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Potential of *Bacillus aryabhattai* and *Pseudomonas fluorescens* for Promoting Plant Growth under Water Stress

Potencial de *Bacillus aryabhattai* e *Pseudomonas fluorescens* na promoção do crescimento vegetal sob estresse hídrico

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ABSTRACT

Drought is one of the major abiotic stresses limiting productivity worldwide, highlighting the need for sustainable strategies to improve crop resilience under water-limited conditions. This study evaluated the effects of the plant growth-promoting bacteria *Bacillus aryabhattai* and *Pseudomonas fluorescens*, applied individually or in combination, on soybean growth under well-watered and water-deficit conditions. The experiment was conducted in a completely randomized design arranged in a 2 × 4 factorial scheme, consisting of two irrigation regimes (well-watered and water deficit) and four biological treatments (control, *B.*

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aryabhattai, *P. fluorescens*, and co-inoculation with both bacteria). Plant growth variables, including shoot and root length, root volume, fresh biomass, and dry biomass, were evaluated after the stress period. Under well-watered conditions, co-inoculation promoted the greatest fresh root biomass and consistently increased shoot biomass compared with the untreated control. Under water-deficit conditions, *B. aryabhattai* alone produced the highest fresh root biomass and root volume, demonstrating superior performance in promoting root development during drought stress. Although most variables did not differ significantly among treatments, inoculated plants consistently exhibited greater vegetative growth than non-inoculated plants. These findings demonstrate the potential of *B. aryabhattai* and *P. fluorescens* as plant growth-promoting bacteria capable of enhancing soybean development, with *B. aryabhattai* showing particular promise for improving plant performance under water-limited conditions.

Keywords: *Glycine max*; plant growth-promoting rhizobacteria; *Bacillus aryabhattai*; *Pseudomonas fluorescens*; drought stress; biological inoculants.

RESUMO

O déficit hídrico é um dos principais fatores abióticos responsáveis pela redução da produtividade, tornando necessária a adoção de estratégias sustentáveis capazes de aumentar a tolerância da cultura ao estresse hídrico. O objetivo deste estudo foi avaliar o potencial de *Bacillus aryabhattai* e *Pseudomonas fluorescens*, aplicados de forma isolada e em coinoculação, sobre o crescimento de plantas de soja cultivadas sob condições de disponibilidade hídrica adequada e déficit hídrico. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 2 × 4, constituído por dois regimes hídricos (sem e com estresse hídrico) e quatro tratamentos biológicos (testemunha, *B. aryabhattai*, *P. fluorescens* e *B. aryabhattai* + *P. fluorescens*). Ao final do período experimental, foram avaliados comprimento da parte aérea e das raízes, volume radicular, massa verde e massa seca da parte aérea e do sistema radicular. Em condições sem estresse hídrico, a coinoculação promoveu maior massa verde de raízes e apresentou tendência de incremento na biomassa da parte aérea. Sob déficit hídrico, *B. aryabhattai* proporcionou os maiores valores de massa verde e volume radicular, evidenciando maior eficiência na promoção do desenvolvimento do sistema radicular sob restrição hídrica. Embora a maioria das variáveis não tenha apresentado diferenças estatisticamente significativas, as plantas inoculadas apresentaram, de forma consistente, desempenho superior ao da testemunha. Os resultados demonstram o potencial de *Bacillus aryabhattai* e *Pseudomonas fluorescens* como bactérias promotoras de crescimento vegetal, destacando *B. aryabhattai* como uma alternativa promissora para aumentar a tolerância da soja ao déficit hídrico.

Palavras-chave: *Glycine max*; bactérias promotoras de crescimento vegetal; *Bacillus aryabhattai*; *Pseudomonas fluorescens*; déficit hídrico; inoculação biológica.

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1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important agricultural crops, playing a strategic role in the production of food, animal feed, biofuels, and industrial products. In Brazil, soybean cultivation initially expanded in the southern region of the country; however, advances in plant breeding, crop management, and the development of cultivars adapted to diverse environmental conditions have enabled its successful expansion into the Cerrado biome and new agricultural frontiers, particularly the MATOPIBA region (FREITAS, 2011).

Brazil is currently the world's largest soybean producer and exporter, contributing substantially to global food security. Nevertheless, achieving high crop productivity depends not only on improved agronomic practices but also on favorable environmental conditions. Among the abiotic factors limiting soybean production, drought stress is one of the most significant due to its high frequency and substantial impact on grain yield. During the 2021/2022 growing season, severe drought events, particularly in southern Brazil, markedly reduced the national average soybean yield despite the country's high overall production, highlighting the vulnerability of the crop to water scarcity (CONAB, 2022).

These adverse climatic conditions were largely associated with the La Niña phenomenon, which alters rainfall distribution across Brazil, resulting in above-average precipitation in some regions and prolonged drought periods in others, especially in the southern states (CONAB, 2022). However, even in regions with adequate annual rainfall, temporary water deficits during critical developmental stages may significantly impair crop performance. Soybean is particularly sensitive to drought during the reproductive phase, and water restriction lasting approximately two weeks may substantially reduce yield potential.

