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Soil and plant nutrition

Ecophysiology, quality, and mycorrhizal dependency in *Musa* spp. (cv. Grand naine) seedlings

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Abstract - The use of alternative technologies involving biological processes, with economic and ecological gains, is desirable for both the expansion of *Musa* spp. farming and the renovation of areas with low yields. Arbuscular mycorrhizal fungi (AMF) can stimulate plant growth, especially by increasing the absorption of phosphorus (P) and other nutrients. This study analyzes the influence of AMF on the growth and physiology of micropropagated *Musa* spp. plants submitted to doses of P. The experimental design was randomized blocks, in a 5 x 5 factorial arrangement, in which the factors were inoculation with AMF (*Glomus clarum*, *Gigaspora margarita*, *Gigaspora albida*, *Clareoideoglomus etunicatum*, and the control without AMF) and five doses of P (0, 50, 100, 200, and 400 mg kg^{-1}), with four replicates. The application of P doses increased growth in micropropagated *Musa* spp. seedlings, regardless of mycorrhizal inoculation. The highest rates of mycorrhizal colonization occurred at the lowest P doses, and the dose of 50 mg kg^{-1} P provided better conditions for mycorrhizal formation in all AMF species under study. The symbioses with AMF, as well as the use of P, increased photosynthesis rate, thus favoring the growth, development, and quality of *Musa* spp. seedlings. Species *G. clarum*, *C. etunicatum*, and *G. margarita* were the most promising for plant growth.
Index terms: mycorrhizae, soil microbiology, bananas, seedling production.

Ecofisiologia, qualidade e dependência micorrízica em mudas de *Musa* spp. cv. Grand naine

Resumo - O uso de tecnologias alternativas que envolvem processos biológicos, com ganhos econômicos e ecológicos, é desejável para a expansão da bananicultura e a renovação de áreas que apresentam baixas produtividades. Os fungos micorrízicos arbusculares (FMAs) têm a capacidade de estimular o crescimento das plantas, especialmente por meio do incremento na absorção do fósforo (P) e de outros nutrientes. Objetivou-se, neste trabalho, verificar a influência dos FMAs sobre o crescimento e a fisiologia de mudas micropropagadas de bananeira submetidas a doses de P. O delineamento experimental adotado foi o de blocos casualizados, em arranjo fatorial 5 x 5, sendo os fatores inoculação com os FMAs (*Glomus clarum*, *Gigaspora margarita*, *Gigaspora albida* e *Clareoideoglomus etunicatum* e o controle sem FMAs), e cinco doses de P (0; 50; 100; 200 e 400 mg kg^{-1}), com quatro repetições. A aplicação de doses de P proporcionou maior crescimento das mudas micropropagadas de bananeira, independentemente da inoculação micorrízica. As maiores taxas de colonização micorrízica foram observadas nas menores doses de P, sendo a dose de 50 mg kg^{-1} de P a que proporcionou melhores condições na formação de micorrizas para todas as espécies de FMAs avaliadas. As simbioses com FMAs e o uso de P proporcionaram aumento da taxa de fotossíntese das plantas, favorecendo o crescimento, o desenvolvimento e a qualidade de mudas de bananeira.
Termos para indexação: micorrizas, microbiologia do solo, banana, produção de mudas.

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Introduction

The search for new planting areas to expand the cultivation of banana (*Musa* spp.), as well as the renewal of areas with low yields, require the use of well-nourished seedlings, with high genetic and phytosanitary quality. In this context, the need for phosphate fertilizers also stands out, especially in Cerrado soils. With these requirements, it is desirable to use alternative technologies that involve biological processes, with economic and ecological gains, favoring crop establishment (GUIMARÃES et al., 2020).

The analysis of the benefit of arbuscular mycorrhizal fungi (AMF) in crops evidences their ability to stimulate plant growth. This occurs especially through the increase in nutrient absorption, mainly phosphorus (P), from the increase in root absorption surface (AUGÉ et al., 2016). This is due, among other factors, to the geometry of the hyphae, which favors their establishment in plant roots (smaller radius), thus covering a larger soil contact surface. Other factors include the kinetics of water and nutrient absorption, and chemical changes in the rhizosphere/hyphosphere that enable higher and better distribution of the absorption area (BUSCOT, 2015; SOUZA, 2015).

Phosphorus signals the establishment of symbiosis between AMF and plants, stimulating hyphae to colonize plant roots, especially when this nutrient is at lower concentration in the soil (BONFANTE and DESIRÒ, 2015; AUGÉ et al., 2016). Research shows the efficiency of AMF in the absorption of nutrients such as phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), and copper (Cu) (SOARES et al., 2012; LEHMANN et al., 2015; DEHGHANIAN et al., 2018).

The symbiosis effect between AMF and plants is predominantly nutritional. Therefore, three mechanisms associated with AMF stand out: increased nutrient mobility, enhanced absorption, and selective absorption (BONFANTE and DESIRÒ, 2015; LATEF et al., 2016; AUGÉ et al., 2016). Notwithstanding, the absorption of each mineral varies depending on soil conditions, host plant, and AMF species in symbiosis. Studies analyzing the effect of AMF on plants also show greater leaf water potential, stomatal conductance, transpiration, and photosynthetic rates. The effects of AMF on plant growth under water scarcity translate into greater root thickness and length, leaf area, and biomass accumulation (KUMAR et al., 2017).

Mycorrhization also favors plant physiology. Oliveira (2016) analyzed plants under salt stress and observed that unlike nonmycorrhizal plants, premycorrhizal plants showed no change in chlorophyll content, a fact already reported by other authors (OLIVEIRA, 2016). Arbuscular mycorrhizal fungi control the synthesis of chlorophyll under adverse conditions - especially salt stress - from the increased absorption of Mg by mycorrhizal plants (KUMAR et al., 2017).

Several AMF species are cataloged, but few studies address the effects and interactions of these microorganisms on plants (OLIVEIRA, 2016) aiming at their subsequent use in the field. Thus, due to lack of evidence, AMF lack host specificity.

The success of mycorrhizal inoculation depends on fungus-plant relationships, which must be carefully studied as AMF species act differently depending on host plant and environmental conditions (OLIVEIRA, 2016; KUMAR et al., 2017).

