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Photosynthetic Performance of *Handroanthus chrysotrichus* Seedlings Grown in Substrate with *Rhizobacteria*

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Summary

Rhizobacteria, regarded as renewable resources, enable a sustainable system for producing vigorous and rapidly growing seedlings. The objective of this study was to evaluate the effect of plant growth-promoting rhizobacteria in the production of *H. chrysotrichus* seedlings. The experimental design was completely randomized. 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 following were evaluated: leaf number, leaf area, as well as chlorophyll content; minimum and maximum fluorescence; and maximum quantum efficiency of photosystem II. The inoculation with *B. amyloliquefaciens* has been found to enhance leaf area. Moreover, *B. amyloliquefaciens* plays a role in maintaining the functionality of the photosystem reaction center. Consequently, it can be concluded that *B. amyloliquefaciens* stands out as the most effective inoculant for golden trumpet for promoting greater efficiency of the photosystem II.

INTRODUCTION

Handroanthus chrysotrichus (Mart. ex DC.) Mattos tree belonging to the Bignoniaceae family, meeting the market demands for urban planting, ecosystem restoration, and integrated systems necessitates the reduction of nursery times, achieved through accelerated growth and the development of high-quality seedlings. This enhanced growth and seedling quality can be attained through the application of plant growth-promoting bacteria. In addition to their growth-enhancing properties, rhizobacteria also play a crucial role in mitigating drought and salinity stress, aiding in the phytoextraction of heavy metals, contributing to nutrient supplementation, fixation, or solubilization, facilitating the production of phytohormones, and controlling pathogens (Dias and Santos, 2022).

Among the most prominent rhizobacteria known for their positive impact on seedling growth and quality across various plant species are *Bacillus subtilis* and *Bacillus megaterium* (Guimarães et al., 2021; Santos et al., 2021, Silva et al., 2022), *Bacillus amyloliquefaciens* (Matsumura et al., 2016; Rios et al., 2018; Wang et al., 2020; Ngalimat et al., 2021), and *Azospirillum brasilense* (Gonzalez et al., 2018; Zeffa et al., 2019; Jarquín-Rosales et al., 2023). Given these considerations, 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.

MATERIALS AND METHODS

The study was carried out between September 2021 and January 2022 in a greenhouse at the College of Agricultural and Veterinary Sciences (UNESP/FCAV), Campus de Jaboticabal, SP, Brazil - under the coordinates 21°15'2" latitude, 48°16'47" longitude and 600 meters of altitude. The Brazil climate of the micro-region by Köppen-Geiger system is tropical savanna Aw type, with dry winter (Andre e Garcia, 2015).

The design of the experiment was entirely randomized. There were five treatments (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens* and *Azospirillum brasilense*, plus the absence of rhizobacteria - control); four repetitions and ten plants per plot. The seeds of golden trumpet tree were collected from existing trees in the Experimental Nursery of Ornamental and Forestry Plants of the Faculty of Agrarian and Veterinary Sciences (UNESP/FCAV) during September 2022. Seeds were sown in tubes with 280 cm^3 of volume capacity placed in polypropylene trays for 54 containers, containing Carolina Soil® as commercial substrate, composed of peat, vermiculite, roasted rice husk, calcined dolomite limestone, NPK 14-16-18 fertilizer and micronutrients (information obtained from the packaging). The trays were suspended on metal mesh benches 70 cm from the ground in a covered greenhouse with the sides protected with black screen that allows 50% of the light to pass through and with a clear plastic layer above the screen cover. The irrigation was performed by automatic micro sprinklers, activated three times a day for 15 minutes each, with a flow rate of 30 L h^{-1}.

The microorganisms are part of the collection of the Laboratory of Soil Microbiology of the Department of Vegetal Production of UNESP-FCAV, Campus de Jaboticabal, where they were grown separately, in nutrient broth medium for seven days in flasks kept in B.O.D. (Eletrolab, model 347 F, Brazil), at 25 °C temperature. After the incubation period, the microorganisms were centrifuged separately at 10,000 rpm for 10 minutes at 28 °C (Novatecnica, model MLW K24, Brazil). The inoculum concentration was standardized according to Barry and Thornsberry (1991) and Sahm and Washington II (1991) at 1 x 107 CFU mL-1 using a spectrophotometer (Micronal, model B382, Brazil) at 695 nm absorbance.

