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Changes in infectivity and effectiveness of *Glomus mosseae* in relation to soil nitrogen nutrition

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Abstract

The object of this study was to investigate the infectivity and efficiency of *Glomus mosseae*, an arbuscular mycorrhizal fungus at different NH_4NO_3 levels (0, 50, 100, 150 mg kg^{-1} soil) at two harvesting times on the host plant (*Phaseolus vulgaris* L.), the common bean. The formation of mycorrhiza is influenced by available P concentration in soil, by the developmental stage of the host and even by the relative amount of N. Fertilization by N can retarts AMF colonization, especially at 50 and 100 mg kg^{-1} soil nitrogen levels, but because of relative lack of P the highest concentration caused no great inhibition. In young plants the P content of shoots showed a negative correlation with N uptake. The number of AM fungal entry points may be an important parameter for evaluation of both infectiveness and effectiveness of the AMF.

Keywords: *Glomus mosseae*, colonization properties, infectivity, effectiveness, N supply

1. Introduction

Nowadays conventional agriculture is gradually being replaced by low input sustainable cropping systems in which rhizosphere microorganisms play an important role. They improve nutrient utilization, vigour of plants as well as alleviate nutrient loading of the environment (Harrier and Watson, 2003; Németh, 2006; Szili-Kovács and Németh, 2006). Arbuscular mycorrhizal fungi (AMF) are important root symbionts and should be regarded as a vital component of the terrestrial ecosystems (Harley and Harley, 1987; Harrier and Watson, 2003; Plenchette et al., 2005). The great majority of economically important crops, vegetables, fruits and ornamentals are more productive when in symbiosis with AM fungi (Harley and Harley, 1987; Tawaraya, 2003). AMF mainly help plants in mineral nutrient acquisition from soil, but other beneficial effect of these fungi have been described (Clark and Zeto, 2000; George, 2000; Harrier and Watson, 2003; Marschner, 1997; Takács and Vörös, 2003).

The one of the most important function of arbuscular mycorrhiza is the improvement of the phosphorus acquisition of their host plants (Marschner, 1997; Parádi et al., 2003). However, high available phosphate concentrations in soils inhibits the developing of root colonozation and the symbiosis (Bruce et al., 1994; Douds et al., 2000; Liu et al., 2003; Marschner and Dell, 1994; Mendoza and Pagani, 1997; Tawaraya, 2003).

In contrast to phosphorus, the effect of nitrogen fertilization on the infectivity and effectiveness of AM fungi is not yet clear. Nitrogen (N) is the main limiting element in plant growth and nutrition (Csathó et al., 2005; Miller and Cramer, 2004). The contribution of arbuscular mycorrhizal fungi with respect to host plant nitrogen supply is contradictory. Nitrogen concentrations in the shoots of mycorrhizal plants are often not affected or sometimes even reduced compared with non-mycorrhizal plants (Takács et al., 2006). In other cases, the total amounts of N in shoots of mycorrhizal plants may be greater than that of non-mycorrhizal plants due to increased shoot growth and biomass production (Ames et al., 1984). According to Marschner and Dell (1994) 80% of the P, 25% of the N, 10% of the K, 25% of the Zn and 60% of the Cu, respectively, can be transported through the AMF hyphae to plant hosts. The most commonly reported beneficial effect of AMF on plant N nutrition is the enhancement of both ammonium (NH_4^+) and nitrate (NO_3^-) uptake (Clark and

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Zeto, 2000; Harrier and Watson, 2003; Johansen et al., 1993; Tobar et al., 1994). Johansen et al. (1996) found a direct NH_4^+ and NO_3^- transport in hyphae. The beneficial effect of AMF root colonization on uptake of organic N compounds has also been proven (Ames et al., 1983; Hawkins et al., 2000).

