# Mycorrhization in oil palm (*Elaeis guineensis* and *E. oleifera* x *E. guineensis*) in the pre-nursery stage

## Micorrización en palma de aceite (*Elaeis guineensis* y *E. oleifera* x *E. guineensis*) en etapa de previvero

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**ABSTRACT**

The symbiosis between oil palm roots and arbuscular mycorrhiza (AM)-forming fungi is an important aspect of the biology of the crop. Here, we describe the mycorrhization process and the effect of an inoculation with *Glomus intraradices* on plant growth during the pre-nursery stage of plants of *E. guineensis* and the interspecific hybrid *E. oleifera* x *E. guineensis,* under the agro-ecological conditions of the Colombian Eastern Plains. The total percentage of mycorrhization over time and the accumulated dry biomass were measured at the end of the pre-nursery stage. The progress of the colonization process of seedlings inoculated with *Glomus intraradices* was compared with the progress of non-inoculated seedlings, in both autoclaved and non-autoclaved soil. Measurements of colonization were performed on semi-permanent micro-preparations of cleared and stained rootlets. Quantifications were done using the intercept field method, which differentiates arbuscules, hyphae, vesicles and spores. Root colonization started 1 month after sowing (mas). At the end of the pre-nursery stage (3 mas), arbuscule and hyphal networks were established, especially in *E. guineensis* seedlings. An increase in dry weight of seedlings of this species was found in response to inoculation with a commercial source of AM. The results suggest that inoculation with AM at early stages of oil palm may potentially increase the vigor of seedlings transplanted to the main nursery.

**Key words:** root inoculation, plantation, Colombia, seedlings.

**RESUMEN**

La simbiosis entre las raíces de palma de aceite y hongos formadores de micorrizas arbusculares (MA) es un aspecto importante de la biología de este cultivo. En este artículo se describe el proceso de micorrización y el efecto de la inoculación con *Glomus intraradices* sobre el crecimiento en previvero de plantas de palma de aceite *E. guineensis* y el híbrido interespecífico *E. oleifera* x *E. guineensis,* bajo las condiciones agroecológicas de los Llanos Orientales de Colombia. El porcentaje de micorrización en el tiempo y la biomasa seca acumulada fueron medidos al final de la etapa de pre-vivero. El progreso del proceso de colonización de plántulas inoculadas con *Glomus intraradices* fue comparado en plántulas inoculadas y no inoculadas tanto en suelo esterilizado como en suelo no esterilizado. Las mediciones fueron llevadas a cabo sobre micro-preparados semi-permanentes de raíces clareadas y teñidas, sobre las cuales la cuantificación se hizo usando el método del campo intercepto, el cual permite diferenciar arbúsculos, hifas, vesículas y esporas. La colonización radicular se inició 1 mes después de siembra (mds) en previvero. Al final de la etapa de previvero, (3 mds), arbúsculos y redes hifales ya estaban establecidas, especialmente en plántulas de *E. guineensis.* Un incremento en peso seco de las plántulas de esta especie fue observada en respuesta a la inoculación. Los resultados sugieren que la inoculación con MA en etapas tempranas del crecimiento de palma de aceite podría incrementar potencialmente el vigor de las plántulas trasplantadas al vivero.

**Palabras clave:** inoculación de las raíces, plantación, Colombia, plántulas.

## Introduction

Improving competitiveness and sustainability are some of the challenges that the oil palm industry is currently facing worldwide. The use of biological soil resources, especially a core group such as arbuscular mycorrhiza-fungi (AM), which strengthen the root system by increasing the water and nutrient absorption surface of roots (Harrison and Van Buuren, 1995; Smith and Read, 2008), is an alternative that can help overcome these challenges. It has been previously shown that oil palm seedlings inoculated with commercial mycorrhizal products, particularly in the nursery stage, show significant increases in dry biomass and accumulation of nutrients, mainly phosphorus (Blal *et al*., 1990; Ramlah and Mohd Tayeb, 1991; Schultz, 2001; Motta and Munévar, 2005). Those results are promising because they support the potential impact of these fungi on the early growth stages of seedlings. However, to our knowledge, none of the previous

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studies have assessed the biology of the symbiosis between AM and oil palm seedlings on a commercial scale in Colombia. Consequently, unanswered questions about the best time and the procedure to inoculate with promising AM strains in commercial plantations in this country remain.

