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Bacillus subtilis Bs10 as an efficient inoculant for growth promotion in soybean plants

Bacillus subtilis Bs10 como um inoculante eficiente para a promoção do crescimento de plantas de soja

Aloisio Freitas Chagas Junior[1]*; Gaspar Moreira Braga Junior[2]; Albert Lennon Lima Martins[2]; Lillian França Borges Chagas[1]; Luciane de Oliveira Miller[3]; Andrea Carla Caldas Bezerra[3]

Highlights

Bacillus subtilis can promote plant growth.
B. subtilis Bs10 was tested for soybean development and nutrition.
The *B. subtilis* Bs10 increased biomass, nodulation, and nitrogen content in soybean.
The doses of *B. subtilis* Bs10 between 200 and 350 mL influenced soybean plant growth.

Abstract

Currently, food with low levels of pesticides, sustainable agricultural practices and increased crop productivity have gained prominence worldwide. Rhizobacterial inoculants are an alternative to manage large crops that favors sustainable plant growth. Thereby, this study aimed to assess the effect of increasing doses of inoculant based on the rhizobacterium *Bacillus subtilis* Bs10 compared to the commercial product based on *B. subtilis* on the development of soybean culture. Two experiments were carried out with two soybean cultivars, M8210 IPRO and M8615 IPRO, assessed at the vegetative and reproductive stages. Doses of *B. subtilis* Bs10 with 0, 100, 200, 300, 400 mL per 50 kg seeds were inoculated in each experiment, as well as an additional treatment with commercial product based on *B. subtilis.* Biomass, nodulation, plant height, internodes, number of pods and number of grains of soybean cultivars were assessed. The inoculation of *B. subtilis* Bs10 significantly ($p < 0.05$) improved the agronomic characteristics of both soybean cultivars with an increase in shoot and root biomass, nodulation, phosphorus content, and accumulation of nitrogen in the aerial part. The doses of *B. subtilis* Bs10 between 200 and 350 mL had significant prominence in increasing the variables studied herein.

Key words: Bioinoculant. Biomass. *Glycine max* (L.) Merr.

[1] Associate Profs. Drs., Universidade Federal do Tocantins, UFT, Gurupi, TO, Brazil. E-mail: chagasjraf@uft.edu.br; lillianfbc@uft.edu.br
[2] Drs. in Vegetable Production, Universidade Federal do Tocantins, PPGPV/UFT, Gurupi, TO, Brazil. E-mail: gasparjr_braga@mail.uft.edu.br; eng.albertlennon@gmail.com
[3] Researcher, JCO Fertilizers, Barreiras, BA, Brazil. E-mail: lucianeom@jcofertilizantes.com.br; andrea@jcofertilizantes.com.br
* Author for correspondence

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Resumo

Atualmente, alimentos com baixo teor de agrotóxicos, práticas agrícolas sustentáveis e aumento da produtividade das lavouras têm ganhado destaque no mundo. Inoculantes de rizobactérias são uma alternativa no manejo de grandes lavouras, o que favorece o crescimento sustentável das plantas. Assim, este estudo teve como objetivo avaliar o efeito de doses crescentes do inoculante à base de Rhizobacteria *Bacillus subtilis* Bs10 em comparação ao produto comercial à base de *B. subtilis* no desenvolvimento da cultura da soja. Dois experimentos foram conduzidos com as cultivares de soja M8210 IPRO e M8615 IPRO e avaliados nas fases vegetativa e reprodutiva. Doses de *B. subtilis* Bs10 com 0, 100, 200, 300, 400 mL por 50 kg de sementes foram inoculadas em cada experimento, bem como tratamento adicional com produto comercial à base de *B. subtilis.* Foram avaliadas biomassa, nodulação, altura de planta, entrenós, número de vagens e número de grãos de cultivares de soja. A inoculação de *B. subtilis* Bs10 melhorou significativamente ($p < 0,05$) as características agronômicas de ambas as cultivares de soja com aumento na biomassa da parte aérea e radicular, nodulação, teor de fósforo e acúmulo de nitrogênio na parte aérea. As doses de *B. subtilis* Bs10 entre 200 e 350 mL destacaram-se significativamente no aumento das variáveis estudadas neste trabalho.

Palavras-chave: Bioinoculante. Biomassa. *Glycine max* (L.) Merr.

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important crops in the world economy. Increase in the productive capacity of soybeans is directly linked to scientific advances and the technologies available to the productive sector (Kamali et al., 2017). However, a larger world demand for food with less pesticides and the environmental concern with more sustainable practices have led to the search for alternatives that contribute to a greater crop productivity.

Beneficial microorganisms can represent an environmentally sustainable option to increase crop productivity and reduce disease incidence, including the plant growth-promoting rhizobacteria (PGPR) group. PGPR are soil bacteria and can promote plant growth as inoculants and control phytopathogens (Kundan et al., 2015; Gagné-Bourque et al., 2015). PGPR can act either directly or indirectly in plants and are linked to the production of antibiotics, siderophores, and hormones, induction of systemic resistance, production of, asymptomatic nitrogen fixation, and phosphate solubilization (Zeilinger et al., 2016; Saravanakumar et al., 2016; Braga et al., 2017).

Moreover, producing low-cost inoculants with PGPR, such as *Bacillus subtilis,* is a technology applied to reduce environmental risks caused by the inappropriate and often excessive use of agrochemicals and pesticides. Such a natural, renewable, and sustainable biotechnological alternative is aimed at enhancing agricultural production, generate more competitive and differentiated products, and reducing costs for agricultural producers. Hence, several studies have assessed the ability of *Bacillus subtilis* species of biocontrol of plant diseases and enhance of plant growth and crop productivity (Ishak et al., 2016; Z. Ahmad et al., 2017; Braga et al., 2018; Diaz et al., 2019).

