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Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants

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Abstract The influence of an arbuscular mycorrhizal (AM) fungus on phosphorus (P) and iron (Fe) uptake of peanut (*Arachis hypogea* L.) and sorghum (*Sorghum bicolor* L.) plants was studied in a pot experiment under controlled environmental conditions. The plants were grown for 10 weeks in pots containing sterilised calcareous soil with two levels of Fe supply. The soil was inoculated with rhizosphere microorganisms only or with rhizosphere microorganisms together with an AM fungus (*Glomus mosseae* [Nicol. & Gerd.] Gerdemann & Trappe). An additional small soil compartment accessible to hyphae but not roots was added to each pot after 6 weeks of plant growth. Radiolabelled P and Fe were supplied to the hyphae compartment 2 weeks after addition of this compartment. After a further 2 weeks, plants were harvested and shoots were analysed for radiolabelled elements. In both plant species, P uptake from the labelled soil increased significantly more in shoots of mycorrhizal plants than non-mycorrhizal plants, thus confirming the well-known activity of the fungus in P uptake. Mycorrhizal inoculation had no significant influence on the concentration of labelled Fe in shoots of peanut plants. In contrast, ^{59}Fe increased in shoots of mycorrhizal sorghum plants. The uptake of Fe from labelled soil by sorghum was particularly high under conditions producing a low Fe nutritional status of the plants. These results are preliminary evidence that hyphae of an arbuscular mycorrhizal fungus can mobilise and/or take up Fe from soil and translocate it to the plant.

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Introduction

Most vascular plant species are colonised by mycorrhizal fungi. In annual plants, arbuscular mycorrhizal (AM) fungi are particularly common. Direct and indirect effects of mycorrhizal fungi on nutrient uptake and plant growth have been extensively studied and reviewed (Smith and Read 1997). Among the direct effects of AM fungi, uptake from soil of mineral elements by the mycorrhizal mycelium and subsequent transfer to the plant have been demonstrated in particular for phosphorus (P) (Sanders and Tinker 1973; Li et al. 1997), potassium (George et al. 1992), nitrogen (Ames et al. 1983), and for metals such as zinc, copper, and cadmium (Guo et al. 1996).

Not much information is available on the effect of AM fungi on iron (Fe) uptake by plants (George et al. 1994). The long-term effect of mycorrhizal colonisation on Fe concentration in the plant tissue and on total plant Fe uptake is variable, and the reported results are inconsistent. In the presence of AM fungi, a decrease in Fe concentration was observed in soybean (Pacovsky and Fuller 1988), whereas for maize an increase of shoot Fe concentration was described (Clark and Zeto 1996b). In another experiment, total Fe uptake by soybean and maize was increased in mycorrhizal plants (Lambert et al. 1979). Effects of AM fungi on plant Fe acquisition strongly depend on experimental conditions, for example, on type of plant and fungus, soil pH (Treeby 1992; Clark and Zeto 1996a), soil supply of P, and on soil temperature (Raju et al. 1990). However, differences in total Fe uptake or tissue Fe concentration between mycorrhizal and non-mycorrhizal plants are not direct evidence for the ability of mycorrhizal hyphae to take up Fe and deliver it to the plant, because other differences between mycorrhizal and non-

mycorrhizal plants (for example, in root morphology) may indirectly affect Fe uptake. Using ^{59}Fe-EDTA on excised roots in short-time experiments (up to 15 min), Cress et al. (1986) showed increased Fe uptake by roots of the grass *Hillaria jamesii* when the roots were mycorrhizal (four different *Glomus* isolates). However, to date the effects of the mycorrhizal hyphae on Fe translocation to the intact plant have not been specifically investigated.

In the present study, by spatially separating the growing zones of roots and hyphae in soil, and by using radiolabelled elements (^{32}P, ^{59}Fe), the contribution of external hyphae to P and Fe uptake by peanut and sorghum was measured. Plants possessing different strategies for the acquisition of Fe (Marschner 1995) were selected for this experiment. Peanut is dicotyledoneous and, thus, is a strategy I plant (Fe-deficiency response: enhanced net excretion of protons from the roots, increased Fe-reducing capacity), while sorghum is graminaceous and, thus, is a strategy II plant (Fe-deficiency response: enhanced release of phytosiderophores from the roots).

