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Artigos

Initial growth and ecophysiological aspects of forest legumes inoculated with mycorrhizal fungi in areas degraded by mining

Crescimento inicial e aspectos ecofisiológicos de leguminosas florestais inoculadas com fungos micorrízicos em áreas degradadas por mineração

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ABSTRACT

The objective of this work was to evaluate the growth in plant height, stem base diameter, survival rate, and ecophysiology of *Acacia mangium* and *Mimosa caesalpiniifolia* plants with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*) in areas degraded by mining in the municipality of Itaporanga D'Ajuda, SE, Brazil. The experiment was conducted with plants grown with spacing of 3 × 3 m in areas degraded by sand and gravel mining in Itaporanga D'Ajuda, using plants of the species *Acacia mangium* and *Mimosa caesalpiniifolia*. Four treatments were used: *Acacia mangium* without AMF; *Mimosa caesalpiniifolia* without AMF; *Acacia mangium* with AMF; *Mimosa caesalpiniifolia* with AMF. Plants from each treatment were evaluated for plant height, stem base diameter, survival rate and ecophysiological parameters at 12 months after planting. The forest legume *Mimosa caesalpiniifolia* inoculated with mycorrhizal fungi (*Rhizophagus clarus*) presented the best results in plant height, survival rate, and photosynthetic efficiency, at twelve months after planting. Plants in the treatment with *Acacia mangium* without inoculation with mycorrhizal fungi (*Rhizophagus clarus*) had the largest stem base diameters and second highest plant heights. However, the lowest survival rate of the plants may have caused overestimation of the results of stem base diameter and plant height.

Palavras-chave: Ecological restoration; Forest seedlings; *Rhizophagus clarus*

RESUMO

O objetivo deste estudo foi avaliar o crescimento em altura, diâmetro, a taxa de sobrevivência e a ecofisiologia de *Acacia mangium* e *Mimosa caesalpiniifolia* inoculadas com fungos micorrízicos arbusculares (*Rhizophagus clarus*) e sem inoculação em área degradada de mineração no município de Itaporanga D'Ajuda, SE. O experimento foi implantado no espaçamento de 3x3 m em uma área degradada por mineração de areia e cascalhos no município de Itaporanga D'Ajuda, SE com as espécies *Acacia mangium* (Acacia) e *Mimosa caesalpiniifolia* (Sabiá). Foram definidos quatro tratamentos: *Acacia mangium* sem FMA; *Mimosa caesalpiniifolia* sem FMA; *Acacia mangium* com FMA; *Mimosa caesalpiniifolia* com FMA. As plantas de cada tratamento foram avaliadas quanto à altura da planta, diâmetro da base do caule, taxa de sobrevivência e parâmetros ecofisiológicos aos 12 meses após o plantio, utilizando 5 plantas selecionadas ao acaso por tratamento. Doze meses após o plantio conclui-se: a leguminosa florestal *Mimosa caesalpiniifolia* inoculada com fungos micorrízicos *Rhizophagus clarus* apresentou os melhores resultados em altura, taxa de sobrevivência e eficiência fotossintética. A *Acacia mangium* sem inoculação com fungos micorrízicos *Rhizophagus clarus* teve o maior diâmetro e a segunda maior altura. Porém a menor taxa de sobrevivência pode ter superestimado os resultados de diâmetro e altura.

Keywords: Restauração ecológica; Mudas florestais; *Rhizophagus clarus*

1 INTRODUCTION

The use of soil recovery methods is useful to promote the recovery by restoration of terrestrial ecosystems. It can be developed through the planting of trees or shrubs using transplant techniques, direct sowing, hydroseeding, and promotion of natural regeneration through the seed bank stored and deposited on the soil. Currently, these are the most efficient methods for the recovering of forests in areas degraded by mining. Despite the large number of available methods, the planting of seedlings of native arboreal species is the most used, since the mined areas usually have a low natural resilience and the planting of seedlings ensures a higher initial control of the emerging plants in the site (MARTINS; LIMA; BARROS JUNIOR; AMORIM; OLIVEIRA; SCHWARTZ, 2020).

