SYMBIOTIC EFFICIENCY OF INOCULATION WITH NITROGEN-FIXING BACTERIA AND ARBUSCULAR MYCORRHIZAL FUNGI IN *Tachigali vulgaris* SEEDLINGS

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ABSTRACT – The present study aimed to evaluate the effect of inoculation with nitrogen-fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) on the development of *Tachigali vulgaris* seedlings under nursery conditions. The seedlings were produced in 1 kg bags on a substrate of sand and vermiculite (1:1), following a completely randomized experimental design in a 3 (NFB) x 2 (with and without AMF) factorial scheme with 3 additional control treatments consisting of: seedlings inoculated only with AMF (mycorrhizal control), non-inoculated seedlings fertilized with N (nitrogenized control) and without N (absolute control). The following variables were evaluated: height, stem diameter (SD), shoot dry mass (SDM), root dry mass (RDM) and nodule dry mass (NDM), P accumulation in the shoot, and root mycorrhizal colonization (RMC). Efficiency and effectiveness were calculated to evaluate the plant response to double inoculation. The treatments showed a significant effect for all variables, except for mycorrhizal colonization, nodule number, and dry mass, with the nitrogen treatment having the highest growth values. Plants submitted to double inoculation showed a higher accumulation of dry matter, height and SD, reaching a 124% higher RDM regarding the absolute control, 90% more SDM, and 207% more NDM regarding the seedlings inoculated only with rhizobia. The positive effect of double inoculation occurred regardless of the strain used. The results indicate that the joint inoculation of NFB and AMF was beneficial for the species, promoting its growth.

Keywords: Symbiosis, Biological nitrogen fixation, Recovery of degraded areas.

EFICIÊNCIA SIMBIÓTICA DA INOCULAÇÃO COM BACTÉRIAS FIXADORAS DE NITROGÊNIO E FUNGOS MICORRÍZICOS ARBUSCULARES EM MUDAS DE Tachigali vulgaris

RESUMO –O presente estudo teve como objetivo avaliar o efeito da inoculação com bactérias fixadoras de nitrogênio (BFNs) e fungos micorrízicos arbusculares (FMAs) no desenvolvimento de mudas de ***Tachigali vulgaris*** *em condições de viveiro. As mudas foram produzidas em sacos de 1 kg em substrato areia e vermiculita (1:1), seguindo delineamento experimental inteiramente casualizado, em esquema fatorial 3 (BFNs) x 2 (com e sem FMAs), com 3 tratamentos testemunhas adicionais consistindo de mudas inoculadas apenas com os FMAs (testemunha micorrizada), mudas não inoculadas fertilizadas com N (testemunha nitrogenada) ou sem N (testemunha absoluta). As seguintes variáveis foram avaliadas: altura, diâmetro a altura do colo (DAC), massa seca de parte aérea (MSPA), raízes (MSR) e nódulos (MSN), acúmulo de P na parte aérea e colonização micorrízica (CM) das raízes. Calculou-se a eficiência e a eficácia para avaliar a resposta das plantas à dupla inoculação. Os tratamentos demonstraram efeito significativo para todas as variáveis, com exceção de colonização micorrízica, número e massa de nódulos secos, sendo o tratamento nitrogenado o que apresentou os maiores valores de crescimento. Plantas submetidas à dupla inoculação apresentaram maior acúmulo de matéria seca, altura e DAC, alcançando MSR 124% maior em relação à testemunha absoluta, 90% mais MSPA*

e 207% mais MSN em relação às mudas inoculadas somente com rizóbio. O efeito positivo da dupla inoculação ocorreu independente da estirpe utilizada. Os resultados indicam que a inoculação conjunta de BFNs e FMAs foi benéfica para a espécie, promovendo o seu crescimento.

Palavras-Chave: Simbiose, Fixação biológica de nitrogênio, Recuperação de áreas degradadas.

1.INTRODUCTION

Several forest planting methodologies have been adopted to recover degraded areas, among which the use of nitrogenfixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) in mutualistic symbiosis with legumes is highlighted (Chaer et al., 2011). NFB can supply a plant with 60 to 100% of its nitrogen needs when in symbiosis, which can reach up to 600 kg of N ha^{-1} per year (Peoples et al., 2009; Döbereiner, 1984). Such an association offers economic and ecological advantages when dispensing with the use of nitrogen fertilizers, which imply in large consumption of fossil fuels to reach the necessary temperatures and pressure in order to be industrially produced by the Haber-Bosch process (Travis, 2017).

