

UTILIZAÇÃO DE BIOESTIMULANTES E MICRORGANISMOS NA MITIGAÇÃO DO DÉFICIT HÍDRICO EM CULTIVO DE FEIJÃO

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1 RESUMO

Os bioestimulantes são substâncias que conferem benefícios fisiológicos e morfológicos às plantas. Objetivou-se com este estudo avaliar a resposta da cultura do feijão à aplicação de bioestimulantes, onde receberam uma dose de uma formulação de bioestimulantes via tratamento de sementes à base de extrato de alga (*Ascophyllum nodosum* + *Kappaphycus alvarezii*) + complexo de aminoácidos contendo (Prolina + arginina) na dose de 2 mL kg⁻¹ de semente, e verificar seu efeito nas condições edafoclimáticas da região do Cerrado. O experimento foi conduzido com plantas de feijão em vasos, em casa de vegetação. Foi utilizado um delineamento inteiramente casualizado (DIC), em esquema fatorial de 4 x 4, sendo 4 tratamentos de sementes (Microrganismos, bioestimulantes, microrganismos + bioestimulantes e testemunha) e 4 níveis de reposição hídrica (25%, 50%, 75% e 100% da capacidade de campo – CC), com 5 repetições, totalizando 16 tratamentos e 80 unidades experimentais (Vasos). Foram determinadas as seguintes variáveis: altura de planta (AP), diâmetro de caule (DC), número de folhas (NF) e área foliar (AF). Os dados encontrados indicam que a aplicação via tratamento semente dos bioestimulantes + microrganismos podem efetivamente aumentar a capacidade de defesa das plantas de feijão contra os danos induzidos pelo déficit hídrico imposto.

Palavras-chave: *Bacillus aryabhattai*; *Bacillus subtilis*; *Trichoderma asperellum*, *Ascophyllum nodosum*; *Kappaphycus alvarezii*.

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USE OF BIOSTIMULANTS AND MICROORGANISMS IN MITIGATION OF WATER DEFICIT IN BEAN CULTIVATION

2 ABSTRACT

Biostimulants are substances that provide physiological and morphological benefits to plants. The aim of this study was to evaluate the response of bean crops to the application of biostimulants, where they received a dose of a biostimulant formulation via seed treatment based on seaweed extract (*Ascophyllum nodosum* + *Kappaphycus alvarezii*) + an amino acid complex containing proline + arginine at a dose of 2 mL kg⁻¹ of seed, and to verify its effect on the edaphoclimatic conditions of the Cerrado region. The experiment was conducted with bean plants in pots in a greenhouse. A completely randomized design (CRD) was used in a 4 x 4 factorial scheme, with 4 seed treatments (microorganisms, biostimulants, microorganisms + biostimulants and control) and 4 levels of water replacement (25%, 50%, 75% and 100% of field capacity - CC), with 5 replicates, totaling 16 treatments and 80 experimental units (Pots). The following variables were determined: plant height (AP), stem diameter (DC), number of leaves (NF) and leaf area (AF). These findings indicate that the application of biostimulants + microorganisms via seed treatment can effectively increase the defense capacity of bean plants against damage induced by imposed water deficit.

Keywords: *Bacillus aryabhattai*; *Bacillus subtilis*; *Trichoderma asperellum*, *Ascophyllum nodosum*; *Kappaphycus alvarezii*.

3 INTRODUCTION

The common bean (*Phaseolus*) bean (*Cucumis vulgaris* L.) belongs to the Fabaceae family, which includes legumes and is among the oldest foods known to humans, being among the first species domesticated by humans (Carvalho *et al.*, 2013; Pinto, 2016). In Brazil, it is considered an excellent source of vegetable protein (16 to 36%), carbohydrates (66 to 77%), iron (2%), lipids (0.66 to 1.43%), and other minerals such as calcium, magnesium, and zinc (Buratto, 2012; Carvalho *et al.*, 2013). In the 2023/24 crop year, the cultivated area dedicated to this crop was 2,856.6 thousand hectares (considering colored common beans, black common beans, and cowpeas), with a production of 3,259 thousand tons, 7.3% greater than the volume harvested in the 2022/23 crop year (Feijão, 2024).

Many factors interfere with plant development. One of the main factors related to crop productivity is the limitation caused by edaphoclimatic factors, as well as the demand for high productivity combined with sustainability issues, such as nutritional

requirements and water restrictions in some regions, hindering the proper development of soybean crops (Carvalho *et al.*, 2013).