Water deficit negatively affects several physiological and biochemical processes, including leaf expansion, photosynthesis, nutrient uptake, stomatal conductance, and root development. Consequently, drought-stressed plants generally exhibit reduced plant height, fewer pods, lower seed weight, and significant yield losses. Previous studies have demonstrated that water stress

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occurring during flowering and grain filling may be as detrimental as prolonged drought throughout the entire crop cycle (GAVA, 2014).

The remarkable expansion of soybean production in Brazil has been closely associated with continuous technological and scientific advances, including improvements in crop management, genetic breeding, and the development of innovative agricultural inputs (FREITAS, 2011). More recently, considerable attention has been directed toward sustainable technologies capable of enhancing plant tolerance to abiotic stresses, among which plant growth-promoting rhizobacteria (PGPR) have emerged as promising biological tools.

Species belonging to the genera *Bacillus* and *Pseudomonas* have received increasing attention due to their ability to colonize the rhizosphere and stimulate plant growth through multiple mechanisms, including phytohormone production, nutrient solubilization, synthesis of bioactive metabolites, stimulation of root system development, and induction of physiological responses associated with drought tolerance. Although the molecular mechanisms underlying these interactions have not yet been fully elucidated, numerous studies have reported significant improvements in plant performance under water-limited conditions following inoculation with these beneficial microorganisms (SILVA, 2019; GAGNÉ-BOURQUE et al., 2015).

Among these beneficial bacteria, *Bacillus aryabhattai* and *Pseudomonas fluorescens* have demonstrated considerable potential for mitigating the adverse effects of drought stress. These microorganisms establish beneficial associations with plant roots, promoting root growth, enhancing nutrient acquisition, and producing metabolites that improve plant adaptation to water-limited environments. Furthermore, they exhibit additional plant-beneficial traits, including phosphate solubilization and biological control of phytopathogens. Consequently, research focusing on these bacterial species has expanded considerably in recent years, driven by the increasing demand for sustainable technologies capable of improving crop resilience and maintaining high productivity under adverse environmental conditions.

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In this context, The present study aimed to evaluate the effects of *Bacillus aryabhattai* and *Pseudomonas fluorescens*, applied individually and in combination, on the early growth and biomass accumulation of soybean plants under contrasting water availability conditions.

2. MATERIALS AND METHODS

2.1 Experimental site

The experiment was conducted at the Experimental Station of the Federal University of Technology – Paraná (UTFPR), Dois Vizinhos Campus, Paraná, Brazil. The experimental area is located at 25°44′05″ S and 53°03′31″ W, at an altitude of approximately 530 m above sea level. According to the Brazilian Soil Classification System, the predominant soils in the region are Oxisols and Nitisols, characterized by deep profiles and good drainage. The regional climate is classified as humid subtropical (Cfa), according to the Köppen classification, with hot summers and no defined dry season. Throughout the experimental period, irrigation was managed under controlled conditions to establish the proposed water stress treatments.

2.2 Experimental design

The experiment was arranged in a completely randomized design following a 2 × 4 factorial scheme with four replications. The first experimental factor consisted of two irrigation regimes: plants maintained under adequate water supply (well-watered condition) and plants subjected to water deficit. The second factor consisted of four biological treatments: untreated control, seed inoculation with *Bacillus aryabhattai*, seed inoculation with *Pseudomonas fluorescens*, and co-inoculation with *Bacillus aryabhattai* plus *Pseudomonas fluorescens*. Consequently, a total of eight treatment combinations were evaluated.

2.3 Plant cultivation and water stress induction

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Soybean seeds were treated with the respective biological agents immediately before sowing. The treated seeds were sown in plastic pots measuring 40 cm in height and 8 L in volume, filled with soil collected from the UTFPR experimental area. Ten seeds were initially sown in each pot, and after seedling emergence, thinning was performed to maintain two uniform plants per experimental unit.

Plants were cultivated under adequate soil moisture until reaching the V5 phenological stage, characterized by five fully expanded trifoliolate leaves. At this stage, irrigation was completely suspended for 15 consecutive days in the water-deficit treatments, whereas plants assigned to the well-watered treatment were maintained near field capacity throughout the experiment.

After the water restriction period, irrigation was resumed in all treatments and maintained under normal conditions for an additional 20 days until the final harvest.

2.4 Plant growth evaluations

At the end of the experimental period, plants were carefully removed from the pots and the root systems were gently washed under running water to remove adhering soil particles. Shoot length (cm) and root length (cm) were determined using a graduated measuring tape. Root volume (mL) was determined by the water displacement method using a graduated cylinder.

Fresh shoot biomass (g) and fresh root biomass (g) were determined immediately after harvest using a precision analytical balance. Subsequently, plant tissues were dried in a forced-air circulation oven until constant weight to determine shoot dry biomass (g) and root dry biomass (g).