In addition to the direct effect on N uptake of the host plant, AMF also influence the rhizosphere microbial community indirectly. Conversely, both soil microorganisms and the soil's fertility status may affect AM formation and activity. AM fungal infection can also enhance nodulation and symbiotic N_2 fixation (Barea et al., 1987; Hamel, 2004), and have a favourable effect on the population of free-living nitrogen fixing bacteria, *Azotobacter* and *Azospirillum* in the rhizosphere (Biró et al., 2000)

The mycorrhizal status of plants may be regulated by nitrogen concentrations in the soil and by nitrogen levels in the host tissues; e.g. several reports indicate that N addition supresses AMF root colonization (Azcón et al., 1982; Mosse and Phillips, 1971). A study of the effect of high-input NH_4NO_3 fertilization on maize root colonization by indigenous AM fungi, in long-term field experiments, revealed that a combination P and K fertilization had no effect or only slightly decreased the infection frequency of AMF (Takács and Vörös, 1998). These observations suggest that indigenous AM fungi may be able to adapt to high nutrient inputs during long-term field experiment (Leake et al., 2005). This was in contrast to observations on lettuce and on coffee seedling (Jackson et al., 2002; Vaast and Zasoski, 1992).

The aim of the present study was to investigate the effect of various nitrogen treatments (0, 50, 100, 150 mg NH_4NO_3 kg^{-1}) on the rate of *Glomus mosseae* colonization in the common bean (*Phaseolus vulgaris* L.) in a pot trials using a calcareous chernozem soil from Nagyhörcsök (Hungary).

2. Materials and Methods

Experimental soils

The calcareous loamy chernozem soil samples, were taken from selected plots of the experimental site of Research Institute at Nagyhörcsök (Hungary), had the characteristics shown in Table 1. The soil intended for AMF inoculation was sterilized twice (1.5 h steaming on 1.2 atm. separated by a 24 h cooling period). A stock solution NH_4NO_3 (34%, 10 ml pot^{-1}) was used to add to the sterilized soils at four levels (0, 50, 100, 150 mg total N kg^{-1} dry soil). In addition P_2O_5 (19%) and K_2O (60%) were added to the each soil at 30 mg total P and K dry soil kg^{-1} (10 ml per pot) level. The soil pH was measured in the control soils and in the pots at the start of the experiment. The NH_4NO_3 fertilizers applied to the pots lowered the initial soil pH with the increasing N rates (Table 1). The soil AL (Ammonium-Lactate)-soluble K_2O- and P_2O_5-content were also measured using the method of Sarkadi et al. (1965). The soil KCl-exchangeable NH_4^+-N and NO_3^--N content was determined as described by Bremner and Keeney (1966) (Table 1).

AMF strain and inocula production

The bean *(Phaseolus vulgaris* L.) plants were inoculated with *Glomus mosseae* (Nicolson and Gerdemann, 1968) strain. The *Glomus mosseae* culture produced by authors was derived from a single spore strain originating from a sandy soil in Őrbottyán (Hungary). The growth medium for the production of AMF inocula was composed of two parts of sand and one part of calcareous chernozem soil (Nagyhörcsök, that was sterilized. For inocula production, pot cultures of AMF infected white clover *(Trifolium repens* L.) were grown for three months in a growth chamber under a controlled climate conditions (temperature between 25°C and 17°C, 16/8 h light/dark period). After assessing the AMF infection generally high (F% = 93.3–100%), the infected roots with attached spores were cut up into small pieces and mixed with the growth medium. The bean seeds were surface sterilized with ethyl alcohol (70%) for 20 s and washed with destilled water 6 times.

AMF inoculation and plant growth

The inocula of *Glomus mosseae*, 5% w/w, was put into a single layer in the middle each pot that contained 250 g soil pot^{-1} and 13 g of soil and sand based inocula. Beans (*Phaseolus vulgaris* L.) (1 seed pot^{-1}) were planted in AMF inoculated and non-inoculated soils and were grown in a growth chamber under controlled conditions (temperature between 25°C and 17°C, 16 (250–270 watts m^{-2})/8 h light/dark period). Three replicates were used per treatment.

Determination of mycorrhizal colonization and plant analysis

The dry biomass production of the bean plants (g pot^{-1}), the N, P, K concentrations of the shoots and the parameters of the mycorrhizal infection were determined after 10 and 20 days growth. Root samples were cleared and stained with acid glycerol aniline blue (Phillips and Hayman, 1970). The root segments were examined using a dissecting microscope at magnification from 40× to 240×. The number of fungal entry point (appressoria), the frequency (F%) of mycorrhizal infection and the absolute arbuscular richness (A%) such as signes of AMF infectiveness were estimated by rating the density of infection on a 30 cm root segments using the five class system (Trouvelot et al., 1986).

