

## Foliar application of biostimulant doses in two phenological stages in common bean culture

Wytalo Oliveira Lopes<sup>1</sup>, Lorena Correia Varão<sup>1</sup>, Wilian Henrique Diniz Buso<sup>1</sup>

<sup>1</sup>Instituto Federal Goiano Campus Ceres, Ceres, Goiás, Goiânia, Brazil. E-mail: [wytalooliveira.agro@outlook.com](mailto:wytalooliveira.agro@outlook.com), [lorennavaraocorreia@hotmail.com](mailto:lorennavaraocorreia@hotmail.com), [wilian.buso@ifgoiano.edu.br](mailto:wilian.buso@ifgoiano.edu.br)

Received: 12/05/2022; Accepted: 27/07/2022.

### ABSTRACT

Salinity is one of the environmental stresses that most affects plants' vital processes, especially germination. Brassinosteroids, including 24-epibrassinolide (EBL), have multiple actions in essential processes in plants. Thus, this research's objective was to evaluate the effects of EBL on tomato seeds' physiological conditioning on germination, growth, and production of dry seedling mass under salinity conditions. The experiment was carried out in a 2x5 factorial scheme, as follows: two concentrations of EBL (0 and 10<sup>-6</sup> M) and five levels of salinity (0.5 control; 1.5; 3.0; 4.5 and 6.0 dS m<sup>-1</sup>), consisting of ten treatments, with four replications of 50 seeds. In isolation, the salt stress reduced the percentage of germination and germination speed index from four to 14 days, besides all the plants' growth traits. In turn, the EBL increased the percentage of germination, germination speed index, seedling length, and radicle dry matter. The application of 10<sup>-6</sup> M of EBL in seed immersion no effect on the seed germination of IPA 6 tomato cultivar under salt stress but increases the root length and the dry matter of the seedlings.

**Keywords:** *Phaseolus vulgaris*, Biostimulant, Phytohormones.

### Aplicação foliar de doses de bioestimulante em duas épocas na cultura do feijoeiro

#### RESUMO

O feijoeiro é uma leguminosa que possui grande relevância na alimentação humana devido às características proteicas de sua semente. O objetivo do trabalho foi avaliar doses de bioestimulante aplicado em dois estádios fenológicos na cultura do feijoeiro comum. O delineamento experimental adotado foi de blocos ao acaso em esquema fatorial 6 x 2. Foram utilizadas seis doses de bioestimulante (0; 0,250; 0,500; 0,750; 1,000; 1,250 L ha<sup>-1</sup>) e dois estádios fenológicos (V3 e R5) com quatro repetições. As variáveis avaliadas foram: número de vagens por planta, número de grãos por vagem, massa de 1000 grãos, altura de plantas e produtividade de grãos. Houve interação significativa do fator dose apenas para a variável produtividade, que atingiu o maior valor com a dose de 0,65 L ha<sup>-1</sup> do bioestimulante. A produtividade alcançada foi de 3.814,50 kg ha<sup>-1</sup>, que representa um ganho de 17,3% em relação a testemunha. Não houve efeito do produto sobre o fator época para nenhuma das variáveis.

**Palavras-chave:** *Phaseolus vulgaris*, Bioestimulante, Fitohormônios.



## 1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a legume that has great relevance in human food due to the protein characteristics of its seed. Brazil is among the largest bean producers in the world, with the crop as one of its main agricultural explorations (Silva et al., 2018; Frasca et al., 2020). According to Conab (2022), the national production of beans for the 2021/2022 harvest was estimated at 3,114.8 thousand tons, 7.6% higher than the one obtained in 2020/2021, adding all the harvests. The higher production is mainly related to the increase in the national average grain yield, which was 11.2% higher than the previous year.

Biostimulants are a group of inputs that producers have well accepted. These products are classified as synthetic substances composed of a set of plant or nutrient regulators, amino acids, and other products, which, when applied exogenously, are capable of expressing effects in plants similar to those of phytohormone groups, such as cytokinin, gibberellin, auxin, abscisic acid, and ethylene (Prieto et al., 2017).

