# Growth and mineral nutrition of banana seedlings cv. Grand Naine inoculated with arbuscular mycorrhizal fungi

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# Abstract

This study aimed to verify the influence of AMF on the levels of macro- and micronutrients and growth of micropropagated banana seedlings submitted to doses of P. The experiment was arranged in randomized blocks, in a 5 x 5 factorial design, with the factors consisting of inoculation with AMF (*G.clarum*, *G. margarita*, *G. albida*, *C. etunicatum*, and the control treatment, without AMF) and doses of P (0, 50, 100, 200, and 400 mg kg<sup>-1</sup>). The association of different species of AMF can favor the growth of banana seedlings, providing better nutrition. The application of doses of P increased the growth of micropropagated banana seedlings regardless of inoculation with AMF. Seedling production associated with *G. margarita* can favor the growth of plants submitted to low doses of P applied to the soil. The interaction between *G. clarum* and the seedling production of Musa spp. did not seem to favor plant growth. Mycorrhizal colonization together with phosphate fertilization contributed to the quality of banana seedlings. The inoculation with *C. etunicatum* favored the increase of K contents in the plant shoot. The increase in the doses of P decreased the levels of N and S for all treatments, causing a decrease in the leaf contents of Mn, B, and Fe. The doses of P did not interfere in the leaf contents of Cu and Zn. The AMF favored the levels of Cu, Zn, Mn, and B in banana leaves and favored the increases of Cu, Fe, Zn, and Mn contents in banana seedlings, varying according with the doses of P.

Keywords: phosphorus levels, plant mineral nutrition, root colonization

## Introduction

In search of new planting areas to expand the cultivation of bananas (Musa spp.) and to renew areas with low yields, it is necessary to use seedlings that are well-nourished and with high genetic and phytosanitary quality. In addition, phosphate fertilizers are necessary, especially in Cerrado soils. With these requirements, it is desirable to use alternative technologies that involve biological processes, providing economic and ecological gains and favoring the crop establishment (Guimarães et al., 2020; Lima et al., 2011).

The use of species of arbuscular mycorrhizal fungi (AMF) has been considered one of the ways to reduce fertilizers and correctives (Miranda, 2008; Cavalcante, 2009), besides increasing the levels of tolerance to abiotic stresses (Colozzi Filho & Nogueira, 2007).

One of the relevant aspects is the relationship between the P availability in the soil and the AMF, which

can affect the efficiency of some species (Cavalcante, 2009). Thus, knowing the doses of P that enable the development of plants in associations with different species of AMF becomes necessary. It is possible to verify their ability to stimulate plant growth mainly by increasing the absorption of nutrients, especially P, and by increasing the absorption surface of plant roots (Berbara et al., 2006). Among the main benefits of this symbiotic relationship for plants, several metabolic changes transmit positive effects on their development and nutrition. Normally, in mycorrhizal plants, there is high enzymatic activity, greater photosynthetic activity and increased production of growth-regulating substances. Through these metabolic changes, plants are more resistant to the effects caused by biotic or abiotic stresses (Nogueira, 2007).

Root colonization of the host through inoculation with AMF depends on symbiotic interaction and the following conditions: soil condition, host development stage, AMF species, and P availability (Miranda, 2008; Mehrota, 2005).

Therefore, this study aimed to study the influence of AMFs and doses of phosphorus on the growth of banana seedlings and on the macro- and micronutrient contents.

# Material and Methods

Micropropagated seedlings of banana cv. Grand Naine obtained in a greenhouse at UEMS (Aquidauana-MS) were used. The experiment was arranged in randomized blocks, in a 5x5 factorial design, using the following AMF in the treatments: *Glomus clarum* (GC), *Gigaspora margarita* (GM), *Gigaspora albida* (GA), *Clareoideoglomus etunicatum* (CE), and the control, without arbuscular mycorrhizal fungi (AMF); and five doses of P (0, 50, 100, 200, and 400 mg kg<sup>-1</sup>), with four replicates, using pots (7 dm<sup>3</sup>) with one plant per pot as the experimental unit.