Another very common symbiotic association not only in the Leguminosae family is with AMF, which possess a most striking beneficial effect in increasing the absorption surface and extension of the root system, thereby enhancing the absorption of nutrients, especially those less mobile in the soil such as phosphorus, zinc and copper (Saggin-Junior and Silva, 2005). The results are more nourished, hydrated and vigorous plants which are more resistant to adverse environmental conditions and pathogens and pests, with differentiated competitiveness in the field (Wagg et al., 2011). This constitutes a relevant factor in the tropics where low fertility soils predominate (Smith and Smith, 2011).

AMF can promote a synergistic effect with NFB in tripartite symbiosis (fungus-plant-bacteria), resulting in greater legume growth when there is a triple association (Bournaud et al., 2018). In addition, the tripartite association has been recognized for its ability to bioremediate contaminated soils, enabling the growth of legumes which will assist by exuding degrading enzymes from persistent organic pollutants (Faria et al., 2017).

Selecting NFB and AMF strains which are symbiotically efficient is of fundamental importance for the recovery technology of degraded areas to be effective due to the fact that many tree legumes have high nodulation specificity (Marques et al., 2001; Chaer et al., 2011) and symbiotic compatibility with AMF (Pouyu-Rojas et al., 2006).

Double inoculation with mycorrhizal fungi and rhizobial strains has been successfully tested for several native forest legumes, however, the experiments in most studies which have evaluated the efficiency of this tripartite symbiosis are conducted in pots with soil in sterile conditions, with a minority of works which have evaluated this interaction in nursery or field conditions (Carvalho and Moreira, 2010; Marques et al., 2001; Patreze and Cordeiro, 2004; Moreira et al., 2010). The purpose of experiments in non-sterile conditions is to find out whether the inoculated microorganisms are natively competitive with those present in the soil and whether they are adapted to the physical, chemical and biological conditions of this soil or substrate (Moreira et al., 2010). The performance of tests in these conditions presents itself as a challenge to research in facing the great number of variables, which includes competition with other microorganisms present in organic or commercial substrates and which can inhibit the establishment and effectiveness of the inoculated microorganism (Freire et al., 2017; Tavares et al., 2016).

Among several forest legumes used in the reforestation of degraded areas, *Tachigali vulgaris* (subfamily Caesalpinioideae) is widely used to recover degraded areas due to its rapid growth, reaching 12 m at 5 years (Dias and Brienza Junior, 1993). It is commonly known as *tachi-do-campo* or tachi, and also has the synonym *Sclerolobium paniculatum*, and is associated with NFB (Faria et al., 1989) and AMF (Marinho et al., 2004), being considered a dependent arbuscular mycorrhizae species (Berbara et al., 2006; Telles et al., 1999). It is classified as initial secondary, is endemic to Brazil, and native to the Cerrado, Caatinga and Amazon Forest biomes (Lima, 2015). It occurs on dry land and in sandy, acidic soils with low chemical fertility and well-drained (Dias et al., 1995), not tolerating low temperatures.

In view of the above, the present study aimed to evaluate the effect of inoculation with NFB and AMF on the growth of *Tachigali vulgaris* seedlings under nursery conditions.

2.MATERIAL AND METHODS

The experiment was carried out in the "Terrace" experimental field nursery of Embrapa Agrobiologia, located in Seropédica, RJ (22° 45' 19.98" S and 43° 40' 04.28" W, altitude 32 m). The region's climate is tropical rainy with a dry winter, being classified as Aw according to the Köppen Classification. The average annual temperature is 23.9 °C, and the average annual precipitation is 1213 mm (Carvalho et al., 2011).

The tachi seeds used in the experiment were collected from the Embrapa Amapá area in Macapá (0° 00' 47.77" S; 51° 05' 04.50" W, 16m altitude) in October 2013. The seeds were immersed in sulfuric acid for 10 min to break their dormancy, following the recommendations by Carvalho and Figueirêdo (1991).

