Evaluation of native mycorrhizae in plantain crop (*Musa* AAB Simmonds) in nursery phase

Evaluación de micorrizas nativas en plantas de plátano Hartón (*Musa* AAB Simmonds) en fase de vivero

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Abstract

The objective was to evaluate the effect of three vesicular arbuscular Mycorrhizal native genera application on growth of Hartón plantain plants in nursery stage. The strains were obtained at Universidad de Córdoba from the Plant Pathology Laboratory collection, which were isolated from plantain crops in Lorica, Los Córdobas and San Bernardo del Viento. A completely randomized experimental design with six treatments (T) was used: *Glomus* sp. (T1), *Acaullospora* sp. (T2), *Scutellospora* sp. (T3), a genera combination of *Glomus*, *Acaullospora* and *Scutellospora* (T4), a chemical treatment (diammonium phosphate, DAP, T5) and absolute control (T6), with ten replicates per treatment. Results showed that more spores and larger infection percentage were obtained with the combination of mycorrhizae genera (T4), followed by T1, T3 and T2 treatments. Besides the above, with the combination of mycorrhizal genera, they were significantly different compared with other treatments in regard to pseudostem diameter, ratio of leaf area:fresh and dry weight of root. Regarding to leaf area and root length, T5 treatment showed the higher measurements.

Key words: *Acaullospora* sp., *Glomus* sp., inoculum, *Scutellospora* sp., sustainable agriculture.

Resumen

En el trabajo se evalúo el efecto de la aplicación de tres géneros nativos de micorrizas vesículo arbusculares sobre el crecimiento de plantas de plátano Hartón (*Musa AAB* Simmonds) en etapa de vivero. Las cepas se obtuvieron de la colección del Laboratorio de Fitopatología de la Universidad de Córdoba, las cuales fueron aisladas de cultivos de plátano en los municipios de Lorica, Los Córdobas y San Bernardo del Viento. Se utilizó el diseo experimental completamente al azar, con seis tratamientos (T): *Glomus* sp. (T1), *Acaullospora* sp. (T2), *Scutellospora* sp. (T3), una combinación de los géneros *Glomus*, *Acaullospora* y *Scutellospora* (T4), un tratamiento químico de fosfato diamónico, DAP (T5) y un control absoluto (T6), con diez repeticiones por tratamiento. Los resultados mostraron que con la combinación de géneros de micorrizas (T4) se obtuvo un mayor número de esporas y un porcentaje más alto de infección, seguido de los tratamientos T1, T3 y T2. Además, con esta combinación se encontraron diferencias significativas en diámetro del pseudotallo, relación de área foliar:peso fresco y seco de raíz, en comparación con los demás tratamientos. El área foliar y la longitud de raíz fueron más altos en el tratamiento T5.

Palabras claves: *Acaullospora* sp., agricultura sostenible, *Glomus* sp., inóculo, *Scutellospora* sp.

Introduction

Mycorrhizae could be considered as organisms formed by the root of a plant and the mycelium of a fungus. They absorb nutrients and water and are extended in the soil giving these elements to the plants, while protecting them from some diseases. The vesicular-arbuscular mycorrhizae (VAM) are the most common type of mycorrhizal association capable of mutualistic symbiosis establishment with roots of most of species of agricultural interest, thus, forming part of the biological microcosm living in the soil (Sánchez, 2007). In the plant-soil microorganism interaction three types of relation are considered: pathogenicity, neutrality and symbiosis. Arbuscular mycorrhizae fungi (AM) and plant growth promoting rhizobacteria or bacteria (PPGR or PGPB) belong to this last group, because they contribute positively to nutrition and plant growth, while acting as efficient biological control agents against pathogens (Jaizme and Rodríguez, 2004). Mycorrizhae infection produces physical, biochemical and physiological changes on the colonizing roots generating a better health status on the plant and contributes to alleviate abiotic stresses like heavy metals and salinity, among others and, biotic stresses by damage compensation, activation of defense mechanisms and changes on the rhizosphere microbiome (Barea *et al.*, 1997). These effects have an additional meaning in tropical, semiarid and marginal soils, where these fungi are the main resource for both ecosystem conservation and production (Janos, 1987).

