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Soils and plant nutrition

# Jatobazeiro seedlings associated with arbuscular mycorrhizal fungi

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**Abstract** - The symbiotic association between native fruit species with Arbuscular Mycorrhizal fungi (AMFs) can contribute to the growth and quality of seedlings in soils with low nutrient availability. Thus, the objective of this study was to evaluate the effects of inoculation with AMFs and phosphate fertilization on the growth and quality of jatobazeiro seedlings (Hymenaea courbaril L.). The experiment was conducted in a completely randomized design in a 5 x 5 factorial scheme. The treatments were constituted by inoculation with the AMFs: *Clareoideoglomus etunicatum*, *Rhizoglomus heterosporum*, *Rhizoglomus clarum*, MIX (mixture of inoculations) and without inoculation with the AMFs, associated with five P doses: 0, 60, 120, 180 and 240 mg kg<sup>-1</sup>, with growth evaluation at 30, 60, 90, 120 and 150 days after transplantation (DAT). At 150 DAT, the production of biomass, the quality of seedlings, the chlorophyll index, the dependence and mycorrhizal efficiency were determined. Phosphorus did not contribute to the jatobazeiro seedlings growth in the conditions of this study. Seedlings with *R. clarum* and MIX had the highest growth at 150 DAT, while seedlings with *R. clarum* also resulted in a greater biomass accumulation. The mycorrhizal dependence and efficiency was affected by phosphate fertilization, evidencing that jatobazeiro is a kind of optional mycorrhizal association.

**Index terms:** native fruit; *Hymenaea courbaril* L.; mycorrhizal efficiency; phosphate fertilization; seedlings quality.

## Mudas de jatobazeiro associadas a fungos micorrízicos arbusculares

Resumo - A associação simbiótica entre espécies frutíferas nativas com fungos Micorrízicos Arbusculares (FMAs) pode contribuir para o crescimento e a qualidade de mudas em solos com baixa disponibilidade de nutrientes. Desse modo, objetivou-se, com este trabalho, avaliar os efeitos da inoculação com FMAs e adubação fosfatada sobre o crescimento e a qualidade de mudas de jatobazeiro (Hymenaea courbaril L.). O experimento foi realizado em delineamento inteiramente casualizado, em esquema fatorial 5 x 5. Os tratamentos foram constituídos pela inoculação com os FMAs: Clareoideoglomus etunicatum, Rhizoglomus heterosporum, Rhizoglomus clarum, MIX (mistura dos inóculos) e sem inoculação com os FMAs, associados a cinco doses de P: 0; 60; 120; 180 e 240 mg kg<sup>-1</sup>, com avaliação de crescimento aos 30; 60; 90; 120 e 150 dias após o transplantio (DAT). Aos 150 DAT, determinaram-se a produção de biomassa, a qualidade das mudas, o índice de clorofila, a dependência e a eficiência micorrízica. O fósforo não contribuiu no crescimento das mudas de jatobazeiro, nas condições deste estudo. Mudas com R. clarum e MIX tiveram maior crescimento aos 150 DAT, enquanto as mudas com R. clarum também resultaram em maior acúmulo de biomassa. A dependência e a eficiência micorrízica foi afetada pela adubação fosfatada, evidenciando que o jatobazeiro é uma espécie de associação micorrízica facultativa. **Termos para indexação:** frutífera nativa; *Hymenaea courbaril* L.; eficiência micorrízica; adubação fosfatada; qualidade de mudas.

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#### Introduction

Jatobazeiro (*Hymenaea courbaril* L. Fabaceae) is a fruit tree species, native to the tropical regions and of natural occurrence of most states in (GONZAGA et al., 2016) Seasonal Semideciduous Forest. Although this species has a wide geographical distribution, it occurs at low population density (SILVA et al., 2014).

Due to its ability to develop in environments with different edaphoclimatic characteristics (CAMARGO et al., 2020), and because it is an important food source for wild animals and human consumption (LORENZI, 2020), the jatobazeiro can be used in reforestation programs for environmental recovery, agroforestry systems and afforestation (SILVA et al., 2017b, HEEMANN et al., 2018; COSTA et al., 2019; LORENZI, 2020). However, there are still incipient studies with technologies for a production model of jatobazeiro seedlings.

