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# Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of *Plantago lanceolata*

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## Abstract

A greenhouse pot experiment with different phosphorus supply was conducted to study growth, photosynthesis and free polyamine (PA) content in *Plantago lanceolata* L. plants in relation to arbuscular mycorrhizal (AM) colonization. Inoculum of *Glomus fasciculatum* (BEG 53) was used. Inoculated plants had high colonization intensities which were related to the P supply. Non-mycorrhizal (NM) plants showed a typical yield response curve for P availability. Dry masses of mycorrhizal (M) plants were higher at the lowest soil P content than those of NM plants, but the opposite was found at the highest P supply. P contents in M plants were always higher. There were no differences in chlorophyll (Chl) concentrations (except the lowest soil P content) and ratios of variable to maximum Chl fluorescence (Fv/Fm) values between M and NM plants, whereas M plants had higher ratios of leaf area to fresh mass (A/f.m.) at low soil P contents and they had significantly higher $CO_2$ fixation capacities per unit leaf area. Free putrescine (Put), spermidine (Spd) and spermine (Spm) contents in NM plants were usually highest at the lowest P supply. The ratios of Put/(Spd+Spm) were identical in M and NM leaves. They were significantly higher, however, in NM roots at the two low P doses. It is concluded, that a P nutritional status might exist, below which PA concentrations and ratio are increased drastically, possibly indicating P deficiency or a certain state of plant development with a higher demand for AM symbiosis.

*Additional key words*: $CO_2$ fixation, growth depression, putrescine, spermidine, spermine, polyamine ratio.

## Introduction

Arbuscular mycorrhiza is the most ancient and widespread type of mycorrhiza symbiosis. It was detected in almost 90 % of the terrestrial plant species. Arbuscular mycorrhizal (AM) fungi were proved to stimulate phosphorus uptake and growth of their host plants. In return, host plants provide saccharides (4 - 20 % of the net carbon fixed) for their fungal partners.

Morphological and physiological characteristics of the host plant are often changed as a result of AM colonization. Most of these changes, like enhanced growth and photosynthesis can be indirectly attributed to the improved mineral nutrition of the host. The positive growth response to mycorrhiza formation usually declines under high P availability and colonized plants may actually grow less than non-mycorrhizal ones. However, irrespectively of the growth effects, the physiology of mycorrhizal plants are prone to significant changes due to other, direct responses of plant metabolism, like changes in hormonal balances, which are often masked by the nutritional benefits (Smith and Read 1997).

Polyamines (PAs) are involved in several aspects of plant and fungal growth and differentiation (Galston and Kaur-Sawhney 1995, Walters 1995, Walden *et al.* 1997). They were reported to minimise the loss of chlorophyll and photochemical activity during senescence (Subhan and Murthy 2001) and heat stress (Murkowski 2001). The profound changes in plant PA metabolism under several environmental challenges including mineral nutrient deficiencies and plant-pathogen interactions were

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*Abbreviations*: A/f.m. - ratio of leaf area to fresh mass; AM - arbuscular mycorrhizal; Chl - chlorophyll; d.m. - dry mass; f.m. - fresh mass; Fv/Fm - ratio of variable to maximum chlorophyll fluorescence; NM - non-mycorrhizal; M - mycorrhizal; PA - polyamine; Put - putrescine; Spd - spermidine; Spm - spermine.

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reviewed by Bouchereau *et al.* (1999). Put is regarded as a stress signal molecule, while Spd and Spm may confer stress tolerance (Walters 2000). Information is very scarce about their role in plant-microbe symbioses. Optimal concentrations of PAs may be essential for the normal development of arbuscular mycorrhiza (El Ghachtouli *et al.* 1996) and exogenously applied PAs increased the frequency of AM colonization in pea (El Ghachtouli *et al.* 1995). They have a role supposed in the molecular signalling events between the symbiotic partners (El Ghachtouli *et al.* 1995) or regulation of nutrient transport (Kytöviita and Sarjala 1997).

In the present study, growth components, total P content, Chl content, Chl fluorescence (photochemical activity), photosynthetic $CO_2$ fixation capacity and concentrations of three free PAs (putrescine, spermidine and spermine) of mycorrhizal (M) and non-mycorrhizal (NM) plants were studied to examine the benefits or drawbacks of the AM colonization for the plant partner under different P supplies. This way could be appropriate to study the distinct influences of P supply and arbuscular mycorrhiza on physiological characteristics (Fitter 1988). We hypothesized that the amount of PAs might be important in the P-deficiency stress and consequently, in the P supply-related interaction between the symbionts.