A completely randomized design in a 3 (NFB strains) x 2 (with and without AMF) factorial scheme was implemented with with three additional control treatments, five repetition, and ten seedlings per repetition, thus totaling 450 seedlings. The tested NFB treatments were: BR 8402 strain (*Bradyrhizobium* sp.), BR 3637 strain (*Ensifer* sp.), BR 5610 strain (*Bradyrhizobium elkanii*), combined with the presence and absence of an inoculant with multiple AMF species. The additional control treatments consisted of seedlings inoculated only with the AMF (TM - mycorrhizal control) and seedlings fertilized only with N (TN - nitrogen) or without N (absolute control - TA).

The inoculants of the bacterial strains were prepared at the Bioprocess Laboratory of Embrapa Agrobiologia using peat as a vehicle, so that there was a minimum 109 CFU g of peat. The inoculation was done by moistening the seeds with distilled water and mixing them with the inoculant until a uniform layer was formed on the seed surface. The seeds were sown directly in the bag in the nursery on the same day of this inoculation.

The mycorrhizal inoculant was prepared at Embrapa Agrobiologia's Mycorrhizal Laboratory. A dose containing approximately 84 spores (4.5 g of inoculant; 12 spores per dose of each species) per container was applied to the sowing hole at the time of planting. The following species were used: *Acaulospora colombiana* (Spain & N.C. Schenck) *Kaonongbua*, J.B. Morton & Bever (2010), *Acaulospora scrobiculata* Trappe (1977), *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & Schuessler (2010), *Dentiscutata heterogama* (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl (2008), *Gigaspora margarita* W.N. Becker & I.R. Hall (1976), *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl (2014) and *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker and F.E. Sanders (1986). All were from the Johanna Döbereiner Biological Resources Center (CRB-JD).

Plastic bags of 12 cm x 18 cm were used as seedling containers and a mixture of sand and vermiculite (1:1; v:v) as substrate. Chemical analysis of the substrate was carried out at Embrapa Agrobiologia, collecting three samples. A nutrient solution containing macro (KCl, $CaCl_2$, KH_2PO_4, $MgSO_4.7H_2O$) and micronutrients (H_3BO_3; $MnSO_4$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $Na_2MoO_4.2H_2O$) was applied to all seedlings (all treatments) every two weeks, following a solution recommendation for pots according to Leonard de Norris, modified to half the salt concentration (Gruzmán and Döbereiner, 1968).

TN seedlings received 8 mg of nitrogen per seed in the form of ammonium nitrate weekly to compare the efficacy of the inoculated bacteria on the mineral source of N. Thus, a total of 308 mg of N per seedling after 270 days of the experiment. The absolute control did not receive any type of inoculant or mineral fertilizer from N.

The following variables were then measured 270 days after the experiment was implemented: height, stem diameter (SD), shoot dry mass (SDM), root dry mass (RDM), nodule dry mass (NDM), and the number of nodules (NN). The height and DD data were obtained from 10 plants at random, and the dry masses and number of nodules from seven plants within each plot.

The shoot P content was determined according to the methodology presented by Silva (1999). Mycorrhizal colonization was carried out from oven-dried roots at 60 °C, being rehydrated with 2% glycerin and heating at 90 °C for 30 min, then leaving them for 12 hours in water. Samples of the fine roots were clarified after rehydration according to the method used by Koske and Gemma (1989), and the staining according to Grace and Stribley (1991), while the mycorrhizal colonization rate was estimated by the gridline intersect method in checkered plates by Giovannetti and Mosse (1980).

The plant response to mycorrhization was calculated according to the formula of Plenchette et al. (1983). The efficiency and efficacy of bacterial inoculation were determined using the following formulas: efficiency = shoot dry matter of the inoculated treatment/shoot dry matter of the absolute control) x 100; efficacy = (shoot dry matter of the inoculated treatment/shoot dry matter of the nitrogenous control) x 100.

The data were tested for normality and homogeneity of variance by the Shapiro Wilk and Bartlett tests at 5% probability, with the mycorrhizal colonization percentage being transformed into arcsen (square root (x/100)). The analysis of variance for the characteristics and the Scott Knott test were performed at 5% probability to compare the treatment means using the Sisvar (Ferreira, 2011) and R (R Development Core Team, 2018) programs.

