

# Mycorrhizal effects on glomalin-related soil protein and chlorophyll contents in coffee plants in the Peruvian Amazon<sup>1</sup>

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## ABSTRACT

The inoculation of vegetatively propagated coffee plants with arbuscular mycorrhizal fungi (AMF) prior to field establishment may positively influence the plant growth and physiology. This study aimed to evaluate the AMF effects on the glomalin-related soil protein and chlorophyll contents in vegetatively propagated coffee plants inoculated in a greenhouse and transplanted to an open field, in the Peruvian Amazon. The experiment consisted of eight treatments, in a 2 × 4 factorial design, being two coffee varieties (Caturra and Pache) and four AMF inocula (control, Moyobamba, El Dorado and Huallaga). The inocula were collected from organic coffee crops and named according to the province from which they were collected. The mycorrhizal colonization and chlorophyll content were statistically higher in the plants inoculated with AMF, if compared to the non-inoculated plants, while the glomalin-related soil protein content ranged from 61.6 to 69.1 mg g<sup>-1</sup> and showed no statistically significant differences among the inocula, although the Moyobamba inoculum showed to be numerically superior. The effect of the coffee variety was not statistically significant among the variables under study.

**KEYWORDS:** *Coffea arabica* L., arbuscular mycorrhizal fungi, sustainable agriculture.

## INTRODUCTION

The production of coffee in the Peruvian Amazon may be affected by climate change, mainly due to expected changes in temperature and rainfall patterns (Robiglio et al. 2017). Furthermore, coffee growers are in a vulnerable situation due to the high pressure from diseases and poor nutrient management of the crops (Ramírez-Builes et al. 2020). In these circumstances, the use of arbuscular mycorrhizal

## RESUMO

Efeitos micorrízicos nos teores de proteína do solo relacionada à glomalina e de clorofila em cafeeiro na Amazônia peruana

A inoculação de cafeeiro propagado vegetativamente com fungos micorrízicos arbusculares (FMA) antes da instalação no campo pode influenciar positivamente no crescimento e fisiologia das plantas. Objetivou-se avaliar os efeitos de FMA nos teores de proteína do solo relacionada à glomalina e de clorofila em cafeeiro propagado vegetativamente, inoculado em casa-de-vegetação e transplantado para campo aberto, na Amazônia peruana. O experimento consistiu de oito tratamentos, em esquema fatorial 2 × 4, sendo duas variedades de café (Caturra e Pache) e quatro inóculos de FMA (controle, Moyobamba, El Dorado e Huallaga). Os inóculos foram coletados em cafezais orgânicos e nomeados de acordo com a província em que foram coletados. A colonização micorrízica e o teor de clorofila foram estatisticamente maiores nas plantas inoculadas com FMA, em comparação com as plantas não inoculadas, enquanto o teor de proteína do solo relacionado à glomalina variou de 61,6 a 69,1 mg g<sup>-1</sup> e não apresentou diferenças estatisticamente significativas entre os inóculos, embora o Moyobamba tenha sido numericamente superior. O efeito da variedade de café não foi estatisticamente significativo nas variáveis estudadas.

**PALAVRAS-CHAVE:** *Coffea arabica* L., fungos micorrízicos arbusculares, agricultura sustentável.

fungi (AMF) represents a promising alternative for a sustainable increase in coffee yield.

AMF are soil microorganisms that form symbiosis with plant roots, developing a mycelial network in the rhizosphere (Marro et al. 2014). They facilitate the absorption of water and nutrients from the soil, and there is evidence that they induce disease tolerance in plants (Begum et al. 2019, Vallejos-Torres et al. 2021). Xu et al. (2018) showed that AMF increase the chlorophyll content and promote

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photosynthetic capacity in maize. Furthermore, they improve the glomalin-related soil protein (GRSP) content - an insoluble glycoprotein that accumulates in plant roots and soil (Seguel et al. 2008, Chen et al. 2018). GRSP favors the stability of soil aggregates, positively influences soil fertility, contributes to soil carbon storage and enhances plant growth under abiotic stress (Irving et al. 2021).

