Mycorrhiza (2009) 19:143–148
DOI 10.1007/s00572-008-0221-2

ORIGINAL PAPER

Expression of the nitrate transporter *nrt2* gene from the symbiotic basidiomycete *Hebeloma cylindrosporum* is affected by host plant and carbon sources

David Rékangalt · Régis Pépin · Marie-Christine Verner · Jean-Claude Debaud · Roland Marmeisse · Laurence Fraissinet-Tachet

Received: 3 October 2008 / Accepted: 10 December 2008 / Published online: 6 January 2009

Abstract Although the function of the extramatrical mycelium of ectomycorrhizal fungi is considered essential for the acquisition of nitrogen by forest trees, gene regulation in this fungal compartment is poorly characterized. In this study, the expression of the nitrate transporter gene *nrt2* from the ectomycorrhizal basidiomycete *Hebeloma cylindrosporum* was shown to be regulated by plant host and carbon sources. In the presence of a low fructose concentration, *nrt2* expression could not be detected in the free-living mycelium but was high in the extramatrical symbiotic mycelium associated to the host plant *Pinus pinaster*. In the absence of nitrogen or in the presence of nitrate, high sugar concentrations in the medium were able to enhance *nrt2* expression. Nevertheless, in the presence of high fructose concentration, high ammonium concentration still completely repressed *nrt2* expression indicating that the nitrogen repression overrides sugar stimulation. This is the first report revealing an effect of host plant and of carbon sources on the expression of a fungal nitrate transporter-encoding gene.

Keywords Nitrate uptake · Nitrate transporter · Nitrogen and carbon regulation · Ectomycorrhiza · *Hebeloma cylindrosporum*

D. Rékangalt · R. Pépin · M.-C. Verner · J.-C. Debaud · R. Marmeisse · L. Fraissinet-Tachet
Université de Lyon,
69622 Lyon, France

D. Rékangalt · R. Pépin · M.-C. Verner · J.-C. Debaud · R. Marmeisse · L. Fraissinet-Tachet (✉)
CNRS, UMR5557, INRA, USC 1193, Ecologie Microbienne,
Bât. Lwoff, Université Lyon 1,
43 boulevard du 11 novembre 1918,
69622 Villeurbanne Cedex, France
e-mail: fraissin@univ-lyon1.fr

Introduction

Ectomycorrhizal fungi colonize the roots of the majority of tree species of the boreal and temperate forests (Smith and Read 1997) and constitute a large proportion of the microbial biomass in many forest soils (Fogel 1980). It is considered that ectomycorrhizas help trees to overcome nutrient limitation of forest soils through the foraging activity of the fungal extramatrical mycelium (Read and Perez-Moreno 2003). The main consequence of this plant/fungal association is the bidirectional exchange of fungus-derived nutrients for plant-derived carbohydrates, enabling the plant colonization of mineral nutrient-poor environments. Extramatrical mycelium from the ectomycorrhizal fungi can proliferate extensively within soils (Högberg and Högberg 2002) and efficiently prospects for nutrient resources which may not be directly accessed by the host plant. Although the function of this symbiotic mycelium exploring the soil is important for nutrient acquisition by forest trees, gene regulation in this fungal compartment is poorly characterized (Morel et al. 2005; Wright et al. 2005).

It has been shown that the ectomycorrhizal agaric *Hebeloma cylindrosporum* improves nitrate nutrition of its natural host plant *Pinus pinaster* (Plassard et al. 2000, 2002). Nitrate uptake is a key step controlling the amount of nitrate incorporated by the fungal cells and its subsequent reduction and finally nitrogen export to the plant cell. The *H. cylindrosporum* nitrate assimilation pathway genes including nitrate transporter (*nrt2*), nitrite (*nir1*), and nitrate (*nar1*) reductases-encoding genes have been cloned and their regulation studied in response to nitrogen sources added to mycelia grown in pure culture (Jargeat et al. 2000, 2003) and in association with *P. pinaster* for the *nir1* gene (Bailly et al. 2007). Their transcriptions are repressed by