It is necessary to design efficient agronomic practices for obtaining the maximum benefit of the AM symbiosis as research results cannot always be reproduced on a commercial scale. This was highlighted by Phosri *et al*. (2010), who explained the need to further investigate, in inoculation schemes, the effect of the application of AM in the nursery stage. Here, we present the first observations of the mycorrhization process starting at the first month after sowing the seeds in the pre-nursery, and ending at the fourth month when the plants are regularly transplanted to the nursery.

The study was performed in a commercial plantation in Colombia. We aimed to describe the mycorrhization process with the conditions under which seedlings actually grow in commercial plantations, in order to determine when the application of effective inoculants should be performed. Generally, oil palm growers apply AM inoculants just before transplanting the seedlings from the pre-nursery to the nursery. However, pre-nursery seedlings may already be colonized with native strains of AM, which can be a potential source of variation in the response to inoculation because of possible competition between these microorganisms and those that are on site before the inoculation.

## Materials and methods

This study describes the mycorrhization process in oil palm seedlings (*Eleais guineensis* and interspecific hybrid *E. guineensis* x *E. oleifera* (OxG hybrid) in the pre-nursery stage, which usually lasts three months. AM-structure presence in the root cortex, observed in micro-preparations of young roots of 1 to 4-month seedlings, was quantified as percentage of colonization. The morphological differentiation of colonization with AM, according to Petterson *et al*. (2004), was used. These structures include intra- and extraradical hyphae, arbuscules, spores and, occasionally, vesicles. Descriptions were made for *E. guineensis* and the interspecific hybrid *E. oleifera* x *E. guineensis*.

### General conditions of the experiment

Two experiments were conducted in a commercial oil palm plantation located near the township of Veracruz, department of Meta, in the Colombian Orinoco region, 305 m a.s.l. (4°13'30'' N, 73°13'36'' W). Due to differences in seed availability and to facilitate the harvest of roots and microscopic observations, we spread the experiments over two consecutive seasons. The first experiment was established in August 2009 for *E. guineensis* and the second one, in February 2010 for the OxG hybrid. During these months, the weather in this part of Colombia is transitional between dry and rainy. Throughout the course of the experiments, the maximum precipitation was 615 mm in April; the minimum was 90 mm during the month of November. The average annual precipitation on the plantation is 3,000 mm. The daily temperature ranged between 20 and 38°C, with a daily average of 27°C.

### Materials

Pre-germinated seeds of *E. guineensis* (material IRHO 7001) and the OxG hybrid (Coari x La Me) were planted in 1 kg-capacity bags with the soil regularly used on the plantation. To differentiate the effect of native mycorrhizae and accompanying microorganisms present at the beginning of the experiment on the response variables, half of the treatments contained sterile soil. Sterilization of the soil was done by autoclaving it in two cycles at 3 MPa and 121°C for 15 min/each cycle. Analysis of three sterilized soil samples and three non-sterile soil samples yielded the results presented in Tab. 1. Because this experiment was conducted on a plantation, sterile soil was applied only as a means of eliminating the native microorganisms at the beginning of the experiment with the objective of providing a low competitive pressure of native microoganisms for the treatment with commercial inoculant. Furthermore, with this treatment, we aimed to investigate whether soil sterilization at the pre-nursery stage may be advantageous combined with inoculation. With the same objective, treatments with sterile soil where watered with distilled water during the first two weeks. Further irrigation events were managed with regular irrigation water from the plantation.

### Treatments and experimental design

Four different treatments were applied at sowing: soil without commercial inoculant (NS UNINOCULATED); soil with commercial inoculant (NS INOCULATED); sterile soil without commercial inoculant (SS UNINOCULATED); sterile soil with commercial inoculant (SS INOCULATED). The inoculum contained 2,000 propagules/mL of *Glomus intraradices* produced *in vitro* and was purchased from a private company. The inoculation was performed in accordance with the manufacturer's recommendations, by immersion of the radicle at the time of planting and re-inoculation by injection two months after planting. Each dose contained 250 propagules. Each treatment was applied to a total of 50 seeds completely randomized in a split-plot design where the main plot was the inoculation

**TABLE 1.** Analysis of the soil used in the experiments, with averages of three replicates for each type of soil (autoclaved and non autoclaved) ± standard deviation. L: Loam. S: Sandy.

