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ARTICLE

Growth and quality of *Cariniana estrellensis* seedlings in response to two bioinputs: rhizobacteria and *Ascophyllum nodosum* extract

Crescimento e qualidade de mudas de *Cariniana estrellensis* em resposta a dois bioinsumos: rizobactérias e extrato de *Ascophyllum nodosum*

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Abstract
Jequitibá-branco is a tree species native to Brazil, widely used in landscaping of squares and parks as well as in reforestation programs. Successful plant establishment in urban environments depends on high-quality seedlings, whose performance can be improved through the use of biostimulants such as plant growth-promoting rhizobacteria and seaweed extracts. Thus, the objective of this research was to study the effects of rhizobacteria and the seaweed extract of *Ascophyllum nodosum* in seedling production of *Cariniana estrellensis* (jequitibá-branco). Two experiments were conducted; in the first, there were six treatments: individual inoculation of the bacteria *Azospirillum brasilense*, *Bacillus amyloliquefaciens, B. pumilus* and *B. subtilis* and the control (absence of inoculation); in the second, there were five treatments: four concentrations of *A. nodosum* extract (3, 6, 9, 12 and 15 mL L^{-1}) and control (without application of the extract). For both experiments, the experimental design was completely randomized; there were four replications and five seedlings per plot. The following characteristics were evaluated: shoot length and root system length, stem diameter, number of leaves, leaf area, shoot dry mass, and root dry mass. The total dry mass and Dickson Quality Index were also calculated. The results obtained were subjected to analysis of variance. It was concluded that the use of the rhizobacteria *A. brasilense*, *B. amyloliquefaciens* and *B. subtilis*, as well as *A. nodosum* extract at a concentration of 6 mL L^{-1}, were the most effective for the production of jequitibá-branco seedlings.
Keywords: Bioinputs, native tree species, rhizosphere, seedling production, seedling quality.

Resumo
O jequitibá-branco é uma espécie nativa do Brasil, utilizada no paisagismo de praças, parques e em reflorestamentos. O sucesso do desenvolvimento das plantas no meio urbano é dependente de mudas de alta qualidade que podem ser incrementadas com o uso de bioestimulantes como as rizobactérias promotoras de crescimento em plantas e os extratos de algas marinhas. Assim, o objetivo desta pesquisa foi estudar os efeitos de rizobactérias e do extrato da alga *Ascophyllum nodosum* na produção de mudas de *Cariniana estrellensis* (jequitibá-branco). Foram dois experimentos; no primeiro, foram seis tratamentos: inoculação individual das bactérias *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *B. megaterium*, *B. pumilus* e *B. subtilis* e o controle (sem inoculação); no segundo, foram seis tratamentos: cinco concentrações de extrato de *A. nodosum* (3, 6, 9, 12 e 15 mL L^{-1}) e o controle (sem aplicação do extrato). Para ambos os experimentos o delineamento experimental foi o inteiramente casualizado; foram quatro repetições e cinco mudas por parcela e avaliou-se as seguintes características: comprimento da parte aérea e do sistema radicular, diâmetro do colo, número de folhas, área foliar, massa seca da parte aérea e de raízes; foram calculados a massa seca total e o Índice de Qualidade de Dickson. Os resultados obtidos foram submetidos à análise de variância. Concluiu-se que o uso das rizobactérias *A. brasilense*, *B. amyloliquefaciens* e *B. subtilis* e o extrato de *A. nodosum* nas concentrações acima de 6 mL L^{-1} foram mais eficientes para a produção de mudas de jequitibá-branco.
Palavras-chave*:* Bioinsumos, espécie nativa, produção de mudas, qualidade de mudas, rizosfera.

Introduction

Trees have great ornamental value and are widely used in landscaping and urban afforestation. When choosing species for urban environments, it is recommended to prioritize those natives to the region; they not only offer superior ecological balance but also provide food and shelter for local fauna. Furthermore, these trees are generally well adapted to the climate and specific conditions of the local environment, which favors vigorous and healthy growth (Batista et al., 2022; Dardengo et al., 2022).

Commonly known as jequitibá-branco, the species *Cariniana estrellensis* (Raddi) Kuntze belongs to the Lecythidaceae family, reaches a height of 35 to 45 m, with an erect trunk; the leaves are simple, elliptical to obovate; the flowers are cream-colored, fragrant and arranged in racemes; and the fruits are ellipsoid, woody pyxidia containing winged seeds. The species is native to Brazil, occurring from southern Bahia to Rio Grande do Sul, in the Atlantic Forest and semi-deciduous forests, and may also appear in gallery forests in Acre and central Brazil, being of ecological, ornamental and economic importance, with applications in urban landscaping, ecological restoration and timber production (Lorenzi, 2016).