AMF isolates were multiplied in association with Brachiaria decumbens in a substrate consisting of a 2:1 (v:v) mixture of soil and sand, being sterilized three times in an autoclave at a temperature of 121°C for one hour. The pots were maintained in a greenhouse for a period of four months. The substrate used in the experiment consisted of a 2:1 (v:v) mixture of soil and vermiculite, with the subsurface horizon consisting of a dystrophic Red Acrisol (Schiavo et al., 2010). The chemical analysis of the substrate showed the following results: pH (H<sub>2</sub>O; 1:2.5) - 4.8; P (Mehlich<sup>-1</sup>) - 3.5 mg dm<sup>-3</sup>; K - 1.6 mmol dm<sup>-3</sup>; Ca - 10.0 mmol<sub>c</sub> dm<sup>-3</sup>; Mg - 7.0 mmol<sub>c</sub> dm<sup>-3</sup>; A1 - 4.0 mmol<sub>c</sub> dm<sup>-3</sup>; and total H - 23 mmol<sub>c</sub> dm<sup>-3</sup>.

The substrate was sterilized in an autoclave at 121 °C for one hour and placed in plastic pots (7 dm<sup>3</sup>) after sterilization. Subsequently, limestone was applied based on soil analysis in order to increase base saturation to 70%. The humidity of the pots was maintained at an approximate value to field capacity for a period of 30 days for the limestone to react with the soil. After this period, doses of P (0, 50, 100, 200, and 400 mg kg-1 of soil) were added using  $KH_2PO_4$  as a source. Due to the increasing doses of P, it was necessary to balance the doses of potassium using KCI as a source. Inoculation was carried out at planting with 50 mL of inoculum consisting of a mixture of soil, spores, and roots of Brachiaria decumbens colonized with AMF, except for the control treatment. Planting was carried out with one seedling per pot and with the inoculum. At 20 and 50 days after planting, fertilization was carried out using 0.616 g N plant<sup>-1</sup>. Plants received manual irrigation.

At 75 days after planting (DAP), the seedlings of

each treatment were removed from the pots and the root system was separated from the shoot. The contents of N, P, K, Ca, Mg, S, Mn, Zn, Cu, Fe, and B were determined.

Seedling growth was evaluated by measuring the seedling height and the pseudostem diameter on the collar of the plant (30, 45, 60, and 75 DAP). At 75 DAP, mycorrhizal colonization was evaluated (Giovannetti & Mosse, 1980). Plant shoots and roots were dried in a forced air ventilation oven at 65 °C for 72 hours. Mycorrhizal dependence (MD) and mycorrhizal efficiency (ME) were determined (Plenchette et al., 1983). The data obtained were subjected to analysis of variance using the software SAEG.

# **Results and Discussion**

There was no regression adjustment for mycorrhizal colonization (Table 1). In the absence and at the dose of 50 mg kg<sup>-1</sup> P, the rate of AMF infection ranged from 75 to 85% and from 60 to 90%, respectively. This confirms greater mycorrhization when plants are subjected to conditions in which doses of P are below the optimal for development. When the dose of P of 100 mg kg<sup>-1</sup> was applied, greater amplitude in the colonization rate (30 at 70%) was observed. At higher doses, the colonization rate depends on the AMF. At this dose, 30% of roots were colonized by GA and 70% by GC.

This pattern was maintained at the doses of 200 and 400 mg kg<sup>-1</sup> P, with values between 45 to 75% and 40 to 75%, respectively. However, GM and GC presented lower amplitude in mycorrhization rates as the doses of P increased, obtaining 75% and 85% for the absence of P and 55% and 75% for the dose of 400 mg kg<sup>-1</sup> P, respectively. There are cases in which 5% of colonization was enough for good development (Karanika et al., 2008), and in other cases, high colonization did not have a positive effect (Trindade et al., 2003). Both fungal species EG and GE presented 75% of root colonization in the absence of P and 40% at the dose of P of 400 mg kg<sup>-1</sup>, respectively.

Root colonization in seedlings by GC was superior compared to GA at the dose of 100 mg kg<sup>-1</sup> P in the soil, with an increase of 133.33%. It was observed that an increase in the doses of P in the soil from 0 to 100 mg. kg<sup>-1</sup> reduced GA colonization.

The plant height of banana seedlings at 30 and 45 days was higher and linear when using GA compared to the other treatments (Figure 1a and 1b). Thus, these seedlings presented greater growth due to symbiosis with GA. However, at 60 and 75 days, the height was similar between seedlings inoculated with the different species (Figure 1c and 1d). At 45 DAP, plants inoculated with GM showed presented greater height compared to the control at the dose of 50 mg kg<sup>-1</sup> P in the soil (Figure 1b).

