Ensaios e Ciência
ISSN 1415-6938 e ISSN 2178-695X

DOI: 10.17921/1415-6938.2025v29n2p505-517

Effect of Hydrocapacity Bacteria Inoculation on Soybean Development Under Water Deficit

Efeito da Inoculação de bactérias Hidrocapacitadoras no Desenvolvimento da Soja sob Déficit Hídrico

Received on: 16/01/2025
Accepted on: 29/05/2025

Romildo Rêgo Sacramento Júnior: Instituto Federal Goiano, Campus Ceres. GO, Brazil.

Igor Araújo: Universidade do Estado de Mato Grosso, Campus Nova Xavantina, Programa de Pós-Graduação em Ecologia e Conservação. MT, Brazil. E-mail: igor.araujo@outlook.com.br

Jessica Maria Israel de Jesus: Universidade de São Paulo. SP, Brazil.

Aleksander Samuel Fidelis Pereira: Instituto Federal Goiano, Campus Ceres. GO, Brazil.

Mônica Lau da Silva Marques: Instituto Federal Goiano, Campus Ceres. GO Goiás, Brazil.

Abstract

Soybean (*Glycine max* L.) is a legume of a great economic importance for Brazil, the world's leading producer and exporter of this crop. In the 2022/2023 season, production reached a record 322.8 million tons. However, due to the El Niño climatic phenomenon, the forecast for the 2023/2024 season indicates a decrease to 306.4 million t, representing a 5.08% reduction. This decline is attributed to delayed and reduced rainfall, highlighting water scarcity as a growing challenge for farmers, particularly in regions prone to climatic extremes. In this context, low-cost strategies such as the use of plant growth-promoting and hydrocapacity microorganisms have been investigated as promising alternatives to mitigate the effects of water deficit. Here, we evaluated the impact of hydrocapacity bacterial inoculation on the vegetative growth of soybeans under water stress conditions. The experiment consisted of four treatments: irrigated inoculated seeds, irrigated non-inoculated seeds, non-irrigated inoculated seeds, and non-irrigated non-inoculated seeds, arranged in a randomized block design with six replicates. We assessed root length, number of nodes, number of leaves, number of flowers/pods, and plant height. The use of a commercial product containing *Bacillus aryabhathai*, *Bacillus circulans*, and *Bacillus haynesii* resulted in significant improvements in the vegetative development of the cultivar CZ37B31I2X, promoting greater root length, number of nodes, flowers/pods, plant height, and dry mass. These results indicate the potential of hydrocapacity microorganisms to enhance soybean resilience under water deficit conditions.

Keywords: *Glycine max* L. Water Deficit. Plant Growth. Agricultural Production. *Bacillus* spp.

Resumo

A soja (*Glycine max* L.) é uma leguminosa de grande importância econômica para o Brasil, maior produtor e exportador mundial dessa cultura. Na safra 2022/2023, a produção atingiu um recorde de

322,8 milhões de toneladas. No entanto, devido ao fenômeno climático El Niño, a previsão para a safra 2023/2024 indica uma redução para 306,4 milhões de toneladas, representando uma diminuição de 5,08%. Esse declínio é atribuído ao atraso e à redução das chuvas, destacando a escassez hídrica como um desafio crescente para os agricultores, especialmente em regiões suscetíveis a extremos climáticos. Nesse contexto, estratégias de baixo custo, como o uso de microrganismos promotores de crescimento e hidrocapacitadores, têm sido investigadas como alternativas promissoras para mitigar os efeitos do déficit hídrico. Neste estudo, avaliamos o impacto da inoculação de bactérias hidrocapacitadoras no desenvolvimento vegetativo da soja sob condições de estresse hídrico. O experimento foi composto por quatro tratamentos: sementes inoculadas irrigadas, sementes não inoculadas irrigadas, sementes inoculadas não irrigadas e sementes não inoculadas não irrigadas, dispostos em delineamento em blocos casualizados com seis repetições. Avaliamos o comprimento radicular, o número de nós, o número de folhas, o número de flores/vagens e a altura das plantas. O uso de um produto comercial contendo *Bacillus aryabhathai*, *Bacillus circulans* e *Bacillus haynesii* resultou em melhorias significativas no desenvolvimento vegetativo da cultivar CZ37B31I2X, promovendo maior comprimento radicular, número de nós, flores/vagens, altura das plantas e massa seca. Esses resultados indicam o potencial dos microrganismos hidrocapacitadores para aumentar a resiliência da soja em condições de déficit hídrico.