ammonium and, surprisingly, do not need nitrate for induction as usually required in saprophytic and pathogenic ascomycete species. In filamentous fungi, the genetics of the nitrate assimilation pathway have been extensively investigated particularly in the ascomycetes *Emericella nidulans* and *Neurospora crassa* (for reviews, see e.g., Crawford and Arst 1993; Kinghorn and Unkles 1994; Marzluf 1997). Genes involved in nitrate assimilation are generally induced by nitrate and nitrite and repressed by reduced nitrogen sources such as ammonium and glutamine. In higher plants, it is well known that the nitrate assimilation pathway is under multifactorial control by various signals sensing the carbon and the nitrogen status of the cells. It is related to the light–dark cycle, sugar, and nitrogen contents (Forde 2000; Foyer et al. 2003; Cardenas-Navarro et al. 1998; Lejay et al. 1999, 2003). In *Chlamydomonas reinhardtii*, three putative nitrate transporter genes of the *nrt2* family are regulated by both nitrogen and carbon supply (Quesada et al. 1998). Although the effects of external nitrogen source on nitrate transporter have been clearly demonstrated in a number of organisms, including higher plants (Forde 2000; Williams and Miller 2001), filamentous fungi, and yeasts (Siverio 2002), the effects of external carbon sources remain to be determined in fungi.

In this study, we compared the *nrt2* gene expression level in the extramatrical symbiotic mycelium associated with *P. pinaster* roots. We also analyzed its expression pattern in the mycelium of *H. cylindrosporum* grown, in pure culture, on different carbon sources at different concentrations in order to mime the sugar supplied to the fungus by the plant and its potential effect on *nrt2* expression pattern.

Materials and methods

Strains and growth conditions

The wild-type haploid h1 strain (mating type *A1 B2*) of *H. cylindrosporum* (Debaud and Gay 1987), and two dikaryotic strains: D2 (h1×h7) and the unrelated GCA6 one (Gryta et al. 2000) were used for this study. Mycelia were grown on a modified Melin–Nokrans medium (Gay et al. 1994) buffered at pH 6.0 with 25 mM MES, solidified with 1.5% agarose and overlaid with a cellophane membrane to allow plant inoculation or mycelium recovery for RNA extraction. All nitrogen sources were added after autoclaving the medium as concentrated filter-sterilized stock solutions. Cultures were carried out in the dark at 22°C. *P. pinaster* (Ait.) Sol. plants were obtained from seeds (CEMAGREF batch) which were surface-sterilized and germinated according to Debaud and Gay (1987). Fungal inoculation was performed by covering over its entire length the main root of a 2-week-old plant by a 7×2-cm mycelium strip. Fifteen days after inoculation, both the inoculated roots and the attached mycelium which had grown out of the initial inoculum were harvested.

