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ARTICLE

Bacteria potential as biostimulants in miniature rose cultivation

Potencial de bactérias como bioestimulantes no cultivo de minirosas

Mariana Martins da Silveira[1,*], André Caturelli Braga[1], Mariana Campos de Lima[1], Antonio Maricélio Borges de Souza[2], Gabriel Longuinhos Queiroz[1], Carlos Henrique Barbosa Santos[1], Everlon Cid Rigobelo[1], and Kathia Fernandes Lopes Pivetta[1]

[1]Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal-SP, Brasil.
[2]Universidade Federal de Viçosa (UFV), Viçosa-MG, Brasil.

Abstract
Miniature rose cultivation has significant ornamental and economic importance. In order to meet the commercial demand for high-quality products, it is necessary to adopt technologies that ensure producer competitiveness. The inoculation of plant growth-promoting microorganisms emerges as a sustainable strategy, favoring healthy plant growth, increasing seedling vigor, and contributing to more efficient management practices. The objective of this study was to evaluate the effect of rhizobacteria on the growth and development of miniature roses 'Two Tone Jewel'. The experiment was conducted in a completely randomized design with six treatments corresponding to the microorganisms *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus subtilis* and the control, without inoculation, with four replications. The evaluated parameters included number of leaves, leaf area, shoot height, number of buds and open flowers, shoot dry mass, root dry mass, and total dry mass. *A. brasilense* showed the highest values in most parameters, together with *B. amyloliquefaciens*, which was distinguished in leaf area and shoot dry mass. Inoculation with *Azospirillum brasilense* and *Bacillus amyloliquefaciens* may have potential for integration into sustainable strategies for miniature rose production; however, no statistically significant effects on growth and flowering were observed under the conditions evaluated.
Keywords: Biostimulants, ornamental horticulture, plant growth-promoting bacteria, *Rosa* spp., seedling vigor.

Resumo
O cultivo de minirosas tem grande relevância ornamental e econômica. A fim de atender à demanda comercial por produtos de alta qualidade, é necessário o uso de tecnologias que assegurem a competitividade do produtor. A inoculação de microrganismos promotores de crescimento se apresenta como uma estratégia sustentável, que favorece o crescimento saudável das plantas, aumenta o vigor das mudas e contribui para práticas de manejo mais eficientes. O objetivo deste trabalho foi avaliar o efeito de rizobactérias sobre o crescimento e desenvolvimento de minirosas 'Two Tone Jewel'. O experimento foi conduzido em delineamento inteiramente casualizado com seis tratamentos correspondentes aos microrganismos *Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus pumilus, Bacillus subtilis* e o controle, sem inoculação, com 4 repetições. Os parâmetros avaliados incluíram número de folhas, área foliar, altura da parte áerea, número de botões e flores abertas, massa seca da parte aérea, massa seca de raízes e massa seca total. *A. brasilense* apresentou os valores mais elevados na maioria dos parâmetros, juntamente com *B. amyloliquefaciens* que se distinguiu em área foliar e massa seca da parte aérea. A inoculação com *Azospirillum brasilense* e *Bacillus amyloliquefaciens* pode apresentar potencial para integração em estratégias sustentáveis de produção de minirosas; entretanto, não foram observados efeitos estatisticamente significativos sobre o crescimento e o florescimento nas condições avaliadas.
Palavras-chave: Bactérias promotoras do crescimento vegetal, bioestimulantes, horticultura ornamental, *Rosa* spp., vigor de plântulas.

Introduction

Ornamental floriculture constitutes one of the highest value-added segments within modern horticulture, characterized by high technological intensity, strong demand for aesthetic standardization, and increasing requirements for environmentally sustainable production systems (Partap et al., 2023).

Unlike food crops, in which yield is the main performance criterion, in ornamental plants commercial quality is determined by a complex set of morphological and physiological attributes, including plant architecture, uniformity, color intensity, number and size of flowers, shoot-root balance and post-production durability (Proietti et al., 2022). In this context, the adoption of technologies that promote balanced growth and abundant flowering while improving production efficiency has become a strategic priority for the sector.

