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PHYSIOLOGICAL PERFORMANCE OF COMMON BEANS SUBMITTED TO DEFICIT IRRIGATION AND BIOSTIMULANTS

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1 ABSTRACT

The common bean is a sensitive plant to the effects of water deficit and physio-biochemical alterations, which influence the yield are observed. Vegetal biostimulants are inputs that present potential to mitigate the effects of water deficit on crop development. This paper aimed to evaluate the physiological and biochemical impact of water deficit on the common bean and the potential of applying biostimulants as a mechanism to tolerate stress. The assay was conducted in an agricultural greenhouse in Botucatu-Brazil, the pots were disposed in a split-plot design in randomized blocks, with four replications. The treatments in the plots corresponded to the water content tension, control (-10 kPa) and water deficit (-40 kPa), in the subplots the biostimulant treatments (B1- control; B2- *Bacillus amyloliquefaciens* BV; B3- *Bacillus amyloliquefaciens* BV 03 + algae extract *Ascophyllum nodosum*). The analyzed variables were leaf pigments, gas exchanges, total soluble proteins, L-proline, specific activity of the enzyme superoxide dismutase, shoot dry matter and crop yield. The water deficit negatively affected all parameters evaluated and the biostimulants in the tested form, did not show efficiency in helping the plants tolerate stress due to drought. We suggest new studies to prove the efficiency of biostimulants for field applications.

Keywords: antioxidative metabolism, plant growth-promoting bacteria, algae extract, leaf gas exchanges, *Phaseolus vulgaris* L.

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DESEMPENHO FISIOLÓGICO DO FEIJOEIRO SUBMETIDO A IRRIGAÇÃO DEFICITÁRIA E BIOESTIMULANTES

2 RESUMO

O feijoeiro é uma planta sensível aos efeitos do déficit hídrico e são observadas alterações físico-bioquímicas que influenciam na produtividade. Bioestimulantes vegetais são insumos que apresentam potencial para mitigar os efeitos do déficit hídrico no desenvolvimento da

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cultura. O objetivo deste trabalho foi avaliar o impacto fisiológico e bioquímico do déficit hídrico no feijoeiro e o potencial da aplicação de bioestimulantes como mecanismo de tolerância ao estresse. O ensaio foi conduzido em casa de vegetação agrícola, em Botucatu-Brasil, os vasos foram dispostos em parcelas subdivididas em blocos ao acaso, com quatro repetições. Os tratamentos nas parcelas correspondem às lâminas de irrigação definidas com base no potencial de água no solo - controle (-10 kPa) e déficit hídrico (-40 kPa), nas subparcelas os tratamentos com bioestimulante (B1- controle; B2- *Bacillus amyloliquefaciens* BV; B3- *Bacillus amyloliquefaciens* BV 03 + extrato de algas *Ascophyllum nodosum*). As variáveis analisadas foram pigmentos foliares, trocas gasosas foliares, proteínas solúveis totais, L-prolina, atividade específica da enzima superóxido dismutase, matéria seca da parte aérea e produtividade da cultura. O déficit hídrico afetou negativamente todos os parâmetros avaliados e os bioestimulantes na forma testada não mostraram eficiência em auxiliar as plantas na tolerância ao estresse por seca. Sugerimos novos estudos para comprovar a eficiência de bioestimulantes para aplicações em campo.

Palavras-chaves: metabolismo antioxidativo, bactérias promotoras de crescimento de plantas, extrato de algas, trocas gasosas foliares, *Phaseolus vulgaris* L.

3 INTRODUCTION

Common beans are one of the main crops grown worldwide and together with rice are the staple food in many countries. The total common bean area in Brazil in 2020/2021 is estimated at approximately 2,98 million hectares (NATIONAL SUPPLY COMPANY, 2021).

In recent years the areas of common bean production are expanding to areas with low levels of rainfall indexes, reinforcing the importance of knowing the crop behavior under adverse conditions, since the common bean is a plant sensitive to water deficit.

The stress due to the lack of water in the soil is one of the main problems faced by the worldwide agriculture and the future scenarios of climate changes, with temperature increase and changes in precipitation patterns, it has been letting producers even more pessimistic (JOSHI *et al.*, 2016). The drought stress unleashes a wide variety of vegetal responses, including morphological, physiological and biochemical also changes at molecular level, molecular level, affecting negatively the growth, yield and survival of the plants (LIU *et al*., 2019; DIAS *et al*., 2018).

The effects and the crop responses to the water deficit (WD) condition depend on the variety, duration, stress intensity and phenological stage in which they are or have been submitted to (DIPP *et al*., 2017). For the common bean, the flowering and grain filling are the most sensitive stages (MATHOBO; MARAIS; STEYN, 2017; ROSALES *et al.*, 2012). According to Mathobo, Marais, and Steyn (2017), if the deficit occurs in the end of the reproductive stage, it may not cause any significant damage to the grain yield, indicating that with the correct irrigation it is possible to save water without yield loss.