Materials and methods

Experimental set-up

In order to investigate specifically the effects of AM fungal hyphae on the nutrition of plants, a hyphal compartment was introduced into the soil 6 weeks after planting peanut and sorghum in a pot experiment. This compartment was isolated from the bulk soil by a fine nylon mesh which prevented roots from penetrating the soil compartment. In contrast, because of their small diameter, mycorrhizal hyphae could cross the net and grow into the compartment (Fig. 1). This spatial separation of hyphae from roots has been used previously in compartmented box systems for the study of the direct effect of AM fungal hyphae on the assimilation of various nutrients by plants (see, for example, Ames et al. 1983; George et al. 1996). Radiolabelled P and Fe were later injected into the hyphal soil compartment. Since both elements have a low mobility in the loess soil used, it was assumed that the labelled elements would be confined mostly to the hyphal soil compartment for the duration of the experiment.

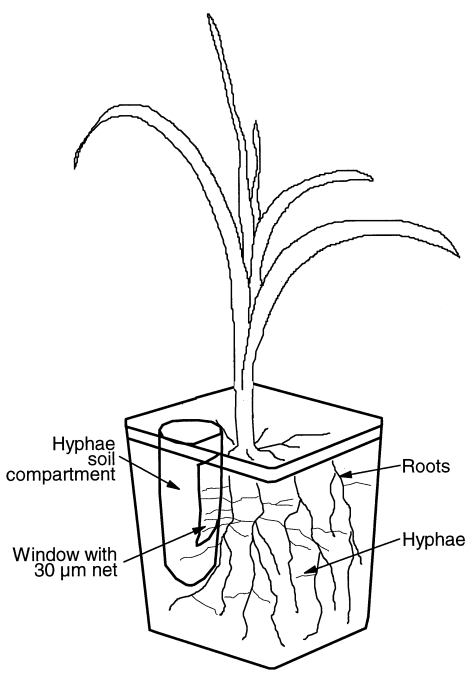

Fig. 1 Schematic drawing of the experimental culture system

Cultivation of plants

Experiments were carried out in plastic containers (900 cm^3) using a sterilised (autoclaved at 105 °C) and subsequently fertilised calcareous subsoil from loess with a pH of 7.3 ($CaCl_2$), poor in extractable Fe (1.4 mg DTPA-extractable Fe per kg soil) and P (3.8 mg $NaHCO_3$–extractable P per kg soil). For a more-detailed soil description see Li et al. (1991b). The four different treatments were inoculation with rhizosphere microorganisms together with an AM fungus (*Glomus mosseae*) and fertilisation with Fe (+Fe+AM) or not (–Fe+AM), or inoculation with rhizosphere microorganisms other than AM fungi and fertilisation with Fe (+Fe–AM) or not (–Fe–AM). Thus, this was a 2-factorial experiment with two levels for each factor; each treatment had three replicates. Non-mycorrhizal controls (–AM) were inoculated with sterilised (autoclaved at 105 °C) inoculum and a filtrate (blue ribbon filter paper, type 589[3], Schleicher and Schuell, Dassel, Germany) of the original inoculum. The mycorrhiza inoculum comprised loess soil containing *G. mosseae* spores and maize root pieces, where approximately 50% of the root length was colonised by *G. mosseae*. The inoculum (10% of soil volume) was mixed with the soil. The fungus was originally isolated by E. Sieverding from a Luvisol near Göttingen, Germany (51° 30′ N, 9° 55′ E) and propagated several times on maize grown in loess soil in a greenhouse for 8 weeks. The isolate BEG no. 107 can be obtained from the authors.

Before the onset of the experiment, each pot was fertilised per kg soil with 100 mg N as NH_4NO_3, 200 mg K as K_2SO_4, 50 mg P (for –AM treatment) or 20 mg P (for +AM treatment) both as $Ca(H_2PO_4)_2$, 100 mg Mg as $MgSO_4$, 10 mg Zn as $ZnSO_4$, 10 mg Cu as $CuSO_4$, and 10 mg Fe (for +Fe treatment only) as FeEDDHA mixed into the total soil volume. More P was applied to the non-mycorrhizal than to the mycorrhizal plants, with the aim of achieving similar plant growth in both treatments in spite of the higher P-uptake capacity of mycorrhizal roots. Starting 2 weeks after planting, some red leaf coloration appeared in plants of all treatments. When 20 ml of a diluted standard nutrient solution (for composition see Walter et al. 1994) without Fe was added once a week to each pot, the leaf symptoms disappeared.