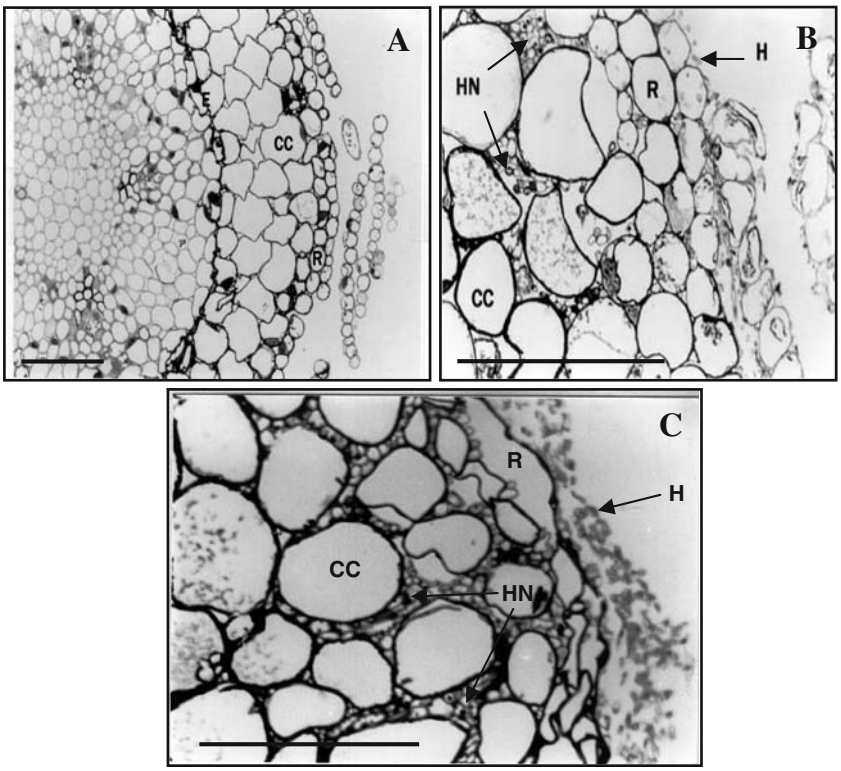

Fig. 1 Colonization of *Pinus pinaster* tap root inoculated (**b**, **c**) or not (**a**) with mycelium of the D2 strain of *Hebeloma cylindrosporum*. Culture media contained 13.9 mM fructose and 3.78 mM of either nitrate (**a**, **b**) or ammonium (**c**). *CC* cortex cells, *R* rhizodermal cells, *E* endoderm, *H* external hyphae, *HN* Hartig net. Bar=100 μm

Effect of carbon supply on *nrt2* gene expression

The effect of carbon supply on *nrt2* gene expression in mycelia associated or not to *P. pinaster* plants was analyzed by growing the dikaryotic D2 mycelium for 15 days on a buffered modified Melin–Norkrans medium containing either a low (2.8 mM) or a high (13.9 mM) fructose concentration without nitrogen (N=0) or supplemented with either 3.78 mM NH_4 (supplied as $(NH_4)_2SO_4$) or NO_3 (as $NaNO_3$). For inoculation, plants were placed in 140 mm diameter Petri dishes on sterile modified Melin–Norkrans medium covered by a cellophane sheet. Mycelium was harvested 15 days after inoculation.

Mycelium of the D2 strain was also grown for 10 days on a buffered modified Melin–Nokrans medium supplemented with 3.78 mM $NaNO_3$ and containing a range from 1 to 15 mM final concentration of carbon source supplied as either fructose, glucose (from 1 to 15 mM) or an equimolar mixture of glucose+fructose (from 0.5 to 7.5 mM each).

Microscopy

Inoculated main roots were processed for microscopy according to a method adapted from Pépin and Boumendil (1982). All operations were performed at room temperature. Samples were fixed for 4 h in 2% glutaraldehyde–0.5% paraformaldehyde in a 0.1-M Mac Ilvaine (citrate-phosphate) buffer at pH 7. They were subsequently washed for 3 h in 0.2 M Mac Ilvaine buffer (pH 7) and then postfixed overnight in 0.5% OsO_4 in 0.1 M Mac Ilvaine buffer (pH 7). After dehydration in a graded series of ethanol solutions and substitution in propylene oxide, the samples were embedded in Epon resin. Polymerization of the resin was carried out at 60°C for 3 days. Then, 0.7 μm semi-thin sections were cut using a Leica Ultracut E ultramicrotome, dried on glass slides, and finally stained with a methylene blue–azure II mixture (Richardson et al. 1960). Microphotographs were taken using Kodak T-max film (100ISO).

Nucleic acid extraction and hybridization

H. cylindrosporum RNA was extracted using the Trizol reagent (Invitrogen). For northern blot analysis, 20 μg of total RNA was fractionated on 1% agarose gels containing 2.2 M formaldehyde. Hybridizations were carried out under stringent conditions to allow hybridization only between homologous sequences. Probes were made from gel purified-DNA fragments and labeled with [α32P] dCTP using the random labeling kit from Boehringer. The *nrt2* DNA fragment was PCR-amplified with the TRN1U (5′-GGTGCTACTTTCGCCATCAT-3′) and the TRN1L (5′-AACGCCTTTCCAACTTCTGT-3′) primers localized in the coding region of the *nrt2* gene. The *nar1* DNA fragment was PCR-amplified with both NAR1U (5′-GCTCTAGAGC CAAGCGCATCGTACCCC-3′) and NAR1L (5′-GAACG AAGAAGAGGTTGCTG-3′) primers localized in the coding region of the *nar1* gene. The 5.8S rDNA gene used as a control was obtained by PCR amplification of the ITS1-5.8S-ITS2 region using both ITS1 (5′-TCCGTAGGTG AACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGAT ATGC-3′) primers (White et al. 1990). Quantification of the hybridization signals was performed using a radioimager and the Molecular Analyst software (BioRad).