| Parameter | Units | Sample preparation | Method for determination | Non –sterilized soil | Sterilized soil |
|---|---|---|---|---|---|
| Texture | | -- | Bouyoucos | S-L | S-L |
| Sand | % | -- | Bouyoucos | 55.82 ± 0.58 | 54.49 ± 1.15 |
| Clay | % | -- | Bouyoucos | 7.48 ± 1.73 | 10.15 ± 0.58 |
| Silt | % | -- | Bouyoucos | 36.73 ± 1.53 | 35.40 ± 1.00 |
| pH | Units | Ratio 1:1 water: soil | Potentiometry | 6.83 ± 0.07 | 7.17 ± 0.07 |
| Electric conductivity | dS $m^{-1}$ | Ratio 1: 1 water: soil | Conductimetry | 1.05 ± 0.10 | 1.18 ± 0.10 |
| CEC | cmol(+) $kg^{-1}$ | Ammonium acetate pH 7 | Volumetry | 8.51 ± 0.80 | 9.55 ± 1.21 |
| Organic carbon | % | Walkley Black method | Volumetry | 2.32 ± 0.35 | 2.52 ± 0.22 |
| Organic matter | % | | | 4.00 ± 0.61 | 4.35 ± 0.38 |
| Potassium | cmol(+) $kg^{-1}$ | Ammonium acetate pH 7 | Atomic absorption | 0.29 ± 0.02 | 0.31 ± 0.02 |
| Calcium | cmol(+) $kg^{-1}$ | Ammonium acetate pH 7 | Atomic absorption | 12.67 ± 1.23 | 13.53 ± 0.91 |
| Magnesium | cmol(+) $kg^{-1}$ | Ammonium acetate pH 7 | Atomic absorption | 1.51 ± 0.16 | 1.40 ± 0.18 |
| Sodium | cmol(+) $kg^{-1}$ | Ammonium acetate pH 7 | Atomic absorption | 0.04 ± 0.01 | 0.043 ± 0.01 |
| Phosphorus | mg $kg^{-1}$ P | Bray II | Colorimetric | 243 ± 2.00 | 248.23 ± 34.76 |
| Spore concentration of HFMA | Spores/10 mL | Sucrose gradient | Direct count on 40x fields | 7.00 ± 2.64 | 0 |

and the subplot was the soil sterilization. The experimental unit consisted of groups of seven seedlings and each treatment contained six experimental units. Samples for observation under the microscope for colonization rate consisted of three randomly selected seedlings. For each plant, three counts were performed. At least five seedlings were randomly selected for determination of dry weight.

### Colonization rate and inoculation effect

The mycorrhization process was measured in root fragments extracted from three plants per treatment. From each plant, 5 g fresh weight of young roots were harvested. Rootlets were cleared and stained in 10% KOH and blue ink, as described by Phillips and Hayman (1970) and Veirgeligh *et al.* (1998), with modifications for oil palm roots as suggested by Sieverding (1991). These modifications included the clearing in 10% KOH in three autoclaving cycles; treatment with 1% HCl solution and bleaching with alkaline hydrogen peroxide solution. Ten root fragments (1 cm each) were placed on glass slides with polyvinyl lacto glycerol (PVLG) and covered with a glass slide, pressing the roots down to make the final semi-permanent micro-preparation. The mycorrhization rate was examined and quantified in at least three slides per plant. Colonization rate was performed by direct microscopic observation at 40x (Carl Zeiss, STEMI DV4, Germany), recording presence-absence of colonization in 50 fields per slide, and differentiating arbuscules, hyphae, spores and vesicles (Brundrett *et al.*, 1996). After the pre-nursery stage, four months after planting, dry biomass of leaves and roots was measured in at least five seedlings per treatment.

### Data analysis

Analyses of variance were conducted to determine the effect of the treatments on the response variables ($P \leq 0.05$). Treatment means were compared by Tukey test ($P \leq 0.05$), using the SAS® v.9.0 software.