The experiment was conducted in a growth chamber with a temperature of 25/20 °C (day/night), 16/8 h day/night regime, and 500 $\mu E\ m^{-2}\ s^{-1}$ photon flux density. Seeds were surface-sterilised by rinsing with 10% H_2O_2 for 2 (sorghum) or 5 (peanuts) min, and then placed directly in the pots. Five (sorghum, cv. Y303A) or three (peanut, cv. ICGV) plants per pot were allowed to grow for a total of 10 weeks. After the sixth week, a soil sample was taken with a small auger and at the same time one plastic tube (centrifugation tube, diameter 3 cm, length 10 cm) was placed into the soil to create a hyphal soil compartment. This tube had two "windows" covered with a nylon net (mesh size 30 µm, Zurich Bolting Cloth, Rüschlikon, Switzerland) as described in George et al. (1996). The tube contained the same sterilised, fertilised soil as described above, with 50 mg P per kg soil as $Ca(H_2PO_4)_2$.

After introduction of the additional compartments, 2 weeks were allowed for growth of hyphae into the tubes. Thereafter, aqueous solutions (5 ml) of the radiolabelled elements ^{32}P [37 kBq in 74.3 µmol $Ca(H_2PO_4)_2$] and ^{59}Fe [12×37 kBq in 0.89 mmol Fe ammonium citrate] were injected into the hyphae compartment.

Plant harvest and analysis

In the soil sample taken after 6 weeks (see above), infection of roots by AM fungi was analysed. The roots were separated from soil, cleared with KOH, stained with trypan blue, and the rate of infection determined using the grid line intersection method (Giovanetti and Mosse 1980).

Plants were harvested 2 weeks after the application of ^{32}P and ^{59}Fe. Roots were not used further for analysis because of their high radioactivity. The shoots were cut into pieces and the γ-radioactivity of the fresh material was counted on a gamma spectrometer to estimate the content of ^{59}Fe. Shoots were then dried, ashed, and dissolved in HCl and the β-radioactivity measured using a liquid scintillation counter in order to calculate the ^{32}P in the shoot material.

Statistical analysis

Means and standard deviations were calculated. The data were subjected to analyses of variance with mycorrhizal inoculation and long-term Fe supply as experimental factors. Where necessary (for data of Fe-uptake from the labelled soil compartment), data were arcsine transformed before analysis to obtain normality.

Results

Six weeks after planting, 40% of sorghum root length and 28% of peanut root length was colonised by AM fungi. Hyphal density in soil was not determined but abundant mycorrhizal hyphae were observed microscopically near the sampled roots and in hyphal compartments of several additional pots not used for the labelling experiment.

Although no Fe was supplied to the –Fe plants, and a low-Fe soil was used, no leaf symptoms of Fe deficiency were observed during the experiment. At harvest, additional Fe supply had not significantly affected shoot growth of sorghum and had decreased shoot growth of peanut by 20% (Fig. 2; Table 1). As expected from the experimental set-up (higher P fertilisation to non-mycorrhizal plants), mycorrhizal colonisation did not increase shoot growth. Non-mycorrhizal (and additionally P-fertilised) sorghum plants had higher (by 28%) shoot growth than mycorrhizal sorghum plants.

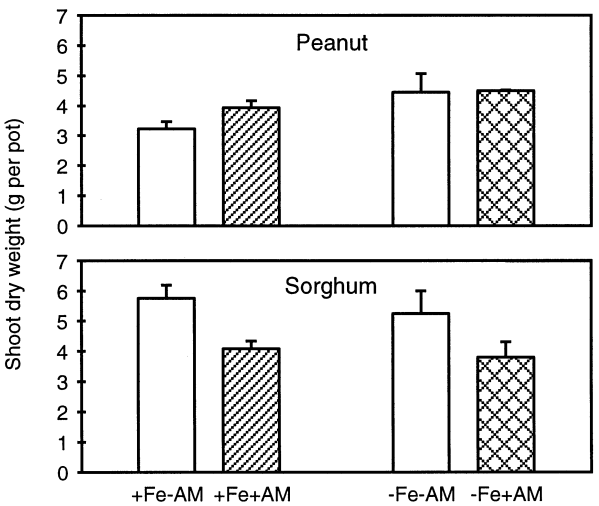

Fig. 2 Shoot dry weight (means and standard deviations) of non-mycorrhizal (*–AM*) and mycorrhizal (*+AM*; *Glomus mosseae*) peanut and sorghum plants. Plants were grown in soil with (*+Fe*) or without (*–Fe*) addition of iron