Inoculation with GA favored seedling height, showing linear growth at 30 and 45 DAP as a function of the doses of P (Figure 1a and 1b). In the subsequent seasons (60 and 75 DAP), there was adjustment of the quadratic regression, with the maximum heights of 26 and 28.5 cm being associated with the doses of 395 and 336 mg kg<sup>-1</sup> P, respectively (Figures 1c and 1d). At 75 DAP, seedlings inoculated with CE and GC also presented adjustment of the quadratic regression as a function of doses of P, with maximum the heights of 29.2 cm and 28.5 cm being associated with the doses of 310 and 296 mg kg<sup>-1</sup> P, respectively. Similar results were also found by Costa et al. (2001) and by Cavalcante et al. (2002) and Costa et al. (2005) with other fruit trees.

 Table 1. Mycorrhizal colonization of banana seedlings inoculated with arbuscular mycorrhizal fungi as a function of different phosphorus doses.

Inoculation <sup>1</sup>	Doses of P (mg kg <sup>-1</sup> )				
	0	50	100	200	400
SFMA	0 b	0 b	0 c	0 b	0 b
GM	75 a	60 a	60 ab	60 a	55 a
GA	75 a	65 a	30 bc	45 a	40 a
GC	85 a	90 a	70 a	75 a	75 a
CE	75 a	70 a	55 ab	45 a	40 a

Means followed by the same letter and lowercase letter in the column do not differ by Tukey's test at 5% probability. 1 NAMF: uninoculated; GM: G. margarita; GA: G.albida; GC: G.clarum; EC: C. etunicatum.

NAMF  $\hat{y} \triangleq = 8.12+0.06p-9.34x10^{-5}p^2 R^2 = 0.78$ GM  $\hat{y} = 9.39+0.08p-1.29x10^{-4}p^2 R^2 = 0.87$ GA  $\hat{y} = 8.96+0.03p R^2 = 0.76$ GC  $\hat{y} = ns$ 

CE  $\hat{y}$  = 8.24+0.06p-1.00x10<sup>-4</sup>p<sup>2</sup>.R<sup>2</sup> = 0.90

NAMFŷ ▲ = 9.06+0.07p-1.01x10<sup>-4</sup>p<sup>2</sup>.R<sup>2</sup>= 0.85 GM ŷ∎= 10.22+0.09p-1.58x10<sup>-4</sup>p<sup>2</sup>.R<sup>2</sup>= 0.88 GA ŷ•= 9.99+0.03p.R<sup>2</sup>= 0.77 GC ŷ×= ns

CE  $\hat{y}$ += 9.39+0.08p-1.24x10<sup>-4</sup>p<sup>2</sup>.R<sup>2</sup>= 0.86



**Figure 1.** Plant height at 30 DAP (a); 45 DAP(b); 60 DAP (c); and 75 DAP (d) of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.

Plants inoculated with GM presented a larger pseudostem diameter compared to uninoculated plants at a dose of 50 mg kg<sup>-1</sup> P in the soil at all periods measured (Figure 2), with increases of up to 48% at 30 DAP (Figure 2a). At the dose of 100 mg kg<sup>-1</sup> P in the soil, inoculation with GM at 30 DAP was superior in relation to the control and to inoculation with GA, with increases of 47 and 38%, respectively; A similar fact occurred at 45 DAP, when increases of 31 and 32%, respectively, were observed for the same AMF aforementioned (Figure 2b). These data are corroborated with Lins et al. (2003) and Trindade et al. (2003).

Pseudostem diameters in the treatment inoculated with GC did not present regression adjustment (Figure 2), as well as the control treatment at 45 DAP (Figure 2b). At 30 DAP (Figure 2a), only CE presented adjustment of the quadratic regression, with the maximum diameter of 23.44 mm being associated with the dose of 278 mg kg<sup>-1</sup> P in the soil.

At 45 DAP (Figure 2b), the inoculation with GM provided the largest diameter, 30mm, with the corresponding dose being relatively low compared to the other treatments, 268 mg kg<sup>-1</sup> P, according to

the regression adjustment. At the same period, plants inoculated with GA showed a linear increase in diameter as the doses of P increased, reaching 27 mm. This same diameter was obtained through inoculation with EC, but at a dose of 298 mg kg<sup>-1</sup> P.

