

## Efficiency of *Bacillus subtilis* Bs10 as a plant growth promoting inoculant in soybean crop under field conditions

Eficiência de *Bacillus subtilis* Bs10 como um inoculante que promove o crescimento de plantas na cultura da soja em condições de campo

Eficiencia de *Bacillus subtilis* Bs10 como inoculante que promueve el crecimiento de plantas en soja en condiciones de campo

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### Abstract

The growth of the Brazilian market for biological defensive follows a worldwide trend of reducing the use of pesticides in crops, due to international and societal demands for a more sustainable agriculture. The objective of the present work was to evaluate the efficiency of *Bacillus subtilis* Bs10 as an inoculant for the soybean crop, through the promotion of plant growth and productive performance in the field. Three field experiments were conducted in the municipalities of Porto Nacional and Formoso do Araguaia, Tocantins, Brazil. The treatments used in the experiments were five doses of the *B. subtilis* Bs10-based product (0, 100, 200, 300 and 400 mL 50 kg of seeds<sup>-1</sup>), plus one treatment with a commercial *B. subtilis*-based product. The inoculation of *B. subtilis* Bs10 provided increases in biomass, agronomic characteristics, plant population and productivity in soybeans under field conditions. There were positive results starting at a dose of 200 mL, with gains in productivity ranging from 28 to 41% in relation to the absolute control treatment, without inoculation of *B. subtilis*, and from 11 to 42% in relation to the treatment with commercial product. The bio formulated *B. subtilis* Bs10 should be recommended as a plant growth promoting inoculant in soybean.

**Keywords:** Rhizobacteria; *Glycine max* L. Merrill.; Biomass; Agronomic characteristics; Productivity.

### Resumo

O crescimento do mercado brasileiro de defensivos biológicos segue uma tendência mundial de redução do uso de agrotóxicos nas lavouras, devido às demandas internacionais e da sociedade por uma agricultura mais sustentável. O objetivo do presente trabalho foi avaliar a eficiência do *Bacillus subtilis* Bs10 como inoculante para a cultura da soja, por meio da promoção do crescimento vegetal e do desempenho produtivo no campo. Três experimentos de campo foram conduzidos nos municípios de Porto Nacional e Formoso do Araguaia, Tocantins, Brasil. Os tratamentos utilizados nos experimentos foram cinco doses do produto à base de *B. subtilis* Bs10 (0, 100, 200, 300 e 400 mL 50

kg de sementes<sup>-1</sup>), mais um tratamento com produto comercial à base de *B. subtilis*. A inoculação de *B. subtilis* Bs10 proporcionou aumentos na biomassa, nas características agronômicas, na população de plantas e na produtividade da soja em condições de campo. Houve resultados positivos a partir da dose de 200 mL, com ganhos de produtividade variando de 28 a 41% em relação ao tratamento controle absoluto, sem inoculação de *B. subtilis*, e de 11 a 42% em relação ao tratamento com o produto comercial. O bio formulado *B. subtilis* Bs10 pode ser recomendado como um inoculante promotor de crescimento de plantas em soja.

**Palavras-chave:** Rizobacteria; *Glycine max* L. Merrill.; Biomassa; Características agronômicas; Produtividade.

### Resumen

El crecimiento del mercado brasileño de defensivos biológicos sigue una tendencia global de reducción del uso de plaguicidas en los cultivos, debido a las demandas internacionales y sociales de una agricultura más sostenible. El objetivo del presente trabajo fue evaluar la eficiencia de *Bacillus subtilis* Bs10 como inoculante para el cultivo de soja, mediante la promoción del crecimiento vegetal y el desempeño productivo en el campo. Se llevaron a cabo tres experimentos de campo en los municipios de Porto Nacional y Formoso do Araguaia, Tocantins, Brasil. Los tratamientos utilizados en los experimentos fueron cinco dosis del producto a base de *B. subtilis* Bs10 (0, 100, 200, 300 y 400 mL 50 kg de semillas<sup>-1</sup>), más un tratamiento con un producto comercial a base de *B. subtilis*. La inoculación de *B. subtilis* Bs10 proporcionó incrementos en biomasa, características agronómicas, población de plantas y rendimiento de soja en condiciones de campo. Se obtuvieron resultados positivos a partir de la dosis de 200 mL, con ganancias de productividad que variaron del 28 al 41% en relación al tratamiento control absoluto, sin inoculación de *B. subtilis*, y del 11 al 42% en relación al tratamiento con productos comerciales. El *B. subtilis* Bs10 bio-formulado se puede recomendar como un inoculante que promueve el crecimiento de las plantas en la soja.

**Palabras clave:** Rizobacterias; *Glycine max* L. Merrill.; Biomasa; Características agronómicas; Productividad.

## 1. Introduction

The growth of the Brazilian biological defensive market follows the global trend of reducing the use of agrochemicals in crops, due to international and societal demands for a more sustainable agriculture, where farmers see in the use of biological products an alternative to reduce the application of pesticides and chemical fertilizers, which is the main management method in major crops, besides reducing the cost of production, associated risks to human health and natural resources, and providing gains in productivity (MAPA, 2019).

The soybeans (*Glycine max* (L.) Merrill) represents the main Brazilian agricultural commodity, being the crop that has grown the most in the last three decades, its importance in the economy is since Brazil is the largest exporter of the complex (grain, bran, and oil) and the first world producer (CONAB, 2020).

Bacteria of the genus *Bacillus* make up a significant part of the biological products marketed worldwide. This is due to their cosmopolitan distribution, versatility in producing various substances of plant interest such as phytohormones and mineral solubilizers (HUANG et al., 2014; KALAYU, 2019; MOHAMED et al., 2018) and the ability to control important pathogens (RAHMAN et al., 2016).