2.5 Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) after verifying the assumptions of normality and homogeneity of variances. Whenever significant differences among treatments were detected, means were compared using Tukey's multiple comparison test at the 5% probability level ($p \leq 0.05$). All

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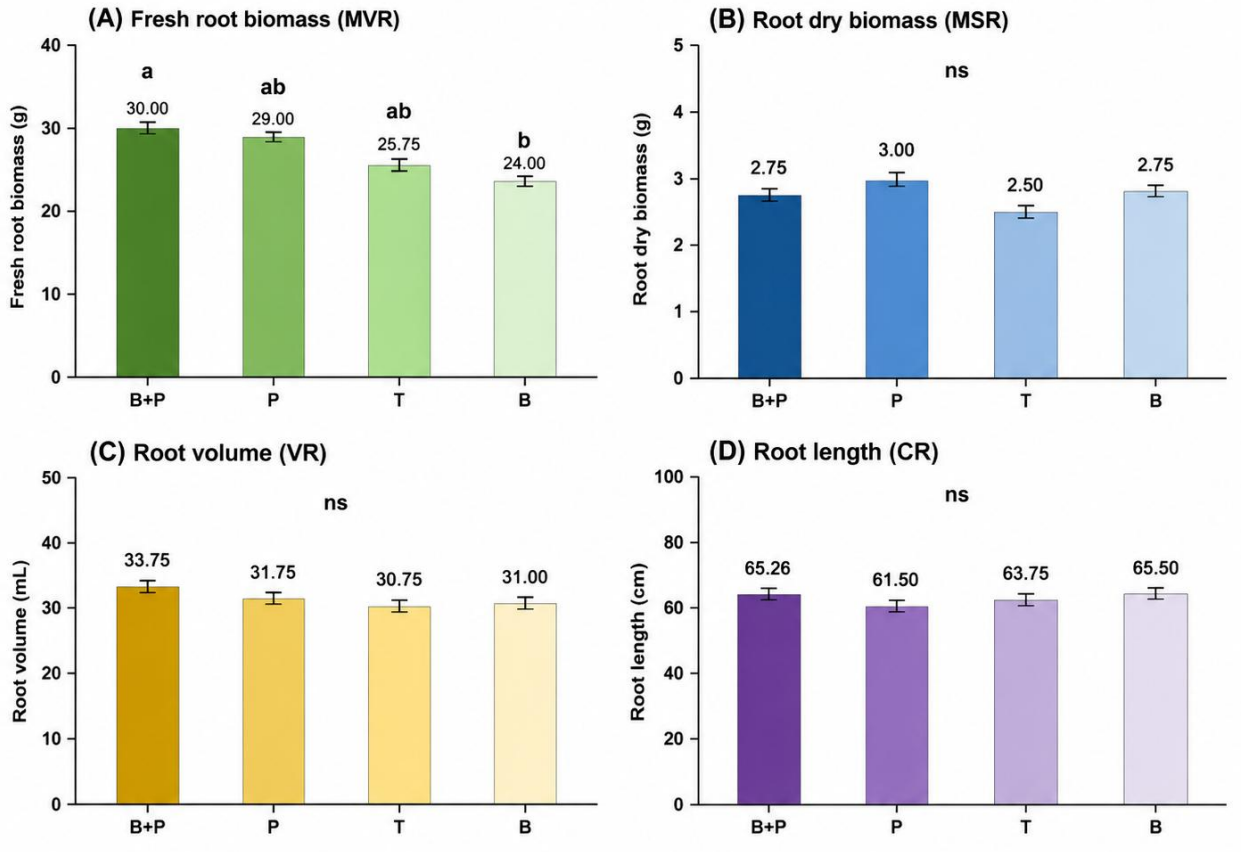
statistical analyses were performed using the RBio statistical software (Bhering, 2017).

3. RESULTS AND DISCUSSION

3.1 Root development under well-watered conditions

Under well-watered conditions, significant differences among treatments were observed only for fresh root biomass, whereas root dry biomass, root volume, and root length were not significantly affected by bacterial inoculation (Figure 1). The co-inoculation of *Bacillus aryabhattai* and *Pseudomonas fluorescens* (B+P) resulted in the highest fresh root biomass, approximately 14% greater than the untreated control.

Figure 1. Root growth parameters of soybean plants grown under well-watered conditions following seed inoculation with *Bacillus aryabhattai* (B), *Pseudomonas fluorescens* (P), their co-inoculation (B+P), or no inoculation (T). (A) Fresh root biomass, (B) Root dry biomass, (C) Root volume, and (D) Root length. Different lowercase letters indicate significant differences according to Tukey's test ($p \leq 0.05$). "ns" indicates non-significant differences.



Source: The authors.

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Although no significant differences were detected for root dry biomass, root volume, and root length, inoculated plants consistently exhibited numerically greater values than the control (Figure 1). These results suggest that bacterial inoculation promoted root development under favorable growing conditions, although the response magnitude was insufficient to produce statistical differences for all evaluated variables.

The increase in fresh root biomass following co-inoculation may be associated with complementary mechanisms of plant growth promotion. Species of *Bacillus* and *Pseudomonas* are recognized for producing phytohormones and other bioactive compounds capable of stimulating root growth, nutrient uptake, and rhizosphere colonization (SILVA, 2019; GAGNÉ-BOURQUE et al., 2015).

Root length did not differ significantly among treatments (Figure 1). A possible explanation is the physical restriction imposed by the experimental pots, which may have limited vertical root growth. Similar observations have been reported by Guerra et al. (2021), who demonstrated that root confinement in small containers restricts root expansion and consequently influences shoot development. Likewise, Campagnol et al. (2012) reported that larger containers promote greater root expansion and improved shoot growth in tomato plants.

