Bioestimulants on common bean (*Phaseolus vulgaris* L.) cultivar TAA Marhe *in vitro*

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Abstract

Plant biostimulants are substances that can increase plant germination growth, and development, acting on the stimulation of cell division. This study aimed to evaluate the *in vitro* action of two commercial biostimulants AgroRaiz® and Stimulate® on the physiological quality of seeds of *Phaseolus vulgaris* cultivar TAA Marhe. Seeds of the TAA Marhe cultivar were used at the usual concentration of both biostimulants AgroRaiz® 0.3 L 100 kg^{-1} and Stimulate® 0.5 L 100 kg^{-1} seeds. Classic germination test, dry matter, root length and accelerated aging test were performed. The experimental design was completely randomized with three treatments and four replications. The usual doses of biostimulators did not influence the germination rate and dry mass by the classical method; however, Stimulate® demonstrated action on mean root length. For the aging assay, AgroRaiz® proved to be superior to Stimulate® in germination and seedling dry matter.

Keywords: gibberellin, auxin, cytokinins, root development, seed germination, bean.

Resumo

Bioestimulantes vegetais são substâncias que podem incrementar a germinação, crescimento e desenvolvimento vegetal, agindo sobre a estimulação da divisão celular. Esse estudo teve por objetivo avaliar a ação *in vitro* de dois bioestimulantes comerciais AgroRaiz® e Stimulate® sobre a qualidade fisiológica de sementes de *Phaseolus vulgaris* cultivar TAA Marhe. Utilizaram-se sementes do cultivar TAA Marhe na concentração usual de ambos os bioestimulantes AgroRaiz® 0,3 L 100 kg^{-1} e Stimulate® 0,5 L 100 kg^{-1} sementes. Teste de germinação clássico, matéria seca, comprimento de raiz e ensaio de envelhecimento acelerado foram realizados. O delineamento experimental foi inteiramente casualizado com três tratamentos e quatro repetições. As doses usuais dos bioestimuladores não influenciaram sobre a taxa de germinação e massa seca pelo método clássico; entretanto, Stimulate® demonstrou ação sobre o comprimeto médio radicular. Para o ensaio por envelhecimento, AgroRaiz® demonstrou ser superior sobre Stimulate® na germinação e matéria seca de plântula.

Palavras-chave: giberelina, auxina, citocininas, desenvolvimento radicular, germinação de sementes, feijão.

Resumen

Los bioestimulantes vegetales son sustancias que pueden aumentar la germinación, el crecimiento y el desarrollo de las plantas, actuando sobre la estimulación de la división celular. Este estudio tuvo como objetivo evaluar la acción *in vitro* de dos bioestimulantes comerciales AgroRaiz® y Stimulate® sobre la calidad fisiológica de semillas de *Phaseolus vulgaris* cultivar TAA Marhe. Se utilizaron semillas del cultivar TAA Marhe a la concentración habitual de ambos bioestimulantes AgroRaiz® 0.3 L 100 kg^{-1} y Stimulate® 0.5 L 100 kg^{-1} semillas. Se realizaron pruebas clásicas de germinación, materia seca, longitud de raíz y prueba de envejecimiento acelerado. El diseño experimental fue completamente al azar con tres tratamientos y cuatro repeticiones. Las dosis habituales de bioestimuladores no influyeron en la tasa de germinación y masa seca por el método clásico; sin embargo, Stimulate® demostró acción sobre la longitud media de la raíz. Para el ensayo de envejecimiento, AgroRaiz® demostró ser superior a Stimulate® en germinación y materia seca de plántulas.

Palabras clave: giberelina, auxina, citoquininas, desarrollo de raíces, germinación de semilla, frijol.

1. Introduction

Beans (*Phaseolus vulgaris* L.) are one of the main agricultural crops in Brazil, given their economic and social importance, constituting one of the main sources of protein, Fe, Ca, Mg, Zn, B vitamins complex, carbohydrates and fiber in the population's diet (Mesquita et al., 2007; Bossonali et al., 2017; Frasca et al., 2020). Its production stands out throughout the national territory, and in the 2017/18 harvest around 3.18 million hectares were cultivated, with an average production of 3.12 million tons (Conab, 2018).