The accession of inoculation technology with arbuscular mycorrhizal fungi (AMFs) has been shown to be efficient in seedlings' production, especially of forest species (SANTOS et al., 2016; RODRIGUES et al., 2018), and can be used as an alternative tool for spreading of jatobazeiro seedlings. Among the advantages of using the AMFs, is the broad spectrum of colonization, since a plant species can be colonized by any kind of AMF (CAVALCANTE et al., 2009), having as main effect, the increase in plant growth in reflection of the greater absorption of nutrients. Especially those with lower mobility, such as phosphorus (P), thus presenting a stimulating effect on the development of plants (PEREIRA et al., 2018; SILVA et al., 2017a).

Generally the fruit and forest species have good response to mycorrhizal inoculation, and P absorption is the most significant effect (DALANHOL et al., 2017). P governs the effects of mycorrhizal symbiosis in the plants, and depending on the amount of available P, it can be of mutualistic, neutralistic or parasitic nature, besides acting directly on the metabolism and growth of plants (MOREIRA et al., 2019; SMITH et al., 2010).

In the literature, some species have responded positively regarding the association with AMF, such as *Jatrophas curcas* L.(OLIVEIRA et al., 2017), Australian cedar (SILVA et al., 2017c), cocoa tree (MENDES et al., 2020) and coffee plant (ARAÚJO et al., 2020). On the other hand, other species were not as responsive as "pitangueira" (DALANHOL et al., 2016), "timbaúba" (LEITE et al., 2017) and paricá (LIMA et al., 2020). Thus, for the use of this biotechnology tool as an efficient agricultural practice it is necessary to conduct studies that seek the best combination among AMF species, with plants of interest, since the AMFs have different efficacy, and each host plant species has variable mycorrhizal dependence (BERUDE et al., 2015).

Based on the above, the AMFs are promising for nursery production, since the use of mycorrhizal fungi inoculation can contribute to the quality and vigor of fruit seedlings, with rapid development, with high aggregate economic value, besides requiring fewer agricultural inputs. Thus, the objective was to evaluate the inoculation effects with arbuscular mycorrhizal fungi and phosphate fertilization on the growth and quality of jatobazeiro seedlings.

#### Material and Methods

The experiment was conducted in the period between May and December 2020, in a protected environment – covered greenhouse with low-density polyethylene (LDPE) transparent plastic, with 150 microns thick and laterally surrounded by structure coated with black nylon screen with 75% shade, without automated irrigation system, at the Agricultural Sciences School of Universidade Federal da Grande Dourados (UFGD) (22°11'53.2"S and 54°56'02.3"W, 400 m), in Dourados/MS. The climate of the region is type Am (Tropical Monsoon), with warm summers and dry winters (FIETZ et al., 2017).

Jatobazeiro seedlings were derived from fruits collected in the remaining area of Cerrado. After manual processing, the seeds were subjected to thermal scarification, to perform the dormancy overcoming and subsequently, were sown in plastic pots of 0.00029 m³ filled with commercial substrate Bioplant®. The trays were placed under a nursery with 70% of shading for 40 days, when the seedlings had a mean height of 15.0 cm. Soon after they were transplanted to pots with volumetric capacity of 0.007 m³.

The substrate used in the experiment was composed of a soil mixture - Oxisol of clay texture and thick sand at the ratio of 2:1 (v/v), with the following chemical attributes before the correction : pH (CaCl<sub>2</sub>)= 4.69; P= 5.57 mg dm<sup>-3</sup>; K= 0.10 cmol<sub>c</sub> dm<sup>-3</sup>; Ca= 2.91 cmol<sub>c</sub> dm<sup>-3</sup>; Mg= 0.85 cmol<sub>c</sub> dm<sup>-3</sup>; S= 7.65 mg dm<sup>-3</sup>; Al= 0.30 cmol<sub>c</sub> dm<sup>-3</sup>; H + Al= 4.44 cmol<sub>c</sub> dm<sup>-3</sup>; CEC= 8.30 cmol<sub>c</sub> dm<sup>-3</sup>; BS= 3.86 cmol<sub>c</sub> dm<sup>-3</sup>; organic matter= 9.79 g dm<sup>-3</sup>; Fe= 41.60 mg dm<sup>-3</sup>; B= 0.25 mg dm<sup>-3</sup>; Cu= 2.40 mg dm<sup>-3</sup>; Mn= 16.50 mg dm<sup>-3</sup>; Zn= 1.45 mg dm<sup>-3</sup>; bases saturation= 45.60.