Therefore, this study aims to assess the effect of doses of the *Bacillus subtilis* Bs10 isolate as an inoculant on promoting plant growth for two soybean cultivars in a greenhouse. Soil physicochemical analyses and biomass performance at different growth stages were assessed, as well as their nutritional status (nitrogen and phosphorus).

Material and Methods

Two tests were carried out in a greenhouse installed at the Experimental Station of the Federal University of Tocantins (UFT), Campus of Gurupi, located in the southern region of the state of Tocantins, Brazil. The geographical coordinates of the experimental station are 11º43'45'' S and 49º04'07'' W with an average altitude of 280 meters and a humid tropical climate classified as small water deficiency (B1wA'a') with savanna vegetation or tropical savanna, according to Köppen-Geiger (Peel et al., 2007).

The strain of *Bacillus subtilis* Bs10 was selected based on its potential as plant growth promoter and had been previously isolated from savanna soils in cultivation areas in the state of Tocantins. The strain was maintained and grown in LB (Luria-Bertani) medium.

The isolate was preliminary identified considering the morphological, structural, and biochemical characteristics, according to the Bergey's Manual of Systematic Bacteriology (James et al., 2005). Subsequently, Helixxa Serviços Genômicos performed the molecular characterization of the 16S rRNA region through Sanger sequencing. Genus and bacterial species were identified by comparing the consensus sequence obtained against the database of the National Center for Biotechnology Information (NCBI) with the BLAST tool (Morgulis et al., 2008). Such a comparison with GenBank database showed 99% identity with coverage (Query) of 99% of the 1429 bp of the consensus sequence generated against the region of the 16S rRNA gene for *Bacillus subtilis.* The *Bacillus subtilis* Bs10 sequence was deposited on the GenBank database as SUB6806180.

The main characteristics of the rhizobacteria isolate *Bacillus subtilis* Bs10 are described on Table 1. The microorganisms used are deposited and preserved in the collection of CBMAI (Unicamp).

Table 1
Code, geographical origin, color, indoleacetic acid production, phosphate solubilization, and taxonomic classification of the isolated rhizobacteria Bs10 used in this study

Isolated/Code[a]	Origin[b]	Color[c]	AIA[d]	Solub.[e]	Taxonimic
Bs10/CBMAI2947	TO/Brazil	White	+	+	*B. subtilis*

[a] Numeric code of rhizobacteria isolate; [b] Geographic source of isolate; [c] Colony color; [d, e] Producer of indolacetic acid and phosphate solubilization, the methodology is described in Braga (2019).

Two experiments were carried out under greenhouse conditions to assess the effect and influence of *B. subtilis* UFT-Bs10 as an inoculant on soybean growth promotion. Table 2 shows the physicochemical characteristics of the soil, classified as medium texture Yellow Sieved Latosol (sieved) (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2011). The soil was used at a depth of 0-20 cm obtained from the UFT experimental station area. Likewise, both experiments were fertilized as recommended for cultivation and soil analysis, using the formulated 5-25-15 at the proportion of 400 Kg per hectare.

The experiments of soybean seeds inoculations were conducted based on a completely randomized design (CRD) with four replications for each evaluation period. The soybean seeds were initially inoculated with rhizobia (*Bradyrhizobium japonicum* SEMIA 5079 and SEMIA 5080) at a concentration of $3x10^9$ CFU mL^{-1} to promote a good nodulation of plant roots, which guarantees nitrogen supply to the culture. Thereafter, six treatments consisting of five doses of the inoculant liquid formulation based on *Bacillus subtilis* Bs10 were developed as follows: 100, 200, 300, and 400 mL per 50 kg of seeds at a minimum concentration of $1x10^8$ CFU mL^{-1}, an absolute control (without inoculation) and a positive control (commercial product) based on *Bacillus subtilis* UFPEDA 764 at the dose recommended by the manufacturer at a concentration of $3x10^9$ CFU mL^{-1}.

The doses of the inoculant *B. subtilis* Bs10 and the commercial product were applied directly to the seeds one hour before planting. Consequently, pots with a capacity of 3.8 L were filled with soil and sown with 10 seeds per pot. After germination, thinning was performed, leaving two plants per pot, which was considered as the experimental unit. Irrigation was performed manually by supplying the plants with water until reaching the field soil capacity.

For biomass productions and plant growth analysis, three assessments were conducted in both experiments, as follows: (1st) vegetative phenological stage of the culture (V3-V5), (2nd) reproductive stage of the culture (R2-R3), (3rd) end of the culture cycle (R8). The first and second assessments were performed at 35 and 64 days after emergence (DAE) for experiment 1 and at 30 and 52 days after plant emergence for experiment 2. The collected material was taken to the Microbiology Laboratory followed by separating the root system from the aerial part of the plants and roots. The parts of the plants were washed under tap water to remove the adhered soil, and the nodules were carefully removed and counted to obtain the number of nodules (NN). Then, the plant material was dried in an oven with forced aeration at 65 °C for 72 hours until reaching constant mass.

Table 2
Physicochemical characteristics of soils used in greenhouse experiments and soybean phenotypic characteristics