Furthermore, the bacteria of this genus can be consider plant growth promoting rhizobacterium (PGPR), because they can colonize the rhizosphere and promote plant growth (KALAM et al., 2020; TAVANTI et al., 2020). Rhizobacteria are essential for nutrient recycling and have potential as biofertilizers (SANTOS et al., 2020) for productivity increment, besides being able to benefit the plant during periods of stress (PII et al., 2015). Studies conducted by Jain et al. (2016), showed that *Bacillus* isolates can increase, in soybean, the fresh weight of the aerial part and root, besides increasing the number of lateral roots. Associated with the diversity of metabolites produced by bacteria of the genus *Bacillus* and the harmlessness to animals and humans, along with the ability to form spores, structures that enable the preparation of formulations with greater stability, makes these bacteria interesting for the development of inoculant products in agriculture (LAGERLÖF et al., 2015).

However, in order to achieve an increase in biomass and consequently productivity for soybeans with greater economic returns, as well as the absence of data on specific biological products that describe the behavior of rhizobacteria in regions of the Brazilian Cerrado, it is necessary to continue the process of generating information from targeted research that

seeks and evaluates innovative management practices, such as the use of the *Bacillus subtilis* Bs10 strain, aiming at efficiency as a plant growth promoter and, consequently, an increase in productivity.

Thus, this study aimed to evaluate the efficiency of *Bacillus subtilis* Bs10 doses, under liquid formulation, as an inoculant for soybean culture, through the promotion of plant growth and the productive performance in the field in three regions in the state of Tocantins.

## 2. Methodology

### *Experimental location and weather conditions*

Three field experiments were carry out in the municipalities of Porto Nacional and Formoso do Araguaia, in the state of Tocantins, Brazil, between the 2015/2016 and 2016/2017 seasons in Porto Nacional and between the 2017 season in Formoso do Araguaia, with some distinct edaphoclimatic characteristics.

Two experiments were conducted in the municipality of Porto Nacional, in the 2015/2016 and 2016/2017 seasons at the ALX Farias Agro Agricultural Research in the Cerrados LTDA Research Station (23° 36' 45.1" S - 51° 11' 01.4" W). The local climate characterization is a humid tropical climate with Aw type classification according to Köppen and Geiger, mean temperature is 26.1 °C and 1622 mm of mean annual rainfall. The experiments were perform in the period from December to April in each crop.

In the municipality of Formoso do Araguaia, Tocantins, an experiment was conduct in the Formoso Project third stage, under the latitude of the geographic coordinates: 11°47'45" S and longitude 49°31'43" W, 240 meters altitude. The local climatic characterization is a humid tropical climate with small water deficit (B1wA'a') according to Köppen and Geiger. The region of Formoso do Araguaia had a mean annual temperature of 24.0 °C and mean rainfall for the experimental period below 80 mm. The experiment was conduct from June to October 2017.

### *Soil characteristics and fertilization*

Soil samples were collect from the experimental areas and analyzed chemically and granulometric according to EMBRAPA (2011). In the experiment in Porto Nacional 2015/2016 seasons the following characteristics were found: Ca+Mg 2.8 cmol dm<sup>-3</sup>; Ca 1.6 cmol dm<sup>-3</sup>; Mg 1.2 cmol dm<sup>-3</sup>; Al 0.00 cmol dm<sup>-3</sup>; H+Al 3.10 cmol dm<sup>-3</sup>; K 27.6 mg dm<sup>-3</sup>; CTC (T) 8.30 cmol dm<sup>-3</sup>; SB 2.8 cmol dm<sup>-3</sup>; P 4.7 mg dm<sup>-3</sup>; V 19.6%; M 0.00%; Mat. Org. 2.81% e 28.1 g dm<sup>-3</sup>; pH H<sub>2</sub>O 5.6; sand 67.0%, silte 14.8% and clay 18.2%. For the 2016/2017 seasons the following characteristics were found: Ca+Mg 2,55 cmol dm<sup>-3</sup>; Ca 1,75 cmol dm<sup>-3</sup>; Mg 1,15 cmol dm<sup>-3</sup>; Al 0,00 cmol dm<sup>-3</sup>; H+Al 1,50 cmol dm<sup>-3</sup>; K 66,3 mg dm<sup>-3</sup>; CTC (T) 7,92 cmol dm<sup>-3</sup>; SB 1,49 cmol dm<sup>-3</sup>; P (Mel) 9,4 mg dm<sup>-3</sup>; V 62%; M 0,00%; Mat. Org. 2,0% e 20,0 g dm<sup>-3</sup>; pH H<sub>2</sub>O 5,5; sand 73,3%, silte 7,2% and clay 19,5. In both areas the soil was classified as a dystrophic Red Yellow Latosol of medium texture.

In the experiment in Formoso do Araguaia, the following characteristics were found: Ca+Mg 4.1 cmol dm<sup>-3</sup>; Ca 4.1 cmol dm<sup>-3</sup>; Mg 2.0 cmol dm<sup>-3</sup>; Al 0.00 cmol dm<sup>-3</sup>; H+Al 4.2 cmol dm<sup>-3</sup>; K 35mg dm<sup>-3</sup>; CTC (T) 8.31 cmol dm<sup>-3</sup>; SB 6.9 cmol dm<sup>-3</sup>; P (Honey) 10.7 mg dm<sup>-3</sup>; V 62.0%; M 0.00%; Org Mat. 3.6%; pH H<sub>2</sub>O 5.7; 79.01% sand, 8.83% silt and 12.16% clay. The soil was classify as Plintosol Haplico (EMBRAPA, 2011) with historical use of soybean and rice.

For the first experiment in Porto Nacional (2015/2016 season), filler dolomitic limestone with RNV of 100% was applied 80 days before planting in the amount of 1.5 Ton ha<sup>-1</sup>. For the 2016/2017 season, no limestone was use. For both experiments, 2015/2016 and 2016/2017 season, a base fertilization was perform with the application of 350 kg ha<sup>-1</sup> of the formulation 05-25-15. In the experiment in Formoso do Araguaia, fertilization was carried out with 350 kg ha<sup>-1</sup> of the NPK 02-25-25 formulation + 50 kg ton<sup>-1</sup> of Br12.