In nature, mycorrhizal fungi are an integral part of crop plants, among them the plantain, ensuring their growth in different conditions and environments. Mycorrhizae impact the crop for its optimal growth on soils with certain levels of fertility. Effects of these microorganisms have consequences on development and nutrition and, can enhance the plant natural resistance on situations of biotic or abiotic imbalances (Domínguez *et al.*, 2004). In the banana crop it has been demonstrated the high efficiency of mycorrhizae under controlled conditions and are used in most of the micropropagated materials (Rizzardi, 1990; Declerck *et al.*, 1994, 1995; Jaizme and Azcón, 1995; Yano-Melo *et al.*, 1999).

Alvear *et al.* (2006) consider that pesticide applications negatively affect biological activity on soils. In the department of Córdoba, Colombia, non-controlled application of fertilizers and other agrochemicals has generated a reduction in soil fertility, negative effects on structural stability, and in the microbiote, that affects production on plants of agricultural interest. Nonetheless this problematic in this department, the studies focused on multiplication and application of native mycorrhizal fungi in plantain crops, are scarce. However, there are specific studies on the estimation of arbuscular mycorrizhal diversity (Corcho and Urrea, 2006), in the populational relation of mycorryzhae associated to this crop, their effects on the physico-chemical properties of soil (Novoa *et al.*, 2006) and in the relation of arbuscular mycorrhizae and nematodes present in the crop rhizosphere (González and Paternina, 2008). The objective of this research was to evaluate the effect of inoculation of different genus of native mycorrhizal and the application of a chemical fertilizer on some morpho-physiological variables of Hartón plantain at early stages of growth.

Materials and methods

The study was done on the plant nursery of the Universidad de Córdoba, Montería, Colombia, 8° 48' N and 75° 58' W. Native strains of vesicular-arbuscular mycorrhizae (VAM) were evaluated in the Phytopathology Lab of the Universidad de Córdoba, that came from Hartón plantain (*Musa AAB*) producing farms in the towns of Lorica, Los Córdobas and San Bernardo del Viento, that have abundant mycorrhizae according to studies of Barrera (2005) and Álvarez and Villadiego (2005).

The obtaining of spores was done in two plantain producing farms on the previously named towns. In each one of them, 10 rhizospheric soil samples were collected at 0.60 m of distance from the plant corm and at a depth of 0.20 m, samples were homogenized and packed on plastic bags of 1 kg capacity with their respective label. In total, 40 samples were taken and moved in styrofoam boxes to the lab where the host root system and soil were processed according to the Ohms and Jenkins methodology (1964).

On samples of soil with roots mycorrhizae were isolated and identified at the morphological level, taking into account spore shape, hyphal connection, number of cell walls and color, and using the pictorial key of Schenck and Pérez (1990). The mycorrhizal-root colonization was assessed by mounting samples to observe on the microscope the internal colonization of the plant tissue by the hyphae, as well as the arbuscules and vesicle formation, according to the Sieverding´s methodology (1983).

For spore multiplication, 1 kg of inoculum of the substrate from each farm was taken. Multiplication was done at the plant nursery, in three 1 x 1 m plots using as host *Brachiaria decumbens* grass established on a sand:lime (1:1) mix. This multiplication process was kept for a 120 days period to obtain inoculum and evaluate the efficiency of the mycorrhizae native genera, being selected those that showed a mature stage and better morphology.