Soil chemical characteristics	Experiment 1	Experiment 2
Calcium + Magnesium (Ca+Mg)	1.8 cmol dm^{-3}	2.5 cmol dm^{-3}
Calcium (Ca)	2.2 cmol dm^{-3}	1.7 cmol dm^{-3}
Magnesium (Mg)	1.1 cmol dm^{-3}	0.6 cmol dm^{-3}
Aluminum (Al)	0.0 cmol dm^{-3}	0.0 cmol dm^{-3}
Hydrogen + Aluminum (H+Al)	1.5 cmol dm^{-3}	2.5 cmol dm^{-3}
Potassium (K)	19.0 mg dm^{-3}	83.54 mg dm^{-3}
Phosphorus (P)	5.7 mg dm^{-3}	4.7 mg dm^{-3}
Cation Exchange Capacity (CEC)	7.51 cmol dm^{-3}	8.31 cmol dm^{-3}
Total Exchangeable Bases (TEB)	3.3 cmol dm^{-3}	2.4 cmol dm^{-3}
Base saturation (BS)	69%	39.4%
Organic matter	29.5%	28.1%
pH H_2O	5.8	5.4
Soil texture characteristics	**Experiment 1**	**Experiment 2**
Sand	70.1%	72.3%
Silt	7.2%	8.2%
Clay	22.7%	19.5%
Soybean phenotypic characteristics	**Experiment 1**	**Experiment 2**
Soybean cultivar	M8210 IPRO	M8615 IPRO
Growth type	Semi-erect and determined	Semi-erect and determined
Flower color	White	White
Average plant height	72 cm	85 cm
Relative Maturation	8.2	5.8
Average cycle	120 days	117 days

The dry material was weighed on a precision analytical balance to obtain shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), and nodule dry mass (NDM). The aerial part biomass from the second assessment was used to assess the nutritional status of the plants, with the levels of nitrogen and total phosphorus in the aerial part determined through the Kjeldahl and colorimetry methods, respectively. Then, the nitrogen accumulation in the aerial part (ANAP) was calculated as ANPA (g pot^{-1}) = SDM x total nitrogen content. Subsequently, plant height (PH), number of internodes (NI), number of pods (NP) and number of grains per plant (NG) were determined in a third assessment.

The data were subjected to analysis of variance using F test. A regression analysis assessed the effect of the different growth promoter doses . A Duncan test was applied to group the means of relative efficiency (RE) of the treatments at 5% significance. All analyses were performed on the software Sisvar, version 5.6. The doses of maximum technical efficiency were obtained by deriving and equating to zero the quadratic functions of the characteristics with significant effects for the quantitative variables. All charts were plotted using the SigmaPlot application.

Results and Discussion

The inoculation by *B. subtilis* Bs10 in increasing doses Experiment 1 for plant growth and production of soybean biomass M8210 IPRO indicated a positive effect of inoculation with *B. subtilis* Bs10 at increasing doses on larger shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), number of nodules (NN), and nodule dry mass (NDM) at 35 and 64 days after emergence (DAE) compared to the commercial product (Figure 1). The 400 mL dose of *B. subtilis* Bs10 provided a significant linear response to SDM with an average value of 1.98 g at 35 DAE, as well as 106, 53% higher than the absolute control (without inoculation – 0 mL dose) and the positive control (commercial product), respectively. In addition, SDM showed a quadratic behavior for the doses tested at 64 DAE, with the regression model suggesting a dose of 246 mL per 50 kg seeds with greater technical efficiency and an estimated SDM value of 9.01 g, as well as increases by 113% and 58.6% in relation to the treatment without inoculation and to the commercial product, respectively.

Moreover, the higher doses of the inoculant *B. subtilis* Bs10 exerted no significant difference on the RDM at 35 DAE (Figure 1). However, the RDM at 64 DAE had a quadratic behavior at the reproductive stage, suggesting a dose of 272 mL of the inoculant per 50 kg seeds with an estimated value of 3.69 g and an increase by 20% in relation to the treatment without inoculation. In addition, the tested doses of *B. subtilis* Bs10 showed no higher RDM than the commercial product. Otherwise, TDM showed a linear response in the soybean vegetative stage (35 DAE) in relation to the applied doses of *B. subtilis* Bs10, however, no significant difference was observed. Additionally, the soybean plants showed a positive quadratic response at 64 DAE for the TDM variable as function of the effects of increasing doses of *B. subtilis* Bs10. The dose with the greatest response suggested by the regression model was 247 mL per 50 kg seeds with an estimated TDM of 12.67 g, increasing from 74.2% and 31.2% compared to the treatment without inoculant (absolute control) and the commercial product (positive control), respectively.