Thus, the present study investigated the influence of AMF and P doses on the growth, ecophysiology, and mycorrhizal dependence of micropropagated banana seedlings.

Materials and methods

The experiment used micropropagated seedlings of the banana cultivar Grand Naine, obtained from the National Center for Research in Cassava and Fruits of Cruz das Almas – CNPMF/EMBRABA, in Bahia State, Brazil. The experiment was conducted from February to June 2017 in a protected environment: a greenhouse covered with low-density polyethylene (LDPE) plastic film 150 microns thick, laterally protected with a black nylon screen providing 75% shading, without automated irrigation. The greenhouse is located in the Faculty of Agricultural Sciences, Federal University of Grande Dourados - UFGD, in Dourados city, Mato Grosso State, Brazil (22° 11’ 53.2” S latitude, 54° 56’ 02.3” W longitude, and 400 m altitude).

According to the Köppen classification, the climate of the region is Cfa type, humid subtropical (PEEL et al., 2007). The average annual air temperature is 22.9 ºC, with a monthly average minimum of 12.3 ºC in July, and a monthly average maximum of 31.7 ºC in January. The experimental design was randomized blocks, in a 5x5 factorial arrangement, with treatments composed of arbuscular mycorrhizal fungi species (*Glomus clarum*, *Gigaspora margarita*, *Gigaspora albida*, and *Clareoideoglomus etunicatum*) plus the control (without AMF) and P doses (0, 50, 100, 200, and 400 mg kg^{-1}), with four replicates. Each experimental unit consisted of a pot containing 7 L of substrate, with one plant per pot.

Arbuscular mycorrhizal fungi isolates were obtained from the collection of the Organic Matter and Soil Microbiology Laboratory at UEMS. The fungi were multiplied in pots with *Brachiaria decumbens*, in a substrate composed of soil and coarse sand at a ratio of 2:1 (v:v), which were mixed in a concrete mixer and sterilized in an autoclave at a temperature of 121 ºC for one hour. The pots were kept in a greenhouse for four months.

The experimental soil is a Dystroferric Red Latosol obtained from the subsurface horizon at a depth of 30 cm, in the soil resting area of the Faculty of Agricultural Sciences at UFGD. The soil has following chemical characteristics: hydrogen potential (pH_{H_2O}) = 5.20; $P_{Mehlich^{-1}}$ = 2.25 mg dm^{-3}; aluminum (Al^{+3}) = 14.40 $mmol_c.dm^{-3}$; hydrogen (H^+) + Al^{+3} = 26.40 $mmol_c.dm^{-3}$; K, Ca^{+2}, and Mg^{+2} = 0.50, 4.30, and 1.60 $mmol_c.dm^{-3}$, respectively; base saturation (V%) =19.53; aluminum saturation (m%) = 69.23.

The substrate was sterilized in an autoclave at 121 ºC for one hour, being then placed in the pots. After soil chemical analysis, the soil was corrected with filler limestone so as to increase base saturation to 70%. The base saturation method was used to calculate the need for soil correction (GUIMARÃES et al., 2020).

For limestone incorporation, the soil of each pot was placed along with limestone in 20-liter plastic bags, which were inflated with air and agitated uninterruptedly for one minute until complete mixture. The soil moisture of each pot was kept close to field capacity for a period of 30 days so that the limestone could react with the soil.

Fertilization was carried out by adding P doses (0, 50, 100, 200, and 400 mg kg^{-1} of soil) according to each treatment, using K_2HPO_4 (dipotassium phosphate) as a source. The increasing P doses made it necessary to balance K doses, which was achieved by using potassium chloride (KCl) (60% K_2O) as a source. In this process, the soil from each pot was again transferred to plastic bags, which were inflated with air and agitated until complete mixing with the minerals.

Inoculation was performed at planting with 50 cm^3 of an inoculum consisting of a mixture of soil and spores and roots of *Brachiaria decumbens* colonized with AMF, except in the control treatment. Planting was carried out by placing a banana seedling (average of 3 cm in height, with two fully open leaves) in each pot. The inoculum was placed under the seedling so that the roots were in contact with it. Nitrogen fertilization was carried out at 20, 70, and 120 days after planting, with 0.70 g N $plant^{-1}$ (GUIMARÃES et al., 2020), using urea as N source.

Seedling growth analysis consisted of periodic measurements of seedling height (cm) - considering the distance from the ground to the insertion of the newest fully opened leaf, with the aid of a measuring tape - and pseudostem diameter (mm) at neck height, with the aid of a digital caliper. These measurements were performed at 30, 60, 90, 120, and 150 days after planting (DAP). Seedling ecophysiology was assessed at 150 DAP, in which the following were evaluated: chlorophyll index, with the aid of a chlorophyll meter (model SPAD-502; Konica-Minolta, Tokyo, Japan); photosynthetic (A) and transpiration (E) rate; stomatal conductance (gs); internal CO_2 concentration (Ci); and water use efficiency - WUE (A/E), rubisco carboxylation efficiency (A/Ci), and intrinsic water use efficiency - iWUE (A/gs), using an infrared gas analyzer (IRGA), brand ADC, model LCi PRO (Analytical Development Co. Ltda, Hoddesdon, UK). The evaluations were performed between 8 and 11 a.m., on fully expanded leaves that were previously marked (second or third fully opened leaf) so that there was a pattern in the measurements.

At 150 DAP, the seedlings from each treatment were removed from the pots, and roots were separated from shoots. After washing, 2 cm-long subsamples of roots were collected and preserved in 50% ethanol for later determination of mycorrhizal colonization. Colonization was determined by the intersection method in reticulated Petri dishes (GIOVANNETTI; MOSSE, 1980), after staining the roots with methyl blue (KOSKE; GEMMA, 1989). Shoots and roots were dried separately in a forced ventilation oven at 65 ºC for 72 hours. The following were also evaluated:

- Dickson quality index (DQI), according to the equation proposed by Dickson et al. (1960):

DQI (%) = TDM / (H / SD + SDM / RDM);

- mycorrhizal dependence (MD), according to the equation proposed by Plenchette et al. (1983):

MD (%) = ((MDM – NDM) / MDM) x 100;

- mycorrhizal efficiency (ME), according to the equation proposed by Plenchette et al. (1983): ME (%) = ((MDM – NDM) / NDM) x 100. Where, TDM = total dry matter, H = shoot height, SD = stem diameter, SDM = shoot dry matter, RDM = root dry matter, MDM: dry matter of mycorrhizal seedlings, and NDM: dry matter of nonmycorrhizal seedlings. The data obtained were subjected to analysis of variance using the SISVAR software (FERREIRA et al., 2011), with the effect of AMF treatments being compared by the Tukey test at 5% probability, and the effects of P doses subjected to regression analysis (adjusted $R^2 > 0.7$).