The successful establishment of vigorous trees, whether in urban environments or reforestation projects depends, in part, on the availability of high-quality seedlings, therefore, investigating additional strategies to improve seedling development may contribute to more efficient nursery production and support the use of this native species in landscaping and reforestation.

Among these strategies, plant growth-promoting bacteria (PGPB) have shown potential to enhance plant development through mechanisms such as phytohormone production, improved nutrient acquisition, and suppression of plant pathogens (Ehinmitan et al.; 2024; Giri et al., 2025). Bacteria belonging to the genera of *Azospirillum* and *Bacillus* have been investigated for their plant growth-promoting potential. Tiepo et al. (2018) demonstrated that *Azospirillum brasilense* inoculation improved photosynthetic performance and reduced oxidative damage in *C. estrellensis* seedlings under water deficit conditions. Calzavara et al. (2021), on the other hand, observed that the responses to bacterial inoculation may not achieve significant improvements in the growth of this species, indicating that the beneficial effects may depend on cultivation conditions.

Seaweed extracts represent another bioinput approach, with *Ascophyllum nodosum* rising in importance, being widely investigated for its potential to regulate physiological and biochemical processes associated with plant growth, nutrient acquisition, and stress responses (Kumari et al., 2023). In native tree seedlings, Bernardes et al. (2023) reported that *A. nodosum* extract positively affected growth and quality of *Alibertia edulis*,

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including increases in root biomass. These findings indicate that seaweed extracts may contribute to nursery seedling development, although their effects on the growth and morphological quality of *C. estrellensis* remain insufficiently documented.

Although PGPB and seaweed extracts differ in origin and modes of action, both may influence root development, biomass accumulation, and other morphological characteristics associated with seedling quality. Thus, investigating both distinct biostimulants addresses a common question regarding their potential to improve the production of *C. estrellensis* seedlings. It was hypothesized that bacterial inoculation and *A. nodosum* extract application, evaluated independently, could promote seedling growth and morphological quality under nursery conditions. Therefore, this study aimed to evaluate, in two independent experiments, the effects of selected plant growth-promoting rhizobacteria and increasing concentrations of *A. nodosum* extract on the growth and quality of *C. estrellensis* seedlings.

Material and Methods

Two independent experiments were carried out in a greenhouse located at the Experimental Nursery of Ornamental and Forest Plants, belonging to the Department of Plant Production, School of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, São Paulo State, Brazil. The greenhouse roof and sides were covered by black shade cloth providing 50% shading, in addition to an upper layer of transparent plastic above the shade covering.

The first experiment, conducted from September to October 2024, evaluated the effect of plant growth-promoting rhizobacteria (PGPR), while the second, conducted from November 2025 to February 2026, investigated the effect of the extract of the seaweed *Ascophyllum nodosum*.

Both experiments followed a completely randomized design, with six treaments, four replications, and five seedlings per plot. Meteorological data corresponding to each experimental period was obtained from the UNESP Agrometeorological Station (UNESP, 2026) (Fig. 1).