In the three experiments the area was prepared by the conventional method, using a harrow, two leveling operations to uniform the area with the use of a leveling grid and the furrowing, adopting a furrow depth of 10 cm and spaced 0.5 m between lines.

### ***Bacillus subtilis* strain**

In the three experiments, the strain of *Bacillus subtilis* Bs10 was use, obtained in Cerrado soil, and identified by morphological characteristics based on specialized literature and through genetic characterization by sequencing the 16S rRNA region, using the Sanger technique. The determination of the bacterial genus and species was compared with the consensus sequence obtained from the NCBI database (2017) using the BLAST tool (Table 1). The main characteristics of the rhizobacteria isolate *Bacillus subtilis* Bs10 were described (Table 1). The microorganisms used are deposit and preserved in the collection of CBMAI (Unicamp).

**Table 1.** Code, and taxonomic classification for the isolated rhizobacteria Bs10 used in this study

Isolated/Code <sup>a</sup>	Origin <sup>b</sup>	Species identification	GenBank Access	Similarity	Color <sup>c</sup>	AIA <sup>d</sup>	Solub. <sup>e</sup>
Bs10/CBMAI2947	TO/Brazil	<i>Bacillus subtilis</i> subsp. <i>subtilis</i>	NC 000964.3	99%	white	+	+

<sup>a</sup> Numeric code of rhizobacteria isolate; <sup>b</sup> Geographic source of isolate; <sup>c</sup> Colony color; <sup>d, e</sup> Producer of indolacetic acid and phosphate solubilization, the methodology is described in Braga Junior (2019). Source: Authors.

### **Treatments used**

The treatments used in the three experiments were five doses of the product based on *Bacillus subtilis* Bs10 (0, 100, 200, 300 and 400 mL 50 kg of seeds<sup>-1</sup>), plus a treatment with a commercial product based on *B. subtilis*.

For the treatments with different dosages was used the inoculant in a liquid formulation, with the microorganism *B. subtilis* Bs10, formulated in the minimum concentration of  $1 \times 10^8$  CFU mL<sup>-1</sup>.

The treatment with the commercial product used in the experiment was a commercial product based on *B. subtilis* isolated UFPEDA 764 at a concentration of  $3 \times 10^9$  CFU mL<sup>-1</sup> in the suspension concentrate formulation, however, recommended as a nematicide. This product was use because it was not found a product based on *B. subtilis* and/or another species belonging to the *Bacillus* genus registered with MAPA Brazil (Ministry of Agriculture, Livestock and Supply) as an inoculant that promotes plant growth in soybean. The dose as recommended by the manufacturer was use.

### **Used soybean cultivars and seed treatment**

The cultivars used in the experiments in Porto Nacional were for the 2015/2016 season M8349 IPRO and the 2016/2017 season M8644 IPRO. For the experiment in Formoso do Araguaia the cultivar 99R03 DuPont Pionner was used.

In the experiments in Porto Nacional, the seeds were previously treat with a product based on thiophanate-methyl, fipronil pyraclostrobin, using 100 mL 50 kg of seeds<sup>-1</sup> in both experiments. In Formoso do Araguaia the seeds were treated with Dermacor and Certeza using 100 and 200 mL 100 kg seeds<sup>-1</sup>, respectively.

In all three experiments, one hour before planting, the seeds of soybean were inoculate by rhizobium (*Bradyrhizobium japonicum*, stirpes SEMIA 5079 and SEMIA 5080) peat type with a concentration of  $10^9$  cel. g<sup>-1</sup>. The inoculant was applied at a rate of 500 g of inoculant for every 50 Kg of seed. Subsequently, the different doses of inoculant based on *B. subtilis* Bs10

and the commercial product were apply directly to the seeds and later the planting was started. In the three experiments 15 seeds were used per linear meter, aiming for a final stand of nine plants per linear meter.

### ***Experimental Design and Evaluations***

The experimental design was in randomized blocks with six treatments and four repetitions, in experimental plots of 24 m<sup>2</sup>, with nine lines with a spacing of 0.5 meters by six meters in length. A useful area of 4.5 m<sup>2</sup> was use within each 24 m<sup>2</sup> experimental plot. During the crop cycle, the necessary phytotechnical and phytosanitary management was carried out.

For the experiments in Porto Nacional in the two seasons, 2015/2016 and 2016/2017, the evaluations were made equally, where the biomass and nodulation evaluations were made at two times, at 30 (vegetative stage V3-V5) and 60 (reproductive stage R2-R3) days after sowing (DAS), in the lines before the border. In the experiment in Formoso do Araguaia the evaluations were made at 30 (vegetative stage V3-V5) and 58 (reproductive stage R2-R3) DAS.

In the three experiments, five plants were randomly collected from each allotment, where the biomass and nodulation parameters were evaluated. The roots were wash under running water to remove all undesirable material, taking care not to lose roots and nodules, with the help of a sieve. The aerial part was separate from the roots with a cut made at the base of the stem, and the nodules were removed and counted. Subsequently, the aerial part and the nodules were placed in paper bags and taken to dry in an oven at 65 °C until reaching a constant weight. After that, the dry matter of shoot, number of nodules and dry mass of the nodules were determined.

For the evaluation of the initial stand, final stand and productivity were used the central useful area referring to 4.5 m<sup>2</sup>. At the end of the crop cycle in the R8 stage, the agronomic characteristics of plant height, number of internodes, number of pods and number of grains were determined in the rows next to the experimental plots, using five random plants per experimental plot.

Grain production was determined in the central rows of the allotment in the useful area of 4.5 m<sup>2</sup>, after the physiological maturity of the plants. The pods were threshed by hand, the grains cleaned and weighed on analytical scales. After weighing, the yield per hectare (kg ha<sup>-1</sup>) were quantify, correcting grain humidity to 13%.