## Materials and methods

*Plantago lanceolata* L. plants were grown in pots (250 g soil) on a γ-irradiated (20 kGy) mixture of loam and sand (1:1) in a greenhouse for three months (January to April). Greenhouse day/night temperature was around 24/17 °C and photosynthetic photon flux density (PPFD) was maximum 200 μmol $m^{-2}$ $s^{-1}$ at the beginning, but increased up to 600 - 700 μmol $m^{-2}$ $s^{-1}$ during the second half of the experiment.

Nutrients ($KNO_3$, $Ca(NO_3)_2$, $MgSO_4$, Fe-EDTA) were provided at the beginning (121, 283, 118 and 0.4 μg $g^{-1}$ (soil), respectively). $KH_2PO_4$ was added in 2 concentrations ($P_1$: 33 and $P_2$: 196 μg $g^{-1}$) or was not added ($P_0$). Plants were watered equally every 2 d with 10 - 20 $cm^3$ of distilled water. Plant-available soil nutrient contents were determined by a *Thermo Jarrell Ash ICAP 61E* ICP-spectrometer (*Thermo Optek Corp.*, Franklin, USA) after ammonium-lactate (P, K) or KCl ($NO_3$-$NO_2$-N) soil-extraction (Buzás 1988). $K_2O$ and $NO_3$-$NO_2$-N contents were 91.9 and 65.5 μg $g^{-1}$. $P_2O_5$ contents were 1.7 ($P_0$), 32.7 ($P_1$) and 135.3 ($P_2$) μg $g^{-1}$. Soil $CaCO_3$ content (determined by a *CM-22* Scheibler-calcimeter, *Labor MIM*, Budapest, Hungary) was 20 μg $g^{-1}$ and soil pH (KCl-extract) was 7.59.

Soil-based inoculum of *Glomus fasciculatum* (30 g) originated from the European Bank of Glomales (BEG 53) was laid into each pot 1 cm below the soil surface. The same amount of γ-sterilized inoculum was laid into the control pots. Seeds were placed above the inoculum layer. Mycorrhizal colonization was visualized according to Phillips and Hayman (1970). Colonization parameters (frequency - F [%], intensity - M [%], arbuscule content - a [%]) were estimated by the method of Trouvelot *et al.* (1986). Dry masses (after drying at 80 °C until constant mass),leaf lengths, highest leaf widths and ratios of leaf area to fresh mass (A/f.m.) were determined. Mycorrhizal dependency values (% difference in dry mass between M and NM plants compared to M plants) were calculated (Plenchette *et al.* 1983). Total percentage plant phosphorus contents (% of d.m.) were measured by an ICP-spectrometer after selenium-sulphuric acid digest (Buzás 1988). Chl concentration as determined according to Porra *et al.* (1989) and Chl fluorescence kinetics parameters (variable to maximum fluorescence ratio, Fv/Fm) were measured by a PAM (Pulse Amplitude Modulation) Chlorophyll Fluorometer (*Heinz Walz GmbH*, Effeltrich, Germany) according to Schreiber (1986). $^{14}CO_2$ fixation capacities of excised leaves were determined using a closed illuminated (irradiance of 1000 μmol $m^{-2}$ $s^{-1}$) glass chamber with 0.1 % $CO_2$ concentration (Láng *et al.* 1985). Radioactivity of the leaf samples was determined by an *LS 5000 TD* liquid scintillation apparatus (*Beckman Instruments*, Fullerton, USA). Quantities of free polyamines were determined by thin-layer chromatography (Rácz *et al.* 1996) and a *Fluoromax-2* spectrofluorometer (*Instruments S.A., Jobin Yvon/Spex Division*, Longjumeau Cedex, France). The ratio Put/(Spd+Spm) was calculated.

The parallel experiments gave similar results, but the second was chosen to present due to the better light conditions of that period. Two-way *ANOVA* was applied for statistical analysis using the software *SPSS 7.5*. Means were compared between treatments by the least significant difference (*LSD*) test or the Student's *t*-test.