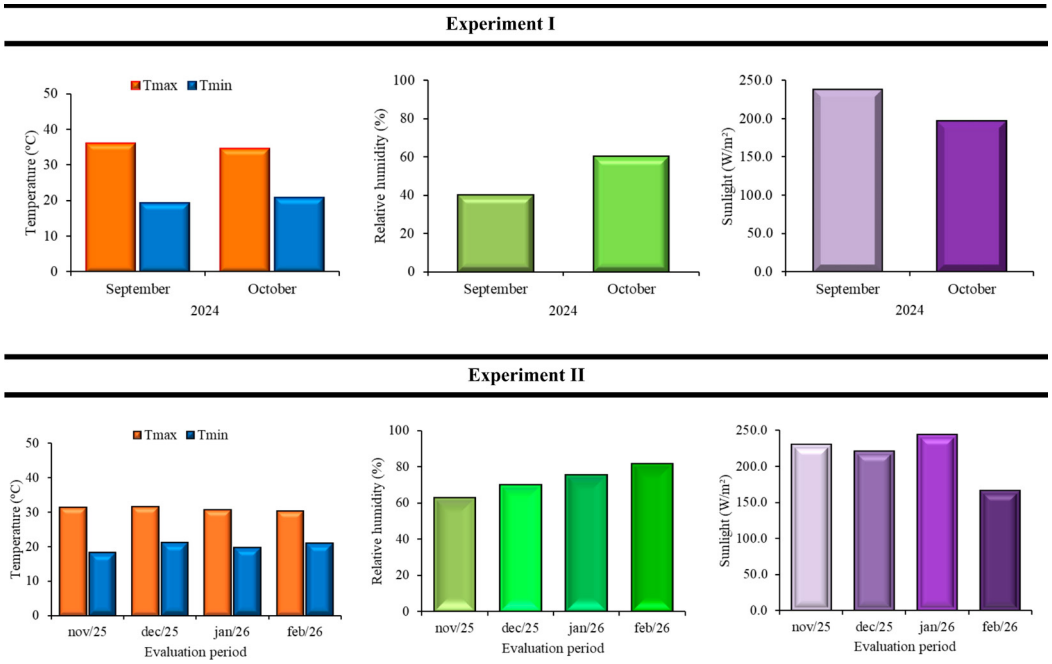

Fig. 1. Climatic data (mean temperature, relative humidity, and sunlight) during the periods from September to October 2024 (Experiment I) and from November 2025 to February 2026 (Experiment II).

The experiments were carried out under different propagation conditions. Experiment I involved seedlings grown from seeds in 280 cm³ tubes containing commercial substrate, whereas Experiment II used previously established seedlings transplanted into 0.4 L plastic bags containing a mixture of subsoil and cattle manure. The experiments also differed in their cultivation period and evaluation times. Therefore, the effects of each bioinput were analyzed independently, without direct statistical comparisons between experiments.

Effect of rhizobacteria

Six treatments were evaluated (1. Absence of microorganisms - control; 2. *Azospirillum brasilense*; 3. *Bacillus amyloliquefaciens*; 4. *B. megaterium*; 5. *B. pumilus*; 6. *B. subtilis*), with four replications and five seedlings per plot (Luo et al., 2022; Dobrzyński et al., 2022; Lee et al., 2024; Ayaz et al., 2024).

Jequitibá-branco seeds were sown in tubes with a volumetric capacity of 280 cm³, arranged in polypropylene trays with the capacity for 54 containers, containing the commercial substrate Carolina Soil®, composed of peat, vermiculite, carbonized rice husk, calcined dolomitic limestone, NPK fertilizer (14-16-18) and micronutrients, according to the manufacturer's specifications. The trays were kept suspended on metal mesh benches at 70 cm above the ground.

Three seeds were distributed per tube and, after emergence, thinning was performed to retain one seedling per container, using as criterion the most vigorous and centrally positioned seedling. Irrigation of the seedlings was performed using micro-sprinklers, activated automatically three times a day, with a duration of 15 minutes each and a flow rate of 30 L h^{-1}.

The microorganisms were produced separately in nutrient broth medium, following the methodology used by Silveira et al. (2025). Inoculum concentrations were standardized to 1 x 10^7 colony-forming units (CFU) mL^{-1} using spectrophotometry (Micronal, model B382, Brazil) at a wavelength of 695 nm. Bacterial inoculation was performed immediately after sowing by applying 1 mL of the solution to the substrate, at the center of the tube, using a mechanical micropipette (VF-1000, Digipet®). Seedlings assigned to the control treatment were not inoculated.

Effect of seaweed extract (*Ascophyllum nodosum*)

Six treatments were evaluated: five concentrations of the extract (3, 6, 9, 12 and 15 mL L^{-1} of water) and the control (without application of the extract), with four replications and five seedlings per plot. (Bernardes et al., 2023)

The extract of the seaweed *A. nodosum* was commercially acquired, and the application and concentrations were based on Ferreira et al. (2025). Three applications of the respective solution concentration were performed via drenching: at the time of transplanting and at 30 and 60 days thereafter.

Seedlings with a height of 18±1 cm were transplanted into plastic bags with a volumetric capacity of 0.4 L, containing as substrate a mix of subsoil + cattle manure (3:1). The substrate showed pH 6.9 ($CaCl_2$), organic matter content of 33 g dm^{-3}, and available P and S contents of

152 and 92 mg dm^{-3}, respectively. Exchangeable cation contents were 32, 18, and 12.8 mmolc dm^{-3} for Ca, Mg, and K, respectively, while Al content was low (1 mmolc dm^{-3}), resulting in low aluminum saturation (m = 1%) and high base saturation (V = 81%). The cation exchange capacity was 77.7 mmolc dm^{-3} and the sum of bases was 63.3 mmolc dm^{-3}. Micronutrient contents were 0.83 mg dm^{-3} B, 2.0 mg dm^{-3} Cu, 33 mg dm^{-3} Fe, 18.4 mg dm^{-3} Mn, and 6.1 mg dm^{-3} Zn. The seedlings were kept suspended on metal mesh benches at 70 cm above ground. Irrigation of the seedlings was carried out by means of micro-sprinklers activated automatically three times a day, with a duration of 15 minutes each and a flow rate of 30 L h^{-1}.

