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ARTICLE

Rhizobacteria in the quality of *Tabebuia* spp. seedlings

Rizobactérias na qualidade de mudas de *Tabebuia* spp.

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Abstract
White trumpet tree (*Tabebuia roseoalba*) and pink trumpet tree (*Tabebuia rosea*) have ecological and ornamental importance and are used in landscaping and environmental restoration programs. However, the cultivation of high-quality seedlings for these species still faces limitations related to root development, vigor, and nutritional management, thereby promoting the use of bacteria as a sustainable strategy to enhance plant performance. In this scenario, the study aimed to determine the effects of single and combined applications of *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* on white and pink trumpet tree seedling growth. Eight treatments were arranged in a completely randomized design, four replications, and ten plants per plot. Characteristics such as height, root length, stem diameter, leaf area, chlorophyll content, dry mass, and Dickson Quality Index were evaluated and means were compared by Tukey's test ($p \leq 0.05$). For the two species evaluated, the use of rhizobacteria promoted greater growth and seedling quality. For *T. roseoalba*, the triple bacterial combination (*A. brasilense* + *B. amyloliquefaciens* + *B. subtilis*) provided the most effective results, followed by the isolated inoculation with *B. subtilis*. For *Tabebuia rosea*, the triple bacterial combination (*A. brasilense* + *B. amyloliquefaciens* + *B. subtilis*) also provided the most effective results, followed by the combinations of *A. brasilense* + *B. amyloliquefaciens* and *A. brasilense* + *B. subtilis*. Thus, microbial inoculation represents an effective and sustainable alternative for the production of higher-quality ornamental forest seedlings.
Keywords: *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, nursery, sustainability.

Resumo
O ipê-branco (*Tabebuia roseoalba*) e o ipê-rosa (*Tabebuia rosea*) apresentam relevância ecológica e ornamental, sendo amplamente utilizados em programas de paisagismo e recuperação ambiental. Entretanto, produzir mudas de qualidade para essas espécies ainda possui limitações relacionadas ao desenvolvimento radicular, vigor e manejo nutricional, o que tem estimulado o uso de bactérias como alternativa sustentável para melhoria do desempenho das mudas. Nesse contexto, o presente estudo objetivou avaliar o efeito da inoculação de *Azospirillum brasilense*, *Bacillus amyloliquefaciens* e *Bacillus subtilis*, aplicadas isoladamente e em combinações, na formação de mudas de ipê-branco e ipê-rosa. Os experimentos foram conduzidos em delineamento inteiramente casualizado; foram oito tratamentos, quatro repetições e dez mudas por parcela. Foram avaliadas características como altura, comprimento radicular, diâmetro do colo, área foliar, teor de clorofila, massa seca e Índice de Qualidade de Dickson. As médias foram comparadas pelo teste de Tukey ($p \leq 0,05$). Para ambas as espécies, a aplicação de rizobactérias proporcionou maior crescimento e qualidade das mudas. Para *T. roseoalba*, a combinação tripla de bactérias (*A. brasilense* + *B. amyloliquefaciens* + *B. subtilis*) proporcionou resultados mais efetivos seguido da inoculação isolada com *B. subtilis*. Para *Tabebuia rosea*, a combinação tripla de bactérias (*A. brasilense* + *B. amyloliquefaciens* + *B. subtilis*) também proporcionou resultados mais efetivos seguido das combinações de *A. brasilense* + *B. amyloliquefaciens* e *A. brasilense* + *B. subtilis*. O uso desses microrganismos, portanto, representa alternativa efetiva e sustentável para a produção de mudas florestais ornamentais de maior qualidade.
Palavras-chave: *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, sustentabilidade, viveiro.

Introduction

The species *Tabebuia roseoalba* and *Tabebuia rosea* are highly valued and frequently used in Brazilian landscaping. *Tabebuia roseoalba* (Ridl.) Sandwith, popularly known as white trumpet tree, is a tree native to South America, with wide distribution in Brazil, particularly in the Cerrado, Caatinga, and Atlantic Forest biomes, with occurrences also in Argentina, Paraguay, and Uruguay (Lorenzi et al., 2016). The species can reach between 7 and 30 meters in height; it presents a straight trunk with corky bark, and its trifoliate compound leaves fall during the dry season, leaving the tree covered by its exuberant flowering of white trumpet-shaped flowers, which generally occurs between July and September (Johanes et al., 2022; Lohmann, 2020).