## Results and discussion

Figs. 1 and 2 show the initial advancement of AM colonization in the root cortex of *E. guineensis* and OxG hybrid plantlets, respectively. Despite the high P soil content (243 and 248 mg $kg^{-1}$, see Tab. 1), due to the application of phosphate fertilizers as a pre-nursery land preparation practice, the symbiosis with AM in oil palm seedlings started for both *E. guineensis* and the OxG hybrid around 1 month after sowing (mas). Colonization rate values ranged between 8.86 and 15.20% for all treatments, except for the SS UNINOCULATED treatment, which showed that colonization began 27 days after sowing (das) for *E. guineensis,* and 47 das for the OxG hybrid as illustrated in Fig. 3. This happened with both the commercial inoculant and native fungi in the soil in the two plant materials (Fig. 3A and B). One month after planting, mainly hyphae were found in the epidermis and root cortex, with a notable frequency of appressoria, widening of the hyphae through which the fungus penetrates into the root tissue. Interestingly, colonization points and profuse networks of hyphae and arbuscules occurred mainly in tertiary roots and rarely in quaternary roots. This contrasts with what had been previously described by Corley and Thinker (2003), who described quaternary roots as the preferred point of colonization for this plant species.

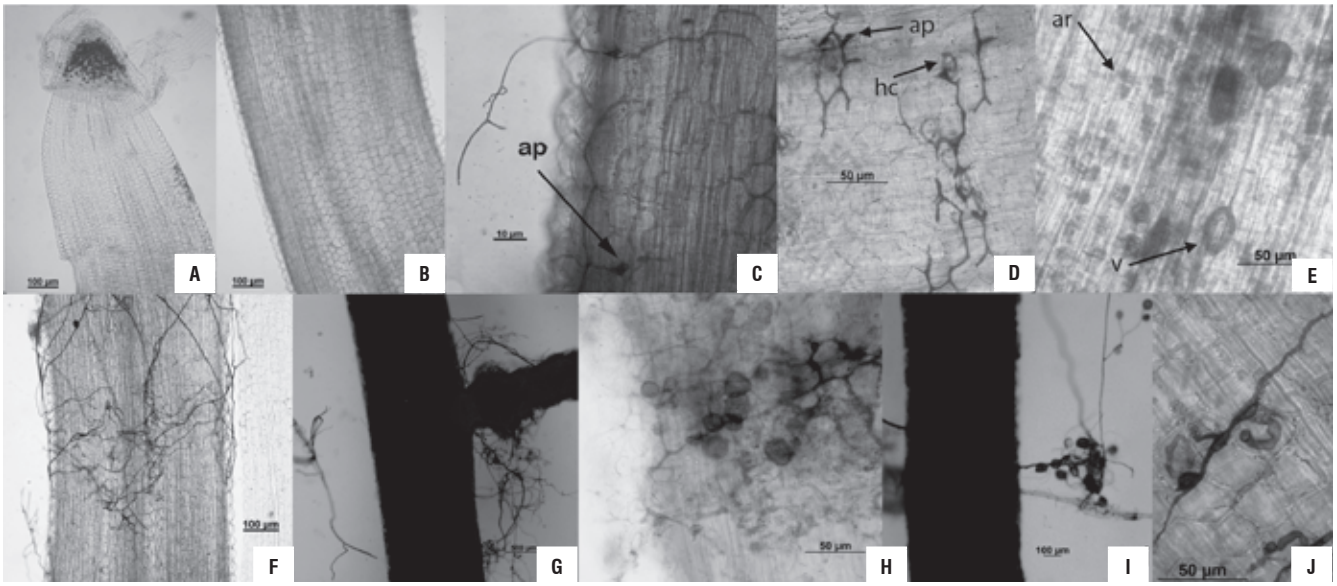


**FIGURE 1.** Mycorrhization process in *E. guineensis* seedlings during the first three months of growth in the pre-nursery stage. A, B, C : Roots 1 month after planting in pre-nursery. D, E : Roots 2 months after planting in pre-nursery. F, G, H, I: Roots 3 months after planting in pre-nursery. A. Apical meristem of tertiary root without points of colonization. B. Tertiary root without points of colonization. C. Onset of the colonization of the root hypodermis. Seedlings with intramatrical hyphal networks (D) and production of *Arum*-type arbuscules (E). F. Extraradical hyphae mainly covering tertiary roots. G. Secondary root with tertiary root covered by extraradical hyphal network at the third month of the pre-nursery stage. H. Intramatrical spores in the tertiary root cortex. I. Extraradical spores surrounding a tertiary root. J. Formation of *Paris-type mycorrhizae.* Vesicle (v). Arbuscule (ar). Appressorium (ap). Hyphal coiling (hc). (Photos: Galindo-Castañeda, T.)