In the crops in general the water deficit can reduce the photosynthesis, promote the stomatal closure, induce the pigment degradation of photosynthesis and oxidative stress through the generation and accumulation of reactive oxygen species (ROS) in the cells (DIAS *et al*., 2018). The plants developed many mechanisms to avoid the formation of ROS or to induce the dismantle of the existing ones. Some of these mechanisms are related to the antioxidative, enzymatic and non-enzymatic metabolism, which can neutralize the free radicals; therefore, reducing the damages associated.

The degree of tolerance to drought in some crops has been correlated to the antioxidant capacity of the species (LAXA *et al*., 2019).

Other mechanism adopted by plants to adapt to drought may include the development of the root system that can increase the efficiency of plants in obtaining water and nutrients (SANTOYO *et al*., 2016). In addition, adjustments in the growth rate, changes in the structure of the plant and osmotic adjustment are other mechanisms also used by the plants to increase the efficiency in the use of water (ANJUM *et al*., 2011). These changes in the vegetal metabolism can be potentialized with the use of biostimulant products, formulated from microorganisms and seaweed extracts. Among them, the plant inoculation is highlighted (by soil or seed) with different species of rhizobacteria, considered high beneficial to plants.

The associations between microorganisms and plants can favor drought adaptation, since in a symbiotic form or not they suggest physiological changes, allowing better flowering, germination, and processes of establishment of the plants (HAYAT *et al*., 2010). Besides, they can induce an increase in the efficiency of the plants to obtain on obtaining nutrients such as nitrogen, phosphorus, and iron (SANTOYO *et al*., 2016), and they can also stimulate the hormonal production, such as of auxins, cytokinins, and gibberellins which accelerate growth and development processes (VURUKONDA; VARDHARAJULA; SHRIVASTAVA, 2016).

Seaweed extracts are also considered as plant biostimulants and are widely used in agriculture, especially the species *Ascophyllum nodosum*. Seaweed extracts are biodegradable, environmentally friendly products, and contain nutrients, amino acids, vitamins and growth promoting substances. Previous studies have pointed out to the possibility of using of seaweed extract to increase plant tolerance to different types of biotic and abiotic stress (SHARMA *et al*., 2014; XU; LESKOVAR, 2015).

This research hypothesizes that rhizobacteria when inoculated in association or not with algae extract may increase the ability of plants to tolerate drought stress. Thus, this study aimed to evaluate the physiological and biochemical effects of water deficiency in common bean crop and the use of rhizobacteria and algae extract as biostimulants in order to mitigate the stress effects.

4 MATERIAL AND METHODS

The experiment was conducted in an agricultural greenhouse with 60 m² of area in Botucatu, Brazil. Temperature and relative humidity measurements were performed with the assistance of an automatic thermohygrometer installed at the central region of the greenhouse to monitor the weather conditions during the experiment. The relative humidity average during all the experimental period was 63.5% and the mean temperature was 26°C, values that are considered appropriate for the development of common bean.

4.1 Treatments and experimental design

The treatments were disposed in a split-plot experimental design in randomized blocks with two water content tension as the plots (control at -10 kPa and water deficit at -40 kPa) and three biostimulants as the subplots (B1- control; B2- *Bacillus amyloliquefaciens* BV 03 (3×10^9 UFC mL^{-1}); B3- *Bacillus amyloliquefaciens* BV 03 + brown algae extract *Ascophyllum nodosum*), with four replications repetitions and each replication presented 10 plants.

The experimental units were constituted of polyethylene pots with capacity of 30 L, in dystrophic Red Latosol in sandy loam soil, with the following characteristics: Organic matter = 7 g dm^{-3};

pH ($CaCl_2$) = 4.2; P (resin) = 2.0 mg dm^{-3}; K^+= 0.4 $mmol_c$ dm^{-3}; Ca^{2+}= 12 $mmol_c$ dm^{-3}; Mg^{+2}= 2.0 $mmol_c$ dm^{-3}; H + Al = 28.0 $mmol_c$ dm^{-3} and CEC (cation exchange capacity) = 41.0 $mmol_c$ dm^{-3}; S = 18.0 mg dm^{-3}; B = 0.19 mg dm^{-3}; Cu = 0.06 mg dm^{-3}; Fe = 11.0 mg dm^{-3}; Mn = 3.1 mg dm^{-3}; Zn = 0.4 mg dm^{-3}; Total sand = 77.4 %; Clay = 17.7%; Silt = 4.9% and 33% of saturation bases. The fertilizations followed the recommendations for common bean crop according to Aguiar *et al*. (2014). The soil water retention values were obtained by Richards method (RICHARDS, 1965) and the curve was adjusted according to Van Genuchten (1980). The adjustment parameters found were θs= 0.3397 (cm^3 cm^{-3}); θr = 0.0498 (cm^3 cm^{-3}); α = 0.5446; n = 1.4674; m = 0.3184.