From an ecological perspective, *T. roseoalba* is classified as a pioneer to early secondary species, with relatively rapid growth, high tolerance to full sunlight, and its capacity to colonize open and degraded areas, acting in soil protection against erosion, nutrient cycling, and the creation of a favorable microclimate for the regeneration of other species. Furthermore, because it flowers during the dry season, over a period of natural resource scarcity, it represents an important source of nectar for pollinators, contributing to biodiversity conservation throughout ecosystems where it occurs (Lorenzi et al., 2016).

Popularly known as pink trumpet tree, *Tabebuia rosea* (Bertol.) Bertero ex A. DC. is a tree belonging to the Bignoniaceae family, native to the Caribbean islands and tropical regions of Central America and northern South America, widely cultivated in Brazil as an ornamental species and in urban forestry and landscaping projects (Lorenzi et al., 2003). The nomenclature adopted follows that used by Herrera (2025) and Plants of the World Online (POWO) of the Royal Botanic Gardens, Kew, which recognizes *T. rosea* as the accepted name for the species, with *T. pentaphylla* listed as a synonym (POWO, 2026).

The species *T. rosea* presents a dense canopy formed by palmately compound leaves with five dark-green, coriaceous, and rigid leaflets (Herrera, 2025). One of the main characteristics of the species, according to Lorenzi et al. (2003), is that its spectacular flowering, in intense pink tones, generally occurs while the tree is still bearing leaves, distinguishing it from other trumpet trees that flower after leaf fall. The flowers attract pollinators such as bees, butterflies, and hummingbirds, contributing to local biodiversity.

Although the ecological and economic importance of these species is recognized, the production of high-quality seedlings in nurseries still faces challenges, such as the standardization of cultivation conditions, substrate nutritional management, and the guarantee of plants with well-developed root systems and sufficient biomass to withstand field conditions (Toca et al., 2022). In this scenario, the search for sustainable alternatives capable of reducing dependence on chemical inputs without compromising seedling vigor and quality has driven research on the use of bioinputs based on plant growth-promoting bacteria (PGPB).

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These microorganisms colonize the rhizosphere and establish beneficial interactions through associations with host plants, exerting direct and indirect effects on plant growth. (Singh et al., 2019). Among the direct mechanisms are atmospheric nitrogen fixation, inorganic phosphate solubilization, production of phytohormones such as auxins, gibberellins, and cytokinins, synthesis of siderophores, and activity of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces ethylene levels and, consequently, plant stress (Liu et al., 2025).

Indirect mechanisms involve pathogen suppression through the production of antimicrobial compounds, competition for niches and nutrients, and induction of systemic resistance in the host plant (Prisa and Jamal, 2025). The incorporation of PGPB into agricultural and forestry practices offers considerable potential for sustainable production, contributing to reduced use of mineral fertilizers and agrochemicals, improved soil health, and increased seedling vigor (Upadhyay et al., 2022).

Among the bacterial groups most frequently evaluated for these purposes are *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis*, which have shown promising results in ornamental and forest species (Campos et al., 2024, 2025). The beneficial effects associated with *A. brasilense* are mainly attributed to its ability to stimulate root architecture through the production of plant-growth regulators and to contribute to nitrogen acquisition. Moreover, this bacterium may increase phosphorus availability and favor plant development under conditions of limited nutrient supply (Fukami et al., 2018; Sun et al., 2022).

In contrast, *B. amyloliquefaciens* is frequently associated with improvements in nutrient cycling, root growth, and stress tolerance due to the synthesis of bioactive metabolites (Luo et al., 2022). Likewise, *B. subtilis* is recognized for enhancing nutrient uptake and suppressing phytopathogens through mechanisms related to biological control and siderophore production (Hashem et al., 2019).

Accordingly, this research examined whether isolated or combined applications of *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* could improve the morphophysiological performance and overall quality of *Tabebuia roseoalba* and *Tabebuia rosea* seedlings produced under nursery conditions.

