Mycorrhiza (1996) 6:247–251

ORIGINAL PAPER

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Effects of N source on pH and nutrient exchange of extramatrical mycelium in a mycorrhizal Ri T-DNA transformed root system

Accepted: 7 March 1996

Abstract The influence of different N sources on medium pH variation and the effect of the external mycelium of arbuscular mycorrhizal fungi on nutrient dynamics were studied using a two-compartment, aseptic Petri plate system. VA mycorrhizal, transformed roots of carrot (*Daucus carota* L.) were cultured in the proximal compartment and external mycorrhizal mycelium in the distal compartment. The medium in the distal compartament contained N either as NO_3^- or as NH_4^+. The pH and the anion and cation concentrations were measured every 15 days in filtrates prepared from the distal compartments. Thirteen weeks after colonization, there was a significant basification or a light acidification of the NO_3^- and NH_4^+ medium, respectively. There was no change in NO_3^- concentration but a significant decrease in NH_4^+ concentration. Treatments containing N as NO_3^- showed no variation in cations such as Ca^{2+} and Mg^{2+} or anions such as PO_4^{2-}, and SO_4^{2-} but showed significant increases in the concentration of K^+. Treatments containing N as NH_4^+ showed no variation in cations or anions, except for increases in the concentrations of K^+ and Cl^-.

Key words *Glomus intraradices* · Nitrogen source · Extramatrical mycelium · Mycorrhizal transformed roots · pH

Introduction

The promotion of plant growth by arbuscular mycorrhizal (AM) fungi has been widely recognized (Bethlenfalvay 1992). This effect of mycorrhizal fungi is mediated by the external mycelium, which absorbs and/or mobilizes nutrients and translocates them into the associated plant roots. Various chemical, physical and biological factors may influence the mobilization and solubilization of ions around the external hyphae, and consequently nutrient acquisition by AM fungi, as well as other processes in the soil mycorrhizosphere, especially in zones not accessible to plant roots.

The effect of nitrogen on the physiology and growth of plants has been well documented for both agricultural and forest ecosystems (Stribley and Read 1980; Bowen and Smith 1981; Smith et al. 1985; Barea 1991). However, the specific effects of the N source on the kinetics and physiology of the external mycelium of AM fungi, and its influence on mycorrhizosphere dynamics, remain poorly understood. AM fungi possess the enzymes required for assimilation of both ammonium (Smith et al. 1985) and nitrate (Ho and Trappe 1975). Furthermore, Johansen et al. (1993) found that N-assimilation by the external hyphae of the AM fungus *Glomus intraradices* (Schenck and Smith) absorbs mineral N from the growth medium and transports it into the host plant. Their results also suggest that the external hyphae can assimilate NO_3^- from the growth medium, but remains unclear whether the external mycelium has the ability to assimilate NH_4^+.

Such changes in N status could modify the pH of the surrounding environment. Stribley (1987) and Li et al. (1991b) suggested that the hyphal environment undergoes a significant decrease in pH with NH_4^+ and increase with NO_3^-. Ortas et al.(1993), working with mycorrhizal sorghum plants, showed a decrease in rhizosphere pH in soils with low P availability, in the presence of both NH_4^+ and NO_3^-, although NH_4^+ had the greatest effect. Under non-sterile conditions in pots allowing the spatial separation of roots, hyphae and soil, Li et al. (1991b) observed a decrease in soil pH at the soil-hyphae and the soil-mycorrhizal root interface when mycorrhizal white clover plants were fertilized with NH_4^+. It is not known whether these mycorrhizos-

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phere pH changes are a consequence of the uptake or release of (in)organic ions to regulate the cellular pH of fungal cells, or are produced indirectly by other mycorrhizosphere components, such as plant roots or other microorganisms. In plant roots, production or excretion of H^+ as well as the production of organic acids may also induce pH changes. In ectomycorrhizal fungi, pH alterations in the vicinity of hyphae have been associated with certain fungi that excrete organic acids (Cairney and Ashford 1989; Lapeyrie et al. 1990); however, the nature and kinetics of the compounds associated with these pH alterations remain unknown.