The application of biostimulants in containing treatments common bean plants (*Phaseolus vulgaris* L.; cv. IAC Imperador) was biostimulants was performed through seed inoculation (SI) one hour before the planting. For treatment B1 the SI was performed with distilled water with the dose of 2 mL kg^{-1} seeds, B2 – biostimulant *Bacillus amyloliquefaciens* BV 03 with the dose of 2 mL kg^{-1} seeds; and B3- *Bacillus amyloliquefaciens* BV 03 + algae extract *Ascophyllum nodosum* in the dosage of 2 mL kg^{-1} seeds each. After the inoculation the planting was performed on August 28 of 2018, it was inserted four seeds per pot predicting subsequent roughing, leading so two plants per pot.

4.2 Crop management and irrigation

The irrigation system was by dripping in which self-compensating medium-flow button type emitters, the flow was set up at 2.0 L h^{-1} and the operation pressure at 1 Bar. The drippers were connected to distributors with two outlets and connected to arrow-type emitters by means of microtubes. An arrow-type emitter was placed per pot, dividing the flow rate by 1.0 L h^{-1} per pot. The initial distribution uniformity coefficient was of 98%, which it is classified as excellent according to classification presented by Bernardo; Soares; Montovani (2006).

The irrigation management was done by using six tensiometers per experimental plot, installed in the depth of 0.15 m. The monitoring was conducted daily with the assistance of a puncture digital tensiometer and the tension values were converted in volumetric soil water content, based on the adjustment equation of the soil water retention curve. On certain days of the trial, the control of the water depths was performed twice a day due to elevated temperatures. The adjustment of the soil water retention curve was performed through the equation proposed by Van Genuchten (1980).

Initially, all treatment combinations received the same water depth, increasing the soil humidity to the tension of -10 kPa, which is considered as corresponding tension to the control humidity, near field capacity. The differentiation of irrigation depths in the plots occurred in the corresponding stage of the beginning of crop flowering, 35 days after emergence (DAE). The threshold tension for the treatment of water deficit was -40 kPa. In order to determine the applied depth and the irrigation time, were used Equations 1 and 2, respectively (BERNARDO; SOARES; MONTOVANI, 2006).

$$I = \frac{(\theta trat - \theta act) * z}{Ef} \quad (1)$$

Where:
I - Irrigation depth (mm);
θtrat - volumetric water content (cm^3 cm^{-3}) corresponding to water treatments;
θact - actual volumetric water content (cm^3 cm^{-3});
z - effective depth of root system (200 mm);
Ef - water application efficiency (98%).

$$IT = \frac{(I * PA)}{(n * q)} * 60 \quad (2)$$

Where:
IT - irrigation time (minutes);
I - gross irrigation depth obtained by Equation 1 (mm);
PA - pot area (m^2);
n - number of emitters per pot;
q – emitter flow (1 L h^{-1}).

Considering the different phenological stages of bean crop, the irrigation depth corresponding to -10 kPa (control) and -40 kPa (WD) were where divided into vegetative phase and reproductive phase. In the vegetative phase, both treatments received 75.5 mm of water between planting and pre-flowering (35 days after emergence). In the reproductive phase, it was provided 149.5 mm for control plants and 48.5 mm for plants submitted to water deficit. In total, the plants in the control treatment received 225 mm of water and the treatment under water stress 124 mm of water. Before the harvest, the irrigation was suspended in both treatments to dry the grains in the plant. The harvest of the treatments was carried out 84 and 77 DAE for the control treatment and the treatment under water deficit, respectively.

4.3 Date of the evaluations

The physiological evaluations were content of leaf pigments (chlorophyll *a*, chlorophyll *b* and carotenoids), photosynthesis (*A*), stomatal conductance (g_s), internal carbon concentration (C_i), transpiration (*E*) and relative water content (RWC). The biochemical evaluations corresponded to the total soluble protein content, specific activity of the enzyme Superoxide Dismutase (SOD, EC 1.15.1.1) and determination of the amino acid content of L-proline.

For content of pigments, relative water content and biochemical evaluations, healthy leaves were collected of the middle third of the plants at 56 DAE, phenological stage R8, corresponding to the grain filling. The determinations of leaf gas exchanges occurred in different periods of the previous evaluations, in which a total of five measurements were performed during the crop cycle. The evaluations occurred when some plants were already submitted to deficit irrigation and in the stages of growth and grain filling of pods. The periods were at 47, 49, 52, 54 DAE. In all evaluations, one leaf per replication was evaluated in each treatment.

4.4 Leaf pigments content

In order to determine the leaf pigments content, leaves were collected during the morning and leaf discs with 1.04 cm² of diameter were removed, each one was kept in 2 mL of dimethylformamide (DMF) for 24h, in the dark, to extract chlorophyll (*a*, *b*) and carotenoids. After that, the readings were performed in a spectrophotometer at wavelengths 480, 646.8 and 663.8 nm. The results were expressed in µg cm-² (LEE; PERAIRE; ZIENKIEWICZ, 1987).

