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SCIENTIFIC ARTICLE

Rhizobacteria in growth and quality of açaí seedlings

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Abstract

The success of any plant development relies on healthy and vigorous seedlings, and the use of rhizobacteria is a sustainable alternative for the production of high-quality seedlings as they positively interfere in plant development. Thus, the objective of this study was to evaluate the effect of rhizobacteria on growth and quality of seedlings of açaí (*Euterpe oleracea* Mart.), a native palm of Brazil, which has significant ornamental value in addition to the ecological and economic role, mainly by providing sweet heart of palm and fruit pulp. The experimental design was entirely randomized. There were five treatments (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens* and *Azospirillum brasilense* plus the absence of microorganisms - control); four replicates and ten plants per plot. The following characteristics were evaluated: shoot height (cm), root length (cm); stem diameter (mm); number of leaves; leaf area (cm^2); shoot, and root as well as total dry matter (g). Shoot/root ratio was determined and Dickson Quality Index. The data were submitted to variance analysis and the means were compared using Tukey's test at 5% probability. Pearson's correlation matrix was also determined. The rhizobacterium *Bacillus subtilis* provided higher growth while *Bacillus amyloliquefaciens* provided lower growth and quality of açaí seedlings.

Keywords: *Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus subtillis*, *Euterpe oleracea.*

Resumo

Rizobactérias no crescimento e qualidade de mudas de açaí

O sucesso do desenvolvimento de qualquer planta é dependente de mudas sadias e vigorosas e o uso de rizobactérias é uma alternativa sustentável para a produção de mudas de alta qualidade pois interferem positivamente no desenvolvimento das plantas. Desta forma, o objetivo deste trabalho foi avaliar o efeito de rizobactérias no crescimento e na qualidade de mudas de açaí (*Euterpe oleracea* Mart.) palmeira nativa do Brasil, que apresenta expressivo valor ornamental além da importância ecológica e também econômica, principalmente pelo fornecimento de palmito doce e polpa dos frutos. O delineamento experimental foi o inteiramente casualizado. Foram cinco tratamentos (*Bacillus subtilis, Bacillus megaterium, Bacillus amyloliquefaciens e Azospirillum brasilense* mais a ausência de microrganismos – controle); quatro repetições e dez plantas por parcela. Foram avaliadas as características: comprimento da parte aérea (cm) e do sistema radicular (cm); diâmetro do coleto (mm); número de folhas; área foliar (cm^2); massa seca da parte aérea, das raízes e total (g) e determinadas: razão parte aérea/raízes e o Índice de Qualidade de Dickson. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey, a 5% de probabilidade. Foi determinada, ainda, a matriz de correlação de Pearson. A rizobactéria *Bacillus subtilis* proporcionou maior e *Bacillus amyloliquefaciens* menor crescimento e qualidade das mudas de açaí.

Palavras-chave: *Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus subtillis*, *Euterpe oleracea.*

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Introduction

Contemporary landscaping increasingly employs native species, adopting a sustainable approach (Araújo et al., 2022). The use of indigenous vegetation in landscaping is of great importance for the conservation of local native diversity, especially as an alternative in the substitution of exotic plants due to their adaptation characteristics to the environment, biological diversity and their major ecological role in landscaping (Prestes et al., 2020), in addition to enhancing the regional landscape identity and promoting the coexistence of fauna that depends on these plants (Araújo et al., 2022).

Therefore, it is important to promote the use of native palms in landscaping, including the species *Euterpe oleracea* Mart., popularly known as açaizeiro or açaí, which has significant ornamental value. The açaí palm is also economically important, with the heart of palm being the most traditional product and the fruit pulp being the one with the greatest economic interest (Silva, 2021).

The açaizeiro is considered among the most promising species (D'arace et al., 2019) and, in parallel with its commercial expansion, there is a growing need for quality seedlings which involves the reduction of nursery time and its good performance in the field (Araújo et al., 2018).

Consequently, the quality of açaí seedlings influences the survival and productivity of the plants after transplanting, and the employment of beneficial microorganisms in the seedling production process, which is directly related to the production of plant hormones, vitamins, or conversion of substances to a form that can be assimilated by the plant, aids in the adaptation of the seedlings (Pio-Gonçalves et al., 2022).

Among these microorganisms, rhizobacteria that promote plant growth stand out, being able to positively interfere in growth and development of plants in several ways, including producing phytohormones, alleviating drought stress, mitigating salinity stress, acting in the phytoextraction of heavy metals, nutrient supplementation and/or pathogen biocontrol (Dias and Santos, 2022).