AMF play an important role as biofertilizers in the management of crops in the field, improving the quality of soil aggregation and promoting the vegetative growth of plants (Diagne et al. 2020). Their benefits on the plants vegetative development and nutrient absorption have been studied in many species of economic interest, such as *Coffea arabica* (Vallejos-Torres et al. 2020), *Solanum lycopersicum* (Bona et al. 2016), *Phaseolus vulgaris* (Ibijbijen et al. 1996) and *Prunus cerasifera* (Berta et al. 1995). In addition, the literature reports that AMF have positive effects on the plants chlorophyll content and photosynthetic capacity. AMF strains of *Glomus clarum* had positive effects on stomatal conductance and photosynthetic activity of *Lycopersicon esculentum* (Dell'Amico et al. 2002), and *Funneliformis mosseae* produced increases in the total chlorophyll content and in the net photosynthesis rate in *Solanum lycopersicum* (Wang et al. 2017). Furthermore, Ruth et al. (2011) reported that AMF have a positive effect on plant water uptake, contributing to approximately 20 % of the total water uptake.

Thus, this study aimed to evaluate the effects of arbuscular mycorrhizal fungi on soil glomalin-related soil protein and chlorophyll contents in coffee plants vegetatively propagated in the Peruvian Amazon.

## MATERIAL AND METHODS

The study was carried out at an experimental plot located in the Jepelacio district, Moyobamba province, San Martín region, Peru, at an altitude of 1,050 m.a.s.l.

A 2 x 4 factorial design, with eight treatments, was used, being two coffee varieties (Caturra and Pache) and four arbuscular mycorrhizal fungi (AMF) inocula (control, Moyobamba, El Dorado and Huallaga). The AMF inocula were collected from soils of organic coffee crops and named according to the province in which they were collected. Three randomly distributed replicates were set up, with each replicate containing four coffee plants for evaluation.

Coffee plants vegetatively propagated by rooting of cuttings were used. For this purpose, 8-cm cuttings with 50 % of leaf area were rooted in microtunnels (Vallejos-Torres et al. 2020). The AMF inocula were obtained from the phytopathology laboratory of the Instituto de Investigaciones de la Amazonía Peruana and multiplied using trap plants such as rice (*Oriza sativa*) and maize (*Zea mays*) (Vallejos-Torres et al. 2019). Table 1 shows the AMF species identified in each AMF inoculum.

After two months, the rooted coffee cuttings were transferred to plastic bags with substrate at a 1:2 ratio of sterile river sand and agricultural soil, and, at the same time, inoculated with the AMF inocula. The inoculation of coffee plants with AMF drew on the application of approximately 2,000 spores of the corresponding mycorrhizal inoculum to each cutting, then these inoculated seedlings were acclimated in a greenhouse.

The coffee seedlings were exposed to an 80-percent-shaded greenhouse for 15 days and subsequently transferred to a 50-percent-shaded greenhouse for another 15 days. After this period, these coffee plants were exposed to sunlight for additional four months, before being transplanted to an open field. The soils in the study area belong to the Tropofluent group, derived from recent sediments and consisting mainly of silt and clay (Peru 1968). The environmental conditions of the research plot were evaluated from October 2019 to March 2020, with temperatures of 21.3-25 °C, relative humidity of 67.2-76.1 % and rainfall of 155.2-227.5 mm.

For field establishment, the distance between rows and plants was 1.5 m. Additionally, 100 g

Table 1. Arbuscular mycorrhizal fungi (AMF) species identified in each AMF inoculum.

AMF inoculum	Identified AMF species
Moyobamba	<i>Glomus sinuosum</i> and <i>Glomus</i> sp.
El Dorado	<i>Acaulospora rugosa</i> , <i>A. spinosissima</i> , <i>A. lacunosa</i> , <i>Glomus sinuosum</i> and <i>Ambispora appendicula</i>
Huallaga	<i>Acaulospora mellea</i> , <i>Acaulospora</i> sp. 1, <i>Acaulospora</i> sp. 2, <i>Glomus macrocarpum</i> and <i>Glomus</i> sp.

of earthworm humus were applied to each plant. The regrowth of some trees was allowed in the experimental plot, so, at 10 months after the establishment of the coffee plants, a shading of approximately 30 % was achieved, homogeneously distributed. The variables under study were evaluated at 10 months after the experiment was established under field conditions.