For the bioassay it was used a completely randomized block design, with six treatments and 10 replicates. Treatments were T1 = *Glomus* sp. (G), T2 = *Acaullospora* sp. (A), T3 = *Scutellospora* sp. (S), T4 = combination of the genera *Glomus*, *Acaullospora* and *Scutellospora* (G + A + S), T5 = chemical or relative (application of 40 g/plant of diammonium phosphate (DAP), and T6 = absolute control.

The experimental unit was composed of a Hartón plantain corm sowed at 10 cm depth on a plastic bag of 8 kg capacity filled with soil previously sterilized and analyzed chemically. To each bag were inoculated 60 mycorrhizae spores for the T1, T2 and T3 treatments and, 20 spores of each genus for the T4 treatment. Plant length and diameter measurements were done every 15 days after inoculation. Four months after inoculation measurements of fresh and dry weight, root length, leaf area with the leaf area integer Li-Cor Li-3100 were done, it was calculated the leaf area ratio (leaf area/total dry matter), number of mycorrhizal spores in the soil and in the plant, and percentage of mycorrhizal colonization in the roots. The collected data were subjected to analysis of variance (Anova) and to mean comparison Tukey´s test ($\alpha = 0.05$) using the software SAS univariate version 9.1 (SAS Institute Inc., 2004).

Results and discussion

Pseudostem length

For this characteristic no differences were found ($P > 0.05$) among the treatments T1, T2, T3, T4 and T5. In all the cases, except the absolute control, T6, plants have a similar pseudostem length during their life cycle (Figure 1), suggesting that plants and mycorrhizae established a symbiosis allowing the last ones to absorb nutrients that contributed to promote plant growth, and, the beneficial effect on plant growth and development of VAM inoculation, alone or combined, is better expressed through the time (Cano, 2001; González and Cuenca, 2008). In this case, probably among the main nutrients absorbed by the inoculated plants are phosphorus and nitrogen which are main components of DAP (T5), that statistically resulted to be the same as the treatments with VAM application. According to Marschner and Dell (1994) and Smith and Read (1997) the previous results are based on the carbohydrate translocation from the plant to the fungus and on the absorbed nutrients by the fungi used by the plant (Figure 1).

Pseudostem diameter

In the Table 1 is observed that no differences between treatments were found ($P > 0.05$). Only 30 days after mycorrhizae inoculation in the treatment 4 (combination of VAM genera) a higher value for this variable was seen, followed by the T1 treatment (*Glomus* sp.). This proves that early mycorrhization contributes to a better plant development through more efficient utilization of certain nutrients, allo

