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Scientific Article

RHIZOBACTERIA INCREASE THE GROWTH AND QUALITY OF *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos SEEDLINGS

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ABSTRACT

Handroanthus chrysotrichus (Mart. ex DC.) Mattos, popularly known as the golden trumpet tree, is a native tree of Brazil with exuberant flowering. It is used for urban afforestation and reforestation of degraded areas, and its wood is destined for several purposes. In order to develop vigorous plants after planting, it is essential to produce high-quality seedlings, which can be obtained using rhizobacteria. As renewable sources, rhizobacteria exhibit growth-promoting properties, facilitate nutrient uptake and/or solubilization, and contributes to the production of phytohormones, while also serving as pathogen controllers and mitigators of abiotic stress. These attributes collectively enable the establishment of a sustainable production system for producing vigorous and rapidly growing seedlings. This study aimed to evaluate the effect of plant growth-promoting rhizobacteria in the production of *H. chrysotrichus* seedlings. The experimental design followed a completely randomized setup. The treatments consisted of microorganisms (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*, and *Azospirillum brasilense*) plus the absence of microorganisms - control; four repetitions and ten plants-per-plot. The parameters evaluated were shoot height; stem diameter; root length; shoot, root, and total dry matter; leaf number and leaf area, as well as chlorophyll content; minimum and maximum fluorescence; and maximum quantum efficiency of photosystem II; shoot height/stem diameter ratio and Dickson's Quality Index. The *B. amyloliquefaciens* inoculation promoted an increase in seedling shoot height, stem diameter, root length, leaf area, shoot, root, and total dry matter and also increased the Dickson Quality Index. It can be concluded that *B. amyloliquefaciens* is the most efficient inoculant for producing golden trumpet tree seedlings, followed by *A. brasilense*.

Keywords: *Azospirillum brasilense*; *Bacillus* spp.; Plant growth-promoting microorganisms.

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RIZOBACTÉRIAS INCREMENTAM O CRESCIMENTO E A QUALIDADE DAS MUDAS DE *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos

RESUMO – Popularmente conhecida como ipê-amarelo, *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos é uma arbórea nativa do Brasil, de florescimento exuberante, usada na arborização de cidades, no reflorestamento de áreas degradadas, além de sua madeira ser destinada para diversos fins. Para o desenvolvimento de plantas vigorosas após o plantio, é importante a produção de mudas de alta qualidade, que podem ser obtidas com o uso de rizobactérias. Consideradas como fontes renováveis, as rizobactérias apresentam propriedades de promoção do crescimento, facilitadoras da absorção e/ou solubilização de nutrientes e contribuem para a produção de fitohormônios, ao mesmo tempo que podem atuarem como controladoras de patógenos e na mitigação de estresse abiótico. Esses atributos juntos possibilitam o estabelecimento de um sistema de produção sustentável para a obtenção de mudas vigorosas e de crescimento rápido. O trabalho teve como objetivo avaliar o efeito de rizobactérias promotoras de crescimento de plantas na produção de mudas de ipê-amarelo. O delineamento experimental foi o inteiramente casualizado. Os tratamentos consistiram nos microrganismos (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens* e *Azospirillum brasilense*) mais a ausência de microrganismos – controle; em quatro repetições e dez plantas por parcela. Os parâmetros avalizados foram: altura da parte aérea; diâmetro do coleto; comprimento do sistema radicular; massa seca da parte aérea, das raízes e total; número de folhas e área foliar, além do teor de clorofila; fluorescência mínima e máxima; e máxima eficiência fotoquímica do fotossistema II; razão altura da parte aérea/diâmetro do coleto e o Índice de Qualidade de Dickson. A inoculação de *B. amyloliquefaciens* promoveu incremento na altura da muda, no diâmetro do coleto, no comprimento de raiz, na área foliar, na massa seca da parte área, das raízes e total e, também, aumentou o Índice de Qualidade de Dickson. Pode-se concluir que *B. amyloliquefaciens* é o inoculante mais eficiente para a produção de mudas de ipê-amarelo, seguido por *A. brasilense*.

Palavras-Chave: *Azospirillum brasilense*; *Bacillus* spp.; Microrganismos promotores de crescimento de plantas.

1. INTRODUCTION

The species *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos, popularly known as the Golden Trumpet Tree, belonging to the Bignoniaceae family, is a Brazilian native tree of wide geographic distribution, present in the Cerrado, Atlantic Forest, and Pampa biomes, with high economic value, widely used in urban afforestation and the recovery of degraded areas. It is also a high-density wood species, highly requested in the construction and furniture industry, which can be cultivated in integrated systems, such as crop-livestock-forestry (CLF) integration, and intercropped with other species in agroforestry systems (AFSs) (Oliveira Junior et al., 2020; Flora…, 2023).