4.5 Gas exchanges parameters

The evaluations of gas exchanges were performed from 9 until 11 a.m., in leaves completed expanded of the middle third of the plants, with the assistance of portable infrared gas analyzer, model LI-COR 6400 IRGA (LI-COR, Lincoln, NE, USA). The microclimate parameters in the chamber were kept constant during the measurements performed in the different treatments, using 1000 µmol of photons m^{-2} s^{-1} (MATHOBO; MARAIS; STEYN, 2017) of PAR (*photosynthetic active radiation*) and relative humidity between 50 and 60%. The following variables were measured: net CO_2 assimilation rate (*A*; µmol CO_2 m^2 s^-), stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}), internal carbon concentration (*Ci*; µmol m^{-2} s^{-1}) and transpiration (*E*; mmol H_2O m^{-2} s^{-1}).

4.6 Relative water content

Relative water content (RWC) was determined by the relation between the vegetal tissue fresh, turgid and dry, according to Barr and Weatherley (1962) and calculated by the Equation 3.

$$RWC = [(FW - DW)/(TW - DW)] * 100 \quad (3)$$

Where:
RWC – Relative water content;
FW – fresh weight (mg);
TW – turgid weight (mg);
DW – dry weight (mg).

4.7 Biochemical analyses

In order to perform the biochemical analyses, leaf samples from the middle third of one plant per replication were collected. After the collection, the leaves were immediately frozen in liquid nitrogen and stored in freezer at -80°C to later trituration.

The samples were processed to obtain two different extracts: the first one for the analyses of protein concentration and enzymatic activities, was obtained through the resuspension of 300 mg of the milled vegetal material in 5.0 mL of potassium phosphate buffer 0.1 M, pH 7.8 in the presence of 300 mg of PVPP (polyvinylpolypyrrolidone). The second extract to determine the L-proline contents was obtained through the resuspension of leaf tissue (500 mg) in 10.0 mL of sulfosalicylic acid (3% in distilled water).

The total soluble protein concentration presents in the extracts was determined in duplicate, following the method described by Bradford (1976) with bovine serum albumin (BSA) as a standard protein.

The L-proline content was determined following the method described by Bates, Waldren, and Teare (1973). To colorimetric test was performed by taking aliquots of 2 mL of the crude extract; 2.0 mL of acid ninhydrin; and 2.0 mL of glacial acetic acid. After heating in boiling water bath, the flasks were immediately cooled with ice bath. The reaction mixture was extracted with 4 mL of Toluene and subsequently a reading was performed at 520 nm in a spectrophotometer. As a reference, standard curve was used with (0, 20, 40, 60, 80, and 100 mg) of L-proline p.a.

The activity of SOD was determined by the addition of 30 μL of crude extract at a solution containing 13 mM of methionine, 75 μM of Nitro blue Tetrazolium Chloride (NBT), 100 nM of Ethylenediaminetetraacetic acid (EDTA) and 2 μM of riboflavin in 3.0 mL of potassium phosphate buffer 50mM, pH 7,8, according to what is described by Del Longo *et al.* (1993). The reaction was initiated by the tubes lighting, in chamber composed by fluorescent tubes (15W), at 25°C. After 5 minutes of incubation, the end of catalysis was determined by light interruption (GIANNOPOLITIS; RIES, 1977). The compound formed by photoreduction of NBT was determined by the increase in absorption, performed by spectrophotometry with readings at 560nm. One unit of SOD was defined as the necessary enzyme quantity to 50% inhibition of photoproduction of the NBT. For the calculation of the specific enzyme activity, we considered the percentage of inhibition obtained, the sample volume and the sample protein concentration ($\mu g\ \mu L^{-1}$).

4.8 Shoot dry matter

The shoot dry matter (dry matter of the leaves + dry matter of the stem) was determined by putting samples in the dryer with forced air circulation at 60ºC ± 5 until constant weight, and later they were weighed in a precision balance.

4.9 Crop Yield

After the harvest, the grains were manually separated from the pods, counted and weighed. The yield estimate (tons/ha) was made considering a population of 240 thousand plants per hectare (0.5 m inter-row and 0.083 m in the row space) and the grain humidity corrected to 13%. The grain humidity after the harvest was obtained by the kiln method at 105 ± 3°C for 24 hours and the data expressed in percentage (wet basis) (BRASIL, 2009).

4.10 Statistical analysis

The experiment was set up in split-plot in a randomized block design with four replications. The treatments in the plots corresponded to the irrigation depths and in the subplots the biostimulants. To perform the statistical analysis, the data obtained were initially submitted to the Kolmogorov-Smirnov normality test using Minitab® 17 software and when the normality of the data was confirmed, they were submitted to variance analysis. When significant in the analysis of variance, the interaction between factors was studied by applying the Tukey test to compare means at 5% probability using Agrostat® software. In the absence of significance in the variance analysis for the interaction between the factors, it was evaluated the average of the main factors.

5 RESULTS AND DICUSSIONS

5.1 Leaf pigments content

The leaf pigment content was obtained by the observation of the chlorophyll contents (*a* and *b*) and the carotenoid content. The chlorophyll *a* content (Table 1) was not significant in the interaction between the factors, and it was significant only to the differentiation of the irrigation water depths, in which the control depth plants presented the highest average pigment concentration. In relation to chlorophyll *b* and carotenoids, there were not differences among the treatments.