Results and discussion

Arbuscular mycorrhizal fungi (AMF) interacted with P doses for mycorrhizal colonization (%) (Figure 1). The roots of plants without AMF inoculation did not show colonization. The highest rates of mycorrhizal colonization occurred at the lowest P doses (without application and with 50 mg kg^{-1}), with a linear decrease in treatments with *G. albida*, *C. etunicatum*, and *G. margarita*. These results corroborate studies that highlight that P regulates symbiosis between AMF and plants, and that colonization percentage correlates inversely with the plant's status regarding this nutrient (LEHMANN et al., 2015; DEHGHANIAN et al., 2018).

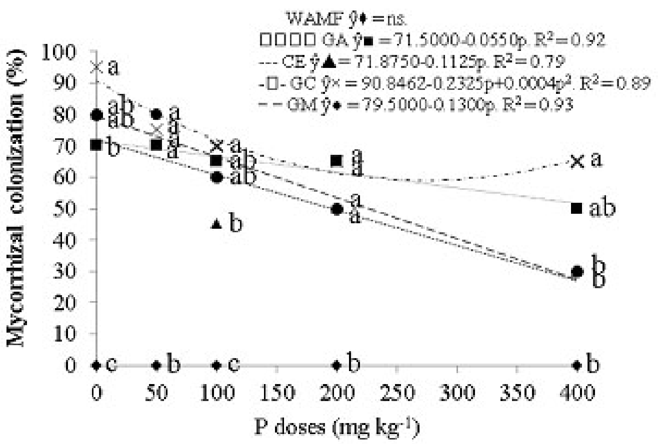

Figure 1. Mycorrhizal colonization (%) in banana seedlings inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus (P). Dourados-MS, UFGD, 2019. Different letters in the points represent the means that differ from each other by the Tukey test at 5% probability. WAMF: without inoculation; GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatum*; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

For *G. albida*, despite the small linear decrease, the colonization rate remained high, even with the increase in P doses (Figure 1). The same happened with *G. clarum*, with quadratic regression adjustment, in which more than 60% of the roots were colonized with the fungus up to the dose of 400 mg kg^{-1} P.

The increase in soil P levels can influence mycorrhizal formation by changing colonization rates in the host plant. Even with the decrease in colonization rate, the inoculated AMF species showed different performances regarding the capacity and dynamics of mycorrhizal colonization of plants depending on soil P supply. Observations indicate that the functional characteristics of each of the AMF species inoculated influence their association with the host and with the soil environment (DALANHOL et al., 2016; OULEDALI et al., 2018). These findings for different AMF species can favor the understanding of the complexity that drives interactions between plants, environments, and AMF, which is desired for the advancement of knowledge about mycorrhizae.

The interaction between AMF and P doses was significant for the growth traits plant height and pseudostem diameter throughout the evaluation period (Figures 2 and 3).

The analysis of plant height at 30 DAP shows that P did not influence the growth of plants with *G. clarum*. Up to that moment, this AMF species favored plant growth at the lowest doses of P, especially 50 mg kg^{-1} (Figure 2A). At 60 DAP, however, P doses influenced plant growth, with linear increase (Figure 2B). From that point onwards there was quadratic regression adjustment. At 150 DAP, the estimated dose of 377 mg kg^{-1} P provided the greatest plant growth, however, without differing from the control (Figures 2C, 2D, and 4E).

With the exception of *G. clarum* at 30 DAP, all microbiological treatments showed significant adjustments for P doses up to 150 DAP. From 90 DAP onwards, all microbiological treatments showed quadratic adjustment (Figure 2). Seedlings colonized by *C. etunicatum* and *G. margarita* show good growth with the lowest estimated doses (about 300 mg kg^{-1} P). However, according to the quadratic adjustment, the dose of 325 mg kg^{-1} P provides greater growth (27.8 cm) of banana seedlings in the proposed conditions.

At 60, 90, and 120 DAP, at a dose of 200 mg kg^{-1} P, the treatment with *G. albida* provided a lower height than treatments with the other fungal species. Species *C. etunicatum* provided higher plant height at the dose of 50 mg kg^{-1} P in relation to the control in the evaluations from 90 DAP onwards. The same behavior occurred for this species at the dose of 200 mg kg^{-1} P at 90 and 120 DAP. A higher plant growth also occurred at 150 DAP in treatments with *G. clarum* and *G. margarita* at the dose of 50 mg kg^{-1}, and in the symbiosis with *G. margarita* at the dose of 100 mg kg^{-1} P.

There was regression adjustment for pseudostem diameter as a function of times and microbiological treatments, with the exception of 30 DAP for the treatments with *G. albida*, *G. clarum*, and *G. margarita* (Figure 3). Plants in symbiosis with *G. clarum* were disadvantaged in pseudostem diameter at the dose of 400 mg kg^{-1} P up to 30 DAP in relation to the other AMF treatments (Figure 3A). At this dose, the symbioses did not favor seedling diameter up to 150 DAP (Figure 3).

This correlates mainly with the higher soil P levels. At the highest P dose (400 mg kg^{-1}), the AMF do not favor plant growth in any way.

In soils with high P contents, AMF species with evolved infectious mechanisms may for some reason not favor plants. In other words, these species are inefficient in exchange, not favoring the growth of the host plant (OULEDALI et al., 2018; LEHMANN et al., 2015; DEHGHANIAN et al., 2018).