Therefore, to meet the market demand for urban planting, recovery areas, or integrated systems, there is a need to reduce the nursery time, which is provided by faster growth and development of good quality seedlings, aiming for better performance in the field after planting. The seedlings' more significant growth and quality can be obtained using plant growth-promoting bacteria (Monteiro et al., 2014).

Rhizobacteria has proven to be effective in agriculture, promoting plant growth. Rhizobacteria possess various traits that contribute to enhanced plant growth. These traits include the production of phytohormones, the capacity to fix atmospheric nitrogen and solubilize phosphate in the soil, the production of siderophores for iron acquisition, ACC deaminase activity aiding stress tolerance, and the production of the phytohormone indole-3-acetic acid (IAA) (Gupta et al., 2015; Chandran et al., 2021). Furthermore, they indirectly promote plant growth by controlling pathogens through competition for nutrients and space, the production of antibiotic substances, and the induction of plant defense mechanisms (Zhu et al., 2023; Riaz et al., 2021).

Several authors reported positive results regarding growth promotion plant improvement of different species with *Bacillus subtilis* and *Bacillus megaterium* (Kondo et al., 2020; Santos et al.,2021; Campos et al., 2023), *Bacillus amyloliquefaciens* (Zhao et al., 2016; Rios et al., 2018; Gamez et al., 2019) and *Azospirillum brasilense* (Gonzalez et al., 2018; Kondo et al., 2020).

Considering the above, the objective of this study was to evaluate the effect of rhizobacteria (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*, and *Azospirillum brasilense*) on the growth and quality of golden trumpet tree (*H. chrysotrichus*) seedlings.

2. MATERIAL AND METHODS

The research was conducted from September 2021 to January 2022 in a greenhouse located at the Faculdade de Ciências Agrárias e Veterinárias (UNESP/FCAV), Câmpus de Jaboticabal, SP, situated at coordinates 21°15'2" latitude, 48°16'47" longitude, and an altitude of 600 meters. The climate of the micro-region, classified by the Köppen-Geiger system, is tropical savanna (Aw type), characterized by dry winters (Andre and Garcia, 2015).

The experiment was conducted using a completely randomized design. It comprised five treatments: *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*, *Azospirillum brasilense*, and a control group without rhizobacteria inoculation. Each treatment had four replications, with ten plants per plot.

The seeds of the golden trumpet tree were harvested from a single mature tree at the Experimental Nursery of Ornamental and Forestry Plants of the College of Agricultural and Veterinary Sciences (UNESP/FCAV) in September 2021. From here, the study was carried out using the same methodology extensively described by Campos et al (2023), Briefly, these seeds were planted in 280 cm^3 tubes within polypropylene trays, each tray holding 54 containers filled with Carolina Soil®. The trays were placed in a greenhouse with a shading cloth that allowed 50% light penetration, elevated 70 cm above the ground on metal mesh benches. Three seeds were initially planted per tube, and upon germination, only the most robust seedling per container was kept for further evaluation, and the remaining two were discarded. Automated irrigation using micro sprinklers was set for three 15-minute sessions per day. The microorganisms were sourced from a collection of the Soil Microbiology Laboratory at UNESP-FCAV were cultivated individually in a nutrient broth for seven days, then centrifuged to standardize an inoculum concentration of 1 x 107 CFU/mL. The microorganism solution was applied in drench twice to the substrate near the seedling stem, at 30 and 60 days after planting. Controls did not receive this inoculation. The experiment was terminated, and all treatments were evaluated at 107 days after planting when the first roots were observed at the bottom of the tubes, and a wide range of plant growth parameters were measured, including shoot height, root length, stem diameter, shoot and root dry matter, leaf number, leaf area, and the Dickson Quality Index was calculated. The average temperature during the experiment was 24.9ºC and relative humidity was 61.4%.

In addition, chlorophyll content (SPAD unit) and fluorescence were also assessed using a ChlorofiLOG, model CFL1030, FALKER®. The minimum fluorescence (F0), maximum fluorescence (FM), and maximum quantum efficiency of photosystem II (FV/FM) were obtained with a handheld chlorophyll fluorometer (OS30p, Opti Science). A bacterial count was performed in the substrate and in the shoot and roots of the treatments where rhizobacteria were applied to observe the bacterial growth after inoculation. Ten grams were added to an Erlenmeyer flask containing 95 mL of 0.1% sodium pyrophosphate saline solution for bacterial count in the substrate. All Erlenmeyer flasks were shaken for 1 hour, and from the contents of the flasks, serial dilutions were prepared following the methodology proposed by Wollum (1983). One hundred microliters (100 µL) of the obtained solutions per dilution were inoculated in Petri plates containing agar or potato dextrose agar medium in three replicates. The Petri plates were then kept in a B.O.D at 30 °C, and the number of Colony Forming Units (CFU) was counted after 24, 48, and 72 hours (Vieira and Nahas, 2005). The leaves and roots were separated and washed under running water for 10 minutes for plant bacterial counts. One gram (1 g) of each vegetative tissue (leaves and roots) was submitted to superficial disinfection to eliminate epiphytic microorganisms by immersing both tissues sequentially in 70% ethanol for 1 minute, sodium hypochlorite solution (2.0-2.5% active Cl) for 3 minutes and 70% ethanol for 30 seconds. Afterward, the tissues were washed thrice with distilled water (Araújo et al., 2002). Once sterilized and rinsed, the tissues were aseptically macerated with a mortar and pestle. Then, dilutions were performed and aliquots of 100 µL were sown on Petri plates containing tryptone soy agar medium for bacteria isolation. The plates were then grown in microbiological incubators at