The plant macro- and microelement concentrations were

Table 1. Chemical and physical properties of the untreated and treated soils used in the pot experiment after different N application rates.

Physical and chemical properties	Nitrogen treatments (mg N kg^{-1} soil)			
	0	50	100	150
Silt (0.02–0.05 mm), %	40			
Clay (<0.002 mm), %	20			
Soil organic matter (OM), %	3.20			
$CaCO_3$ content, %	6.34			
pH (H_2O), mg kg^{-1} soil^{a}	7.96	7.9	7.85	7.76
pH (KCl), mg kg^{-1} soil^{b}	7.68	7.62	7.61	7.57
KCL-NO_3-N, mg kg^{-1} soil^{c}	19.90 a	64.70 b	89.70 c	125.0 d
KCL-NH_4-N, mg kg^{-1} soil^{d}	23.30 a	29.00 b	51.70 c	72.04 d
AL-P_2O_5, mg kg^{-1} soil^{e}	350±52			
AL-K_2O, mg kg^{-1} soil^{f}	394±44			

^{a}pH$_{H2O}$ = 1:2.5, soil:H_2O; ^{b}pH KCl = 1:2.5, soil:1 M KCl; ^{cd}KCl-exchangeable nitrate-N (KCl-NO_3-N), ammonium-N (KCl-NH_4-N); ^{ef}Available P and K contents using the Ammonium-Lactate (AL) methods determined by inductively coupled plasma emission spectrometry (ICP) after digestion of soil samples by HNO_3+H_2O_2; Different lower case letters indicate significant differences between non-fertilized and fertilized soils at $P<0.05$. Data are means of 3 replicates.

Table 2. *G. mosseae* infection level (F%), arbuscular richness (A%) and number of fungal entry points (appressoria) according to the method of Trouvelot et al. (1986).

NH_4NO_3 treatments	Extent of AMF root colonization					
	F%		A%		Number of appressoria	
	10 days old	20 days old	10 days old	20 days old	10 days old	20 days old
0 mg kg^{-1}	75.54 a	82.2 ab	50.75 a	28.33 a	89 a	168 ab
50 mg kg^{-1}	38.8b c	83.3 ab	13.5 b	44.54 b	33 b	211 ac
100 mg kg^{-1}	27.76 b	74.43a	11.05 b	32.69 ab	30 b	142 b
150 mg kg^{-1}	49.99 c	89.98 b	21.03 b	37.52 ab	45 b	239 c
Mean	48.04	82.48	23.76	35.77	49	179

F%: Infection frequency of AMF; A%: arbuscular richness in the root system; fungal entry-points were expressed per 30 cm root length; Different lower case letters indicate significant differences between non-fertilized and fertilized soils at $P<0.05$. Data are means of 3 replicates.

assessed after wet digestion of the air-dried plant samples with cc. HNO_3 + cc. H_2O_2. Shoot macro- and microelement contents were measured by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

Statistical analysis

Experimental data were statistically analysed by one way ANOVA using SPSS 9.0 for Windows to detect significant differences between treatment means. The significance threshold level was set at $P<0.05$.

3. Results

The effectiveness of infection of *Glomus mosseae* strain was evident from the response of the bean plants to the presence of AMF in the root system.

Root colonization of AMF, ranged from 11% to 90% were generally high in root system of beans grown in every soil treatment (Table 2). In 10-day-old plants, the maximum values of *G. mosseae* root colonization were detected in the control (0 mg N kg^{-1}) soils. In 10-day-old plants grown in N treated soils, the frequency of *G. mosseae* infection (F%), the arbuscular richness (A%) and the number of fungal entry points significantly decreased with increasing nitrogen levels. In the 20-day-old plants, the low N input slightly increased the root colonization in comparison with control plants, especially with respect to appressoria numbers. However there were no significant differences in frequency of infection (F%) and arbuscular richness (A%) at any nitrogen treatments in the root of the 10- or 20-day-old plants. At both harvesting times the 100 mg N kg^{-1} fertilization hardly inhibited the root colonization of AM fungi. An increasing root colonization was found at the highest N fertilizer rate (150 mg N kg^{-1}) compared with 100