The maximum diameters were obtained in the treatments with GM and GA at 60 DAP (Figure 2c), 35.6 and 36.6 mm, respectively, and at 75 DAP, approximately 42 mm for both (Figure 2d), with the average dose of 268 mg kg<sup>-1</sup> P in the GM treatment and 375 mg kg<sup>-1</sup> P in the GA treatment. Thus, the use of AMF, except for GC, favored the diameter of banana seedlings, with GM being highlighted.



**Figure 2.** Plant diameter at 30 DAP (a); 45 DAP(b); 60 DAP (c); and 75 DAP (d) of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.

Seedlings inoculated with GM and CE did not show regression adjustment for root dry matter (Figure 3a). In this variable, the maximum accumulation was obtained with GA (15.14 g) at a dose of P corresponding to 398 mg kg<sup>-1</sup>. For shoot dry matter (Figure 3b), treatments with AMFs presented increases compared to the control, maintaining a linear growth and reaching 16.15g. Inoculation with GA provided greater dry matter accumulation (19.71 g), presenting linear growth as the doses of P increased. Inoculation with GM, GC, and CE provided an accumulation of 17.9, 17.2, and 17.3 g of shoot dry matter with the calculated doses of 322; 358; and 304 mg kg<sup>-1</sup> P, respectively.

Seedlings inoculated with GA presented greater

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total dry matter accumulation (Figure 3c). GM, GC, CE, and control treatments presented total dry matter accumulation in the range of 29.41; 29.93; 28.27; and 27.62 g of dry matter with doses of 324; 359; 302; and 370 mg kg<sup>-1</sup> of P.

Plants inoculated with GA presented greater root, shoot, and total dry matter accumulation when subjected to the dose of 400 mg kg<sup>-1</sup> P in the soil, presenting increases of 34.14; 22.04; and 27.08%, respectively (Figure 3). At this same dose, mycorrhizal formation with GA also favored increases in root and total dry matter compared to inoculation with GM and CE, presenting increases of 33.1 and 50.2% and 21.63 and 33.75%, respectively (Figure 3a and 3c). GA only presented superior results for shoot dry matter compared to the treatment with CE, showing an increase of 23.19% (Figure 3b).

At a dose of 50 mg kg<sup>-1</sup> P in the soil, seedlings inoculated with GM and GA presented superior results for shoot dry matter (increases of 73.98 and 59.72%, respectively), while GM also presented superior results for total dry matter (increase of 62.97%), compared to uninoculated seedlings. The increase observed for root and total dry matter may be associated with the adaptive strategy of the plant to obtain inorganic P (IP) (Ferrol et al., 2018). According to these authors, as the rate of IP uptake by the roots is much higher than its diffusion rate in the soil solution, a PI depletion zone surrounding the root system is generated. Thus, in order to increase IP uptake, root branching, root hair length, and mycorrhizal associations may increase.



**Figure 3.** Root dry matter (a); shoot dry matter (b) and total dry matter (c) of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.

There was no regression adjustment for the root dry matter of seedlings inoculated with GM and CE (Figure 3a). For this variable, maximum accumulation was obtained with GA (15.14 g) at a dose of P corresponding to 398 mg kg<sup>-1</sup>. For shoot dry matter (Figure 3b), treatments with AMFs presented increases compared to the control, maintaining a linear increase and reaching 16.15 g. GA provided greater accumulation (19.71 g), with linear increase as the doses of P increased. GM, GC, and CE provided a shoot dry matter accumulation of 17.9; 17.2; and 17.3 g with the calculated doses of 322; 358; and 304 mg kg<sup>-1</sup> P, respectively.

For total dry matter (Figure 3c), regression adjustment was observed for all treatments in relation to the doses of P. Greater accumulation was observed in seedlings inoculated with GA. Seedlings inoculated with GM, GC, and CE and the control treatment presented dry matter accumulation of 29.41; 29.93; 28.27; and 27.62 g at the doses of 324; 359; 302; and 370 mg kg<sup>-1</sup> P. Thus, even under conditions of greater P availability, banana seedlings still need symbiosis with arbuscular mycorrhizal fungi to increase IP acquisition due to the formation of the depletion zone (Ferrol et al., 2018). Greater mycorrhizal dependence and efficiency were observed mainly at the lowest doses of P, with variation occurring as a function of the fungus inoculated and the doses of P (Figure 4). The genus *Gigaspora* predominates in soils with high acidity and low fertility. However, Silveira et al. (2003) observed that the genus *Gigaspora* was the most suitable to the variations in nutrient availability.