At 60 DAP (Figure 3B), at the dose of 100 mg kg^{-1} P, plants colonized by *G. albida* had a larger pseudostem diameter than plants colonized by *G. clarum*, which had the lowest averages. Moreover, the symbiosis of *G. margarita* at the same dose, at 150 DAP, favored a larger pseudostem diameter in relation both to the control and to the treatment with *G. clarum* (Figure 3E).

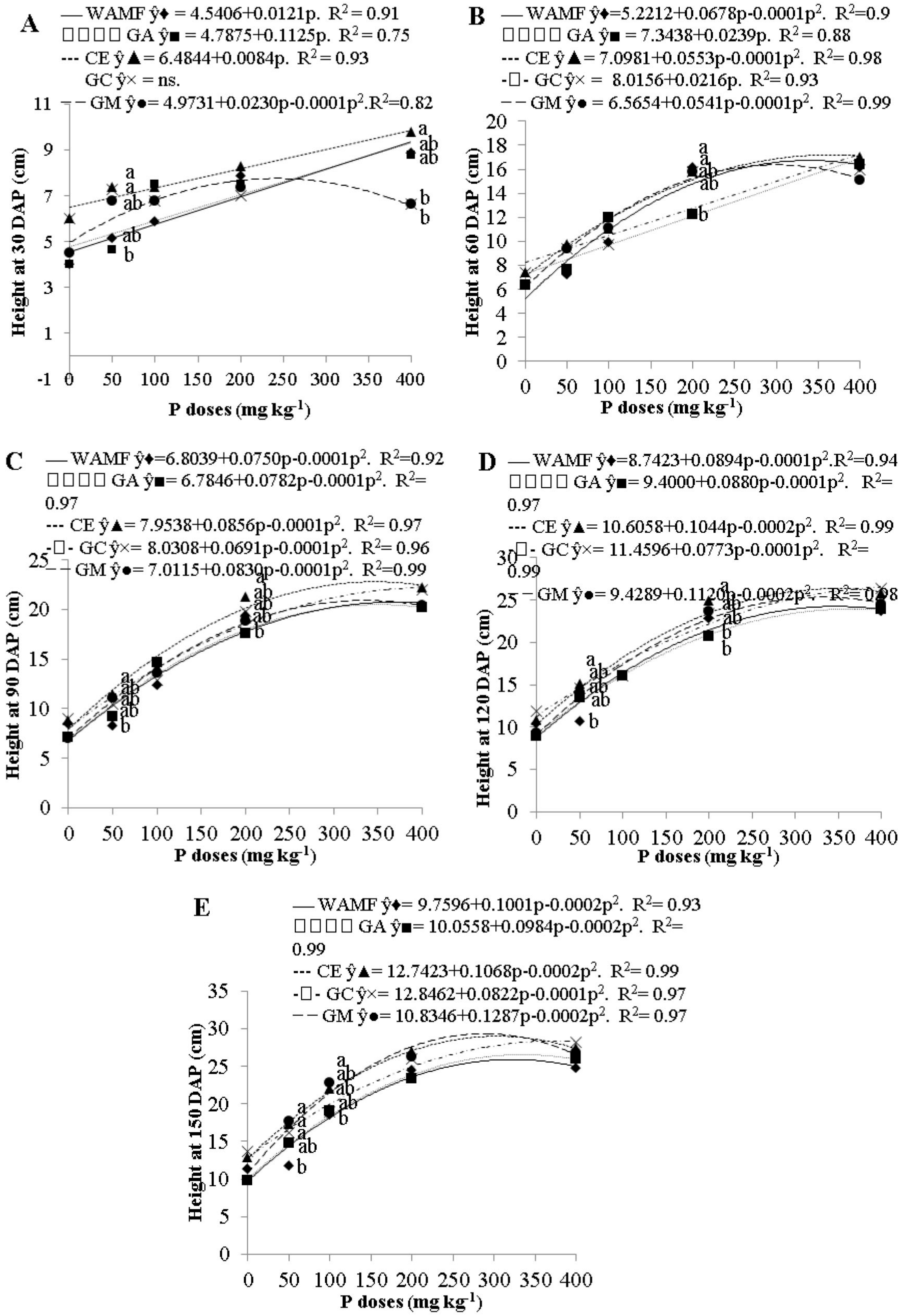

Figure 2. Height of banana seedlings (cm) at 30 days after planting (DAP) (A); 60 DAP (B); 90 DAP (C); 120 DAP (D); 150 DAP (E), inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus (P). Dourados-MS, UFGD, 2019. Different letters in the points represent the means that differ from each other by the Tukey test at 5% probability. WAMF: without inoculation; GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatum*; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

