# Effect of indigenous mycorrhizal colonization on phosphorus-acquisition efficiency in soybean and sunflower

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## Abstract

Despite a general consent about the beneficial contribution of arbuscular mycorrhizal fungi (AMF) on natural ecosystems, there is an intense debate about their role in agricultural systems. In this work, soybean (*Glycine max* L.) and sunflower (*Helianthus annuus* L.) field plots with different P availabilities were sampled across the Pampean Region of Argentina (> 150 samples from Mollisols) to characterize the relationship between available soil P and indigenous mycorrhizal colonization. A subsequent pot experiment with soybean and sunflower was carried out to evaluate the effect of P supply (0, 12, and 52 mg P $kg^{-1}$) and AMF inoculation on AMF colonization and crop responsiveness to P in a Mollisol. Both crops showed high AMF colonization in the field (average: 55% for soybean and 44% for sunflower). While mycorrhizal colonization in soybean was significantly and negatively related to available soil P, no such trends were apparent in sunflower. Also, total biomass was 3.5 and 2.0 times higher in mycorrhizal than in nonmycorrhizal pot-grown soybean under low- and medium-P conditions, respectively. Sunflower, on the other hand, did not benefit from AMF symbiosis under medium and high P supply. While mycorrhization stimulated P-uptake efficiency in soybean, the generally high P efficiency in sunflower was not associated with AMF symbiosis.

**Key words:** arbuscular mycorrhizae / Argentina / *Glycine max* / *Helianthus annuus* / Mollisol

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## 1 Introduction

Most (80%) higher plants are able to form symbiotic associations with mycorrrhizae, especially arbuscular mycorrhizal fungi (AMF; *Brundrett*, 2002). Mycorrhizal fungi have the potential to enhance uptake of relatively immobile plant nutrients, in particular phosphorus (P; *Bolan*, 1991), through the development of a network of extraradical hyphae that allows an increase in the soil volume exploited by plant roots (*Miller* et al., 1995). Mycorrhizal colonization is strongly influenced by the available soil P (*Covacevich* et al., 2007). Comparisons of AMF colonization of soybean plants growing in low- and high-P conditions have revealed reductions of 12%–71% in P-sufficient plants, depending on the genotype, soil P level, and growth stage (*Kelly* et al., 2001; *Khalil* et al., 1999). The contribution of AMF to plant P nutrition is evident in low-P soils (*Covacevich* and *Echeverría*, 2009), but such benefit may not be expected under nonlimiting conditions.

Although there is a general consent about the beneficial contribution of AMF in natural ecosystems, there is an intense debate about their real role in agricultural systems (*Ryan* and *Graham*, 2002). In such sense, the responses of crops to AMF are usually unpredictable and the effects of AMF colonization are controversial (*Peng* et al., 1993). Soybean, sunflower, and maize are among the most important grain crops all over the world and the main summer crops in the Pampean Region, the most important agricultural area of Argentina. In this region, which is dominated by highly productive Mollisols, almost 70% of the soils are P-deficient (*Echeverría* and *García*, 2005), which constitutes one of the main local constraints to crop yield. In a recent work, we found that a more favorable root morphology allows soybean and sunflower to acquire more P per unit of carbon invested belowground than maize (*Fernandez* et al., 2009). To extend these observations, further understanding of other traits that contribute to the P efficiency of soybean and sunflower is necessary. Understanding the differential responses of crops to low soil-P availability can help to design agricultural practices conservative in this element. The objectives of this work were: (1) to assess the indigenous mycorrhizal colonization in soybean and sunflower in Mollisols of the Pampean Region; (2) to evaluate AMF colonization and their contribution to P efficiency in soybean and sunflower at contrasting P levels.

## 2 Material and methods

### 2.1 Field study

In order to assess the indigenous mycorrhizal colonization in Mollisols, soybean (*Glycine max* L.) and sunflower (*Helianthus annuus* L.) plots with different levels of available