The cultivation of miniature roses (*Rosa* spp.) is of great importance in the ornamental floriculture sector, due to its high commercial value and wide acceptance in the consumer market (Michonneau et al., 2021). The production of high-quality ornamental plants depends on technologies capable of promoting vigorous growth, uniformity, and high floral output (Wani et al., 2023).

The intensive production of miniature roses under protected cultivation generally involves the frequent use of soluble mineral fertilizers and growth regulators aiming to accelerate development and maximize flowering (Çelikel et al., 2019). Although efficient in the short term, these practices may increase production costs, raise the risk of substrate salinization, and reduce environmental impacts. Additionally, there is increasing pressure from the consumer market for products cultivated under more improved production practices with reduced reliance on synthetic chemical inputs (Partap et al., 2023).

In this scenario, the use of plant growth-promoting microorganisms (PGPMs) emerges as a promising biotechnological alternative. The interaction between these biostimulants and ornamental plants involves complex physiological, biochemical, and molecular processes (Kisvarga et al., 2022).

Among the direct mechanisms, the production of indole-3-acetic acid (IAA) plays a key role by altering root architecture, promoting greater branching and increasing the absorptive surface (Satria and Armansyah, 2026). This effect is particularly relevant in potted systems, where root volume is limited (Liu et al., 2025).

Improved nutrient acquisition may result in greater leaf expansion, increased photosynthetic rate, and higher biomass accumulation (Scudeletti et al., 2023). Phosphorus solubilization via the release of organic acids enhances the availability of this essential element for ATP and phospholipid synthesis, directly affecting energy metabolism and cell expansion (Dutta et al., 2025).

Furthermore, the production of siderophores improves iron acquisition, an essential element for chlorophyll synthesis and the functioning of the photosynthetic system (Mani et al., 2025). In addition, some species produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, reducing ethylene levels under stress conditions and

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supporting continued growth in intensive protected cultivation systems (Awlachew and Mengistie, 2022). Similarly, indirect mechanisms also play a relevant role. Among these are the induction of systemic resistance against pathogens, competition for ecological niches in the rhizosphere, and the production of antimicrobial metabolites stand out, which are particularly beneficial under stress conditions (Diaz et al., 2025).

The genus *Azospirillum* is widely studied for its ability to associate with different plant species and promote growth through phytohormone production and improved nutrient use efficiency (Giri et al., 2025; Santos et al., 2025). In several crops, inoculation with *Azospirillum* is associated with increased leaf area, higher chlorophyll content, and greater biomass accumulation (Sun et al., 2022). In ornamental plants, although still less explored, this genus shows significant potential to improve traits related to vigor and flowering.

Species of the genus *Bacillus* also stand out due to their ability to form endospores, which confers greater environmental stability and viability in commercial formulations (Ullah et al., 2024). According to Mohanty et al. (2021), besides phosphorus solubilization and plant hormone production, several *Bacillus* species produce bioactive compounds capable of stimulating primary and secondary plant metabolism. In ornamental systems, this may result in greater leaf expansion, improved architecture, and increased emission of floral buds.

Although there has been progress in understanding these biostimulants in extensive food crops, as reported by Xiao et al. (2023) and Upadhyay et al. (2022), studies focused on ornamental plants are still limited. Ornamental plant physiology presents important particularities: the balance between vegetative growth and flowering is determinant for commercial value, and excessive vegetative growth responses may be undesirable if they compromise the plant's aesthetic proportion (Jegadeeswari et al., 2025; Proietti et al., 2022). Therefore, understanding how different microorganisms simultaneously influence vegetative and reproductive variables is essential for the safe adoption of these technologies in the sector.

Another relevant aspect concerns production system sustainability. The incorporation of bioinputs may reduce dependence on mineral fertilizers, increase nutrient use efficiency, and promote more resilient systems (Mohammadi et al., 2024). In the context of global agricultural intensification, microorganism-based technologies represent a major innovation frontier in horticulture (Prisa and Jamal, 2025).