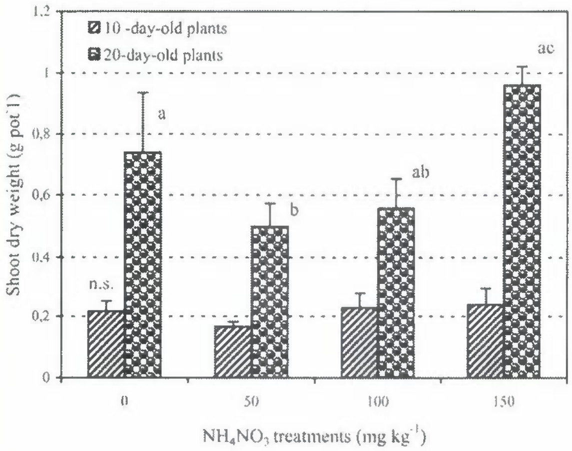

Figure 1. Effect of N fertilization on biomass production in common beans inoculated with *Glomus mosseae* and grown for 10 and 20 days (shoot dry weight in g pot^{-1}). Data are the mean of 3 replicates. Different letters indicate significant differences between non-fertilized and fertilized soils at $P<0.05$.

mg N kg^{-1} dose. We conclude that the number of appressoria and the arbuscular richness (A%) was more sensitive to nitrogen treatments than the frequency of infection (F%). The frequency of infection (F%), amount of arbuscules (A%) and appressoria numbers increased with the length of the growth period.

There were no significant differences detected in shoot production of 10-day-old mycorrhizal plants among different N application rates (Fig. 1). At the second harvesting time, 20 days, the 50 mg N kg^{-1} soil treatment caused significant decrease in shoot production compared to the control plants (0 mg N kg^{-1}). However at the highest N (150 mg N kg^{-1}) dose the shoot dry weight was higher than the shoot production of control beans colonized with *G. mosseae*.

At ten days old, the amount of N in the bean shoots significantly increased with increasing N levels in the soils (Fig. 2A). At the second harvesting time, significant differences were only found between the control and the treated plants. There were no significant differences detected in shoot P concentrations in 10-day-old