Results

nrt2 expression in *H. cylindrosporum* symbiotically associated or not with the plant

In order to test the effect of host plant on the fungal *nrt2* gene expression, we first developed a technique of

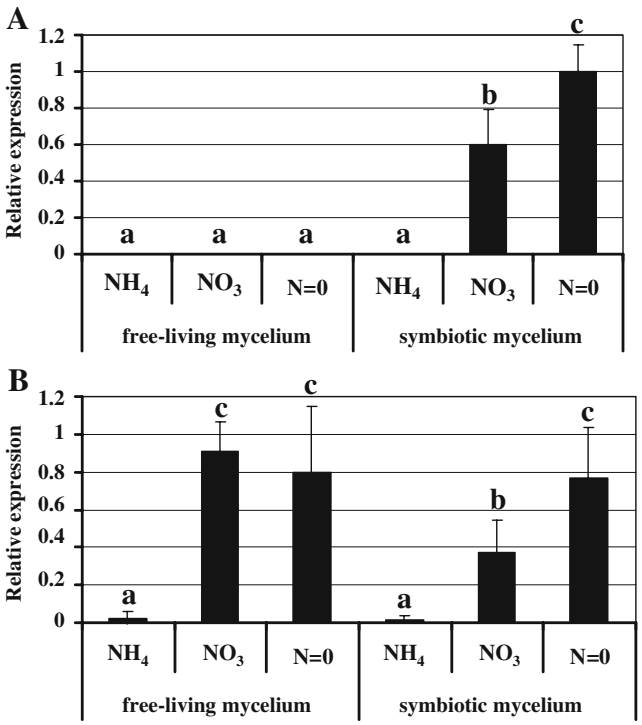

Fig. 2 Effects of fructose concentrations and nitrogen sources on *nrt2* gene expression in mycelia of *H. cylindrosporum* symbiotically associated or not to *Pinus pinaster* roots. Mycelia of the D2 strain were grown for 15 days in association or not with the plant on a medium containing either 2.8 mM (**a**) or 13.9 mM (**b**) fructose, without nitrogen (N=0) or supplemented with 3.78 mM of either NH_4 or NO_3. *nrt2* expression was analyzed by northern blot hybridization using 20 μg of total RNA extracted from ten mycelia. Membranes were successively hybridized to the *nrt2* and the 5.8S rDNA probes. Hybridization signals for the *nrt2* transcripts were normalized using the hybridization signals produced by the 5.8S rDNA probe. This experiment was repeated three times independently. *Different letters* indicate significant differences ($p<0.05$) as determined by Fischer's PLSD test

inoculation which allowed infection of seedling tap roots over their entire length. *P. pinaster* main roots were entirely covered by a 7×2-cm *H. cylindrosporum* mycelial strip from the D2 strain, on a culture medium supplemented with fructose as this monosaccharide was shown to be the most suitable carbon source for in vitro mycorrhizal syntheses (Combier et al. 2004). The growing mycelium that had emerged from the inoculum was harvested 15 days after inoculation to study *nrt2* expression. Microscopic observations of cross sections made through the tap root confirmed fungal infection characterized by the presence of a Hartig net surrounding rhizodermal and outer cortical cells (Fig. 1). Intraradical fungal growth was particularly massive on ammonium-containing medium (Fig. 1c) compared to a nitrate containing one (Fig. 1b) or a medium without N (data not shown).

Plant effect was studied 15 days after inoculation by comparing the *nrt2* expression in the extramatrical mycelium symbiotically associated to the roots of *P. pinaster* seedlings and in similar mycelia that had been transferred in control Petri dishes without plant. The effect of a factorial combination of two fructose concentrations (2.8 and 13.9 mM) and of three N sources (either no N, 3.78 mM NO_3 or NH_4) added to the media was studied (Fig. 2). No *nrt2* expression was observed in the free-living mycelium growing on a low fructose concentration (Fig. 2a). On the opposite, in the extramatrical mycelium living in symbiosis with the plant root, *nrt2* expression was detected on both low and high fructose concentrations in media with either no N or nitrate as N source (Fig. 2a, b). This experiment was repeated with two other fungal strains: the haploid homokaryotic strain h1 (related to the D2 strain) and the unrelated dikaryotic GCA6 one. *nrt2* gene regulation pattern was similar in all three strains: on low fructose concentration *nrt2* gene was not expressed in the free-living mycelium and only showed expression when the fungus was associated to the plant root (data not shown). On the opposite, in the presence of high fructose concentration in the medium (13.9 mM), we did not observe any significant effect of the plant on nitrate transporter gene expression (Fig. 2b). In both the free-ling mycelium and the extramatrical symbiotic mycelium, very little *nrt2* messenger RNA (mRNA) accumulated in presence of ammonium while a strong accumulation was observed in the absence of a N source or in presence of nitrate.