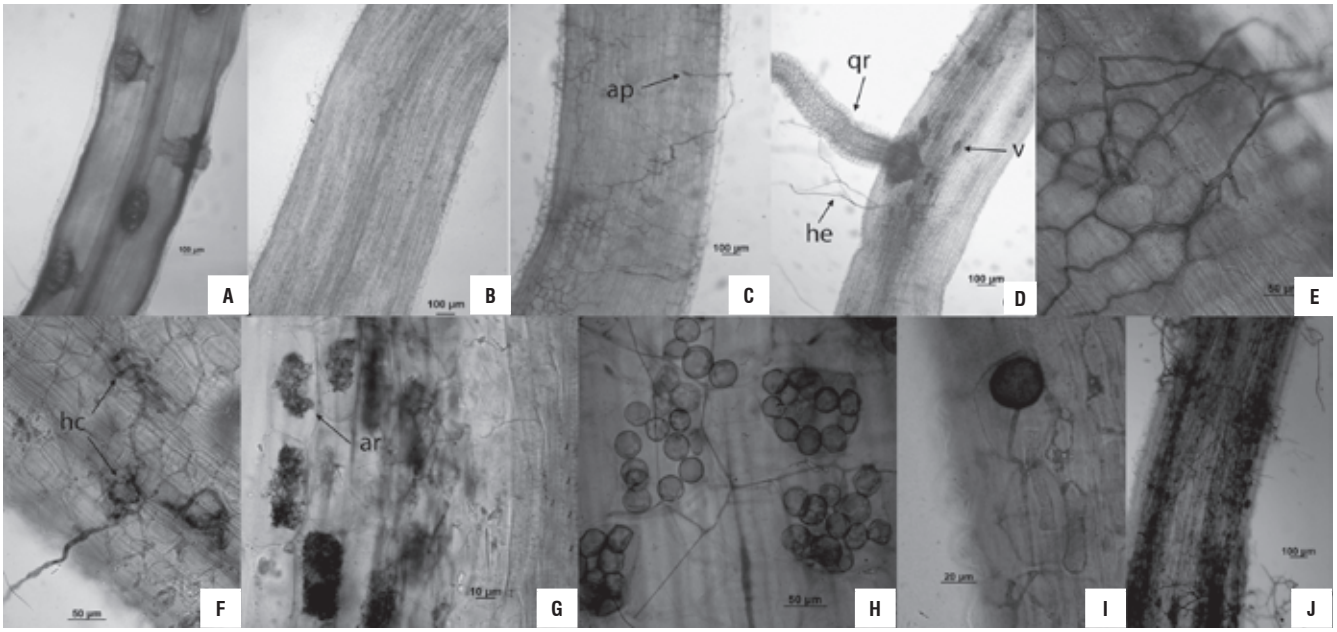


**FIGURE 2.** Mycorrhization process in OxG hybrid seedlings during the first four months of growth in pre-nursery. A, B, C. Roots 1 month after planting in pre-nursery. D, E, F. Roots 2 months after planting in pre-nursery. G, H, I. Roots 4 months after planting in pre-nursery. A. Lignified tertiary roots. B. Non-lignified tertiary root without points of colonization. C. Tertiary root with initial points of colonization in the hypodermis. D. Tertiary root mycorrhized with quaternary root. E. Intramatrical hyphal networks in the cortex. F. Beginning of formation of hyphal coiling of *Paris*-type morphology. G. Detail of *Arum*-type arbuscules formed after inoculation with *Glomus intraradices.* Note the vascular bundles adjacent to arbuscule network and hyphae H, I. Intramatrical spores present in roots inoculated with *G. intraradices*. J. Fully mycorrhized tertiary root. Appressorium (ap). Arbuscule (ar). Vesicle (v). Extraradical hyphae (he). Quaternary root (qr). Hyphal coiling (hc). (Photos: Galindo-Castañeda, T. )