Water deficit stress is among the most important abiotic factors influencing plant growth and production (Wu *et al.*, 2016). It is important to emphasize that the damage caused by water stress during crop development (including during the processes of germination, growth, and reproduction) can be irreversible. During the germination phase, water stress affects the uniformity and speed of germination and the percentage of germinated seeds, resulting in crop growth loss in the field (Castro; Silva, 2023). Subsequent water stress during the vegetative growth phase has a more pronounced effect on photosynthesis and negatively affects plant productivity (Vurukonda). *et al.*, 2016).

Given these circumstances, it is necessary to consider new approaches to mitigate the climatic effects that directly affect bean plants (Das; Albinsson, 2023). Sustainable strategies have thus been created to address these challenges as a way to attempt climate change mitigation for

efficient protection from the plant itself through the use of biostimulant substances and the soil microbiota (Cavalcante *et al.*, 2020).

The presence of natural extracts provides physiological benefits to plants. Among these, we can mention the characteristics of hormonal balance and osmoprotection, which act inside plant cells, protecting them against dehydration and thus maintaining their metabolic activities at an adequate level, even in adverse situations (Prieto *et al.*, 2017; Cavalcante *et al.*, 2020).

Biostimulants are defined as mixtures of two or more plant regulators with other substances (amino acids, natural extracts, nutrients and vitamins) (Traon. *et al.*, 2014; Silva *et al.*, 2017; Yakhin *et al.*, 2017), which can, depending on their composition, concentration, and proportion of substances, stimulate plant growth through increased cell division, cell elongation, and cell differentiation and thus increase the capacity for nutrient and water absorption, directly affecting development (seed germination, growth, flowering, fruiting, and senescence) and crop productivity (Kolling *et al.*, 2016; Silva *et al.*, 2017; Cavalcante *et al.*, 2020).

In soil, plants interact with the microorganisms present there, offering a very specific environment for the natural development and growth of microbial communities. During the evolutionary process, these communities developed skills and strategies that enabled their interaction with plants (Deng *et al.*, 2022). These interactions can benefit plants without causing harm, or they can cause harm when pathogenic microorganisms cause biochemical and physiological imbalances in plants, generating different types of diseases (Álvarez *et al.*, 2016).

Among the various microorganisms that have been characterized as capable of increasing plant growth, bacteria of the genus *Bacillus* and fungi of the genus

Trichoderma stand out because they can fix and solubilize essential nutrients and provide metabolites involved in the mechanism of tolerance to abiotic stresses (Chagas *et al.*, 2017; Su *et al.*, 2017; Sousa *et al.*, 2023).

The hypothesis of this study is that the application of biostimulants and specific microorganisms via seed treatment can reduce the negative effects of water deficit on common bean (*Phaseolus*) crops. (commonly known as **Phyllanthus vulgaris* * L.), promoting greater resistance to water deficit, in addition to improving plant growth and development. This intervention, applied under controlled greenhouse conditions and with different levels of water replenishment, will allow us to assess whether biostimulants and microorganisms contribute to maintaining the metabolic activity of plants under adverse conditions typical of the Brazilian Cerrado. In this context, the present study aimed to evaluate the influence of water regimes on the biometric variables of common bean crops via seed treatment.

4 MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at Fazenda Fontes do Saber, at the Faculty of Agronomy of the University of Rio Verde – UniRV (17°47'14.3"S 50°57'53.9"W), between the months of May and June 2024.

The soil used in the pots was collected at the college in a cultivation area in the 0.0–0.20 m layer, classified as a typical dystrophic red latosol (LVdf), with a very clayey texture and cerrado phase (Santos *et al.*, 2018). Before the experiments were performed in the pots, soil sampling was carried out for physicochemical characterization, where analyses were performed according to the methodologies described by Teixeira *et al.* (2017) (Table 1).