The soil correction was performed, according to Raij (2011), it was applied 1 kg per m³ of hydrated lime with RPTN of 95%, in order to raise the base saturation (V %) to 65%. After the correction, the substrate was sterilized in autoclave for an hour, at a temperature of 121 °C and pressure of 1 atm, in order to ensure that only the mycorrhizal fungi of the inoculum will be present in the substrate. Soon after sterilization, the substrate was placed in plastic pots of 0.007 m³ capacity and taken to the greenhouse house.

The experimental design was completely randomized, and the treatments were arranged in a 5 x 5 factorial scheme, with subdivided plots in time for the growth variables and chlorophyll index. The treatments consisted of the combination of seedlings without AMFs inoculation (control) and inoculated with *Clareoideoglomus etunicatum*, *Rhizoglomus heterosporum*, *Rhizoglomus clarum* and MIX (mixture of the three AMFs species), associated with the combination of five doses of P: 0, 60, 120, 180 and 240 mg kg<sup>-1</sup> soil, with four replications. Each experimental unit was constituted by a pot containing 0.007 m<sup>3</sup> of substrate, with two seedlings per pot.

The phosphorus doses were established based on the study of Bassan et al. (2020), in which the source used was the  $\mathrm{KH_2PO_4}$  (dipotassium phosphate). Due to the increasing doses of P, it was necessary to balance the doses of K, with 0.49; 0.37; 0.25; 0.12 and 0.00 g dm<sup>-3</sup> of KCl, in the preparation of the substrate.

In the seedlings transplantation, the AMFs were inoculated in the substrate from the mixture of soil and colonized roots  $(5x10^{-5} \text{ m}^3 \text{ pot}^{-1} \text{ of inoculum})$ , at an approximate depth of 3 cm, near the seedlings root system (BRITO et al., 2017).

The irrigation was periodically performed according to the seedlings needs, to maintain 75% of the water retention capacity in the soil. At 112 days after transplantation nitrogenized fertilization was performed with 0.70 g of N plant<sup>-1</sup>, having urea as source.

Every 30 days, starting at 30 until 150 days after seedlings transplantation (DAT), the following morphological characteristics were evaluated: i) plant height - PH (cm), determined by the root neck distance until the highest leaf inflexion, with the aid of a measuring tape; ii) root neck diameter - RND (mm), inserted at 1.0 cm above the substrate level, with the use of digital caliper; iii) rate between height and neck diameter - HNDR, calculated through the formula: HNDR = (PH/RND). The chlorophyll index was also evaluated with SPAD-502 chlorophyll meter (Konica-Minolta, Tokyo, Japan), the evaluations were performed between 8 and 10 o'clock in the morning, always in the first pair of leaves, so that the measurements were always performed in the same leaves.

After 150 DAT, seedlings were removed from the pots, separating them in shoot (leaves + stems) and root system. The shoot and roots were weighed in a precision analytical scale (0.0001 g), for determining the shoot fresh mass – SFM and the root fresh mass – RFM. Subsequently, they were placed separately in Kraft® paper bags and submitted to drying in an air forced circulation greenhouse at  $60^{\circ} \pm 5$  for 72 hours to obtain the shoot dry mass - SDM; root dry mass - RDM; total plant dry mass - TDM, and the shoot and root ratio - SRR was calculated. The seedlings quality pattern – DQI was calculated according to Dickson et al. (1960).

The mycorrhizal dependence – MD and the mycorrhizal efficiency – ME were obtained from the data of the mycorrhizal seedlings dry masses (MDM) and the non-mycorrhizal seedlings dry masses (NDM), through the formulas: iv) MD (%) = (MDM - NDM) / MDM) x 100 and v) ME (%) = ((MDM - NDM) / NDM) x 100 (PLENCHETTE et al., 1983).

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All data were submitted to the Shapiro Wilks normality test (P < 0.05), and transformed when they did not show normal by the formula: ARCOSENO $\sqrt{x/100}$ . The data were submitted to the analysis of variance (ANOVA) and, when significant by the F test ( $P \le 0.05$ ), the means were compared by the Tukey test for AMFs and the regression analysis for phosphorus doses ( $P \le 0.05$ ), using the SISVAR software (FERREIRA, 2011). The growth characteristics and the chlorophyll index were analyzed in subdivided plots at time and, when significant (F test), the means were submitted to regression analysis ( $P \le 0.05$ ). The MD and ME results were presented in a descriptive way.

From the data of 150 DAT, it was determined the Pearson linear correlation ( $P \le 0.05$ ) and the cluster analysis by the most distant neighbor method (Euclidian distance), using the PAST 3.21 *software*.