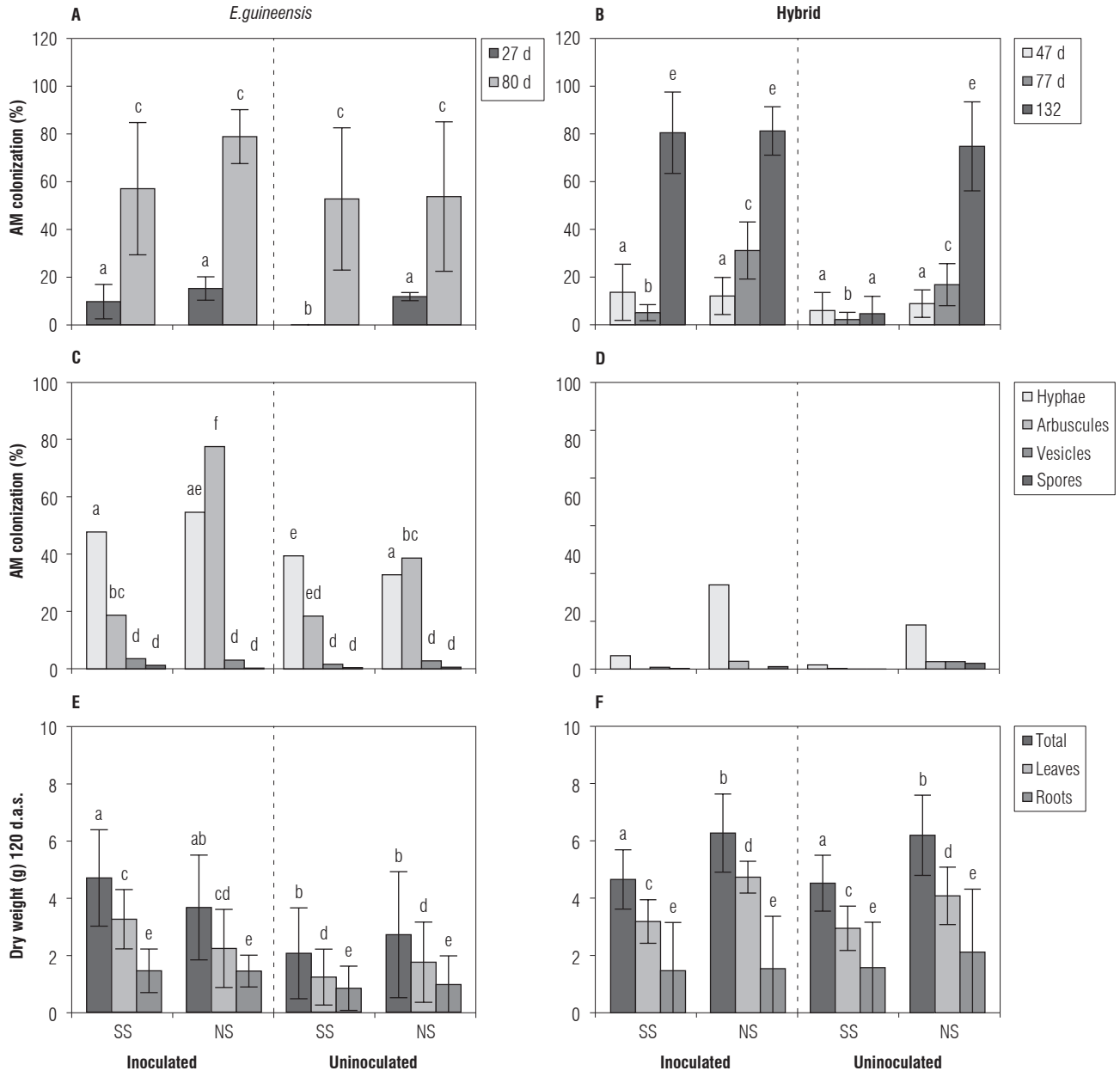


**FIGURE 3.** Root colonization rates and dry weight response to inoculation with *G. intraradices* in seedlings of *E. guineensis* and the OxG hybrid. A, B: Root colonization rate by AM in pre-nursery. Columns represent averages with respective standard deviation (n = 3). C, D: Root colonization rate by AM structures before the end of the pre-nursery stage. Bars represent averages with respective standard deviation (n = 3). E, F: Dry weight at the end of the pre-nursery stage. Bars represent averages with their respective standard deviations (n = 5 for *E. guineensis* n = at least 3 for OxG Hybrid). Bars with the same letter are not significantly different (Tukey test $P \leq 0.05$). d = das. SS = sterilized soil. NS = non-sterile soil.

Two months after sowing, *Arum*-type and *Paris*-type mycorrhizae were found and their arbuscules and coiled hyphae, respectively, were observed within cortical cells (Fig. 1D, E and J; Fig. 2E and F). The two morphology types were found simultaneously, mainly in NS treatments. Some roots even presented two types of morphology in the same fragment examined. After 2 months, intraradical hyphal networks increased in proportion (data not shown), and still, vesicles and spores were not found by the end of the second month.