Table 1 *P* values from analysis of variance for main and interaction effects. Values in bold letters indicate values of $P \leqslant 0.05$

Source of variation	Shoot dry weight	Shoot concentration of element taken up from the labelled compartment	
		P	Fe
Peanut			
Fe supply (A)	**0.002**	0.384	0.255
AM colonisation (B)	0.099	**0.042**	0.739
A × B	0.141	0.902	0.732
Sorghum			
Fe supply (A)	0.208	0.895	**0.002**
AM colonisation (B)	**<0.001**	**<0.001**	**<0.001**
A × B	0.749	0.685	**0.017**

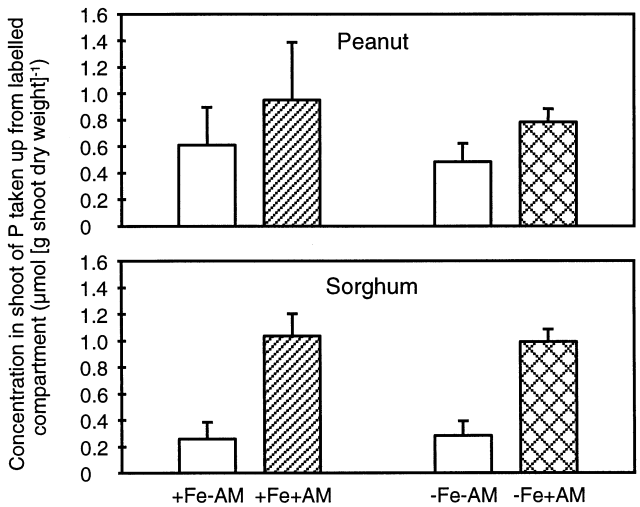

Fig. 3 Concentrations in shoot of P taken up from the labelled compartments (means and standard deviations) in shoots of non-mycorrhizal (*–AM*) and mycorrhizal (*+AM*; *Glomus mosseae*) peanut and sorghum plants 2 weeks after supply of ^{32}P to hyphal soil compartments. Plants were grown in soil with (*+Fe*) or without (*–Fe*) addition of iron

The concentration in shoots of P taken up from the labelled compartment (Fig. 3; Table 1) and also the total amount of P taken up into shoots from the labelled compartment (data not shown) were significantly higher in mycorrhizal than non-mycorrhizal plants. This was observed in both sorghum and peanut. The concentration of P in shoots of non-mycorrhizal plants was higher in peanut than in sorghum (Fig. 3).

In peanut, the concentration in the shoots of Fe taken up from the labelled compartment was highly variable and was not significantly affected by mycorrhizal colonisation or long-term Fe supply (Fig. 4, Table 1). However, in sorghum, the Fe concentration in shoots

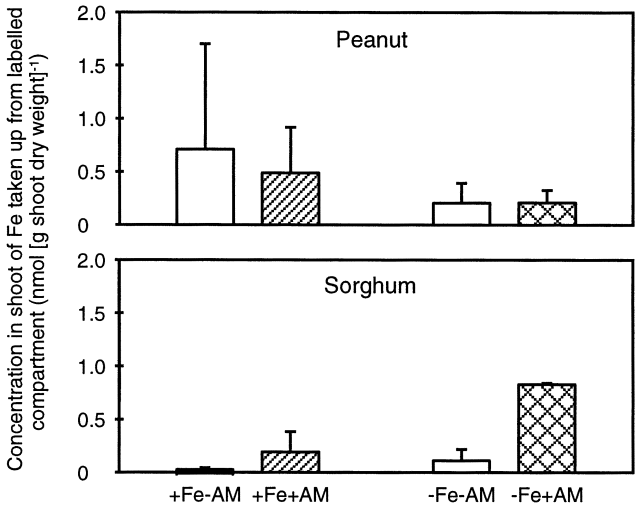

Fig. 4 Concentrations in shoot of Fe taken up from the labelled compartments (means and standard deviations) in shoots of non-mycorrhizal (*–AM*) and mycorrhizal (*+AM*; *Glomus mosseae*) peanut and sorghum plants 2 weeks after supply of ^{59}Fe to hyphal soil compartments. Plants were grown in soil with (*+Fe*) or without (*–Fe*) addition of iron

(Fig. 4) and also the total amount of Fe taken up from this compartment (data not shown) were significantly higher in mycorrhizal than non-mycorrhizal plants. More labelled Fe was taken up by mycorrhizal sorghum when the plants was not supplied with additional Fe during growth (significant interaction of AM colonisation and Fe supply; Table 1).