Material and Methods

Independent experiments were established for each species following identical methodological procedures. The experiments were conducted from October 22 to December 3, 2025, encompassing the period from sowing to the final evaluation. The experimental arrangement comprised eight bacterial inoculation strategies: 1: (control without inoculation), 2: (*Azospirillum brasilense*), 3: (*Bacillus amyloliquefaciens*), 4: (*Bacillus subtilis*), 5: (*A. brasilense* + *B. amyloliquefaciens*), 6: (*A. brasilense* + *B. subtilis*), 7: (*B. amyloliquefaciens* + *B. subtilis*), and 8: (*A. brasilense* + *B. amyloliquefaciens* + *B. subtilis*). Treatments were distributed in a completely randomized design with four replicates composed of ten seedlings each. The mean value of the ten seedlings within each replicate was used for statistical analysis, with each replicate considered the experimental unit.

Bacterial suspensions were prepared separately before application and adjusted to a concentration of 1×10^8 CFU mL^{-1}. Seed inoculation was performed at sowing and repeated at two-week intervals during the cultivation period. At each application, 1 mL of the respective bacterial suspension was supplied to each container. For combined treatments, 1 mL of each bacterial suspension was applied to each container; therefore, treatments containing two bacteria received a total of 2 mL of bacterial suspension, whereas the treatment containing three bacteria received a total of 3 mL. The inoculation methodology was adapted from Silveira et al. (2025). Seedlings assigned to the control treatment received no bacterial inoculation.

The experiments were established inside a polyethylene-covered greenhouse. Environmental conditions were monitored throughout the cultivation period using a digital thermo-hygrometer. Measurements of air temperature and relative humidity were recorded twice daily, and weekly mean values were subsequently calculated (Fig. 1).

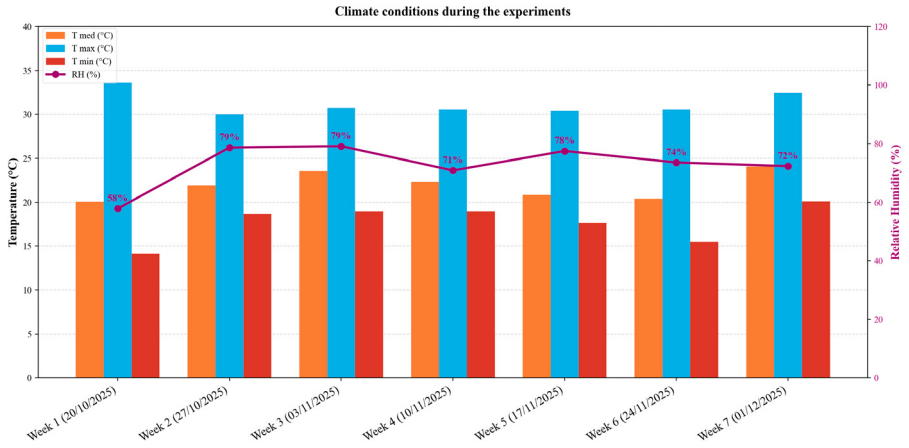


Fig. 1. Mean weekly climatic data referring to average temperature (T med), maximum temperature (T max), and minimum temperature (T min) in °C, and relative humidity (RH) in %.

Seeds were sown in polypropylene trays containing 54 plastic tubes, each with a volume of 280 cm^3. The containers were filled with a commercial substrate (Carolina Soil®) formulated with sphagnum peat, vermiculite, carbonized rice husk, dolomitic limestone, mineral fertilizer, and micronutrients. After emergence, seedlings were thinned to maintain only one healthy and centrally positioned individual per container.

Water was provided through an automatic micro-sprinkler system activated three times daily. Each irrigation cycle lasted 15 min, corresponding to a discharge rate of approximately 30 L h^{-1}.

Seedling evaluations began when roots reached the lower portion of the containers, indicating adequate development for biometric analysis. The variables measured included shoot height, stem diameter, number of leaves, leaf area, chlorophyll index, and root length. Height was measured from the substrate surface to the apex of the youngest fully expanded leaf. Stem diameter was recorded at the collar region using a digital caliper. Root length was determined after careful removal of the substrate surrounding the root system. Leaf area was measured with a LI-COR® leaf area meter, while chlorophyll content was estimated using a portable chlorophyll meter.