Table 1. Chlorophyll *a*, Chlorophyll *b* and Carotenoid content (μg cm-2 of leaf) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	**Biostimulants**			
	B1	**B2**	**B3**	**Average**
		Chlorophyll *a*		
C	12.3	12.3	12.7	12.6 a
WD	10.0	11.9	10.9	10.9 b
Average	11.16 A	12.12 A	11.55 A	
		Chlorophyll *b*		
C	4.65 ^{NS}	4.71	4.84	4.74
WD	4.41	5.00	5.50	4.97
Average	4.53	4.85	5.17	
		Carotenoid		
C	3.18 ^{NS}	2.81	3.10	3.03
WD	2.40	2.89	2.78	2.68
Average	2.79	2.85	2.94	

^{NS}- Non-significant by Tukey's test at 5% of probability.
Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

The evaluations of pigment rates indicate that the imposed water deficit condition was responsible for the decrease of chlorophyll *a* content in the bean leaves. Ammar *et al*. (2015) also observed the same effect for the fava bean and argue that the decline can be due to oxidative damage to chloroplasts, that may lead to the destruction of chlorophyll molecules. The photosynthetic pigments are important to the plants in the absorption of light energy and production by reducing potential during the photosynthetic process (JALEEL *et al*., 2009). The chlorophylls *a* and *b* are the most important photoreceptor pigments and more susceptible to degradation when the plants are submitted to water deficit. Therefore, in case of these pigments are degraded, it may cause a negative influence on the photosynthetic rates, consequently affecting the growth and yield of crops (FAROOQ *et al*., 2009).

5.2 Gas exchanges parameters

During the different crop stages, different parameters of gas exchange were evaluated. The CO_2 assimilation rate (*A*) is presented in Table 2. In the evaluation 47 DAE, when the water deficit had already been started, there was significant effect to the treatment interaction. At this evaluation, the increase of *A* in the control (C) irrigation water depth was approximately 40 and 32% for treatments B2 and B3, respectively, in the control (C) irrigation water depth compared to the control B1. However, in WD depth, this effect was not observed among the treatments. Also, in the evaluation 49 DAE, it was possible to observe decreasing of photosynthetic rates when the plants were submitted to water deficit. A similar behavior was observed for the subsequent evaluations (52 and 54 DAE) when the control irrigation water depth presented higher averages of *A* and the different treatments with biostimulants did not influence the measurement in these evaluation periods.

Table 2. CO_2 assimilation rate (A; µmol CO_2 m^2 s^{-1}) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	**47 DAE**			
	B1	**B2**	**B3**	**Average**
C	12.5 Ba	20.8 Aa	18.2 Aa	17.2
WD	10.6 Aa	9.3 Ab	8.2 Ab	9.4
Average	11.6	15.0	13.2	
Treatments	49 DAE			
	B1	B2	B3	Average
C	20.9	20.0	17.9	19.6 a
WD	5.8	4.8	6.4	5.7 b
Average	13.4 A	12.4 A	12.2 A	
Treatments	52 DAE			
	B1	B2	B3	Average
C	18.8	15.8	16.7	17.1 a
WD	9.8	8.8	6.9	8.5 b
Average	14.3 A	12.3 A	11.8 A	
Treatments	54 DAE			
	B1	B2	B3	Average
C	17.6	17.2	15.7	16.8 a
WD	9.5	8.9	11.2	9.8 b
Average	13.5 A	13.0 A	13.5 A	

^{NS}- Non-significant by Tukey's test at 5% of probability.
Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

At all evaluation times for stomatal conductance (g*s*) no significant effect was found for the interaction between the factors (Table 3). The analysis of variance showed that there was a significant effect only on the irrigation water depths for all evaluations, and the control treatment presented the highest stomatal conductance value.

Table 3. Stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	**47 DAE**			
	B1	**B2**	**B3**	**Average**
C	0.469	0.498	0.338	0.435 a
WD	0.077	0.066	0.057	0.067 b
Average	0.273 A	0.282 A	0.197 A	
Treatments	49 DAE			
	B1	B2	B3	Average
C	0.682	0.628	0.466	0.593 a
WD	0.052	0.047	0.054	0.051 b
Average	0.367 A	0.338 A	0.260 A	
Treatments	52 DAE			
	B1	B2	B3	Average
C	0.272	0.245	0.306	0.274 a
WD	0.080	0.065	0.049	0.065 b
Average	0.176 A	0.155 A	0.177 A	
Treatments	54 DAE			
	B1	B2	B3	Average
C	0.486	0.370	0.309	0.389 a
WD	0.091	0.080	0.129	0.102 b
Average	0.288 A	0.228 A	0.219 A	

^{NS}- Non-significant by Tukey's test at 5% of probability.
Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

In the evaluations 47, 52, 54 DAE for the transpiration (E), the analysis of variance showed significant effect only for the irrigation water depth treatment, with the highest averages for the control water depth (Table 4). In contrast, the evaluation at 49 DAE showed significant effect for the interaction between these treatments. In general, as in the other assessments, *E* was reduced due to water deficit and the treatments B1 and B2 were superior when the plants were not under water restriction. However, when submitted to water deficit the biostimulants treatments did not differ between each other significantly.