The rhizobacterium *Bacillus subtillis* acts in disease biocontrol, stimulates plant growth, can solubilize phosphorus from soil, increase nitrogen fixation, produce siderophores that promotes its growth and suppress pathogens, and can also increase tolerance to stresses (Hashem et al., 2019).

Bacillus megaterium is also related to the ability to solubilize inorganic phosphorus which increases the amount of available phosphorus and promotes plant growth (Huang et al., 2019). *B. subtilis* and *B. megaterium* have shown evident plant growth promotion effect for some species (Guimarães et al., 2021; Santos et al., 2021; Silva et al., 2022).

Other rhizobacterium, such as *Bacillus amyloliquefaciens*, also shows good results in growth promotion of some plant species (Farzand et al., 2020; Wang et al., 2020; Abreu et al., 2022) promoting resistance against diseases (Ngalimat et al., 2021). While *Azospirillum brasilense* assists plant growth mainly through the production of phytohormones, particularly indoleacetic acid, as well as by nitrogen fixation (Nguyen et al., 2019).

The range of interferences in microbial life is wide. Abiotic factors, such as temperature, nutrients, pH, salinity, energy sources, and toxic elements; as well as biotic factors represented mainly by microbial genetics and the interaction between microorganisms have a restraining power on the survival and activity of microorganisms (Furtak and Galazka, 2019; Cavalcante et al., 2022).

Thus, the objective of this study was to evaluate the effects of rhizobacteria on the growth and quality of açaí (*Euterpe oleracea* Mart.) seedlings.

Material and Methods

The present study was carried out between November 2021 and March 2022, in a greenhouse located in the state of São Paulo under the coordinates 21°15'2" S, 48°16'47" W and 600 meters of altitude. The region climate is tropical savanna *Aw* type (with dry winter and rainy summer) (Andre and Garcia, 2015). The data with average, maximum, minimum temperatures and average relative humidity during the period of the experiment are shown in Figure 1 (UNESP, 2022).

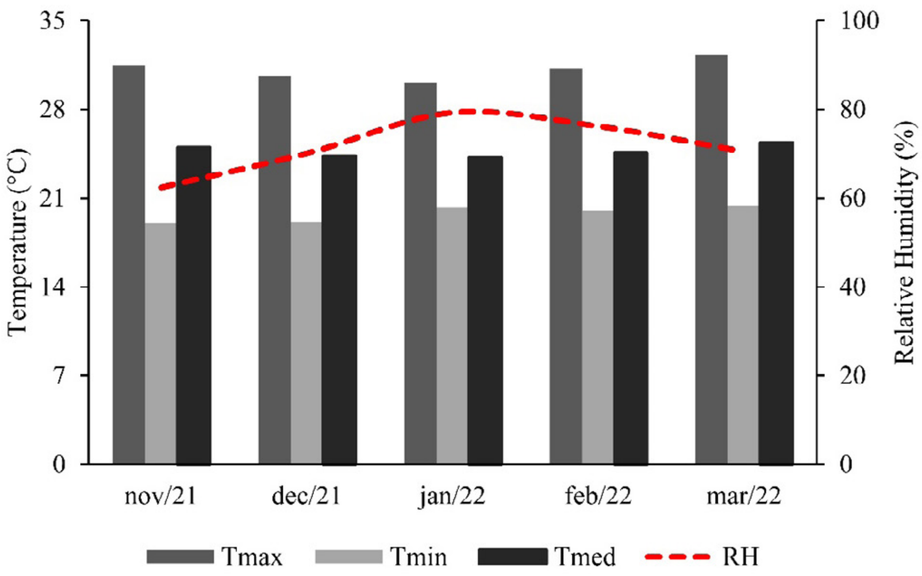

Figure 1. Data of maximum (Tmax), minimum (Tmin) and average (Tmed) temperature, and average relative humidity (RH) obtained during the period of November 2021 to March 2022.

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.

Açaí seedlings, obtained from seeds at UNESP/FCAV, with lengths of 5 cm ± 1 cm, were planted in tubes with volumetric capacity of 280 cm³ 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), and then placed in polypropylene trays with capacity for 54 tubes.

The trays were placed 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 also with clear plastic above the screen cover. The irrigation was performed by automatic micro sprinklers activated twice a day for 15 min each, with a flow rate of 30 L h^{-1}.