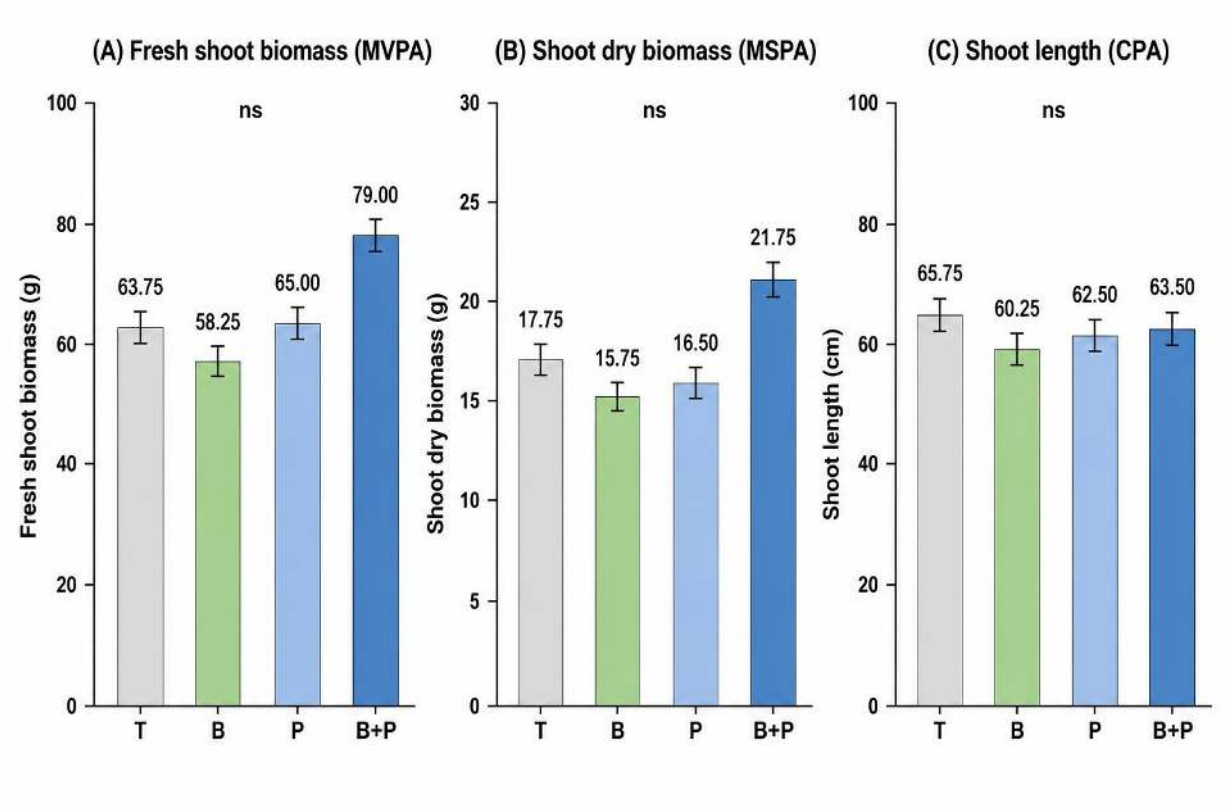
3.2 Shoot growth under well-watered conditions

No significant differences were observed among treatments for shoot fresh biomass, shoot dry biomass, or shoot length under well-watered conditions (Figure 2). Nevertheless, plants receiving the combined inoculation of *B. aryabhattai* and *P. fluorescens* exhibited the highest numerical values for fresh and dry shoot biomass, with increases of approximately 19% and 18%, respectively, compared with the untreated control.

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Figure 2. Shoot growth parameters of soybean plants grown under well-watered conditions following seed inoculation with Bacillus aryabhattai (B), Pseudomonas fluorescens (P), their co-inoculation (B+P), or no inoculation (T). (A) Fresh shoot biomass (MVPA), (B) Shoot dry biomass (MSPA), and (C) Shoot length (CPA). Bars represent treatment means. ns indicates no significant differences among treatments according to Tukey's test ($p \leq 0.05$).



Source: The authors.

Although these differences were not statistically significant, the positive numerical responses indicate a beneficial effect of bacterial inoculation on soybean vegetative growth. Such responses are consistent with the capacity of plant growth-promoting rhizobacteria to improve nutrient acquisition and stimulate plant metabolism under favorable environmental conditions (SILVA, 2019; GAGNÉ-BOURQUE et al., 2015).

3.3 Root development under water deficit

Water deficit significantly affected root development, with differences observed for fresh root biomass and root volume (Figure 3). Plants inoculated with

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Bacillus aryabhattai exhibited the highest values for both variables, surpassing the untreated control by approximately 30%.

Root growth represents one of the primary adaptive mechanisms that enable plants to tolerate water deficit by increasing soil exploration and improving water uptake. Although root dry biomass did not differ significantly among treatments, plants inoculated with *B. aryabhattai* showed values approximately 38% greater than those observed for the control (Figure 3), reinforcing the positive influence of this bacterium on root development.