To determine biomass accumulation, shoots and roots were separated and oven-dried at 70 °C until reaching constant weight. Root dry mass, shoot dry mass, and total dry mass were then recorded using an analytical balance. Seedling quality was subsequently estimated through the Dickson Quality Index (DQI).

Data from each species were evaluated independently using analysis of variance procedures. Treatment means were separated by Tukey's multiple comparison test whenever the F-test indicated significance at the 5% probability level. Statistical analyses were performed with AgroEstat software (Barbosa and Maldonado Junior, 2015). In addition, violin plots were generated in Python to illustrate data distribution and treatment effects.

Results

For *T. roseoalba* (white trumpet tree), bacterial inoculation generally promoted better seedling performance than the uninoculated treatment (Fig. 2). The control group displayed lower values for most measured attributes, whereas leaf number (Fig. 2c) and leaf area (Fig. 2d) remained unaffected by the inoculation treatments.

Among inoculated seedlings of *T. roseoalba*, shoot height (Fig. 2a) and root length (Fig. 2f) showed comparable responses, with all bacterial treatments surpassing the control. The consortium composed of *A. brasilense*, *B. amyloliquefaciens*, and *B. subtilis* produced the highest chlorophyll index (Fig. 2e), shoot dry mass (Fig. 2h), and total dry mass (Fig. 2i). For stem diameter (Fig. 2b), root dry mass (Fig. 2g), and Dickson Quality Index (Fig. 2j), the triple consortium and the isolated application of *B. subtilis* generated the most favorable results.

A comparable trend was observed for *T. rosea* (pink trumpet tree), where inoculated seedlings generally achieved greater values than the non-inoculated control (Fig. 3). Significant treatment effects were detected for most variables, whereas stem diameter (Fig. 3b) and chlorophyll index (Fig. 3e) remained statistically unchanged among treatments.