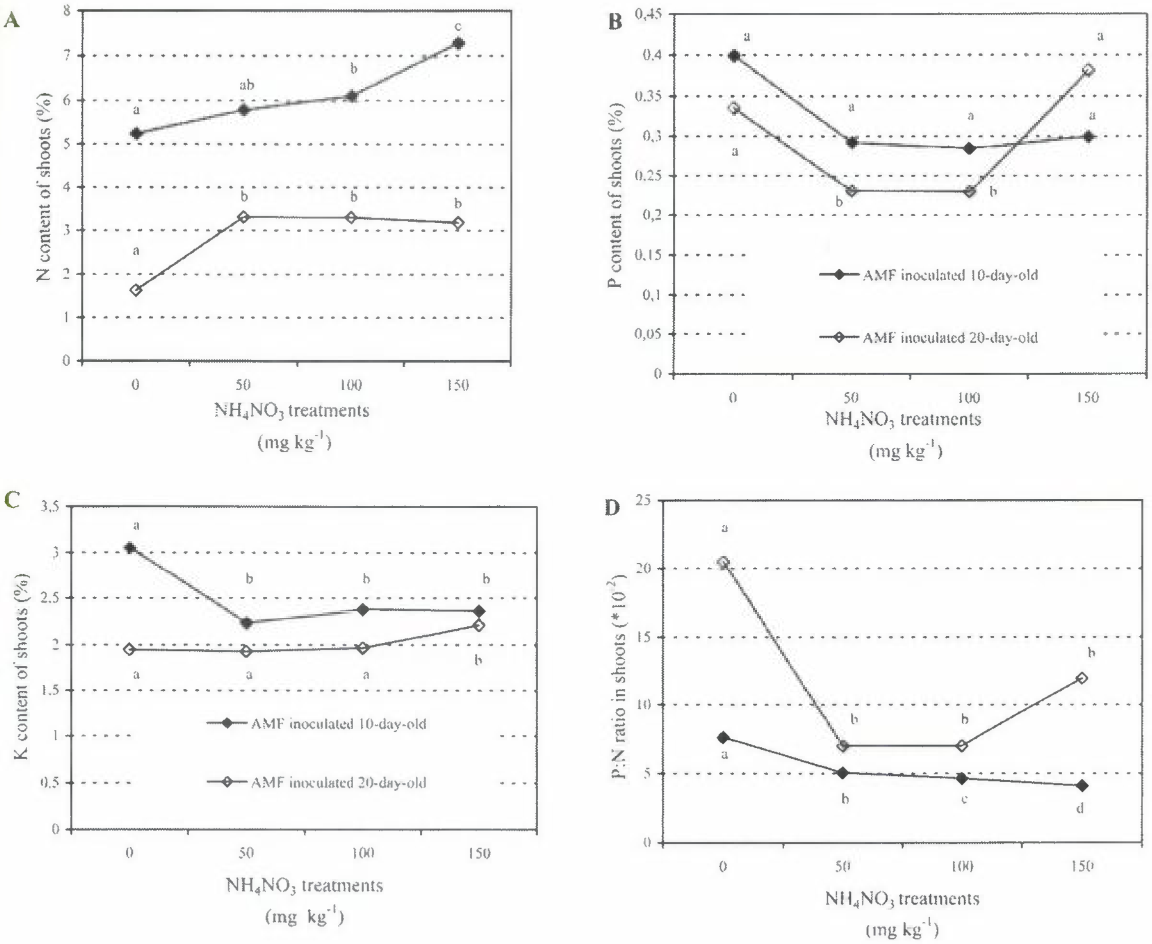

Figure 2. A–D: Effect of N fertilization on the N, P, K contents (%) and N:P of shoots inoculated with *Glomus mosseae* at case of 10- and 20-day-old plants. Data are the means of 3 replicates. Different letters indicate significant differences between non-fertilized and fertilized soils at $P<0.05$.

Table 3. Results of correlation analyses between soil treatments, infectivity and effectiveness of *G. mosseae* inoculation in the common bean (*Phaseolus vulgaris* L.).

Correlations			10-day-old plant				
	Nt	F%	A%	Apr.	DW	P	N
NH_4NO_3 treatments (Nt)	–	–0.538	–0.588	–0.566	0.333	–0.733**	0.962**
Frequency of infection (F%)	0.135	–	0.954**	0.889**	0.105	0.915**	–0.33
Arbuscularity (A%)	–0.143	0.674*	–	0.875**	0.112	0.925**	–0.437
Appressorium number (Apr)	0.320	0.785**	0.786**	–	0.212	0.876**	–0.425
Dry weight (DW)	0.375	0.474	0.118	0.434	–	0.057	0.33
P content in shoot (mg kg^{-1}) (P)	0.230	0.305	–0.172	0.312	0.789**	–	–0.591*
N content in shoot (mg kg^{-1}) (N)	0.719**	0.196	0.402	0.347	–0.213	–0.365	–
Correlations			20-day-old plant				

*Correlation is significant at P<0.05; **Correlation is significant at P<0.01.

mycorrhizal plants among different N application rates (Fig. 2B). In 20-day-old plants the P content of shoots was less than controls only for plants grown in 50 and 100 mg N kg^{-1} soils.

A different tendency was found with respect to the K percent of bean shoots (Fig. 2C). The shoot K concentrations of 10-day-old beans significantly decreased in N treated samples as compared to control plants. A significantly higher shoot K contents was found in 20-day-old plants in pots treated 150 mg N kg^{-1}.

The P: N ratios of shoots ranged from 0.0411 to 0.076 at younger plants (Fig. 2D), while at the second harvesting time it was higher and ranged from 0.0697 to 0.2053. The maximal P:N ratio was found in shoots of 20-day-old plants grown in control soil. However the minimal level of P:N ratio was detected at the highest N level at 10-day-old plants. The P:N ratio in shoots of 10-day-old plants were significantly suppressed with increasing N rates (Fig. 2D). In older plants the P:N ratio of shoots diminished with N levels, however at the highest N level the tendency reversed, but these differences were not significant.

At both sampling times, the parameters of AMF root colonization were positively correlated (Table 3). The effectiveness of the fungal partner was also indicated by the impact of AMF colonization on nutrient levels in the plants. Significant differences were found in the infectiveness and effectiveness of *G. mosseae* at various harvesting times (Table 3). Phosphate uptake was increased by the presence of mycorrhizas (Correlation coefficient = 0.915**) especially in 10-day-old plants. Nitrogen uptake of the host increased with higher soil N level and it showed negative correlation to the P uptake (Correlation coefficient = –0.591*; –0.365). There was no relationship found between number of appressorium and biomass production of bean. The correlation between the growth or N uptake of host and AMF root colonization changed with age of bean.