Seedling evaluations

For the rhizobacteria experiment, when root began to appear at the lower base of the tubes (42 days), the seedlings were removed from the containers and the roots were washed to remove the substrate; and for the seaweed extract experiment, when roots reached the bottom of the container (91 days), evaluated in sampled seedlings.

The following were evaluated in the laboratory: shoot length - SL (cm), measured from the substrate level to the apex of the last leaf, and the root system length - RSL (cm), both obtained using a millimeter ruler; stem diameter - SD (mm), determined at the substrate level using a digital caliper with 0.01 mm precision (Western® PRO DC-6); number of leaves - NL; leaf area - LA (cm^2), using an electronic leaf meter (LI-COR®, model 3100); shoot dry mass - SDM and root dry mass - RDM (g $plant^{-1}$), obtained after drying in a forced-air circulation oven at 70 °C until constant weight, and subsequently weighed on a precision balance (0.001 g).

Total dry mass - TDM (g $plant^{-1}$) was calculated as the sum of SDM and RDM, and the Dickson Quality Index (DQI) was also determined, obtained by the formula proposed by Dickson in 1960 and applied in several studies, as described by Souza et al. (2022), where:

$$DQI = TDM(g) \div [SL(cm) \div SD(mm) + SDM(g) \div RDM(g)]$$

Statistical analysis

The data were subjected to analysis of variance; for the study of the effect of rhizobacteria, the means were compared by Tukey's test at 5% probability, and for the seaweed extract, when significant, polynomial regression analysis was performed in order to verify the effect of the variables as a function of increasing extract concentrations, using the AgroEstat software (Barbosa and Maldonado Junior, 2015).

Results and discussion

Effect of rhizobacteria

The analysis of biometric indicators shows that the rhizobacteria were effective in promoting the growth and quality of *Cariniana estrellensis* seedlings, although the magnitude of responses varied among bacterial species and evaluated characteristics (Fig. 2). *Azospirillum brasilense* and *Bacillus subtilis* promoted favorable responses in shoot length, leaf area, and shoot dry mass, whereas *B. amyloliquefaciens,* and *B. subtilis* showed the highest root system dry mass and Dickson Quality Index (DQI) values. All inoculated treatments increased root system length in comparison to the non-inoculated control, while stem diameter and number of leaves remained unaffected.

Means followed by the same lowercase letter on the column do not differentiate between themselves by the Tukey's test at 5% probability.

The lower performance of the control treatment can also be observed in Fig. 3. These results reinforce the considerations of Upadhyay et al. (2022), who report that rhizobacteria interact beneficially with plant roots, stimulating growth.