#### Results and discussion

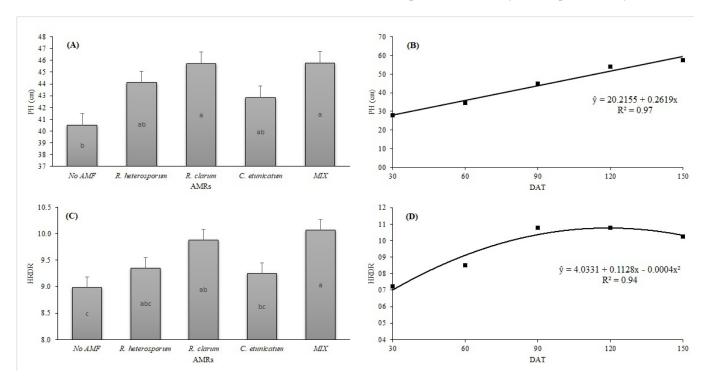
In general, there was no significant effect of the interaction among the P doses (phosphorus) and the arbuscular mycorrhizal fungi (AMFs) for the growth characteristics in jatobazeiro seedlings. Phosphate fertilization did not provide a significant effect on seedlings. There was an effect of the evaluation time interaction only with AMFs for the root neck diameter, for the other characteristics there was no interaction between the period and the other studied factors. The seedlings quality standard, represented by the DQI, root fresh mass and shoot / root ratio were also not influenced by phosphorus doses and for the inoculation with the AMFs.

Jatobazeiro seedlings did not answer the tested P doses, which may indicate that the doses applied to the soil were superior optimal for the initial development of this species. This result may be associated with the fact that the species is classified as climax, little demanding in nutrients and more adapted to acid and low fertility soils, therefore less responsive to fertilizers supply and consequently little affected by P availability in the soil (SANTOS et al., 2008; ALVES et al., 2015; SOARES et al., 2013).

In addition, the jatobazeiro has large seeds, which contributes to higher P content in the form of reserve compounds, which possibly contributed to meet the nutritional demands of this nutrient during the initial seedlings growth (FARIA et al., 2013; LACERDA et al., 2011). Thus, the content of 5.57 mg dm<sup>-3</sup> of P, present in the soil used in the experiment (before fertilization), which increased after soil correction, associated with P present in the seeds, as reserve compound, were possibly sufficient to meet the P needs, since the seedlings that did not receive phosphate fertilization, did not differ from fertilized seedlings, suggesting high phosphorus use efficiency in the initial growth phase.

The height of jatobazeiro seedlings was influenced by the AMFs species and evaluation time, isolated, and the seedlings cultivated with the MIX and *R. clarum* inoculums had higher means (45.77 and 45.73 cm,

respectively), differing statically from those without inoculation (Figure 1A). As for evaluation periods, the seedlings showed linear growth, with higher height (57.40 cm) at 150 DAT (Figure 1B). For the height / root neck diameter ratio (HRDR), the responses were similar to the PH for the AMFs, i.e., seedlings with MIX and R. clarum, had higher means (10.06 and 9.87 cm, respectively), differing statistically from the seedlings cultivated with C. etunicatum and those without AMF (Figure 1C). On the other hand, for the evaluation period, we observed the quadratic response, with maximum value (11.98) at 141 DAT (Figure 1D). The root neck diameter (RND) was adjusted to the linear model, with higher value at 150 DAT, especially in the seedlings with AMFs (Table 1), and this increase contributes in the transport of water and nutrients to the seedlings in initial formation. In addition, this increase in RND accompanied by PH at 150 DAT favored the morphometric stability, here represented by the HNDR.



**Figure 1.** Plant height - PH (A-B) and height/ root neck diameter ratio - HRDR (C-D) of jatobazeiro seedlings in response to inoculation with arbuscular mycorrhizal fungi (AMFs) and the days after transplantation (DAT). Equal letters do not differ among themselves, by the Tukey test (P > 0.05).

**Table 1.** Root neck diameter (mm) of jatobazeiro seedlings inoculated with arbuscular mycorrhizal fungi (AMFs), due to the days after transplantation (DAT).