The main difficulty in studying extramatrical mycelium is the lack of a system to clearly demonstrate the influence of different environmental factors on the mycelium or the effects of the mycelium on its surroundings. St-Arnaud et al. (1995) developed a two-compartment, aseptic Petri plate system that allows maintenance of the mycorrhizal roots on one side of the plate and the development of the external hyphae alone on the other side, thereby reducing the influence of the roots on fungus growth. This system can be used to study specific effects of the N source on the kinectics and physiology of the external mycelia of AM fungi, as well as effects of the fungus on the ionic environment, without interference by other non-volatile mycorrhizosphere components. The objectives of the present work were to investigate the influence of different N sources on medium pH variation and the effect of the external mycelia of AM fungi on nutrient kinetics using the system described above.

Materials and methods

The two-compartment Petri plate system consists of VA mycorrhizal, transformed carrot roots (*Daucus carota L.*) in the proximal compartment and external mycorrhizal mycelium in the distal compartment (St-Arnaud et al.1995).

Mycorrhizal inoculum

The minimal medium of Becard and Fortin (1988) was used as the standard growth medium for the establishment and maintenance of the dual cultures. Cloned *Agrobacterium rhizogens*–transformed carrot roots colonized with the AM fungus *Glomus intraradices* were routinely subcultured or propagated by transferring medium blocks of approximately 1 cm^2 containing both colonized roots and spores onto fresh minimal media.

Culture conditions

In the proximal compartment, the standard growth medium contained (in mg l^{-1}): 731 $MgSO_4 \cdot 7H_2O$, 80 KNO_3, 65 KCl, 4.8 KH_2PO_4, 288 $Ca(NO_3)_2 \cdot 4H_2O$, 8 NaFeEDTA, 0.75 KI, 6 $MnCl_2 \cdot 4H_2O$, 2.65 $ZnSO_4 \cdot 7H_2O$, 1.5 H_2BO_3, 0.13 $CuSO_4 \cdot 5H_2O$, 0.0024 $Na_2MoO_4 \cdot 2H_2O$, 3 glycine, 0.1 thiamine hydrochloride, 0.1 pyridoxine hydrochloride, 0.5 nicotinic acid, 50 myo-inositol and 10000 sucrose. In the distal compartment, two different treatments were used: (1) the standard medium mentioned above, with N in the form of NO_3^-, and (2) a modified medium with N in the form of NH_4^+ and containing (in mg l^{-1}) 731 $MgSO_4 \cdot 7H_2O$, 130 KCl, 4.8 KH_2PO_4 184.0 $CaCl_2$, 1420 $(NH_4^+)_2SO_4$, as well as the other components of the standard growth medium. The pH of both media was adjusted to 5.5 with HCl or NaOH and 4% Gel Gro (ICN Biomedicals) was added as the gelifying agent before sterilization at 121 °C for 15 min. Blocks of approximately 1 cm^2 of inoculum were placed in the proximal compartment, and the plates were sealed thoroughly with parafilm and incubated in the dark at 26 °C.

Experiment 1

In order to observe pH variations with the two different sources of nitrogen, the pH of the distal compartments was determined every 15 days in a solution obtained by filtering the media under gentle pressure through a Texel geotextile cloth (Beauce, Quebec) using a 20-ml syringe.

Experiment 2

In order to measure changes in anion and cation concentrations in the distal compartment, NH_4^+, K^+, Mg^{2+}, Ca^{2+} NO_3^-, PO_4^{2-}, and SO_4^{2-} were determined by HPLC in the filtrates mentioned above. Ions were separated on a Dionex (Oakville, Ontario) HPLC system using a pulsed electrochemical detector in the conductivity mode. Anions were separated on a 4×250-mm IONPAC AS4A-SC column, with a guard column (IONPAC AG4A-SC) and an on-line anion self-regenerating supressor (ASRS-1) to improve the signal-to-noise ratio, using an aqueous bicarbonate buffer (1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$) at 2.0 ml/min. This allowed complete separation of all major anions in 10 min. The integration response was linear for concentrations up to 10-fold that normally found in the minimal medium. Cations were separated on a 4×250-mm IONPAC CS-12 analytical column, with a guard column (IONPAC CG-12) and an on-line cation self-regenerating supressor (CSRS-1) to reduce the signal-to-noise ratio, using aqueous methanesulphonic acid (20 mM) at 1.0 ml/min; this separated the cations present within 12 min.