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a constant temperature of 30 °C for 24 hours for bacterial growth, and CFU count was performed after 24, 48, and 72 hours. Data were analyzed using AgroEstat® (Barbosa & Maldonado Júnior, 2015) statistical software with Tukey's test for comparison, and the boxplots was performed using R software v4.1.2 (2021).

3. RESULTS

The rhizobacteria *Bacillus amyloliquefaciens* and *Azospirillum brasilense* showed higher means for shoot height, root dry matter, and total dry matter; the average values between them were 12.03 cm, 0.607 g, and 0.913 g, respectively. There were no differences in the stem diameter and root length among *B. amyloliquefaciens*, *A. brasilense*, and *Bacillus megaterium*. Thus, the rhizobacterium *B. amyloliquefaciens* stood out for shoot dry matter (0.342 g), followed by *A. brasilense*. There was no difference between the control and *Bacillus subtilis* for growth parameters (Figure 1).

Therefore, *B. amyloliquefaciens* proved

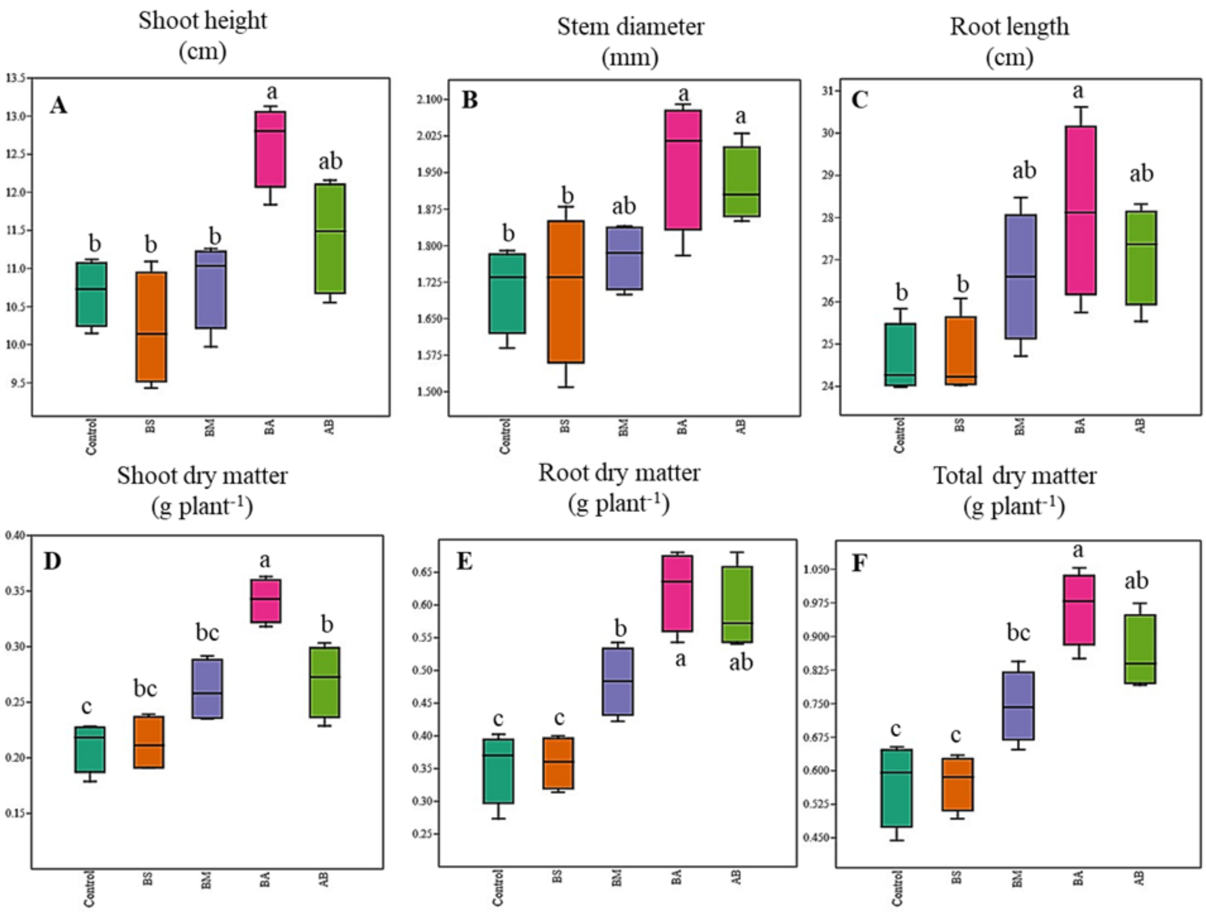

Figure 1. Boxplots of growth evaluation of *Handroanthus chrysotrichus* seedlings without inoculation (Control) and inoculated with *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) and *Azospirillum brasilense* (AB). A – shoot height (cm), B – steam diameter (mm), C – root length (cm), D – shoot dry matter (g plant^{-1}), E - root dry matter (g plant^{-1}), F – Total dry matter (g plant^{-1}). Means followed by the same lowercase letter do not differ by Tukey's test ($p < 0.05$). Jaboticabal, SP, 2022.