Table 1. Chemical and granulometric analysis of the soil collected for use in the trial, 2024 crop season, Rio Verde – GO

Prof. ¹	pH	P	K	Here	Mg	Al	H+Al
cm	CaCl ₂	mg dm ⁻³			cmol _c dm ⁻³		
0-20	6.32	1.50	132	2.81	0.69	0.00	2.31
Prof.	MO	SB	CTC	V	M	Ass	Faith
cm	g dm ⁻³	cmol _c dm ⁻³		%		mg dm ⁻³	
0-20	24.15	3.84	6.15	62.42	0.00	5.3	67.3
Prof.	Mn	Zn	Sand	Silt	Clay	Textural class	
cm	mg dm ⁻³			%			
0-20	79.3	3.1	32.09	21.94	45.97	Clayey	

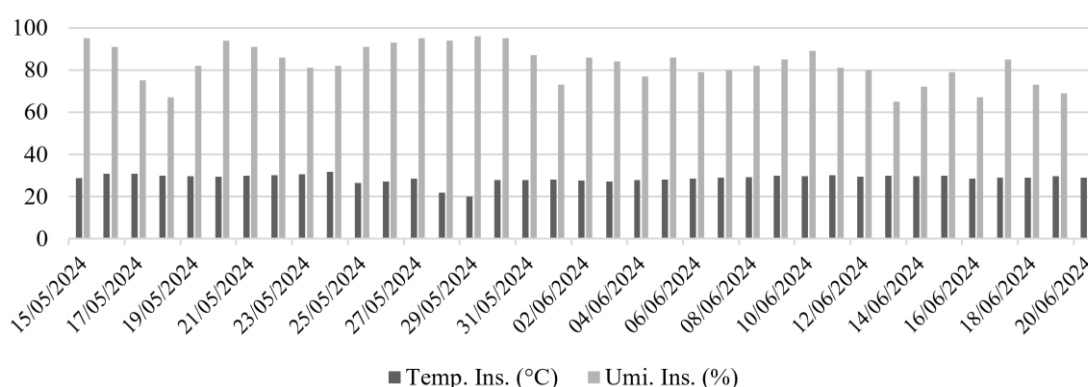
¹ pH of the soil solution, determined in calcium chloride solution; OM: organic matter, determined by the colorimetric method; P: phosphorus, melhich; K⁺: potassium, melhich; Ca²⁺ and Mg²⁺: exchangeable contents of calcium and magnesium, respectively, in KCl; S-_{SO42-}: sulfur in the form of sulfates, extracted by calcium phosphate and determined by the colorimetric method; Al₃₊: Exchangeable aluminum, extracted by 1 mol L⁻¹ potassium chloride solution; H+Al: total soil acidity, determined in the SMP buffer solution at pH 7.5; SB: sum of bases (K⁺ + Ca²⁺ + Mg²⁺); CEC: cation exchange capacity (K⁺ + Ca²⁺ + Mg²⁺ + H+Al); V: soil base saturation (SB/CEC ratio); m: aluminum saturation [Al³⁺/(SB+Al³⁺) ratio]; Cu, Fe, Mn and Zn: copper, iron, manganese and zinc, extracted by the melhich solution.

Source: Authors (2025)

In accordance with the classification of Alvares *et al.* (2013) and Köppen and Geiger (1928), the region's climate is classified as Aw (tropical), with rainfall occurring from October to April and drought occurring from May to September. The maximum temperature ranges from 35°C to 37°C, and the minimum temperature ranges

from 12°C to 15°C (in winter, there are occurrences of up to 5°C). Annual rainfall reaches approximately 1,800 mm but is poorly distributed throughout the year (Silva *et al.*, 2017).

During crop development, local climate data were monitored (Figure 1).

Figure 1 Daily data of instantaneous temperature and relative humidity during the period of the experiment, 2024 crop season, Rio Verde – GO

Source: INMET Normal Automatic Station – Rio Verde – GO

Soil correction was performed in the pots, and the soil was incorporated into the soil at a depth of 0–20 cm. The amount of fertilizer used corresponded to 4 t ha⁻¹ of dolomitic limestone. Planting fertilization

was carried out in the pots and incorporated into the soil at a depth of 0–10 cm. The amount of fertilizer used corresponded to 120 kg ha⁻¹ of P₂O₅ using MAP as the source and 130 kg ha⁻¹ of K₂O using KCl as the source. A

solution to supply the micronutrients Mn, Zn and B was applied at a rate of 1 kg ha⁻¹, and manganese sulfate, zinc sulfate and boric acid were used as the sources. Topdressing was carried out at the V3 stage, at a rate of 120 kg ha⁻¹ of N, on the surface of the pot, using urea as the source. Soil correction and fertilization were carried out in accordance with the needs of the crop, on the basis of soil analysis, following the recommendations of Sousa and Lobato (2004).