4. Discussion

Our results indicate that the effect of N addition (0, 50, 100, 150 mg N kg^{-1} soil) on *G. mosseae* colonization depended on the vegetative stage and nutrition demand of the host (Bruce et al., 1994). Other authors have also found that fertilization by N can either enhance or decrease AMF colonization (Table 2) (Johansen, 1999; Ortas and Rowell, 2004; Treseder and Allen, 2002). At early developmental stages application of N reduced development of infection units and the spread of the *G. mosseae* within bean roots. After 20 days, an increase in number of appressoria also contribute to the high values of infection frequency. The number of AM fungal entry points may be an important parameter for evaluation, not only of infectiveness, but also the effectivennes of AMF. In contrast to our observations Ortas and Rowell (2004) found an increased in percentage of infection at 50 mg NO_3^- or NH_4^+ N kg^{-1} soil at two harvesting times of sorghum plants in soils with different pH. However, in agreement with our results, the 100 mg N kg^{-1} soil treatment reduced the root colonization of indigenous fungi with both applied N sources. Carpio et al. (2005) also reported that the suppressive effect of high input fertilization was more pronounced in case of colonization with single species than with multispecies inoculum. The critical level (also called ideal or optimal) of N in many plants is approximately 3 percent (Plank, 1979), but in tissues of very young plants it can be 4 percent or more. In our experiment, the 10-day-old shoot N contents of mycorrhizal bean plants was very high (Fig. 2A). In contrast, the N content of older plants were relatively low.

For legumes, the critical level of P generally ranges from 0.25% to 0.30% or slightly higher and it is from 1.75% to 2.00% for K. At both sampling times the P percentage of shoots were lower in N fertilized soils (50, 100, 150 mg N kg^{-1} soil) (Fig. 2B). The P and especially K contents of bean shoots were initially high and decreased with age (Fig. 2B–C).

The P:N ratio of host tissue is an indicator of the physiological and nutritional state of plants (Miller et al., 2002) it can be an important factor determining arbuscular mycorrhiza development and functioning. Mycorrhizal plants typically have a higher P:N and C:N ratios than non-mycorrhizal plants (Tobar et al., 1994). In our experiment the P:N ratio in shoots of young plants were supressed with N addition (Fig. 2D). Low P:N ratios indicated the P limiting for plants, while high P:N ratio caused N deficiency (Sylvia and Neal, 1990). Higher AMF colonization significantly increased P uptake at the highest N level in our study as well as in others (Mosse and Phillips, 1971) (Table 1 and Fig. 2B). The beans had sufficient N, but plant growth was limited by minimal P at 50 or 100 mg N kg^{-1} soil rates at both harvesting times. Therefore AMF colonization, enhanced P uptake and caused an increased biomass production and probably carbon supply to the fungal partner (Bucking and Shaker-Hill, 2005). Maximum root colonizations by *G. mosseae* was also observed when there was a high P:N ratio in shoot tissues (Smith et al., 1986).

AM fungi are obligate symbionts depending on the photosynthetic products of host. Approximately 50% of the carbon translocated from leaf tissues to AMF colonized roots (Pfeffer et al., 1999). N fertilization modifies the costs and benefits balance in mycorrhizal symbiosis based on the bidirectional organic and inorganic nutrients transport (Corkidiki et al., 2002).

The benefit of AMF enhanced P uptake is more than the carbon cost and the investment in transport of photosynthetic assimilates from leaves tissues to root and to fungi. When host are not nutrient limited the AMF were initially C-limited and the colonization decreased. In our experiment with common bean plants, the lowest *G. mosseae* colonization was detected at 100 mg N kg^{-1} level. We suggest that at the highest N fertilization (150 mg kg^{-1}) the host should become P- or other macronutrient-limited. The AMF colonization was altered to improve the nutrient transport from the soil via fungal hyphae to the plant. In conclusion, the symbiosis between bean hosts and AM fungi dynamically changes according to the soil nutrient supply and demands of developing plant.

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