Figura 1. Boxplots da avaliação do crescimento de mudas de *Handroanthus chrysotrichus* sem inoculação (Controle) e inoculadas com *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) e *Azospirillum brasilense* (AB). A – altura da parte aérea (cm), B – diâmetro do caule (mm), C – comprimento da raiz (cm), D – matéria seca da parte aérea (g planta^{-1}), E - matéria seca da raiz (g planta^{-1}), F – Matéria seca total (g planta^{-1}). Médias seguidas pela mesma letra minúscula não diferem pelo teste de Tukey ($p < 0,05$). Jaboticabal, SP, 2022.

to be effective in growth when compared to the control; these rhizobacteria promoted gains of 18.3% in shoot height, 15.3% in stem diameter, 14.5% in root length, 62.1% in shoot dry matter, 76.2% in root dry matter and 68.7% total dry matter. For *A. brasilense*, the gains compared to the control were 6.9% for shoot height, 12.3% for stem diameter, 10.4% for root length, 27.7% for shoot dry matter, 67.1% in root dry matter, and 50.4% in total dry matter. The rhizobacteria *B. megaterium* showed gains of 8.2% in root length, 23.6% in shoot dry matter, 36.5% in root dry matter, and 30% in total dry matter compared to the control.

There was no significant difference between treatments for leaf number, chlorophyll content, and FV/FM ratio (Figure 2). For leaf area, the *B. amyloliquefaciens* species had the highest mean, 60.2 cm²; the gain on leaf area from inoculation was 215 %, compared to control. The F0 and FM, *B. amyloliquefaciens*, and *A. brasilense* had the highest mean values, 114, 476, and 114, 439, respectively.