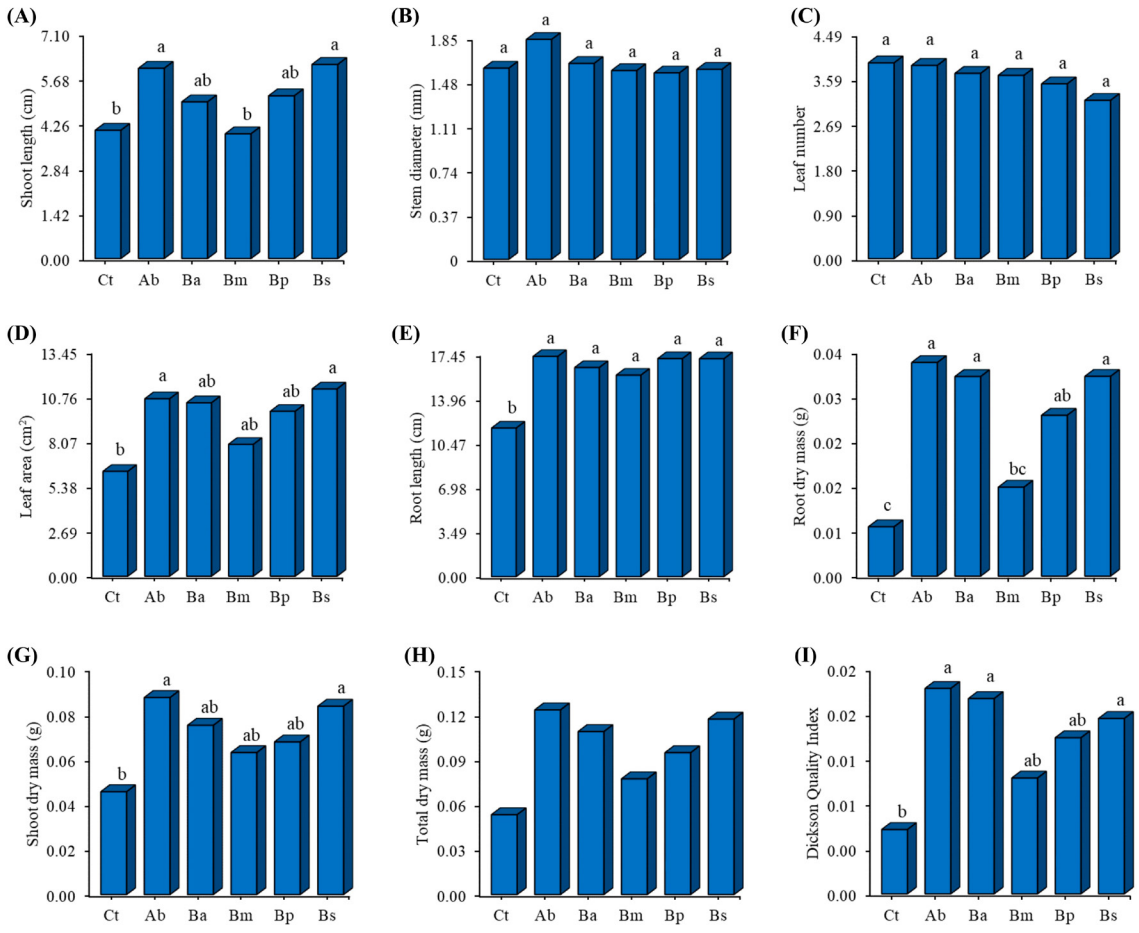

Fig. 2. Shoot length (A), stem diameter (B), number of leaves (C), leaf area (D), root system length (E), root dry mass (F), shoot dry mass (G), total dry mass (H) and Dickson Quality Index (I) of jequitibá-branco seedlings (*Cariniana estrelellensis*) inoculated or not (control - Ct) with the bacteria *Azospirillum brasilense* (Ab), *Bacillus amyloliquefaciens* (Ba), *Bacillus megaterium* (Bm), *Bacillus pumilus* (Bp) e *Bacillus subtilis* (Bs).



Fig. 3. Visual appearance of jequitibá-branco seedlings (*Cariniana estrellensis*) inoculated or not (control) with the rhizobacteria *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *B. megaterium*, *B. pumilus* e *B. subtilis*.

The favorable responses of *Cariniana estrellensis* to *A. brasilense* may be associated with its ability to influence plant hormonal balance, particularly through the production of auxins, which stimulate root development and, consequently, contribute to nutrient acquisition and shoot growth (Giri et al., 2025).

There were no significant differences among treatments for the number of leaves in jequitibá-branco (Fig. 2C). Similarly, Campos et al. (2023), Campos et al. (2024), and Silveira et al. (2025) also did not observe differences in the number of leaves among treatments (rhizobacteria and control) for açaí palm (*Euterpe oleracea*), golden trumpet tree (*Handroanthus chrysotrichus*), and zinnia (*Zinnia peruviana*), respectively, indicating that this specific characteristic does not appear to reflect effective responses.

Taiz et al. (2017) state that higher values of number of leaves and leaf area indicate better absorption of solar radiation, thus, greater photosynthetic capacity of the seedlings, allowing the development of other organs to be accelerated. In this study, there were no differences among treatments for number of leaves; for leaf area, however, there was a superiority of seedlings inoculated with *A. brasilense* and *B. subtilis* (Fig. 2D) which was sufficient to allow greater photosynthetic efficiency, showing that these bacteria acted more efficiently in plant metabolism, potentially, contributing to biomass accumulation (Ren et al., 2025).