Given these considerations, it is necessary to evaluate, under commercial protected cultivation conditions, the effects of different plant growth-promoting rhizobacteria on morphological and reproductive parameters of miniature roses. Such understanding may improve production management and support the development of biotechnological strategies aligned with environmental efficiency and competitiveness in the ornamental sector.

Thus, the present study aimed to evaluate the effects of different bacterial biostimulants on vegetative development, biomass accumulation, and flowering of miniature rose cultivar Two Tone Jewel, providing a scientific basis for their integration into environmentally responsible ornamental production systems.

Materials and Methods

The experiment was conducted in a commercial miniature rose production area located in the municipality of Holambra, São Paulo State, Brazil, under protected cultivation conditions. Seedlings of the cultivar Two Tone Jewel were used, vegetatively propagated by cuttings.

The experiment was established using standardized herbaceous cuttings obtained from previously cultivated plants during the pinching operation, a routine practice used to promote branching and plant development. The cuttings were selected for uniform size and subsequently planted in pots, with five cuttings per pot, to establish a new generation of plants.

Fig. 1 presents climatic data recorded inside the experimental greenhouse, including mean leaf temperature, mean substrate temperature, and relative air humidity. The data presented correspond to two-week means.

Climatic variables were monitored using a data acquisition system installed inside the experimental greenhouse. The system recorded air temperature, wet-bulb temperature, CO_2 concentration, leaf temperature, substrate temperature, and photosynthetically active radiation (PAR), while vapor pressure deficit was calculated by the monitoring system. The equipment was provided through a rental service; therefore, information regarding the manufacturer, model, and specific sensor specifications was not available.

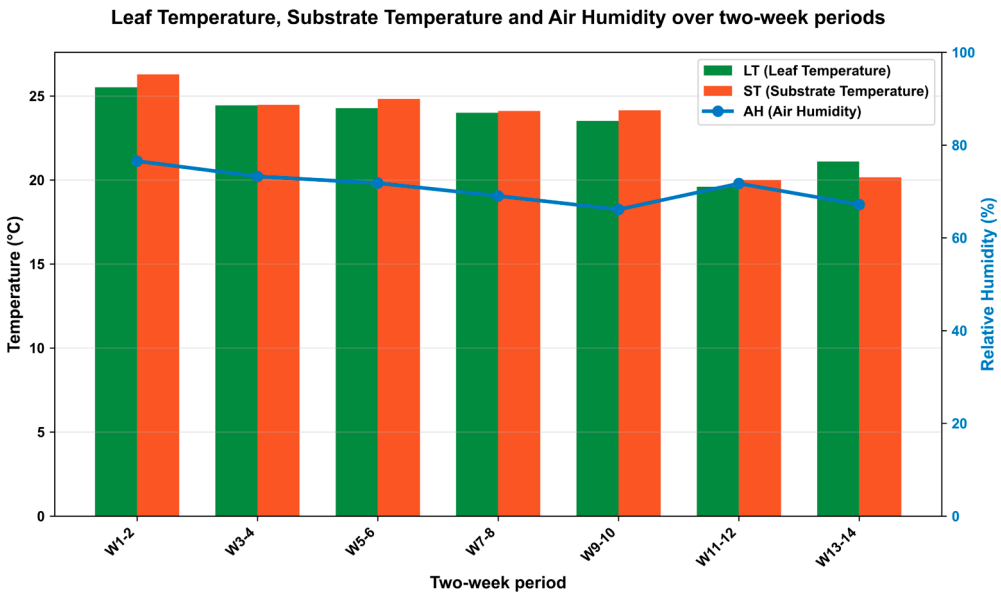

Fig. 1. Two-week averages of leaf temperature (LT), substrate temperature (ST), and relative air humidity (AH) recorded in the greenhouse during the mini rose cultivation period (March–June). Data were monitored at 5-min intervals. The labels W1-2 to W13-14 represent consecutive two-week periods.

Irrigation was carried out using the flooding method and managed according to the grower's standard production practices, based on continuous monitoring of the substrate solution throughout the experimental period. The plants were grown in a commercial substrate for ornamental plants (Mecplant Flores, MECPLANT), composed of pine bark, limestone, and simple superphosphate.