### ***Statistical analysis***

The data were submit to variance analysis with F test, and the effect of the doses of growth promoter was submit to regression analysis, the means of the treatments were grouped by Tuckey test at 5% significance, using the Sisvar application. The doses of maximum technical efficiency were obtain by deriving and equating to zero the quadratic functions of the characteristics that presented significant effects for the quantitative variables. The graphs were plot using the SigmaPlot application.

## **3. Results**

### ***Experiment 1: Porto Nacional 2015/2016***

For the evaluated variables of dray matter of shoot, number of nodules, dray matter of nodules, at 30 and 60 DAS, a positive quadratic behavior was observed regarding the inoculation of increasing doses of *B. subtilis* Bs10 (Fig. 1). For the dray matter of shoot, at 30 and 60 DAS, soybean plants obtained higher average dry biomass in the doses of 242 and 305 mL, respectively, providing an increase at 30 DAS of 31 and 25%, and at 60 DAS of 24.9 and 10.9%, compared to the treatment without inoculation and the commercial product, respectively (Figure 1).

As for nodulation, at 30 DAS there was no significant difference between the doses tested (Figure 1). At 60 DAS there was a significant difference, where maximum efficiency in the production of number of nodules was found in the dose 400 mL, with an increase of 65 and 23% compared to the treatment without inoculation and the commercial product, respectively. Regarding the dry matter of nodules, at 30 DAS the dose that provided greater efficiency in the increment was 315 mL, resulting in an increase of 50 and 20.6% compared to the treatment without inoculation and the commercial product, respectively (Figure 1). At 60 DAS the dose that provided the maximum efficiency in the increase of dry matter of nodules was 283 mL, with 30.8 and 16.2% more than the treatment without inoculation and the commercial product, respectively (Figure 1).

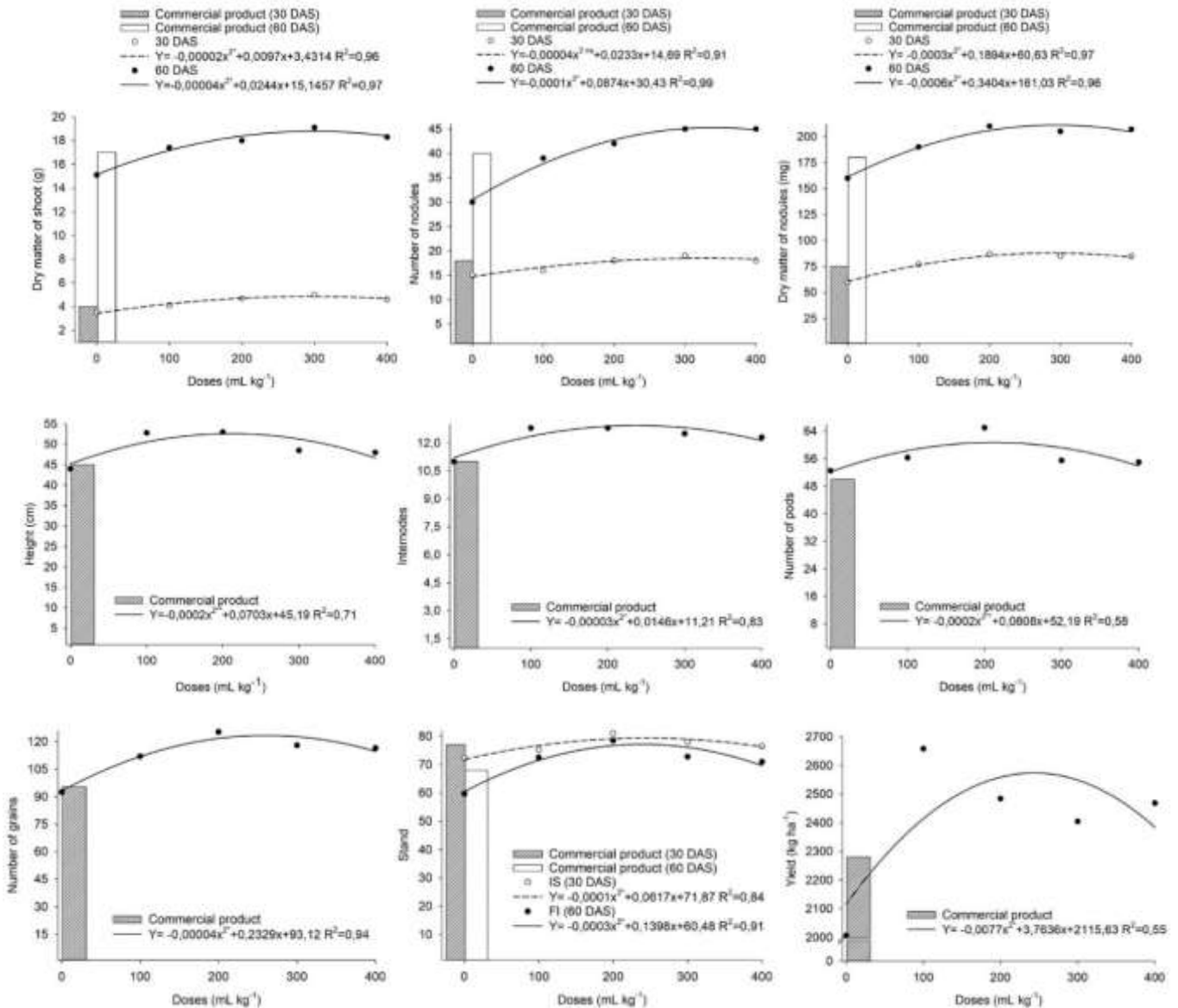
As for plant height, internodes, number of pods, and number of grains, for all were observed significant positive quadratic responses referring to inoculated doses of *B. subtilis* Bs10 (Fig. 1). In the plant height variable, the 175 mL dose was the dose of maximum efficiency providing the highest average value in the height of soybeans plants, 16.5 and 14% higher than found in the treatment without inoculation and commercial product, respectively (Figure 1). For internode quantity, the greatest response was found at the 243 mL dose, with increases of 17% in relation to the treatment without inoculation and the commercial product (Figure 1).