Palavras-chave: *Glycine max* L. Déficit hídrico. Crescimento Vegetal. Produção Agrícola. *Bacillus spp.*

1 Introduction

Soybean (*Glycine max* L.) is a legume of great economic importance, with a taproot system composed of a well-developed main root and secondary or lateral roots that form branches (Kaspar; Brown; Kassmeyer, 2004; Lynch 2013; Kim *et al*., 2023). The center of origin for this crop is attributed to the Asian continent but nowadays currently, soybeans are the result of numerous scientific advances, especially in genetic improvement that help to growth soybean worldwide.

Brazil, the top global producer and exporter of soybeans, supplies soybean meal, oil, and derivatives for human and animal nutrition and biodiesel production. Thus, soybean cultivation plays a crucial role in the Brazilian economy. Brazil's 2022/2023 soybean harvest set a record at 322,8 million tons. However, the 2023/2024 forecast predicts a 5,08% drop to 306,4 million tons in National Supply Company (CONAB, 2024). This decline is attributed to the El Niño climate phenomenon, which affected agricultural conditions in the country (Cunha, 2000).

El Niño raises sea temperatures in the Equatorial Pacific, disrupting rainfall in Brazil by reducing it in the central region and increasing it in the south. This exacerbates water scarcity for soybeans, affecting yield and physiology through reduced photosynthesis and root development (Taiz *et al*., 2017). Strategies like drought-tolerant varieties and efficient irrigation are crucial to mitigate these impacts (Lam *et al*., 2018). Soybean production in Brazil experiences significant fluctuations due to prolonged drought periods. Globally, this variation can reach about 50% (Wang *et al.,* 2003). To tackle climate challenges, Brazilian producers must innovate. A proven, low-cost solution is using growth-promoting and hydrocapacitating microorganisms. Studies indicate that inoculation with *Bacillus* genus bacteria has been beneficial in mitigating the effects of drought on soybeans (Rodrigues, 2019).

Bacillus enhances plant growth through symbiosis, pathogen control, reduced water stress, improved metabolism, and increased phytohormones (Argentel-Martinez *et al*., 2025; Kumar *et al.,* 2016; Kim *et al.,* 2017). Given soybeans' economic importance, strategies to address production challenges are crucial. Advances in biotechnology enable lab cultivation of soil-native bacteria for crop applications. This reduces reliance on agrochemicals and offers a sustainable alternative. Therefore, the objective of this work is to evaluate the effect of inoculation with hydro capacitating bacteria on the vegetative development of soybeans.

2 Material and Methods

2.1 Study area

The experiment began on January 9, 2024, in a greenhouse located in the municipality of Confresa – MT, at coordinates 10º 38’ 48” S, 51º 34’ 53” W, at an altitude of 240 meters. The environment was maintained with a natural light cycle, and the region's climate is classified as tropical semi-humid.

2.2Experimental design

The cultivar used was CZ37B31I2X. The seeds were pre-treated with specific industrial seed treatment products: Standak Top (Pyraclostrobin 25 g L^{-1}; Thiophanate-methyl 225 g L^{-1}; Fipronil 250 g L^{-1}), Poncho (Clothianidin 600 g L^{-1}), and Votivo Prime (*Bacillus firmus* strain I-1582 240 g L^{-1}).

We used 24 white plastic trays, each with a capacity of 7 liters. The substrate used was non-autoclaved sand. The experiment was structured with four treatments: 1) Inoculated Seed with Irrigation (ISI), 2) Non-Inoculated Seed with Irrigation (NISI), 3) Inoculated Seed without Irrigation (ISWI), and 4) Non-Inoculated Seed without Irrigation (NISWI). The experimental design was conducted in randomized blocks with six replicates. In each replicate, 13 soybean seeds were planted, totaling 312 seeds.

The randomization of treatments was performed using the website https://www.4devs.com.br/sorteador. The randomization results are presented in Table 1.