The solutions containing *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Bacillus pumilus*, and *Bacillus subtilis* were cultivated separately in nutrient broth for seven days, in flasks maintained in a B.O.D. incubator (Eletrolab, model 347 F, Brazil), at 25 °C.

After incubation, the cultures were centrifuged at 5,300 ×g for 10 minutes at 28 °C (Novatecnica, model MLW K24, Brazil). The inoculum

concentration was standardized to 1 × 10^7 CFU mL^{-1}, according to the recommendations of Barry and Thornsberry (1991) and Sahm and Washington II (1991), using a spectrophotometer (Micronal, model B382, Brazil) with absorbance reading at 695 nm.

The experimental design was completely randomized. Six treatments were used: control (without inoculation), *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Bacillus pumilus*, and *Bacillus subtilis*. Four replications were used, totaling 24 experimental plots. Each plot consisted of six pots, containing five seedlings per pot. All seedlings were evaluated. For each pot, the measurements obtained from the five seedlings were averaged, and subsequently, the mean of the six pots was calculated to obtain a single value for each plot. Thus, the plot was considered the experimental unit for statistical analysis. The inoculation was performed in the week of planting the cuttings, by applying 1 mL of the bacterial suspension directly to the substrate, in the region of the base of the cuttings.

Close to the commercialization period, the plants were removed from the containers and rinsed to remove excess substrate from the roots. In the laboratory, a new washing was carried out to remove all remaining substrate from the roots.

Subsequently, shoot length (cm) was evaluated, measured from the substrate level to the apex of the last leaf using a millimeter ruler; the number of fully expanded leaves, determined by visual counting; leaf area (cm^2), using an electronic leaf area meter (Li-3100C, LI-COR®, Lincoln, Nebraska, USA); the number of flowers (open and buds); shoot dry mass (g) and root dry mass (g), obtained after drying in a forced-air circulation oven at 70 ºC until constant weight (FANEM, model 520), and subsequently weighed on a precision balance of 0.001 g (SHIMADZU®, model AY220); and total dry mass (g), obtained by summing shoot and root dry masses.

At the end of the experimental period, root tissues were collected to evaluate the presence of viable bacterial populations associated with the plants. The roots were separated from the aerial parts and washed with running water. Subsequently, 1 g of root tissue was surface-disinfested with 70% ethanol for 1 min, followed by 2%-2.5% sodium hypochlorite for 3 min and 70% ethanol for 30 s, and then washed three times with distilled water. The tissues were aseptically macerated using a sterile mortar and pestle, followed by serial dilutions. Aliquots of 100 µL from the appropriate dilutions were plated onto tryptone soy agar in triplicate and incubated at 30 °C in a B.O.D. chamber. Colony-forming units (CFUs) were counted after 24, 48, and 72 h and expressed as CFU mL^{-1}. The data obtained were subjected to analysis of variance (α=0.05), and the means were compared by Tukey's test at 5% probability, using AgroEstat software (Barbosa and Maldonado Junior, 2015). Additionally, boxplot graphs were generated and Pearson correlation analysis was performed at 5% probability using the Python programming language.

Results and Discussion

Effects of rhizobacteria on biometric parameters

Rhizobacterial treatments resulted in significant differences among some growth parameters of *Rosa hybrida* 'Two Tone Jewel' (Fig. 2). *Azospirillum brasilense* showed the highest numerical values for leaf number (A), buds and open flowers (C), and total dry mass (G), while *Bacillus amyloliquefaciens* showed the highest numerical value for shoot dry mass (E). Nevertheless, none of the inoculated treatments differed significantly from the non-inoculated control. The numerical response observed for *A. brasilense* may be associated with the widely documented ability of *Azospirillum* species to produce indole-3-acetic acid (IAA), a key phytohormone in the regulation of plant growth (Giri et al., 2025).