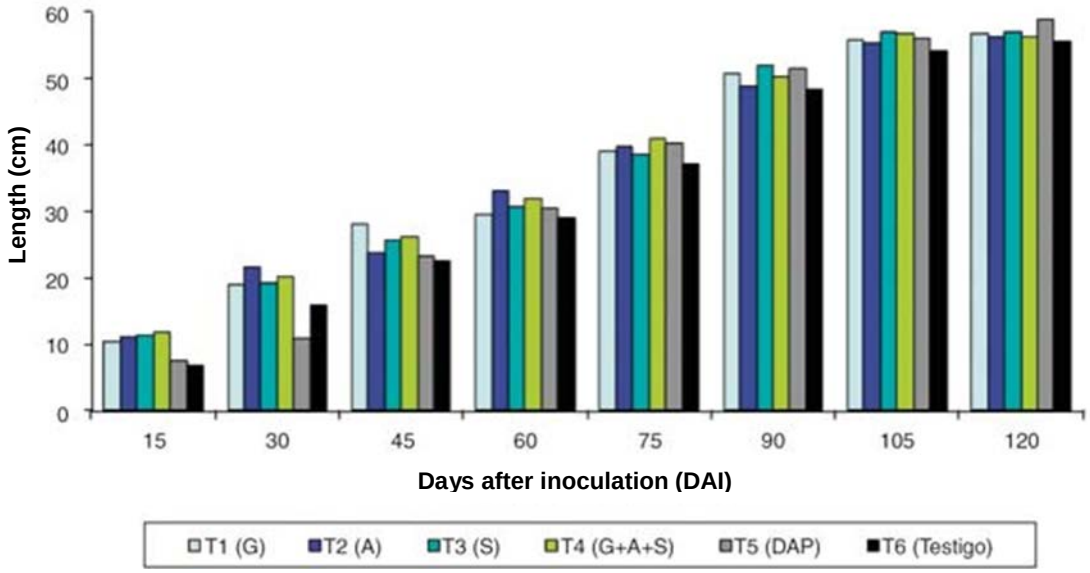

Figure 1. Effect of the application of native VAM genera on pseudostem length of Harton plantain (*Musa* AAB Simmonds). T1: *Glomus* sp., T2: *Acaullospora* sp., T3: *Scutellospora* sp., T4: combination of genera; T5: soil with application of phosphoric fertilizer; T6: absolute control.

wing stress reduction that has beneficial consequences on plantain plants of different origin (Jaizme and Rodríguez, 2004) at the nursery phase (Chagüezá, 2011).

It is possible that the fast plant adaptation to the substrate conditions, especially pH 5.63, favored development of native *Glomus* sp. and *Scutellospora* sp. genera, since according to Guerrero *et al.* (1996) the optimal values range from 5.5 to 6.5.

In the period between 45 and 120 days there were no differences on pseudostem diameter among treatment, however the treatments inoculated with mycorrhizae, T1 (*Glomus* sp.), T2 (*Acaullospora* sp.), T3 (*Scutellospora* sp.), T4 (VAM genera combination) and T5 (application of DAP) excelled the absolute control, suggesting the importance of the VAM

Table 1. Effect of inoculation with native genera of VAM on the pseudostem diameter (cm) of Hartón plantain (*Musa* AAB Simmonds) plants in nursery conditions.

Treatments	**Days after inoculation**							
	15	**30**	**45**	**60**	**75**	**90**	**105**	**120**
T1: *Glomus* sp. (G)	1.74	2.48 a*	2.62	2.86	3.08	3.22	3.31	3.33
T2: *Acaullospora* sp.(A)	1.93	2.05 ab	2.61	2.65	2.95	3.22	3.23	3.26
T3: *Scutellospora* sp.D E	1.52	2.40 a	2.70	2.74	3.02	3.09	3.26	3.28
T4: Mix (A+G+S)	2.16	2.53 a	2.71	2.90	3.10	3.23	3.32	3.36
T5: (DAP)	2.01	2.42 a	2.49	2.64	2.98	3.15	3.19	3.30
T6: (Absolute control)	1.15	1.30 b	2.31	2.62	2.93	3.03	3.10	3.14
CV (%)	14.2	15.1	12.7	21.3	17.6	9.1	24.8	2.6
(Treatments)	ns	**	ns	ns	ns	ns	ns	ns

G: *Glomus* sp.; A: *Acaullospora* sp.; S: *Scutellospora* sp.; G+A+S: mix of *Glomus* sp. + *Acaullospora* sp.+ *Scutellospora* sp.; DAP: soil with application of 40 g of diammonium phosphate per plant.

*Averages with different letter on the same column are statistically different, Tukey ($P \leq 0.05$); **: F test highly significant ($P \leq 0.01$); ns: F test no significant; CV: coefficient of variation.

on plantain growth. Results from banana (*Musa* AAA) obtained by Jaizme *et al.* (2004) demonstrate that mycorrhizal effect during vegetative phase is maintained during the nursery phase, even under the commercial fertilization regime.