Table 1 - Randomization results for treatment allocation, conducted using the website https://www.4devs.com.br/sorteador

1 – ISI	3 – ISWI	4 – NISWI
3 – ISWI	2 – NISI	3 – ISWI
4 – NISWI	4 – NISWI	1 – ISI
2 – NISI	1 – ISI	2 – NISI
2 – NISI	2 – NISI	2 – NISI
4 – NISWI	1 – ISI	4 – NISWI
1 – ISI	3 – ISWI	3 – ISWI
3 – ISWI	4 – NISWI	1 – ISI

Source: research data.

Approximately 500g of seeds were inoculated. The inoculation process was carried out manually using a 1L graduated cup and a 10 mL graduated syringe.

First, inoculation was performed with *Bradyrhizobium japonicum* (SEMIA 5079 and SEMIA 5080) at 7×10^9 CFU mL^{-1}. The dose used was the manufacturer's recommended 4.8mL/kg of seed. After the first inoculation, the planting of treatments 2 – NISI (Non-Inoculated Seed with Irrigation) and 4 – NISWI (Non-Inoculated Seed without Irrigation) was carried out. Immediately after planting, the seeds of treatments 1 – ISI (Inoculated Seed with Irrigation) and 3 – ISWI (Inoculated Seed without Irrigation) were inoculated with the hydrocapacitating agent, a commercial formulation containing three species of bacteria: *Bacillus aryabhattai* CBMAI1120 2.1×10^{12}, *Bacillus circulans* CCT0026 3.0×10^{11}, and *Bacillus haynesii* CCT7926 8.8×10^{11}. The dose used was the manufacturer's recommended 2mL/kg of seeds.

Planting took place on January 9, 2024, at 4:40 PM. To avoid contamination of the seeds, the sand was moistened manually using gloves. The seeds were then sown in rows, simulating the typical planting arrangement in a crop field. During plant development, irrigation of treatments ISI and NISI was carried out with a 5-liter watering can, while treatments ISWI and NISWI received irrigation from a second 5-liter watering can. For all treatments, irrigation was performed daily in the afternoon.

On January 13, 2024, plant emergence was observed, at 4 Days After Planting (DAP). At 7 DAP, counting and calculations were performed to assess germination, comparing plants inoculated with the hydro capacitating agent to those that were not. When the plants predominantly reached the V4 to V5 growth stages, on February 12, 2024 (34 DAP), irrigation was stopped for treatments ISWI and NISWI. This interruption aimed to analyze how long the plants could survive without irrigation and to determine if there were differences in survival duration between inoculated and non-inoculated treatments.

Finally, on March 29, 2024 (56 DAP), plants from treatments ISI and NISI, which were still receiving irrigation, as well as those from treatments ISWI and NISWI, which had their irrigation suspended, were harvested. The root length (RL) in centimeters was measured with a 30 cm graduated ruler, the number of nodes (NN), the number of leaves (NL), the number of flowers/pods (NF), and plant height (PH) were also evaluated.

2.3 Data analysis

To assess differences between treatments, analysis of variance (ANOVA) was used. Means were compared using Tukey's test at a 5% significance level, with the help of the statistical program Sisvar version 5.3. In cases where interactions between the analyzed variables were observed, data breakdowns were performed for a more detailed evaluation.

3 Results and Discussion

Significant differences were recorded between the irrigated and non-irrigated plant treatments, as well as between the inoculated plants and those that did not receive this treatment. The interaction between the factors of inoculation and irrigation also showed a significant effect (Table 2).

Table 2 - Analysis of Variance for Soybean Seeds, Confresa, MT, 2024

Source of Variation	RL	NN	NL	NP	PH	DM
Inoculation	0.00*	0.69*	0.01*	0.68^{ns}	0.02*	0.04*
Irrigation	0.00*	0.00*	0.09*	0.41^{ns}	0.00*	0.00*
Inoculation*Irrigation	0.01*	0.00*	0.00*	0.00*	0.00*	0.25^{ns}
Error						
CV%	34.33	12.27	18.32	44.37	15.60	12.72
Overall Mean	42.03	5.97	4.99	1.59	22.33	14.49

*Significant; ns: Not significant by the F Test at 5% error probability (P < 0.05). CV% = Coefficient of Variation. RL: Root Length (cm); NN: number of nodes; NL: number of leaves; NF: number of flowers/pods; PH: plant height (cm); DM: dry mass (g).
Source: research data.