Phytohormones are organic compounds produced naturally by plants, which in small amounts can regulate various morphophysiological processes of plants, modifying or improving the organism and anatomy of plants (Santos et al., 2020). As these compounds can guarantee improvements in the grain yield characteristics of several crops, studies involving plant regulators have become widely used in agriculture (Aguiar et al., 2015).

The use of biostimulants is highlighted in agriculture for the benefits they provide to cultivated species since these substances applied in foliar spraying, in seed treatment, or directly in the soil can improve the absorption and efficiency of nutrients, resulting in increases in grain yield (Frasca et al., 2020). Aguiar et al. (2015), evaluating the application of biostimulant in bean crops in a protected environment, reported that the use of the biostimulant Stimulate<sup>®</sup> provided significant increases in the number of pods per plant, 100-grain weight, and grain yield.

Among the possible hormones used in the composition of biostimulants, the main ones responsible for plant growth are auxins, cytokinin, and gibberellins (Pavezi et al., 2017). Auxins act on plants by promoting rooting and the formation of root primordia. Cytokinins

are responsible for stimulating cell division and the process of cytokinesis, while gibberellins control the germination process, foliage formation, reproduction, and fruit formation (Almeida and Rodrigues, 2016). These compounds also improve plant water absorption, favoring vital processes and improving grain yield and quality under adverse conditions (Ferreira et al., 2013).

Thus, this study aimed to evaluate the effect of doses of biostimulant applied in two phenological stages in the common bean crop.

## 2. Material and Methods

The experiment was carried out in the experimental area of the Instituto Federal Goiano, Campus Ceres, GO, at 15°21'00" S, W 49° 35' 57" W, and an altitude of 564 m, under a central pivot system. The climate in the region is Aw, according to the Köppen classification, characterized as humid tropical with a rainy season in the summer and a dry season in the winter. Soil preparation was carried out, consisting of a heavy harrow and an operation with a discing harrow one day before the experiment was set up. The sowing fertilization was calculated following the recommendations proposed by Sousa and Lobato (2004), according to the chemical characteristics of the soil (Table 1), with 40 kg ha<sup>-1</sup> of N, 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, and 40 kg ha<sup>-1</sup> of K<sub>2</sub>O. Also, topdressing fertilization with N (urea) was carried out at the V4 stage (third fully developed trifoliolate).

The common bean (Pérola cultivar) sowing took place on 05/07/2019, in which the conventional system was adopted with a spacing of 0.50 m between rows, distributing 15 seeds per meter. The randomized block design arranged in a 6 x 2 factorial scheme with four replications was used. Six doses of biostimulant (0; 0.250; 0.500; 0.750; 1.000; 1.250 L ha<sup>-1</sup>) applied at two phenological stages (V3 and R5) were evaluated.

The biostimulant Stimulate<sup>®</sup> was used, which is composed of kinetin 0.09 g L<sup>-1</sup> (0.009% m v<sup>-1</sup>), gibberellic acid, with GA<sub>3</sub> 0.05 g L<sup>-1</sup> (0.005% m v<sup>-1</sup>), indole-3-butyric Acid 0.05 g L<sup>-1</sup> (0.005% m v<sup>-1</sup>) and inert ingredients 999.80 g L<sup>-1</sup> (99.88% m v<sup>-1</sup>). The application of treatments during the experiment was carried out with a backpack sprayer to spray the product on the plants.

**Table 1.** Result of the soil chemical and particle-size analysis at the 0-20 cm depth before the installation of the experiment.

Sand	Silt	Clay	pH in H <sub>2</sub> O	O.M.	Ca	Mg	Al
g kg <sup>-1</sup>				g dm <sup>-3</sup>	cmol <sub>c</sub> dm <sup>-3</sup>		
482	40	478	5.82	22	3.85	1.94	0.00
H+AL		K	CEC	K	P	BS	
cmol <sub>c</sub> dm <sup>-3</sup>		mg dm <sup>-3</sup>					
3.80		0.56	10.15	180.00	30.00	62.57%	

The cultural treatments were the ones usually used in common bean crops in the region. All phytosanitary control was performed to conduct the experiment according to the technical recommendations for the culture to control weeds, insects, and diseases. The seeds were treated with fungicides containing Fludioxonil and Thiamethoxam, both at a dose of 200 mL per 100 kg of seed. For weed management, the post-emergence herbicides Fomesafen at a dose of 1 L ha<sup>-1</sup> and Fluazifop-p-butyl at a dose of 0.75 L ha<sup>-1</sup> were used when the crop had a fully formed second trifoliate.