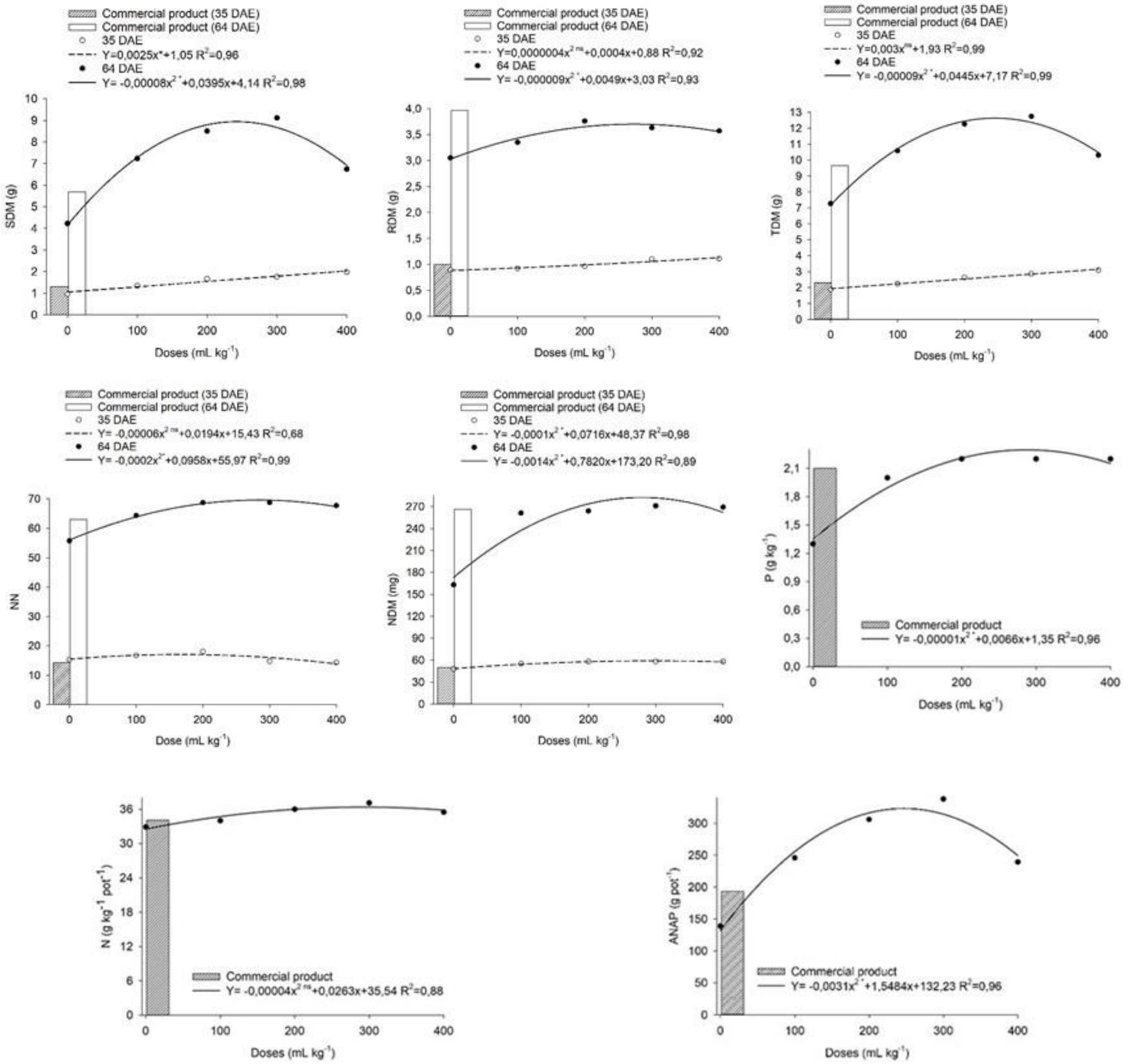

Figure 1. Shoot dry mass (SDM), root dry mass, (RDM), total dry mass (TDM), number of nodules (NN), nodule dry mass (NDM), phosphorus content (P), nitrogen content (N), and nitrogen accumulation in the aerial part (ANAP) in soybean cv. M8210 IPRO inoculated by increasing doses of *Bacillus subtilis* Bs10 and commercial product based on *B. subtilis.*

Furthermore, the variable number of nodules (NN) showed positive quadratic behavior by increasing the inoculant doses at 35 and 64 DAE (Figure 1). The dose of 162 mL per 50 kg seeds provided a higher NN at 35 DAE, however, no significant difference was observed. In contrast, the dose of 239 mL per 50 kg seeds suggested by the regression model produced a greater NN at 64 DAE with an estimated value of 67.44 nodules in two plants, and an increase by 19.28% and 5.4% compared to the treatment without inoculation and the commercial product, respectively. Likewise, the NDM had a positive quadratic response from inoculating the doses of *B. subtilis* UFT-Bs10, with the most responsive doses with the greatest increase in nodule biomass according to the mathematical model as 358 and 279 mL per 50 kg seeds at 35 DAE and 64 DAE, respectively. These average NDM values were higher than the commercial product.

A quadratic response of the phosphorus (P) content in the aerial part of the soybean plants cv. M 8210 IPRO occurred from increasing doses of *B. subtilis* Bs10, with the highest P content found at the 330 mL dose, causing higher P content of 86.9 and 15.71% than at the dose 0 mL and the commercial product, respectively (Figure 1). Higher doses would generate lower P content in the aerial part of the soybean plants. Moreover, the nitrogen (N) content in the aerial part of the plants showed a quadratic behavior, and no significant difference was observed for the tested inoculant doses. Consequently, the nitrogen accumulation in the aerial part (ANAP), as well as the P and N contents, showed a positive quadratic behavior, with the highest ANAP value found at the dose of 249 mL with an estimated average value of 325.58 g per pot, provides an increase by 134.39 and 68.08% compared to the treatment without inoculation and the commercial product, respectively (Figure 1).

As illustrated in Figure 2, the assessment in the end of the crop cycle (stage R8) indicated that the plant height (PH) variable had a positive quadratic response in relation to the doses of *B. subtilis* Bs10, thus generating a larger soybean size. Thus, the dose of 315 mL obtained the highest response and an estimated height of 58.70 cm, corresponding to an increase of 11.8 and 5.7% compared to the treatment without inoculation and the commercial product, respectively. In addition, the internodes showed positive quadratic behavior and there was no significant difference between the doses tested, although all doses tested showed higher mean internode values than the commercial product.