Carioca beans (*Phaseolus*) were used. The carioca bean (BRS Estilo cultivar), developed by Embrapa® was sown on May 15, 2024. The carioca beans were sown manually at a depth of 3 cm in the pots, with a total of 5 plants per pot. Thinning of the plants in the pots was carried out, with 2 plants per pot remaining 7 days after emergence (DAE).

The crop was monitored throughout the entire growing cycle. Weed control was carried out by manually removing emerged plants from the pots. Insecticide application was necessary at 14 days after emergence (DAE) to control white weed (*Bemisia tabaci* breed B), the commercial product Engeo Pleno S® (thiamethoxam + lambda-cyhalotrin) was used at a dosage of 100 mL ha⁻¹, and no fungicide or other phytosanitary product application was necessary until the end of 30 DAE.

Five-liter pots were used, filled with soil to within 2 cm of the rim. The soil was sifted to remove clods, and a total of 5 kg of soil (air-dried fine earth - TFSA) was placed in each pot.

A randomized design (CRD) was used in a 4 x 4 factorial scheme, with 4 seed treatments (microorganisms, biostimulants, microorganisms + biostimulants and

control) and 4 water replacement levels (25, 50, 75 and 100% of field capacity – FC), with 5 repetitions, totaling 16 treatments and 80 experimental units (Pots).

The treatments involving the application of microorganisms included a dose of commercial seed products containing the microorganisms (*Bacillus aryabhattai*, *Bacillus subtilis*, and *Trichoderma asperellum*). All treatments involved the application of the commercial liquid inoculant Nodumax®, which is based on *Bradyrhizobium japonicum* (at a concentration of 7.2×10^9 and a dose of 2 mL kg⁻¹ of seed).

The commercial products used were Arid® based on *Bacillus aryabhattai* (at a dose of 2 mL kg⁻¹ of seed), Batrix® based on *Bacillus subtilis* (at a dose of 2 mL kg⁻¹ of seed), and Triene® based on *Trichoderma asperellum* (at a dose of 2 mL kg⁻¹ of seed).

The treatments involving biostimulant applications involved a dose of a biostimulant formulation via seed treatment based on algae extract (*Ascophyllum nodosum* + *Kappaphycus alvarezii*) + amino acid complex containing proline + arginine at a dose of 2 mL kg⁻¹ of seed.

The soil water retention capacity (field capacity - FC) was determined by weighing all the pots with dry soil after waterlogging and subsequent drainage. The amount of water to be applied in each treatment was controlled by daily weighing of the pots using a portable electronic scale, and the water lost through evapotranspiration was replenished until the pot weight reached the predetermined value (gravimetric method). The data on water replacement depth applied during the trial are described in Table 2.

Table 2. Chemical and granulometric analysis of the soil collected for use in the trial, 2024 crop season, Rio Verde – GO

HR *	CC **		Full blade				
	L	%	L day ⁻¹	L month ⁻¹	mm day ⁻¹	mm month ⁻¹	mm cycle ⁻¹
25%	0.35	7	0.125	9	2.5	75	250
50%	0.70	14	0.250	18	5.0	150	500
75%	1.05	21	0.375	27	7.5	225	750
100%	1.40	28	0.500	36	10.0	300	1000
Average	0.85	17.5	0.312	22.5	6.25	187.5	625

* RH – Water replacement; ** CC – Field capacity.

Source: Authors (2025)

When the bean plants were 7 days after planting (DAP), thinning was carried out; 2 plants were left per pot, and water replacement treatments were applied.

Biometric data collection occurred 30 days after emergence (DAE). The following variables were determined: plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA).

Two plants were sampled (in duplicate). Plant height, expressed in centimeters, was assessed by measuring the length of the main stem from the collar to the last node (apex of the plant), and stem diameter, expressed in millimeters, was measured at the height of the plant's collar using a digital caliper. The number of leaves produced on the main stem was counted.

The determination of the leaf area (cm²) was carried out by measuring the greatest length and width of all the leaves. The collected data were subsequently applied to the empirical equation proposed by Adami *et al.* (2008) (Equation 1). The estimates of the leaf area of each leaf were summed to obtain the leaf area per plant in cm².

$$AF = (C \times L) \times 0.7104 \times NF \quad (1)$$

In what way:

C – Maximum length (cm);

L – Maximum width (cm);

NF – Number of sheets (dimensionless).