For number of pods the 202 mL dose was the one with the greatest increase, producing an estimated average value of 60.3 pods plant<sup>-1</sup> about 14.8 and 20.6% more than the treatment without inoculation and the commercial product, respectively (Figure 1). In the number of grains variable, the maximum efficiency for the characteristic was found at the dose of 291 mL (Figure 1).

Regarding initial stand, final stand, and soybean yield in the 2015/2016 season, significant positive quadratic behaviors were observed in relation to *B. subtilis* Bs10 doses (Figure 1). For initial stand the 308 mL dose was the most efficient, having an initial stand about 12.4% compared to the treatment without inoculation, the treatment with commercial product showed an initial stand close to that found by the maximum efficiency dose of *B. subtilis* Bs10 (Figure 1). In the final stand, the dose that provided the largest stand was 233 mL, yielding a final stand 28.4 and 12.7% more compared to the treatment without inoculation and the commercial product, respectively (Figure 1).

For yield, the 244 mL dose provided the highest yield, with an estimated production of 2575.5 kg ha<sup>-1</sup> resulting in an increase of 28.3% and 12.9% relative to the treatment without inoculation and the commercial product, respectively (Figure 1).

**Figure 1.** Dry matter of shoot, number of nodules, dry matter of nodules, height, internodes, number of pods, number of grains, stand and yield of soybean cv. M8349 IPRO, inoculated with increasing doses of *Bacillus subtilis* Bs10 and commercial product based on *B. subtilis* at 30 and 60 days after sowing (DAS), Porto Nacional - TO, 2015/2016 season.



Source: Authors.

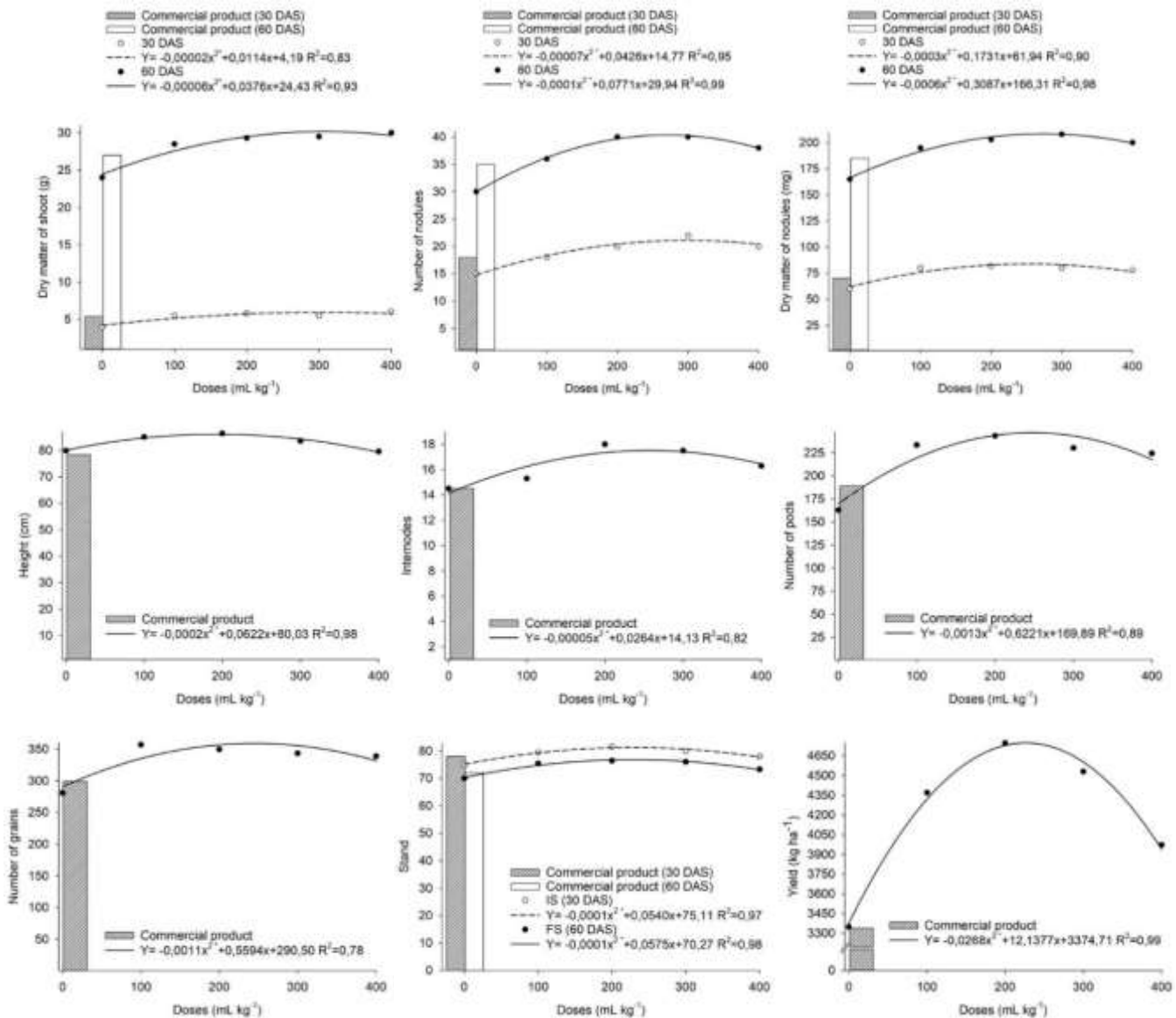
### Experiment 2: Porto Nacional 2016/2017

For the second experiment conducted in Porto Nacional, 2016/2017 season, for the characteristics of biomass and nodulation of soybean plants, the inoculation of *B. subtilis* Bs10 doses provided greater increment (Figure 2). For the evaluated variables dry matter of shoot, number of nodules, dry matter of nodules, at 30 and 60 DAS, a positive quadratic behavior was observed, with significant difference, referring to inoculated doses of *B. subtilis* Bs10 (Figure 2).