Effect of different carbon sources concentrations on *nrt2* expression

In a second experiment, we tested the effect of a concentration range of different carbon sources; from 1 to 15 mM of either fructose, glucose, or an equimolar mixture of fructose+glucose with nitrate as nitrogen source. For all the tested sugars, *nrt2* mRNA accumulation was positively correlated to sugar

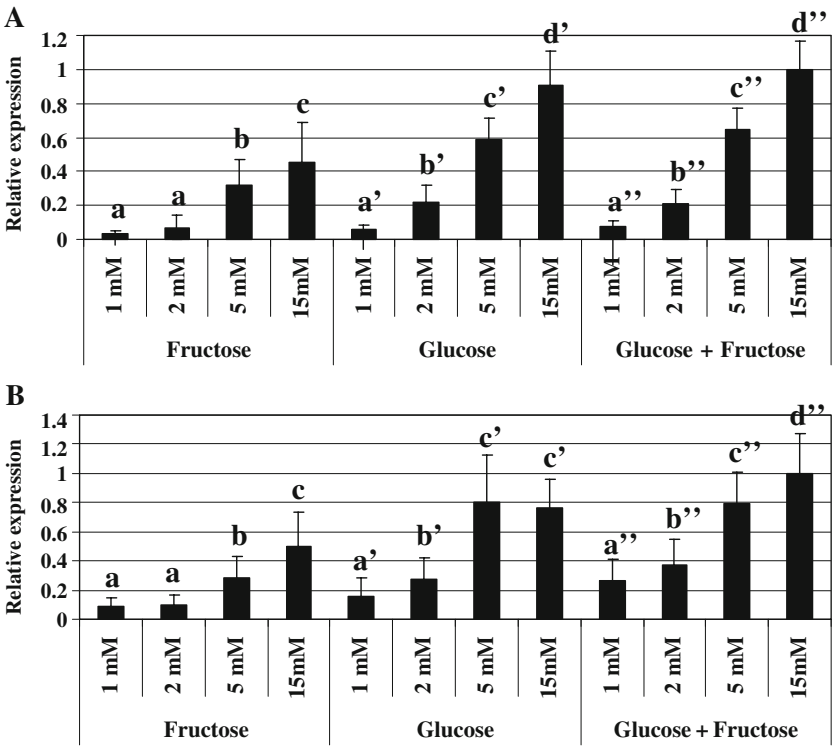

Fig. 3 Effects of different carbon sources and concentrations on *nrt2* (**a**) and *nar1* (**b**) gene expression. Mycelia of the D2 strain were grown for 10 days on media containing a range from 1 to 15 mM (final concentrations) of either fructose, glucose, or an equimolar mixture of glucose +fructose supplemented with 3.78 mM NO_3. *nrt2* and *nar1* gene expressions were analyzed by northern blot hybridization as described in Fig. 2. Northern blots were repeated three times. *Different letters* indicate significant differences ($p<0.05$) as determined by Fischer's PLSD test

concentration (Fig. 3a). The same expression pattern was obtained for the *nar1* gene encoding the nitrate reductase from *H. cylindrosporum* (Fig. 3b).

Discussion

In this study, we evaluated the impact of several factors, host plant and carbohydrate availability, the latest being rarely taken into consideration, in the context of regulation of genes participating to the acquisition of soil nitrogen resources by mycorrhizal fungi. In this respect, regulation pattern of the *nrt2* gene whose protein product is located at the interface between the soil and the fungal cytoplasm to allow nitrate to enter into the cytoplasm is likely to reflect the capacity of the cell to use this nutrient. In the context of mycorrhiza functioning, the dependency of a fungal nitrogen assimilation pathway upon immediate monosaccharide availability could tell us how basal fungal metabolism is dependent upon the plant sugar supply. Indeed, *H. cylindrosporum* cannot degrade and use in pure culture most of the major complex carbohydrates which constitute the plant litter (cellulose, hemicellulose, pectin; unpublished results) and is likely to rely on immediate plant C supply for its survival and foraging activity in soil.