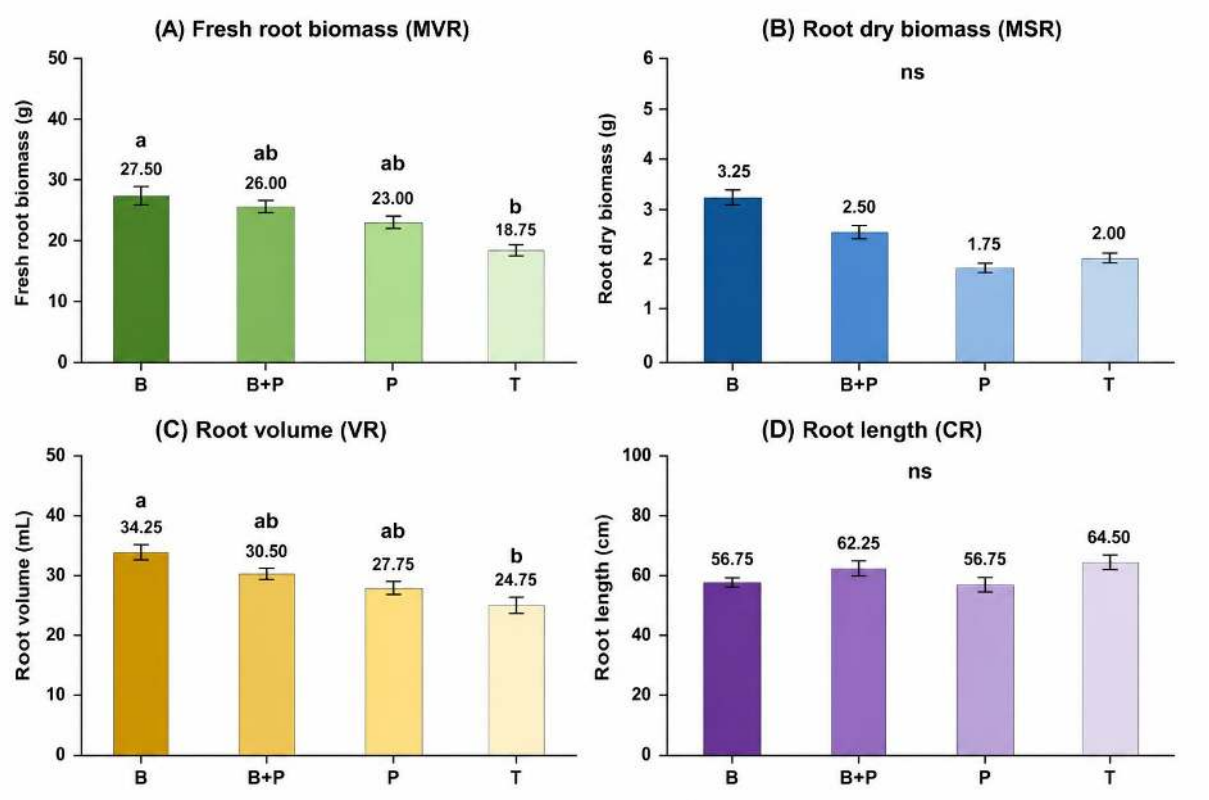
These findings agree with those reported by Sousa et al. (2023), who demonstrated that *Bacillus aryabhattai* enhances plant performance under water-limited conditions through mechanisms involving phytohormone regulation, including gibberellins, cytokinins, abscisic acid, and auxins, thereby contributing to drought tolerance.

Interestingly, the co-inoculation treatment also promoted greater root growth than the untreated control, although its performance was slightly lower than that obtained with *B. aryabhattai* alone (Figure 3). This response suggests that, under drought conditions, the mechanisms activated by *B. aryabhattai* may have played a more prominent role than those associated with the bacterial consortium.

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Figure 3. Root growth parameters of soybean plants grown under water-deficit conditions following seed inoculation with *Bacillus aryabhattai* (B), *Pseudomonas fluorescens* (P), their co-inoculation (B+P), or no inoculation (T). (A) Fresh root biomass (MVR), (B) Root dry biomass (MSR), (C) Root volume (VR), and (D) Root length (CR). Bars represent treatment means. Different lowercase letters indicate significant differences according to Tukey's test ($p \leq 0.05$). **ns** indicates no significant differences among treatments.



Source: The authors.