The rhizobacteria used in this study are part of the collection of Soil Microbiology Laboratory of the Plant Production Department of UNESP-FCAV, Jaboticabal Campus, 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. After the incubation period, the bacteria were centrifuged separately at 10,000 rpm for 10 min 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 seedlings were planted and again at 60 days, by applying 1 mL of the solution directly into 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: shoot height (SH, cm), measured at the substrate level to the tip of the last leaf and root length (RL, cm), both using a ruler in centimeters; stem diameter (SD, mm), determined at the substrate level using a digital caliper accurate to 0.01 mm (Western® PRO DC-6); number of leaves (NL), verified by visual counting of fully expanded leaves; leaf area (LA, cm^2), measured using an electronic leaf area meter (Li-3100C, LI-COR®, Lincoln, Nebraska, USA); shoot (SDM, g) and root dry matter (RDM, g), obtained after drying the shoots and roots in a forced air circulation oven at 70 °C, until reaching constant weight, and weighing them on a precision scale (0.001 g) (SHIMADZU®, model AY220); and the total dry matter (TDM, g), obtained by the sum of SDM and RDM.

From these measurements, the following seedling quality variables were determined: a) shoot/root ratio, obtained from the relation between SDM and RDM; b) Dickson's quality index (DQI), obtained by the formula proposed by Dickson in 1960 and applied in several research studies as described by Souza et al. (2022), where: DQI = [TDM (g)/[SH (cm)/SD (mm) + SDM (g) /RDM (g)].

The obtained data were submitted to analysis of variance, and the means were compared using Tukey's test at 5% probability using the AgroEstat® estatistical software. Correlation analysis was also performed between the variables.

Results and Discussion

The evaluated characteristics in this study presented in table 1 are related to the growth and quality of açaí seedlings after inoculation or not (control treatment) of rhizobacteria. When considering that plant growth is defined as the irreversible increase in weight and volume of cells, tissues and organs, the quality of seedlings can be evaluated using growth indicators, such as shoot height, stem diameter and shoot, root and total dry matter; in addition to these, several metrics are used to evaluate the quality of forest seedlings such as shoot and root dry matter ratio and the Dickson Quality Index (Avelino et al., 2021).

Table 1. Means of shoot height (SH, cm), stem diameter (SD, mm), number of leaves (NL), leaf area (LA, cm^2), root length (RL, cm), shoot dry matter (SDM, g), root dry matter (RDM, g), total dry matter (TDM, g), shoot dry matter/root dry matter ratio (SDM/RDM) and Dickson's quality index (DQI) in açaí (*Euterpe oleracea*) seedlings inoculated or not (control) with growth-promoting rhizobacteria.

Treatments	SH	SD	NL	LA	RL
1. Control	20.88 ab	3.48 b	1.80 a	28.52 ab	24.01 ab
2. *Bs*	22.20 a	4.14 a	1.93 a	33.33 a	26.03 a
3. *Bm*	22.09 a	3.88 ab	1.73 a	30.75 a	23.25 ab
4. *Ba*	18.59 b	3.46 b	1.65 a	23.80 b	21.02 b
5. *Ab*	20.20 ab	3.78 ab	1.70 a	13.38 c	23.29 ab
CV (%)	6.50	5.81	7.55	10.89	5.84
	SDM	RDM	TDM	SDM/RDM	DQI
1. Control	0.3214 ab	0.2507 ab	0.5722 ab	1.31 a	0.0781 abc
2. *Bs*	0.3684 a	0.2785 a	0.6468 a	1.34 a	0.0966 a
3. *Bm*	0.3553 ab	0.2423 ab	0.5976 ab	1.49 a	0.0831 ab
4. *Ba*	0.2588 c	0.1667 b	0.4255 c	1.62 a	0.0617 c
5. *Ab*	0.3066 bc	0.2052 ab	0.5118 bc	1.56 a	0.0744 bc
CV (%)	7.31	18.22	9.45	21.00	12.30

Means followed by the same letter in column do not differ by Tukey's test at 5% probability.
Bs - Bacillus subtilis; Bm - Bacillus megaterium; Ba - Bacillus amyloliquefaciens; Ab - Azospirillum *brasilense*. CV (%): coefficient of variation, expressed as percentage.