Table 4. Transpiration (E; mmol H_2O m^{-2} s^{-1}) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	**47 DAE**			
	B1	**B2**	**B3**	**Average**
C	7.12	7.01	5.69	6.61 a
WD	1.80	1.48	1.31	1.53 b
Average	4.56 A	4.24 A	3.50 A	
Treatments	49 DAE			
	B1	B2	B3	Average
C	9.97 Aa	9.58 Aa	6.61 Ba	8.72
WD	1.65 Ab	1.54 Ab	1.69 Ab	1.63
Average	5.81	5.56	4.15	
Treatments	52 DAE			
	B1	B2	B3	Average
C	4.36	4.25	4.87	4.49 a
WD	1.62	1.40	1.09	1.37 b
Average	2.99 A	2.82 A	2.98 A	
Treatments	54 DAE			
	B1	B2	B3	Average
C	7.68	6.53	5.69	6.63 a
WD	2.24	2.13	2.96	2.45 b
Average	4.96 A	4.33 A	4.33 A	

^{NS}- Non-significant by Tukey's test at 5% of probability.
Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

The measurements of gas exchanges related to CO_2 assimilation rate (A) presented in Table 2 indicate that the biostimulants in evaluation 47 DAE were capable to influence positively the net assimilation rates of common bean in the absence of water stress. Marcos *et al.* (2015) verified that endophytic bacteria are capable to increase the photosynthetic rate in cultivars of sugar cane in the absence of stress. Naveed *et al*. (2014) for corn crop, verified that the use of growth-promoting rhizobacteria was capable of increasing of the photosynthetic rates in plants under water stress conditions as well as in the absence of stress. The capacity of the plants of increasing their photosynthetic rates when inoculated with growth promoting bacteria, has been discussing by several authors (KUMAR; VERMA, 2018; NGUMBI; KLOEPPER, 2016; VURUKONDA et al., 2016).

The decrease in the photosynthetic rates of the plants due to water deficit imposition for common bean crop was also observed by Sartori *et al*. (2019), Lanna *et al.* (2018), and Mathobo, Marais, and Steyn (2017). These results can be associated partly with the chlorophyll molecules degradation according to reports and mainly as a response to stomatal regulation mechanism, which indeed was detected by the considerable reduction in the stomatal conductance (g_s) in the same periods evaluated here (Table 3). The stomata closure blocks the carbon input in leaves and its assimilation is decreased in favor of the photorespiration. This condition was also reported by Soureshjani *et al*. (2019) when evaluating the common bean crop.

Besides influencing the common bean photosynthesis rates, the stomatal regulation of plants when submitted to water stress condition affects the transpiration (*E*), factor observed in this study, as summarized in Table 4. All the measurements presented decrease in leaf transpiration as a response to water deficit. The plants, in general, when they are submitted to drought stress conditions, tend to reduce the loss of water to the environment by transpiration through the stomatal closure mechanism. The control of water loss is an adaptative vegetal capacity in order to keep the water content in the cells and their metabolic activities active during the period in which the water availability in the soil is reduced (TAIZ *et al.*, 2017). Reductions on transpiration rates of common bean under drought stress were also observed by Sartori *et al*. (2019) and Mathobo, Marais, and Steyn (2017).

For CO_2 concentration (C*i*) (Table 5), the significant effect on the 47 and 52 DAE evaluations was recorded only for the irrigation water depths with higher averages in the control treatment. In the evaluation at 49 DAE there was a significant effect on the average of biostimulant treatments, the B2 average was the highest, but not statistically different from B1. Still in the 49 DAE evaluation, the depth of irrigation was significant, and the control irrigation water depth higher than WD.

Table 5. Intercellular carbon concentration (Ci; μmol m^{-2} s^{-1}) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	**47 DAE**			
	B1	**B2**	**B3**	**Average**
C	314.0	266.9	255.5	278.79 a
WD	143.9	131.2	132.4	135.83 b
Average	228.95 A	199.03 A	193.94 A	
Treatments	49 DAE			
	B1	B2	B3	Average
C	304.11	301.01	287.94	297.68 a
WD	180.85	199.03	173.02	184.31 b
Average	242.48 AB	250.03 A	230.48 B	
Treatments	52 DAE			
	B1	B2	B3	Average
C	246.10	254.86	267.49	256.15 a
WD	137.92	134.21	113.28	128.47 b
Average	192.01 A	194.53 A	190.39 A	
Treatments	54 DAE			
	B1	B2	B3	Average
C	296.75 Aa	272.58 Aa	266.78 Aa	278.69
WD	189.14 Ab	193.24 Ab	218.17 Ab	200.22
Average	242.94	232.9	242.53	

^{NS}- Non-significant by Tukey's test at 5% of probability.
Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

For the evaluation 54 DAE, significant effect was found for the interaction among the treatments and similar behavior was observed in other assessments, in which it was verified decrease in the values of *Ci* when the plants were submitted to the water deficit treatment of, but and it did was not identified any difference in relation to the treatments with biostimulants.

