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Short communication

The effect of different nitrogen sources on the symbiotic interaction between *Sorghum bicolor* and *Glomus intraradices*: Expression of plant and fungal genes involved in nitrogen assimilation

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ABSTRACT

In the arbuscular mycorrhizal (AM) symbiosis, plants take up part of the nitrogen (N) through a mycorrhizal pathway. In this study, we assessed the effect of different N sources on the expression of genes coding for enzymes and transporters of the mycorrhizal N uptake pathway, using *Sorghum bicolor* and *Glomus intraradices* as a model. Some of the genes investigated were differentially regulated in the intraradical and in the extraradical mycelium depending on the N source. In AM roots, some fungal and plant genes were co-regulated, suggesting an interdependence of both partners in the mycorrhizal N uptake pathway. Mycorrhizal N transfer may have a preference for glycine (plant growth and N uptake stimulation).

In almost all ecosystems under temperate climates and in cultivated soils (except in nitrogen-poor and cold ecosystems), plants and arbuscular mycorrhizal (AM) fungi take up ammonium (NH_4^+) and nitrate (NO_3^-) rather than amino acids or other N-based organic molecules (review by Courty et al., 2014). The symbiotic nitrogen (N) transfer was shown to be highly efficient in AM symbiosis (Koegel et al., 2013a) and an important component of plant N nutrition. Tanaka and Yano (2005) showed that the amount of N delivered to maize plants by the AM fungus depended on the N form; the rate of transfer was 10 times higher for NH_4^+ than for NO_3^- in *Glomus aggregatum*-inoculated maize plants. Typically, NH_4^+ taken up by the extraradical mycelium (ERM) is assimilated through the glutamine synthase/glutamate synthase (GS/GOGAT) cycle, asparagine synthetase and the urea cycle. N is then transferred from the ERM to the intraradical mycelium (IRM) in the form of arginine (Govindarajulu et al., 2005; Cruz et al., 2007; Tian et al., 2010). Once in the arbuscules, arginine is broken down to release NH_4^+ through the catabolic arm of the urea cycle, which is then transferred to the plant, while the fungus retains the arginine-derived carbon (Bago et al., 2001; Govindarajulu et al., 2005; Kobae et al., 2010; Tian et al., 2010). In arbuscule-containing cells, several fungal NH_4^+ transporters (AMT) expected to be export carriers (AMT1 and AMT2 from *Glomus intraradices*; Lopez-Pedrosa et al., 2006; Pérez-Tienda et al., 2011) and several AM-inducible plant AMT expected to be import carriers (i.e. AMT3; 1 from *Sorghum bicolor*; Koegel et al., 2013b) were strongly expressed and involved in the transport of the NH_4^+ from the fungus to the plant.

Using an vitro system of Ri T-DNA-transformed carrot roots colonized by *G. intraradices*, Tian et al. (2010) characterized the expression pattern of 11 fungal genes involved in N assimilation and transfer after the addition of NO_3^- to the fungal ERM and IRM. They found that genes for ammonium assimilation and arginine synthesis were upregulated in the ERM, and genes for arginine

Abbreviations: AL, arginosuccinate lyase; AMT, ammonium transporter; AMTF, aminomethyltransferase; AspS, asparagine synthetase; ASS, argininosuccinate synthase; CAR, arginase; CPS, carbamoyl-phosphate synthase; GDH, glutamate deshydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase; NT, nitrate transporter; NR, nitrate reductase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; URE, urease.

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catabolism were upregulated in the IRM under these conditions. Sorghum (*Sorghum bicolor*), inoculated with *G. intraradices*, was used to study the effect of different N sources on the nutritional status of the plants as well as on the expression of genes coding for enzymes and transporters involved in the mycorrhizal N assimilation pathway, both in the plant and in the fungus. An overview of the enzymes and transporters studied is given in Fig. S1. An experiment was conducted using compartmented microcosms (Koegel et al., 2013a), where one plant and one hyphal compartment are connected but separated by two 21 μm nylon meshes and an air gap in between (Fig. S2). Using this system, the AM roots (plant + IRM) and the ERM were isolated separately, and thus we studied fungal gene expression in IRM and ERM separately, as well as the potential co-regulation of fungal and plant genes within the AM.

S. bicolor plants in the plant compartment of these microcosms were inoculated with *G. intraradices* BEG-75. Both compartments of the microcosms were irrigated with distilled water twice a week. In addition, the fungal compartments were amended weekly with 8 mL of a Hoagland solution containing different nitrogen sources (−N, NO_3^-, NH_4^+, urea, glycine and arginine). Details of plant growth conditions for tissue analysis are given in Appendix S1. A total of 36 microcosms was prepared and harvested 13 weeks after inoculation. For each microcosm, four subsamples of 50 g soil were taken from the fungal compartment, and four subsamples of 100 mg of fresh AM roots from the plant compartment (one for root colonization and three for RNA extraction). The remainder of the root samples and the shoot material were dried for total N and C content analysis. RNA extraction from soil was done using Geneclean Turbo Kit (MP biomedicals, Santa Ana, CA, USA). RNA extraction from roots and cDNA synthesis from root and soil samples was performed as described by Courty et al. (2009). cDNAs were used as templates in real time quantitative PCR reactions with gene-specific primers (Table S1, Appendix S1).