**Figure 4.** Mycorrhizal dependence (a) and mycorrhizal efficiency (b) in banana seedlings. GM: G. margarita; GA: G.albida; GC: G. clarum; EC: C. etunicatum.

Normally, high doses of P are considered inhibitory for the development of mycorrhizal association. However, this depends on the characteristics of the fungus and the host plant. In this study, it was evident that the level of inhibition depended on the AMF species, as well as that in commercial cultivation conditions, in which orchards are established after soil correction and fertilization, the use of species adapted to greater soil fertility, such as GA, can be a viable alternative.

The endophytes GA and EC presented inverse mycorrhizal dependence and efficiency, with the first inoculum being less efficient at the dose of 0 mg kg<sup>-1</sup> P and contributing positively at the dose of 400 mg kg<sup>-1</sup> P in the soil, while the second presented the inverse result. This means that plants inoculated with these fungi presented lower dry matter accumulation compared to the control. Thus, in the respective doses, these species did not find favorable conditions to provide benefits to banana seedlings, characterizing this interaction as parasitic. The lower response provided by CE may be related to increased doses of P, being also reported by Schiavo et al. (2010).

The N contents of the shoot of banana seedlings were not influenced by inoculation with AMFs, although decreasing as the doses of P increased, showing no regression adjustment (Figure 5a). According to George (2000), normally the N concentrations in the plant shoots are not influenced by the association with AMFs.

It was observed that both N and S contents decreased as the doses of P increased, which may have been related to the effect of dilution of nutrients in the plant. A plant with a larger structure, when subjected to the same conditions that other smaller plants are subjected, may present increased nutrient distribution in its dry matter. The contents of P in the plant tissue increased in response to the doses of P applied. There was no effect of inoculation with AMF, although inoculation with AMF presented P contents higher than the control at the dose of mg kg<sup>-1</sup> P, with an increase of up to 39%, corroborating the results found by Trindade et al. (2003).

Plants inoculated with GC showed an increase (18% and 17%, respectively) in K contents in relation to the control and GM at the dose of 100 mg kg<sup>-1</sup> P. Increases of 20.7 and 17.8% of the doses of K were observed in relation to the control when the plants were submitted to inoculation with CE at the doses of 0 and 200 mg kg<sup>-1</sup> P, respectively. Mg and Ca contents did not change with inoculation, showing a quadratic effect as the doses of P increased (Figure 6a and 6b). This null effect in relation to the AMF may be related to the soil correction with

limestone, inhibiting the absorption of these nutrients by the AMF.

In the Mg contents (Figure 6a), all inoculums with AMF as a function of doses of P, presented quadratic adjustment of the regression, with the maximum content being obtained for inoculation with GA, approximately 9.42 g kg<sup>-1</sup> with the dose of 259 kg<sup>-1</sup> P, while the treatment without AMF was not significant. All inoculums with AMF favored Mg contents. The same occurred for the Ca content (Figure 6b), although GM was not significant and GA provided a maximum concentration of 5.1 g kg<sup>-1</sup> with 257 mg kg<sup>-1</sup> P. On average, both Mg contents as Ca contents were favored by a dose of 256 mg kg<sup>-1</sup> P, reaching maximum foliar contents of 9.40 and 5.10 g kg<sup>-1</sup>, respectively (Figure 6a and 6b).



**Figure 5.** Nitrogen Content (a) of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.



**Figure 6.** Magnesium (a) and Calcium (b) contents of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.

N, P, K, Ca, Mg, and S contents were influenced by the doses of P (Figure 7a, 7b, 7c, 7d, 8a, and 8b), and only the N contents did not differ between inoculums with AMF (Figure 7a). These results show the importance of AMF for the absorption of nutrients other than P. N contents showed quadratic adjustment of the regression as a function of doses of P, obtaining maximum accumulation at the highest dose of P used with GA inoculation. On average, among the other treatments with AMFs, it is possible to obtain an accumulation of 673 mg plant<sup>-1</sup> with 318 mg kg<sup>-1</sup> P. N accumulation was affected by increasing P doses, with higher contents being obtained at high doses of P associated with the application of AMF. According to Clark & Zeto (2000), N is more absorbed in plants with mycorrhizal associations, although George (2000) reported the absence of this effect or even a decrease in the absorption of this nutrient.