## Results

**Root colonization, growth, plant P content, photosynthesis:** Inoculated plants were heavily colonised by the AM fungus at every soil P contents, but intensity of colonization and arbuscule content decreased with increasing P supply (Table 1). Effect of AM colonization on growth parameters had a strong interaction with P availability. NM plants showed a typical dry mass response curve for P supply (Fig. 1), as well as leaf length

and highest width (data not presented). As regards M plants, however, growth parameters remained almost the same and significantly higher than those of NM ones at the lowest soil P dose. This difference not only disappeared at $P_1$, but a negative growth response to AM colonization occurred at the highest P content. The tendency is reflected by mycorrhizal dependency values of whole plants: 40.1 %, 7.4 %, and -31.9 % for $P_0$, $P_1$, and $P_2$ soils, respectively. Specific leaf areas of M plants were significantly higher at the low soil P contents, but

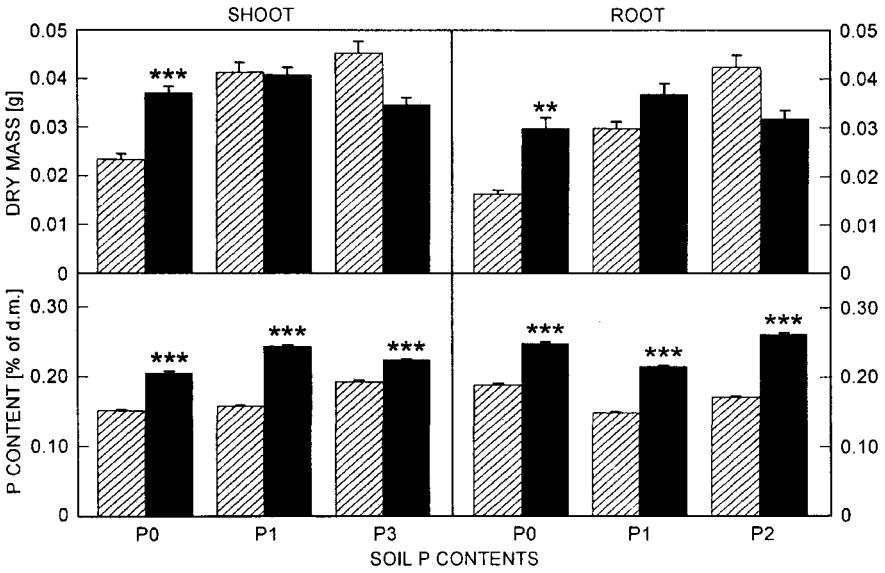


Fig. 1. Mean dry masses and total phosphorus contents of shoots and roots of mycorrhizal (M) and non-mycorrhizal (NM) *Plantago lanceolata* plants. SE is shown by *vertical bars*. Statistically significant differences between M (*closed columns*) and NM (*hatched columns*) plants under the same phosphorus supply are indicated as ** - $P \leq 0.01$, *** - $P \leq 0.001$.

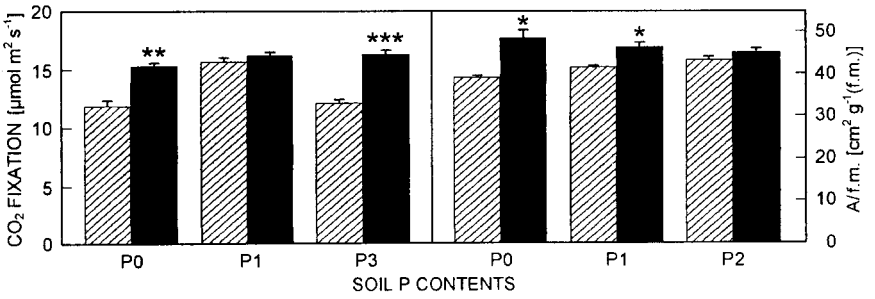


Fig. 2. Mean $CO_2$ fixation capacities and ratios of leaf area to fresh mass (A/f.m) of mycorrhizal (M) and non-mycorrhizal (NM) *Plantago lanceolata* plants. SE is shown by *vertical bars*. Statistically significant differences between M (*closed columns*) and NM (*hatched columns*) plants under the same phosphorus supply are indicated as * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$.