In Table 1 it can be observed that *Bacillus subtilis* stood out with higher means in all studied characteristics, although it did not differ from the control and other bacteria for some characteristics. This bacterium, therefore, showed greater efficiency in producing phytohormones and enzymes, beneficial for seedling development (Mazzuchelli et al., 2014) that promoted both growth and quality of açaí seedlings. Castro et al. (2019; 2020) also observed positive effect of *B. subtilis* in promoting growth of açaí seedlings.

The rhizobacterium *Bacillus megaterium* also showed satisfactory results for açaí seedlings; similarly, Guimarães et al. (2021), Santos et al. (2021) and Silva et al. (2022), reported that employment of *B. subtilis* and *B. megaterium* has contributed to the increment of plant growth.

As for the inoculation with *Bacillus amyloliquefaciens*, the lowest means were obtained for most of the studied characteristics, although this species shows efficiency when associated with other plant species as observed by Chauhan et al. (2019) that by inoculating this bacterium in rice (*Oryza sativa*) seedlings noticed an increase in growth of shoots and roots.

This difference in response may be related to the cultivation method; many studies such as conducted by Chauhan et al. (2019) on rice, are conducted with crops grown into the ground, which is rich in microorganisms, and there is little information on the association of these microorganisms in plants raised in containers holding organic or inert substrates.

Sometimes, when the inoculation of rhizobacteria is performed directly on the soil, incompatibility of the introduced microorganisms with the native microbiota associated with the rhizosphere of the plant has been observed, which generates competition between them affecting the positive effects of the target microorganism (Kumari et al., 2019) and, consequently, inadequate development of the seedlings or, when there is compatibility, generating benefits. However, little is known about the establishment dynamics of microorganisms on substrates composed of organic and inert compounds.

As the development of the açaí seedlings took place in substrate, the main factor that may have influenced the bacteria was the environmental conditions. However, there was not much variation for temperature and relative humidity during the experiment (Figure 1). The unsatisfactory effect, especially for the rhizobacterium *Bacillus amyloliquefaciens*, which presents positive effects for other plant species, may then be due to the tube limitation, the temperature that may not have been ideal for this bacterium and mainly for not having good interaction with this studied plant species.

The superiority of seedlings inoculated with *B. subtilis* followed by *B. megaterium* and *Azospirillum brasilense* in Table 1, indicating that these seedlings have greater potential for success after planting when compared to those that did not receive rhizobacteria (control) or those where *B. amyloliquefaciens* was applied. Silva et al. (2020) when evaluating the stem diameter, they observed that larger diameters is an indicator for analyzing the survival and growth conditions of seedlings after planting.

Higher values of leaf area and number of leaves indicates better sunlight absorption, thereby better photosynthetic capacity of the seedlings allowing the development of other organs to be faster (Taiz et al., 2017). There was no difference between treatments for number of leaves (Table 1), however, there was superiority of seedlings inoculated with *B. subtilis* and *B. megaterium* for leaf area which reflected in other characteristics; this shows that these bacteria acted more efficiently in plant metabolism (Ngalimat et al., 2021), resulting in higher quality seedlings.

There was no difference between treatments for shoot dry matter and root dry matter ratio (Table 1). This ratio is an efficient characteristic for evaluating forest seedling lot quality and is directly related to field establishment and competitiveness under environmental stress conditions such as drought stress (Grossnickle and Macdonald, 2018; Avelino et al., 2021). However, even though there were no difference between treatments for this quality evaluation characteristic, other parameters such as root dry matter and Dickson Quality Index showed superiority for seedlings inoculated with *B. subtilis* (Table 1).

Root dry matter is acknowledged as one of the easiest indicators that best measures seedling establishment in the field because it directly influences water and nutrient uptake (Shen et al., 2019; Avelino et al., 2021). Consequently, seedlings with higher root dry matter will be more effectively established in field because of their ability to adapt after transplanting (Avelino et al., 2021).

The results for Dickson Quality Index strengthen the superiority of the seedlings inoculated with *B. subtilis* (Table 1). The Dickson Quality Index is based on the relationship between several growth indicators to determine the quality of the seedlings, i.e., shoot dry matter, root dry matter, total dry matter, shoot height and stem diameter. For this reason, the Dickson Quality Index formula highlights the balance between growth and survival potential of post-planting plants, and by taking into account several morphological characteristics, the potential errors that could be faced when using one or two indicators will be minimized. Thus, it is an excellent indicator of seedling quality since it includes in its calculation the sturdiness and biomass allocation balance (Mañas et al., 2009; Avelino et al., 2021).