Brazil is the world's largest producer and consumer of common bean, with approximately 2.67 million tons produced in 2015. Beans constitute a primary source of important nutritional sources in the diet of the Brazilian population, with per capita consumption estimated at 17.8 kg $year^{-1}$. Rainfed systems cover 1.66 million ha^{-1}, corresponding to 90.3% of the Brazilian bean production area (Embrapa Arroz e Feijão, 2017).

In the State of Goiás, Brazil, the focus of this work, the state has the fourth largest producer of common bean, with a cultivated area of 130,260 ha^{-1} in 2015 (Silva et al., 2008; Cabral et al., 2011; Embrapa Arroz e Feijão, 2017), agricultural production is concentrated in the same geographic area, extending, however, over three distinct seasons: rains, with sowing from November 1 to December 31; dry, sown from January 1 to February 28; and autumn winter, from May 1st to June 30th. Although the first two crops have sequential sowing dates, they are characterized by different climatic conditions and, consequently, different productive performance. The harvest in the dry season has lower potential productivity when compared to the rainy season, due to the decrease in accumulated radiation and a higher frequency of minimum temperatures below the optimum during the crop cycle (Heinemann; Stone, 2015).

The difference in productivity between the Autumn-Winter crop and the other two, under rainfed conditions, is due to the occurrence of stresses that limit bean productivity (McClean et al., 2011; Nkhata et al., 2021). Common bean cultivation under rainfed conditions suffers restrictions on productivity, both abiotic and biotic. The most widespread abiotic restrictions are: low soil fertility, water deficit and nitrogen deficiency, due to its limited biological fixation (Devi et al., 2012; Francisco et al., 2016). In addition, several bacterial, fungal and viral diseases reduce the productivity of the bean crop (Singh; Schwartz, 2010; Devi et al., 2012; Souza et al., 2013; Barcelos et al., 2014). The development of stress-resistant cultivars has been a successful strategy to overcome their negative impacts on productivity (Araújo, 2015; Júnior et al., 2019). Among abiotic stresses, water deficit can play an important role in reducing common bean productivity. In the rainy and dry seasons, in the State of Goiás, it is still not clear how this stress varies in space and time. As the majority of common bean production takes place under low-cost agriculture on small-scale farms, this information is critical for developing technologies and knowledge to improve productivity (De Luque; Creamer, 2014).

Due to the growing demand for food, new technologies have been used in production systems, with the aim of improving the agronomic development and productivity of common bean, in addition to minimizing costs and increasing the viability of the crop in regions with crop restrictions (Oliveira et al. al., 2015; Samireddypalle et al., 2019). The use of biostimulants stands out due to the benefits caused to the crop. They are synthetic, natural substances (extracts) and/or microorganisms that, when applied to the leaf surface, seeds and soil, stimulate the absorption and efficiency of nutrients and, consequently, provide increases in productivity (Ferreira et al., 2007; Araújo et al., 2020).

The treatment of seeds with biostimulants, micronutrients and amino acids aims to facilitate emergence, promote hormonal balance, expression of their genetic potential, stimulate the development of the root system and the initial growth of seedlings, in addition to making them less susceptible to stress at this stage. initial stage of culture establishment (Ferreira et al., 2007; Bezerra et al., 2007; Binsfeld et al., 2014).

The biostimulant is designated as a mixture of bioregulators with micronutrients (Castro; Vieira, 2001). Biostimulants have been used in order to increase the growth and production of agricultural crops. These substances, when applied directly to plants, promote changes in physiological processes, such as cell division and elongation. In addition, they have a similar mode of action to plant hormones, although they are synthetic substances and exogenous application (Castro; Vieira, 2001; Tecchio et al., 2006).

Most biostimulants are bioregulators with an effect on auxins, cytokinins and gibberellic acid (Silva et al., 2008). Auxins promote the formation of lateral and adventitious roots, in addition to being involved in membrane permeability and having a characteristic action on cell elongation. Cytokinins regulate cell division and differentiation. And gibberellins are involved in seed germination, both in overcoming quiescence and in controlling the hydrolysis of reserves (Taiz; Zeiger, 2013).

Biostimulants may contain micronutrients, with cobalt (Co), zinc and molybdenum (Mo) being the most common. Co participates in the symbiotic process, since it is a component of vitamins B12, precursors of leghemoglobin, a molecule responsible for the adequate supply of O_2 to N_2-fixing bacteria (Marschner, 2011). Zn helps in the synthesis of substances that act on growth and enzymatic systems, in addition to being essential for activating metabolic reactions and participating in the synthesis of tryptophan and this precursor of indoleacetic acid. Mo plays an indispensable role in the assimilation of nitrate absorbed by plants as a cofactor of the nitrate reductase enzyme and as a cofactor of the nitrogenase involved in N_2 fixation (Silva et al., 2008).