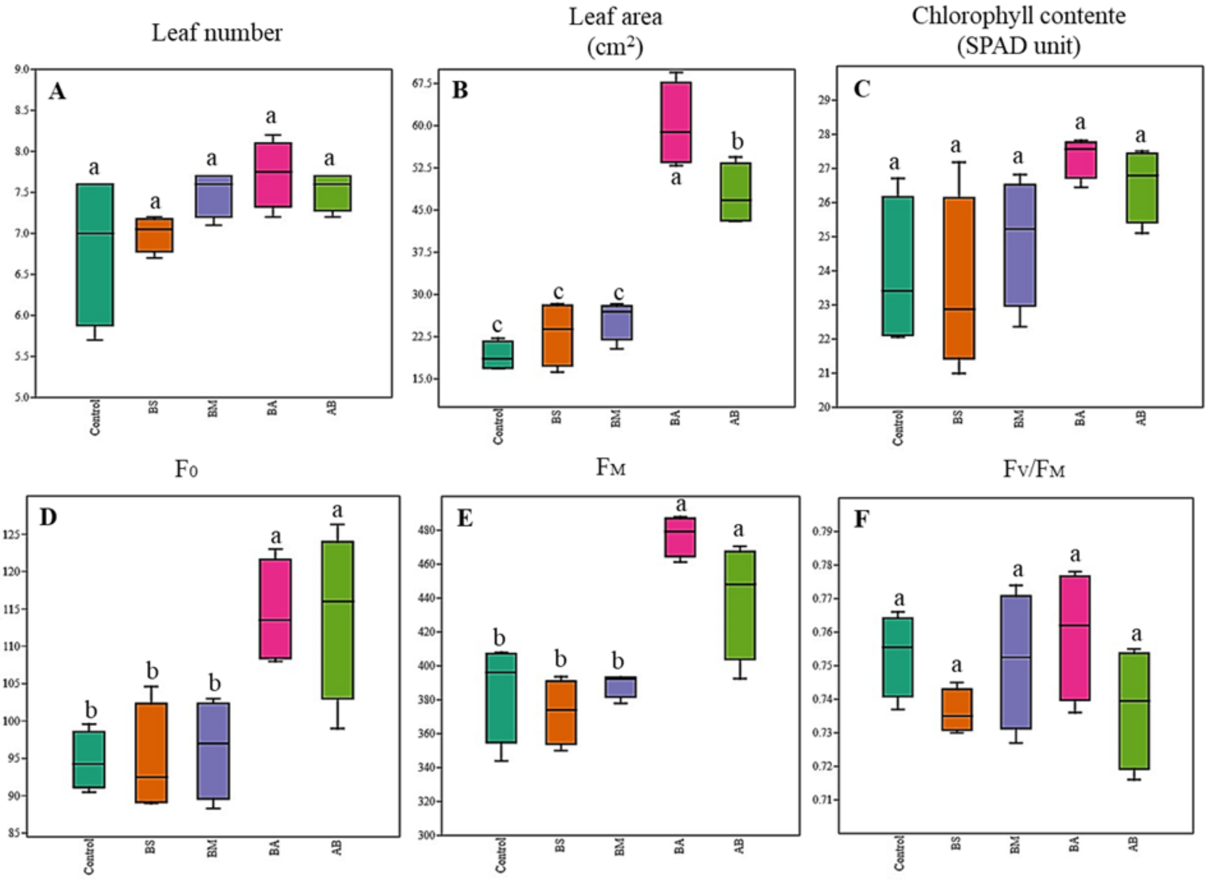

Figure 2. Boxplots of photosynthetic evaluation of *Handroanthus chrysotrichus* seedlings without inoculation (Control) and inoculated with *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) and *Azospirillum brasilense* (AB); A - leaf number, B - leaf area (cm²), C – chlorophyll content (SPAD unit), D - minimum fluorescence (F0), E - maximum fluorescence (FM), F - maximum quantum efficiency of photosystem II (FV/FM). Means followed by the same lowercase letter do not differ by Tukey's test ($p < 0.05$). Jaboticabal, SP, 2022.

Figura 2. Boxplots da avaliação fotossintética de mudas de *Handroanthus chrysotrichus* sem inoculação (Controle) e inoculadas com *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) e *Azospirillum brasilense* (AB); A - número de folhas, B - área foliar (cm²), C – conteúdo de clorofila (unidade SPAD), D - fluorescência mínima (F0), E - fluorescência máxima (FM), F - eficiência quântica máxima do fotossistema II (FV/FM). Médias seguidas pela mesma letra minúscula não diferem pelo teste de Tukey ($p < 0,05$). Jaboticabal, SP, 2022.

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There was no significant difference in the SH/SD ratio. For DQI, *B. amyloliquefaciens* and *A. brasilense* showed the highest averages, 0.142 and 0.140, respectively. (Figure 3). The average DQI gain using *B. amyloliquefaciens* and *A. brasilense* compared to the control was 70.2%.

Regarding the bacteria count in the substrate, the highest number was observed for *B. amyloliquefaciens* (9,34 x 106) and *A. brasilense* (8,35 x 106), followed by *B. megaterium* (Figure 4). The number of bacteria colonies in the roots was greater

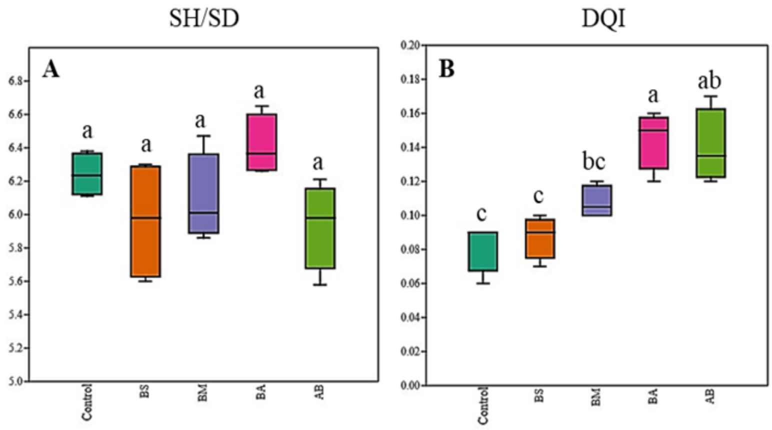

Figure 3. Boxplots of seedling quality evaluation of Handroanthus chrysotrichus without inoculation (Control) and inoculated with *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) and *Azospirillum brasilense* (AB); A - shoot height/stem diameter ratio (SH/SD), B - Dickson Quality Index (DQI). Means followed by the same lowercase letter do not differ by Tukey's test ($p < 0.05$). Jaboticabal, SP, 2022.