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soil P were sampled across a variety of sites of the Pampean Region. Field sampling was designed to represent the local variability in soils and agricultural management. The plots sampled included different initial soil P levels, crop rotations, tillage practices (tillage and no-tillage), and fertilization history. There was no irrigation, and soluble fertilizers were applied. Roots were sampled from plants at reproductive stages of development: R2 in full-season soybean, R1 in double-cropped soybean, and R5 in sunflower (37, 42, and 76 plots, respectively). Winter wheat is currently used by local farmers in rotations with double-cropped soybean, while full-season soybean has no preceding winter crop. Roots were obtained from 0–20 cm soil samples taken either on the row line or between row lines. To avoid thicker roots, soil samples were taken at 5 cm from the plant stem. Roots were washed and sieved (0.6 mm), and 1 g subsample of fine roots was taken and cut into 1–2 cm long segments to determine the level of root colonization. Roots were stained according to *Phillips* and *Hayman* (1970). First, roots were cleared in 10% KOH and then rinsed in water and acidified in 10% HCl. Roots were then stained with lactic-glycerol-Trypan Blue solution, rinsed, and stored in a lactic and glycerol solution. The root segments obtained were spread out on a Petri dish with a gridline marked at the bottom. The percentage of roots colonized by mycorrhizal fungi was determined by the gridline intersect method across 100 root intersections observed under a binocular microscope (40×). At the same sites where roots were obtained, composite soil samples (0–20 cm depth) were taken around the plant to measure available soil P (Bray 1).

### 2.2 Greenhouse experiment

Soybean and sunflower performance under the influence of P availability and AMF was compared in a pot experiment. The soil was a silt loam Typic Argiudoll taken at 5–20 cm depth. The soil was collected in Alberti (35°02′ S, 60°16′ W), Buenos Aires, Argentina and was not sampled in the field study reported here. Soil pH was 5.5, organic-matter content 3.6%, and available P (Bray 1) 8.4 mg $kg^{-1}$. To reduce indigenous AMF community, the soil was air-dried and exposed to solar radiation in a greenhouse for 6 months. Mycorrhizal inoculum was collected from agricultural soils planted with soybean and sunflower with low or medium levels of available P (from 3.9 to 13.3 mg $kg^{-1}$). A composite sample was mixed thoroughly and half of it was steam-pasteurized for 1 h at 100°C for three consecutive days to be used in the control treatment. The inoculum consisted of a mixed indigenous population, in which the dominant genera were *Glomus spp., Acaulospora spp.,* and *Scutellospora spp*. (2 spores [g soil]$^{-1}$).

Treatments were arranged in a factorial randomized complete block design with three factors and four replications. Factors were plant species, P level, and inoculum. Plant species were soybean (*G. max* L. cv. Don Mario 4800 RR) and sunflower (*H. annuus* L. cv. Paraíso 20). The P levels were 0, 12, and 52 mg P $kg^{-1}$ added to the growth media as $KH_2PO_4$. The inoculum factor was composed of two levels: natural soil (AMF treatment) and steam-pasteurized soil (non-AMF treatment). Each pot received 810 g of fresh-weight inoculum (9% dry weight of growth medium). Controls received a similar amount of steamed inoculum. The mixed mycorrhizal inoculum, which included colonized roots, soil spores, and extraradical mycelia, was placed in a layer, 6 cm below the surface soil. Plastic 7 L pots were filled with 9 kg growth medium prepared with a mix of soil and river sand (1:2 soil-to-sand v : v). A preplant basal fertilization was applied as follows (quantities are per pot): 2.8 g N (urea), 2.3 g K ($K_2SO_4$), 0.5 g Mg ($MgSO_4$), 0.5 g Ca ($CaSO_42H_2O$), 1.5 mg Cu ($CuSO_45H_2O$), 5 mg Zn ($ZnSO_47H_2O$), 2 mg B ($H_3BO_3$), 11 mg Fe ($FeSO_47H_2O$), 15 mg Mn ($MnSO_4H_2O$). To compensate for K added as $KH_2PO_4$, 900 and 730 mg K (KCl) were added to the low and medium P treatments, respectively. Soybean received no nitrogen fertilizer but was inoculated with *Bradyrhizobium japonicum* strains, at a rate of 3 mL $kg^{-1}$ of seed ($10^9$ bacteria $mL^{-1}$, Nitragin Cell Tech). Three seeds were sown in each pot. Seedlings were thinned to one per pot 5 d after sowing. Pots were kept between 60% and 100% water-holding capacity. Plants were grown in springtime under natural light and a temperature range of 20°C to 30°C.