The application of arbuscular mycorrhizal fungi (AMF) to soils affected by mining provides a better root and arbuscular development of the vegetation and favors the survival and resistance of plants and mineral nutrition, mainly phosphorus, of arboreal species. AMF affects the plant growth and contributes to increase carbon

fixation and soil structuring (OLIVEIRA; SILVA; ALMEIDA; SAI; RAYMUNDO JUNIOR, 2011; BRAGHIROLLI; SGROTTI; PESCADORII; UHLMANNIII; STÜRMER, 2012).

The success of the initial establishment of seedlings of trees is related to the capture and use of primary resources, light and nutrients. The selection of arboreal heliophile species with high carbon assimilation potential and nutrient and light use efficient would facilitate the revegetation of degraded areas, mainly those with high irradiance and low soil nutrient availability (SANTOS JUNIOR; GONÇALVES; FELDPAUSCH, 2006). Heliophile and pioneer species with fast growth intercropped with slow-growth species are used in the initial processes of restoration and reforesting, the first assist in control and decrease soil erosive processes and the second present greater soil cover, which is effective to maintain soil physical characteristics (MACHADO; CONFESSOR; RODRIGUES, 2014).

Mimosa caesalpiniifolia is among the heliophiles species that can grow in gravel soils and has presented rusticity and resistance with a relatively fast and good development in its native region, the Northeast of Brazil (LORENZI, 2016). It is commonly used as a forage for feeding animals (caprine and ovine), live fence, and agroforestry projects in shrub and degraded areas, and as an alternative cover plant for areas used for economic purposes, due to its high potential for wood, stake, and log productions (LORENZI, 2016).

Acacia mangium Willd. is a heliophile and selective hygrophyte species from the Fabaceae family (LORENZI, 2016). Plants of this species are tolerant to open areas and are used for planting in degraded areas; in addition, they are plants that can fix nitrogen (LORENZI, 2016).

In this context, the objective of this work was to evaluate the growth in plant height, stem base diameter, survival rate, and ecophysiology of *Acacia mangium* Willd and *Mimosa caesalpiniifolia* Benth plants with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*) in areas degraded by mining in the municipality of Itaporanga D'Ajuda, SE, Brazil.

2 MATERIAL AND METHODS

The experiment was implemented in a degraded mining area in the Itália Farm in the municipality of Itaporanga D'Ajuda, state of Sergipe, Brazil (11°05'58.8"S and 37°15'57.7"W), with the planting of seedlings in September 2018. The climate of Itaporanga D'Ajuda is Am, tropical wet, according to the Köppen classification, presenting mean annual temperature of 25ºC, mean maximum annual temperature of 26ºC, and mean minimum annual temperature of 23ºC. The mean annual rainfall depth is 1200 mm, which is concentrated form April to August, and present low incidence from December to March.

The mining area was degraded for the removal of sand and gravel until 2007, when it was deactivated. This area presented plain topography, no vegetation, and soils with no horizons, only a C layer (subsoil). The chemical analysis of this substrate showed the following characteristics: pH (in water) = 5.19; P and K = 1.40 and 3.10 mg dm^{-3}, respectively; Ca + Mg and Al = 0.38 and 0.31 cmolc dm^{-3}, respectively, and 2.02 g dm^{-3} of organic matter content. The texture was classified as sandy.

The seedlings were produced in a forest nursery of the Department of Forest Sciences of the Federal University of Sergipe. The seedlings were grown for 3 months in the nursery and then planted. The ground of the area was scarified and the places of pits were marked with a spacing of 3 × 3 m. Pits of 40 × 40 cm were manually opened and the planting was carried out. The control of cutter ants was done three months before the planting, after the planting, and over the twelve following months, using granulated baits and powdered ant killer application in the anthills.

The experiment was conducted using two heliophile and pioneer forest species for restoration of degraded areas by mining: *Acacia mangium* and *Mimosa caesalpiniifolia*, with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*). Thus, the treatments were *Acacia mangium* without AMF (AM), *Mimosa caesalpiniifolia* without AMF (MC), *Acacia mangium* with AMF (AM+AMF), and *Mimosa caesalpiniifolia* with AMF (MC+AMF).

The experiment was conducted in the field using a randomized block design. The planting was arranged in four blocks per treatment, with four treatments and 15 seedlings per block, totaling 60 plants per treatment. The inoculation was carried out using 5 g of inoculum of mycorrhizal fungi (*Rhizophagus clarus*) in the substrate of the seedlings. The inoculum was acquired from the company Embrapa Agrobiology, RJ, Brazil.