The microorganisms were inoculated twice, once at 30 days after the seeds were sown and again at 60 days, by applying 1.0 mL of the solution directly to the substrate near the stem, using a mechanical micropipette (VF-1000, Digipet®). The seedlings belonging to the control treatment were not inoculated. When the roots began to appear at the bottom of the tubes, the following characteristics were evaluated: Leaf number (LN), verified by visual counting of fully expanded leaves; and leaf area (LA, cm^2), measured using an electronic leaf area meter (Li-3100C, LI-COR®, Lincoln, Nebraska, USA). The chlorophyll content was measured with the ChlorofiLOG, model CFL1030, FALKER®; 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). The obtained data were submitted to analysis of variance and the means were compared using Tukey's test at 5% probability using the *R* statistical software (R Core Team, 2016).

RESULTS

No significant distinctions were observed among the treatments in terms of leaf number, chlorophyll content, and FV/FM ratio (**Fig. 1A,C,F**). However, concerning leaf area, *B. amyloliquefaciens* exhibited the highest average, while for F0 and FM, the combined application of B. *amyloliquefaciens* and A. *brasilense* yielded the highest mean values (**Fig. 1 B,D,E**).

To visually assess the golden trumpet tree seedlings (**Fig. 2**). Remarkably, at 107 days after sowing, the rhizobacterium B. *amyloliquefaciens* showcased superiority among all treatments.

DISCUSSION

The response of golden trumpet tree seedlings to rhizobacteria inoculation, particularly with *Bacillus amyloliquefaciens*, displayed superior performance across all assessed traits. *Azospirillum brasilense* also exhibited favorable results, underscoring the significant contribution of these two bacteria to the enhancement of seedling growth and quality. The promotion of plant growth by bacteria is influenced by various factors, primarily encompassing nutrient mobilization and solubilization, as well as phytohormone production (Ahemad and Kibret, 2014). While previous studies have highlighted the effective promotion of both plant quality and growth through the inoculation of *Bacillus subtilis* and *Bacillus megaterium* (Guimarães et al., 2021; Santos et al., 2021; Silva et al., 2022), it's worth noting that these species exhibited relatively modest outcomes in the present study. Notably, *Bacillus subtilis*, in particular, demonstrated lower mean values across most of the assessed characteristics.