Statistics

Six replicate Petri plates were used for each treatment to calculate means and 95% confidence limits. Data were subjected to an analysis of variance.

Results

Experiment 1

Seven weeks after inoculation, the distal compartment filtrates from the NO_3^- and NH_4^+ treatments showed slight differences in pH. Thirteen weeks after inoculation, when the external hyphae had completely colonized the distal compartments, a significant ($P \leq 0.05$) basification was observed in the NO_3^- treatments. After the same period, filtrates from NH_4^+ treatments were slightly acidified (Fig. 1).

Experiment 2

The nitrogen kinetics in the distal compartments of the NO_3^- and NH_4^+ treatments showed different patterns. The NH_4^+ concentrations had significantly decreased

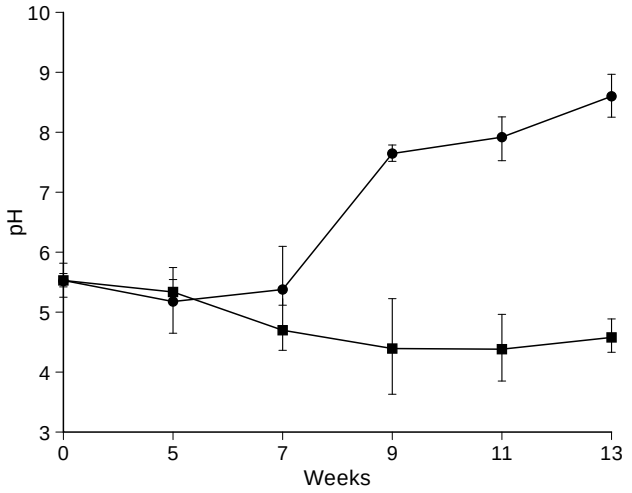

Fig. 1 pH variation with time in filtrates obtained from the distal compartments of Petri plates containing N as NO_3^- –●– or NH_4^+ –■– ; *bars* 95% confidence limits

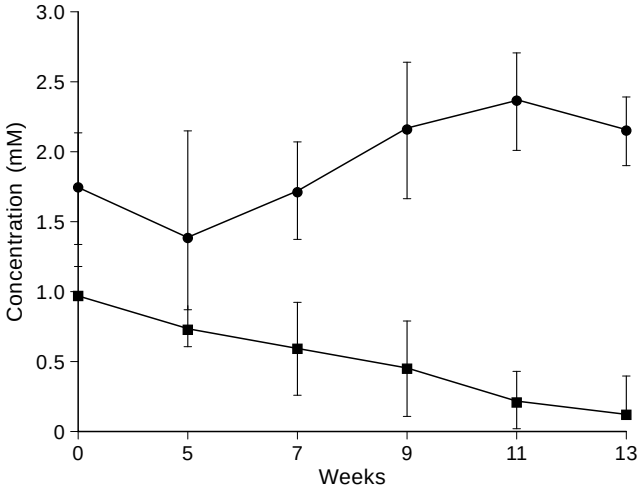

Fig. 2 Variation with time in the concentrations of NO_3^- –●– or NH_4^+ –■– in filtrates obtained from the distal compartments of Petri plates; *bars* 95% confidence limits

($P \leq 0.05$) by 13 weeks after inoculation (Fig. 2), suggesting assimilation of NH_4^+ by the external mycelium. Mean NO_3^- concentrations in the NO_3^- treatments had not decreased by 13 weeks of inoculation (Fig. 2).

Regardless of the treatment, the cations Ca^{2+} and Mg^{2+} showed no significant variation ($P \geq 0.05$) after 13 weeks of incubation. However, the K^+ concentration in the filtrate from the NO_3^- treatment had increased significantly ($P \leq 0.05$) after 13 weeks of culture; in the NH_4^+ treatment, K^+ increased only slightly during the same period (Fig. 3).