Root length differed significantly between the inoculation and irrigation conditions (Table 3). It is evident that the inoculated plants outperformed the non-inoculated ones. In the treatments with daily irrigation, the plants inoculated with *Bacillus* spp. achieved superior results compared to the non-inoculated ones, and the same was observed in the treatments where irrigation was interrupted.

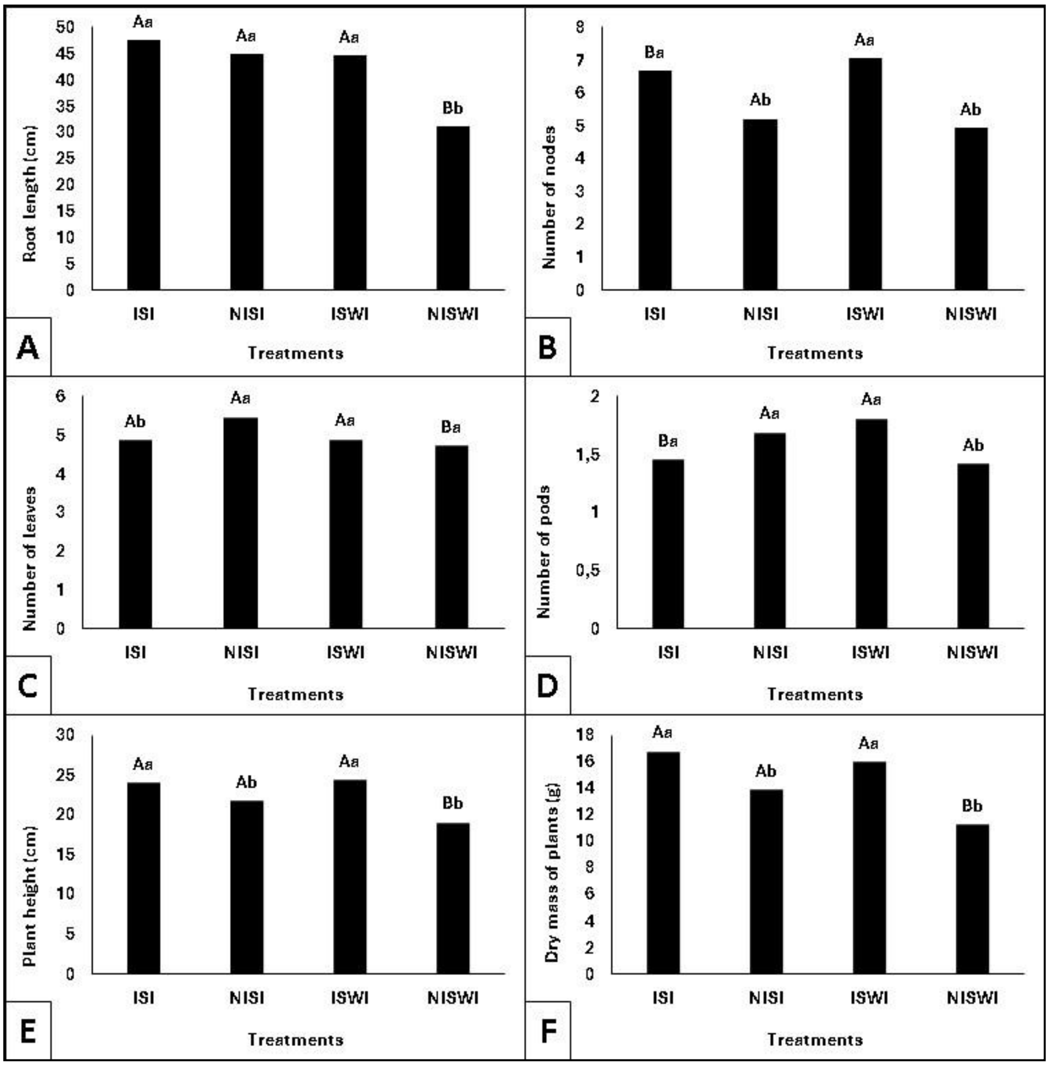
Table 3 - Root Length of Soybeans, Confresa, MT, 2024

Treatments	Means	
	Inoculation	No Inoculation
Irrigation	47.46Aa	44.96Aa
No Irrigation	44.71Aa	31.01Bb

*Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation. Means followed by uppercase letters in the columns and lowercase letters in the rows do not differ from each other by the Tukey test at 5% (P < 0.05).
Source: research data.