Root wet and dry weight

Root wet and dry weight had differences ($P < 0.05$) among treatments (Table 2). Treatments with mycorrhizal inoculation exceeded the treatment with fertilizer application (T5) and the absolute control (T6), standing out T4 (VAM genera combination) treatment which registered the highest values. Results demonstrate the effectiveness of arbuscular mycorrhizae on root growth and agree with Usuga-Osorio *et al.* (2008) who observed the highest mycorrhizal association with the plants when using native inoculum coming from the same agroecosystem of banana crop. Sánchez and Sieverding (1999) consider that absorption of less labile ions, like phosphorus, depends on the volume of soil explored by the roots. In this case the mycorrhized root has an advantage over the non-mycorrhized, because the external mycelium extends to a larger distance than the root hairs which favors growth and production of dry matter.

Root length

For this characteristic there were found significant effects ($P < 0.05$) among treatments. In Table 3 is observed that T5 treatment (DAP application) showed the highest growth, followed by T6 treatment (absolute control), suggesting a low correlation between mycorrhization and root length on plantain plants. Elsen *et al.* (2001) observed similar results on banana studies and indicate that in the establishment of the symbiosis, mycorrhizea colonize biotrophically the root cortex and develop a mycelia outside the matrix, thus, helping the plant to absorb more efficiently water and nutrients without enlarging the root length. The same authors indicate that when the mycorrhizal colonization is not efficient, there are negative or neutral effects on root growth. Contrary, Jaizme and Rodríguez (2004) observed significant effects on the root length increment in *Musa* AAA clon Gran Enano, compared to the control without VAM inoculation, that allowed more dense roots with higher capacity to absorb nutrients and to explore fertile horizons, as well as, with a better anchorage for the plant. This means, according to Elsen *et al.* (2001) and Declerck *et al.* (1995), that mycorrhization is more effective and does not depend on species, varieties or cultivars.

Leaf area

The highest value for leaf area ($P < 0.05$) was found in the plants of T5 treatment (DAP application) (Table 4), followed by treatment T4 (combination of mycorrhizae spores). These results show that the last treatment presented a better adaptation to the substrate in comparison to the other genera individually ino

Table 2. Effect of the inoculation with native genera of VAM in root wet and dry weight (g) Hartón plantain (*Musa* AAB Simmonds) plants in nursery conditions.

Treatments	Root wet weight (g)	Root dry weight (g)
T1: *Glomus* sp (G)	94.7 a*	35.0 b
T2: *Acaullospora* sp(A)	66.6 cc	22.8 c
T3: *Scutellospora* spD	79.2 b	26.4 cb
T4: Mix (A+G+S)	105.1 aa	47.3 a
T5: (DAP)	66.2 cc	22.0 c
T6: (Control absoluto)	57.6 cc	19.2 c
CV (%)	13.5	16.1
(Treatments)	**	**

G: *Glomus* sp.; A: *Acaullospora* sp.; S: *Scutellospora* sp.; G+A+S: mix of *Glomus* sp. + *Acaullospora* sp.+ *Scutellospora* sp.; DAP: soil with application of 40 g of diammonium phosphate per plant.

*Averages with different letter on the same column are statistically different, Tukey ($P \leq 0.05$); **: F test highly significant ($P \leq 0.01$); ns: F test no significant; CV: coefficient of variation.

Table 3. Effect of the inoculation of VAM native genera in root length (cm) of Hartón plantain (*Musa* AAB Simmonds) plants in nursery conditions.

Treatments	Root length (cm)
T1: *Glomus* sp (G)	16.5 c*
T2: *Acaullospora* sp(A)	21.7 b
T3: *Scutellospora* spD	19.3 cb
T4: Combination (A+G+S)	16.2 c
T5: (DAP)	31.6 a
T6: (absolute control)	22.7 b
CV (%)	9.40
Treatments	**

G: *Glomus* sp.; A: *Acaullospora* sp.; S: *Scutellospora* sp.; G+A+S: mix of *Glomus* sp. + *Acaullospora* sp.+ *Scutellospora* sp.; DAP: soil with application of 40 g of diammonium phosphate per plant.