3.RESULTS

The chemical analysis of the substrate showed the following characteristics: pH in water (2:1) = 4.74±0.02; P and K (Mehlich I extractor) = 1.75±0.17 and 12.42±0.49 mg L^{-1}; Ca, Mg and Al (1 M KCl) = 0.16±0.01. 0.44±0.01 and 1.01±0.02 $cmol_c$ dm^{-3}; C (0.0667 mol L^{-1} (potassium dichromate extractor) = 1.4 g dm^{-3}; H+Al (0.5 mol L^{-1} (calcium acetate extractor) = 3.57±0.06 $cmol_c$ dm^{-3}; N (Kjeldhal) = 0.02±0.00 g dm^{-3}.

The treatments showed a highly significant effect ($p<0.01$) for all variables evaluated, except for the number of nodules, nodule dry mass and mycorrhizal colonization, which did not differ between treatments. The analysis of variance showed a significant difference for the height, SD, SDM, RDM, and total P variables (Figure 1).

The tachi seedlings performed better when doubly inoculated (with NFB and AMF, regardless of the bacterium) when compared to uninoculated or mono-inoculated treatments (absolute control, mycorrhizal control and seedlings inoculated only with NFB), being significantly inferior to the nitrogen control. This difference was observed for the variables height, stem diameter (SD), shoot dry mass (SDM) and root dry mass (RDM) (Figure 1).

The analysis of variance showed a significant interaction between AMF and NFB inoculations for height, showing a difference in efficiency between NFB due to the addition of AMF. The BR 3637 and BR 5610 strains produced taller seedlings when inoculated together with AMF than when inoculated alone (Fig 1B). The same was not verified for the BR 8402 strain, which showed no statistical difference in height between mycorrhizal or non-mycorrhizal plants. For the stem diameter and height variables, the double inoculation favored seedling development when inoculated with BR 8402 and BR 3637 strains, but not with BR 5610 (Fig 1A).

There was no interaction between AMF and NFB for the SDM and RDM variables, meaning that the double inoculation favored accumulating dry mass, regardless of the bacterial strain, indicating a general benefit promoted by the AMF inoculation in the biological nitrogen fixation process and seedling growth promotion (Figure 1).

Mycorrhizal colonization (MC) ranged from 17.85% in the nitrogenous control to 48% in the mycorrhizal control (Table 1). According to Carneiro et al. (1998), these values can be considered low (< 20%) and medium (> 20% and < 50%). There was no statistical difference between treatments for this variable, with the data showing a high coefficient of variance, even with the transformation of the data (CV = 48.25).

A higher P concentration was observed in the shoot in the nitrogen control (6.10 mg/plant). The second highest value was in the BR 8402 strain + AMF (2.94 mg/plant), then the BR 3637 strain + AMF (2.62 mg/plant) and the mycorrhizal control (2.61 mg/plant). The other treatments showed values below 2.1 mg/plant (Figure 1E). The only statistically significant difference was observed in the nitrogen control regarding the other treatments.

The fact that all treatments presented mycorrhizal colonization and nodulation, regardless of inoculation, hid the real quantification of the symbiotic efficiency of AMF and NFB. However, this was expected since it would be difficult to establish native communities in non-sterile conditions. Despite this unfavorable control condition, an efficiency rate of over 200% was observed for BR 8402 (Table 1). The efficiency and effectiveness of double-inoculated treatments were higher than treatments inoculated with NFB alone. The best results were obtained with the BR8402 strain + AMF, with efficiency and effectiveness values of 248.10 and 34.97%, respectively, but without statistical difference regarding the other strains + m, observed by the shoot