With respect to nitrogen regulation, our results are in accordance with the results of Jargeat et al. (2000, 2003) and Bailly et al. (2007) who demonstrated that in *H. cylindrosporum*, the different genes of the nitrate assimilation pathway, including *nrt2* are repressed in the presence of NH_4 but do not need NO_3 for induction. High expression levels under nitrogen-deprived conditions, in the absence of the corresponding substrate was also reported for different N-regulated genes from other ectomycorrhizal species; *Tuber borchii* for its nitrate transporter *TbNrt2* (Montanini et al. 2006) and *Amanita muscaria* for its amino-acid transporter *AmAAP1* (Nehls et al. 1999).

In addition to regulation by N sources of the *nrt2* gene, for the first time we identified a regulation by carbon sources. In pure culture, a minimum sugar concentration appears necessary for a sustained transcription of the *nrt2* gene. High level of transcription in the presence of high sugar concentration is, however, prevented by the presence of ammonium. In yeast, gene regulation by glucose is a well-described phenomenon (Özcan et al. 1996; Geladé et al. 2003), whereas in filamentous fungi, gene regulation by carbon sources has extensively been studied essentially in the context of polysaccharide degradation (Felenbock and Kelly 1996). Evidence for similar sugar-dependent mechanisms of gene regulation was reported in the ectomycorrhizal basidiomycete *A. muscaria* with the up-regulation by increased hexose concentrations of *AmMst1* encoding a hexose transporter (Nehls et al. 1998). However, carbon regulation of genes otherwise regulated by N-sources has seldom been reported with the exception of extracellular protease encoding genes which are down-regulated by carbon sources (Jaray and Buxton 1994; Naik et al. 1997; Emri et al. 2006).

Down-regulation under C shortage of a gene participating to NO_3 assimilation could be anticipated, as nitrate reduction to ammonium is a costly process in term of reductive power needed. A tight coupling between nitrate reduction and carbon supply is a well-described process in green plants where down regulation of the nitrate regulation pathway under carbon shortage (i.e., in the dark) is achieved at both the post-translational and transcriptional levels (Lejay et al. 2003; Kang and Turano 2003; Smith et al. 2003).

Interestingly, down-regulation of *nrt2* in the presence of low carbon concentrations in the medium could be compensated by the presence of the host plant. This could suggest that, under our experimental conditions, a functional symbiosis was established leading to the transfer of sugar from the plant to the fungus allowing the latter to reduce nitrate into ammonium, which is incorporated onto carbon skeletons for the synthesis of essential amino acids. This represents a direct demonstration of the dependency of basal fungal metabolism upon its active host plant.

References

Bailly J, Debaud JC, Verner M-C, Plassard C, Chalot M, Marmeisse R, Fraissinet-Tachet L (2007) How does a symbiotic fungus modulate the expression of its host-plant nitrite reductase? New Phytol 175:155–165 doi:10.1111/j.1469-8137.2007.02066.x

Cardenas-Navarro R, Adamowics S, Robin P (1998) Diurnal nitrate uptake in young tomato (*Lycopersicon esculentum* Mill.) plants: test of a feedback-based model. J Exp Bot 49:721–730 doi:10.1093/jexbot/49.321.721

Combier JP, Melayah D, Raffier C, Pépin R, Marmeisse R, Gay G (2004) Nonmycorrhizal (Myc-) mutants of *Hebeloma cylindrosporum* obtained through, insertional mutagenesis. Mol Plant Microbe Interact 17:1029–1038 doi:10.1094/MPMI.2004.17.9.1029

Crawford NM, Arst HNJ (1993) The molecular genetics of nitrate assimilation in fungi and plants. Annu Rev Genet 27:115–146 doi:10.1146/annurev.ge.27.120193.000555