The collected data were subjected to statistical analysis, including analysis of variance using the F test ($p < 0.05$), and cases of significance were subjected to Tukey's mean test ($p < 0.05$) for qualitative data, as well as regression analysis ($p < 0.05$) for quantitative data, using the statistical software SISVAR® version 5.6 (Ferreira, 2019).

5 RESULTS AND DISCUSSION

Summary of the analysis of variance for the variables plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA), evaluated as a function of treatment (TREAT) and water replenishment (WR), as well as the interaction between them (TREAT × WR). The TREAT factor was significant for the number of leaves (NL) ($p < 0.01$) and LA ($p < 0.05$), indicating that the different treatments influenced these specific variables. The WR factor was significant ($p < 0.01$) for all the variables, suggesting that water availability was a determinant of crop growth and development. The interactions between the TREAT and WR factors, as well as the repetitions, were not significant for the variables analyzed (Table 3).

Table 3. Summary of the analysis of variance for the variables plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA) as a function of the source of variation, 2024 Crop Year, Rio Verde – GO

FV	GL	Medium squares			
		AP	A.D	NF	AF
TREATMENT	3	49.855125 ^{ns}	0.332458 ^{ns}	22.618833 ^{**}	134218,188125 [*]
HR	3	302.551458 ^{**}	0.985125 ^{**}	62.098833 ^{**}	509474,310125 ^{**}
TRAT x RH	9	15.248903 ^{ns}	0.588347 ^{ns}	7.701611 ^{ns}	41264,384903 ^{ns}
REP	4	39.345813 ^{ns}	0.362046 ^{ns}	3.886750 ^{ns}	37507,620438 ^{ns}
Residue	60	24.838346	0.362046	4.784750	45577,374238
CV (%)		23.04	15.01	20.53	40.07

^{ns} not significant and *, ** significant at 5% and 1% probability, respectively, according to the F test. FV – source of variation; GL – degrees of freedom; CV – coefficient of variation; REP – repetition; RH – fluid replacement; and TRAT – biostimulant based on microorganisms (*Bacillus aryabhattai* + *Bacillus subtilis* + *Trichoderma asperellum*) and biofertilizer based on algae extract (*Ascophyllum nodosum* + *Kappaphycus alvarezii*) + amino acid complex (proline + arginine).

Source: Authors (2025)

The average values of the variables plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA) as a function of the applied treatments: microorganism-based biostimulants (Micro), biofertilizer (Bio), a combination of both (Micro + Bio), and a control. In terms of the number of leaves (NL), the Bio treatment resulted in the greatest average (11.63), followed by the Micro (11.23) and

Micro + Bio (10.61) treatments, while the control resulted in the lowest value (9.56). With respect to the leaf area (LA), all the treatments with biostimulants and microorganisms (Micro, Bio, and Micro + Bio) presented greater values than the control did, with the highest average observed for the Micro treatment (575.95 cm²) (Table 4).

Table 4. Mean comparison test for the variables plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA) as a function of the treatments, 2024 crop season, Rio Verde – GO

Treatments	AP	A.D	NF	AF
	cm	mm		cm ²
Micro	19.87	3.84	11.23 ab	575.95 a
Bio	20.87	3.92	11.63 a	575.35 a
Micro + Bio	21.64	4.07	10.61 ab	489.10 a
Witness	23.26	4.09	9.56 b	426.79 a
DMS	4.1658	0.5029	1.8283	178.4491
Standard error	1.1144	0.1345	0.4891	47.7374