Figura 3. Boxplots da avaliação da qualidade das mudas de Handroanthus chrysotrichus sem inoculação (Controle) e inoculadas com *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) e *Azospirillum brasilense* (AB); A - razão altura da parte aérea/diâmetro do caule (SH/SD), B - Índice de Qualidade de Dickson (DQI). Médias seguidas pela mesma letra minúscula não diferem pelo teste de Tukey ($p < 0,05$). Jaboticabal, SP, 2022.

when *B. amyloliquefaciens* (4,77 x 104) was inoculated, followed by *B. megaterium* (4,18 x 104). For the shoots, the number was greater for *B. amyloliquefaciens* (8,61 x 103)

It is possible to compare the visual aspect of the golden trumpet tree seedlings in Figure 5 and, of all treatments, at 107 days after sowing, the superiority of the rhizobacterium *B. amyloliquefaciens*.

4. DISCUSSION

In recent years, plant growth-promoting rhizobacteria (PGPR) have garnered significant attention due to their capacity to enhance plant growth. In sustainable agriculture, soil microbial diversity is fostered by practices that enhance nutrient mobilization, solubilization, and disease suppression (Kumari et al., 2019). However, the practical application of these beneficial bacteria sometimes fails due to their inability to colonize the rhizosphere of inoculated plants, so root colonization by inoculant strains appears to be an essential step in the interaction between beneficial bacteria and host plants (Ahemad & Kibret, 2014). One such practice involves the introduction of beneficial bacteria into the soil using microbial inoculants, which can be based on single-species inoculants or various species of PGPRs and then can be used to enhance the availability and uptake of mineral nutrients, resistance to plant diseases, and reduction of abiotic stress to promote healthier plant growth (Santoyo et al., 2021).

The study demonstrated the golden trumpet tree seedlings showed significative responses to the rhizobacteria inoculation, especially with the *Bacillus amyloliquefaciens* was observed the highest means in shoot height (12.03 cm), stem diameter (1,97mm), root length (28,2 cm), shoot dry matter (0.342

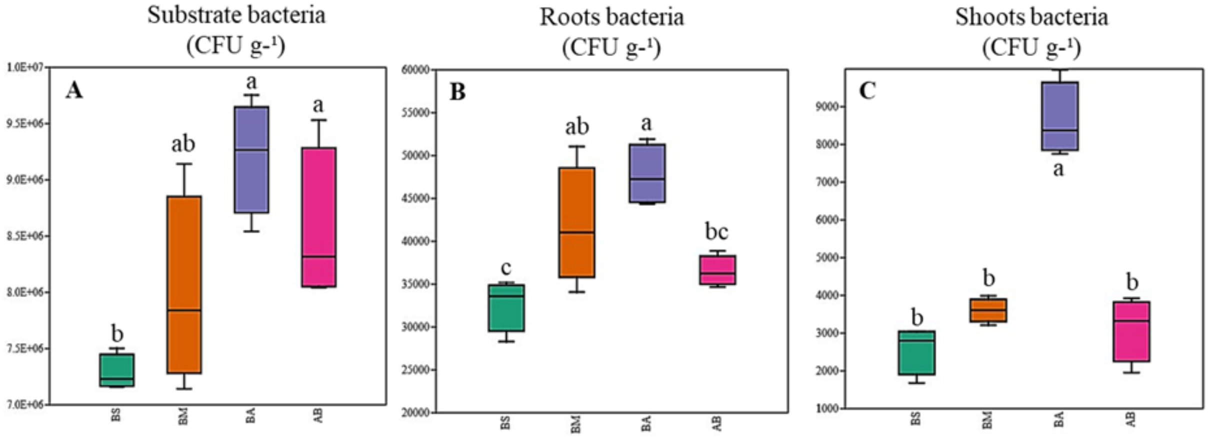

Figure 4. Boxplots of colony-forming units per gram (CFU g^{-1}) in *Handroanthus chrysotrichus* seedlings inoculated with *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) and *Azospirillum brasilense* (AB); A - CFU in the substrate, B - CFU in the roots, C - CFU in the shoots. Means followed by the same lowercase letter do not differ by Tukey test ($p < 0.05$). Jaboticabal, SP, 2022.

Figura 4. Boxplots das unidades formadoras de colônias por grama (UFC g^{-1}) em mudas de *Handroanthus chrysotrichus* inoculadas com *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) e *Azospirillum brasilense* (AB); A - UFC no substrato, B - UFC nas raízes, C - UFC nas partes aéreas. Médias seguidas pela mesma letra minúscula não diferem pelo teste de Tukey ($p < 0,05$). Jaboticabal, SP, 2022.

Figure 5. *Handroanthus chrysotrichus* seedlings at 107 days after sowing. Control (A) *Bacillus subtilis* (B), *Bacillus megaterium* (C), *Bacillus amyloliquefaciens* (D) and *Azospirillum brasilense* (E). Jaboticabal, SP, 2022.

Figura 5. Mudas de *Handroanthus chrysotrichus* aos 107 dias após a semeadura. Controle (A) *Bacillus subtilis* (B), *Bacillus megaterium* (C), *Bacillus amyloliquefaciens* (D) e *Azospirillum brasilense* (E). Jaboticabal, SP, 2022.

g), root dry matter (0.607 g) and, total dry matter (0.913 g) followed by *Azospirillum brasilense*, thus making evident the action of these two bacteria in growth improvement of their seedlings (Figure 1).

