus to the plant cells occurs in form of ammonium (Govindarajulu *et al.* 2005, Jin *et al.* 2005). However, it has been proposed that -depending on the nutritional and photosynthetic status of the plant - there could be two ways of nitrogen release from the fungus to the plant (Chalot *et al.* 2006). The “traditional view” suggests an organic N-transfer under high C availability, whereas under C depletion the synthesis of organic N would be down regulated in the fungus, leading to the transfer of inorganic N.

A third important mineral element is given by sulfur. Interestingly a recent study (Allen and Shachar-Hill 2008) showed that AM fungi transfer also significant amounts of sulfur to the plant. However, so far no plant transcripts coding for S-transporters have been shown to be regulated during the AM interaction. Lastly, it can be questioned whether potassium nutrition might be also improved by AM fungi. Similar to sulfur, so far no transcript data are available to support the potassium transfer during AM symbiosis.

Taking advantage of a previous micro array experiment (Guether *et al.* 2009a), an updated view on the most expressed nutrient transporters during the AM interaction in the model legume *Lotus japonicus* will be provided.

Results

In the micro array experiment on RNA derived from *Lotus japonicus* roots colonized by the fungus *Gigaspora margarita,* many genes showed a significant altered expression (Guether *et al.* 2009a). Among these genes, 47 putative transporters were identified; out of them 29 might be important for nutrient acquisition processes (Table 1). Some of these transcripts have been quantified using quantitative RT-PCR and showed very high over-expression rates (Guether *et al.* 2009a, Guether *et al.* 2009b).

A bioinformatic analysis of the putative phosphate transporter sequence showed an almost 100% identity to the MtPT4 phosphate transporter in *Medicago truncatula* (Guether *et al.* 2009a) which has been shown to be mycorrhiza-specific and essential for the functioning of the symbiosis (Javot *et al.* 2007).

Because the putative ammonium transporter resulted to be the strongest regulated gene in our array experiment (31,000 fold), the full coding sequence was isolated and characterized in detail. The localization of the LjAMT2;2 transcripts via laser microdissection revealed their accumulation in arbusculated cells (Guether *et al.* 2009b). Heterologous complementation of a yeast mutant, impaired in its ammonium uptake system, demonstrated that the LjAMT2;2 protein is a functional ammonium transporter (Guether *et al.* 2009b) and depends on an acidic pH. Further the transport experiments using *Xenopus* oocytes indicated that the LjAMT2;2 protein transports NH_3 instead of NH_4^+. Our results therefore suggest that the transporter binds charged ammonium in the apoplastic interfacial compartment and releases the uncharged NH_3 into the plant cytoplasm.

Table 1 Putative nutrient transporters regulated in roots of *Lotus japonicus* 28 days post infection (dpi) with the AM fungus *Gigaspora margarita* as revealed by a micro array analysis.

Putative annotation	Number of genes
Phosphate transporters	1
Peptide transporters	7
Ammonium transporters	1
Nitrate transporters	4
Amino acid transporters	3
Potassium transporters	1
Sulfate transporters	3
Aquaporins	3
Sugar transporters	2
Zinc transporter	1
Other metal ion transporters	1
Water channels	2

The list of the other putative transporter sequences revealed that 10 out of the 29 genes code for amino acid and peptide transporters (Table 1). Some of them exhibited strong expression changes close to the levels reported from the phosphate and ammonium transporters.
Another important result coming from the micro array data was the identification of sequences for putative potassium and sulfur transporters, which so far have never been described as regulated in AMs.

Discussion

The micro array experiment convincingly demonstrates how AMFs impact mineral plant nutrition, changing the expression of a huge number of nutrient transporters.
An improved P-nutrition is thought to be the main benefit for plants in AM interactions. Based on the almost identical amino acid sequence to the MtPT4 transporter (Harrison *et al.* 2002) our results suggest that the transcript coding for a strongly up-regulated phosphate transporter represents the mycorrhiza-specific LjPT4 phosphate transporter in *Lotus japonicus*.
Nitrogen is an even more important macro-nutrient for plants than phosphorous. Plants have developed numerous systems for the uptake of organic and inorganic N-sources which are present in the soil. Additionally, plant nitrogen nutrition can be improved via symbioses with N-fixing rhizobacteria and mycorrhiza. The AM interaction, representing the most widespread mycorrhizal form on the planet, has been shown to transfer substantial amounts of inorganic N from the fungus to the plant only in recent times (Govindarajulu *et al.* (2005), Jin *et al.* 2005). Our study provides evidence that the mycorrhiza specific LjAMT2;2 ammonium transporter takes up inorganic N at the plant fungus interface and thus is responsible for the

N-transfer from the fungus to the host plant. As demonstrated by experiments in *Xenopus* oocytes the LjAMT2;2 protein binds charged ammonium in the apoplastic interfacial compartment but releases the uncharged NH_3 into the plant cytoplasm. Such findings may have important practical implications in the management of ammonium fertilizers (Guether *et al.* 2009b).

Given that the transported N-forms may be quite diverse, the identification of strongly regulated genes involved in the transport of AAs and peptides will present a good starting point to reply to the question: does an alternative to the inorganic N-transfer exist in AMs?

The discovery of transcripts for putative nitrate, potassium and sulfur transporters is quite surprising given that the plants were grown under not limiting conditions of NO_3^-, K^+ and SO_4^{2-}. We suggest that the improved P nutrition requires an increased need for these compounds. Preliminary experiments on the transporter expression gives a support to the hypothesis. In comparison to the mycorrhiza specific phosphate and ammonium transporters, which transcripts are rarely detectable in non-mycorrhizal roots, the sulfate-, nitrate- and potassium transporters show relatively high expression values in control roots and are moderately regulated upon mycorrhization (M.Guether, unpublished). A good understanding of their regulation will be crucial, since nitrate, potassium and sulfur are key components of mineral fertilizers used in agriculture.

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