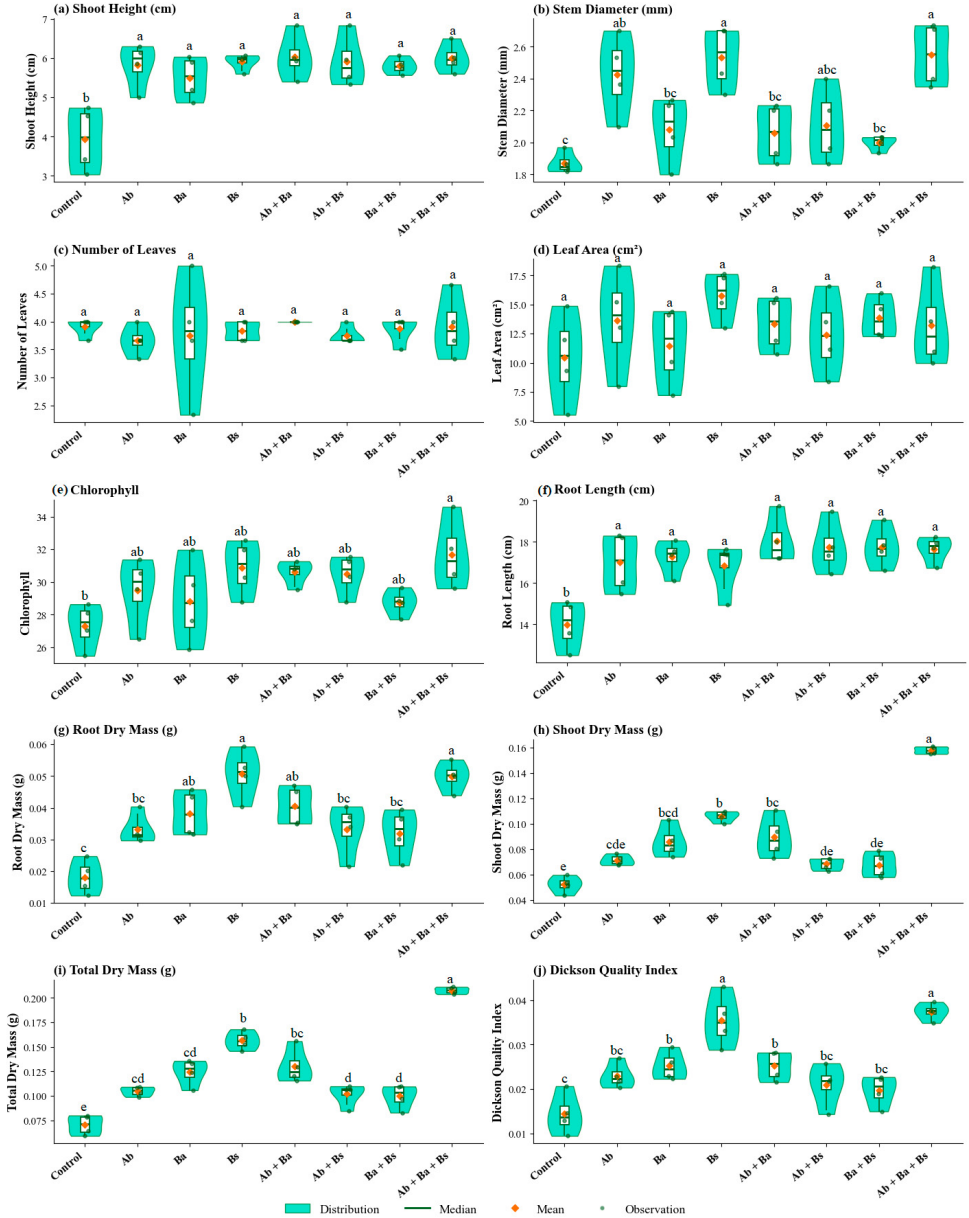


Fig. 2. Growth and quality parameters of *Tabebuia roseoalba* seedlings following inoculation with *Azospirillum brasilense* (Ab), *Bacillus amyloliquefaciens* (Ba), and *Bacillus subtilis* (Bs), applied individually or in combination. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