The use of rhizobacteria has also been reposted for some forest species. The inoculation of *Azospirillum brasilense*, *Bacillus subtilis*, and *B. megaterium* enhances the growth of the primary root and increases the levels of phosphorus and nitrogen in *Araucaria angustifolia* seedlings (Kondo et al., 2020). Campos et al. (2023) identified that the inoculation of *Bacillus subtilis* led to more significant growth and improved quality of *Euterpe oleracea* Mart. (Açaí) seedlings. Luciano et al. (2023) showed that the inoculation of *B. subtilis* in the substrate resulted in significant developments in the height and biomass of *Eucalyptus urograndis*. In the quality of *Corymbia citriodora* seedlings, *Trichoderma* and *B. subtilis* application showed positive results compared to the control for the Dickson Quality Index and initial growth (Ribeiro et al., 2023).

The low interaction between *B. subtilis* and *B. megaterium* with the golden trumpet tree may be related to the plant itself. Research has shown that plant species can respond differently to the association with growth-promoting microorganisms since the amount and composition of released substances in the exudates of plant species can vary, directly affecting the inoculum responses (Zamioudis and Pieterse, 2012; Venturi and Keel, 2016; Velloso et al., 2019; Santos et al., 2020). Another reason for the observed divergent response may be related to the cultivation method, as shown by Rios et al. (2018) and Santos et al. (2021), who conducted plantings in soil rich in microorganisms. However, there needs to be more information on the association of these microorganisms in plants grown in containers containing substrates.

The parameter leaf area corroborated the growth behavior of golden trumpet tree seedlings (Figure 2B). A greater leaf area (60.2 cm²) was observed with *B. amyloliquefaciens* inoculation. On the other hand, the lowest means of leaf area, observed for the control, *B. subtilis* (23,1 cm²) and *B. megaterium* (25,6 cm²), contributed to lower growth and development of the seedlings due to the low light energy accumulation. The leaf area also increased by 80% in *Musa acuminata* Colla plants when inoculated with *B. amyloliquefaciens* (105.96 cm²) compared to non-inoculated control plants (58.86 cm²) (Gamez et al., 2019). In the species *Codonopsis pilosula* (Franch.) Nannf., the leaf area of plants inoculated with *B. amyloliquefaciens* increased by 43%, compared to the control (Zhao et al., 2016). This indicates increased photosynthetic potential of seedlings, with a larger surface area for light absorption and, consequently, greater carbon assimilation (Tang et al., 2015).

For the fluorescence analyses, the minimum (F0) represents the fluorescence with all reaction centers open. It indicates the fluorescence emitted by chlorophyll, a Photosystem II light-harvesting complex molecule. In contrast, the maximum fluorescence (FM) represents the complete reduction of the primary quinone by the incidence of a light pulse on its reaction center, which results in maximum fluorescence (Taiz et al., 2017). For FM, gains of 23.5% were obtained with *B. amyloliquefaciens* rhizobacterization and 13.9% with *A. brasilense*, compared to the control (Figure 2E). This parameter can be attributed to their ability to establish greater and better photosynthetic activity under the same climatic conditions, which reduces the risks of photoinhibition damage, even though there was no difference between treatments for chlorophyll content (Stirbet et al., 2018).

However, there was no difference between treatments for leaf number (Figure 2A), chlorophyll content (Figure 2C), and efficiency of photosystem II (FV/FM) (Figure 2F). In contrast, the chlorophyll content and photosynthetic rate of *C. pilosula* plants inoculated with *B. amyloliquefaciens* increased by 16% and 22%, respectively, compared to the control (Zhao et al., 2016). The rhizobacteria *B. amyloliquefaciens*, together with *B. subtilis*, also positively influenced the performance of the photosynthetic mechanism in *Capsicum chinense* Jacq. (Samaniego-Gámez et al., 2016). Gonzalez et al. (2018) found an increase of chlorophyll in 25% of leaves when *A. brasilense* was inoculated into *Prosopis articulata* S. Watson. Therefore, the absence of discernible variances in physiological parameters after the application of rhizobacteria may be ascribed to the optimal abiotic conditions prevailing in the experimental setting, which favor the growth of seedlings. In this context, it is noteworthy to highlight that rhizobacteria exhibit heightened effectiveness in environments characterized by water scarcity as opposed to well-irrigated conditions, thereby enhancing photosynthetic processes, and mitigating oxidative stress (Zhao et al., 2023).