P contents increased linearly in response to the doses of P (Figure 7b). In general, treatments presented values of increasing order according to the increase in the dose of P. Plants inoculated with GA presented a higher P content compared to the control and CE at the dose of 400 mg kg<sup>-1</sup> P, with increases of 22 and 19.7%, respectively. This increase can be attributed to the increase in the absorption surface of the roots associated with the mycorrhizal fungus and the increase in the soil volume explored.

Inoculation with CE favored the K content at the dose of 200 mg kg<sup>-1</sup> P, providing an increase of 36.5% compared to the treatment with GA (Figure 7c). Although it did not show significant differences, GA provided a K content increase of 20.2% compared to the control at the dose of 400 mg kg<sup>-1</sup> P. Although inoculation with GA presented an increase of 43.23%, it did not differ from the control for Ca contents (Figure 7d). Only GM was superior to the treatment with CE. Ca contents of Ca in the plant tissue increased linearly as a function of doses of P and inoculation with GA and CE.

Mg and S contents were influenced by inoculation of seedlings with GA at a dose of 400 mg kg<sup>-1</sup> P (Figure 8a and 8b). For Mg content, GA presented the highest increase (Figure 8a) in relation to the other inoculums with AMF. For the S content, GA presented higher accumulation only in relation to GM (Figure 8b). Mg and S contents of plants inoculated with GA, and only the S contents of the control and GM increased linearly as a function of the doses of P and AMF, thus showing the benefits of the application of P and the association with AMF.

For S contents (Figure 8b), CE provided increases of 49; 82.2; 68.8; and 41.7% at the dose of 200 mg kg<sup>-1</sup> in relation to the control treatments, GM, GA, and GC, respectively, showing promising results for inoculation at the dose of 200 mg kg<sup>-1</sup> P. Marschner (1995) evaluated the root morphology as the most important factor in the efficiency of P absorption. The main benefit that AMF attribute to the host is the increase in the absorption and translocation of nutrients, especially those with low mobility. Thus, better development of banana seedlings can be favored by the increase in nutrient accumulation (Leal et al., 2005).

Cu and Fe contents in micropropagated banana seedlings did not show regression adjustments according to the doses of P (Figure 9a and 9b). Inoculation with GA in plants submitted to the dose of 0 mg kg<sup>-1</sup> P increased the foliar contents of Cu. The same occurred in the treatment with GC at doses of 50 and 100 mg kg<sup>-1</sup> P. Treatments with GA and GC at doses of 0, 50, and 100 mg kg<sup>-1</sup> P maintained the banana seedlings with Cu contents above the minimum required by the culture, being greater than 6 mg kg<sup>-1</sup>.

Fe contents (Figure 9b) at the dose of 0 mg kg<sup>-1</sup> P

and without AMF were higher than the contents of other microbiological treatments, with significance compared to the treatment with EC. The increase of the dose of P from 0 to 400 mg kg<sup>-1</sup> in the substrate reduced Fe contents in the plant shoots by 53%.

Mn contents were influenced by the microbiological treatments and doses of P (Figure 9c). In general, there was a decrease in the contents of this micronutrient as the doses of P increased. Only the treatment with GC did not show regression adjustment for Mn contents. CE favored the increase in Mn concentrations when plants were subjected to doses of 50 and 100 mg kg<sup>-1</sup> P. However, the best fit (1773.08 mg kg<sup>-1</sup> Mn) was obtained at the calculated dose of 128.37 mg kg<sup>-1</sup> P.

P concentrations in the substrate had no influence on the foliar contents of Zn (Figure 9d). However, the symbiosis of plants with GA at a dose of 400 mg kg<sup>-1</sup> P favored Zn accumulation in leaf tissues, reaching contents close to the adequate in the plant shoots. The average increase in Zn was 87.2% in relation to other treatments.

B contents decreased as the dose of P increased (Figure 10a), presenting adjustment of the quadratic regression for the treatment without AMF (best fit of 6.75 mg kg<sup>-1</sup> B with the corresponding dosage of 331.35 mg kg<sup>-1</sup> P) and adjustment of the linear regression for the treatment with CE. Factors that are related to the inhibition of some elements due to excess or lack of others may be related to these results (Sousa & Lobato, 2004). At doses of 0 and 50 mg kg<sup>-1</sup> P, the highest B contents were obtained for the treatment without AMF, with a maximum increase of 53% compared to other microbiological treatments. At the dose of 400 mg kg<sup>-1</sup> P, the treatment with GM was superior to the others, with an increase of 87.5%. The microbiological treatment with GM maintained the foliar contents of B within the ideal range for the crop.