Although *B. megaterium* and *B. pumilus* generally showed less pronounced responses in some growth characteristics, their effects were not consistently inferior to those of the other rhizobacteria. Particularly, all bacterial treatments promoted similar increases in root system length (Fig. 2E), indicating that root elongation and biomass accumulation may respond differently to inoculation. Variations in bacterial performance may reflect the differences in microbial abilities and interactions with the host plant and substrate environment (Yaghoubi Khanghahi et al., 2025). Kondo et al. (2024) also demonstrated that the positive response of *Azospirillum brasilense* ad *Bacillus spp.* inoculation in *Araucaria angustifolia* seedlings varied according to the developmental stage and cultivation conditions.

For root dry mass, *A. brasilense*, *B. amyloliquefaciens* and *B. subtilis* stood out (Fig. 2F). Altough statistically significant differences were observed, root dry mass values remained approximately between 0.01 and 0.04 g per seedling, while DQI values were below 0.024 (Fig. 2F and 2I). These relatively small absolute values reflect an early developmental stage at which seedlings were evaluated, 42 days after sowing. Root dry mass is recognized as one of the simplest indicators and one that best determines seedling establishment in the field, as it directly influences water and nutrient uptake (Avelino et al., 2021; Irik and Bikmaz, 2024), while DQI integrates biomass accumulation and morphological balance in the assessment of seedling quality (Alonso et al., 2024) Thus, seedlings with greater root dry mass and DQI ought to be more effective in the field due to their capacity for acclimatization after transplanting (Manokari et al., 2023).

For shoot dry mass (Fig. 2G), the superiority of seedlings inoculated with *A. brasilense* and *B. subtilis* was observed, and for total dry mass, the rhizobacterium *A. brasilense* stood out (Fig. 2H).

The difference in responses among bacterial species is consistent with the importance of plant-microorganism specificity. Campos et al. (2023) reported beneficial effects of *B. megaterium* in *Euterpe oleracea* seedlings, contrasting with its more limited responses in some characteristics of *C. estrellensis* here observed. Furthermore, Campos et al. (2025) reported favorable responses to *A. brasilense* in *Handroanthus impertiginosus* seedlings, supporting its potential application in forest nursery conditions; however, little is known about the dynamics of microorganism establishment in substrates composed of organic constituents and, even more so, when they are inert.

The possibilities of microbial life interferences are vast. Abiotic factors, such as temperature, nutrients, pH, salinity, energy sources and toxic elements; as well as biotic factors, mainly represented by microbial genetics and the interaction between microorganisms, exert a limiting power over the survival and activity of microorganisms (Cavalcante et al., 2022; Cunha et al., 2024), and also the dosage, form and frequency of bacterial inoculation.

Thus, the beneficial effects of the association of the rhizobacteria *A. brasilense*, *B. amyloliquefaciens* and *B. subtilis* on the growth and development of jequitibá-branco seedlings, verified in this study (Fig. 2), may be related to the dosage and method of application of these bacteria, the composition and management of the substrate, and also the temperature and relative humidity conditions (Fig. 1) during the development of the research, which favored the development and association of these bacteria with jequitibá-branco.

Effect of *Ascophyllum nodosum* seaweed extract

The application of *Ascophyllum nodosum* extract resulted in the development of *C. estrellensis* seedlings, particularly through changes in root system characteristics (Fig. 4). Root dry mass, total dry mass, and DQI exhibited positive linear relationships within the evaluated range. Root dry mass increased from approximately 0.54 g in non-inoculated seedlings to 1.81 g at 15 mL L^{-1}, while total dry mass increased from 2.16

g to 3.66 g and DQI increased from 0.25 to 0.63. Root system length showed a quadratic response to the increasing extract concentrations. Although the highest observed mean occurred at 6 mL L^{-1} (27.10 cm), it was close to that recorded at 12 mL L^{-1} (26.82 cm), while the regression estimated a maximum near 11 mL L^{-1}; reflecting the distinction between treatment means and the overall trend represented by the regression model. In contrast, the remaining characteristics, there was no regression fit, demonstrating that there were no significant differences between the treatments. These findings indicate that biomass accumulation did not follow the same response patterns as root elongation; they also suggest that the positive effects of seaweed extract were more pronounced in root development and related biomass and quality indicators than in shoot growth.