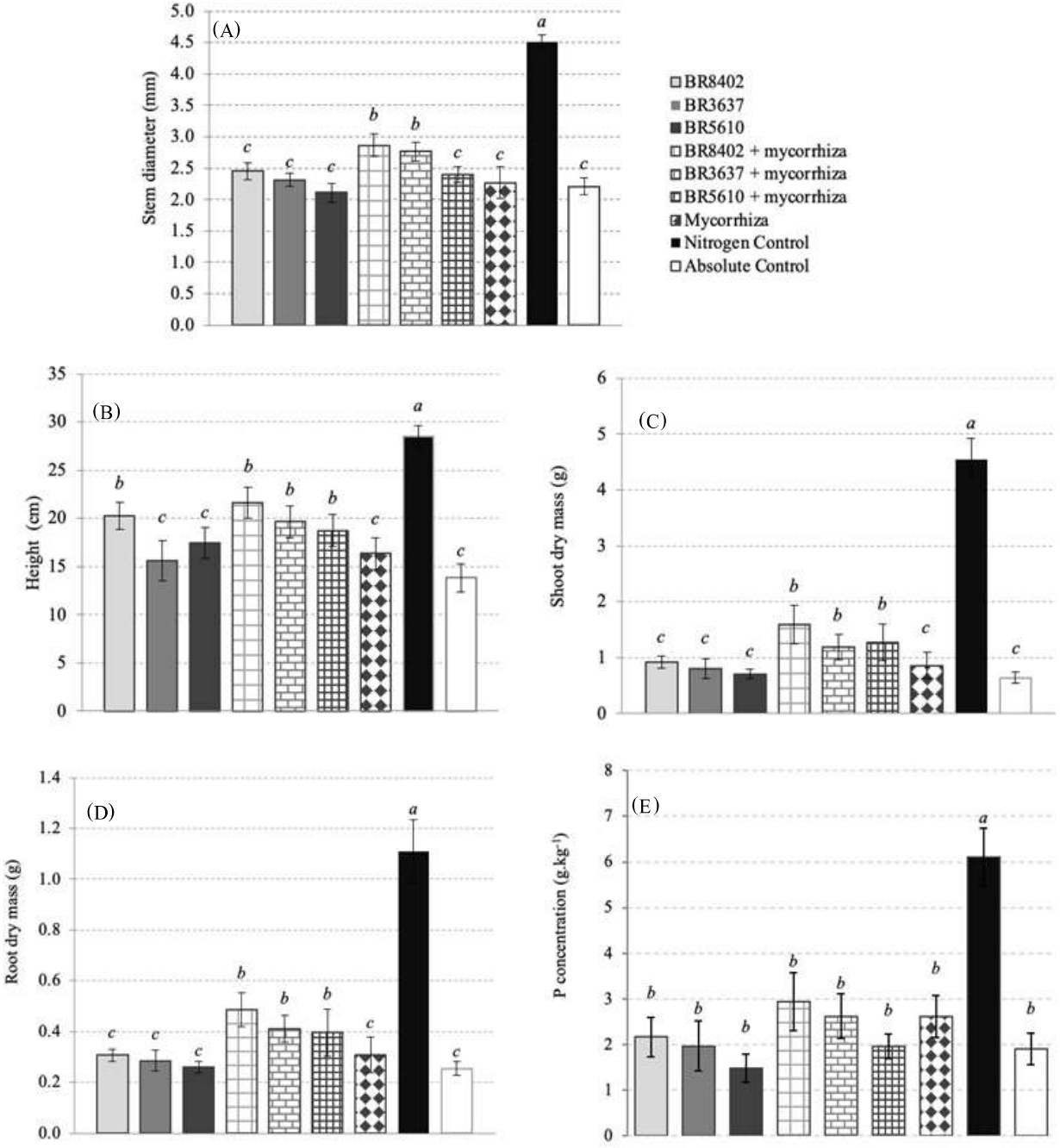

Figure 1 – A-E. Stem diameter, height, shoot dry mass, root dry mass, and P concentration in the shoot of *Tachigali vulgaris* seedlings inoculated with three N_2-fixing bacteria strains combined with arbuscular mycorrhizal fungi inoculant (+ mycorrhiza) and controls without inoculation and without fertilization (absolute), or fertilized with N after 270 days in a nursery. Averages followed by the same letter do not differ by the Scott Knott test at 5% probability. The error bar indicates the standard error.