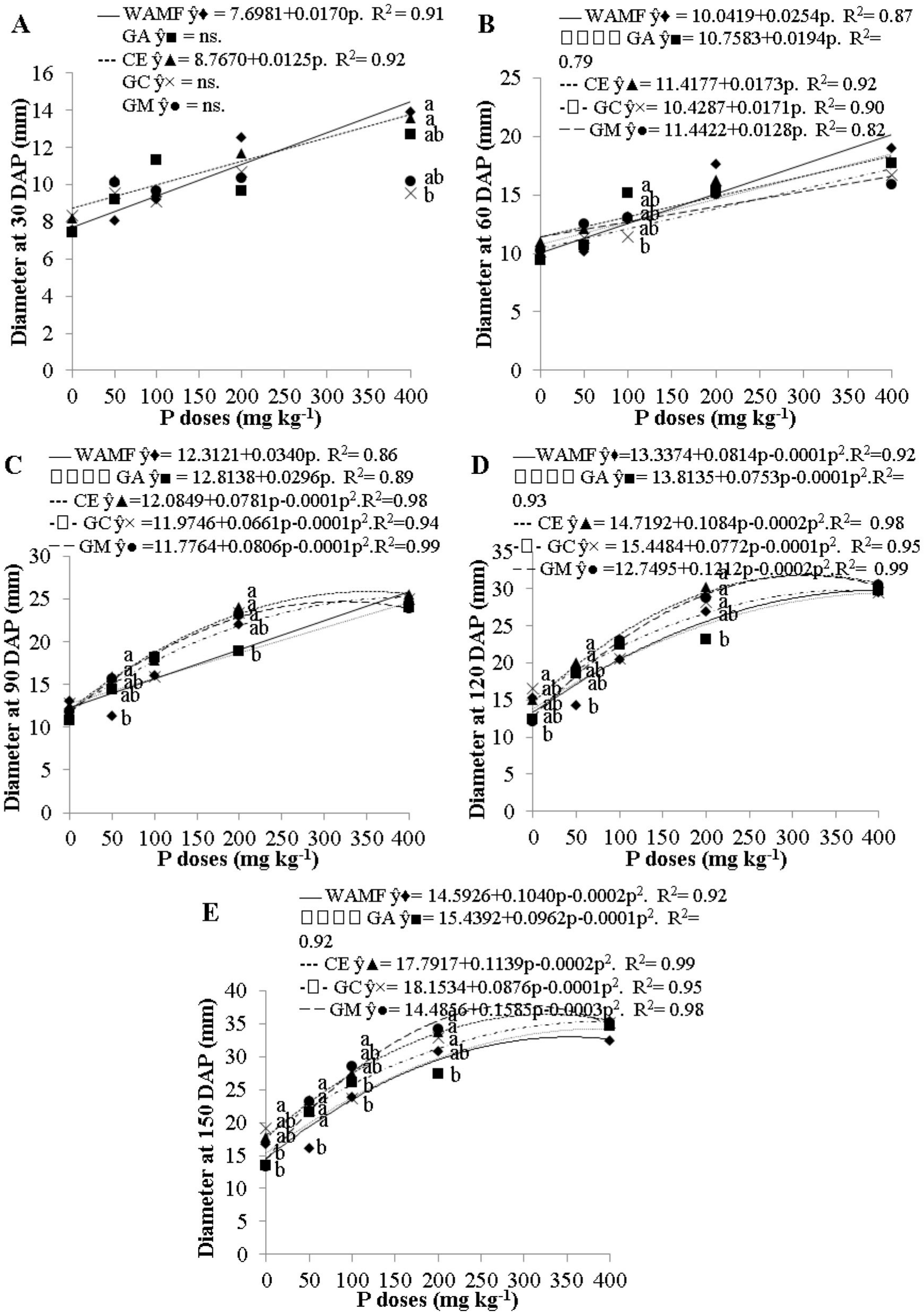

Figure 3. Diameter of the pseudostem of banana seedlings (mm) at 30 days after planting (DAP) (A); 60 DAP (B); 90 DAP (C); 120 DAP (D); 150 DAP (E), inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus (P). Dourados-MS, UFGD, 2019. Different letters in the points represent the means that differ from each other by the Tukey test at 5% probability. WAMF: without inoculation; GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatum*; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

Treatments with *C. etunicatum* favored pseudostem diameter at the doses of 50 and 200 mg kg^{-1} P in evaluations from 90 DAP, with increases of 44% and 7%, respectively, at 150 DAP (Figure 3C, 3E). Species *G. margarita* maintained similar symbiosis results at the same doses, at 90, 120, and 150 DAP (Figure 3C, 3D, 3E). At 150 DAP, in addition to providing greater increases in pseudostem diameter at the doses of 50 and 200 mg kg^{-1} P (43% and 11%, respectively), the treatment with *G. margarita* also favored the stem diameter of plants subjected to 100 mg kg^{-1} P, with a 20% increase.

Species *G. clarum* also showed efficiency in symbiosis for pseudostem diameter in relation to the control when submitted to the doses of 0, 50, and 200 mg kg^{-1} P, at 120 and 150 DAP, with an increase of 15%, 36%, and 7%, respectively, in the last assessment.

Symbiosis with *G. margarita* led to larger pseudostem diameters, reaching 37.82 mm with the lowest estimated P dose (294.5 mg kg^{-1}) according to the quadratic regression adjustment. Similar diameters were obtained by the regression adjustment, but with P doses above 320 mg kg^{-1} P; thus, a better response depends on soil P application.

Arbuscular mycorrhizal fungi can favor the diameter of banana seedlings. Species *G. albida* is less favorable in this regard, whereas species *G. clarum*, *C. etunicatum*, and *G. margarita* are more promising, showing a greater increase in seedling diameter. Seedlings with larger diameter have a better establishment and growth when taken to the field. Thus, seedlings with AMF can stand out when taken to the field, with a higher survival rate (OULEDALI et al., 2018).

For the variables root dry matter, shoot dry matter, and total dry matter of banana plants, AMF factors interacted significantly with P doses (Figure 4).

Plants in symbiosis with *G. clarum* maintained higher root dry matter at the dose of 200 mg kg^{-1} P, with an increase of 28% for this variable (Figure 4A). For this same species, the doses of 200 and 400 mg kg^{-1} P increased shoot dry matter by 10% and 8%, respectively (Figure 4B), and the dose of 200 mg kg^{-1} P increased total dry matter by 21% (Figure 4C).

Species *C. etunicatum* also favored shoot dry matter at the doses of 200 and 400 mg kg^{-1} P (Figure 4B), with an increase of 7% and 14%, respectively, in relation to the treatment with *G. albida*. For total dry matter, the dose of 50 mg kg^{-1} P stood out in relation to the control, with a 68% increase (Figure 4C).

Although only plants colonized with *C. etunicatum* showed significant differences in relation to the other AMF plants for total dry matter, at the dose of 50 mg kg^{-1} P, treatments with the other AMF species maintained increases above 18%.

Plant dry matter showed quadratic regression adjustment (Figure 4), regardless of microbiological treatments. However, plants associated with AMF needed lower P doses to increase their dry matter, with an increase of up to 15% in total dry matter and a reduction of 11% in P.

Low soil P availability can limit plant growth, in the same way that high doses can inhibit mycorrhizal development, which depends on mutualistic associations. The intensity of limitation depends on characteristics inherent to the fungal species and the host plant (PATERSON et al., 2016; SHELDRAKE et al., 2017). Corroborating the results of the present study, other studies mention variations in the responses of plants associated with AMF, which may occur as a function of the experimental conditions. When AMF species colonize the roots of a certain plant species, they increase their dry matter and shoot nutrient content (DALANHOL et al., 2016; OULEDALI et al., 2018). Reports also point to changes in nutrient content due to the inoculation of different AMF species; however, the occurrence of mycorrhizae does not always correlate with changes in plant dry matter.