The mycorrhizal colonization was determined using the equation proposed by Trouvelot et al. (1986), adapted to coffee plants by Vallejo-Torres et al. (2021). The chlorophyll concentration was determined using a portable non-destructive measurement equipment (Konica Minolta SPAD 502). To determine the glomalin-related soil protein (GRSP), soil samples were taken before irrigation at a depth of 0-10 cm. The total glomalin fraction was extracted according to Wright & Upadhyaya (1998) and quantified by the Bradford (1976) method, with the absorbance reading performed at 595 nm, using a spectrophotometer. For this, a standard curve of bovine serum albumin was prepared from a solution of 1 g L<sup>-1</sup>, using 6 concentrations of this solution between 0.05 and 0.5 g L<sup>-1</sup>. The GRSP concentrations were expressed as mg g<sup>-1</sup> of soil.

The obtained data were recorded in an Excel spreadsheet and subjected to normality and homogeneity tests using the Shapiro-Wilk and Levene tests, respectively. Then, the data were subjected to analysis using the JMP Statistical Discovery software ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

The inoculated mycorrhizae colonized the roots of the coffee plants. The AMF inocula showed significant effects on mycorrhizal colonization, but the coffee varieties did not show significant statistical differences ( $p < 0.05$ ) (Table 2). The Huallaga and El Dorado mycorrhizal inocula stood out significantly, with 64.3 and 62.8 % of mycorrhizal colonization, respectively. The control treatment showed the lowest value for mycorrhizal colonization (37.4 %) (Figure 1). The mycorrhizal colonization percentages were higher than those previously reported for coffee plants under field conditions. For example, Vallejos-Torres et al. (2019) found that different mycorrhizal inocula colonized coffee plants in a range of 39.26-48.52 %. Likewise, the study performed by Vallejos-Torres et al. (2021) reported that AMF

Table 2. P-values of mean comparisons for coffee varieties, arbuscular mycorrhizal fungi (AMF) and coffee varieties-AMF interaction.

Means comparison	Mycorrhizal colonization (%)	Chlorophyll content (SPAD units)	GRSP** (mg g <sup>-1</sup> )
Coffee varieties	0.6274	0.0081	0.0982
AMF	< 0.0001*	< 0.0001*	0.0139
Coffee varieties-AMF	0.8156	0.0646	0.0343

\* Significant differences detected using an alpha p-value < 0.05. \*\* GRSP: glomalin-related soil protein.

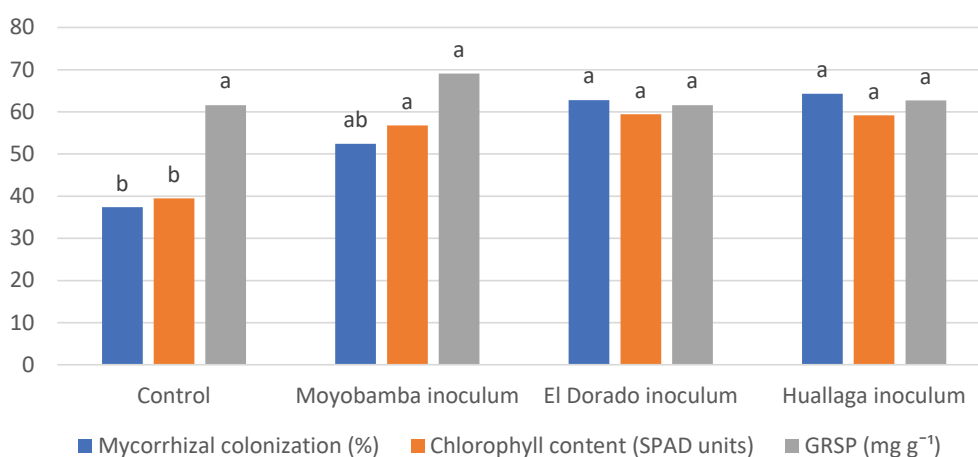


Figure 1. Effects of arbuscular mycorrhizal fungi (AMF) on responses of mycorrhizal colonization, glomalin-related soil protein (GRSP) and chlorophyll in coffee plants.

inocula colonized between 27.5 and 28.51 % of the roots of coffee plants.