Inoculum	DAT						Equation
	30	60	90	120	150	$\mathbb{R}^2$	
N/ AMF	3.75	3.85	4.16	4.97	5.55	L. 0.92	ŷ= 3.038+0.015*x
Rh	3.92	4.18	4.25	5.16	5.79	L, 0.90	$\hat{y}=3.251+0.015*x$
Rc	3.88	4.25	4.16	4.97	5.56	L, 0.88	$\hat{y}$ = 3.344+0.013*x
Се	3.97	4.07	4.12	5.03	5.82	L, 0.84	$\hat{y}$ = 3.212+0.015*x
MIX	3.94	4.00	4.15	5.01	5.48	L, 0.88	$\hat{y}$ = 3.290+0.013*x

Rh= R. heterosporum; Rc= R. clarum; Ce= C. etunicatum; MIX = inoculation mix; L= linear regression; \* (P < 0.05).

The seedlings reached higher height at 150 DAT (Figures 1B and D; Table 1), this late growth is associated with the fact that jatobazeiro presents slow vegetative growth, in the initial phase (CARVALHO, 2003). Growth characteristics of the shoot are usually considered for classification and selection of seedlings, and their period in the nursery, to be taken to the field (ABREU et al., 2020; ARAÚJO et al., 2017; BRACHTVOGEL; MALAVASI, 2010). In the study with jatobazeiro, the seedlings had better development at 150 DAT, since they had higher distribution of photoassimilates between the shoot and the roots, although studies in field conditions are suggested.

The SPAD chlorophyll index, which is based on the intensity of the green color, is essential to evaluate the photosynthetic efficiency of the mycorrhized seedlings. Although the AMFs may increase the fixed carbon (C) during photosynthesis, stimulating the photosynthetic rates (JESUS, 2020), in the jatobazeiro seedlings there was only response of the evaluation time, (Figure 2), presenting quadratic adjustment, and until 90 DAT, the maximum calculated value was 48 SPAD, and reduction after this period.

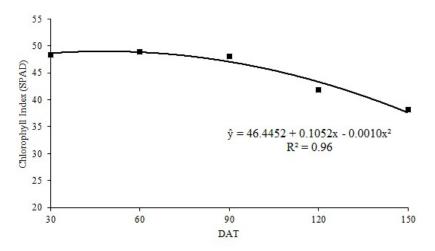


Figure 2. Green color index in jatobazeiro seedlings, in response to the days after transplantation (DAT).

Perhaps, the reduction in the SPAD values is associated with the fact that the plants presented in these later periods yellowish leaves, due to the senescence of some upper leaf limbs. It is noteworthy that the evaluations of these characteristics were always performed in the first pair of leaves, i.e., they were with yellowish coloration, and some seedlings had already lost at least one of their leaves. In addition, it is suggested that this result may be associated initially, with the lack of N after the seedlings transplantation. As a result, at 112 DAT fertilization was performed with N, but it was not a sufficient period for the seedlings to have increased the intensity of green coloration.

The shoot fresh mass (SFM) was influenced by the AMFs species (Figure 3A), and non-inoculated seedlings had the highest means (22.78 g), not statistically differing from the seedlings with *R. heterosporum*. The seedlings inoculated with *R. clarum* and *R. heterosporum* had higher average of shoot dry matter (8.72 and 8.55 g, respectively), and those with *R. clarum* also presented higher values of root dry mass (3.45 g) and total (12.27 g) (Figures 3B, C and D), demonstrating that these AMFs species favor the efficiency of the metabolism and production of photoassimilates.

The increase in the biomass is due to the efficiently absorption of soil nutrients by the mycorrhizal association compared to the non-inoculated ones (BERUDE et al., 2015). Similarly, Brito et al. (2017) observed higher biomass production in *Schizolobium parahyba* seedlings, when associated with *Rhizophagus clarus* and MIX. *Mimosa scabrella* and *Mimosa bimucronata* also had good results when they were inoculated with *R. clarus*, *R. irregulars*, *Acaulospora morrowiae*, *Acaulospora colombiana*, and *Dentiscutata heterogama* (STOFFEL et al., 2016).

Although the Dickson quality index (DQI) was not significant due to the studied factors, the seedlings had a general average of 0.87, indicating satisfactory results. Gomes and Paiva (2011) describe that in order for the seedlings to be considered of high quality, they should present DQI > 0.20. However, it is noteworthy that the values can vary according to several factors, for example: age of the seedlings, species, successional group, among others. Jatobazeiro seedlings had good quality standard, regardless of the use of phosphorus and AMF, suggesting physiological plasticity of this species to cultivation conditions.