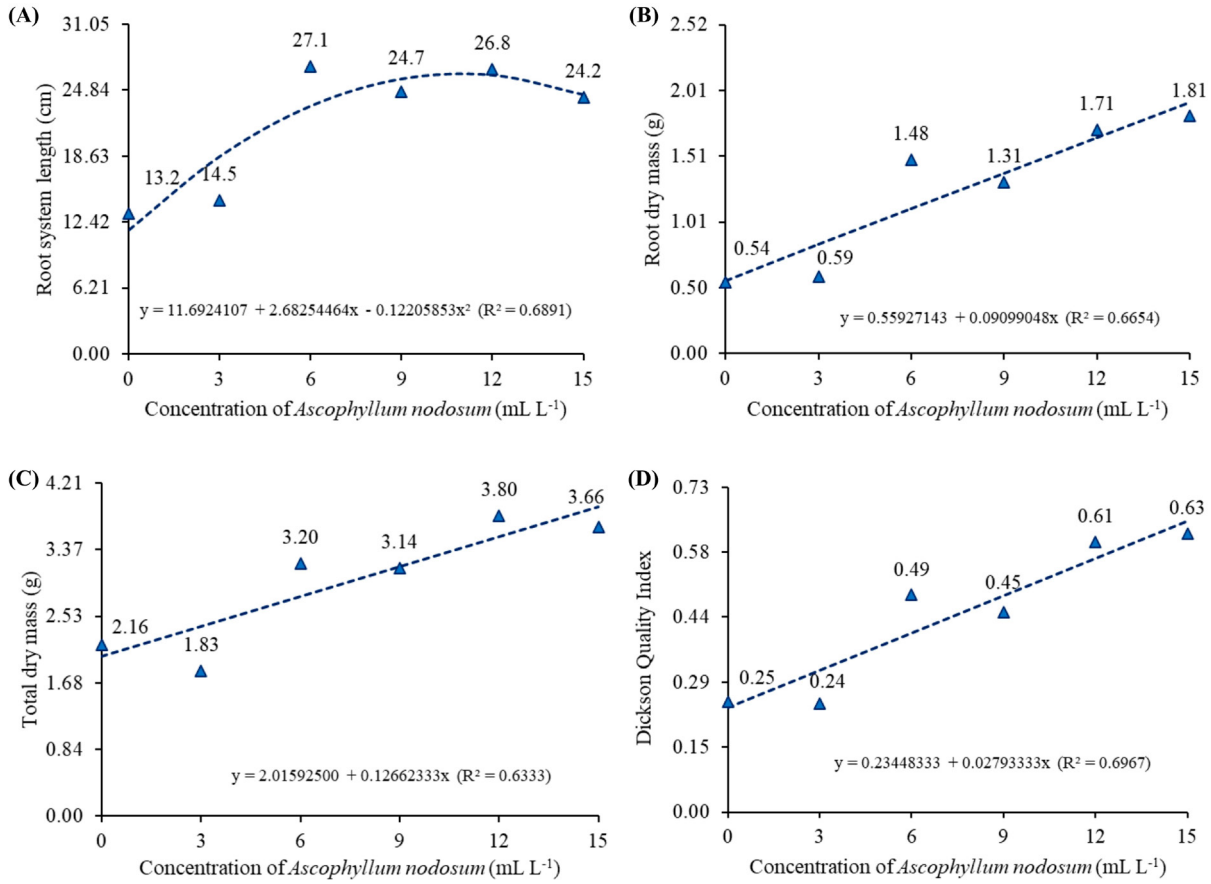

Fig. 4. Root system length (A), root dry mass (B) e total dry mass (C) e Dickson Quality Index (D) of jequitibá-branco seedlings (*Cariniana estrellensis*) treated or not (control) with *A. nodosum* seaweed extract.

The regression models explained a moderate proportion of the variation among treatment means with coefficients of determination of 0.6891 for root system length, 0.6654 for root dry mass, 0.6333 for total dry mass, and 0.6967 for DQI (Fig. 4). Thus, approximately 63% to 70% of the variation in the plotted means was accounted for the fitted equations.

The quadratic response in root system length suggests a reduction in the rate of improvement at higher concentrations, whereas the linear positive relationships for biomass and DQI indicate an overall increasing trend within the range evaluated. However, these models do not establish 6 mL L^{-1} as minimum effective concentration or identify an optimal concentration for all characteristics.

Although *A. nodosum* extract contains several plant growth-regulating compounds (Feitosa et al., 2024), its application can modulate the physiological behavior of plants in distinct ways, as observed in this study.

The superiority of the root system of jequitibá-branco seedlings treated with the highest concentrations of *A. nodosum* extract can also be observed in Fig. 5. This increase may be associated with the nutrients present in the extract, which favor the increase in root length and volume, expanding the explorations of the rhizosphere (Bernardes et al., 2023).