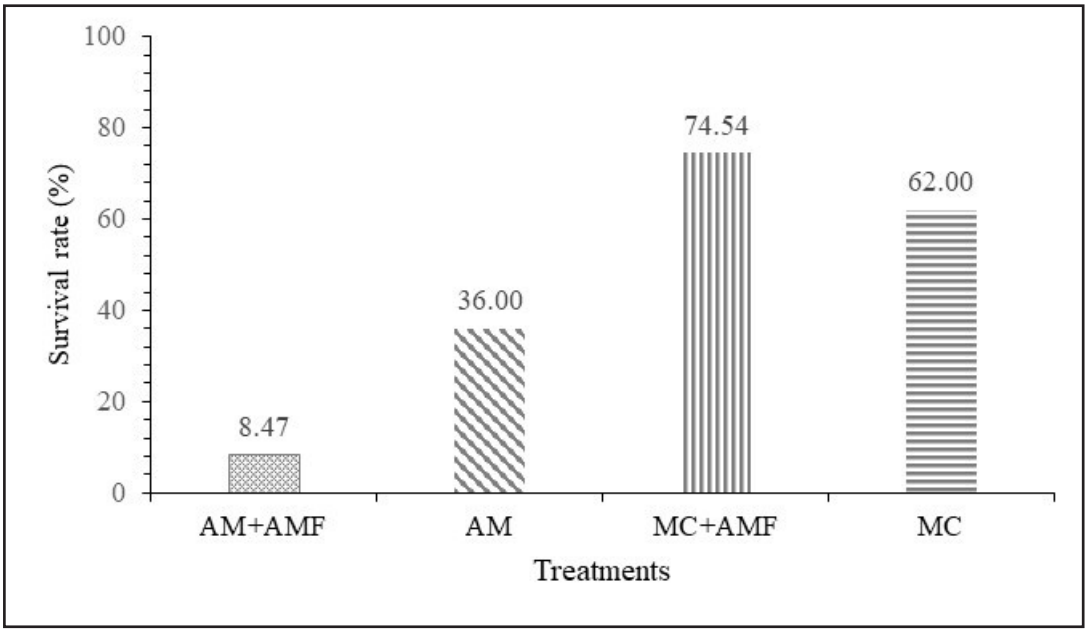
The seedlings were measured for plant height, stem base diameter, and survival rate at 12 months after planting in the field. The morphological parameters measured were total plant height (cm) from the stem base to the upper bud, measured using a tape ruler, and stem base diameter (mm), measured using a digital caliper (DELARMELINA; CALDEIRA; FARIA; GONÇALVES; ROCHA, 2014). The survival rate of the forest species used in the treatments was determined by comparing the total number of planted plants to the number of alive plants at 12 months after planting. The data of plant height and stem base diameter measured at 12 months were subjected to analysis of variance and significant means were compared by the Tukey's test at 5% significance level using the SIGMA PLOT 11.0 program.

The chlorophyll fluorescence was analyzed between 8:00h and 12:00h, considering fully expanded leaves, using a non-modulated fluorimeter (OS-30P; ADC BioScientific, Hoddesdon, UK). Five plants of each treatment were randomly selected in a completely randomized design and the following parameters were obtained at 12 months of planting: initial fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv) determined by the increase in F0 fluorescence to Fm, and maximum quantum efficiency of PSII [Fv/Fm=((Fm-F0)/Fm)]. The fluorescence data measured at 12 months were subjected to analysis of variance and significant means were compared by the Tukey's test at 5% significance level using the SIGMA PLOT 11.0 program.

3 RESULTS

The results found for survival rate showed that the treatments with the species *Mimosa caesalpiniifolia* (MC and MC+AMF) had higher survival rates than the treatments with the species *Acacia mangium* (AM and AM+AMF). The treatment MC+AMF presented higher survival rate than the treatment MC. The treatments with *Acacia mangium* presented very low survival rates (AM and AM+AMF) (Figure 1).