The carbon internal concentration (*Ci)* was influenced only by water deficit imposed to the plants, in which it was observed decrease of measured values after the beginning of stress (Table 5). The *Ci* rate is regulated by stomatal and non-stomatal factors. When the water stress imposed is moderate, the predominance of stomatal limitation to photosynthesis by the decrease of CO_2 input in plants occurs, resulting in low values of C*i* as it was showed in this research. In severe stress situations, the tendency of increasing *Ci* in cells occurs, due to limitations in the photosynthetic activity of plants caused by damage in the photochemical and biochemical reactions of this process (SHI *et al.*, 2014). Lanna *et al.* (2018) evaluated the gas exchanges in leaves of different cultivars of common bean and also observed variations on *Ci* with the imposition of water deficit.

5.3 Biochemical analyses

The total soluble protein content (Table 6) found, it did not differ statistically among the treatments with biostimulants and the applied irrigation depths. The L-proline rates (Table 7) and the specific enzyme activity SOD (Table 8) in the vegetal tissue are considered indicators of physical stress tolerance, like the water deficit. These variables were significantly changed in relation to the irrigation depths, in which the higher activity of enzyme SOD and the accumulation of amino acid L-proline occurred in the treatments under irrigation deficit (WD).

Table 6. The total soluble protein content (mg g^{-1} fresh weight) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	Biostimulants			
	B1	B2	B3	Average
C	11.09 ^{NS}	10.98	7.90	9.99
WD	8.02	8.42	6.74	7.73
Average	9.55	9.70	7.32	

^{NS}- Non-significant by Tukey's test at 5% of probability.
DAE - days after emergence; C - irrigation water depth with -10 kPA in soil; WD - irrigation water depth with -40 kPa in soil.

Table 7. L-proline rate (µmol g^{-1} fresh weight) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	Biostimulants			
	B1	B2	B3	Average
C	0.634	0.556	0.555	0.582 b
WD	0.690	0.695	0.812	0.732 a
Average	0.662 A	0.625 A	0.684 A	

Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

Table 8. The specific activity of SOD (UI µg protein^{-1}) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	**Biostimulants**			
	B1	**B2**	**B3**	**Average**
C	1.636	1.719	1.965	1.773 b
WD	2.409	2.817	2.750	2.659 a
Average	2.023 A	2.268 A	2.359 A	

Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey’s test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

The biochemical evaluations demonstrated that the common bean when submitted to water deficit, it has metabolism changed as a strategy to attenuate the deleterious effects of stress. During the common bean cultivation, it was detected the increase of L-proline rate in common bean leaves with the imposition of water deficit (Table 7). The accumulation of organic solutes in the vegetal cells is somewhat desirable, as a component of osmotic adjustment. In conditions of drought stress, these osmolytes decrease the osmotic potential, favoring the water absorption by the cells and tissues and the cellular turgor maintenance (LAXA *et al.,* 2019). Ammar *et al.* (2015) studied the effects of water stress in fava bean and verified that this condition increased the amino acid L-proline rates.

The specific activity of the enzyme superoxide dismutase (SOD) was affected by the water treatments, it was observed high activity in plants under drought stress (Table 8). During the period in which the plants are submitted to drought stress, it can occur an increase in the production of oxygen-reactive species (ROS), which are highly harmful forms to the cells. When they are produced, they cause lipid peroxidation, deterioration of membranes, protein damage and DNA modification, it may be leading to cellular death (DIAS *et al.,* 2018). In order to control the ROS level and reduce the effects associated to oxidative stress, the vegetal tissues synthesize a complex of antioxidative enzymes, among them the SOD. The enzyme SOD is the first enzyme involved in the plant protection system, acting in the dismutation of the superoxide radical ($O_2^{\bullet -}$) into hydrogen peroxide (LAXA *et al.,* 2019). Soureshjani *et al.* (2019) reported activity increase of SOD in cultivars of common bean submitted to drought stress.

The treatments with biostimulants, despite of not differing significantly in the biochemical evaluations, presented higher averages in B3 for proline when compared to the control. Several authors attribute the capacity of growth promoting rhizobacteria in the osmotic adjustment and in the antioxidative metabolism of plants as one of the main mechanisms used to assist a vegetal in the drought tolerance (KUMAR; VERMA, 2018; NGUMBI; KLOEPPER, 2016; VURUKONDA et al., 2016). Researching the role of the rhizobacteria *Bacillus amyloliquefaciens* NBRI-SN13 in the improvement of effects of several type of physical stress, among them the water stress, in rice plants, Tiwari *et al.* (2017) observed increments of proline rates compared to uninoculated plants. The authors also mention that the accumulation of osmolytes in the cells increases the plant tolerance to stress conditions.

5.4 Relative water content

The results for relative water content (RWC), summarized in Table 9, indicate that there was no significant effect on the interaction between factors and the main effect in the irrigation depths was statistically different ($p<0,05$). The plants treated with biostimulants did not show significant differences. Although not significant, the treatment B3 presented higher values than the control**.**

Table 9. Relative water content (%) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation depths in the common bean crop at 56 DAE.