For the anions Cl^- and SO_4^{2-} in the filtrates obtained from NH_4^+ treatments, a significant increase ($P \leq 0.05$) from 4.32 to 6.27 mM Cl^- was observed, but there was no significant change ($P \geq 0.05$) in SO_4^{2-} concentration during the same period (Fig. 4). Likewise, the filtrates obtained from the NO_3^- treatments showed

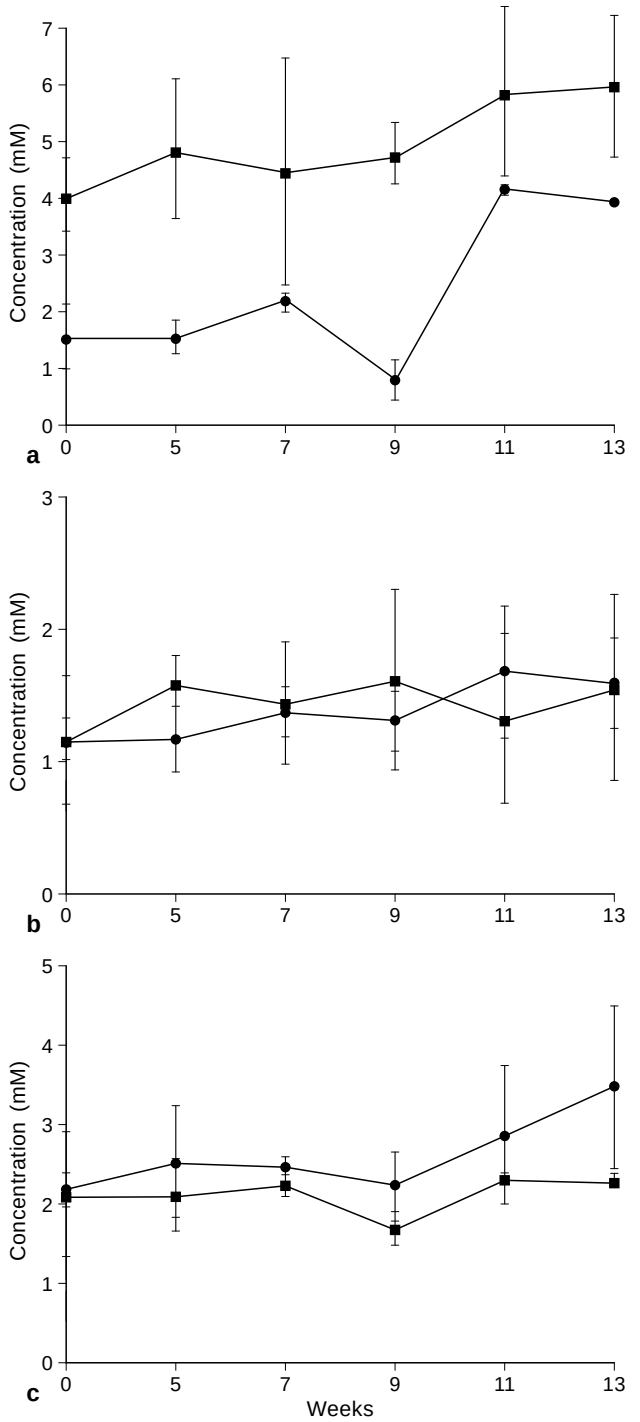

Fig. 3 Variation with time in the concentrations of the cations K^+ (**a**), Ca^{2+} (**b**), and Mg^{2+} (**c**) in filtrates obtained from the distal compartments of Petri plates containing N as NO_3^- –●– or NH_4^+ –■– ; *bars* 95% confidence limits

no significant variation ($P \geq 0.05$) in the levels of Cl^- or SO_4^{2-}. However, the initial concentrations of the anions were lower (0.91 mM Cl^-, 2.82 mM SO_4^{2-}) in the NO_3^- than in the NH_4^+ compartment (4.32 mM Cl^-, 4.13 mM SO_4^{2-}). Irrespective of the treatment, the concentration of PO_4^{2-} was always very low (0.00–0.15 mM) and