### 2.3 Measurements

Plants were harvested 53 d after sowing. The shoot was cut at ground level and dried at 60°C for 3 d to determine dry weight. Roots were carefully washed and sieved to remove soil and sand particles, and 1 g subsample of fine roots was taken randomly from the root system to determine the level of mycorrhizal colonization as described above. Diameter was quantified from remaining roots with the computer image-analysis software ROOTEDGE. Roots were dried to determine dry weight. Subsamples (70 mg) of ground tissue from different plant organs (root, leaf, and stem) were ashed at 500°C for 24 h. The ashes were dissolved in 8 mL of 0.1 M HCl, and the P concentration was measured colorimetrically. Phosphorus-acquisition efficiency was calculated by the amount of absorbed P per unit root biomass and per unit root length.

### 2.4 Statistical analysis

Data collected were statistically analyzed by factorial Analysis of Variance (ANOVA), and the protected least-significant-difference (LSD) procedure was used for mean separation when the F test was significant ($p < 5\%$). A log transformation prior to ANOVA was conducted on the total P uptake data to achieve homogeneity of variance around the means. The relationship between AMF colonization and available soil P was described through regression models fitted using the Table Curve 2D version 5.01 software (Systat, Richmond, California, USA).

## 3 Results

### 3.1 Mycorrhizal colonization in the field

In the field, soybean showed higher AMF colonization than sunflower (55% *versus* 44%, respectively; Fig. 1). Mycorrhizal colonization in soybean was significantly and negatively related to available soil P, following exponential functions (Fig. 1a). Full-season soybean showed higher AMF coloniza-

 

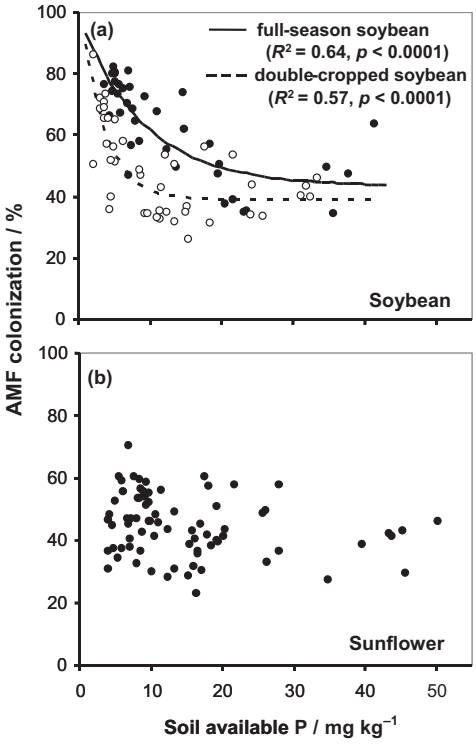


**Figure 1:** Relationship between arbuscular-mycorrhizal-fungi (AMF) colonization and soil available P (Bray 1) across a variety of sites of the Pampean Region for soybean (a) ($y = 43 + 56 \cdot e^{-0.11x}$ and $y = 39 + 69 \cdot e^{-0.31x}$ for full-season soybean and double-cropped soybean, respectively) and sunflower (b)

tion than double-cropped soybean along the range of available soil P. Two significantly different functions ($p < 0.1\%$) were fitted to represent these relationships. Values above 23 and 9.4 mg P (kg soil)$^{-1}$ did not alter AMF colonization in full-season soybean and double-cropped soybean, respectively (Fig. 1a). For sunflower, we found no significant relationship between AMF colonization and the soil P level (Fig. 1b).

Soybean showed a higher AMF colonization in soils with less than 10 mg P (kg soil)$^{-1}$ than sunflower (72%, 57%, and 58%, for full-season soybean, double-cropped soybean, and sunflower, respectively). No significant differences were observed between crops in soils with soil P levels higher than 20 mg P (kg soil)$^{-1}$. No significant effects were found in AMF colonization in relation to the other factors of management practices considered.

## 3.2 Mycorrhizal colonization in the greenhouse experiment

Almost negligible AMF colonization ([1.8 ± 0.71]%) was observed in plants grown in the noninoculated soil. Plants grown in the inoculated soil showed AMF colonization that ranged between 11% and 55%, depending on the species and P level. Soybean roots had twofold higher AMF colonization than sunflower at low and medium P levels (Tab. 1). The percentage of colonization was lower in high-P plants, and there were no significant differences between species.