Discussion

Mycorrhizal colonisation did not increase shoot growth of either host plant in this experiment because more P was provided to non-mycorrhizal plants to overcome the limited ability of non-mycorrhizal roots to absorb P from soil. The decrease in growth observed in mycorrhizal sorghum has been observed previously in a pot experiment and is probably due to the energy cost of the fungus without a corresponding increase in plant P uptake (Raju et al. 1990). Also, Fe supply to soil did not increase shoot dry weight although a low-Fe soil was used. This effect is often observed in pot experiments with low-Fe soil. Optimal soil temperature and moisture conditions in pot experiments help the plant roots to mobilise scarcely available soil Fe sources. Under natural conditions, plant Fe deficiency often occurs at low soil temperature or at high bicarbonate concentrations in moist soils (Marschner 1995). For studies under controlled conditions, nutrient solutions (Cress et al. 1986) or plant mutants with a limited capacity to mobilise Fe from soil (Clark and Zeto 1996a) can be used to induce severe Fe deficiency in plants.

The significantly higher uptake of P from the labelled compartment in mycorrhizal than non-mycorrhizal plants suggested that mycorrhizal hyphae had grown into the hyphal soil compartments, absorbed ^{32}P, and subsequently delivered this ^{32}P to the plant. Using similar experimental techniques, similar results have been obtained by other authors with other host plant-fungus combinations (for example, Jakobsen et al. 1992; Hetrick et al. 1994; Cui and Caldwell 1996). As expected, long-term Fe supply to the plant did not affect P uptake by the hyphae. Some ^{32}P was also found in shoots of non-mycorrhizal plants, in particular in peanut (Fig. 3), very likely as a result of root hairs growing into the hyphal soil compartment. Many plant species can grow several-millimetre-long root hairs (Föhse et al. 1991) and these can take up nutrients from a hyphal soil compartment (Li et al. 1991a).

The results of this experiment suggest that Fe can also be taken up by hyphae of an arbuscular mycorrhizal fungus and subsequently delivered to the host plant (sorghum; Fig. 4). It is further possible that hyphae of mycorrhizal sorghum plants were able to mobilise soil Fe into a plant-available form. In peanut plants, concentrations in the shoot of Fe taken up from the labelled compartment were highly variable and did not differ significantly between mycorrhizal and non-mycorrhizal plants. Differences in ^{59}Fe acquisition may be due to a different response of the two plant species to Fe deficiency. Alternatively, less hyphal growth and longer root hairs may have precluded a mycorrhizal effect on ^{59}Fe uptake in peanut plants under these specific experimental conditions. Hyphae and root hair growth was not determined in the present experiment because we refrained from soil manipulations after harvest due to the high soil radioactivity.

The hyphal uptake of Fe by mycorrhizal sorghum plants appeared to be regulated by the Fe nutritional status of the plants: with low long-term Fe supply, plants obtained more ^{59}Fe via their mycorrhizal hyphae. Thus, mycorrhizal hyphae may help the plant to obtain sufficient Fe when growing in low-Fe soils. Conventional pot experiments comparing mycorrhizal and non-mycorrhizal plants have rarely shown increased Fe concentrations in mycorrhizal plants; even a decrease in Fe concentrations has been observed in mycorrhizal plants (Kothari et al. 1990). However, these experiments were usually carried out with plants adequately supplied with Fe. In an experiment with a maize cultivar susceptible to Fe deficiency, mycorrhizal colonisation significantly increased plant Fe concentration (Clark and Zeto 1996b).

It has been speculated that mycorrhizal fungi produce Fe chelating compounds, for example siderophores (Cress et al. 1986), as do other fungi, including ectomycorrhizal fungi (Szaniszlo et al. 1981). This was not studied in the present experiment but could conveniently be measured, for example, in monoxenic cultures of Ri T-DNA-transformed roots and mycorrhizal fungi (St-Arnaud et al. 1996). In a natural soil environment, however, mycorrhizal hyphae compete for Fe with other soil microorganisms and with the root itself

(George et al. 1994). Therefore, the actual contribution of mycorrhizal fungi to plant Fe uptake will be variable and depend not only on the plant-fungus combination but also on the chemical, physical, and biological soil conditions.

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