Arbuscular mycorrhizal fungi and P doses had significant and positive effects on ecophysiological characteristics such as photosynthesis and chlorophyll index (Figure 5).

Plant transpiration rates did not vary as a function of microbiological treatments when evaluated within each dose (Figure 5A). The doses of 0, 50, and 100 mg kg^{-1} P led to the lowest transpiration rates.

For transpiration rate, P provided a quadratic regression response in the treatments with *G. albida* and *G. margarita*, and the estimated doses of 255 and 332 mg kg^{-1} P provided the highest rate, 4.04 and 4.77 mmol m^{-2} s^{-1}, respectively. In plants colonized with *C. etunicatum* and *G. clarum*, the increase in P doses increased transpiration rate.

Water-deficient plants subjected to high temperatures use stomatal closure control to increase soil water use efficiency. This and other studies make clear that phytotechnical variables related to plant growth generally correlate with transpiration (PINHEIRO et al., 2014; OULEDALI et al., 2018).

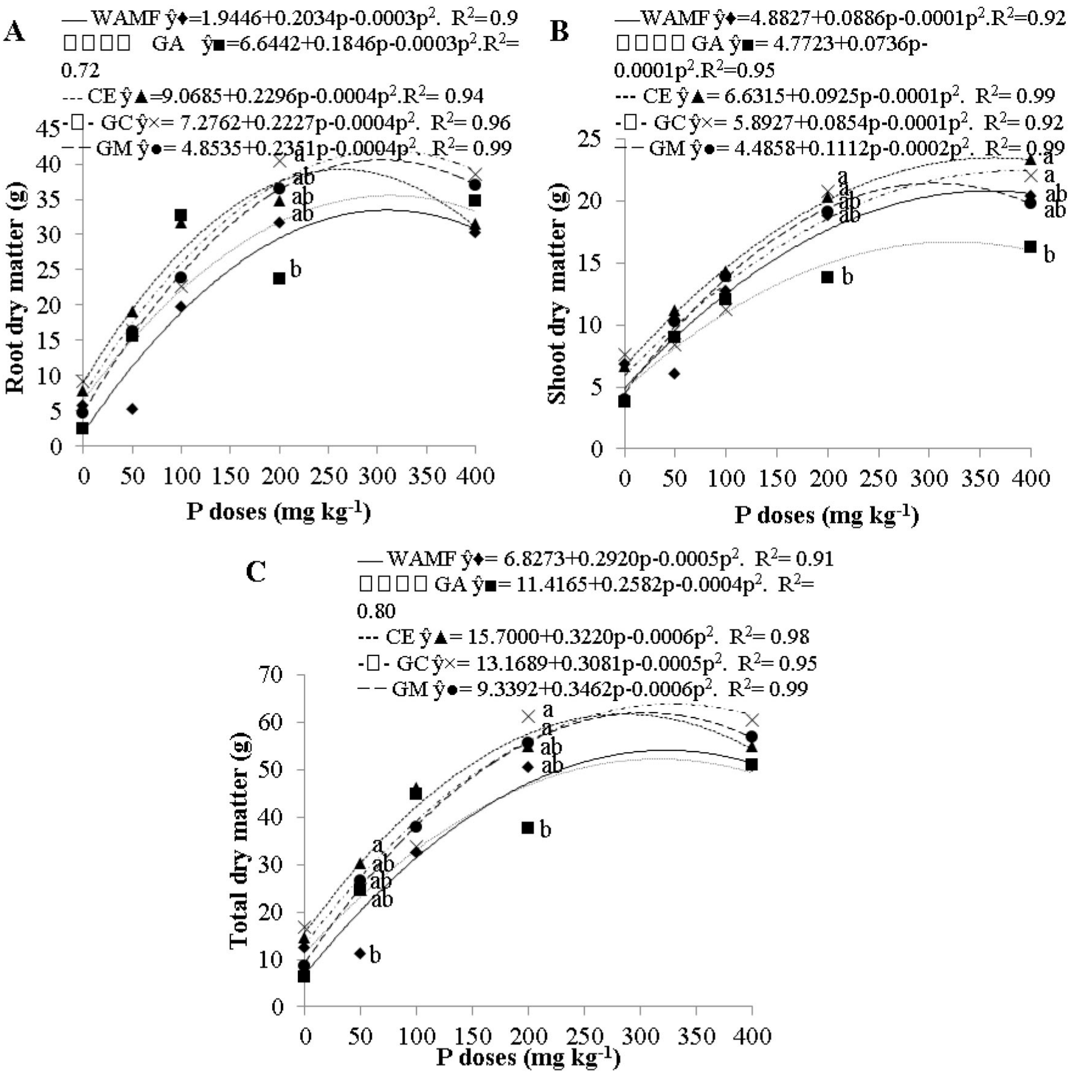

Figure 4. Root dry matter (g plant^{-1}) (A); shoot dry matter (g plant^{-1}) (B); and total dry matter (g plant^{-1}) (C) of banana seedlings inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus (P). Dourados-MS, UFGD, 2019. Different letters in the points represent the means that differ from each other by the Tukey test at 5% probability. WAMF: without inoculation; GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatu*m; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

Plants treated with 0 and 50 mg kg^{-1} P had a reduction in photosynthesis in relation to plants treated with the highest P doses within each microbiological treatment. Plants grown with *G. albida*, *C. etunicatum*, and *G. margarita* had a quadratic regression response, with higher photosynthesis rate, reaching 8.60, 9.62, and 10.90 µmol m^{-2} s^{-1} with the estimated doses of 252, 294, and 304 mg kg^{-1} P, respectively. In the treatment with *G. clarum*, the highest P dose was the one that increased photosynthesis the most, with linear growth. There was no regression adjustment for the control treatment, in which photosynthesis rate means remained lower than in the treatments with *G. margarita* at the doses of 100 and 400 mg kg^{-1} P. Therefore, the symbiosis with AMF and the use of P increase photosynthesis rate, favoring plant growth and development (Figure 5B).