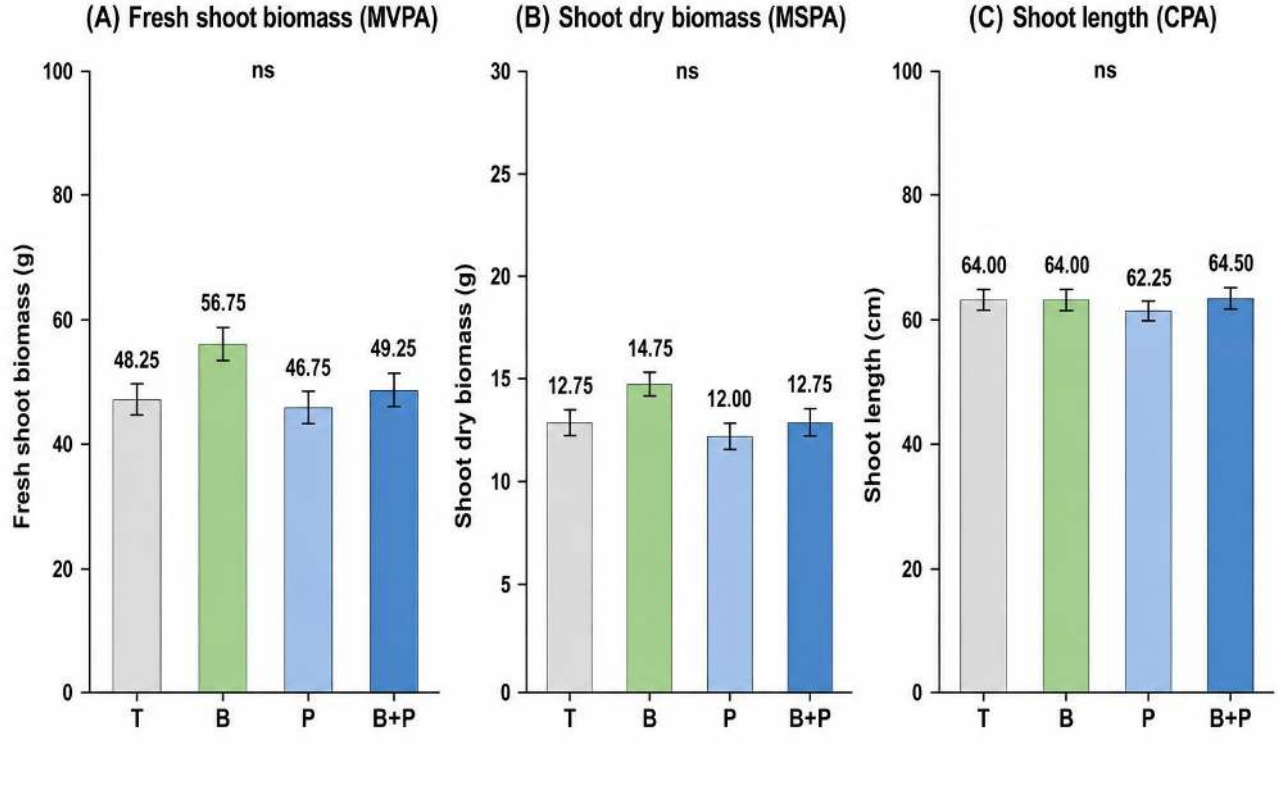
3.4 Shoot growth under water deficit

No significant treatment effects were detected for shoot fresh biomass, shoot dry biomass, or shoot length under water deficit (Figure 4). However, plants inoculated with *Bacillus aryabhattai* consistently exhibited the highest numerical values for both fresh and dry shoot biomass, with increases of approximately 15% and 13%, respectively, relative to the untreated control.

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Figure 4. Shoot growth parameters of soybean plants grown under water-deficit conditions following seed inoculation with *Bacillus aryabhattai* (B), *Pseudomonas fluorescens* (P), their co-inoculation (B+P), or no inoculation (T). (A) Fresh shoot biomass (MVPA), (B) Shoot dry biomass (MSPA), and (C) Shoot length (CPA). Bars represent treatment means. **ns** indicates no significant differences among treatments according to Tukey's test ($p \leq 0.05$).



Source: The authors.

Although the differences were not statistically significant, these results indicate that *B. aryabhattai* contributed to maintaining shoot growth under drought conditions. Similar observations were reported by Spanevello (2023), who demonstrated that the co-inoculation of *Bacillus aryabhattai* with other plant growth-promoting bacteria improved soybean development under drought conditions associated with the La Niña phenomenon.