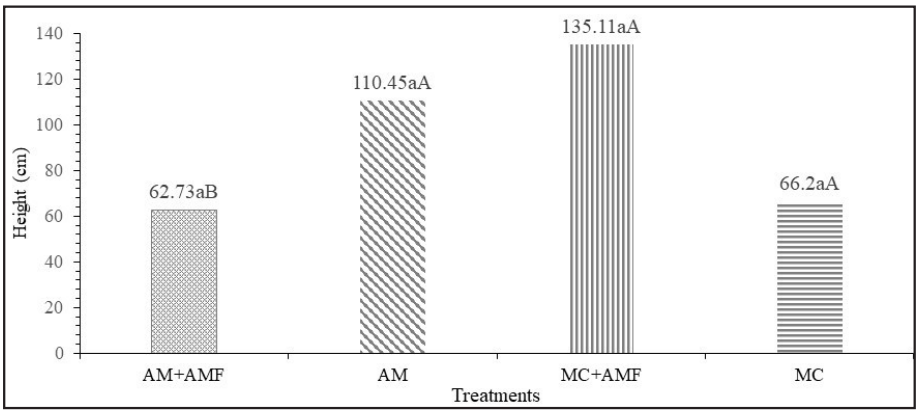
Figure 1 – Survival rate of plants of the *Mimosa caesalpiniifolia* and *Acacia mangium* species with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophaguss clarus*), at 12 months after planting



Source: Authors (2021)

The mean heights of the plants in each treatment are presented in Figure 2. The mean plant heights found were similar for plants with inoculation of each species, and for non-inoculated plants with *Rhizophagus clarus*. The comparison of heights of plants with and without inoculation with *Rhizophagus clarus* showed that MC+AMF had higher heights than AM+AMF (Figure 2).

Figure 2 – Mean height of *Mimosa caesalpiniifolia* and *Acacia mangium* plants with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*), at 12 months after planting

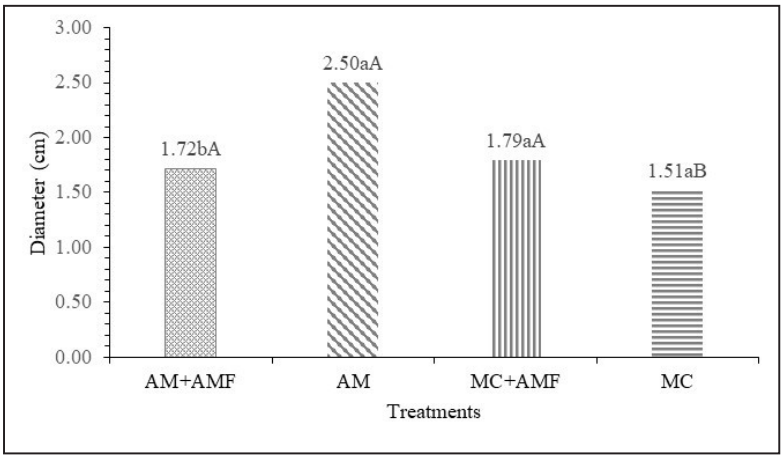


Source: Authors (2021)

In where: Means between species followed by same uppercase letter are not different by the Tukey's test at 5% significance level. Means in each species followed by the same lowercase letter are not different by the Tukey's test at 5% significance level.

The stem base diameter of each species with and without inoculation showed that AM had higher stem base diameter than AM+AMF. The treatment AM presented a higher mean stem base diameter than MC (Figure 3).

Figure 3 – Mean stem base diameter of *Mimosa caesalpiniifolia* and *Acacia mangium* plants with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*), at 12 months after planting



Source: Authors (2021)

In where: Means between species followed by same uppercase letter are not different by the Tukey's test at 5% significance level. Means in each species followed by the same lowercase letter are not different by the Tukey's test at 5% significance level.

The stem base diameter of plants in the treatment MC+AMF was not significantly different, presenting a higher mean for MC, denoting the effect of the symbiosis with *Rhizophagus clarus* on the development of the stem base (Figure 3). The highest Fm values were found for the treatments MC and MC+AMF, with highly significant difference from the treatments AM and AM+AMF (Table 1).