Treatments	**Biostimulants**			
	B1	**B2**	**B3**	**Average**
C	58.3	61.8	65.1	61.73 b
WD	66.8	67.4	71.3	68.52 a
Average	62.54 A	64.62 A	68.20 A	

Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

The physiological and biochemical effects of water deficiency and the application of biostimulants were reflected in the relative water content (RWC) (Table 9). The RWC is considered a good criterion to measure the water status of a plant because it is directly involved with the metabolic activity of tissues (JALEEL *et al.*, 2009). The results for the RWC, it can be associated with the stomatal regulation of plants under stress, that in response to the imposed stress, they trend to decrease tissue water loss via transpiration. In addition, the increase in the concentration of L-proline observed in plants under water stress (Table 7) and its likely effect on osmotic adjustment, it helped to maintain cell turgor even in plants under water restriction.

In relation to the bioestimulant actions, despite not having statistical difference, plants treated with these agricultural inputs presented higher averages of RWC, what is an indicative that they present potential to assist the plants to keep the cellular turgor. Grover et al. (2014) reported that sorghum plants treated with *Bacillus* spp lineage KB 129 cultivated under water stress presented increase of 24% in RWC, compared to untreated plants. Sarma and Saikia (2014) studied the effect of bacteria *Pseudomonas (P. aeriginosa*) genus and checked an increase in the water relative content in mung bean (*Vigna radiata*) cultivated under water deficit.

5.4 Shoot dry matter and crop yield

The shoot dry matter evaluation of the common bean is presented in Table 10. The interaction between the factors by the variance analysis was not significant ($p> 0.05$). The evaluation of the main factors shows that the depth of irrigation WD caused a 40% reduction when compared to treatment C (control). The average of the biostimulant treatments did not show significant difference among them.

Table 10. Shoot dry matter (g plant $^{-1}$) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	Biostimulants			
	B1	**B2**	**B3**	**Average**
C	18.2	17.7	18.1	17.7 a
WD	11.1	11.4	9.8	10.7 b
Average	14.6 A	14.5 A	13.9 A	

Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

The yield data of common beans as a function of irrigation water depths and application of biostimulants are presented in Table 11. No significant effect was found for the interaction among the treatments ($p > 0.05$). The evaluation of the means showed that the biostimulants did not present significant difference among them. The WD irrigation water depth promoted a 50% reduction in crop yield compared to treatment C (control).

Table 11. Yield (ton/ha) due to biostimulants application (B1- control, B2- Bacillus amyloliquefaciens BV 03, B3- Bacillus amyloliquefaciens BV 03 + Ascophyllum nodosum) and irrigation water depths in the common bean crop at 56 DAE.

Treatments	Biostimulants			
	B1	**B2**	**B3**	**Average**
C	4.56	4.18	4.46	4.40 a
WD	2.26	2.17	2.29	2.22 b
Average	3.41 A	3.17 A	3.34 A	

Averages followed by the same letter in lower case in the column and upper case in the lines do not differ statistically at the level of 5% of probability through Tukey's test. DAE - days after emergence; C – control irrigation water depth with -10 kPA in soil; WD- irrigation water depth with -40 kPa in soil.

The imposition of water deficit during the early flowering phase of the bean, it promoted a high reduction in the accumulation of shoot dry matter (dry matter of the leaves + dry matter of the stem) and in the total productivity (Table 10 and Table 11). The reduction in shoot dry matter accumulation and productivity are explained by the physiological changes suffered by plants when subjected to drought stress. The main changes, that were observed in this work, were the degradation of chlorophyll molecules, which together with stomatal closure drastically reduced the photosynthetic rate of plants. Under conditions of reduced photosynthesis, plants tend to have low production and accumulation of biomass especially from leaves, in addition to promoting the senescence of the existing ones. The reduction in the accumulation of leaf biomass in plants under drought stress, it decreases the area of light absorption for photosynthesis, which may reflect a drop in productivity, as observed in this research. The intensity and time of exposure to stress, the genotype and phenological stage in which stress is imposed influence the intensity of production losses by crops. The effects of water stress on beans in reducing dry matter accumulation and yield were also observed by Soureshjani *et al.* (2019) and Mathobo, Marais, and Steyn (2017).

6 CONCLUSION

In general, the present study demonstrated the sensitivity to drought stress of common beans cultivar IAC Imperador with physiological and biochemical changes that culminated in productivity losses.

The research demonstrated that biostimulants composed of rhizobacteria (Bacillus amyloliquefaciens BV 03) associated or not with algae extract (Ascophyllum nodosum) applied by seed treatment under the cultivation conditions and doses used, were not efficient to attenuate the physiological and biochemical effects promoted by drought stress.

With the increasing use of these products in worldwide agriculture, we suggested the development of more studies, using different cultivars and under diverse cultivation conditions in order to validate or not the efficiency of bioestimulants in mitigating the effects of water deficit.

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