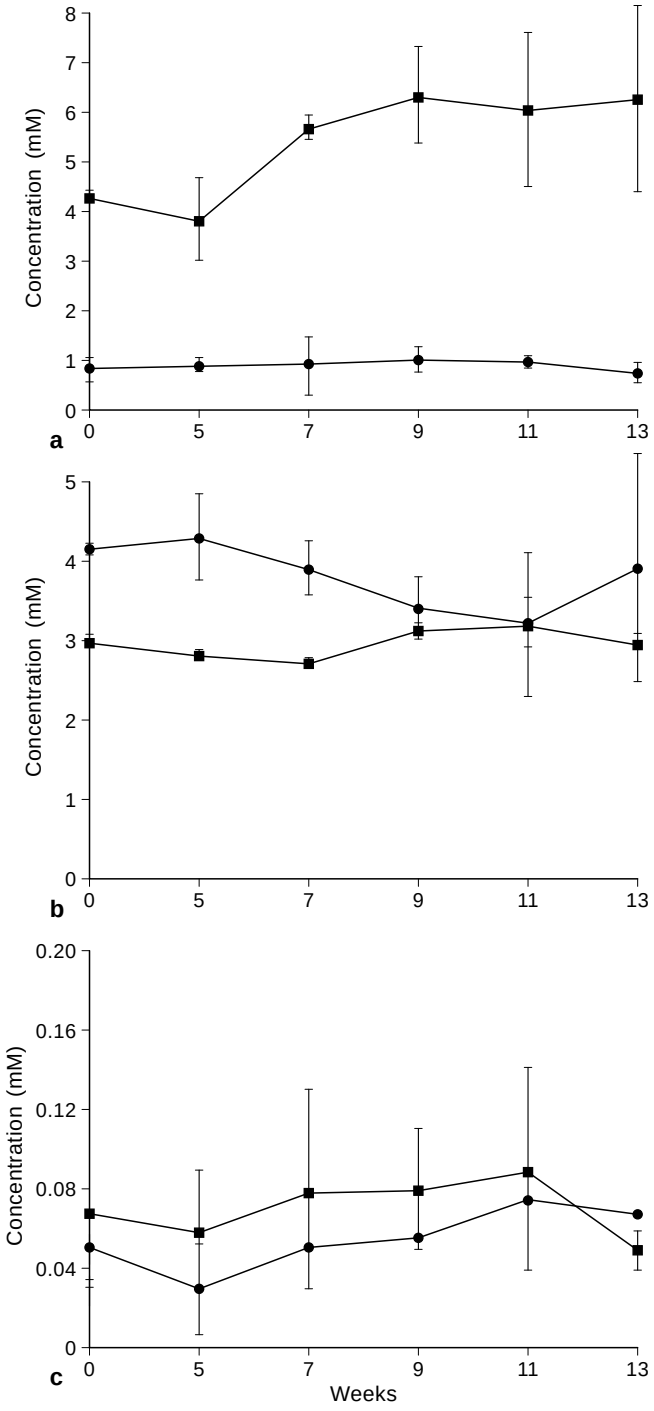

Fig. 4 Variation with time in the concentrations of the anions Cl^- (**a**), SO_4^{2-} (**b**) and PO_4^{2-} (**c**) in filtrates obtained from the distal compartments of Petri plates containing N as NO_3^- –●– or NH_4^+ –■– ; *bars* 95% confidence limits

showed no significant variation ($P \geq 0.05$) during the experiment.

Discussion

AM fungi appear to possess enzymes required for the assimilation of both NO_3^- (Ho and Trappe 1975) and NH_4^+ (Smith et al. 1985; Barea 1991). Our results using root organ cultures, show that NO_3^- absorption by external mycelia growing in a minimal medium containing NO_3^- as the source of N is negligible and, therefore, that NO_3^- reduction is unlikely. In contrast, NH_4^+ absorption by external mycelia can deplete the NH_4^+ of the. Johansen et al. (1993), found that external hyphae of the AM fungus *G. intraradices* were able to assimilate NO_3^- from the growth medium and transport it to the host plant. In contrast, Tobar et al. (1994a,b), working with lettuce plants under water-stressed conditions, presented evidence that the external mycelia of AM fungi can absorb and transport N from both a NO_3^- source and an NH_4^+ source. However, our results suggest that VA mycorrhizal fungi play a more active role in NH_4^+ uptake.