Debaud J-C, Gay G (1987) *In vitro* fruiting under controlled conditions of the ectomycorrhizal fungus *Hebeloma cylindrosporum* associated with *Pinus pinaster*. New Phytol 105:429–435 doi:10.1111/j.1469-8137.1987.tb00880.x

Emri T, Molnar Z, Veres T, Pusztahelvi T, Dudas G, Pocsi IS (2006) Glucose-mediated repression of autolysis and conidiogenesis in *Emericella nidulans*. Mycol Res 110:1172–1178 doi:10.1016/j.mycres.2006.07.006

Felenbock B, Kelly JM (1996) Regulation of carbon metabolism in mycelial fungi. In: Brambl R, Marzluf GA (eds) The mycota III: biochemistry and molecular biology. Springer, Berlin, Germany, pp 369–380

Fogel R (1980) Mycorrhizae and nutrient cycling in natural forest ecosystems. New Phytol 86:199–212 doi:10.1111/j.1469-8137.1980.tb03189.x

Forde BG (2000) Nitrate transporters in plants: structure, function and regulation. Biochim Biophys Acta 1465:219–235 doi:10.1016/S0005-2736(00)00140-1

Foyer CH, Parry M, Noctor G (2003) Markers and signals associated with nitrogen assimilation in higher plants. J Exp Bot 54:585–593 doi:10.1093/jxb/erg053

Gay G, Normand L, Marmeisse R, Sotta B, Debaud JC (1994) Auxin overproducer mutants of *Hebeloma cylindrosporum* Romagnesi have increased mycorrhizal activity. New Phytol 128:645–657 doi:10.1111/j.1469-8137.1994.tb04029.x

Geladé R, Van de Velde S, Van Dijck P, Thevelien JM (2003) Multi-level response of the yeast genome to glucose. Genome Biol 4:233–237 doi:10.1186/gb-2003-4-11-233

Gryta H, Debaud J-C, Marmeisse R (2000) Population dynamics of the symbiotic mushroom *Hebeloma cylindrosporum*: mycelial persistence and inbreeding. Heredity 84:294–302 doi:10.1046/j.1365-2540.2000.00668.x

Högberg MN, Högberg P (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol 154:791–795 doi:10.1046/j.1469-8137.2002.00417.x

Jaray G, Buxton F (1994) Nitrogen, carbon and pH regulation of extracellular acidic proteases of *Aspergillus niger*. Curr Genet 26:238–244 doi:10.1007/BF00309554

Jargeat P, Gay G, Debaud JC, Marmeisse R (2000) Transcription of a nitrate reductase gene isolated from the symbiotic basidiomycete fungus *Hebeloma cylindrosporum* does not require induction by nitrate. Mol Gen Genet 263:948–956 doi:10.1007/PL00008695

Jargeat P, Rekangalt D, Verner M-C, Gay G, Debaud J-C, Marmeisse R, Fraissinet-Tachet L (2003) Characterisation and expression analysis of a nitrate transporter and nitrite reductase genes, two members of a gene cluster for nitrate assimilation from the symbiotic basidiomycete *Hebeloma cylindrosporum*. Curr Genet 43:199–205

Kang J, Turano FJ (2003) The putative glutamate receptor I.I (AtGLRI.I) functions as a regulator of carbon and nitrogen metabolism in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 100:6872–6877 doi:10.1073/pnas.1030961100

Kinghorn JR, Unkles SE (1994) Inorganic nitrogen assimilation: molecular aspects. In: Martinelli SD, Kinghorn JR (eds) *Aspergillus*: 50 years on. Elsevier, Amsterdam, pp 181–194

Lejay L, Tillard P, Mepetit M, Olive FD, Filleur S, Daniel-Vedele F, Gojon A (1999) Molecular and functional regulation of two NO3 − uptake systems by N- and C-status of *Arabidopsis* plants. Plant J 18:509–519 doi:10.1046/j.1365-313X.1999.00480.x

Lejay L, Gansel X, Cerezo M, Tillard P, Müller C, Krapp A, von Wirén N, Daniel-Velede F, Gojon A (2003) Regulation of root ion transporters by photosynthesis: functional importance and relation with hexokinase. Plant Cell 15:2218–2232 doi:10.1105/tpc.013516

Marzluf GA (1997) Genetic regulation of nitrogen metabolism in the fungi. Microbiol Mol Biol Rev 61:17–32