Table 1. Frequency (F) and intensity (M) of AM colonization [%] and arbuscule content (a) of the colonized root fragments [%] in mycorrhizal *Plantago lanceolata*. Means ± SE, $n = 8$. Statistically significant differences ($P \leq 0.05$) are indicated with different letters. Colonization parameters were calculated according Trouvelot *et al.* (1986). Soil phosphorus contents are explained in Materials and methods.

| Soil P ontent | F [%] | M [%] | a [%] |
|---|---|---|---|
| $P_0$ | 100.0 ± 0.0 a | 84.0 ± 2.9 a | 51.6 ± 4.7 a |
| $P_1$ | 95.0 ± 1.9 ab | 78.0 ± 4.4 ab | 47.5 ± 3.2 ab |
| $P_2$ | 93.3 ± 3.2 b | 70.9 ± 6.1 b | 36.7 ± 3.4 b |

the difference declined gradually with increasing P supply (Fig. 2). P contents of M shoots and roots were significantly higher than those of NM ones in all cases (Fig. 1). M plants had significantly higher $CO_2$ fixation capacities calculated per unit leaf area basis (and also per leaf fresh mass, dry mass and Chl content basis - data not presented) except $P_1$ soil (Fig. 2). NM and M plants had the same Fv/Fm ratios and Chl contents per unit leaf area were different only in those grown at the lowest P supply (Table 3).

**Polyamine contents:** Contents of the three free polyamines measured were similar in leaves and roots in NM and M plants when their growth parameters were

similar ($P_1$), irrespectively of their different P contents (Fig. 3). However, leaves of M plants had significantly lower PA concentrations in case of $P_0$ and $P_2$. As regards the lowest soil P content, there were significantly higher PA concentrations not only in NM leaves, but also in NM roots, except Spm (Fig. 3). Most dramatic differences were recorded in the case of Put, while the alterations of Spd and Spm were smaller. Consequently, differences in PA concentrations between M and NM plants were strongly influenced by P supply (Table 2).

Put/(Spd+Spm) ratios in NM and M leaves were similar in every case (Fig. 3). On the contrary, AM colonization had a strong effect on the ratio in the roots (Table 2). NM plants had significantly higher ratios under the two lowest P doses, but this difference disappeared at the highest soil P content (Fig. 3).