In the dry matter of shoot variable, at 30 DAS the dose with the greatest response to the increment of this characteristic was 285 mL, with an average value of 5.81 g, which is 45.2% higher than the treatment without inoculation. The

commercial product obtained a value of 5.4 g, close to that found by the dose of maximum efficiency, proving to be efficient in increasing this characteristic (Figure 2). At 60 DAS the dose of maximum efficiency for dray matter of shoot was 313 mL, with an increase of 25.8 and 11.8% compared to the treatment without inoculation and the commercial product, respectively.

**Figure 2.** Dry matter of shoot, number of nodules, dry mass of nodules, height, internodes, number of pods, number of grains, stand and yield of soybean cv. M8349 IPRO, inoculated with increasing doses of *Bacillus subtilis* Bs10 and commercial product based on *B. subtilis* at 30 and 60 days after sowing (DAS), Porto Nacional - TO, 2016/2017 season.



Source: Authors.

As for number of nodules, at 30 DAS the dose with the greatest effect on increasing nodules in soybean roots was 304 mL, resulting in an increase of 41.6 and 18% compared to the treatment without inoculation and the commercial product,



respectively (Figure 2). At 60 DAS the dose of 385 mL provided the highest number of nodules, leading to an increase of 49.3 and 28% compared to the treatments without inoculation and commercial product, respectively.

For dry matter of nodules, at 30 DAS the dose corresponding to 228 mL provided greater increment (Figure 2). At 60 DAS the dose of 257 mL was the most efficient for the characteristic, with an increase of 24.8 and 11.3% in relation to the treatment without inoculation and the commercial product, respectively.

For the variables of plant height, internodes, number of pods, and number of grains, evaluated after the plants were harvest, they were observe in all quadratic positive significant responses regarding the doses (Figure 2). The dose of greatest efficiency in increasing the size of soy plants was 155 mL, resulting in plants with a height of 6.4 and 8.2% higher than the treatment without inoculation and the commercial product, respectively. For the number of internodes, the dose that provided the best result was 264 mL, with an increase in the number of internodes of 19.8% compared to the treatment without inoculation and commercial product (Figure 2).

Regarding number of pods, the highest was found at the dose of 239 mL, with a mean value of 49.8 and 29.3% more in relation to the treatment without inoculation and the commercial product, respectively (Figure 2). For number of grains, the dose corresponding to 254 mL was the most efficient in increasing the number of grains, 28.7% and 20.9% higher than the values found by the treatment without inoculation and the commercial product, respectively (Figure 2).

For the evaluated characteristics of initial stand, final stand and yield, positive quadratic responses were observed, with significant difference, referring to the inoculation of doses of *B. subtilis* Bs10 (Figure 2). In initial stand and final stand, the doses of maximum efficiency were the doses of 270 mL and 287 mL, respectively (Figure 2). Regarding yield, the dose of 226 mL was the most efficient in the production of soybean grains, with an average yield of 4748.9, representing an increase of 41.8 and 42,3%, in relation to the treatment without inoculation and the commercial product, respectively (Figure 2).

### **Experiment 3: Formoso do Araguaia**

For the characteristic of dry matter of shoot, at 30 DAS there was no significant difference, whereas at 58 DAS a significant positive quadratic behavior was observed, where the greatest increment was provided by the dose of 222 mL, with an estimated mean value of 10.9 g, superior 28.2 and 19.7% to the values found in the treatment without inoculation and commercial product, respectively (Figure 3).

As for number of nodules, at 30 DAS a significant positive quadratic response was observed, the maximum efficiency dose for the characteristic was 372 mL, with an increase of 86.3 and 16.4% compared to the treatment without inoculation and the commercial product, respectively (Figure 3). At 58 DAS a linear response was observed, increasing the number of nodules as the doses of *B. subtilis* Bs10 were increased, where the dose of 400 mL provided an increase of 33.3 and 14.2% compared to the treatment without inoculation and the commercial product, respectively (Figure 3).