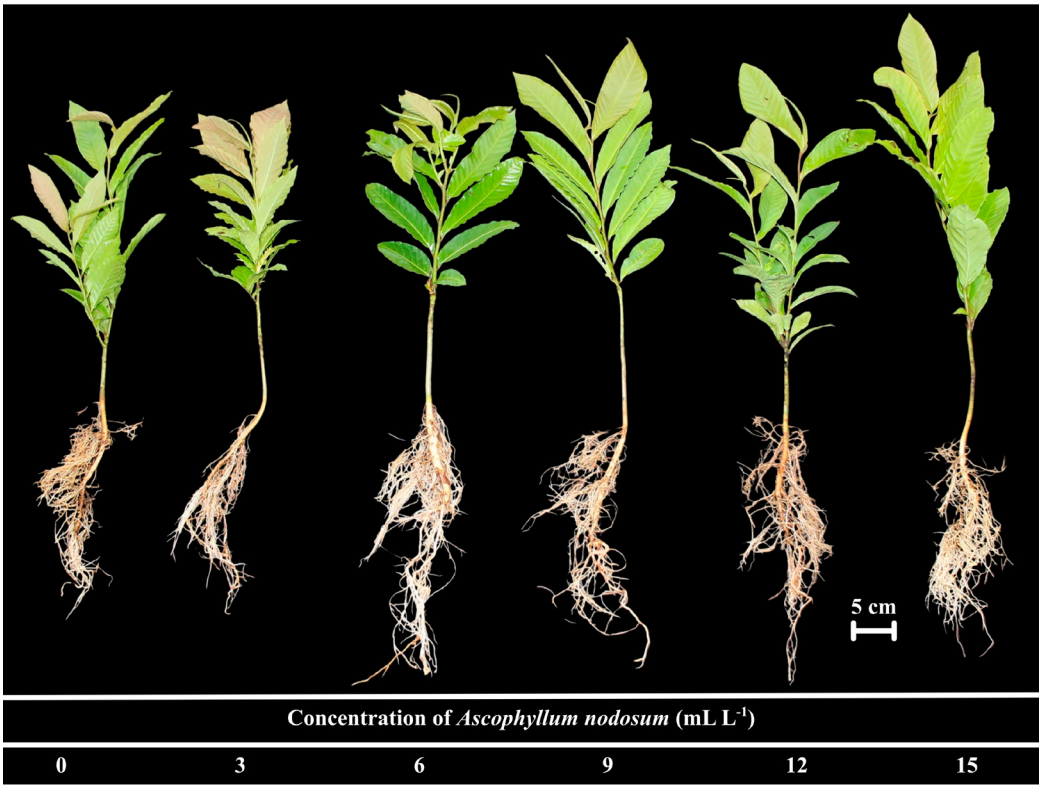

Fig. 5. Visual appearance of jequitibá-branco (*Cariniana estrellensis*) seedlings treated (3, 6, 9, 12 and 15 mL L^{-1}) or not (control - 0) with *A. nodosum* seaweed extract.

Furthermore, this increase may be related to the rise in exogenous phytohormones present in the seaweed extract, which promotes cell division (Bernardes et al., 2023; Carvalho et al., 2024). In this context, the extract influences hormone synthesis, especially auxins and cytokinins, promoting the formation and growth of the root system (Silva et al., 2020).

Conclusions

The rhizobacteria *Azospirillum brasilense*, *Bacillus amyloliquefaciens* and *B. subtilis* promoted the most favorable growth and seedling quality responses. The application of *A. nodosum* extract primarily influenced root development, with positive linear responses in root dry mass, total dry mass, and Dickson Quality Index within the evaluated concentration range. However, differences in experimental periods, cultivation systems, and seedling developmental stages limit direct comparisons between the two approaches. Future studies should evaluate these biostimulants under standardized conditions, investigate their physiological mechanisms, and assess whether the observed benefits translate into an improved seedling establishment and performance under field conditions.

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Author Contribution

MTSB: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Visualization, Writing – Original Draft. **ACB:** Data Curation, Investigation, Validation, Writing – Review & Editing. **MMS:** Conceptualization, Investigation, Methodology, Validation, Writing – Review & Editing. **GLQ:** Formal Analysis, Writing – Review & Editing. **AMBS:** Data Curation, Formal Analysis, Visualization, Writing – Review & Editing. **CHBS:** Data Curation, Investigation, Methodology, Resources, Validation. **ECR:** Investigation, Methodology, Resources, Validation. **KFLP:** Conceptualization, Formal Analysis, Funding Acquisition, 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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