However, Dickson's Quality Index provides different values influenced by several factors, but it has been used as the main indicator of the quality of forest seedlings as it has high correlation with post-planting survival in field (Avelino et al., 2021).

Analyzing Pearson's correlation matrix (Figure 2), significant positive and negative correlations were found among the characteristics. For growth and quality traits of seedlings the highest positive correlation values (above 0.80) were observed between number of leaves and Dickson Quality Index, stem diameter and shoot dry matter, shoot height and shoot dry matter, and shoot height and root dry matter.

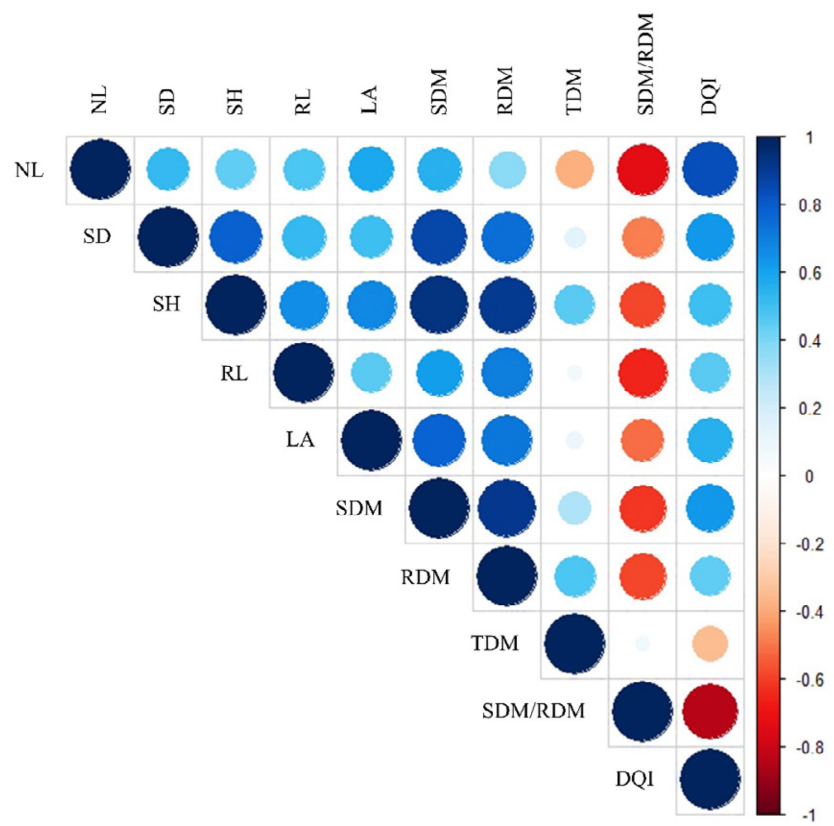

Figure 2. Pearson's correlation matrix between the analyzed variables of acai (*Euterpe oleracea*) seedlings inoculated or not (control) with growth-promoting rhizobacteria.

Significant at 5% probability. Where: NL= number of leaves; SD = stem diameter; SH = shoot height; RL = root length; LA = leaf area; SDM = shoot dry matter; RDM = root dry matter; TDM = total dry matter; SDM/RDM = shoot dry mass/root dry mass ratio; DQI = Dickson's quality index.

Most traits also showed strong and moderate correlation. Total dry matter showed moderate correlation with root dry matter and shoot height, and negative correlation with number of leaves, for other traits it was close to zero. The shoot dry matter/root dry matter ratio was negatively correlated with all traits except total dry matter, where there was no correlation.

The growth variables were positively related to the Dickson Quality Index except total dry matter. This result may indicate that the allocation of dry matter has little interference in the DQI values for this species.

Conclusions

The rhizobacterium *B. subtilis* promoted greater growth and quality of açaí seedlings while the species *B. amyloliquefaciens* showed the lowest values for the evaluated characteristics.

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Author Contribution

TSC: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization and writing-original draft. **MPP:** Conceptualization, data curation, investigation, methodology, visualization and writing-original draft. **GRV:** Conceptualization, investigation, methodology, visualization, writing-review and editing and translation. **AMBS:** Data curation, software, investigation, formal analysis, visualization original draft. **CHBS:** Conceptualization, resources, data curation, investigation, visualization and validation of the methodology. **ECR:** Funding acquisition, methodology, project administration, resources, supervision, validation and writing-review and editing. **KFLP:** Funding acquisition, methodology, project administration, resources, supervision, validation and writing-review and editing.

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