The treatment ISI (Inoculated Seed with Irrigation) showed greater root length compared to the NISI treatment (Non-Inoculated Seed with Irrigation), although there was no statistically significant difference between them (Figure 1A). Nonetheless, we observed a better response from plants inoculated with plant growth-promoting bacteria (PGPB). However, when comparing the ISWI treatment (Inoculated Seed without Irrigation) with the NISWI treatment (Non-Inoculated Seed without Irrigation), there was a significant difference in root length, suggesting that PGPBs contributed to plants growth even under water deficit conditions (Figure 1A).

Figure 1 - Root length of soybean (A), number of soybean nodes (B), number of soybean leaves (C), number of soybean pods (D), plant height of soybean (E), and dry mass of soybean plants (F). *Treatments: Inoculated Irrigated Seed (IIS); Non-Inoculated Irrigated Seed (NIIS); Inoculated Seed Without Irrigation (ISWI); and Non-Inoculated Seed Without Irrigation (NISWI). Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation, according to Tukey's Test at 5% ($P < 0.05$)



Source: research data.

We recorded higher values for the number of nodes in the inoculated plants, showing, on average, approximately two more nodes than the non-inoculated plants (Table 4). When analyzing the treatments with and without irrigation, both inoculated and non-inoculated, we found that the hydrocapacitor assists in node formation under water deficit conditions, especially in inoculated plants, in contrast to the non-inoculated and non-irrigated treatment (NISWI) (Figure 1B).

Table 4 - Evaluation of the Number of Nodes in Soybeans, Confresa, MT, 2024

Treatments	**Means**	
	Inoculation	**Nº Inoculation**
Irrigation	6.68Ba	5.21Ab
No Irrigation	7.06Aa	4.92Ab

*Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation. Means followed by uppercase letters in the columns and lowercase letters in the rows do not differ from each other by the Tukey test at 5% ($P < 0.05$).
Source: research data.

We observed that the number of leaves differed significantly among the non-inoculated irrigated plants, which had, on average, one more leaf compared to the other treatments (Table 5). The non-inoculated irrigated treatment (NISI) performed better in terms of the number of leaves compared to the inoculated irrigated treatment (ISI). For the non-irrigated treatments, we observed a significant difference related to inoculation, indicating that inoculation influences this parameter in soybean plants (Figure 1C).

Table 5 - Number of Leaves of Soybeans, Confresa, MT, 2024

Treatments	**Means**	
	Inoculation	**Nº Inoculation**
Irrigation	4.87Ab	5.48Aa
No Irrigation	4.89Aa	4.73Ba

*Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation. Means followed by uppercase letters in the columns and lowercase letters in the rows do not differ from each other by the Tukey test at 5% ($P < 0.05$).
Source: research data.

The number of pods did not differ significantly among the irrigated treatments. However, a considerable discrepancy was observed in the inoculated plants under water deficit, which showed a higher number of flowers/pods (Table 6). Irrigation did not influence the number of pods parameter among the inoculated or non-inoculated soybean seed treatments (Figure 1D). However, when comparing the inoculated non-irrigated treatment, it is noticeable that the hydrocapacitating significantly helped the plants in this parameter, especially under water deficit conditions (Figure 1D).

Table 6 - Number of Soybean´s Pods, Confresa, MT, 2024

Treatments	Means	
	Inoculation	Nº Inoculation
Irrigation	1.46Ba	1.69Aa
No Irrigation	1.81Aa	1.42Ab

*Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation. Means followed by uppercase letters in the columns and lowercase letters in the rows do not differ from each other by the Tukey test at 5% ($P < 0.05$).
Source: research data.

The plants subjected to the treatments that received inoculation were taller compared to the non-inoculated plants (Table 7). The inoculated plants showed a difference of three to four centimeters more compared to the non-inoculated plants (Table 7). Soybean plant height, both inoculated and non-inoculated, is directly related to irrigation (Figure 1E). Although inoculation was effective even in the inoculated non-irrigated treatment, it was found that these complexes of growth-promoting bacteria adapt well to adverse climatic conditions (Figure 1E).