**Table 1:** Effects of P level and arbuscular-mycorrhizal-fungi (AMF) inoculation on mycorrhizal colonization, total plant biomass, total P uptake, root diameter, acquisition efficiency per unit root biomass and per unit root length, for soybean and sunflower in the greenhouse experiment.

| Species | P added / mg kg$^{-1}$ | | Mycorrhizal colonization | Total plant biomass | Total P uptake | Root diameter | Efficiency of P uptake per unit root biomass | Efficiency of P uptake per unit root length |
|---|---|---|---|---|---|---|---|---|
| | | | / % | / g plant$^{-1}$ | / mg plant$^{-1}$ | / mm | / mg P (g root)$^{-1}$ | / mg P (m root)$^{-1}$ |
| | 0 | NM§ | 0.94 | 2.01 | 1.90 | 0.30 | 3.97 | 0.06 |
| | | M | 46.09 | 7.15 | 12.08 | 0.38 | 7.29 | 0.12 |
| Soybean | 12 | NM | 2.14 | 4.56 | 5.45 | 0.27 | 7.90 | 0.11 |
| | | M | 55.76 | 9.09 | 18.57 | 0.40 | 8.04 | 0.19 |
| | 52 | NM | 1.57 | 20.57 | 49.85 | 0.28 | 17.86 | 0.24 |
| | | M | 21.19 | 20.65 | 52.47 | 0.39 | 11.50 | 0.28 |
| Sunflower | 0 | NM | 3.21 | 0.51 | 0.38 | 0.17 | 3.55 | 0.02 |
| | | M | 25.85 | 0.92 | 0.78 | 0.27 | 3.05 | 0.05 |
| | 12 | NM | 1.30 | 4.51 | 9.07 | 0.27 | 13.54 | 0.17 |
| | | M | 21.85 | 2.87 | 4.68 | 0.32 | 6.70 | 0.11 |
| | 52 | NM | 2.04 | 28.52 | 95.62 | 0.27 | 28.60 | 0.34 |
| | | M | 11.55 | 31.81 | 89.96 | 0.34 | 15.12 | 0.24 |
| **ANOVA F values** | | | | | | | | |
| Species (S) | | | 62.66*** | 0.96 n.s. | 40.47*** | 31.97*** | 40.47*** | 4.29* |
| P level (P) | | | 19.76*** | 415.23*** | 410.58*** | 6.54** | 0.74 n.s. | 58.97*** |
| Mycorrhizae (M) | | | 379.69*** | 9.24** | 24.56*** | 70.83*** | 0.32 n.s. | 13.29*** |
| S × P | | | 8.16*** | 35.94*** | 48.93*** | 7.71** | 1.74 n.s. | 5.70** |
| S × M | | | 71.92*** | 1.85 n.s. | 10.96** | 1.91 n.s. | 7.49** | 6.23* |
| M × P | | | 23.38*** | 0.10 n.s. | 8.89*** | 0.03 n.s. | 1.32 n.s. | 8.96*** |
| S × M × P | | | 6.59*** | 3.96* | 2.75 n.s. | 2.04 n.s. | 1.05 n.s. | 0.30 n.s. |

§ NM: nonmycorrhizal, M: mycorrhizal. *$p < 5\%$, **$p < 1\%$, ***$p < 0.1\%$, n.s. not significant at $p < 5\%$

 

### 3.3 Plant biomass and phosphorus uptake

Plants in pots were grown with a complete basal fertilization (with nutrients other than P), and no symptoms of nutrient deficiency were observed. Soybean growth responded to mycorrhizal colonization better than sunflower, and its response to AMF greatly depended on the soil P level (Tab. 1). Under low-P conditions, total biomass of mycorrhizal soybean plants was 3.5 times higher than their nonmycorrhizal counterparts, whereas under medium-P conditions, mycorrhizal plants doubled the total biomass as compared to nonmycorrhizal plants, and under high-P conditions, soybean plants did not show a significant effect of AMF. Conversely, there was no significant response to AMF in sunflower under any of the three P levels.

The benefit from mycorrhizae on total P uptake (mg plant$^{-1}$) varied considerably between species (Tab. 1). Mycorrhizal inoculation significantly increased P uptake of soybean (average 41%) but exerted no significant effect on sunflower.

### 3.4 Parameters related to phosphorus efficiency

Mycorrhizal inoculation led to a consistent increase in the average root diameter of the two species (Tab. 1). For this parameter, ANOVA showed a significant species × P level interaction. The P level did not affect soybean root diameter (average 0.34 mm), but sunflower root diameter increased at high P levels. Sunflower plants at low P showed the lowest root diameter (average 0.22 mm).