The irrigation was done via a central pivot system with a two-day irrigation interval. Irrigation management was carried out with a Class A evaporation pan. The applied depth was corrected by the Kc of the crop according to the phenological stage of the common bean. The harvest of the experiment took place on 08/28/2019, carried out in a semi-mechanized way, with the uprooting of the plants manually, followed by threshing with the plants being threshed on a thresher. The yield of each plot was then weighed to estimate yield (kg ha<sup>-1</sup>).

The variables analyzed were plant height (measured from the ground level to the end of the main

branch), number of pods per plant, number of grains per pod, 1000-grain weight, and grain yield. Data on yield components and agronomic traits were subjected to analysis of variance. The means were compared by the Tukey test at 5%. A regression analysis was performed on the variables analyzed according to the doses of biostimulant. The R statistical software was used.

### 3. Results and Discussion

The results of the analysis of variance are shown in Table 2. It is observed that there was no significant interaction between the phenological stages and biostimulant doses (SxD) for any of the variables evaluated. Thus, the variables were analyzed individually. Regarding the isolated effect of the factors, the biostimulant doses significantly affected the grain yield (GRY). No significant effect of the phenological stage was observed for any of the variables studied. In the regression analysis, significance was observed only for the quadratic adjustment (Table 2). Evaluating the results for plant height (Table 3), there is a statistical difference between the stages.

**Table 2.** Mean squares of the variables analyzed, plant height (PH), number of pods per plant (NPP), number of grains per pod (NGP), 1000-grain weight (1000Q), and grain yield (GRY) for the phenological stage (S), biostimulant doses (D), and SxD interaction of common bean with biostimulant application at two phenological stages.

Variables	Error mean square <sup>1</sup>			Regression	
	Stage	Dose	SxD	Linear	Quadratic
PH	0.2206 <sup>ns</sup>	0.0225 <sup>ns</sup>	0.0457 <sup>ns</sup>	0.022835 <sup>ns</sup>	0.021341 <sup>ns</sup>
NPP	6.3955 <sup>ns</sup>	8.0994 <sup>ns</sup>	8.9743 <sup>ns</sup>	3.8953 <sup>ns</sup>	17.7421 <sup>ns</sup>
NGP	0.0739 <sup>ns</sup>	0.1023 <sup>ns</sup>	0.3216 <sup>ns</sup>	0.26644 <sup>ns</sup>	0.018428 <sup>ns</sup>
1000W	536.210 <sup>ns</sup>	144.0759 <sup>ns</sup>	202.341 <sup>ns</sup>	360.13 <sup>ns</sup>	38.67 <sup>ns</sup>
GRY	886086.7 <sup>ns</sup>	1234597.8*	340256.7 <sup>ns</sup>	35843 <sup>ns</sup>	2083553*
DF	1	5	5	-	-

ns = not significant, \* significant at 5% by the Tukey test. DF= degrees of freedom

**Table 3.** Plant height (PH), number of pods per plant (NGP), number of grains per pod (NGP), 1000-grain weight (1000W), and grain yield (GRY) of common bean with the application of biostimulant at two phenological stages.

Phenological stage	PH (m)	NPP	NGP	1000W (g)	GRY (kg ha <sup>-1</sup> )
V3	1.23 b	14.23 a	6.66 a	272.88 a	3,434.41 a
R5	1.37 a	13.50 a	4.74 a	266.20 a	3,705.96 a
Biostimulant doses (L ha <sup>-1</sup> )	PH (m)	NPP	NGP	1000W (g)	GRY (kg ha <sup>-1</sup> )
0	1.25	12.32	4.60	272.57	3,400.57
0.25	1.24	14.29	4.72	276.69	3,277.55
0.50	1.39	14.20	4.53	266.57	3,778.61
0.75	1.29	15.26	4.74	268.15	4,277.41
1.00	1.32	13.08	4.81	265.59	3,269.22
1.25	1.31	14.00	4.81	267.69	3,417.70
CV (%)	13.42	16.49	8.92	5.01	18.03

Means followed by the same letter in the column do not differ at 5% probability by the Tukey test.