Figura 1 *– A-E. Diâmetro a altura do colo, altura, massa seca de parte aérea, massa seca de raíz, concentração de P na parte aérea de mudas de* ***Tachigali vulgaris*** *inoculadas com três estirpes de bactérias fixadoras de N_2 combinadas com inoculante de fungos micorrízicos arbusculares (+ micorrizas) e testemunhas sem inoculação e sem adubação (absoluta) ou adubada com N (nitrogenada) após 270 dias em viveiro. Médias seguidas de mesma letra, por variável, não diferem entre si pelo teste de Scott Knott, a 5% de probabilidade. A barra de erro indica o erro padrão.*

Table 1 – Growth of *Tachigali vulgaris* seedlings inoculated with three rhizobia strains with and without mycorrhizae in the nursery after 270 days.
Tabela 1 *– Resultados do desdobramento fatorial do crescimento de mudas de* ***Tachigali vulgaris*** *inoculadas com três estirpes de rizóbio com e sem micorrizas no viveiro após 270 dias.*

Strain	No mycorrhizae	With mycorrhizae	Overall mean	No mycorrhizae	With mycorrhizae	Overall mean
	Height (cm)			SD (mm)		
BR 8402	20.28^{aA}	21.66^{aA}	20.97^{A}	2.45^{bA}	2.87^{aA}	2.66^{A}
BR 3637	15.64^{aA}	19.65^{aA}	17.65^{A}	2.32^{bA}	2.77^{aA}	2.54^{A}
BR 5610	17.46 ^{aA}	18.74^{aA}	18.10^{A}	2.11^{aA}	2.40^{aB}	2.26^{B}
Overall mean	17.80^{a}	20.02^{a}		2.29^{b}	2.68^{a}	
CV(%)	19.81			12.48		
	RDM (g)			SDM (g)		
BR 8402	0.31^{bA}	0.49^{aA}	0.40^{A}	0.92^{aA}	1.59^{aA}	1.26^{A}
BR 3637	0.29^{aA}	0.41^{aA}	0.35^{A}	0.80^{aA}	1.19 ^{aA}	1.00^{A}
BR 5610	0.26^{aA}	0.40^{aA}	0.33^{A}	0.70^{aA}	1.27 ^{aA}	0.99^{A}
Overall mean	0.28^{b}	0.43^{a}		0.81^{b}	1.35^{a}	
CV (%)	34.66			48.30		
	NDM (mg)			Nnod (n)		
BR 8402	32.32^{aA}	59.56^{aA}	45.94^{A}	8.24^{aA}	17.84 ^{aA}	13.04^{A}
BR 3637	30.72^{aA}	56.44^{aA}	43.58^{A}	8.24 ^{aA}	15.28 ^{aA}	11.76^{A}
BR 5610	31.84^{bA}	66.16^{aA}	49.00^{A}	9.60 ^{aA}	18.00 ^{aA}	13.80^{A}
Overall mean	31.63^{b}	60.72^{a}		8.69^{b}	17.04^{a}	
CV (%)	51.18			57.91		
	Mycorrhizal colonization (%)			P total (g kg^{-1})		
BR 8402	32.89^{aA}	24.81 ^{aA}	28.85^{A}	2.16^{aA}	2.94 ^{aA}	2.55^{A}
BR 3637	22.82^{aA}	34.47 ^{aA}	28.65^{A}	1.97 ^{aA}	2.62 ^{aA}	2.30^{A}
BR 5610	37.92^{aA}	23.49 ^{aA}	30.71^{A}	1.48 ^{aA}	1.96 ^{aA}	1.72^{A}
Overall mean	31.21^{a}	27.59^{a}		1.87^{a}	2.51^{a}	
CV (%)	41.75			40.34		
	Efficiency (%)			Efficacy (%)		
BR 8402	143.78	248.10	195.94	20.27	34.97	27.62
BR 3637	124.94	186.03	155.48	17.61	26.22	21.91
BR 5610	109.79	198.50	154.14	15.48	27.98	21.73
Overall mean	126.17	210.87		17.78	29.72	

Averages followed by the same letter, lower case on the line (for the same treatment) and upper case on the column (for the same strain), do not differ by the F and Scott Knott tests at 5% probability, respectively.
Legend: Stem Diameter (SD), root dry mass (RDM), shoot dry mass (SDM), nodule dry mass (NDM), number of nodules (Nnod), mycorrhizal colonization, P concentration in the shoot (total P).
Médias seguidas de mesma letra, minúscula na linha (para o mesmo tratamento) e maiúscula na coluna (para a mesma estirpe), não diferem entre si pelos testes F e de Scott Knott a 5% de probabilidade, respectivamente.
Legenda: Diâmetro a altura do colo (DAC), massa seca de raíz (MSR), massa seca de parte aérea (MSPA), massa seca de nódulo (MSNod), número de nódulos (Nnod), Colonização micorrízica, concentração de P na parte aérea (P total).

dry mass results. The lowest values were obtained by the treatment inoculated with the BR 5610 strain without AMF with values of 109.79 and 15.48%, respectively.