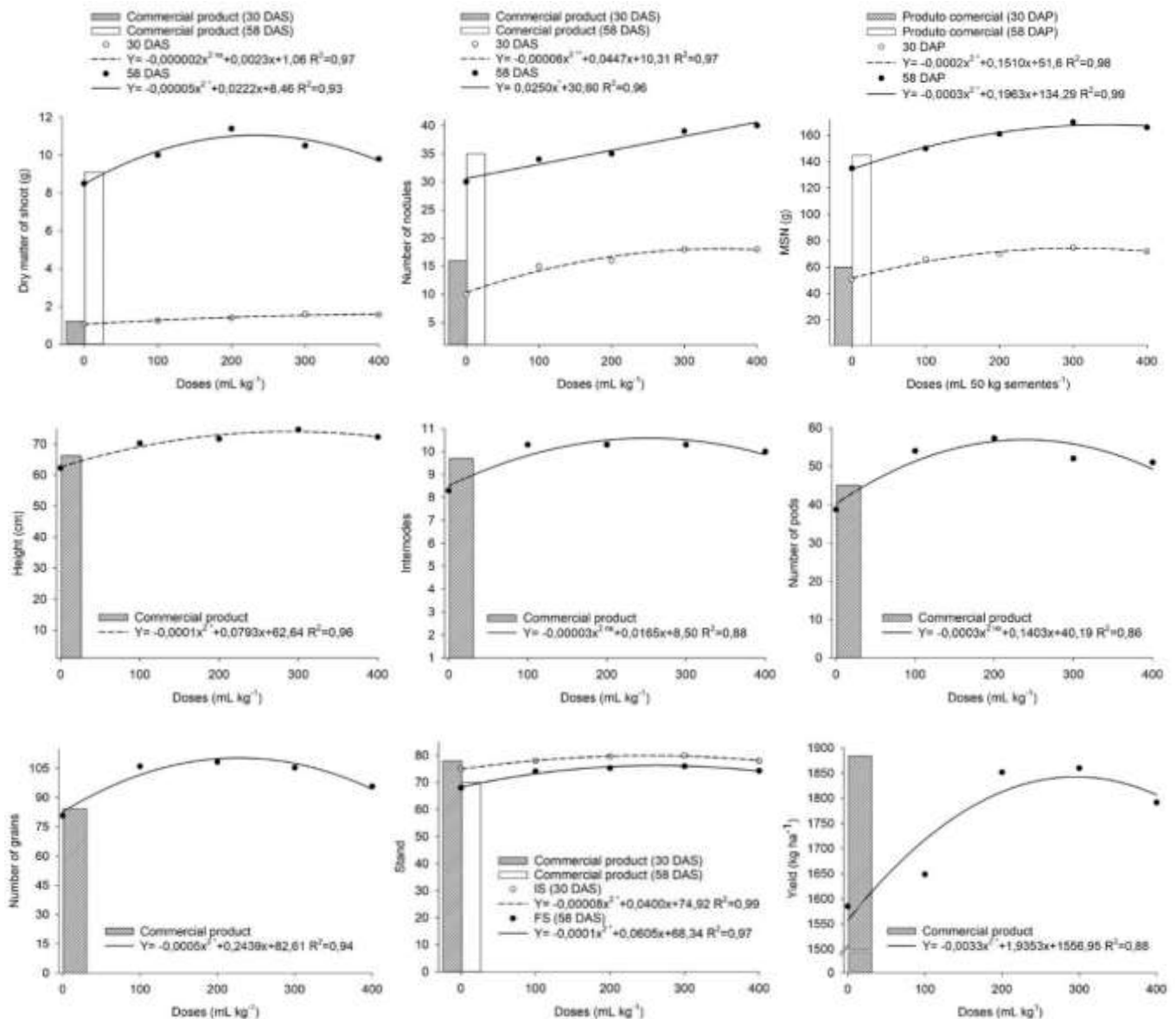
For the variable dry matter of nodules, at 30 DAS the dosage that proposed the biggest medium value for the characteristic was that of 337mL, while the 58 DAS, in the soybean reproductive stage, the maximum efficiency for the variable was found at 327ml dosage, with an average value 23,2 and 14,6% higher in relation to the treatment without inoculation and a commercial product, respectively (Figure 3).

For the agronomic characteristics evaluated at the end of the soybean culture circle, as for plant higher, internodes, number of pods and number of grains were observed quadratic responses referring to inoculation of increasing doses of *B. subtilis* Bs10 (Fig. 3). For the internodes variables and number of pods there was no significant difference between the treatments (Figure 3). Regarding plant height, the dose that provided the largest average soybean plant size was 396 mL, with an increase in relation to the treatment without inoculation and a commercial product of 25.6% and 18%, respectively (Figure

3). For the number of grains at a dose of 244 mL, with an average value of number of grains 39.1 and 33.2% higher in comparison to values found in the treatment without inoculation and a commercial product, respectively (Figure 3).

For the variables of initial stand, final stand and soybean productivity, positive quadratic behavior was observed for both, with a significant difference in relation to the doses of *B. subtilis* Bs10 (Figure 3). The doses of 250 and 302 mL were the ones with the greatest responses for initial stand and final stand, respectively. As for productivity, the dosage of maximum efficiency at grain production was 293 mL, with average productivity of 1840.6 kg ha<sup>-1</sup>, this value is lower than that found by commercial product that was 1884.3 kg ha<sup>-1</sup>, and upper 11.6% in relation to treatment without inoculation (Figure 3).

**Figure 3.** Dry matter of shoot, number of nodules, dry matter of nodules, height, internodes, number of pods, number of grains, stand and yield of soybean cv. M8349 IPRO, inoculated with increasing doses of *Bacillus subtilis* Bs10 and commercial product based on *Bacillus subtilis* at 30 and 60 days after sowing (DAS), Formosa of Araguaia - TO.



Source: Authors.

#### 4. Discussion

Based on the results presented in the three field experiments conducted in the municipalities of Porto Nacional (2015/2016 and 2016/2017 season) and Formoso do Araguaia (2017 season), regarding the growth promotion of soybean plants provided by the inoculant based on *B. subtilis* Bs10, for the characteristics of biomass and nodulation, agronomic characteristics and productivity, there were positive results, superior when compared to the treatment without inoculation and the commercial product tested.

This increase may be related to the ability of some microorganisms, including rhizobacteria such as *B. subtilis*, to stimulate vegetal growth. According to Machado et al. (2016), there are two routes of action: one being the direct one, through the production of substances that will be taken advantage of by plants as phytohormones (MILANI, 2017) and solubilization and availability of nutrients such as phosphate and nitrogen (MISHRA et al., 2016; MOHAMED et al., 2018; KALAYU, 2019) and the other indirect one, through the antagonistic effect to phytopathogens (DALAL; KULKARNI, 2014; ABBAS et al., 2019).

Saeid et al. (2018), evaluated the phosphate solubilization capacity of three *Bacillus* species, including *B. subtilis*, in relation to four sources of phosphorus, where they verified the efficiency of the species solubilization, being the production of organic acids responsible for the release of phosphorus, where the solubilizing exudates produced by *Bacillus* isolates were composed of five organic acids: gluconic, lactic, acetic, succinic, and propionic. Bratkova et al. (2014) observed in their work the ability of *B. subtilis* isolates to solubilize phosphate, and verified the increase in watercress biomass with inoculation, indicating the isolates as good solubilizers of unavailable phosphorus, with potential to be used as plant biofertilizer and for soil fertility improvement. García-Lopes and Delgado (2016) observed in their work that the isolate of *B. subtilis* QST 713 increased phosphorus uptake by 40% by cucumber plants.