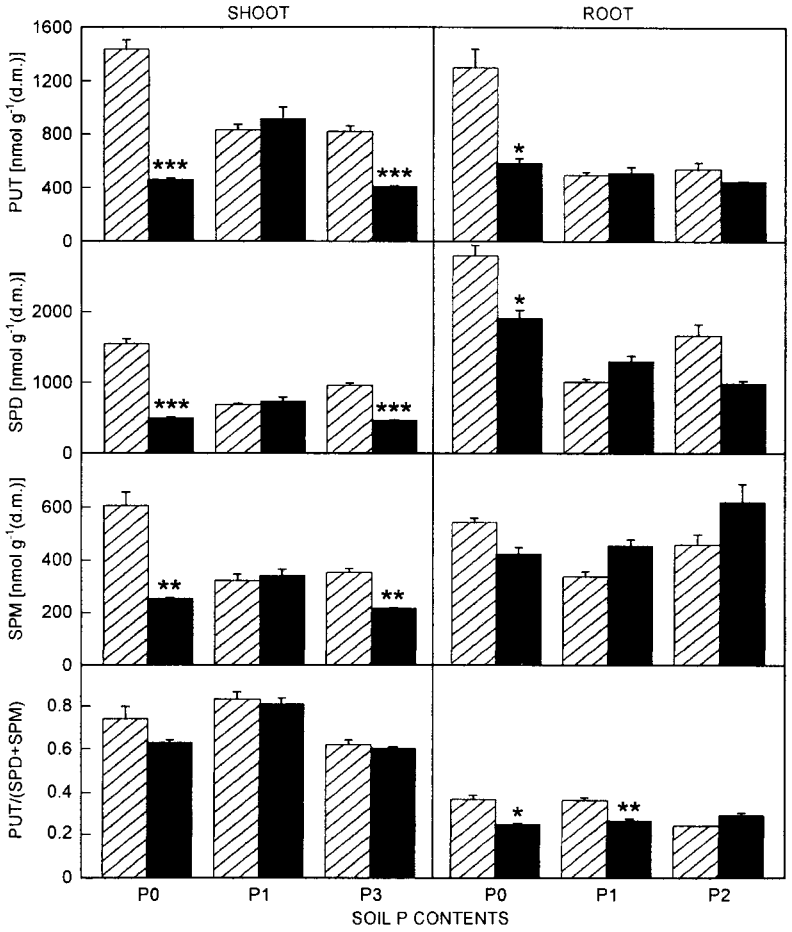


Fig. 3. Mean putrescine (PUT), spermidine (SPD), spermine (SPM) contents and polyamine ratios (PUT/(SPD+SPM)) of leaves and roots of mycorrhizal (M) and non-mycorrhizal (NM) *Plantago lanceolata* plants. SE is shown by *vertical bars*. Statistically significant differences between M (*closed columns*) and NM (*hatched columns*) plants under the same phosphorus supply are indicated as * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$.

## Discussion

AM colonization intensity and arbuscule content decreased with increasing P availability (Table 1), which is a well known phenomenon (Menge *et al.* 1978) and the result of the host dependent regulation of symbiosis (Koide and Schreiner 1992). Positive growth responses of M plants under the lowest P supply ($P_0$) are in accordance with other results (Smith and Read 1997, Nelson and Achar 2001). The significantly lower Chl content per unit leaf area in M plants grown at $P_0$ (Table 3) is possibly correlated with their higher A/f.m., since no alterations were found in the Chl contents per leaf fresh mass unit (data not presented). Differences in the maximum quantum yield of PS II estimated by Fv/Fm values and the Chl contents (except $P_0$) were always negligible between M and NM plants (Table 3), as it was indicated earlier (Fay *et al.* 1996). Therefore, any increase in the photosynthetic $CO_2$ fixation of M plants were most probably the consequence of their significantly higher

shoot P concentrations (Fig. 1). According to Black *et al.* (2000), under low irradiance, enhancement of photosynthesis in M plants is principally a result of their increased P supply and positive effect of sink activity of mycorrhizal colonization on $CO_2$ fixation seems to be undetectable. $P_i$ concentrations in chloroplasts limit the rate of photosynthesis (Sivak and Walker 1986), and low P supply can reduce the activity of photosynthetic enzymes (Lauer *et al.* 1989, Freeden *et al.* 1990). Nevertheless, enhanced carbon-sink activity of the AM fungus (Wright *et al.* 1998), changes in leaf age (Black *et al.* 2000), or increased stomatal conductance (Ebel *et al.* 1997) cannot be ruled out as stimulating factors.

Table 2. Significance (*ANOVA*) of effects of mycorrhizal infection, phosphorus supply and their interactions in shoots or leaves and roots of *Plantago lanceolata* plants: ns - non significant, * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$.

| | F [%] | M [%] | a [%] | |
|---|---|---|---|---|
| Phosphorus | ns | ns | * | |
| | Shoot d.m. | Root d.m. | Shoot P % | Root P % |
| Mycorrhiza | ns | ns | *** | *** |
| Phosphorus | ** | *** | *** | *** |
| Interaction | ** | ** | *** | *** |
| | A/f.m. | $CO_2$ fixation | Chl a+b | Fv/Fm |
| Mycorrhiza | ** | *** | ** | ns |
| Phosphorus | ns | ** | ns | ** |
| Interaction | ns | * | ns | ns |
| | Leaf Put | Root Put | Leaf Spd | Root Spd |
| Mycorrhiza | *** | * | *** | * |
| Phosphorus | ** | ** | ** | *** |
| Interaction | *** | * | *** | * |
| | Leaf Spm | Root Spm | Leaf PA ratio | Root PA ratio |
| Mycorrhiza | *** | ns | ns | ** |
| Phosphorus | * | ns | ** | ns |
| Interaction | ** | ns | ns | ** |

Table 3. Chlorophyll *a+b* content [μg $cm^{-2}$] ($n$ = 8) and Fv/Fm ratio ($n$ = 25) of mycorrhizal (M) and non-mycorrhizal (NM) *Plantago lanceolata* plants. Means ± SE. Statistically significant differences ($P \leq 0.05$) within one phosphorus content are indicated with different letters. P contents are explained in Materials and methods.