The use of P as a consequence of mycorrhizal formation translates into an improvement in the physiological development of host plants, with an increase in photosynthesis rate (OLIVEIRA, 2015). The present study evidenced this fact for plants treated with *G. margarita* at the dose of 100 mg kg^{-1} P. In this symbiosis, the plant provides the fungus with sugars synthesized as a result of photosynthesis and, on the other hand, the fungus facilitates the acquisition of water and nutrients to the plant by increasing its absorption area (FARIAS et al., 2014; OLIVEIRA, 2015).

Microbiological treatments did not influence the stomatal conductance of plants (Figure 5C), although colonization with *G. margarita* increased it by 42% at the dose of 200 mg kg^{-1}, and by 35% at the dose of 400 mg kg^{-1} P in relation to the control. The use of P positively influenced stomatal conductance in microbiological treatments with *C. etunicatum* and *G. margarita*, indicating a favorable relationship of these species with P (AUGÉ et al., 2016).

The use of AMF positively influenced chlorophyll index at the doses of 0 and 50 mg kg^{-1} P (Figure 5D). In the treatment without P application, *G. clarum* increased the chlorophyll content of plants by 17%. At the dose 50 mg kg^{-1} P, the increase was slightly smaller, but positive: 8% for plants with *G. albida*, and 10% for plants with *C. etunicatum* and *G. margarita*. The development of symbiosis includes changes in the plasma membrane, more specifically in the periarbuscular membrane. This process favors the activity of aquaporins, which are plasma membrane proteins that participate in the absorption of water and solutes by the cell (WU et al., 2019; FRACASSO et al., 2020).

Other physiological characteristics under study, such as internal CO^2 concentration, rubisco carboxylation efficiency, intrinsic water use efficiency, and water use efficiency, did not change as a function of microbiological treatments and P doses, with an overall average of: 271.5 µmol mol^{-1}; 0.030 µmol µmol mol^{-1}; 62.47 µmol CO_2 mmol^{-1} H_2O; and 2.41 µmol CO_2 mmol^{-1} H_2O, respectively.

The Dickson quality index (IQD) tended to increase with the increase in soil P doses, with quadratic adjustment except for *G. albida* (Figure 6). At the dose of 100 mg kg^{-1} P, symbiosis with both *G. albida* and *C. etunicatum* was enough to maintain the average of seedling quality values equal to or higher than the average of seedlings from the control treatment at the doses of 200 and 400 mg kg^{-1} P.

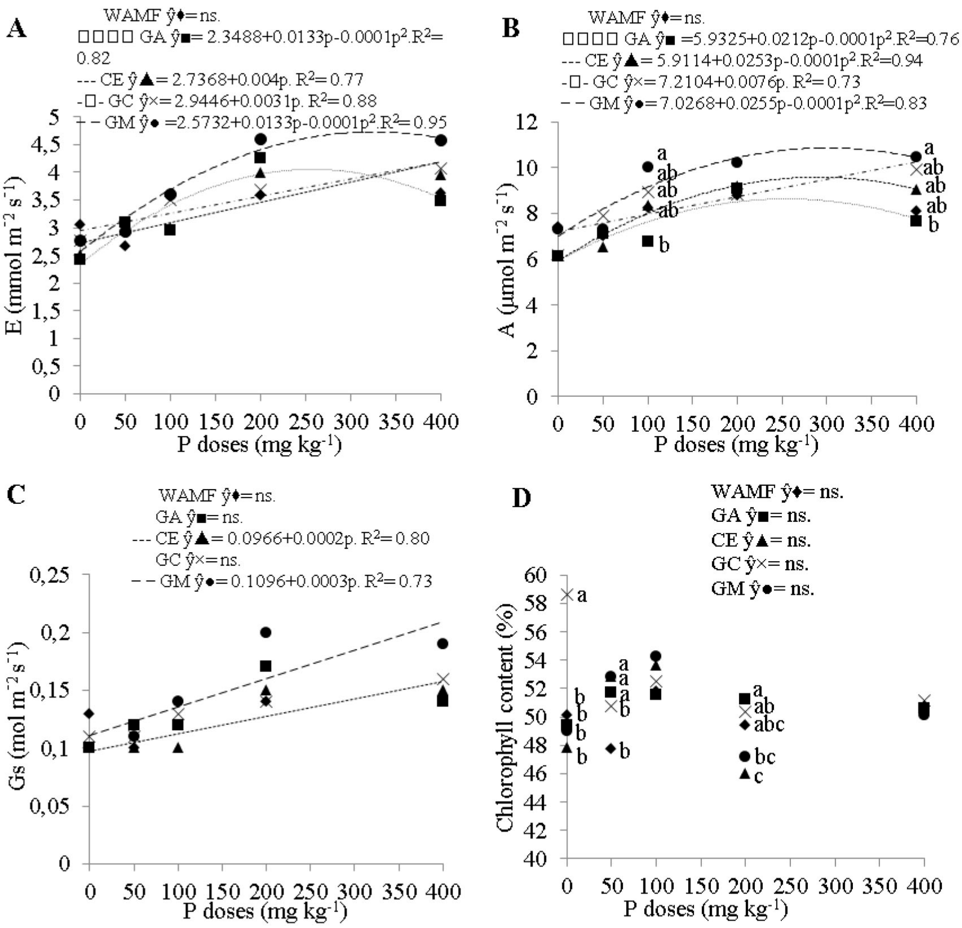

Figure 5. Ecophysiological characteristics: transpiration - E (mmol m^{-2} s^{-1}) (A); photosynthesis - A (µmol m^{-2} s^{-1}) (B); stomatal conductance - Gs (mol m^{-2} s^{-1}) (C); and chlorophyll content (%) (D) of banana seedlings inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus (P). Dourados-MS, UFGD, 2019. Different letters in the points represent the means that differ from each other by the Tukey test at 5% probability. WAMF: without inoculation; GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatum*; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