Table 1 – Parameters of chlorophyll fluorescence in forest species with and without inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*), at 12 months after planting in a degraded area by mining

	AM	**MC**	**MC+AM**	**MC**
F_m	491,0b	562,0a	464,6b	653,3a
F0	146,0b	160,0a	143,0b	163,6a
F_v	345,0c	402,0b	346,0c	489,6a
F_v/F_m	0,70b	0,71b	0,70b	0,75a

Source: Authors (2021)

In where: Means in each species followed by the same lowercase letter are not different by the Tukey's test at 5% significance level.

The treatments MC and MC+AMF presented the highest F0, significantly differing from the other treatments (Table 1). These results can be attributed to the action of phosphorus in the plant; this nutrient is a structural constituent of several components and products of the photosystems, such as NADP reductor, the initial dissipation component (F0).

The treatment MC+AMF presented significantly higher Fv than the other treatments (Table 1). The maximum quantum efficiency of photosystem II (PSII) (Fv/Fm) of the treatment MC+AMF was significantly higher than those of the other treatments (Table 1).

4 DISCUSSION

Several factors can affect the survival rate of plant species, including the soil nutrient availability, water stress, and air temperature. However, according to Dantas (2016), the determinant factor for the survival rate of a forest species is the capacity of adaptability and resilience of the plant species to the place in which it is grown.

The results of survival rate found for AM and AM+AMF were lower than those found by Dantas (2016) in a recovering degraded area, 72.2% after 5 years. Therefore, the higher survival rate of *Mimosa caesalpiniifolia* when compared to *Acacia mangium* may be due to the origin of the species. *Mimosa caesalpiniifolia* is native to Brazil and adapted to the edaphoclimatic conditions of the Northeast region of Brazil, whereas *Acacia mangium* is an exotic species native to Australia.

The mortality rate, plant height, and stem base diameter of the species was different and dependent on the degradation degree of the environment and resilience of the plants. Carneiro, Pereira, Araújo and Silva (2012) reported that plant species have different characteristics that are dependent on ecophysiological characteristics. Gomide, Santos, Siqueira and Soares (2009) and Schiavo (2010) reported that the inoculation of forest legumes with AMF improves their development due to the symbiosis between fungus hyphae and roots of the host plant, increasing the contact zone with soil particles and extraction of essential nutrients in the rhizosphere (P, K, Mg, Ca).

Plants in the treatment MC+AMF presented a better development, with higher plant heights at 12 months after planting than those in the other treatments. *Mimosa caesalpiniifolia* plants inoculated with *Rhizophagus clarus* have presented fast development, rusticity under adverse conditions, tolerance to gravel and degraded soils, and better nutrient absorption (SILVA, 2016, ATTIAS; SIQUEIRA; BERGALLO, 2013), which makes it ideal for the restoration of degraded areas by mining (COSTA; GAMA-RODRIGUES; ZAIA; GAMA-RODRIGUES, 2014).

The mean stem base diameter of plants in the treatment AM was higher than those found by Souza, Venturin, Grisi, Nogueira and Silva (2001), who evaluated the development of *Acacia mangium* with phosphate soil fertilizer application, at 12 months after planting in a degraded area by sand mining. Schiavo, Martins and Rodrigues (2010) evaluated a plantation of *Acacia mangium* for restoration of a of clay mining area, with and without inoculation with mycorrhizal fungi, and found

higher mean stem base diameter for non-inoculated plants, which presented 3.2 cm, contrasting with the 2.7 cm of inoculated plants, at 220 days after planting (SCHIAVO; MARTINS; RODRIGUES, 2010); these results were similar those found in the present study, but showed higher means. Zuba Junior, Sampaio, Pereira, Prates, Fernandes and Alvarenga (2010) evaluated an intercropping with pioneer species in a degraded area in Montes Claros, MG, Brazil, and found a mean stem base diameter of 4.0 cm at 12 months for *Acacia mangium*, which was higher than that found in the present study for AM and AM+AMF.

The presence of more adapted and competitive native mycorrhizal fungi in the area probably associated more easily with *Acacia mangium* plants without inoculation, improving the plant height and stem base diameter. Mendes, Chaves, Pontes Neto, Silva and Figueiredo (2013) evaluated a reforesting area with legumes in the Agreste region of the state of Pernambuco, Brazil, and found a mean stem base diameter of 1.49 cm and a mean plant height of 70.73 cm at 270 days for *Mimosa caesalpiniifolia* seedlings grown a disturbed ecosystem inoculated with *Rhizophagus clarus*; these results were lower than those found in the present study. This denotes the importance of inoculation with *Rhizophagus clarus* for the development of *Mimosa caesalpiniifolia* in degraded ecosystems, as in the present study.