| Soil P content | | Chl *a+b* | Fv/Fm |
|---|---|---|---|
| $P_0$ | NM | 23.4 ± 1.5a | 0.78 ± 0.009 |
| | M | 17.9 ± 1.6b | 0.79 ± 0.006 |
| $P_1$ | NM | 23.3 ± 1.5 | 0.77 ± 0.006 |
| | M | 19.1 ± 1.4 | 0.77 ± 0.005 |
| $P_2$ | NM | 22.1 ± 1.5 | 0.80 ± 0.005 |
| | M | 20.6 ± 1.8 | 0.79 ± 0.005 |

Total P contents of *Plantago lanceolata* (Fig. 1) were in the range of the previously estimated 0.1 - 0.5 % of plant dry mass (Bielski 1973). Furthermore, shoot P contents were within 0.16 - 0.25 % of dry mass, which was considered to be a critical range for a growth response to additional P for many crop species (Singh *et al.* 2000). The positive response of dry masses (Fig. 1) and leaf parameters (data not shown) to P supply in control plants showed that P was truly limiting in this study. Inhibition of leaf growth rate by P deficiency is a well-known phenomenon (Marschner 1995). Since there was no growth depression in M plants at the lowest soil P content in contrast to other results (Bethlenfalvay *et al.* 1983), P supply must have been high enough not to induce nutrient competition between the host and its mycobiont.

Although P concentrations were significantly enhanced in M plants in every case, a negative growth response occurred at the highest soil P dose, as documented earlier (Bethlenfalvay *et al.* 1983). Those plants showing high mycorrhizal dependency at low P were more susceptible to growth depressions as a result of AM colonization at high P (Graham and Eissenstat 1994), similarly to *Plantago lanceolata* in the present study. Further, at the beginning of the experimental period, PPFD was presumably not enough for M plants to utilise the high carbon fixation capacities (measured at high irradiance) efficiently under the greenhouse conditions. This, in turn, must have limited plant carbohydrate production. Low PPFD can lead to negative growth responses in AM symbiosis (Son and Smith 1988).

Role of polyamines in the AM symbiosis was firstly shown by El Ghachtouli *et al.* (1995), who detected that exogenously applied PAs increased the frequency of AM colonization in *Pisum sativum*. According to El Ghachtouli *et al.* (1996), inhibitors of PA biosynthesis inhibited both root growth and AM colonization, which were reversed by added Put. They did not detect, however, any difference in PA concentrations as a result of AM inoculation. In contrast, Goicoechea *et al.* (1998) observed, that AM alfalfa (*Medicago sativa*) plants maintained higher Spd and Spm concentrations under water stress. Kytöviita and Sarjala (1997) showed that ectomycorrhizal root tips of pine (*Pinus sylvestris*) contained consistently higher concentrations of Put, than non-symbiotic ones. In contrast to the earlier results, it was found in the present study, that M plants had lower free PA concentrations than NM ones in many cases and differences seem to be related to P supply. However, we can provide no data on whether these alterations are the result of an induced PA synthesis or a shift from the conjugated and bound forms, as it was suggested earlier (Fester *et al.* 1999).

PA concentrations of NM plants are strongly increased at the lowest P supply both in the leaves and roots (Fig. 3), so it is very probable that these differences originate mostly from the plant partner also in the roots. Increment in Put concentration can be observed under

several stress conditions including mineral nutrient (K) deficiency (Bouchereau *et al.* 1999), but it has not been indicated yet in case of P limitation. The ratio of Put to Spd+Spm was also documented to be a good indicator of many stress conditions (Bouchereau *et al.* 1999). Despite the great differences in concentrations, there were not any significant alterations in PA ratios between NM and M leaves (Fig. 3). On the contrary, significantly higher ratios were found in the NM roots grown at $P_0$ and $P_1$ soils (Fig. 3), where growth parameters of NM plants were below or the same than that of M ones.

It is concluded that P deficiency stress, as well as the demand for and dependency on the AM symbiosis may be reflected by the concentrations and ratio of polyamines. A given state of P deficiency stress appears to exist, below which PA concentrations and PA ratio are increased drastically in the lack of AM colonization. Since PAs might mediate the action of phytohormones and influence the colonization (El Ghachtouli *et al.* 1996), they might also be a component of the regulation of AM colonization by the P nutritional status of the host plant. It seems to be the first indication of the possible role of polyamines in the P nutrition-dependent regulation of AM symbiosis.

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