*Averages with different letter on the same column are statistically different, Tukey ($P \leq 0.05$); **: F test highly significant ($P \leq 0.01$); ns: F test no significant; CV: coefficient of variation.

Table 4. Effect of inoculation of VAM native genera in leaf area (cm^2) of Hartón plantain (*Musa* AAB Simmonds) plants in nursery conditions.

Treatment	Leaf area (cm^2)
T1: *Glomus* sp (G)	841 b*
T2: *Acaullospora* sp(A)	742.4 b
T3: *Scutellospora* spD	839.7 b
T4: combination (A+G+S)	913.7 b
T5: (DAP)	1368.0 a
T6: (Absolute control)	740.0 b
CV (%)	15.3
Treatments	**

G: *Glomus* sp.; A: *Acaullospora* sp.; S: *Scutellospora* sp.; G+A+S: mix of *Glomus* sp. + *Acaullospora* sp.+ *Scutellospora* sp.; DAP: soil with application of 40 g of diammonium phosphate per plant.

*Averages with different letter on the same column are statistically different, Tukey ($P \leq 0.05$); **: F test highly significant ($P \leq 0.01$); ns: F test no significant; CV: coefficient of variation.

culated, thus, they are a good alternative to obtain better plantain plants on soil with low phosphorus and organic matter content, like the ones in the coffee region of Colombia where it is recommended to apply mixes of mycorrhizal fungi of the *Glomus*, *Acaulospora* and *Entrophosforase* genera at the sowing time of plantain (Ruiz *et al.*, 2006).

In the same Table 4 it is observed that inoculated plants with *Glomus* (T1), *Acaullospora* (T2) and *Scutellospora* (T3) genera had greater leaf area values compared to the absolute control (T6), indicating that symbiosis with mycorrhizae produces physical, biochemical and physiological changes on colonized roots that lead to a better general status of the plant and its different organs (Barea *et al.,* 1997). This is important in plantain plants because of their special characteristics as perennial species that needs an initial phase for root development needed for absorption processes, and leaves with a suitable leaf area for photosynthesis (Cayón *et al.*, 1998). As it is known, arbuscular mycorrhizae fungi application favors the development of this surface on mycotrophic crops, like plantain and banana, improving photosynthetic efficiency in leaves and increasing plant growth.

Leaf area:leaf weight ratio

Relation between leaf area (cm^2) and leaf weight (g) was different among treatments ($P < 0.05$) (Table 5). This ratio was higher in plants of the T4 (combination of VAM genera) because the VAM fungi, besides of their direct effect on plant nutrition, induce physiological changes by which the proportion of total dry matter per plant that is converted to leaves (Jarma *et al.*, 1999) and the photosynthesis and carbon fixation rates are increased (Blanco and Salas, 1996).

It is important to highlight that plant development in all the treatments with mycorrhizae exceeded the plants in the absolute control and equaled the ones receiving the chemical treatment (Table 5), which, according to Thaker and Jasrai (2002), is due to the improvement of the plant physiological system, especially at the nursery phase. Leigh *et al.* (2009) considered that the benefits of mycorrhizal fungi on plant crops are closely related to a better use of soil nutrients and water, by complex interactions between these microorganisms structures and the roots of the host plant.

Number of spores of mycorrhizae

The highest number of spores was observed in

Table 5. Effect of inoculation of VAM native genera on the leaf area ratio (cm^2/g) of Hartón plantain (*Musa* AAB Simmonds) plants in nursery conditions.

Treatment	Foliar area (cm^2)
T1: *Glomus* sp (G)	4.3 ab
T2: *Acaullospora* sp(A)	4.1 b
T3: *Scutellospora* spD	4.9b ab
T4: Combination (A+G+S)	9.8 a
T5: (DAP)	5.3 ab
T6: (Absolute control)	3.6 b
CV (%)	8.70
Treatments	**

G: *Glomus* sp.; A: *Acaullospora* sp.; S: *Scutellospora* sp.; G+A+S: mix of *Glomus* sp. + *Acaullospora* sp.+ *Scutellospora* sp.; DAP: soil with application of 40 g of diammonium phosphate per plant.