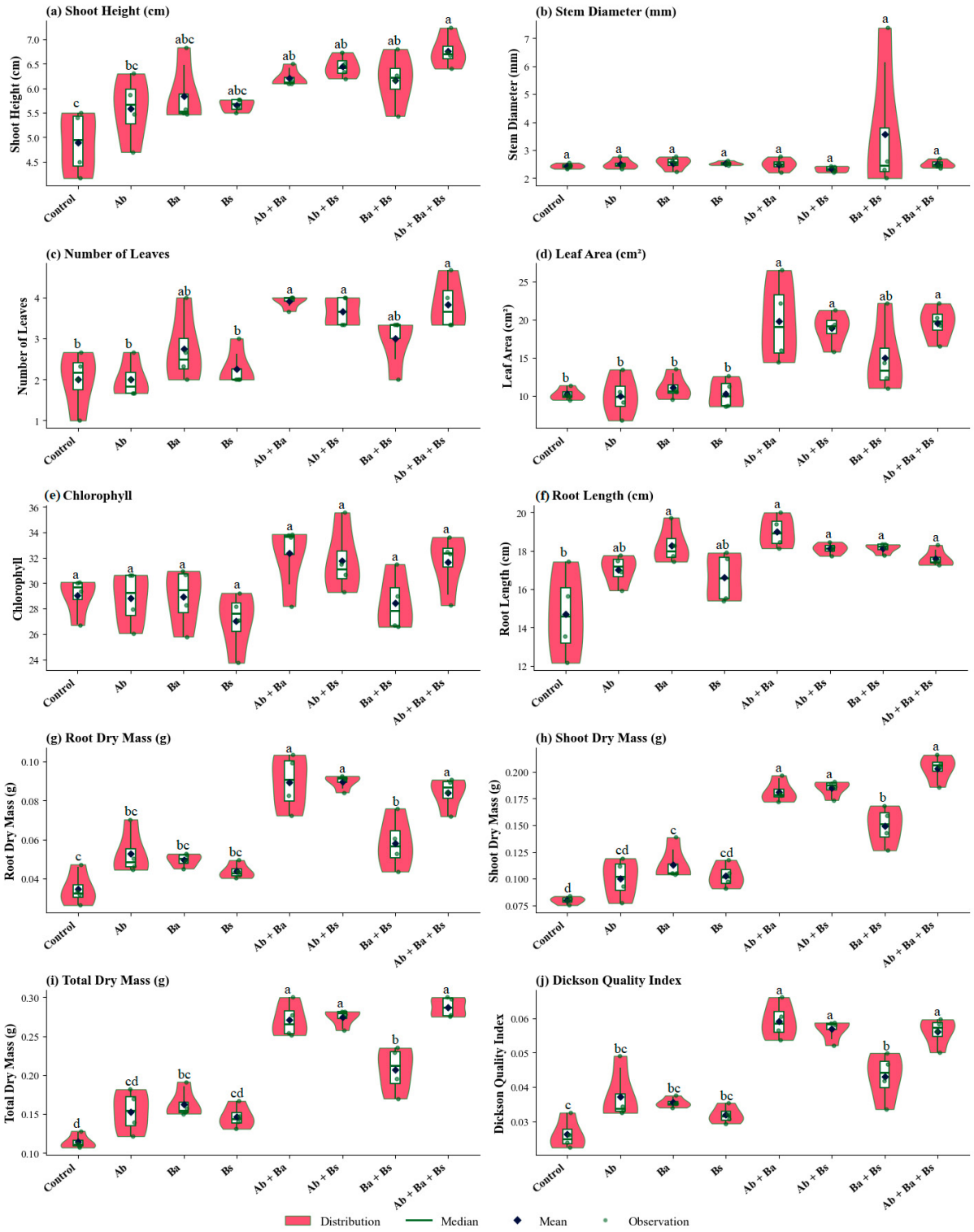


Fig. 3. Growth and quality parameters of *Tabebuia rosea* seedlings under individual and combined bacterial inoculations of *Azospirillum brasilense* (Ab), *Bacillus amyloliquefaciens* (Ba), and *Bacillus subtilis* (Bs). Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

The three-species consortium resulted in the greatest shoot height values for *T. rosea* seedlings (Fig. 3a). For leaf number (Fig. 3c), leaf area (Fig. 3d), root dry mass (Fig. 3g), shoot dry mass (Fig. 3h), total dry mass (Fig. 3i), and Dickson Quality Index (Fig. 3j), similar responses were obtained with the triple consortium and with the combinations *A. brasilense* + *B. amyloliquefaciens* and *A. brasilense* + *B. subtilis*. Root elongation (Fig. 3f) was enhanced by the three-species consortium, the two combinations involving *A. brasilense*, and the isolated inoculation with *B. amyloliquefaciens*, all of which outperformed the remaining treatments.

Discussion

For both *Tabebuia* species, inoculation with rhizobacteria resulted in greater shoot development (Figs. 2a and 3a) and enhanced root elongation (Figs. 2f and 3f). This behavior may be associated with mechanisms commonly described for rhizobacteria, especially in processes related to phytohormone production, particularly auxins, which stimulate cell division and elongation, favoring root growth and, consequently, greater substrate exploration (Fukami et al., 2018; Upadhyay et al., 2022).

Stem diameter presented higher values in the treatments with isolated *B. subtilis* and with the combination of all bacteria for *T. roseoalba* (Fig. 2b). The increase in this variable is particularly relevant in forest species, since seedlings with larger stem diameter tend to present greater tolerance to water stress and better field establishment, and this parameter is widely used as an indicator of forest seedling quality (Souza et al., 2022).

The treatment containing the three rhizobacteria promoted the highest chlorophyll content for *T. roseoalba* (Fig. 2e), which may be associated with differences in plant nutritional status; however, no significant differences were observed for *T. rosea* (Fig. 3e).

For number of leaves (Fig. 2c) and leaf area (Fig. 2d), *T. rosea* responded more efficiently to bacterial inoculation than *T. roseoalba*. However, although no differences were observed for these characteristics in *T. roseoalba*, a significant increase in biomass accumulation was verified (Figs. 2g, h, and i), mainly in the treatments containing *B. subtilis* and the combination of all three bacteria. The combination of all bacteria promoted the highest values for shoot dry mass, total dry mass, and DQI, while isolated *B. subtilis* presented similar performance for root dry mass and DQI.