Physiological variables (F0, Fm, and Fv/Fm) are used to identify possible changes in the photosystem II functioning when plants are subjected to biotic or abiotic stress (KALAJI; SCHANSKER; LADLE; GOLTSEV, 2014; GOTTARDINI; CRISTOFORI; CRISTOFOLINI; NALI, 2014). F0 refers to the minimum sign of fluorescence at the time that the quinones (Qa) of the reaction centers of the PSII are oxidated. The results showed higher F0 for plants in treatments MC and MC+AMF (Table 1). This result was possibly due to the Qa oxidation inefficiency or decreases in receptors of electrons (HAZRATI; TAHMASEBI-SARVESTANI; MODARRES-SANAVY; MOKHTASSI-BIDGOLI; NICOLA, 2016). However, F0 is related to possible environmental changes, especially light.

Fm represents the energy lost by electrons to reach the Qa (first stable receptor of electrons). It is measured by the use of a light pulse on leaves previously adapted to the dark (VIEIRA; PORTES; STACCIARINI-SERAPHIN; TEIXEIRA, 2010; MURCHIE; LAWSON, 2013). Favorable conditions that increase Fm are, in general, expected, but this increase may be followed by a decrease in F0, which would increase the variable fluorescence (Fv), obtained by the Fm/Fv ratio (ECCO; SANTIAGO; RICHART; LIMA; BORSOI, 2017). The higher the Fv, the higher the plant's capacity in transfer energy of electrons ejected from molecules of pigments for the formation of NADPH, ATP, and Fdr and, consequently, the higher the CO_2 assimilation capacity in the biochemical stage of photosynthesis (BAKER, 2008).

The Fv to Fm ratio is the quantum maximum efficiency of the photosystem when reaction centers are opened (GORBE; CALATAYUD, 2012). The values of Fv/Fm are, in general, within 0.75 and 0.85 (SILVA; OLIVEIRA JÚNIOR; GONZAGA; SENA; MACIEL; FIAES; MATTOS; CARNELOSSI, 2019). Decreases in Fm/Fm denote the occurrence of damages by photoinhibition in plants due to stress (SILVA; MARTINS; MIRANDA NETO; DEMOLINARI; LOPES, 2016; HAZRATI; TAHMASEBI-SARVESTANI; MODARRES-SANAVY; MOKHTASSI-BIDGOLI; NICOLA, 2016).

MC+AMF was the only treatment than presented Fv/Fm values within the ideal range. This denotes that plants in treatment MC+AMF presented no damages to photosystem and are more efficient in capturing energy by opened reaction centers of the PSII than those in the other treatments (Table 1). The inoculation with arbuscular mycorrhizal fungi (*Rhizophagus clarus*) for *Mimosa caesalpiniifolia* plants increased phosphorus absorption, indicating that this inoculation increases the quantity of energy used by the plant to photochemical processes, such as CO_2 fixation, and decrease NADPH (ECCO; SANTIAGO; RICHART; LIMA; BORSOI, 2017). P participates in several metabolic processes, including the photosynthetic process, since it is involved in the production and consumption of ATP and NADPH molecule and activation of some essential enzymes to the photosynthetic process (SHUBHRA; GOSWAMI; MUNJAL, 2004).

The lower Fv/Fm found in the other treatments may be related to the low leaf P concentration, since the deficiency of this nutrient can cause stress due to high irradiance (SANTOS JUNIOR; GONÇALVES; FELDPAUSCH, 2006). A pronounced P deficiency may result in decreases in photochemical efficiency of the PSII.

5 CONCLUSIONS

Mimosa caesalpiniifolia plants inoculated with the mycorrhizal fungi *Rhizophagus clarus* presented to best results for plant height, survival rate, and photosynthetic efficiency.

Acacia mangium plants without inoculation with the mycorrhizal fungi *Rhizophagus clarus* had the highest stem base diameters and the second highest plant heights. However, the lowest survival rate found for these plants may have caused an overestimation of the results for stem base diameter and plant height.

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