Table 7 - Plant Height of Soybeans, Confresa, MT, 2024

Treatments	Means	
	Inoculation	Nº Inoculation
Irrigation	24.08Aa	21.80Ab
No Irrigation	24.38Aa	19.07Bb

*Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation. Means followed by uppercase letters in the columns and lowercase letters in the rows do not differ from each other by the Tukey test at 5% ($P < 0.05$).
Source: research data.

Inoculated plants had a greater dry mass compared to non-inoculated plants (Table 8). The inoculated plants showed superior performance in both irrigated and non-irrigated treatments, exhibiting a larger mass compared to non-inoculated plants (Figure 1F).

Table 8 - Dry Mass of Soybean Plants, Confresa, MT, 2024

Treatments	Means	
	Inoculation	Nº Inoculation
Irrigation	16.78Aa	13.93Ab
No Irrigation	15.97Aa	11.30Bb

*Uppercase letters compare inoculation within each irrigation. Lowercase letters compare irrigation within each inoculation. Means followed by uppercase letters in columns and lowercase letters in rows do not differ from each other by the Tukey test at 5% ($P < 0.05$).
Source: research data.

The results of the present study indicate that inoculation with *Bacillus* spp. promoted more pronounced root development compared to non-inoculated plants, regardless of irrigation conditions. This effect may be related to the ability of *Bacillus* spp. to enhance plant growth, either through the production of plant hormones such as auxins or by improving nutrient uptake, such as nitrogen and phosphorus, which are often limited under water stress conditions (Lee; Ka; Song, 2012). The observation that the greatest root length occurred in both continuous irrigation and water stress conditions suggests that inoculation not only enhances growth under optimal conditions but also provides an adaptive advantage in water-scarce environments.

Furthermore, the results indicate that the interaction between inoculation and irrigation conditions is crucial for optimizing plant growth. The greater efficiency of inoculated plants under limited irrigation situations is particularly relevant for sustainable agricultural practices, especially in regions subject to water restrictions (Chagas Junior *et al*., 2022). This suggests that the use of *Bacillus* spp., based inoculants may be a promising strategy to mitigate the impacts of water stress, contributing to the maintenance of agricultural productivity in scenarios of climate change. These findings highlight the importance of exploring the use of beneficial microorganisms in agriculture, especially in crops facing environmental challenges. However, future studies should consider additional variables, such as soil quality and nutrient concentration, to better understand the exact mechanisms of action of these inoculants and their impact in different agronomic contexts.

Additionally, inoculation with *Bacillus* spp. promoted a significant increase in the number of nodes compared to non-inoculated plants, regardless of irrigation conditions. This increment, averaging two more nodes in inoculated plants, suggests that the microorganisms used directly influence the structural development of plants, possibly through the production of growth-promoting substances such as phytohormones (Park *et al.,* 2017; Kumar *et al*., 2020; Liu *et al*., 2022). Furthermore, the treatments with and without irrigation revealed an interesting impact from the use of hydro-retainers, especially under water deficit conditions. These results suggest that the combination of inoculants and hydro-retainers could be an effective strategy to mitigate the effects of water stress in crops, promoting structural development even under adverse conditions.

The use of hydrocapacity appears to act synergistically with the inoculants, enhancing the benefits of inoculation under low water availability conditions. This can be explained by the hydrocapacity ability to retain water in the soil, providing a more consistent water supply for the plants, which, in combination with the growth-promoting effects of *Bacillus* spp., results in more robust development. Previous studies, such as those by (Kalam; Basu; Podile, 2020; Kumar *et al*., 2019; Lee *et al.*, 2012), have indicated the role of growth-promoting microorganisms in improving plant development under stress conditions, such as drought, due to the production of phytohormones like indole acetic acid (IAA) and gibberellins. In this study, the results reinforce these findings, demonstrating that the number of nodes can be considered an indicator of structural development enhanced by these beneficial interactions.