It has been established that the N source can greatly influence rhizosphere pH (Römheld 1990; Trolldenier 1992). In addition, several studies have shown that AM fungi can modify the surrounding pH as well as the ionic environment. Our results show that under aseptic conditions the associated external mycelia of mycorrhizal Ri T-DNA-transformed carrot roots produce different effects on the pH of the media, depending on N source. External mycelium grown on minimal media containing NO_3^- as a source of N significantly increased the pH of the medium, while the same mycelium grown in minimal medium containing NH_4^+ slightly decreased the pH. Li et al. (1991b) observed a decrease in soil pH at both the soil-hyphae and the soil-mycorrhizal root interface, but could not determine whether these changes were produced by the external mycelium or by other mycorrhizosphere variables, such as the plant roots or other rhizosphere microorganisms.

Gianinazzi-Pearson and Smith (1993) associated a decrease in the pH of the hyphal environment with NH_4^+ uptake and a moderate increase in pH with NO_3^- uptake. However, in the present study, we found no correlation between increase of pH and NO_3^- uptake by AM fungi that would indicate a specific contribution of the external mycelium to pH changes at the mycorrhizosphere level.

It is generally accepted that pH has an important effect on the solubility and uptake of mineral nutrients by plant roots. The mobilization of ions such as K^+ (Kothari et al. 1991) and PO_4^{2-} (Li et al.1991a,b) through AM hyphae has been documented. However, the contribution of AM fungi to surrounding ion kinetics is not yet well understood. Our results show no alteration in the concentrations of Ca^{2+} or Mg^{2+} with NO_3^- treatment after 13 weeks, but a strong increase in K^+ concentration was detected after 11 weeks incubation. Changes in K^+ concentration throughout the experiment were closely related with variation in medium pH. The kinetics of K^+ observed in the proximal compartment (data not shown) suggest that this ion is absorbed by mycorrhizal roots in the proximal side and released by the external hyphae. Cations in plants may be associated with organic anions in plant tissues (Watanabe et

al. 1971; Buwalda and Goh 1982), and their flux is involved in pH maintenance within the plant (Raven and Smith 1976). Rygiewicz et al. (1984) suggested that ectomycorrhizal fungi can act as rhizosphere buffers; hence, for an equal amount of absorbed NO_3^-, the surrounding pH changes less than with non-mycorrhizal plants. It appears that AM fungi function in a similar fashion. The negative flux of K^+ observed in the distal side of the Petri plates is believed to be involved in the maintenance of the pH in the proximal side surrounding the mycorrhizal transformed roots. The concentrations of K^+ in the NH_4^+ treatment increased slightly during the last 4 weeks of the experiment. This change may also be associated with efflux of cations maintaining the pH on the proximal side of the Petri plates containing minimal media with NO_3^- as the N source.

The concentration ofs PO_4^{2-} and SO_4^{2-} in the NO_3^- and NH_4^+ treatments did not change throughout the experiment; however, an increase in Cl^- concentration was observed in the NH_4^+ treatment. This increase may be associated with the release of protons to equilibrate internal pH during NH_4^+ uptake by external mycelia.

The present study shows, for the first time under aseptic conditions and with the physical separation of fungus and host, the effect of NH_4^+ and NO_3^- on the pH and ion kinetics mediated by external mycelia of an AM fungus. How the changes recorded influence other events such as the mobilization and solubility of nutrients and microbial activity in the mycorrhizosphere deserves further study. The results confirm the potential of the two-compartment Petri plate system for the study of external mycelia of AM fungi.

Acknowledgements Financial support for J.V. was provided by the Dirección General de Asuntos del Personal Académico de la UNAM, México. We would like to extend our gratitude to Peter Moutoglis for technical support and valuable suggestions.

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