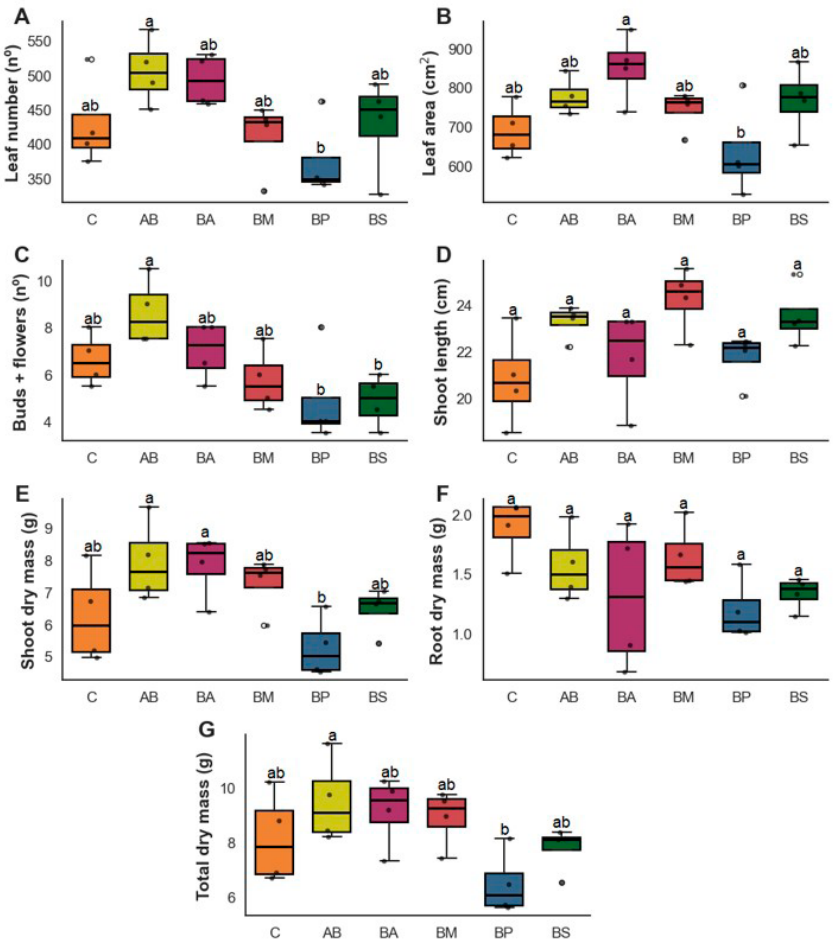

Fig. 2. Growth parameters of *Rosa hybrida* 'Two Tone Jewel' following inoculation with plant growth–promoting rhizobacteria. **(A)** Leaf number, **(B)** Leaf area, **(C)** Buds + flowers, **(D)** Shoot length, **(E)** Shoot dry mass, **(F)** Root dry mass, and **(G)** Total dry mass. Treatments consisted of *Azospirillum brasilense* (AB), *Bacillus pumilus* (BP), *Bacillus subtilis* (BS), *Bacillus megaterium* (BM), *Bacillus amyloliquefaciens* (BA) and a non-inoculated control (C). Different letters indicate significant differences among treatments according to Tukey's test ($p \leq 0.05$).

The hormones produced in response to bacterial stimulation may promote the formation of lateral roots and root hairs, increasing the effective absorption surface of the root system and potentially enhancing water and nutrient uptake, consequently contribuiting tovegetative vigor and shoot development (Awlachew and Mengistie, 2022). In ornamental crops such as miniature roses, increased vegetative growth may influence important commercial attributes, such as plant architecture, canopy density, and visual uniformity, essential characteristics for the quality of potted plants (Kisvarga et al., 2022).

On the other hand, plants treated with *Bacillus pumilus* generally showed lower numerical values for several growth variables, such as number of leaves (Fig. 2A), leaf area (Fig. 2B), and number of buds and open flowers (Fig. 2C). Although showing low values, no difference was observed from the values obtained in the control treatment. Variability in plant responses to inoculation with plant growth-promoting rhizobacteria is common and may be related to differences in rhizosphere colonization efficiency or to compatibility between microbial species and the plant host (Jaén-Contreras et al., 2022).