Microbiological treatments with arbuscular mycorrhizal fungi favored the foliar contents of Cu, Zn, Mn, and B and the Cu, Zn, Fe, and Mn contents in the banana seedlings, varying according to the doses of P applied (Figure 9; 10; 11). Although P is not classified among the macronutrients most absorbed by bananas (Guimarães et al., 2020), studies carried out in this crop with the inoculation of AMF have shown increases in P absorption, besides showing increases in the absorption of other nutrients (Leal et al., 2005).

The increase in the doses of P provided an increase in the Cu contents of the plants (Figure 10b), with adjustments of the quadratic regression in the treatments without AMF, GM, and CE, obtaining Increases of 375%,

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420%, and 488%, respectively. The adjusted mean dose of P is 311 mg kg<sup>-1</sup>, presenting a greater response to the Cu content in plants (0.106 mg plant<sup>-1</sup>). In the treatment with GA, Fe contents were directly influenced by the increasing P doses (Figure 11a), reaching a maximum dose of 400 mg kg<sup>-1</sup> P. In the treatment with GM, the highest content (1.55 mg plant<sup>-1</sup>) can be obtained with 270.5 mg kg<sup>-1</sup> P.

Uninoculated microorganisms (without AMF) and organisms inoculated with GM and CE favored the Mn contents in the presence of P application in the soil (Figure 11b). However, the greatest increase (26.02 mg plant<sup>-1</sup>) was obtained at the dose of 221 mg kg<sup>-1</sup> P for the treatment with CE. This same microbiological treatment favored greater Mn accumulation at the dose of 200 mg kg<sup>-1</sup> P. Zn contents within the microbiological treatments were only influenced by the NAMF and CE treatments in relation to the increase in the doses of P (Figure 11c). The highest content of this nutrient (0.246 mg plant<sup>-1</sup>), for both treatments, can be obtained at the average dose of 272 mg kg<sup>-1</sup> P, according to the regression adjustment presented. At the dose 400 mg kg<sup>-1</sup> P, the treatment with GA provided the greatest Zn increase (0.462 mg plant<sup>-1</sup>). For B contents, only the treatment with GM presented regression adjustment with the doses of P, obtaining the highest content (0.202 mg plant<sup>-1</sup>) at the dose of 305.8 mg kg<sup>-1</sup>. At the dose of 400 mg kg<sup>-1</sup> P, the treatment with GM presented the highest B content (0.194 mg plant<sup>-1</sup>), not differing only from the contents obtained in the treatment with GA.

The increases in micronutrients in treatments with AMF can be attributed to the increase in the absorption surface of roots associated with mycorrhizal fungi and the increase in the soil volume explored. The importance of using AMF in the production of banana seedlings is evident. The efficiency of the mycorrhizal association and its intensity vary due to the nutritional requirement of the crop, soil fertility, and interaction with other microorganisms.



**Figure 7.** Nitrogen (a); phosphorus (b); potassium (c); and calcium (d) contents of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.



**Figure 8.** Magnesium (a) and sulfur (b) contents of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.



**Figure 9.** Copper (a); iron (b); manganese (c) and zinc (d) contents of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.



**Figure 10.** Boron (a) and copper content (b) of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.



**Figure 11.** Iron (a); manganese (b); \inc (c); and boron (d) contents of the shoot of banana seedlings inoculated with AMF as a function of different doses of P. Different letters in the points differ by the Tukey's test at 5% probability. NAMF: uninoculated; GM: G. margarita; GA: G. albida; GC: G.clarum; CE: C. etunicatum; ns: not significant.

## Conclusions

The response of Musa spp. in association with arbuscular mycorrhizal fungi depends on the availability of phosphorus in the soil and the fungal species.

The production of seedlings associated with GM can favor the growth of plants subjected to low doses of

P applied to the soil.

The interaction of GC with Musa spp. in the production of seedlings does not seem to favor plant growth.

Mycorrhizal colonization together with phosphate fertilization contributed to the nutritional and agronomic

quality of banana seedlings.

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