At the end of the 3 mas, when it is recommended that seedlings be taken to the nursery, the symbiosis in *E. guineensis* had already been established through hyphae and arbuscules; the production of vesicles and some intramatrical spores had begun (Fig. 3A and C), with colonization

values up to 78%. The lowest colonization percentage (52%) occurred in seedlings with the SS UNINOCULATED treatment.

Under the described experimental conditions, the OxG hybrid plants presented all the structures typical of AM colonization as depicted in Fig. 2 and 3D in a delayed fashion compared to *E. guineensis*. While in the former, the colonization rates reached values of up to 30%, the latter showed values between 50 and 70% by the end of the third month. To achieve colonization rates of over 70% in OxG hybrid seedlings, it was necessary to wait one more month in pre-nursery (132 d Fig. 3B) as compared to *E. guineensis*. A specific study with simultaneously sowed plants of both *E. guineensis* and the hybrid OxG would provide more conclusive insights about the relative duration of the colonization process in the two plant materials. However, this is a preliminary result that could be taken into account as reference.

The dry plant biomass and colonization percentages presented in Fig. 3 show the potential benefits of inoculation and soil sterilization as possible practices in pre-nurseries for improving plant vigor. Although no significant differences were found in total colonization rate values of *E. guineensis* between treatments at 80 d after planting, dry weight values did show significant differences at 120 d ($P \leq 0.05$; $r^2 = 0.87$), as illustrated in Fig. 3A and E. Seedlings inoculated in SS and NS treatments increased biomass accumulation by 216 and 150%, respectively, when compared with non-inoculated seedlings (Fig. 3E). Importantly, this increase was not associated with an increase in dry root biomass, which showed no significant differences between treatments. Because plants that accumulated the highest biomass were those with the SS INOCULATED treatment, we anticipate that inoculation in sterilized soil may represent an effective inoculation technique for promoting plantlet growth after the completion of the pre-nursery stage. In the presence of native microorganisms, with non-sterilized soil, the efficiency of this inoculum was reduced by 32%, which is still a promising result when compared with non-inoculated plants.

Compared to *E. guineensis*, the hybrid plants presented different results in response to soil sterilization and root inoculation. The application of the commercial inoculant had no significant effect on total root colonization values of the OxG hybrid at any time during the pre-nursery stage. This contrasts with the effect of soil sterilization on the colonization rate at 77 d after planting ($P \leq 0.05$; $r^2 = 0.78$), when colonization rates were 2.2 and 5.1% for plants with SS, while for plants with NS the respective values ranged between 16.8 and 31.1% (Fig. 3B). However, after four months in pre-nursery, the colonization was similar in INOCULATED and NS UNINOCULATED, reaching values of between 74.7 and 81.2%. These results show that both native fungi and fungi present in the inoculum contributed equally to the increase in colonization values of these plants at the end of the fourth month, but not at the end of the third month. Close to the end of the pre-nursery stage, an effect of soil sterilization on leaf dry weight ($P \leq 0.05$; $r^2 = 0.82$) and total weight ($P \leq 0.05$; $r^2 = 0.68$) were found as can be seen in Fig. 3F. However, no effect was found due to inoculation. This result may indicate an important contribution of native microorganisms to biomass accumulation of the OxG hybrid, while inoculation of these plants increases the levels of colonization in the third month, it does not seem to produce effects on biomass dry weight.

## Conclusions

This paper demonstrates, for the first time in Colombia, that AM colonization of oil palm seedlings is an active and progressive process throughout the pre-nursery stage as shown by the quantification of colonization percentages. A detailed examination of the symbiosis development indicates that all the structures typical of AM symbiosis such as arbuscules, vesicles, intra- and extraradical hyphae and spores, developed during this first stage of growth. At the end of the first month, appresoria and intraradical hyphae where common in the root cortex. Then, at the end of the second month, the establishment of intraradical hyphal networks began. Although the formation of arbuscules begins at this time, only until the 3 mas did mycorrhizae develop abundant arbuscule networks in this study. A few spores were observed just before transplanting to the main nursery. The design of inoculation practices must consider these results, as it would be desirable to inoculate the seedlings starting the pre-nursery stage with inoculants with high concentrations of selected and efficient AM species.