4.DISCUSSION

Franco et al. (1995) observed that tachi seedlings showed a very slow initial growth rate under field conditions, even with the addition of a bovine manure compound. These observations, combined with the fact that tachi positively responded to mycorrhization, would explain the great difficulty in producing seedlings observed in substrates which are less rich in organic matter and without AMF inoculation. Carpanezzi et al. (1983) recommend that the time necessary for the seedlings of this species to reach the ideal size for planting (with a height of 20 to 25 cm) should be 150 to 180 days after sowing. This slow growth was observed in this study, in which the best treatment (nitrogen control) reached 30 cm in height after nine months in the nursery.

The double inoculation of tachi seedlings proved to be effective for accelerating their growth, which was reflected in greater height, SD, shoot dry mass (SDM), root dry mass (RDM) and nodule dry mass (NDM). RDM reached 124% higher values in the treatments with double inoculation regarding the absolute control, and the SDM was up to 90% higher with double inoculation regarding the seedlings inoculated only with NFB. The plant inoculated with the BR 5610 strain plus AMF showed 207% higher NDM than the plant inoculated with the strain alone, although this variable has not been statistically differentiated, probably due to the high coefficient of variation (51.18%). The double inoculation was no better than the nitrogen control, as expected.

Schiavo and Martins (2003) also observed a positive effect of double inoculation in *Acacia mangium* Willd seedlings, providing an increase of 54% in the shoot dry mass regarding the absolute control. Double inoculation in *Anadenanthera peregrina* var. *falcata* (Benth.) Altschul. seedlings provided about 60% greater biomass than the control after ten months (Gross et al., 2004). Marques et al. (2001) verified an increase of 56% in the dry mass of *Centrolobium tomentosum* Guillem. ex Benth. plants doubly inoculated compared to uninoculated ones.

It should be emphasized that this does not always happen, as there are cases in which the relationship between these two endosymbionts can generate null or antagonistic effects on plant growth (Xavier and Germida, 2003; Santos et al., 2008), and even colonization of plant nodules by AMF and consequent reduction in NFB in some cases (Carvalho and Moreira, 2010).

The BR 8402 strain + AMF showed efficiency and effectiveness of 248% and 35%, respectively (Table 1). This index is based on the shoot dry mass ratio (%) of the inoculated plant with that of the absolute control (efficiency) and nitrogen (efficiency). However, it is observed that there was no statistical difference between the shoot dry mass of the three doubly inoculated strains, demonstrating that the joint inoculation of NFB and AMF is generally beneficial for the studied species, regardless of the strain.

The nitrogen control reduced nodule production, but did not completely inhibit it, which may indicate that the dose used of 308 mg of N per bag of seedling (1 kg) in this experiment was not sufficient to supply the demand for N of the species. NFB symbiosis has a considerable cost to the plant in terms of photo-assimilated carbon (C). It is widely documented in the literature that the plant negatively regulates symbiosis with NFB when supplied with high levels of N, and there is additionally feedback control called self-regulation related to nodulation which consists of a process in which the nodule establishment systematically suppresses the subsequent formation of other nodules, preventing the plant from being nodulated indefinitely (Carvalho and Moreira, 2010). The ideal N level found by Simões et al. (2016) for tachi was 150 mg dm^{-3}, even though they did not evaluate the nodulation, and the studied period was only 100 days. A dose of 405 mg dm^{-3} of N would be necessary for seedlings of 270 days (which was the case of the present study), considering the linear growth of the species.