3.5 Agronomic implications of bacterial inoculation

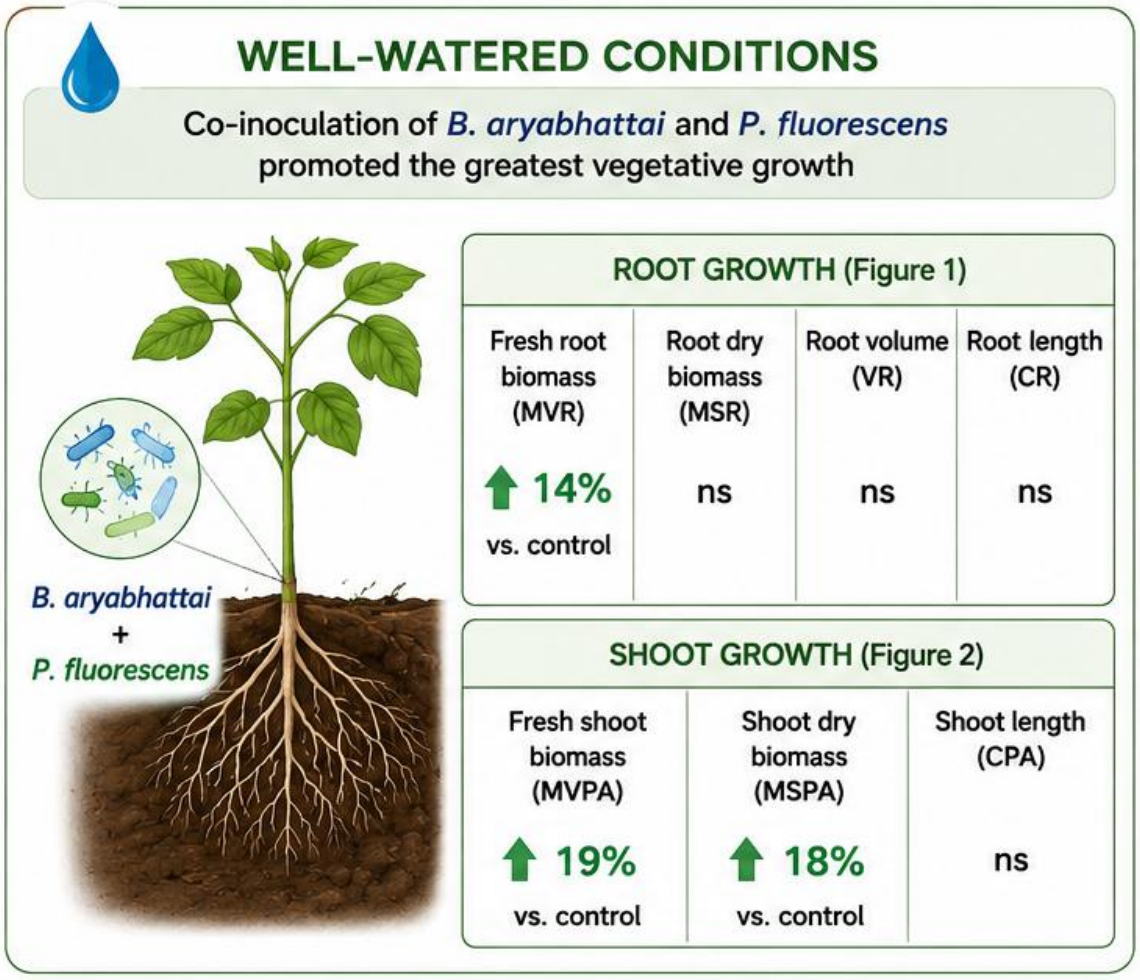
A comparative evaluation of both irrigation regimes demonstrated that bacterial performance varied according to environmental conditions. Under

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adequate water availability, co-inoculation with *B. aryabhattai* and *P. fluorescens* generally resulted in the greatest values for root and shoot growth variables (Figures 1 and 2). A schematic summary of the main responses observed under well-watered conditions is presented in Figure 5. Conversely, under water deficit, inoculation with *B. aryabhattai* alone consistently produced the best root development, particularly for fresh root biomass and root volume (Figures 3 and 4). The overall effects of bacterial inoculation under drought conditions are summarized in Figure 6.

Figure 5. Schematic representation of the agronomic effects of seed co-inoculation with *Bacillus aryabhattai* and *Pseudomonas fluorescens* on soybean plants grown under well-watered conditions. Co-inoculation promoted greater root fresh biomass and consistently increased shoot biomass compared with the untreated control, indicating enhanced vegetative development under favorable moisture conditions.