The application of the biostimulant in the reproductive stage (R5) provided greater plant height (1.37 m) and was statistically superior to the application in the vegetative stage (V3). This effect can be explained by the presence of kinetin in the product, which is part of the group of cytokinins, hormones whose functions include increasing the growth of lateral buds, thus interfering with apical dominance (Taiz et al., 2017). Contrary to these results, Abrantes et al. (2011), studying the application of a biostimulant in two bean cultivars in Cerrado soil, did not find statistical differences in plant height in response to different application stages (V4 and R5) and observed taller plants (49.4 cm) with the application in the vegetative stage than the reproductive stage (47.7 cm).

The doses studied did not differ statistically for PH, as there was no adjustment in the regression analyses (Tables 2 and 3). Similarly, Oliveira et al. (2013), evaluating the interaction between salinity and biostimulant in cowpea, did not observe a significant effect of the biostimulant on plant height in any of the evaluations. There was no difference between the application at different phenological stages for the average number of pods per plant (NPP) (Table 3). There were no adjustments for the doses by the regression analysis (Table 2), but the dose of 0.75 L ha<sup>-1</sup> showed an increase of 23.05% concerning the NPP value of the control.

This increase may be due to the indole-3-butyric acid in the biostimulant, which can delay flower abscission, stimulate flower setting without fertilization, and promote plant growth through cell elongation (Andrei, 2005). These results corroborate those found by Alleoni et al. (2000), who worked with the foliar application of biostimulant on beans and observed an increase in NPP of 4.7% concerning the control, despite not finding the statistical difference between treatments with the control.

For the number of grains per pod (NGP), there was no statistical difference between the applications at different phenological stages (Table 3); however, it is observed that the application in the vegetative stage provided 40.5% more NGP than the application in the reproductive stage. This may be due to a greater rooting of plants in their vegetative phase due to indole-3-butyric acid (auxin) in the biostimulant. More rooted plants can absorb water and nutrients from the soil, which improves the allocation of substances to the preferred drains of plants, such as grains, preventing embryo abortion (Dourado Neto et al., 2014).

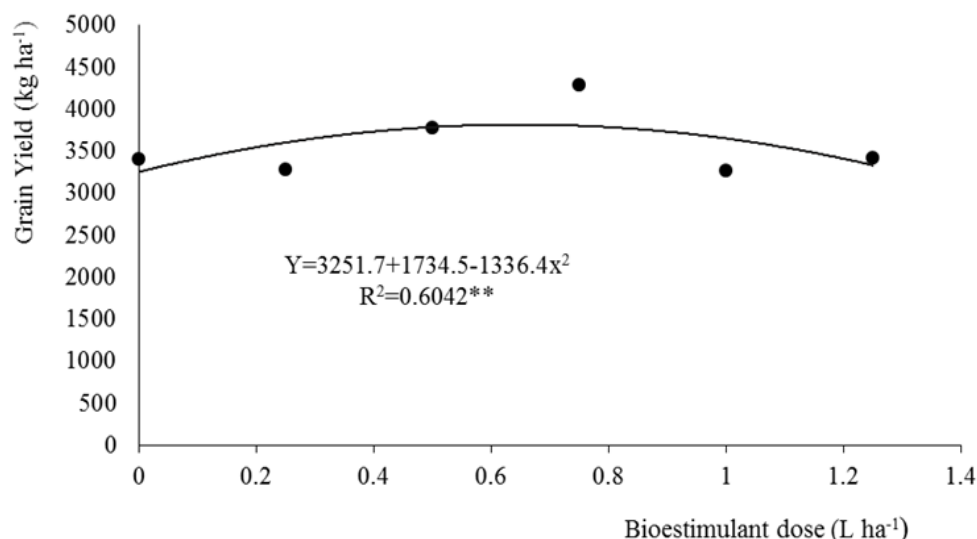
Alleoni et al. (2000) observed an increase in the number of grains per pod (1.7%) concerning the control only in the foliar application of 0.75 L ha<sup>-1</sup> of biostimulant at the beginning of bean flowering