*Averages with different letter on the same column are statistically different, Tukey ($P \leq 0.05$); **: F test highly significant ($P \leq 0.01$); ns: F test no significant; CV: coefficient of variation.

plants of the T4 treatment (Figure 2). In the treatments with individual VAM application, T1 (*Glomus* sp.) stood out, evidencing that this genus is widely distributed on soils, independently of the fertility degree (Guerrero *et al.*, 1996). This author points outs that *Glomus* has a wide range of adaptation to different abiotic conditions, among them, pH between 5.0 and 6.5. On the other side, Sánchez and Sieverding (1999) and Sánchez (2007) found similar results to the ones of this research and observed that *Glomus* is highly infective with high adaptability to diverse soil conditions.

Mycorrhizal colonization percentage

In this characteristic, again the plants on the T4 treatment showed the highest average value, 91.1% (Figure 3). Treatment with *Glomus* sp. (T1) presented the highest capacity of infection and of forming special structures, 90% of colonization, than the other individually inoculated genera. Treatments with *Acaullospora* sp. (T2) and *Scutellospora* sp. (T3) showed colonization percentages of 72.3 and 78.3%, respectively.

The above agrees with the mycorrhizal effectiveness and the adaptation of *Glomus* sp. To different substrate conditions (Barea, 1997). The high percentage of mycorrhizal colonization demonstrates the existence of an effective symbiosis between the inoculated treatments and Hartón plantain plants. The values of this study are higher than the ones found by Usuga-Osorio *et al.* (2008) who observed a general association expressed by a 37.76 ± 21.86% on different plants, including banana, cultivated on different substrates. On the other hand, Jaizme and Garzon (1995)