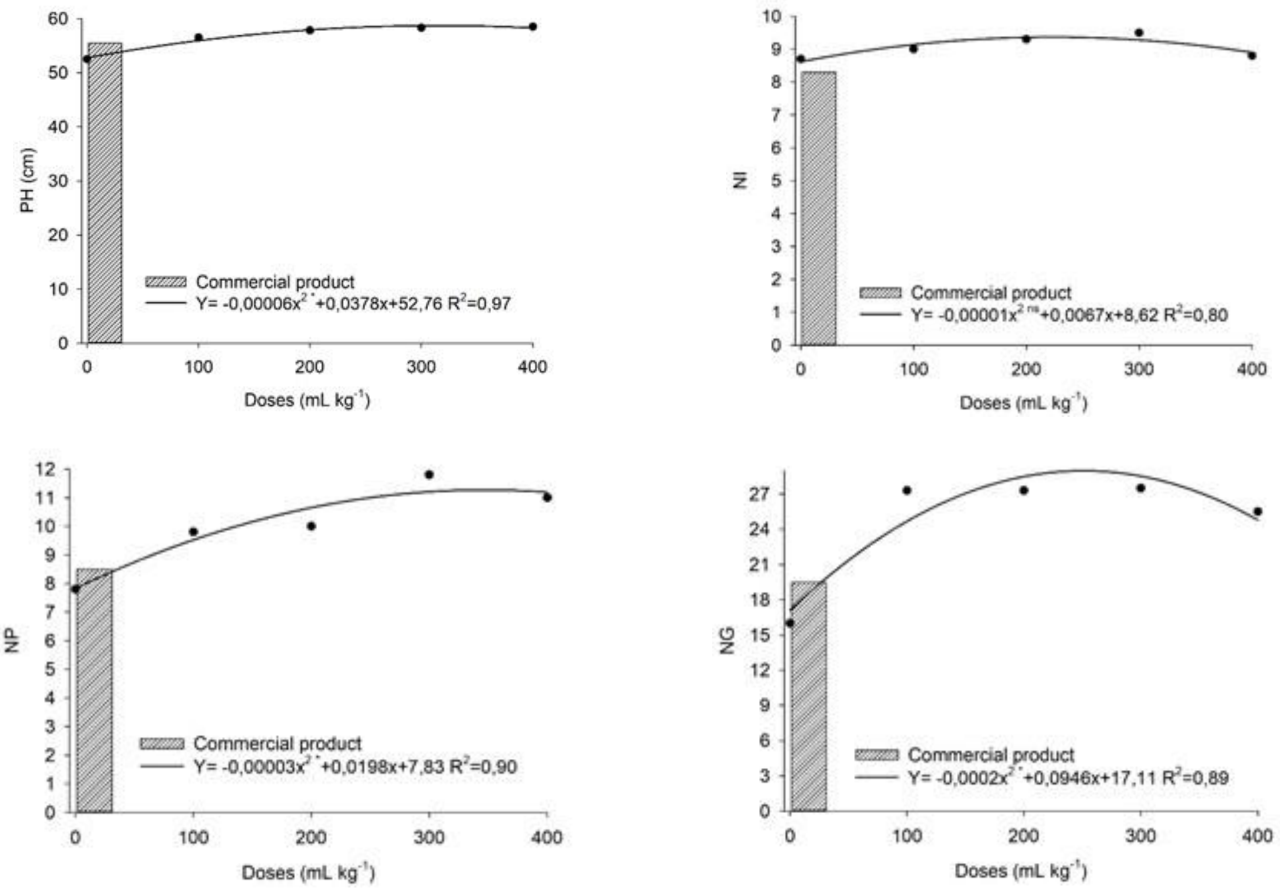

Figure 2. Plant height (PH), number of internodes (NI), number of pods (NP) and number of grains per pod (NG) in soybean plants M8210 IPRO at stage R8 inoculated with increasing doses of *Bacillus subtilis* Bs10 and commercial product based on *B. subtilis.*

Likewise, the variable number of pods (NP) showed a positive quadratic behavior at increasing doses of *B. subtilis* Bs10, with the dose with best response being predicted by the model at 330 mL, providing an increase by 42.17 and 30.47% compared to the treatment without inoculation and the commercial product, respectively. Consequently, the number of grains (NG) showed quadratic responses, with the largest NG being produced at a dose of 236.5 mL with an increase by 76.81 and 45% compared to the treatment without inoculation and the commercial product, respectively (Figure 2).

Experiment 2 indicated that the biomass and nodulation characteristics at 30 and 52 DAE provided a greater increase in the variables assessed of soybean plants M8615 IPRO with the inoculation of the doses of *B. subtilis* Bs10 for plant growth and production of soybean biomass M8210 IPRO (Figure 3). The SDM variable at both 30 and 52 DAE showed a positive quadratic response at increasing doses of *B. subtilis* Bs10, with 330 and 271 mL at 30 and 52 DAE, respectively, as the doses that provided the largest shoot biomass. Higher doses were not efficient in obtaining the highest SDM value, which led biomass to decrease. The most efficient doses at 30 and 52 DAE had an estimated SDM value of 1.98 g and 5.57 g, respectively, corresponding to SDM increases of 122 and 45.58% at 30 DAE and 74 and 61, 44% at 52 DAE compared to the treatment without inoculation and the commercial product, respectively.

Likewise, the RDM showed quadratic behavior in relation to the doses inoculated by *B. subtilis* Bs10 at 30 and 52 DAE. The assessments showed that the doses of 280 and 223 mL at 30 and 52 DAE promoted the greatest increase in RDM, with an estimated average value of 0.88 and 3.05 g in two plants, respectively. This resulted in an increase in the RDM of 79.59 and 60% at 30 DAE, and 125 and 83.73% at 52 DAE compared to the treatment without inoculation and the commercial product, respectively (Figure 3). Moreover, the TDM variable also had a significant quadratic response to the increasing doses of *B. subtilis* Bs10 at 30 and 52 DAE (Figure 3). The highest averages were found at the doses of 230 and 235 mL at 30 and 52 DAE, respectively, with average values higher than those of the commercial product. All tested doses promoted higher mean values for SDM, RDM, and TDM for both assessment compared to the treatment without inoculation and the commercial product.

In contrast, the number of nodules (NN) had a quadratic response in relation to the doses tested at 30 and 52 DAE without significant difference (Figure 3). The MSN variable showed a linear response at 30 and 52 DAE, showing that the nodules increased their mass with the increase in *B. subtilis* Bs10 doses. Thus, we also found a greater efficiency in increasing the size of the nodules by inoculating the doses of *B. subtilis* Bs10 compared to the commercial product.

In turn, phosphorus content (P), nitrogen content (N) and nitrogen accumulation in the aerial part (ANAP) showed positive quadratic responses for the variables (Figure 3). The N content had no significant difference, however, the inoculations of the *B. subtilis* Bs10 doses provided a higher N content, with the highest value found at the 262 mL dose. Conversely, the P and ANAP contents had the highest averages at the doses of 210 and 294 mL, with an average value of 4.02 g per kg and 244.46 g per pot, respectively. Consequently, these values provided a content of P and ANAP 29.67 and 41.46% higher than that of the commercial product, respectively.