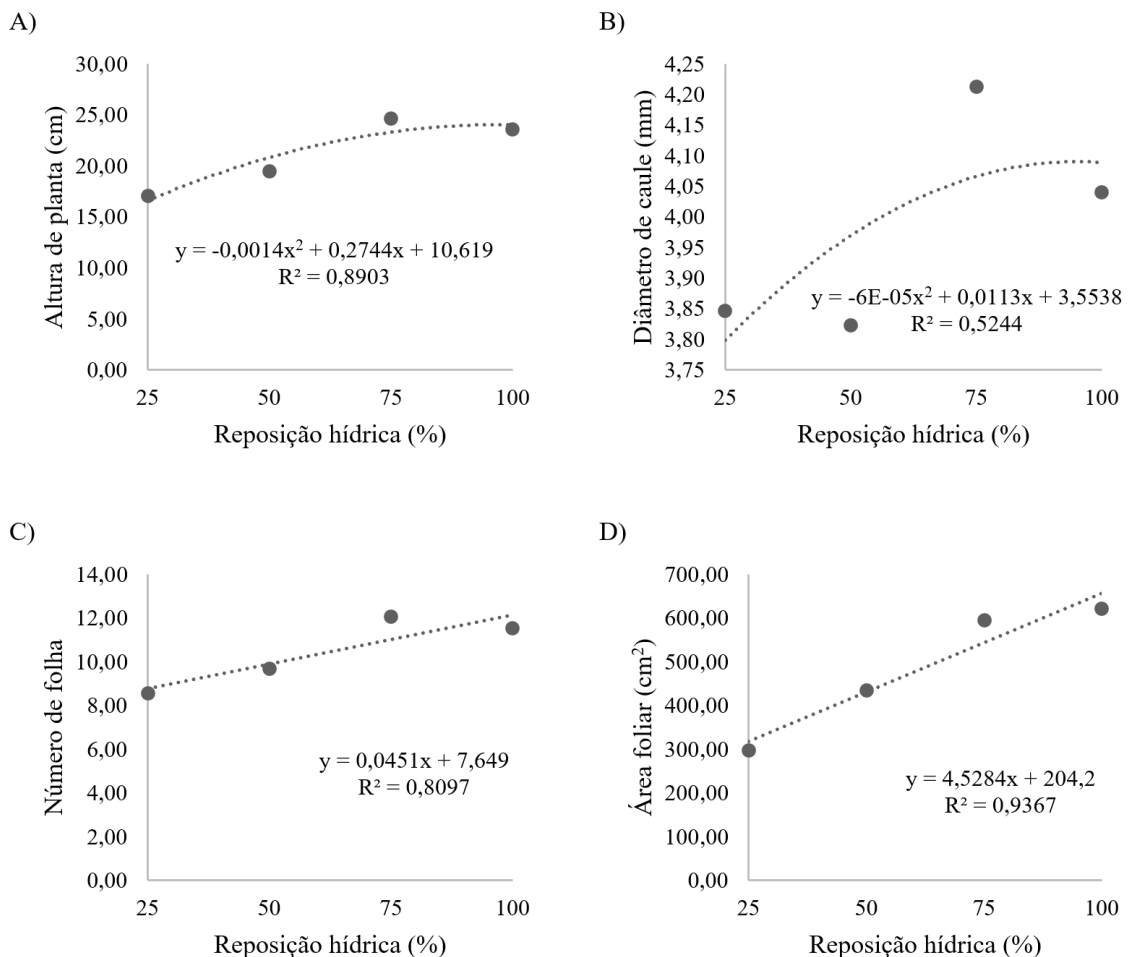
Means followed by the same letter in the columns do not differ from each other according to Tukey's test ($p < 0.05$). Microbiostulants based on microorganisms (*Bacillus aryabhattai* + *Bacillus subtilis* + *Trichoderma*). *Asperellum*; Bio – Biofertilizer based on algae extract (*Ascophyllum nodosum* + *Kappaphycus alvarezii*) + amino acid complex (proline + arginine).

Source: Authors (2024)

The responses of the plants to different water replenishment levels for the variables of plant height, stem diameter, number of leaves, and leaf area allowed visualization of the effects of water dosage on bean plant morphology (Figure 2). With respect to plant height, the maximum efficiency point was 98% water replenishment, with an increase of 60.4% (24.06 cm) (Figure 2A). With respect to stem diameter, the maximum efficiency point was 94.16% water replenishment, with an increase of 5.14 mm (30.86%) (Figure 2B). The number of leaves increased with 100%

water replenishment, resulting in 12.16 leaves at the 100% water replenishment level, with an increase of 4.50 leaves for each 25% water replenishment level. For each 25% increase in the water level, a gradual increase in the number of leaves was observed, as described by the equation in the graph. (Figure 2C). The leaf area increased with 100% water replacement, resulting in a value of 657.04 cm². For each 25% increase in water depth, the leaf area progressively increased, as indicated by the linear trend of the graph equation. (Figure 2D).