Leaf area (Fig. 2B) showed significant differences among bacterial treatments, with the highest numerical value observed in plants treated with *Bacillus amyloliquefaciens*. However, it differed only from the treatment with *Bacillus pumilus* inoculation, which obtained the lowest value. Greater leaf expansion may indicate a potential increase in the photosynthetic capacity of the plants (Joshi et al., 2023). Species of the genus *Bacillus* are recognized for producing metabolites such as phytohormones, siderophores, and organic acids that enhance nutrient availability in the rhizosphere, especially phosphorus and micronutrients (Bisht et al., 2025; Mani et al., 2025). Increased nutrient availability may stimulate processes of cell division and expansion in developing leaves, resulting in greater leaf surface area (Mohanty et al., 2021).

Inoculation with these biostimulants also influenced reproductive traits of miniature roses. However, it didn't differ significantly from that of the non-inoculated control, plants treated with *Azospirillum brasilense* showed a higher numerical value for the number of buds and flowers (Fig. 2C) among the bacterial treatments. The stimulation of reproductive development may be associated with improved nutritional status of the plants and hormonal modulation promoted by rhizosphere microorganisms. Rhizobacteria capable of producing auxins, cytokinins, and gibberellins may influence physiological processes related to floral differentiation and bud formation (Prisa and Jamal, 2025).

Additionally, the greater numerical values observed for some vegetative traits in inoculated plants may indicate a potential increase the availability of carbohydrates that support reproductive development. The allocation of photoassimilates to floral structures is a central factor in ornamental crops, in which the abundance and uniformity of flowers determine the attractiveness of the final product (Jegadeeswari et al., 2025). Thus, the higher numerical value increase in flowering observed in plants inoculated with *A. brasilense* highlights the potential of this microorganism to simultaneously improve vegetative vigor and reproductive performance in mini rose cultivation.

Similar results were reported by Silveira et al. (2025), who observed a significant increase in initial establishment and biometric traits of *Zinnia elegans* after inoculation with plant growth-promoting rhizobacteria, demonstrating the potential of these microorganisms in modulating plant growth in ornamental species. Similarly, Campos et al. (2023) demonstrated that rhizobacterial inoculation improved growth and seedling quality of *Euterpe oleracea*, indicating that plant–microorganism interactions may enhance physiological processes related to nutrient uptake and phytohormone synthesis.

Root dry mass did not show significant differences among treatments. This result suggests that rhizobacteria may have promoted changes in root system architecture without necessarily increasing total root biomass. These functional modifications in the root system may contribute to the numerical differences in shoot growth and biomass accumulation observed among the bacterial treatments. Consistently, Campos et al. (2025) reported that bacterial inoculation increased growth and seedling quality of *Handroanthus impetiginosus*, supporting that growth promotion mediated by beneficial microorganisms is often associated with the production of plant growth regulators, such as auxins, as well as nutrient solubilization in the rhizosphere (Prisa and Jamal, 2025).

Visual representation of plant morphology at the end of the experimental period

A representative plant from each treatment is presented in Fig. 3 to provide a visual representation of plant morphology at the end of the experimental period. For photographic documentation, plants from each treatment were arranged in ascending order according to plant size, and the plant positioned in the middle of the distribution was selected.

Fig. 3. Representative photographs of *Rosa hybrida* 'Two Tone Jewel' plants grown under different rhizobacterial inoculation treatments: control (C), *Azospirillum brasilense* (Ab), *Bacillus amyloliquefaciens* (Ba), *Bacillus megaterium* (Bm), *Bacillus pumilus* (Bp) and *Bacillus subtilis* (Bs). Images illustrate typical plant morphology observed for each treatment at the end of the experiment.

Plants inoculated with *Azospirillum brasilense* and *Bacillus amyloliquefaciens* visually exhibited denser canopies and a greater number of reproductive structures compared to the control treatment. These visual differences corroborate the quantitative results observed in growth and biomass analyses reported by Campos et al. (2024), who also observed a significant increase in the vegetative development of *Handroanthus chrysotrichus* seedlings after inoculation with rhizobacteria, a result mainly attributed to the stimulation of plant activity and greater efficiency in nutrient assimilation.