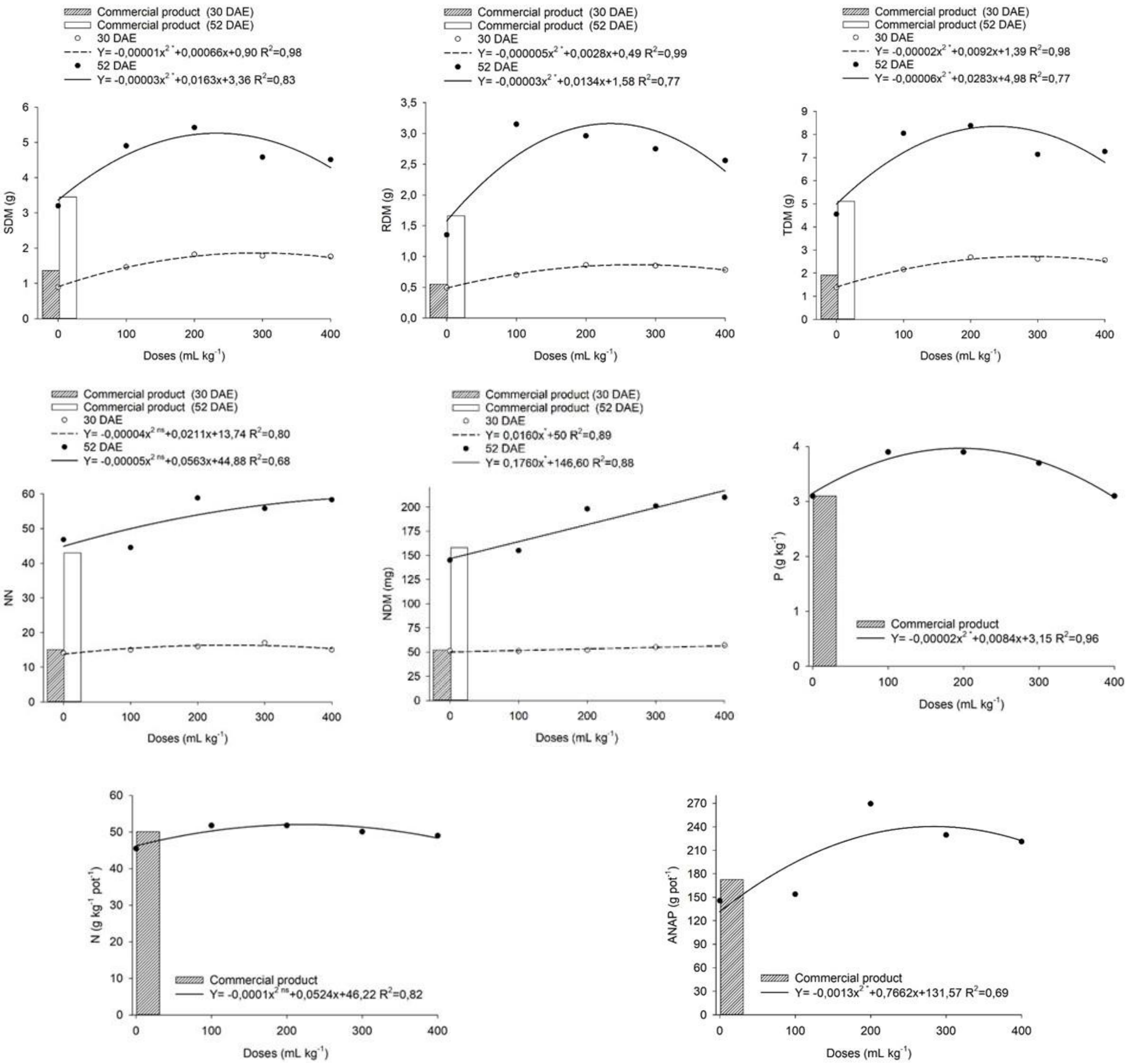

Figure 3. Shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), number of nodules (NN), nodule dry mass (NDM), phosphorus content (P), nitrogen content (N), and nitrogen accumulation in the aerial part (ANAP) of soybean M8615 IPRO at 30 and 52 days after emergence (DAE) inoculated by *Bacillus subtilis* Bs10.

The agronomic characteristics assessed at stage R8 showed that the plant height (PH) and the number of pods (NP) had no significant difference in relation to the doses of *B. subtilis* Bs10 (Figure 4). The number of internodes showed a positive quadratic response due to the increasing doses of *B. subtilis* Bs10, with 271 mL as the dose with the best response to the number of internodes, with an average value of 10.8 internodes, 30.56% higher than the commercial product.

The lowest mean values for the variable were found in the commercial product treatment and in the treatment without inoculation. Thus, the NG variable had a quadratic response, increasing the NG with the inoculation of *B. subtilis* Bs10 doses. The dose of 235 mL presented the maximum point for variable NG, with an estimated value of 48.09 pods, 21.74 and 33.58% higher than the commercial product and the treatment without inoculation, respectively (Figure 4).