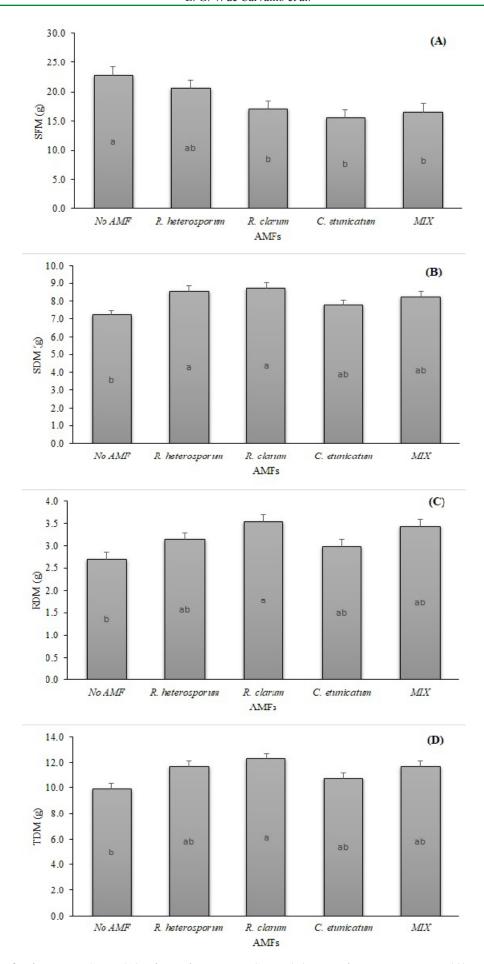
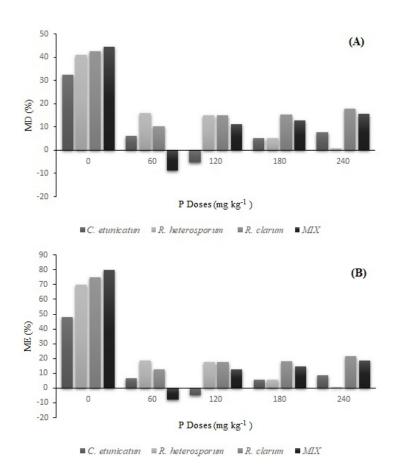


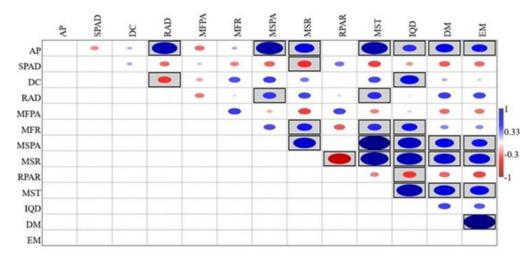
Figure 3. Shoot fresh mass – SFM (A), shoot dry mass – SDM (B), root dry mass – RDM (C) and total dry mass – TDM (D) of jatobazeiro seedlings in response to inoculation with arbuscular mycorrhizal fungi (AMFs). Equal letters do not differ among themselves, by the Tukey test (P > 0.05).

It can be observed positive and negative significant correlations of the Pearson correlation analysis, at all intensity levels (heat map) (Figure 5). The strong positive correlations (~1.0) occurred between: PH and NDR; PH and TDM; PH and MSD; MST and MSPA; EM and DM,

reinforcing that the increase in the growth characters favored the increase of photoassimilates, resulting in higher biomass production, and negative (~1.0) between: RPAR and MSR.



**Figure 4**. Mycorrhizal dependence – MD (A) and mycorrhizal efficiency – ME (B) in jatobazeiro seedlings, in response to inoculation with arbuscular mycorrhizal fungi and phosphorus doses.



**Figure 5**. Heat map of the bivariate Pearson correlation analysis among the evaluated characteristics in jatobazeiro seedlings, in response to inoculation with arbuscular mycorrhizal fungi and phosphorus doses.

The DQI, the MD and the ME presented intermediate level positive correlations (~0.50 to 0.33), with the PH, SDM, RDM and TDM, suggesting that even without significant difference, the correlation indicates that the AMFs participate in improvements of the jatobazeiro seedlings vigor.

The positive correlation between DQI with PH and RND is associated with the fact that they are characteristics that constitute seedlings quality, since they relate to the balance capacity, which correlated positively with the seedlings biomass. The DQI considers the biomass distribution balance in the plant with the growth of the shoot, and the higher its value, the greater its vigor, and it is a much employed evaluation in the nursery (MEDEIROS et al., 2018).