For *T. rosea*, bacterial inoculation exerted a stronger influence on vegetative expansion. The combinations involving *A. brasilense*, either with *B. amyloliquefaciens* or *B. subtilis,* as well as the triple consortium, consistently promoted greater leaf production, larger leaf area, increased biomass accumulation, and higher DQI values (Figs. 3c, 3d, and 3g–j). The expansion of leaf area is physiologically important because it enlarges the surface available for light interception and carbon assimilation, thereby increasing photoassimilate production (Scudeletti et al., 2023). This mechanism likely contributed to the greater biomass accumulation observed in this species.

Root-related traits were also strongly influenced by bacterial inoculation. In both species, increases in root length and root dry mass were evident when compared with the control treatment (Figs. 2f, 2g, 3f, and 3g). Such responses are highly desirable in container-grown forest seedlings, as root development directly determines the efficiency of water and nutrient uptake and plays a fundamental role in establishment after transplanting (Satria et al., 2026).

The largest values of shoot and total dry mass were generally associated with combined inoculations rather than single-strain applications. In *T. roseoalba*, the three-species consortium produced the most expressive gains (Figs. 2h and 2i), whereas in *T. rosea* similar responses were obtained with the triple consortium and with the two combinations containing *A. brasilense* (Figs. 3h and 3i). These treatments also generated the highest DQI values (Figs. 2j and 3j), indicating the production of seedlings with a more balanced biomass distribution and greater overall vigor. The observed responses to combined inoculations may be associated with interactions among rhizobacteria and their complementary effects on plant development, as proposed by Oliveira et al. (2025). However, differences in the total inoculum volume among treatments should also be considered when interpreting these responses.

Because DQI integrates several morphological and biomass-related variables into a single index, it is regarded as one of the most reliable indicators of seedling quality (Souza et al., 2022). Therefore, the higher DQI values obtained in inoculated treatments demonstrate that bacterial inoculation improved not only growth but also the structural balance of the seedlings.

The favorable performance of treatments containing *B. subtilis* in *T. roseoalba* may be explained by the biological characteristics of this bacterium. Previous studies have highlighted its ability to mobilize phosphorus, produce siderophores, increase nutrient uptake efficiency, and synthesize metabolites capable of stimulating plant development (Hashem et al., 2019; Mani et al., 2025). These mechanisms may have contributed to the superior biomass production and quality indices observed in the present study, although they were not directly evaluated.

The findings obtained here are consistent with previous reports involving rhizobacterial inoculation in ornamental and forest species. Campos et al. (2024; 2025) described improvements in growth and seedling quality of *Handroanthus chrysotrichus* and *H. impetiginosus* following bacterial inoculation, attributing these responses to enhanced nutrient availability and hormone-mediated stimulation within the rhizosphere. The present results extend this evidence by demonstrating that microbial consortia can provide additional benefits beyond those obtained with isolated strains, particularly in *T. rosea*.

Observations obtained from the photographic records were consistent with the numerical results (Fig. 4). Seedlings subjected to bacterial inoculation displayed more developed root systems, characterized by greater ramification and apparent substrate exploration capacity than those observed in the control treatment. These characteristics were especially noticeable in the combined treatments, which also exhibited superior values for root growth and quality-related variables. The visual differences reinforce the positive influence of rhizobacteria on seedling establishment potential.