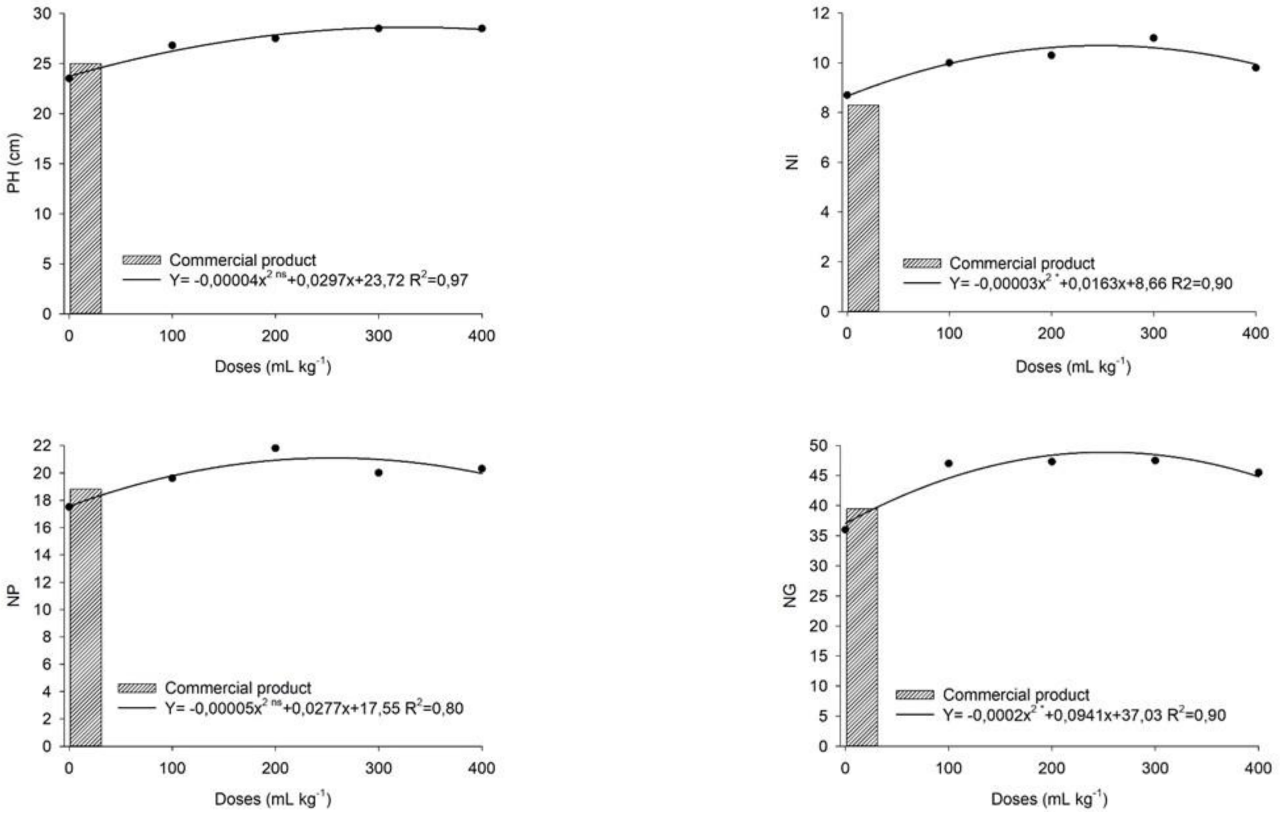

Figure 4. Plant height (PH), number of internodes (NI), number of pods (NP), and number of grains per pod (NG) in soybean plants M8615 IPRO at stage R8 inoculated with increasing doses of *Bacillus subtilis* Bs10 and commercial product based on *B. subtilis.*

Inoculation with *B. subtilis* Bs10 proved able to provide a greater increase in biomass and nodulation based on the results in cv. M8210 IPRO and cv. M8615 IPRO (Figures 1 and 3), as well as greater plant productive potential. Such an increase is possibly linked to the bacterial action mechanisms to promote plant growth, including improving plant nutrition, production, and regulation of phytohormones, in addition to suppression of disease-causing organisms. Thus, such mechanisms may have acted either directly or indirectly using one or more mechanisms (Kundan et al., 2015; Olanrewaju et al., 2017).

Mazzuchelli et al. (2014) inoculated *B. subtilis* in corn seeds and obtained a 14% increase in shoot biomass. Raasch et al. (2013) found that treatments with inoculation of *B. subtilis* in substrate and mini cuttings obtained an increase by 39.7 to 45.1% in SDM in the production of eucalyptus seedlings. These results corroborate those found herein in relation to the positive effect of *B. subtilis* Bs10 inoculation on the higher aerial part biomass with percentage increases in SDM greater than those found by those authors (Figures 1 and 3).

Such an increase in SDM and RDM promoted by the inoculation of *B. subtilis* Bs10 may have occurred due to the capacity of this rhizobacterium to produce plant hormones. Plant hormones or plant growth regulators can be produced by *B. subtilis* such as indoleacetic acid (IAA), gibberellins, and cytokinins (Cerqueira et al., 2015; Kumari et al., 2018). The main effect of such hormones is acting on cell elongation, cell division in tissue culture, in rooting, enhancing the formation of the root system and roots, thus possibly resulting in greater shoot and root biomass (Taiz et al., 2017).

Ishak et al. (2016) found two isolates of *B. subtilis* to be able to produce vegetable hormones of the auxin group, gibberellins, and cytokines, such as 1-naphthalene acetic acid, tryptamine, 3-indole propionic acid, indole-3- acid butyric, indoleacetic acid, gibberellic acid, and trans-zeatin. Z. Ahmad et al. (2017) verified the ability of *B. subtilis* strain 330-2 to produce indole-3-acetic acid, siderophores, lytic enzymes, and solubilized different sources of organic/inorganic phosphates and zinc, in addition to increasing RDM and SDM by 36.79 and 12.21% in rice and 41.66 and 29.15% in corn, respectively, at 21 and 40 DAP. Saharan and Nehra (2011) observed that *Bacillus* species contributed to improving different root parameters, such as rooting, root length, and dry matter content, while inoculation with isolates producing IAA enhanced the absorption of some nutrients, thus promoting the growth of sweet potatoes and greater rooting of eucalyptus seedlings. Such results corroborate those found herein. Such an increase in the RDM caused by the inoculation of *B. subtilis* provides a greater exploitation of the soil by the roots and can guarantee a greater supply of water and nutrients to the plant, thus generating greater increase in the SDM.