The mycorrhizal colonization of plant roots and surrounding soil through the development of fungal extraradicular hyphae improves the stability of soil aggregates and the ability of plants to uptake nutrients (Andrade et al. 2009, Vallejos-Torres et al. 2021). Mycorrhizal colonization is correlated with the diversity of AMF species; therefore, the use of AMF inocula containing various species of mycorrhizal fungi is recommended to increase the nitrogen and phosphorus plant uptake and improve the vegetative growth (Delavaux et al. 2017, Begum et al. 2019). This is corroborated by the results of the present study, since the two inocula (El Dorado and Huallaga) with five AMF species showed a higher colonization than the inoculum (Moyobamba) with two species.

The AMF inocula p-value was 0.0139. This small p-value may be an evidence that not all means are equal; however, the differences were not statistically significant. Similar responses were observed for the coffee varieties and for the interaction between both factors ( $p < 0.05$ ) (Table 2). Although the differences were not statistically significant, the application of the Moyobamba AMF inoculum numerically presented the highest content of GRSP ( $69.1 \text{ mg g}^{-1}$ ), if compared to the other AMF inocula (El Dorado inoculum with  $61.6 \text{ mg g}^{-1}$  and Huallaga inoculum with  $62.7 \text{ mg g}^{-1}$ , respectively) (Figure 1). Moreover, the coffee varieties factor was not statistically significant, with similar numerical values.

According to Rillig et al. (2001), tropical soils have glomalin in concentrations over  $60 \text{ mg g}^{-1}$ . The results obtained in the present study in the Peruvian Amazon, a tropical region, are consistent with those authors, and the treatments in which the coffee plants were not inoculated with AMF showed  $61.6 \text{ mg g}^{-1}$  of GRSP in the soil, whereas those inoculated with the Moyobamba AMF inoculum showed the highest value ( $69.1 \text{ mg g}^{-1}$ ). Different results were reported by Hossain (2021), who conducted a review article on glomalin content in various environments and found that Treseder & Turner (2007) reported  $13.50 \text{ mg g}^{-1}$  of glomalin in tropical rainforest soils, while Rillig et al. (2003) reported the lowest glomalin content in a desert soil ( $0.007 \text{ mg g}^{-1}$ ). The glomalin content found in the present study was much higher than that reported in the literature, and this corroborates that

there is colonization in non-inoculated coffee plants in the field. This is probably due to the presence of native AMF in the soil of the coffee crops used in this study.

The chlorophyll content was influenced by the AMF inoculated into the coffee plants, this being the factor that showed significant differences. On the other hand, the coffee varieties factor did not have statistically significant effects on the chlorophyll content for the coffee crop soils in question ( $p < 0.05$ ) (Table 2). The three AMF inoculated into the coffee plants induced a higher leaf chlorophyll content, if compared to plants that were not inoculated with AMF (Figure 1). Although there was no interaction between the AMF and the coffee varieties factors, Figure 2 shows the chlorophyll values of each AMF treatment and each variety, validating that, regardless of the coffee variety, the plants not inoculated with AMF had a lower chlorophyll content.

Aseri et al. (2008) reported that *Punica granatum* plants inoculated with *Azotobacter chroococcum* and *Glomus mosseae* had an increase in the chlorophyll content, whereas Vafadar et al. (2014) suggested that an increased chlorophyll content influences the photosynthetic capacity of *Stevia rebaudiana*. Furthermore, in coffee plants, the AMF inoculation increases the photosynthetic rate and stomatal conductance (Cruz et al. 2020). These reports, complemented with the results of the present study, allow inferring that the increase of the chlorophyll content in coffee leaves due to inoculation with AMF is related to a higher photosynthetic rate, positively affecting the physiology of coffee plants.