In ornamental production systems, plant visual architecture constitutes a decisive factor for the commercial acceptance of the product, making these effects particularly relevant for the floriculture sector (Mittal et al., 2026).

Correlations among growth variables

Strong positive correlations were observed between number of leaves and leaf area, indicating that plants producing a greater number of leaves also tend to exhibit a larger photosynthetic surface (Fig. 4). Similarly, shoot dry mass showed strong positive correlations with leaf area and number of leaves. These associations suggest that the evaluated vegetative traits were related to biomass accumulation. However, correlation analysis reflects associations among variables and does not establish causal relationships or provide evidence of treatment effects.

Total dry mass showed an almost perfect correlation with shoot dry mass, demonstrating that most of the biomass in miniature roses is allocated to aerial tissues. The number of buds and flowers showed moderate positive correlations with biomass variables, suggesting that

reproductive development is partially related to the accumulation of vegetative resources. Plants with greater photosynthetic capacity therefore tend to sustain higher flower production (Zhou et al., 2026).

Persistence and colonization of rhizobacteria

At the end of the experimental period, viable bacterial populations were recovered from root tissues of the inoculated plants (Fig. 5). The maintenance of viable microbial populations in association with plant roots may contribute to interactions between microorganisms and plants, which is considered relevant to the performance of microbial inoculants (Oliveira et al., 2025).

Differences in CFU counts were observed among treatments (Fig. 5). These differences indicate variation in the number of viable bacterial populations recovered from the root tissues; however, their biological significance should be interpreted with caution, as CFU counts alone do not necessarily reflect the colonization capacity or functional activity of the microorganisms.

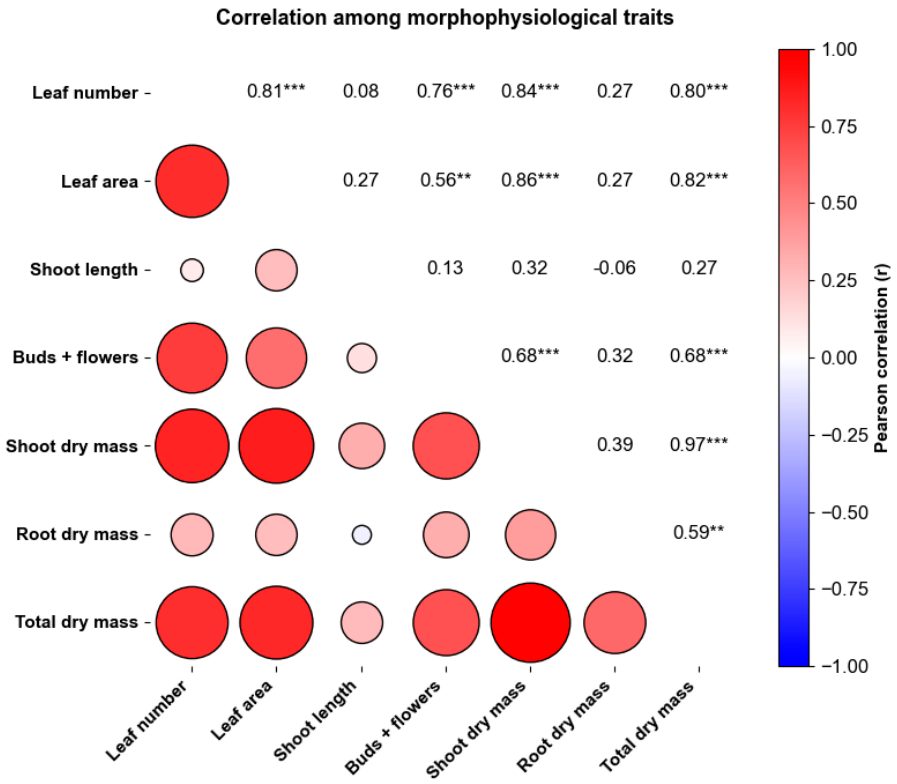

Fig. 4. Pearson correlation matrix showing relationships among morphological and biomass parameters measured in *Rosa hybrida* 'Two Tone Jewel' plants subjected to rhizobacterial inoculation treatments. Color intensity indicates the strength and direction of correlations (positive or negative). Asterisks (*) indicate statistically significant correlations according to Pearson's test ($p \leq 0{,}05$).