With regard to efficiency of P uptake per unit root length and efficiency of P uptake per unit root biomass, the two species responded to AMF inoculation in different ways (Tab. 1). Phosphorus-acquisition efficiency per unit root biomass was negatively affected by mycorrhizal colonization in sunflower (average 46%), but not affected in soybean. When P-acquisition efficiency was calculated per unit root length, significant increases were observed in soybean in response to AMF regardless of the P level (average 36%), but not in sunflower.

## 4 Discussion

Synchronized soil and root sampling in the field allowed us to characterize the relationship between available soil P and indigenous mycorrhizal colonization in a wide range of agricultural plots of the Pampean Region. The percentage of root AMF colonization in the field was high even in P-rich soils, where values were above 30%. The AMF-colonization threshold values found in soybean (23.0 and 9.4 mg Bray 1 P [kg soil]$^{-1}$) were lower than those previously found in wheat (27 mg Bray 1 P kg$^{-1}$) (*Covacevich* et al., 2007). Soybean AMF thresholds were close to the critical range of soil P for fertilization recommendations commonly accepted for soybean (9 to 21 mg Bray 1 P kg$^{-1}$; *Dodd* and *Mallarino*, 2005; *García* et al., 2005), which would be advantageous because the increase in AMF-colonization levels occurs just at the point where soil P becomes a growth-limiting factor. Arbuscular-mycorrhizal-fungi colonization in soybean plants grown in the greenhouse also showed a high sensitivity to available soil P. Whereas sunflower showed a relationship between AMF colonization and soil available P in the greenhouse, this relationship was not found in the field.

Soybean was clearly more responsive to AMF symbiosis than sunflower. This can be associated with its higher AMF colonization, especially under P-deficient conditions. The AMF symbiosis exerted a positive effect on biomass accumulation and P uptake under low and medium P levels. The high response of soybean to AMF has also been demonstrated before (*Kelly* et al., 2001; *Nurlaeny* et al., 1996). On the other hand, sunflower did not show consistent benefits from AMF symbiosis. The few studies on the role of AMF in sunflower have shown divergent results (*Thompson,* 1987; *Sakurai* et al., 2001).

In a previous paper, we found that soybean and sunflower are able to absorb more P per unit of carbon invested belowground than maize (*Fernández* et al., 2009) and observed that this higher efficiency was related to a more favorable root morphology and architecture (*i.e.,* root shallowness, higher specific root length) of soybean and sunflower. Results from the experiments presented here extend those observations by showing that the high P-acquisition efficiency of soybean is also related to the large benefits from AMF symbiosis. In contrast, the high acquisition efficiency of sunflower reported earlier (*Fernández* et al., 2009) is not associated with AMF symbiosis.

Arbuscular mycorrhizal fungi consistently increased the mean root diameter of the two species studied. This response to mycorrhizal colonization has already been reported in several species (*Nurlaeny* et al., 1996; *Peng* et al., 1993), especially when mycorrhizal plants are grown at low P availability. In soybean, the increased root diameter may have been compensated by the effectiveness of hyphae of AMF plants to absorb P (*Deressa* and *Schenk*, 2008). Sunflower had thinner roots than soybean, which may have contributed to its P-acquisition efficiency. In fact, sunflower was more effective at absorbing P in the absence of mycorrhizae (Tab. 1).

There are three practical implications that arise from our work: (1) Important interspecific variability in AMF responsiveness exists between crops known to be P-efficient; (2) an important benefit from AMF, reflected by total biomass accumulation, was observed in soybean in a not highly P-deficient soil (our medium-P treatment, Tab. 1), and (3) maximum growth was observed in our high-P treatment with or without AMF. It has been pointed out that the quantitative contribution of AMF to the nutrition of economically relevant crops in soils that are not highly P-deficient is not yet clear and that AMF do not necessarily enhance P uptake sufficiently to maximize crop yields (*Ryan* and *Graham*, 2002). In Mollisols, there is a great potential for AMF symbiosis, as suggested by the high levels of colonization observed. Specially, AMF symbiosis may contribute to the higher suitability of soybean to grow in low-P soils and to explain the lower critical P levels of soybean compared to other crops. It is worth noting that critical P levels for the same crop are rather constant along soils belonging to the same or closely related taxonomic order (*Dodd* and *Mallarino*, 2005; *Echeverría* and *García*, 2005; *Robinson*, 1978). There is no reason to expect that the AMF

 

thresholds found in Mollisols of the Pampean Region of Argentina would differ greatly from those found elsewhere in the world.

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