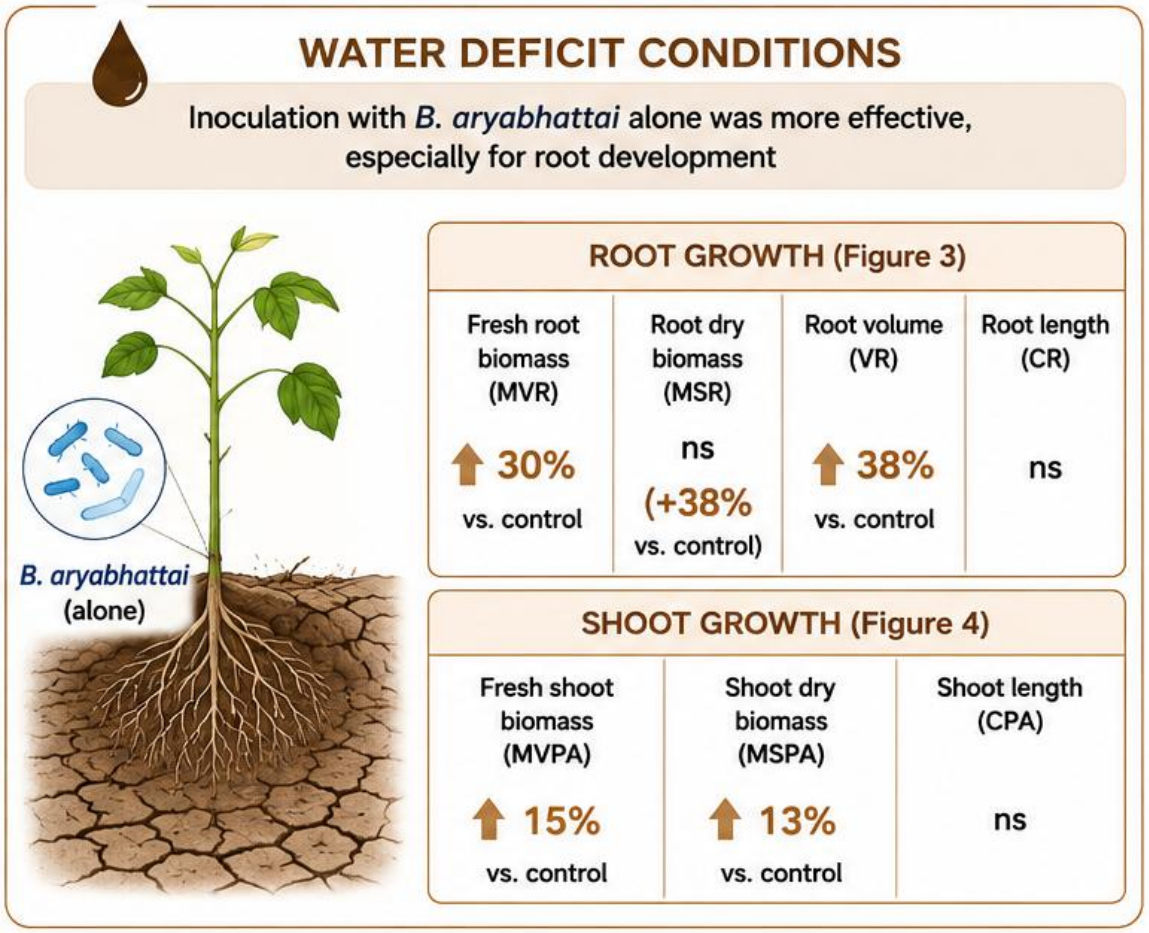


Source: Created by the authors with the assistance of ChatGPT (OpenAI, GPT-5.5), based on the experimental results obtained in this study.

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Figure 6. Schematic representation of the agronomic effects of *Bacillus aryabhattai* inoculation on soybean plants grown under water-deficit conditions. Inoculation enhanced root development, particularly fresh root biomass and root volume, and contributed to the maintenance of shoot biomass under drought stress, demonstrating its potential to improve soybean tolerance to water limitation.



Source: Created by the authors with the assistance of ChatGPT (OpenAI, GPT-5.5), based on the experimental results obtained in this study.

These results indicate that the effectiveness of plant growth-promoting bacteria depends on environmental conditions. Under favorable moisture conditions, the combined activity of *B. aryabhattai* and *P. fluorescens* appears to enhance vegetative growth. In contrast, under drought stress, *B. aryabhattai* alone demonstrated greater capacity to mitigate the adverse effects of water limitation (Figure 5 and 6).

The beneficial effects of *Pseudomonas fluorescens* observed under well-watered conditions are supported by the findings of Klein and Klein (2021) and

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Guimarães et al. (2021), who reported improvements in soybean root growth, biomass accumulation, and grain yield following seed inoculation with this bacterium. Under drought conditions, however, the superior performance of *B. aryabhattai* may be explained by its ability to stimulate biofilm formation, regulate phytohormone production, and enhance antioxidant activity, mechanisms previously described by Kavamura (2012) and Park et al. (2017).

Overall, although relatively few variables exhibited statistically significant differences, the consistent numerical improvements observed in root and shoot growth demonstrate the potential of these bacterial inoculants for soybean cultivation. In particular, the responses obtained under water deficit reinforce the potential use of *Bacillus aryabhattai* as a sustainable biological strategy to improve crop tolerance to drought stress.

4. CONCLUSION

The present study demonstrated that seed inoculation with Bacillus aryabhattai and Pseudomonas fluorescens, applied individually or in combination, positively influenced soybean growth under the experimental conditions evaluated. Under well-watered conditions, co-inoculation generally resulted in the greatest values for shoot and root growth variables, particularly fresh root biomass, suggesting that the combined use of these plant growth-promoting bacteria may favor vegetative development when water availability is not limiting. Under the water-deficit regime adopted in this study, Bacillus aryabhattai consistently promoted greater root fresh biomass and root volume than the non-inoculated control, indicating a positive effect on root development under water restriction. Although several additional growth variables exhibited numerically higher values in inoculated plants, these responses should be interpreted with caution when statistical significance was not detected.

The results indicate that inoculation with plant growth-promoting bacteria contributed to maintaining plant growth under contrasting water availability conditions, particularly by stimulating root system development, which may represent an important characteristic for plant establishment under limited water

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availability. Nevertheless, the present findings should be interpreted as evidence of growth promotion under the evaluated experimental conditions rather than definitive proof of increased drought tolerance, resilience, or improved water-use efficiency. Such physiological responses were not directly assessed in this study and, therefore, cannot be inferred solely from morphological growth variables.

Overall, the study provides evidence that Bacillus aryabhattai and Pseudomonas fluorescens have potential as plant growth-promoting bacteria for soybean cultivation, with Bacillus aryabhattai showing particularly promising responses for root development under the water-restriction conditions evaluated, while co-inoculation tended to promote greater vegetative growth under well-watered conditions. However, additional studies are required to confirm these responses under a broader range of environmental conditions. Future investigations should include complete factorial analyses of plant responses, quantification of soil water status, confirmation of bacterial colonization, evaluation of physiological and biochemical indicators associated with drought responses, assessment of nodulation and plant–microorganism interactions, as well as greenhouse and field trials involving different cultivars, soil types, and environmental conditions. Such studies will be essential to clarify the mechanisms underlying the observed growth responses and to determine the practical agronomic applicability of these microorganisms as biological tools for soybean production under water-limited environments.

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