Moreover, the availability and solubilization of nutrients such as phosphorus and nitrogen is another mechanism by which the *B. subtilis* Bs10 strain may have acted in increasing biomass. M. Ahmad et al. (2018) observed the ability of isolate Q3, identified as *B. subtilis,* to solubilize phosphate, while Satapute et al. (2012) analyzed the capacity of the *B. subtilis* AS-4 isolate as a nitrogen-fixing bacteria (NFB), which can be exploited as an inoculant in the soil. Such a greater increase in the root and availability of P and N provided by the inoculation of *B. subtilis* may result in greater absorption of nutrients and larger production of nodules for the plant. Thus, the larger the soybean roots, the greater the number of sites for infection and nodule formation will be available, thus resulting in a greater number of nodules. This may explain the results found herein, where inoculation with the doses of *B. subtilis* Bs10 provided higher NN, NDM, P, and ANAP contents in both the tested soybean cultivars (Figures 1, and 3).

Araújo et al. (2010) conducted an experiment with cowpea beans and found that the co-inoculation of *B. subtilis* and *Bradyrhizobium* caused an increase in cowpea nodulation, which enhanced the NN and NDM possibly due to the influence of *B. subtilis* on promoting nodulation by the *Bradyrhizobium* inoculated. This co-inoculation resulted in a greater NDM, similarly to the results that obtained herein, where the inoculation of the doses of *B. subtilis* Bs10 co-inoculated with *Bradyrhizobium japonicum* favored the nodulation of the soybean cultivars, not causing a negative effect compared to the inoculation of *Bradyrhizobium* only. Such an influence may be related to the contribution to increasing the competitiveness of the inoculated bacteria, the larger number of infection sites, and the inhibitory growth action of pathogenic fungi in the roots, in addition to the *B. subtilis* isolate being non-toxic to *Bradyrhizobium.* Lima et al. (2011) observed a significant increase in ANPA in corn plants inoculated in the seed by *B. subtilis.*

Results by Diaz et al. (2019) for *Bacillus* spp. as plant growth-promoting bacteria in cotton under greenhouse conditions confirm that *B. subtilis* isolates 248, 290, and 263 may represent a good alternative as plant growth-promoting endophytes to cotton crops, as they positively affected several of the parameters assessed, such as root and shoot dry matter and phosphorus content in the soil.

Ratz et al. (2017) found a higher P content in the aerial part of corn plants inoculated with *B. subtilis* isolates. These results are in line with those found herein, where inoculations of *B. subtilis* Bs10 doses provided a higher content of P and ANAP in both soybean cultivars studied (Figures 1 and 3). Such an availability and solubilization provided by *B. subtilis* are given through the production of enzymatic phosphatase complexes, mainly acid phosphatases, action of low molecular weight organic acids, by acting on the solubilization of P and through the activity of the enzyme nitrogenase for N, transforming P and N into forms available to plants (Olanrewaju et al., 2017).

Furthermore, such a greater increase in biomass and nodulation provided by the inoculation with *B. subtilis* Bs10 resulted in a higher yield in the culture by the end of the cycle, with a significant increase in PH, NP, and NG for cv. M8210 IPRO (Figure 2), and internodes and NG for cv. M8615 IPRO (Figure 4).

The agronomic characteristics of plant height, architecture, and pod are attributes defined by genetic expressions that may or may not be influenced by some factors, such as microorganisms established in the soil. This may explain the absence of significant difference in the inoculated doses of *B. subtilis* Bs10 for the number of internodes in the cv. M8210 IPRO (Figure 2), and for PH and NP in cv. M8615 IPRO (Figure 4). Ratz et al. (2017) inoculated *B. subtilis* in the soil and in the soybean seed, however, no significant difference was found for the PH variable.

Consequently, most of the variables analyzed herein in soybean cultivars with inoculation of the best dose of *B. subtilis* Bs10 showed greater increase in relation to the inoculation of the commercial product control. This may have occurred because the *B. subtilis* Bs10 isolate proved to be an isolate with more specific and efficient mechanisms in promoting plant growth in relation to the commercial product isolate, since the commercial product isolate is recommended with targeted mechanisms as a biological nematicide, but which also works to promote better plant development.

In addition, increasing doses of *B. subtilis* Bs10 showed positive quadratic behavior for most of the variables assessed herein in relation to the inoculation for both of the tested cultivars, with the greatest results found between the 200- and 350-mL doses. These results corroborate those found by Costa et al. (2019), who used inoculation of the same doses used herein (0, 100, 200, 300, 400 ml) based on *B. subtilis* isolated Pant001 in two soybean cultivars. They observed positive quadratic behavior for most of the variables assessed, including RDM, SDM, and PH, indicating that applying high doses prevent soybeans from developing, which can be justified by the suppression of microorganisms in the treatment of seeds.

The *B. subtilis* Bs10 strain was efficient in the variables analyzed in relation to dose 0 (absolute control, without inoculation) and positive control (commercial product). The efficiency of using *B. subtilis* in plant growth is related to the biological characteristics of this microorganism, which expresses facilities for maintaining its viability in bioformulates; in addition to the potential for increasing productivity and reducing diseases, thus demonstrating the importance of the results for the strain of *Bacillus subtilis* Bs10.

Conclusions

The inoculation of *Bacillus subtilis* Bs10 provided increases in biomass, nodulation, nitrogen content, nitrogen accumulation in the aerial part, and in the agronomic characteristics assessed in soybean. The doses of *Bacillus subtilis* Bs10 between 200 and 350 mL per 50 kg seeds showed the maximum efficiency and increase for the variables of SDM, RDM, TDM, NN, NDM, PH, internodes, NP, NG, P content, N content, and ANAP in soybean culture. In addition, the inoculation of *Bacillus subtilis* Bs10 proved more efficient than the commercial product in promoting plant growth in soybeans.

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