The 18-48% values of mycorrhizal colonization found in the present study were lower than those of other studies developed for this species which found more than 60% of mycorrhizal colonization (Caldeira et al., 1999; Marinho et al., 2004). This suggests that the inoculated fungi were not totally efficient for tachi or did not perfectly adapt to the substrate. However, very low rates of colonization for some plants are sufficient to promote good development, so that the colonization percentage does not directly and linearly reflect the efficiency of mycorrhizal symbiosis, as it is greatly altered by time and other factors which regulate the mycorrhizae development (McGonigle, 2001; Kiriachek et al., 2009).

The colonization percentage can also be affected by factors such as P content available in the substrate, plant age, soil pH, root density, AMF propagules in the soil, mycorrhizal dependence on the plant species, nitrogen concentration in the soil, and soil management, among others (Duponnois et al., 2008). The pH of 4.7 found in the vermiculite sand substrate used in the experiment can be considered low for the development of some AMF species (Wang et al., 1993). Very acidic soils can interfere with the spore germination, the growth of the germ tube and the colonization of arbuscular-vesicular mycorrhizal fungi roots, with this influence being variable according to the fungus species (Siqueira et al., 1984). The Glomeraceae group, formed by the *Glomus*, *Funneliformis*, and *Rhizoglomus* genera, is more affected by soils with pH below 6.5 compared to the Gigasporaceae group, formed by the *Gigaspora*, *Dentiscutata*, and *Scutellospora* genera (Stürmer et al., 2018).

Tachi is considered a species with high mycorrhizal dependence, and it would be expected that it would not be able to develop in the absence of mycorrhizal fungi, and that it would also stop responding to mycorrhization only when the availability of phosphorus (P) was very high (Berbara et al., 2006). Teles et al. (1999) found that AMF inoculation favored an accumulation of total dry matter in the tachi and nodulation up to the dose of 40 mg of P kg^{-1} and 80 mg of P kg^{-1}, respectively. The mycorrhizal or non-mycorrhizal seedlings were equal in growth after this dosage, and this experiment was evaluated up to 140 days. Dias et al. (1995) observed a critical P level of 26.10 mg dm^{-3} of substrate for this species. In the present work, 50 mg of P per seedling in the form of dipotassium and monopotassium phosphate over 270 days in the form of a nutrient solution was applied to a substrate which contained 1.7 mg L^{-1}. The applied amount of P may have contributed to inhibiting colonization given the low nutritional requirement of the species. Other species which have intermediate mycorrhizal dependence, such as *Mimosa artemisiana* Heringer & Paula, fail to respond to inoculation when the available P (Mehlich I extractor) in the soil is greater than 187 mg kg^{-1} and has a greater response to AMF inoculation with P available (Mehlich I extractor) in the soil of 56 mg kg^{-1} (Hentz et al., 2013). Burity et al. (2000) observed that the addition of 40 kg ha^{-1} of P for *Mimosa caesalpiniifolia* Benth. increased the AMF colonization of roots. Chaves et al. (1995) observed that the addition of 200 mg dm^{-3} P did not affect colonization for *Dalbergia nigra* (Vell.) Fr.All. ex Benth.

Recent studies have indicated that mycorrhizal-dependent species such as *Piptadenia gonoacantha* (Mart.) J. F. Macbr. and *Acacia holosericea* A.Cunn. ex G.Don. nodulate in the absence of AMF, but the nodules are not efficient in absorbing atmospheric N (Bournaud et al., 2018; Oliveira Júnior et al., 2017; Duponnois et al., 2008). These same studies indicate that mycorrhizal colonization is dependent on rhizobia, and the efficacy of the nodule and plant growth are dependent on the presence of specific combinations of rhizobia strains and AMF. Although the present study did not go into methodological depth in evaluating the efficacy of the nodule, it still corroborates this evidence by verifying a significant gain in the dry mass of the double inoculated treatments, and therefore confirming the need to produce seedlings with both symbionts. It is suggested that new strains of bacteria be tested, preferably isolated from tachi roots in their natural habitat, since the strains used herein were not collected from the natural biome of this tree, and there may be even more gains with species-specific bacteria, and the same gain could be obtained with a selection of AMF strains for this tree.

5.CONCLUSIONS

Tachigali vulgaris grows better when inoculated with rhizobia and mycorrhizal fungi than when inoculated alone with one of these symbionts.

The positive effect of double inoculation occurred regardless of the bacteria strain used.

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