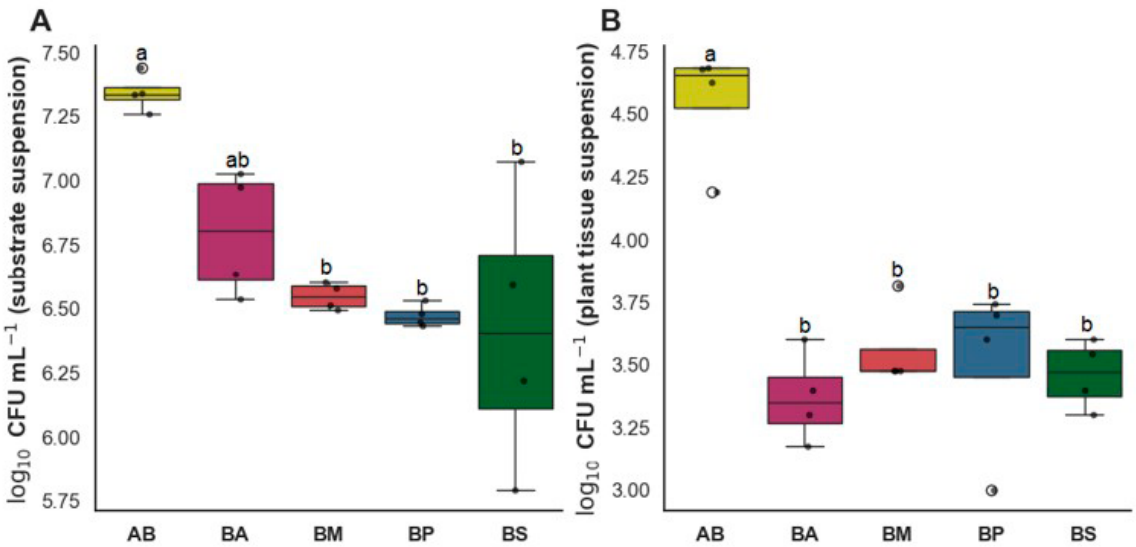

Fig. 5. Boxplots showing colony-forming units (CFU) of rhizobacteria recovered after the experiment. **(A)** CFU extracted from the substrate and **(B)** CFU extracted from plant tissues of *Rosa hybrida* 'Two Tone Jewel'. Treatments included *Azospirillum brasilense* (AB), *Bacillus amyloliquefaciens* (BA), *Bacillus megaterium* (BM), *Bacillus pumilus* (BP) and *Bacillus subtilis* (BS); the control treatment was not included in this analysis. Values are expressed as $\log_{10}$ CFU mL^{-1}. Boxplots represent the median (central line), interquartile range (box), and minimum-maximum values (whiskers), with individual replicates shown as points. Different letters indicate significant differences among treatments according to Tukey's test ($p \leq 0{,}05$).

Conclusions

Bacterial inoculation resulted in variable responses in miniature rose 'Two Tone Jewel', depending on the microorganism and growth variable evaluated. *Azospirillum brasilense* presented higher mean values for leaf number, number of buds and flowers, and total dry mass, while *Bacillus amyloliquefaciens* presented higher mean values for leaf area and shoot dry mass. However, these responses were not consistently different from the uninoculated control. Shoot length and root dry mass were not significantly affected by bacterial inoculation. Overall, the results indicate that the effects of bacterial inoculation varied among the evaluated traits, and further studies are needed to determine the potential of these microorganisms as biostimulants for miniature rose production.

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Author Contribution

MMS: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft. **ACB:** Data Curation, Investigation, Validation, Writing – Review & Editing. **MCL:** Conceptualization, Investigation, Methodology, Validation, Writing – Review & Editing. **AMBS:** Data Curation, Formal Analysis, Visualization, Writing – Review & Editing. **GLQ:** Formal Analysis, Writing – Review & Editing. **CHBS:** 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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