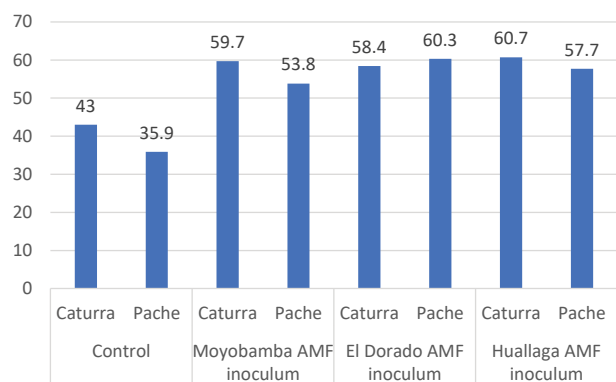


Figure 2. Effects of the arbuscular mycorrhizal fungi (AMF) on the chlorophyll content in Caturra and Pache coffee plants at 10 months after the experiment was established under field conditions.



Figure 3 shows a scatterplot matrix of the three studied variables after the inoculation of coffee plants with AMF. The mycorrhizal colonization ranged 30-70 %, and the GRSP and chlorophyll contents ranged 50-80 mg g<sup>-1</sup> and 30-65 SPAD units, respectively. If a correlation coefficient is close to 0, the trend is weaker, that is, there is a greater data dispersion. This weak trend is observed in the correlation between mycorrhizal colonization and GRSP content, with a correlation coefficient equal to 0.0135. In contrast, the variables mycorrhizal colonization and chlorophyll content showed a strong positive correlation (0.8134). Moreover, the correlation coefficient between the variables GRSP content and chlorophyll content is equal to 0.1972, indicating a low positive correlation (Figure 3).

The results of the present study indicate that the application of AMF in coffee clonal plants, i.e., genetically uniform, increases the chlorophyll content, in a way that the commercial production of AMF inoculants represents a sustainable and environmentally friendly alternative that may help with the establishment of coffee plants under

field conditions, thus favoring the soil fertility and structure, mainly in the Amazon, where soils with low fertility levels are common.

## CONCLUSIONS

1. The mycorrhizae inoculation into vegetatively propagated coffee plants produced an increase of chlorophyll under field conditions, while the glomalin-related soil protein did not show significant differences between arbuscular mycorrhizal fungi (AMF) inoculated and non-inoculated plants;
2. The mycorrhizal colonization was higher in coffee plants subjected to the El Dorado and Huallaga arbuscular mycorrhizal fungi inocula, when compared to those subjected to the Moyobamba AMF inoculum. This may be because these first two AMF inocula comprised five fungi species, while the Moyobamba inoculum had two;
3. The study demonstrated the positive effect of inoculation of these fungi in a nursery, followed by an increase of chlorophyll content in plants in the field, thus positively influencing the plant photosynthetic capacity.

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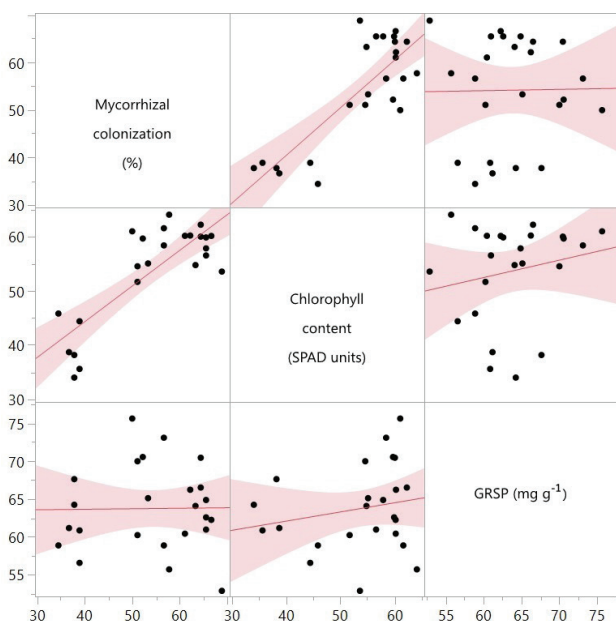


Figure 3. Correlation coefficients among mycorrhizal colonization, glomalin-related soil protein (GRSP) content and chlorophyll content in coffee plants inoculated with arbuscular mycorrhizal fungi (AMF). Correlation coefficient between mycorrhizal colonization and chlorophyll content: 0.8134; between mycorrhizal colonization and GRSP: 0.0135; between chlorophyll content and GRSP: 0.1972.

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