There are details regarding the root morphology and anatomy of oil palm observed in this study that may be considered in the development of AM-based technologies. For example, it is noteworthy that mycorrhizae were found usually in the tertiary roots and to a lesser extent in quaternary roots. In oil palm, the anatomy of quaternary roots differs from that of the tertiary roots in that they have no vascular bundles, no lignified endodermis and do not form gaps in the cortical parenchyma (Hartley, 1983; Corley and Thinker, 2003). The lack of vascular vessels and lignified structures is typical of the primary role of absorption of the

quaternary roots, which explains the absence of root hairs in oil palm roots (Hartley, 1983). However, when observing quaternary roots (Fig. 2D) and comparing them with the fine hyphae of the AM, it seems that having a profuse network of hyphae, from the symbiosis with AM, might be of important benefit to the seedlings, as the thinner hyphae, compared to the quaternary roots, could represent significant increases in water and nutrient uptake surfaces of abundantly mycorrhized seedlings.

Observations made in this study may have implications for the development of technologies like *in vitro* propagation of plants, inoculation practices and nursery management. For example, the development of the different AM structures inside oil palm seedling roots at the end of the first month in pre-nursery suggests that contact and penetration of the fungus through the epidermis and towards the cortex by means of the appressoria begin very early in the development of the plantlet, during the first mas. Given the anatomy of the tertiary roots of oil palm, it is expected that the entry points of these hyphae are the short cells of the hypodermis (Hartley, 1983; Petterson *et al*., 2004). This result may be taken into account for the development of plantlets propagated *in vitro* which may benefit from an early inoculation with AM. Direct observations of the cells that accept the symbiont may be needed for such studies. Another interesting result is that the establishment of intraradical hyphal networks began at the second month. Although the formation of arbuscules begins at this time, only until the third month did mycorrhizae develop complex arbuscule networks in this study. These mycorrhizae structures have been proposed as the main point of interchange of water, nutrients and photosynthates between fungus and plant. Thus, after the second month, when arbuscule production starts, symbiosis may start being beneficial for the plant. The last affirmation is reasonable considering that the seedling depends on the supply of nutrients by the seed during the first two months after planting (Corley and Thinker, 2003). After two months, the plant begins to depend on soil nutrients, the moment at which the plant should allow the development of arbuscules, as shown in Fig. 3C and D. This is an indication that the plant may be delaying colonization because it may not need it during the first two months of growth. On the other hand, the application of fertilizers should be re-assessed in pre-nursery if early nutrition is enhanced with inoculation of efficient AM. We anticipate interesting results of experiments under high and low P contents during the 3 mas.

This work is evidence that inoculation with selected strains present in commercial products is an alternative for increasing the vigor of *E. guineensis* seedlings to be transplanted to the nursery. It was also found that inoculation of these seedlings in the pre-nursery stage could enhance the benefits of the symbiosis, by allowing greater colonization of roots before the native mycorrhizae or other microorganisms enter the roots. In contrast, for the hybrid plants, the colonization and increase in dry weight did not differ with the application of commercial inoculants used in this experiment, suggesting that native microorganisms are potentially beneficial to seedling vigor. However, these results may be complemented with replicates during consecutive seasons and by comparing the colonization process of *E. guineensis* and hybrids simultaneously.

Finally, it is interesting to note that despite the high concentrations of phosphate in the soil, mycorrhization was observed in the experiments. Previously, Motta and Munevar (2005) presented evidence of mycorrhization in soils with concentrations of phosphorus of 53 mg $kg^{-1}$. That is, despite the phosphorus fertilizer applied to the soil in palm plantations, AM fungi are able to initiate contact with the root and colonize the tissues of young plants. In addition to the results presented in this study, Ramlah and Mohd Tayeb (1991) found that oil palm seedlings have growth increases in response to mycorrhization under phosphate concentrations of up to 300 mg $kg^{-1}$. These pieces of evidence have practical implications: on the one hand, phosphate applied to the soil in commercial oil palm plantations might not inhibit the symbiosis. On the other hand, soils could be biologically enriched with selected AM, despite the agronomic history of many soils previously treated with high level of phosphorus fertilizers. However, it is necessary to determine the maximum P concentration at which mycorrhizal symbiosis in oil palm is beneficial for pre-nursery stages. It is also important to perform a selection of efficient strains of native AM that can compete efficiently with natural soil microorganisms.


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