Plant root colonization was in the order of 80% for all nitrogen sources but significantly lower in the −N treatment (Fig. 1a). When the hyphal compartment received any one of the nitrogen sources, the plants accumulated much more dry weight than in the −N treatment (Fig. 1b). Compared to the other N sources, glycine significantly increased the total N content of the AM plants (Fig. 1c), indicating that glycine is a particularly well-assimilated N source for *G. intraradices*.

The expression level of 19 fungal genes coding for transporters and enzymes of the N pathway between the ERM and in the IRM, following the rationale of Tian et al. (2010) but also including the fungal AMTs and nitrate reductases, was compared (Table 1). The expression of six genes was detected only in the IRM, independently of the N source (*GiAMT1*, *GiAMT2*, *GiAspS*, *GiNR3*, *GiOAT1* and *GiOAT2*), suggesting specific roles at the

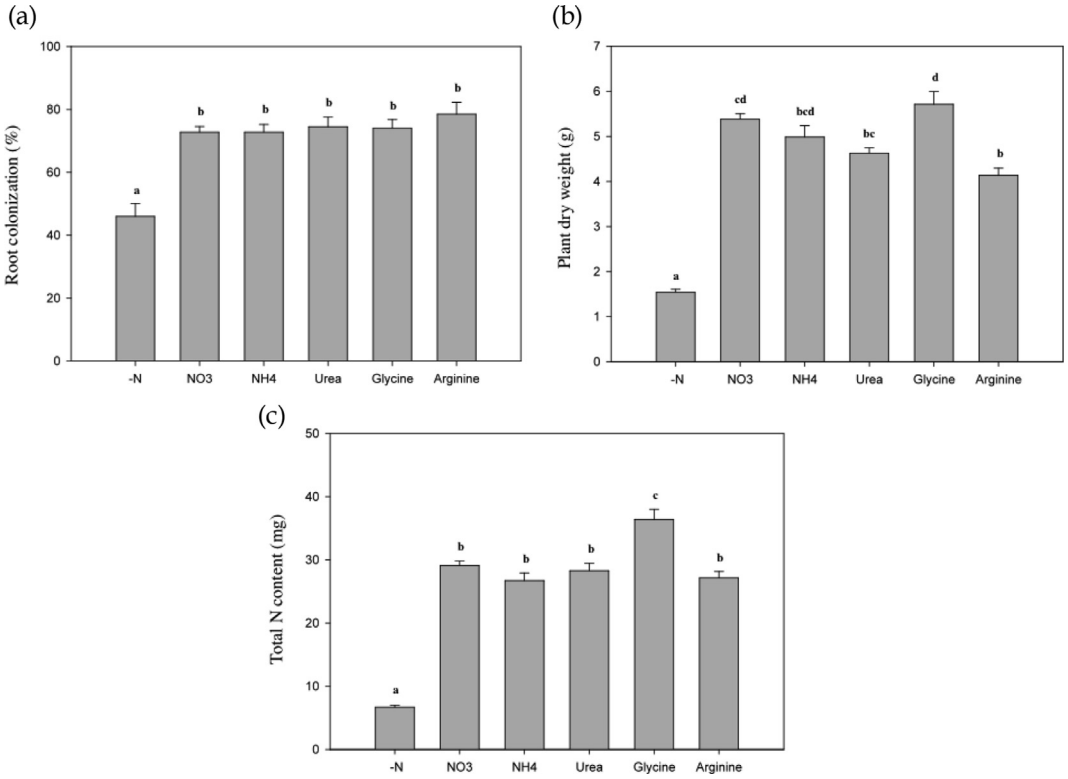

Fig. 1. Percentage of root length colonized (a), dry weight (b) and total N content (c) in plants colonized by *Glomus intraradices* in the different N treatments (−N, NO_3^-, NH_4^+, urea, glycine and arginine), measured 13 weeks after inoculation. Root colonization was quantified according to the grid intersection method as described by Brundrett et al. (1984) and as detailed by Koegel et al. (2013b). Total colonization comprised intersections containing hyphae, vesicles, spores or arbuscules. Procedure for total N and C analysis was described in Courty et al. (2011). Values are mean of six replicates. Differences between means of variables were performed with a one-way ANOVA (Scheffe's F-test), using SPSS 18.0. Different letters indicate statistically different values ($p < 0.05$).