Figure 2. Plant height (A), stem diameter (B), number of leaves (C) and leaf area (D) as a function of water replenishment, 2024 crop season, Rio Verde – GO



, and * indicate significance at the 5% and 1% probability, respectively, according to the F test. TRAT – Biostimulant based on microorganisms (*Bacillus aryabhattai* + *Bacillus subtilis* + *Trichoderma asperellum*) and biofertilizer based on algae extract (*Ascophyllum nodosum* + *Kappaphycus alvarezii*) + amino acid complex (proline + arginine).

Source: Authors (2024)

The plant height (PH) significantly varied when the treatments involving biostimulants and microorganisms were compared. In the treatments with microorganisms and biostimulants, as well as in the combination of both, the plants were taller than those in the control, suggesting that the treatments favor vegetative growth. These results indicate that the application of biostimulants and microorganisms may have contributed to the synthesis of plant hormones, such as auxins and cytokinins, which are essential for cell growth. Among the treatments, the use of isolated biostimulants stood out, suggesting that this intervention offers specific advantages in promoting cell elongation in bean plants.

When the effect of water replenishment on plant height was analyzed, it was found that plants subjected to the highest levels of water replenishment (75% and 100% of field capacity) reached greater heights than those that received less water. This effect was expected, since adequate water availability is essential for nutrient transport and biochemical processes involved in growth. Under water deficit conditions, plants generally reduce their height development as a strategy to decrease the evapotranspiration surface, which is reflected in the results, as plants subjected to 25% and 50% replenishment experienced reduced heights.

The biostimulants and microorganisms applied in this study may have mitigated the effects of water deficit, contributing to a height development closer to the ideal, even under conditions of moderate stress, suggesting that these bioactive substances may act as mediators of resilience to water stress.

In the case of stem diameter (SD), the analysis of the treatment effects revealed that the application of biostimulants and microorganisms resulted in a slight, but consistent, increase in diameter compared with that of the control. Stem diameter is an

important indicator of structural robustness, providing greater support and resistance for plants. Although the differences between treatments were not highly significant, the treatment with associated biostimulants and microorganisms resulted in the greatest increase in the diameter, suggesting that the combination of these agents can strengthen the supporting structure of plants.

The effects of different levels of water replenishment on stem diameter were also notable, with plants subjected to relatively high levels of water replenishment exhibiting relatively large diameters. Under water stress conditions, the diameter tends to decrease, since the plant allocates fewer resources to structural growth, concentrating on survival. Thus, the increase in diameter with greater water replenishment indicates that plants that benefit from sufficient water achieve a more robust structure, which may imply a greater capacity for nutrient transport and greater stability.

In terms of the number of leaves (NL), the treatments with biostimulants and microorganisms were superior to the control, with the treatment with isolated biostimulants standing out. The number of leaves is an important indicator of a plant's photosynthetic capacity, as a greater number of leaves tends to increase the area available for capturing sunlight. This characteristic is fundamental for plant development, especially under water stress conditions, since more numerous and well-formed leaves contribute to more efficient photosynthesis, promoting greater resilience.

Furthermore, the different levels of water replenishment influenced the number of leaves, with higher averages observed in the treatments with water replenishment rates of 75% and 100%. Under conditions of lower water availability (25% and 50%), the number of leaves was reduced, reflecting an adaptive response of the plant to water restriction, where there is less investment in foliage to reduce water loss. This adaptation

confirms the hypothesis that plants under water stress develop reduced foliage to conserve water resources and maintain vital functions.

Leaf area (LA) was positively affected by treatment with biostimulants and microorganisms, with treatments using isolated and combined biostimulants standing out. Leaf area plays a crucial role in photosynthesis and evapotranspiration and is an indicator of a plant's ability to capture light and regulate its temperature. Compared with the control plants, the plants that received biostimulants and microorganisms presented greater leaf areas, suggesting that these treatments promote leaf development, which increases metabolic efficiency, which is especially important during periods of water deficit.

Analysis of water replenishment levels revealed that the leaf area was significantly greater in plants subjected to higher replenishment levels (75% and 100%). Under water stress conditions, the leaf area decreased, which is expected, as the plant tends to limit its leaf growth to reduce water loss through transpiration. This adaptive behavior has also been observed in previous studies, in which water stress led to a decrease in leaf area as a plant survival strategy.

Finally, the importance of leaf area in plant metabolism, especially under water deficit conditions, is reinforced by the plant's ability to maintain an adequate leaf area, which facilitates photosynthesis and allows for continued development, even under water limitations. The biostimulants and microorganisms tested in this study have the potential to mitigate the effects of water stress and promote the maintenance of leaf area, which can ultimately contribute to the final productivity of bean crops.