The mycorrhizal symbiosis can be of a mutualistic, neutralistic or parasite nature, depending on the species of AMF involved and the availability of P in the soil (SMITH et al., 2010). The establishment of mycorrhizal associations/colonizations should not be used as the sole parameter or efficiency guarantee, since certain AMFs species can be highly infective, but inefficient in providing nutrients to the plant, not favoring its growth.

The response in the plant growth and in the mycorrhization, at different soil fertility levels, is dependent on plant species. According to Ouledali (et al., 2018), these differences can be defined genetically by the plant species, being variables or dependent on the interaction between the genomes and by the soil conditions, by the response and mycorrhizal efficiency. Thus, it is essential to know the dependence and the mycorrhizal efficiency of the host plant, thus defining, to what extent the host plant will respond to the colonization with AMF and which are the species that will bring better results to the symbiosis, in a given condition (SCHIAVO et al., 2010).

Without P, the mycorrhizal dependence (MD) and mycorrhizal efficiency (ME) of the inoculated seedlings with C. etunicatum was 32% and 48%, respectively, occurring reduction up to 120 mg kg<sup>-1</sup> of P (Figures 4A and B), reflecting in -5% values for both characteristics. However, from 180 mg kg<sup>-1</sup> of P, the values increased, reaching up to 7% of MD and 8% of ME, cultivated with 240 mg kg<sup>-1</sup> of P.

The MD and the ME of the seedlings with MIX had similar responses to those with C. etunicatum. Without P, the MD and ME was 44 and 79%, respectively. With 60 mg kg<sup>-1</sup> of P, the MD and ME declined to -8%. From 120 mg kg<sup>-1</sup> of P, the MD and ME values increased, reaching up to 15% of MD and 18% of ME associated with the higher dose of P.

Jatobazeiro seedlings with *R. clarum* without P had MD of 42% and ME of 74%, which reduced with 60 mg kg<sup>-1</sup> of P, and increased with 120 mg kg<sup>-1</sup> of P, reaching up to 17% and 21% of MD and ME, respectively, with 240 mg kg<sup>-1</sup> of P. Already the seedlings with *R. heterosporum* 

had decreased MD and ME as increase in the doses of P, varying from 42% and 0.87% without and with 120 mg kg<sup>-1</sup> of P for the MD and from 70% to 0.88% for ME.

According to the MD category proposed by Habte and Manjunath (1991), the jatobazeiro seedlings had moderate MD (25 to 50%), without P for all AMFs species. Seedlings with *R. clarum* and *R. heterosporum* in the other doses of P were marginally dependent (MD< 25%). Com120 and 60 mg kg<sup>-1</sup> of P, seedlings with *C. etunicatum* and MIX, respectively, did not present responses to mycorrhizal inoculation. However, the increase of these doses the seedlings became marginally dependent, since the MD values increased (Figures 4A), suggesting that the effect of the mycorrhizal association in the jatobazeiro is affected by the phosphate fertilization in the conditions in which this study was performed.

The MD and ME negative values in the seedlings with *C. etunicatum* + 120 mg kg<sup>-1</sup> of P and MIX + 60 mg kg<sup>-1</sup> of P (Figure 4A and B), indicate that the seedlings had lower dry mass accumulation compared to those without AMF. According to Bassan et al. (2020), these responses indicate that the AMFs inoculums may not have found favorable conditions for their development and to benefit the seedlings.

Ramírez-Gil (2019), in their study with *Gmelina* arborea, also verified moderate MD in the low and moderate P conditions, when inoculated with *Rhizoglomus* fasciculatum, R. aggregatum and R. irregulare and marginal dependence when inoculated with *Glomus* fasciculatum and Entrophospora colombiana. In the high P condition (0.20 mg kg<sup>-1</sup>), all AMFs species inoculated in *Gmelina* arborea had negative MD values.

In a study with *Peltophorum dubium*, the AMFs *Glomus clarum* and *Gigaspora margarita* had high MD and ME with 60 and 120 mg kg<sup>-1</sup> of P (BASSAN et al., 2020), indicating that for facultative mycorrhizal association species, high P levels in the soil make the adversary mycorrhizal association to plant, as observed with the jatobazeiro, where the MD and ME decreased with the application of phosphate fertilization. Where in the condition of high P concentration (120 mg kg<sup>-1</sup>), the fungus continued demanding photoassimilates for its survival, while the plant, due to the moderate or facultative mycorrhizal dependence, was less benefited by the absorption of water and nutrients by the fungus.

In general, jatobazeiro seedlings had satisfactory ME values, especially those cultivated with the AMFs *R. heterosporum*, *R. clarum* and MIX, which promoted values higher than 70% without P (Figure 4B), reflecting in the biomass and height production.