Moreover, the results showed a significant difference in the number of leaves between treatments, with non-inoculated and irrigated plants (NISI) averaging one more leaf compared to the other treatments. This result may seem contradictory at first glance, as inoculation with *Bacillus* spp. was expected to promote an increase in the number of leaves, especially under irrigation conditions. However, it is possible that the effect of inoculation did not manifest in the number of leaves under ideal irrigation conditions, perhaps because, in environments with good water availability, plants do not rely as much on the benefits provided by growth-promoting microorganisms, such as phytohormones or improved nutrient absorption. In this context, non-inoculated plants may have invested their resources more efficiently in leaf growth (Passos *et al*., 2023). For treatments without irrigation, inoculation had a notable effect, significantly influencing the number of leaves in soybean plants. This suggests that under water stress conditions, inoculation may play an important role in maintaining and developing the leaf apparatus, likely due to its ability to mitigate the effects of water deficit (Solanha *et al*., 2023). *Bacillus* spp. may help plants tolerate water stress through the production of substances that increase resilience, such as indole acetic acid (IAA) and gibberellins, which are known to promote plant growth even under challenging conditions. These findings align with previous studies, such as those by (Lee; Ka; Song, 2012; Liu *et al*., 2022; Vasques; Nogueira; Hungria, 2024), which highlight the role of growth-promoting bacteria in helping plants adapt to water stress, favoring physiological and structural aspects such as leaf and root formation. In the present experiment, the beneficial effect of inoculation was more evident under water scarcity conditions, suggesting that the microorganisms used may provide an adaptive advantage in these scenarios.

The number of pods per flower on soybean plants did not differ significantly between irrigated treatments, regardless of inoculation. This result suggests that under ideal irrigation conditions, both inoculated and non-inoculated plants can achieve similar performance regarding flower formation, indicating that constant water availability may suppress the additional effects of inoculation on reproductive development. On the other hand, a notable discrepancy was observed in inoculated plants that were under water deficit, where the number of flowers was considerably higher compared to non-inoculated plants.

This indicates that inoculation with *Bacillus* spp. provided a protective effect under water stress conditions, favoring flower formation. This effect may be associated with the production of phytohormones known to promote growth and reproductive development in plants, especially under stress. Since irrigation did not significantly affect pod numbers between inoculated and non-inoculated treatments, it suggests that *Bacillus* spp. inoculation provides a specific protective effect under water stress, beyond general plant development.

This was further evident with the use of the hydro-retainer, which helped inoculated plants maintain a high number of flowers under water deficit conditions. The hydro-retainer likely improved soil moisture retention, helping inoculated plants use residual water more effectively. Combined with the microorganisms' growth-promoting effects, this led to more flowers. Furthermore, the use of technologies such as hydro-retainers, which improve water availability in scarcity situations, can enhance the positive effects of inoculation.

The efficiency of inoculation even in the absence of irrigation suggests that growth-promoting bacteria can provide an adaptive advantage to plants, allowing them to continue developing in scenarios of water deficit. This finding is particularly relevant in the context of climate change, where water availability may be uncertain and the capacity of crops to adapt to challenging environmental conditions will be key to maintaining productivity.

Inoculated plants exhibited significantly higher dry mass compared to non-inoculated plants, regardless of irrigation conditions. This superior performance of inoculated plants in both irrigated and non-irrigated treatments suggests that inoculation with *Bacillus* spp. consistently promotes benefits in biomass accumulation, reinforcing the role of these growth-promoting bacteria in improving plant performance (Azizoglu *et al*., 2024 Oliveira *et al*., 2020).

The increase in dry mass observed in inoculated plants may be associated with the ability of *Bacillus* spp. to enhance nutrient absorption and water use efficiency, in addition to stimulating the production of phytohormones that favor plant growth. The higher dry mass in inoculated plants under water stress conditions is particularly relevant as it indicates that inoculation may help mitigate the negative effects of water deficit, allowing plants to maintain their growth and biomass accumulation even in adverse situations. These results corroborate previous studies, such as those by Chagas Junior *et al*. (2022), which also reported an increase in dry mass in beans and soybeans inoculated with *Bacillus* spp.

4 Conclusion

Inoculation with *Bacillus* spp. represents a promising strategy to enhance soybean resilience in drought-prone regions, offering a viable alternative to minimize productivity losses caused by water scarcity. The application of bioinoculants, combined with technologies such as hydrocapacitors, can be a crucial tool for agricultural sustainability and the adaptation of soybean crops to the conditions imposed by climate change.

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