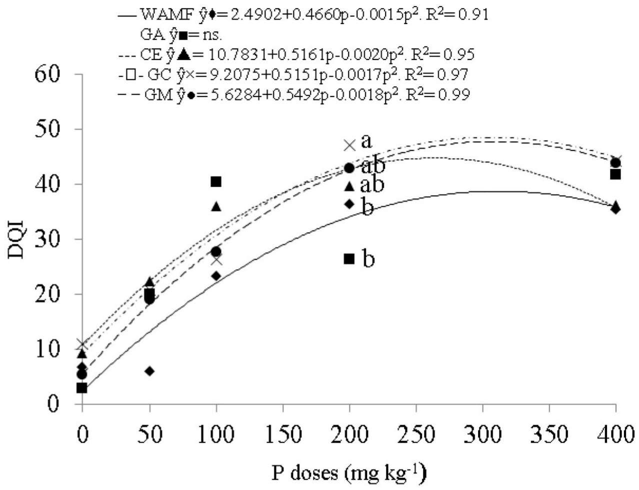

Figure 6. Dickson quality index in banana seedlings inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus. Dourados-MS, UFGD, 2019. Different letters in the points represent the means that differ from each other by the Tukey test at 5% probability. WAMF: without inoculation; GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatum*; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

Except for the treatments with *G. margarita* at dose 0 and *G. albida* at 0 and 200 mg kg^{-1} P, banana seedlings colonized by AMF maintained a higher mean DQI than control seedlings within each dose. For this variable, the conditions provided by the treatment with 50 mg kg^{-1} P stood out, where the symbiosis of the AMF species under study favored the appearance of more promising plants, with quality similar to that of control plants treated with 100 mg kg^{-1} P. At the dose of 200 mg kg^{-1} P, plants grown with *G. clarum* maintained a higher DQI, mainly in relation to the treatment with *G. albida*.

Symbiosis with AMF can increase plant growth by increasing root thickness and length, leaf area, biomass, water uptake, and macro- and micronutrient uptake through dissemination of hyphal networks and excretion of glomalin, external to the roots of plants (AUGÉ et al., 2016; OULEDALI et al., 2018).

A study with *Dioscorea alata* L. addressed the specific requirement of P and N (nitrogen) for biomass accumulation (SOUSA et al., 2018), with increasing linear QDI responses to different P doses, especially when accompanied by nitrogen fertilization (PINHO et al., 2019). Research shows that inoculation with AMF improve the uptake and ratio of N and P in plants, directly influencing their growth (AUGÉ et al., 2016; KANDHASAMY et al., 2020).

The present study showed greater mycorrhizal dependence and efficiency especially at the lowest P doses, with the degree of variation depending on the associated fungus and on P doses (Figure 7).

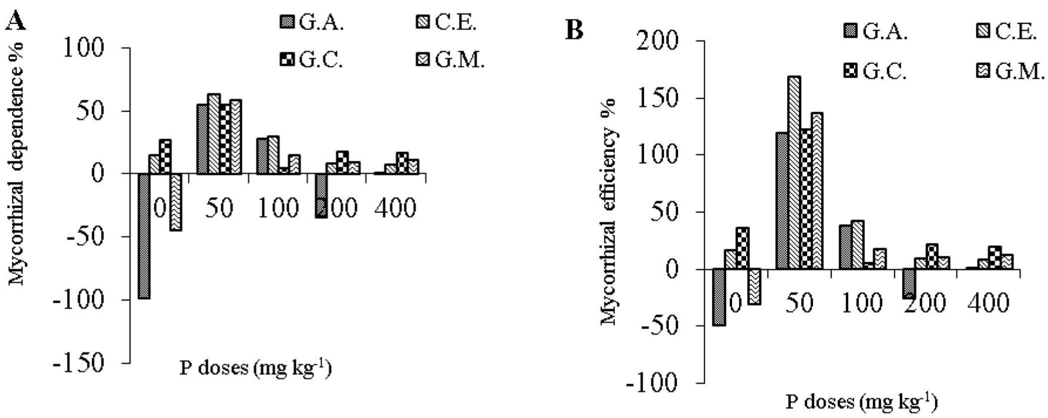

Figure 7. Mycorrhizal dependence (%) (A) and mycorrhizal efficiency (%) (B) in banana seedlings inoculated with arbuscular mycorrhizal fungi, under doses of phosphorus. Dourados-MS, UFGD, 2019. GA: *Gigaspora albida*; CE: *Claroideoglomus etunicatum*; CG: *Glomus clarum*; GM: *Gigaspora margarita*; ns: not significant.

The response in plant growth and mycorrhization, at different levels of soil fertility, depends on plant species. These differences can be genetically defined by the plant (dependence on mycorrhizae, or dependence on the interaction between fungus and plant genomes and edaphic conditions). Mycorrhizal efficiency, in turn, refers to responsiveness (OULEDALI et al., 2018).

The endophyte *G. albida* presented negative levels of mycorrhizal dependence and efficiency at the doses of 0 and 200 mg kg^{-1} P. The same occurred for the treatment with *G. margarita* at dose 0. This means that plants inoculated with these fungi showed lower dry matter accumulation than the control. Thus, in the respective doses, these species did not find suitable conditions to favor banana seedlings. This happened because these inocula are favored by the increase of soil P doses to more balanced levels, with application requirement (50 mg kg^{-1}, ideally) for optimal mycorrhization, with mutualistic symbiosis. Higher P levels reduce banana dependence on symbiosis and reduce the efficiency of mycorrhizal colonization.

The highest mycorrhizal dependence and efficiency occurred at the dose of 50 mg kg^{-1} P, which points to a positive effect of symbiosis with this level of P, favoring the growth and development of banana seedlings in relation to the control. Dalanhol et al. (2016) evaluated an AMF mixture in *Eugenia uniflora* seedlings and attributed the low efficiency of AMF to the high levels of P present in the substrates used. This also occurred in the present study at the doses of 200 and 400 mg kg^{-1} P. Overall, the dose of 50 mg kg^{-1} P provided better conditions for mycorrhizal formation for all AMF species under study.

Conclusions

The dose of 50 mg kg^{-1} P provided better conditions for mycorrhizal formation for all AMF species under study, with high mycorrhizal dependence and efficiency.

The effect of mycorrhizal formation on the agronomic and ecophysiological characteristics of plants depends on AMF species, nutrition, and host species.

Phosphorus application provides proper growth of banana seedlings in soil with low content of this element. The symbiosis with AMF and the use of P increase photosynthesis rate, favoring the growth, development, and quality of banana seedlings.

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