Several studies were carried out with the aim of studying the effect of biostimulants and nutrients applied to seed treatment. However, the results are still contradictory (Ferreira et al., 2007; Turan et al., 2021), since positive and negative effects were observed, depending on the culture studied, the vigor of the seeds, the variables studied and the products and doses used (Ferreira et al., 2007; Bertolin, 2010; Santos et al., 2013; Binsfield, 2014). However, there is a lack of information on the effects of biostimulants on seed germination and initial seedling growth, as well as on the knowledge of the physiological effects involved (Binsfeld et al., 2014).

This study aimed to evaluate the biostimulators AgroRaiz® and Stimulate® on initial parameters of germination and seedlings of *Phaseolus vulgaris* cultivar TAA *in vitro*.

2. Material and Methods

2.1 Experiment location

The test was conducted in the first half of 2021 at the Laboratório de Irrigação e Hidráulica of the Instituto Federal Goiano campus Rio Verde, Goiás State, Brazil, in the Southwest region with the following geographic coordinates (17°45'08" S and 50°55'53" W) and altitude 754 m above sea level.

2.2 Expperimental design

The experiment was set up in a completely randomized block design. Being employed 3 treatments with 7 repetitions each, totaling 21 experimental units. Each experimental unit consisted of 4 rolls of germination paper (28 cm x 38 cm), weight 65 g, neutral pH (Empatia Mundomix, Bioscience, Brazil) containing 50 seeds each. The treatments were composed taking into account the application of biostimulants (AgroRaiz® and Stimulate®) in bean seed treatment and with the absence of both biostimulants.

2.3 Bioestimulants and chemical constitution

The biostimulants used in the experiment were: AgroRaiz® (TOC 10%, N 6.5%, and 4.0%, amino acids L 14%) which was used at a dose of 0.3 L 100 kg^{-1} of seeds and the Stimulate® (kinetin 0.09 g L, gibberellic acid 0.05 g L, 4-indol-3-ylbutyric acid 0.05 g L^{-1}) at a dosage of 0.5 L 100 kg^{-1} seeds.

2.4 Bean cultivar tested

The bean cultivar TAA Marhe, early cycle of 65 to 75 days of high productivity, type 1, semi-erect, light-colored in sandy soils and reddish in soils with little straw, was used.

2.5 Preparation and inoculation

The bean seeds were transferred to transparent plastic packages, and later, the treatment using the biostimulants was added, separately with the aid of a micropipette with a capacity of 1000 µL. A volume of 580 µL was added for the biostimulant AgroRaiz®, and the volume of 960 µL for the biostimulant Stimulate®, separately. Then, the inoculated seed samples were moved until the complete homogenization of the treatments.

2.6 Germination and aging tests

The length of classically germination roots (CG) was carried out between germinating papers, with two leaves for the base and one for the cover, moistened with distilled water, in a proportion of 2.5 times the dry mass of the paper. Then, the leaves were arranged in order to form "rolls", being subsequently wrapped in transparent polyethylene packages and placed in a B.D.O type germinator at a constant temperature of 25 ºC. In each roll, 50 seeds were packed and each repetition consisted of 2 rolls, totaling 100 seeds per repetition. The germination evaluation was carried out at seven and ten days after sowing, and the results were expressed in percentage (%) of normal and abnormal seedlings and dead seeds for control and biostimulants (Brasil, 2009; Silva et al., 2019).

The dry mass of seedlings for classical (DM*) and accelerated aging (DM**) germination and germination for accelerated aging (AAG) were determined by the mass of 50 seedlings. Each seedling was separated and washed in running water. Then, the seedlings were dried in an oven with forced ventilation at 60 °C until reaching the constant mass in grams (g) determined on an analytical balance as determined by Silva et al. (2019).

2.7 Analyzed parameters

Root length (CGRL) was evaluated after counting ten days. The evaluation was carried out on the eighth day, sectioning the root of the normal seedlings, measuring the lengths with the aid of a millimeter ruler. The results were expressed in centimeters (cm) (Beutler; Centurion, 2004).