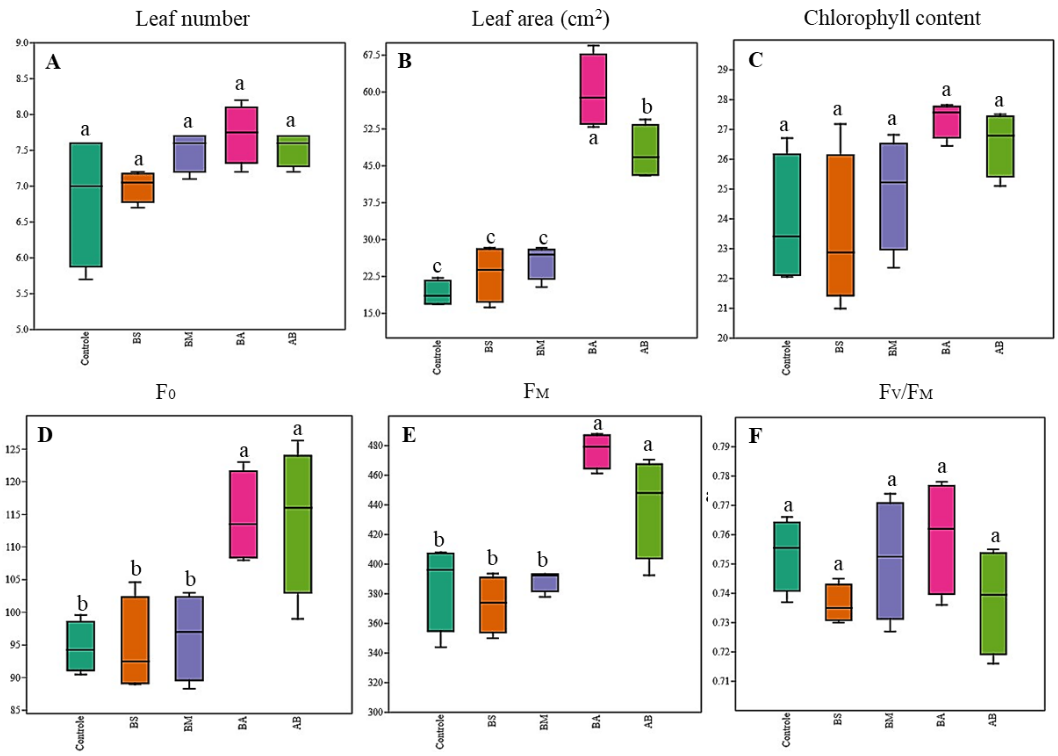

Figure 1. Boxplots of photosynthetic evaluation of *Handroanthus chrysotrichus* seedlings without inoculation (Control) and inoculated *with Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) and *Azospirillum brasilense* (AB); A) leaf number, B) leaf area, C) chlorophyll content, D) minimum fluorescence (F_0), E) maximum fluorescence (F_M), F) maximum quantum efficiency of photosystem II (F_V/F_M). Means followed by the same lowercase letter do not significantly differ by Tukey's test ($p < 0.05$). Jaboticabal, SP, Brazil, 2022.

Figure 2. *Handroanthus chrysotrichus* seedlings at 107 days after sowing. A) Control, B) *Bacillus subtilis*, C) *Bacillus megaterium*, D) *Bacillus amyloliquefaciens,* and E) *Azospirillum brasilense.* Jaboticabal, SP, Brazil, 2022.

The physiological variables examined in this study provide insights into the growth patterns of golden trumpet tree seedlings. Although there were no significant differences among treatments in terms of leaf number, the inoculation of B. *amyloliquefaciens* resulted in a larger leaf area. This observation suggests an enhanced photosynthetic potential among seedlings with increased surface area for light absorption, thereby facilitating greater carbon assimilation (Taiz et al., 2017). In contrast, the lower mean values for leaf area observed in the control group, as well as with B. subtilis and B. *megaterium* inoculations, contributed to reduced growth and development of the seedlings due to limited accumulation of light energy.

In fluorescence analyses, it's important to note that the minimum (F0) represents the fluorescence when all reaction centers are open, signifying the fluorescence emitted by the chlorophyll a molecules within the Photosystem II light harvesting complex. Conversely, the maximum fluorescence (FM) signifies the complete reduction of the primary quinone as a result of a light pulse incident on its reaction center, resulting in maximum fluorescence (Taiz et al., 2017). Consequently, the superior results achieved by the rhizobacteria B. *amyloliquefaciens* and A. *brasilense* in this study can be attributed to their capacity to establish a greater and more efficient photosynthetic activity under the same climatic conditions, thereby reducing the risk of photoinhibition damage, even though there were no discernible differences among treatments in terms of chlorophyll content. In a study conducted by Samaniego-Gámez et al. (2016), it was observed that the rhizobacterium B. *amyloliquefaciens*, in conjunction with B. *subtilis*, had a positive impact on the photosynthetic performance of *Capsicum chinense* Jacq. Additionally, Gonzalez et al. (2018) that the inoculation of A. *brasilense* with *Prosopis articulata* S. Watson resulted in an increase in chlorophyll levels in 25% of the leaves.

CONCLUSIONS

Based on the results obtained, rhizobacteria exerted a significant influence on the growth, quality, and photosynthetic capacity of the studied golden trumpet tree species. Consequently, the utilization of these beneficial microorganisms in the cultivation of golden trumpet tree seedlings holds great promise.

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