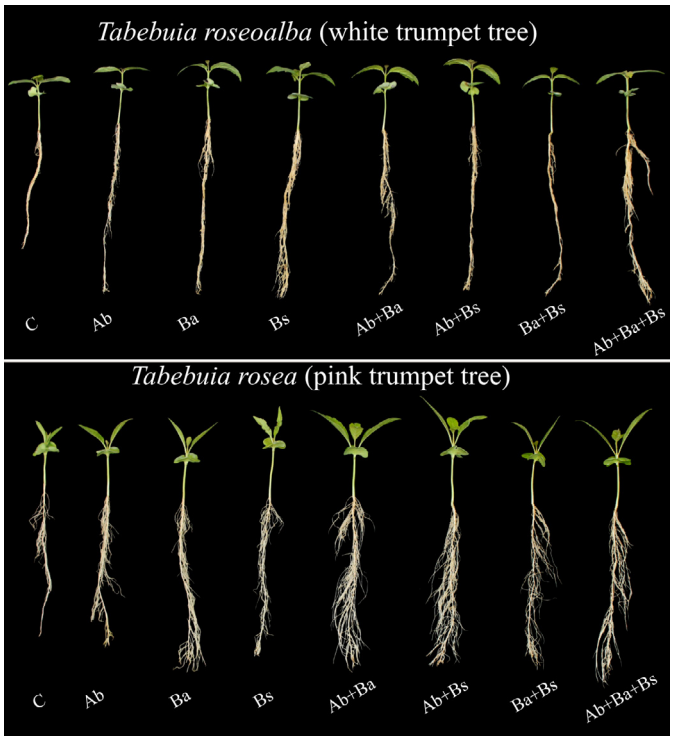


Fig. 4. Morphological appearance of *Tabebuia roseoalba* and *Tabebuia rosea* seedlings under the evaluated inoculation treatments with *Azospirillum brasilense* (Ab), *Bacillus amyloliquefaciens* (Ba), and *Bacillus subtilis* (Bs).

For *T. roseoalba*, treatment effects were more clearly expressed belowground than in shoot morphology. Seedlings inoculated with *B. subtilis*, either alone or combined with other bacteria, exhibited root systems with greater uniformity and apparent thickness. The consortium composed of the three bacterial species produced the most extensive root architecture, including a higher abundance of fine roots, a characteristic commonly associated with improved resource acquisition. In *T. rosea*, morphological differences among treatments were more evident. Compared with the control, seedlings receiving bacterial combinations showed more vigorous shoot growth and a substantially larger root system.

Treatments containing *A. brasilense* generally produced the most expressive responses among the evaluated inoculation strategies. This outcome may be associated with the functional complementarity established between microorganisms presenting different ecological roles in the rhizosphere. While *A. brasilense* contributes mainly through nitrogen-related processes and stimulation of root growth via phytohormones, *Bacillus* species are frequently linked to nutrient mobilization, siderophore synthesis, and antagonism against soilborne microorganisms (Fukami et al., 2018; Hashem et al., 2019; Luo et al., 2022). The simultaneous occurrence of these processes may have favored plant development more efficiently than isolated inoculations. Similar trends have been documented by Kisvarga et al. (2022) and Oliveira et al. (2025), who emphasized the relevance of combining microorganisms with distinct functional attributes.

Conclusions

Rhizobacterial inoculation positively influences the growth and quality of *Tabebuia roseoalba* and *Tabebuia rosea* seedlings under nursery conditions.

For *T. roseoalba*, the most consistent responses are obtained with the consortium formed by *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis*, as well as with the isolated application of *B. subtilis*, resulting in greater biomass accumulation and improved DQI values.

For *T. rosea*, bacterial associations containing *A. brasilense* promote improvements in vegetative growth, root development, biomass production, and overall seedling quality, highlighting the advantages of combined inoculations over individual applications.

The findings indicate that bacterial inoculation has potential to improve the growth and quality of *T. roseoalba* and *T. rosea* seedlings under nursery conditions. Further studies using standardized total inoculum doses are needed to distinguish the effects of bacterial combinations from those associated with differences in total inoculum dose.

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Author contribution

JPAF: Conceptualization, Data Curation, Investigation, Methodology, Visualization, Writing – Original Draft. **MMS:** Data Curation, Investigation, Validation, Writing – Review & Editing. **ACB:** Conceptualization, Investigation, Methodology, Validation, Writing – Review & Editing. **AMBS:** Data Curation, Formal Analysis, Visualization, Writing – Review & Editing. **GLQ:** Formal Analysis, Writing – Review & Editing. **ETF:** Data Curation, Investigation, Methodology, Resources, Validation. **ECR:** Investigation, Methodology, Resources, Validation. **KFLP:** Conceptualization, Formal Analysis, Methodology, Project Administration, Supervision, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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