To compare the results with those of similar studies in which biostimulants and microorganisms were used in the management of water deficit, several researchers reported that these compounds

can increase the resilience of plants to water stress, increasing water retention, strengthening the cell wall, and promoting metabolic pathways that maintain redox homeostasis (Nephali *et al.*, 2021). In the Cerrado, owing to its seasonal water restrictions, these biostimulants become sustainable alternatives to mitigate the negative impacts of water deficit and promote agricultural productivity even under adverse conditions (Bonatelli, *et al.*, 2021).

Biostimulants based on algae and bacterial extracts promote better nutrient absorption and increase root diameter, making plants more robust and efficient in terms of the use of water and nutrients. Hamid *et al.* (2021) demonstrated that bioactive compounds, such as amino acids and plant hormones, can induce biochemical changes in plants, such as increased osmolyte production, which protects cells against water stress. This is crucial for production systems in the Cerrado, where adaptation to a dry climate is vital for regional food security.

Ascophyllum extracts *nodosum* are promising tools for use in crops such as beans, as they stimulate photosynthetic processes and increase resistance to water deficit. In addition, these products aid in plant development, reduce productivity loss under stress conditions and help to sustain agricultural production in areas susceptible to water scarcity, such as the Brazilian Cerrado (Goñi; Quille; O'connell, 2018).

Good practices suggest the use of a combination of biostimulants and specific microorganisms to optimize productivity under water-restricted conditions. Kaushal *et al.* (2023) reported that the use of these substances, combined with water management techniques, can maximize crop performance, promoting both growth and plant resilience in the face of climatic challenges.

Thus, the current literature reinforces that integrated management with

biostimulants and microorganisms is fundamental for sustainable and resilient agriculture in the Cerrado, providing viable alternatives for coping with climate variations and maintaining productivity.

The experimental limitations of this study include its execution in a controlled greenhouse environment, which may not fully reflect field conditions. Small-scale experiments, such as this one, often present limitations in terms of climatic and edaphic variability, which are essential elements for evaluating the real impact of biostimulants and microorganisms on water deficit management. According to Nephali *et al.* (2021), controlled conditions limit the observation of complex interactions between plants, soil and climate, which are crucial in situations of water stress in open fields.

Another limiting factor is the control of edaphoclimatic variables in the greenhouse environment, where soil uniformity and fixed temperature can mask fluctuations that directly influence the performance of applied treatments. Hamid *et al.* (2021) reported that the presence of varied natural conditions is essential to validate the effectiveness of biostimulants and microorganisms, as environmental variability can directly influence the response of plants to applied treatments.

In future research, the development of large-scale field studies, especially in Cerrado areas, which face unique climatic and edaphic challenges, is recommended. Studies conducted in the field allow for greater representativeness of real growing conditions, providing a more accurate scenario for evaluating the effectiveness of biostimulants and microorganisms (Goñi; Quille; O'Connell, 2018).

Furthermore, studies are needed to evaluate the application of different formulations and concentrations of biostimulants and microorganisms over extended cultivation cycles. According to Kaushal *et al.* (2023), the combination of different types of biostimulants and

microorganisms, applied at varying doses and throughout growing cycles, can increase crop resilience and better adapt plants to seasonal and long-term stresses.

Finally, future studies could explore the variability of species and their response to biostimulants, since different cultures may react differently to combinations of microorganisms and biostimulants. Bonatelli *et al.* (2021) reported that the differential behavior of agricultural species in response to biostimulants indicates the need to test the specific adaptation of each crop, promoting more efficient precision agriculture in the context of the Cerrado.

6 CONCLUSION

Compared with the number of leaves (NL), the average height (PH) and stem diameter (SD) were not *significantly different between the two treatments; the average height and stem diameter were greater for the biostimulant-treated plants than for the micro+ bio and microtreated plants (Ascophyllum nodosum + Kappaphycus alvarezii). + amino acid complex containing (proline + arginine).*

The leaf area variable was significant for the treatments, with an average of 516.79 cm².

In terms of water replenishment, all the variables significantly increased, but only plant height resulted in a 2% decrease in water use and a 5.84% decrease in stem diameter, with increases of 60.4% for plant height and 30.86% for stem diameter.

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