Seedling dry mass (SDM) and accelerated aging germination (SDMAA), were determined by the mass of 50 seedlings. Each seedling was separated and washed in running water. Then, the seedlings were dried in an oven with forced ventilation at 60 °C until reaching the constant mass in grams (g) determined on an analytical balance as determined by Silva et al. (2019).

2.8 Statistical analysis

The results were submitted to analysis of variance, and when significance was found for a given variab le, the Tukey a test $p < 0.05$ was used to compare the means between treatments. Statistical analysis was performed using the Sisvar 5.6 program (Ferreira, 2019).

3. Results and Discussion

Between treatments without and with biostimulants on the CG and DM*, our findings did not demonstrate a significant difference for the variety of *P. vulgaris* tested. The CGRL showed a significant difference only for the treatment of bean seeds with the biostimulant Stimulate® (Table 1). Regarding the results of AAG, post aging germination showed a significant difference for the control and both biostimulants, with a higher germination rate for the control. Among the biostimulants, promising results were observed for AgroRaiz®, which demonstrated greater aptitude on AAG over the biostimulant Stimulate. When verifying the DM** content, a pattern can be observed on the results based on the AAG rate, where the control followed by the biostimulant AgroRaiz® presented greater mass (Table 1).

Comparative studies on the bean culture show a number of diversified results between the various types and chemical composition of biostimulants and cultivars. Ramos et al. (2015) using the biostimulant Stimulate® by seed hydration method 0.75 L 100 kg^{-1} of seeds, where they found germination rates between 82-95% higher for the bean cultivars BRS Horizonte, Pérola and BRS Pontal than ours found to cultivate TAA Marhe. Although the three cultivars showed statistical difference between the treatments, in particular the control and those treated with biostimulant, with mean germination ranging from 83-98% for classic germination and between 59-92% for germination due to aging, also higher than our results. As for the dry biomass content, BRS Horizonte presented a higher mass with 23.44 mg per seedling, statistical difference was not observed for the cultivars Pérola and BRS Pontal. For root length, the BRS Horizonte cultivar showed a superior result with 6.47 cm when compared to the Pérola and BRAS Pontal cultivars, 5.22 and 5.62 cm, respectively. Our results prove to be superior to the study by Ramos and collaborators using Stimulate®, since the cultivar used differs from those used by the researchers.

Other studies, such as the study by Abrantes et al. (2011), the researchers evaluated the biostimulant Stimulate® where they observed that the dose of 2 L ha^{-1} through the leaves promoted an increase in the vigor of winter bean seeds. Silva et al. (2008) evaluated nine *P. vulgaris* cultivars in the State of Goiás, and observed that the best germination rates were for the Xamedo and BRS Radiante cultivars above 80% recommended value. Castro et al. (2005) obtained a significant increase in the dry biomass of common bean roots using a growth regulator up to a maximum concentration of 10 mL kg^{-1} of seed. Silva (2001) working with Stimulate on bean seeds at doses ranging from 1 to 5 mL in 0.5 kg of bean $seeds^{-1}$, obtained satisfactory results on the germination rate, dry biomass of the roots and number of normal seedlings and reduction of abnormal seedlings.

Table 1. Average results of classical germination (CG), accelerated aging germination (AAG), classical germination root length (CGRL), and dry matter (classical DM*) and accelerated germination (DM**) of bean cultivar TAA Marhe seeds treated with bioestimulants AgroRaiz® e Stimulate®.

Treatments	Germitest			Accelerated Aging	
	CG	DM*	CGRL	AAG	DM**
Control	41,14^{ns}	59,57^{ns}	9,22 b	41,71 a	69,92 a
AgroRaiz®	41,57^{ns}	62,11^{ns}	9,63 ab	27,28 b	65,88 b
Stimulate®	44,42^{ns}	62,66^{ns}	10,82 a	9,00 c	61,56 c
CV (%)	7,38	8,76	10,40	19,59	3,48

Note: *Dry matter classical germination. **Dry matter germination by accelerated aging. (ns): not significant. CV: coefficient of variation. Results with statistical difference were evaluated by Tukey's test at $p < 0.05$ for comparison between treatment means. Source: Authors, 2021.

4. Conclusions

The use of AgroRaiz® and Stimulate® biostimulants did not influence the germination rate and dry mass of seeds germinated by the classic method when compared to the control. The germination test and dry mass by accelerated aging, AgroRaiz® showed greater aptitude when compared to Stimule®. The biostimulant Stimulate® showed greater root length *in natura* by classical germination.

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