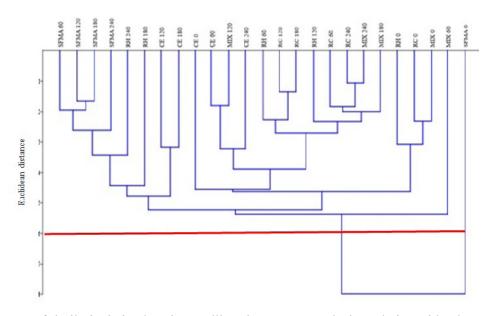
Thus, the jatobazeiro can be classified as a species of facultative mycorrhizal association. In the low P availability in the soil, the mycorrhizal association intensifies, however, when there are high P concentrations in the soil, the association decreases, since the plant already has sufficient P for its development (ROCHA et al., 2006; SOUZA et al., 2017).

The efficiency of the mycorrhizal to promote plants' growth, varies according to the AMF combination with the plant species, associated with biotic and abiotic factors of the system, such as the soil type and the use of phosphate fertilization, may present different degrees of efficiency, being even ineffective or temporary parasite (SMITH et al., 2010; FEITOSA; SANTOS, 2017; BASSAN et al., 2020).

In studies with *Toona ciliata*, Silva (et al., 2017a) observed that in the conditions of low P in the soil, the AMFs *C. etunicatum* and *A. colombiana*, had a high ME, while under high P availability (250 mg dm<sup>-1</sup>) these same inoculums had a reduction in the percentage of ME, around 20%, similar to those observed in this study. Indicating that even in extremely high P concentrations, some AMFs species, when associated with certain species, still promote benefits to the plant, even if reduced.

From the results observed with jatobazeiro, the importance of experiments that enable to know the mycorrhizal efficiency among different plant species and AMF are evident. Despite the lack of specificity, the mycorrhizal symbiosis depends on the genetic control, and this is performed by fungus and plant, as well as environmental conditions (DECLERCK et al., 1995; STOFFEL et al., 2016). It is noted that both the MD and the ME are relative and vary according to the AMFs species and the plant to be inoculated (ROCHA et al., 2006).

The dendrogram obtained from the multivariate analysis of the hierarchical grouping demonstrated that the tested treatments were divided into two groups, from a subjective cut at the distance 6 (Figure 6). The first group is composed of the treatments containing the AMFs inoculations, with and without phosphate fertilization, and by the treatments without inoculation with AMFs, but they received the phosphate fertilization, indicating that these treatments are similar. The second group was composed only by treatment in which there was no inoculation with AMFs and neither the realization of the phosphate fertilization.



**Figure 6**. Dendrogram of similarity in jatobazeiro seedlings in response to the inoculation with arbuscular mycorrhizal fungi (NAMF= no arbuscular mycorrhizal fungus; RH= *R. heterosporum*; RC= *R. clarum*; CE= *C. etunicatum*; MIX= inoculation mixture) and phosphorus doses (0, 60, 120, 180 and 240 mg kg<sup>-1</sup> soil).

Considering the grouping based on the evaluated characteristics, it is observed that the jatobazeiro seedlings without mycorrhizal inoculation, but received P fertilization, presented performance similar to the seedlings that were inoculated with the AMFs, independent of the phosphorus doses. This result is important, since with the inoculation of AMFs there is lower dependence on external inputs, in this case, the phosphate fertilization, reducing production costs.

Thus, it can be verified that the AMF *R. clarum* species and the inoculum mixture (MIX), were efficient in the growth promotion, while the *R. clarum* species also

provided higher biomass increment of the jatobazeiro seedlings. Based on the literature presented and before the experimental observations, it is recommended the use of the AMF *R. clarum* for the production of jatobazeiro seedlings, and that the minimum stay time of the seedlings in the nursery conditions, are around the 150 DAT. In future perspectives, it is suggested that other studies should be performed, such as quantification of the nutrient levels in the seedlings, besides testing other nutrients in the formation of jatobazeiro seedlings, even without soil correction.

#### **Conclusions**

Under the conditions in which this study was performed:

Jatobazeiro seedlings inoculated with AMF *Rhizoglomus clarum* had higher biomass production;

The phosphate fertilization did not contribute to the production of jatobazeiro seedlings;

Jatobazeiro seedlings had satisfactory mycorrhizal efficiency values for the tested AMFs species, and the species has optional mycorrhizal association.

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