There was no difference between the treatments for shoot height/stem diameter ratio in the production of golden trumpet tree seedlings. This ratio expresses the robustness of the plant, where a lower value indicates a higher capacity of the plant to survive and establish itself in the field (Cargnelutti et al., 2017). Thus, by analyzing this characteristic, no superiority is observed for any bacterium; however, when only the stem diameter is evaluated, the superiority of *B. amyloliquefaciens* and *A. brasilense* is observed, which may also explain the better performance of the seedlings inoculated with both species since the stem diameter is the most reliable post-planting performance predictor, indicating the plant quality (Ritchie et al., 2010). The species *H. chrysotrichus* is typically propagated by seed (Lorenzi, 2020). Therefore, the seeds used in this experiment are derived from a single plant, ensuring that the progeny comprises half-siblings and decreasing the genetic component as a factor for variability.

The results obtained for Dickson's Quality Index (DQI) reinforce the superior quality of the seedlings inoculated with *B. amyloliquefaciens* followed by *A. brasilense*. The index is a complete parameter to indicate the seedling performance, so the higher the DQI, the higher the seedling quality within that lot (Lisboa et al., 2018).

The DQI formula uses root dry matter, recognized as one of the simplest indicators that best determines the establishment of seedlings in the field, as it directly impacts the water and nutrient uptake (Shen et al., 2019; Avelino et al., 2021). In this way, seedlings with more outstanding root dry matter will be more successful in the field due to their better acclimation capacity after transplanting (Avelino et al., 2021). This characteristic showed similar results to the DQI in the present study, further strengthening the quality superiority of golden trumpet tree seedlings inoculated with *B. amyloliquefaciens* followed by *A. brasilense*.

The higher dry root matter of the seedlings inoculated with *B. amyloliquefaciens* and *A. brasilense* may be due to their bigger length, as this trait showed similar results for both bacteria and also an increase in root hairs, as can be seen in Figure 5 letter D. When bacteria are present in the roots, they cause changes in the density and length of the root hairs, increasing the root surface area, allowing better use of nutrients and water (Hakim et al, 2021). Such effects are associated with the ability of rhizobacteria to release growth-promoting substances such as indole acetic acid, gibberellins, and cytokinins (Ahemad & Kibret, 2014).

The highest number of colony units in the substrate (Figure 4A) was observed when *B. amyloliquefaciens*, and *A. brasilense* were inoculated, confirming that these rhizobacteria had more significant proliferation in this substrate in association with the species, as well as showing the best growth and quality results for the golden trumpet tree seedlings. James Jr. et al. (1985) observed increased plant colonization by certain bacteria and highlighted that some bacterial strains establish irreversible bonds in roots compared to non-root-colonizing bacteria. It was observed with *Azospirillum* inoculation in corn, which increased plant colonization and promoted root growth (Fallik et al., 1988).

The starting point in plant growth promotion is the colonization of the rhizosphere and plant tissues, such as roots and leaves (Bulgarelli et al., 2013). In this study (Figure 4B), the treatment with *B. amyloliquefaciens* showed the highest number of colony units in the roots (4.7 x 104), followed by *A. brasilense* (3.6 x 104), indicating a site preference for colonization of these bacteria in golden trumpet tree. *B. amyloliquefaciens* inoculation also presented the highest number of colonies in the shoots, with 8,61 x 103 CFU g^{-1} (Figure 4C), making the positive interaction of this rhizobacteria with the studied tree even more evident.

Based on the obtained results, it was possible to observe that the tested rhizobacteria interfered in the growth, quality, and photosynthetic capacity of the studied species and therefore the use of these beneficial microorganisms in the production of golden trumpet tree seedlings is promising, agreeing with Yarte et al. (2022) who reported improvement in the seedlings production of pink trumpet tree (*Handroanthus impetiginosus* (Mart. ex DC.) Mattos) with rhizobacteria inoculation.

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5. CONCLUSION

Using rhizobacteria to cultivate golden trumpet trees improved growth parameters such as plant height, stem diameter, root length, plant biomass, and leaf area. The inoculation with *Bacillus amyloliquefaciens* proved to be the most efficient inoculant. Additionally, *Azospirillum brasilense* bacteria can also be an alternative in the production of seedlings of this species.

Based on these findings, the application of isolated rhizobacteria is recommended to optimize the production of golden trumpet tree seedlings. The enhanced performance of the seedlings leads to shorter production times in nurseries and results in more robust and healthier seedlings, consequently increasing the survival rate post-planting.

Furthermore, rhizobacterization does not necessitate changes in the standard routine of seedling production in nurseries, making it a convenient and effective method for improving seedling quality.

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AUTHOR CONTRIBUTIONS

Conceptualization: Campos TS, Vieira GR; Performed the analysis: Vieira GR, Campos TS, Santos CHB; Analysis of results: Souza AMB, Campos TS; Statistical analysis: Souza AMB; Santos CHB; Writingoriginal draft: Campos TS, Vieira GR, Souza AMB; Writing-review & editing: Campos TS, Rigobelo EC, Pivetta KFL; Supervision and coordination of research: Pivetta KFL, Rigobelo EC.

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