(reproductive stage); however, they also did not observe the statistical difference between treatments concerning the control. Almeida et al. (2014) also did not observe any difference between biostimulant applications in the vegetative and reproductive stages of common beans for the number of grains per pod. Analyzing the effect of the biostimulant doses on the NGP, it is noted that it did not adjust significantly to the linear and quadratic models (Table 2). Abrantes et al. (2011) state that they did not observe the influence of different doses (0; 0.5; 1.0; 1.5; and 2.0 L ha<sup>-1</sup>) and stages (V4 and R5) of application of the biostimulant on the number of grains per pod in beans.

Although the variable 1000-grain weight did not show a statistical difference between the applications at different stages and doses studied (Table 3), the application of the bioregulator at the V3 stage resulted in an increase in the 1000-grain weight of 2.5% concerning the application in R5. Meanwhile, the dose of 0.25 L ha<sup>-1</sup> increased 1.5% over the control for this variable. Dourado Neto et al. (2014) also did not report significant differences in the number of pods and 1000-grain weight when analyzing the efficiency of biostimulants in the common bean crop.

For the grain yield variable (Table 3), the application in the reproductive period (3,705.96 kg ha<sup>-1</sup>) resulted in an increase in yield of 7.9% concerning the application in the vegetative period (3,434.41 kg ha<sup>-1</sup>). This may be related to the fact that applying gibberellin can improve the setting of flowers and the development of fruits of different cultures (Rodrigues and Leite, 2004), which can generate grain yield increases when applied at flowering. Abrantes et al. (2011) reported that the application of biostimulants in beans in the reproductive stage presented grain yield on average 822 kg ha<sup>-1</sup> higher than in the vegetative stage.

The highest grain yield, 3,814 kg, was achieved with a dose of 0.65 L ha<sup>-1</sup> of the biostimulant, representing a gain of 17.3% compared to the control (Figure 1). Similar values for grain yield were found by Perin et al. (2016) in an experiment with beans, which used 500 mL 100<sup>-1</sup> kg of biostimulant seed in the seed treatment and observed grain yield of 4140.2 kg ha<sup>-1</sup>, being statistically higher than the control (2884.79 kg ha<sup>-1</sup>), which represents a 43.5% increase in grain yield. Alleoni et al. (2000) also observed increases in common bean grain yield up to 7.4% concerning the control with the foliar application of 0.75 L ha<sup>-1</sup> of biostimulant; however, they did not find statistical differences between the treatments and the control. Ferreira et al. (2013), evaluating the application of biostimulants in common beans cultivated in the winter season, did not observe any influence of the product on grain yield in the two years of cultivation.



**Figure 1.** Common bean grain yield (kg ha<sup>-1</sup>) according to the biostimulant doses (L ha<sup>-1</sup>).

The results of the present study are similar to those found by Oliveira et al. (2015), who observed that the foliar application of biostimulant at doses of 0.5 to 0.75 L ha<sup>-1</sup> increased the grain yield of cowpea beans. According to Leite et al. (2009), the agronomic efficiency of bioregulators is closely related to the application dose since excessive doses can cause a toxic effect on the plant and reduce the effect of plant hormones.

#### 4. Conclusions

The use of biostimulant at a dose of 0.65 L ha<sup>-1</sup> results in higher grain yield under the conditions in which the research was carried out, regardless of the phenological stage applied.

#### Authors' Contribution

Wytalo de Oliveira Lopes contributed to the experiment setup, evaluations, data collection, tabulation, and manuscript writing. Lorena Correa Varão helped in the experiment setup, data reviewing, and manuscript writing. Wilian Henrique Diniz Buso assisted in the experiment setup, data reviewing, manuscript writing, data analysis, elaboration of graphs, and guided the first author.

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