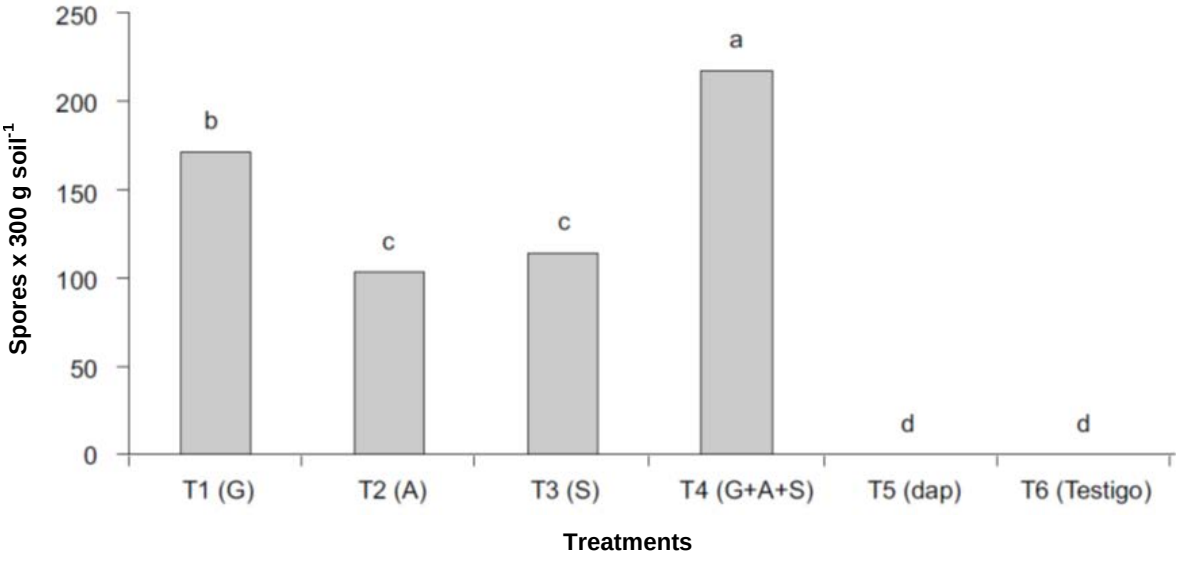

Figure 2. Number of isolate spores per treatment, 120 days after seedling inoculation on Hartón plantain (*Musa* AAB Simmonds). T1: *Glomus* sp., T2: *Acaullospora* sp., T3: *Scutellospora* sp., T4: genera combination; T5: soil with phosphoric fertilization (DAP); T6: absolute control.

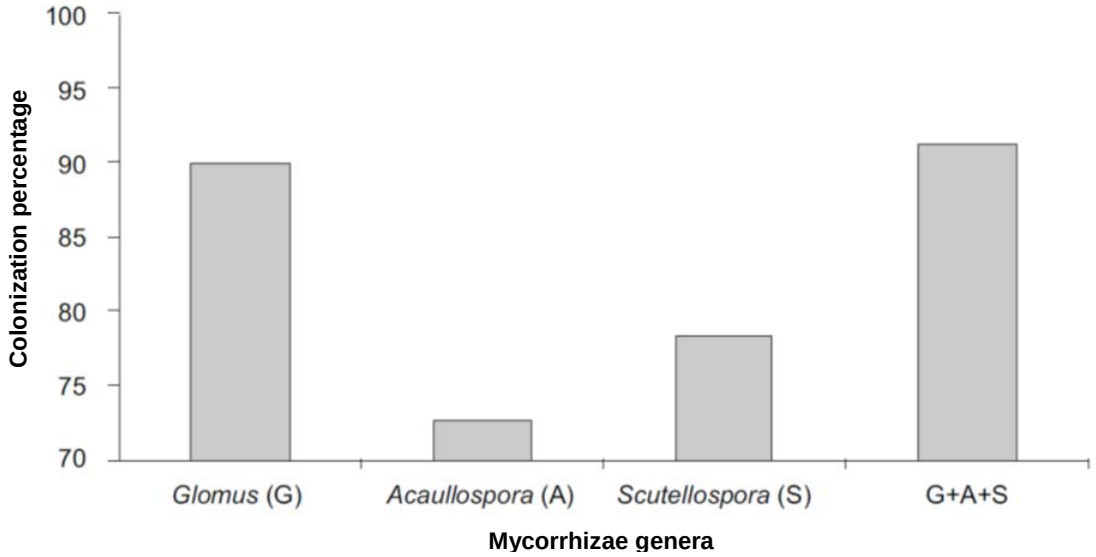

Figure 3. Effect of the inoculation of native mycorrhizae genera in the colonization percentage in roots of Hartón plantain (*Musa* AAB Simmonds) 120 days after inoculation under nursery conditions.

found colonization values of 71 and 75% for *Glomus manihotis* and *Glomusintra radices*, respectively.

Conclusions

- Harton plantain plants on early growth stages at the nursery presented simbiosis with the combination of native vesicular-arbuscular mycorrhizae of the *Glomus*, *Acaullospora* and *Scutellospora* genera, resulting on a higher spore number and higher root colonization percentage.
- The highest colonization percentage with the inoculation of individual native mycorrhizae was obtained with the *Glomus* genus.
- The proportion of total dry matter that is converted to leaves in the Harton plantain at the nursery phase, measured by the leaf area relation, is favored with the native mycorrhizae application. Plant leaf area is stimulated by the application of diammonium phosphate (DAP), although the application of combination of VAM native genera (T4) presented a better adaptation to the substrate conditions in comparison to the other genera inoculated individually, therefore, it is an alternative to obtain good plantain plants on low fertility soils.
- The variables length, root dry and wet weight, were positively affected by the application of mycorrhizae, in comparison to the application of DAP and the absolute control.

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