Montanini B, Viscomi AR, Bolchi A, Martin Y, Siverio JM, Balestrini R, Bonfante P, Ottonello S (2006) Functional properties and differential mode of regulation of the nitrate transporter from a plant symbiotic ascomycete. Biochem J 394:125–134 doi:10.1042/BJ20051199

Morel M, Jacob C, Kohler A, Johansson T, Martin F, Chalot M, Brun A (2005) Identification of genes differentially expressed in extraradical mycelium and ectomycorrhizal roots during *Paxillus involutus–Betula pendula* ectomycorrhizal symbiosis. Appl Environ Microbiol 71:382–391 doi:10.1128/AEM.71.1.382-391.2005

Naik RR, Nebes V, Jones EW (1997) Regulation of the proteinases B structural gene PRB1 in *Saccharomyces cerevisiae*. J Bacteriol 179:1469–1474

Nehls U, Wiese J, Guttenberger M, Hampp R (1998) Carbon allocation in ectomycorrhizas: identification and expression analysis of an *Amanita muscaria* monosaccharide transporter. Mol Plant Microbe Interact 11:167–176 doi:10.1094/MPMI.1998.11.3.167

Nehls U, Kleber R, Wiese J, Hampp R (1999) Isolation and characterization of a general amino acid permease from the ectomycorrhizal fungus *Amanita muscaria*. New Phytol 144:343–349 doi:10.1046/j.1469-8137.1999.00513.x

Özcan S, Leong T, Johnston M (1996) Rgt 1p of *Saccharomyces cerevisiae*, a key-regulator of glucose induced genes, is both an activator and a repressor of transcription. Mol Cell Biol 16:6419–6426

Pépin R, Boumendil J (1982) Préservation de l'ultrastructure du sclérote de *Sclerotinia tuberosa* (champignon discomycètes); un modèle pour la préparation des échantillons imperméables et hétérogènes. Cytologia (Tokyo) 47:359–377

Plassard C, Bonafos B, Touraine B (2000) Differential effects of mineral and organic N sources, and of ectomycorrhizal infection by *Hebeloma cylindrosporum*, on growth and N utilization in *Pinus pinaster*. Plant Cell Environ 23:1195–1205 doi:10.1046/j.1365-3040.2000.00630.x

Plassard C, Guérin-Laguet EA, Véry A-A, Casarin V, Thibaud J-B (2002) Local measurements of nitrate and potassium fluxes along roots of maritime pine. Effects of ectomycorrhizal symbiosis. Plant Cell Environ 25:1–10 doi:10.1046/j.0016-8025.2001.00810.x

Quesada A, Hidalgo J, Fernandez E (1998) Three *Nrt2* genes are differentially regulated in *Chlamydomonas reinardtii*. Mol Gen Genet 258:373–377 doi:10.1007/s004380050743

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? New Phytol 157:475–492 doi:10.1046/j.1469-8137.2003.00704.x

Richardson KC, Jarret L, Finke H (1960) Embedding in epoxy resin for ultrathin sectioning in electronic microscopy. Stain Technol 35:313–323

Siverio JM (2002) Assimilation of nitrate in yeasts. FEMS Microbiol Rev 26:277–284 doi:10.1111/j.1574-6976.2002.tb00615.x

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, San Diego, CA, USA

Smith CS, Weljie AM, Moorhead GBG (2003) Molecular properties of the putative nitrogen sensor PII from *Arabidopsis thaliana*. Plant J 33:353–360 doi:10.1046/j.1365-313X.2003.01634.x

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for polygenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, USA, pp 315–322

Williams LE, Miller AJ (2001) Transporters responsible for the uptake and partitioning of nitrogenous solutes. Annu Rev Plant Physiol Plant Mol Biol 52:659–688 doi:10.1146/annurev.arplant.52.1.659

Wright DP, Johansson T, Le Quéré A, Söderström B, Tunlid A (2005) Spatial patterns of gene expression in the extrametrical mycelium and mycorrhizal root tips formed by the ectomycorrhizal fungus *Paxillus involutus* in association with birch (*Betula pendula*) seedlings in soil microcosms. New Phytol 167:579–596 doi:10.1111/j.1469-8137.2005.01441.x