Ribeiro et al. (2018) reported in their work the ability of *B. subtilis* isolates to produce high concentrations of indoleacetic acid (IAA) and solubilize phosphate in vitro and the positive effect on millet growth and nutrient uptake, resulting in an increase in the dry weight of the aerial part, root and in the content of N, P and K, indicating the strains with potential to be used as plant inoculants in highly weathered tropical soils. Tahir et al. (2017), in their study found that plant growth promotion was mediated by the volatiles of *B. subtilis* isolate (SYST2) by increasing the rate of photosynthesis and regulating the production of phytohormones, as well as altering the expression of genes related to auxin, gibberellin, expansin, cytokinin, and ethylene production.

Thus, due to the ability of *B. subtilis* to be efficient in the production of phytohormones and phosphates solubilization (BRAGA JUNIOR et al., 2017; MOHAMED et al., 2018; SAEID et al., 2018; KALAYU, 2019; SAXENA et al., 2019), it is possible to relate positive increase of biomass in the field experiments with inoculation of *B. subtilis* Bs10 to these mechanisms.

In relation to number of nodules and dry matter of nodules, the inoculation of *B. subtilis* Bs10, in the different field experiments, did not negatively interfere in the nodulation. In the three trials, there was a significant increase in nodulation and dry matter of nodules. Atieno et al. (2012) in an experiment using two soybean cultivars observed that the co-inoculation of *B. japonicum* and *B. subtilis* obtained a higher yield in number of nodules and dry matter of nodules compared to exclusive inoculation of *B. japonicum*.

Rocha (2019) observed that inoculation of *B. subtilis* promoted significant increments for the number of pods per plant and the number of grains per plant in cowpea beans and common beans. Ratz et al. (2017) verified that the inoculation of *B. subtilis* did not promote a significant increase in height in soybean plants but increased the number of pods and grains per plant. These results corroborate those found in the present study.

In the three experiments carried out the inoculation of *B. subtilis* Bs10 provided a bigger stand for soybean culture. This increase in the stand provided by inoculation of *B. subtilis* Bs10 is possibly relate to increased germination, improvement and rapid development of seedlings and the suppression of soil-borne phytopathogens, such as *Fusarium* and *Rhizoctonia*, leading to a lower incidence of seedling tipping. According to Lanna Filho et al. (2010), the rapid development of the seedling conditions it to reach the adult stage faster, remaining less time in the field, which favors the escape against pathogens present in the soil and in the external environment, it can also promote greater resistance to adverse abiotic conditions by being nutritionally balanced.

The inoculation of *B. subtilis* Bs10 provided an increase of productivity at three fulfilled experiments, the increase of the trial had a variation of 186 to 23.3 a 1398 kg ha<sup>-1</sup>. That raise is a result of the benefits and action mechanisms by which the inoculation with *B. subtilis* Bs10 proportionate throughout the plant's growth. Batista (2017) verified a development in soybean productivity which received the inoculation of *B. japonicum* SEMIA 5080 and of *B. subtilis* RZ2MS9, corresponding to an increase of 35 and 11 sacks per hectare more than the control. These results corroborate those found in this study.

Positive quadratic responses were observed about the inoculation of the doses of *B. subtilis* Bs10 for most variables analyzed at the three trials, promoting an upgrade at variables, showing a positive effect of inoculation in the different locations and experiments. However, the optimal dose varied in the experiments and according to the parameters evaluated, which for most of the parameters at three experiments, doses from 200 mL promoted increases at the variables. Rocha (2019) by working testing doses of 100, 200, 300, 400 mL 50 kg seeds<sup>-1</sup> of *B. subtilis* inoculated in common beans and cowpea, noticed an increase in the pods and grains numbers where the great dose varied with the parcel at *Vigna unguiculata* species and for *Phaseolus vulgaris* the great dose was by 400 mL.

The results of this research showed the efficiency and stability of the inoculant made from *B. subtilis* Bs10, which upgraded the biomass, agronomic characteristics, plant's population, and the productivity at two distinct product regions, in two crops, with different soybean cultivars.

Other studies show the positive effects of inoculating seeds with *Bacillus*, such as Santos et al. (2021) where he concluded that the inoculant based on *Bacillus subtilis* and *B. megaterium* is efficient for grain yield, for increasing the crude protein content and for improving the digestibility of the vegetable fiber of *Avena sativa* L. in dosages above of 150 mL ha<sup>-1</sup>, and Guimarães et al. (2021) using also inoculant containing the isolates of *B. subtilis* (B2084) and *B. megaterium* (B119), applied via seed inoculation, at a dose of 100 mL per 60,000 seeds, with half the dose of phosphorus recommended for the culture of corn, showing efficiency, resulting in productivity statistically superior to the control without inoculation.

The success of *B. subtilis* at the promotion of plant's growth is intrinsically related with the biological characteristics of those microorganisms, that presents easiness for the maintenance of its viability in bio formulates, for their characteristics of resistance on adverse environmental conditions and on industrial processes, which became important characteristics for the products development (LANNA FILLHO et al., 2010).

The efficiency of certain *B. subtilis* isolates in promoting satisfactory results when applied under different environmental conditions (soil, pH, fertility, and temperature) is directly linked to the fact that this microorganism produces spores and metabolites that are highly resistant and tolerant to adverse conditions, such as heat and cold, nutritional deficiency, desiccation, or moisture, as well as extreme pH conditions, pesticides, fertilizers, and storage time. Even though *B. subtilis* has this mechanism to produce spores resistant to adverse conditions, it is recommend to use native isolates from soils in the region where it will be used, as was the case in the present study where the isolate *B. subtilis* Bs10 native from soil from the Cerrado, region of Tocantins.

Candidates for plant growth promoters need, according to Santoyo et al. (2012), gather characteristics such as fast growth and colonization of different environments, competitive capacity, use of different sources of nutrients, survival under stress, synthesis of metabolites with high antimicrobial activity and, finally, capacity to promote growth in plants.

Thus, the *B. subtilis* Bs10 strain tested in different field trials has the potential to be explored as a bio formulated plant growth promoter.

## 5. Conclusion

*Bacillus subtilis* Bs10 inoculation provided increases in biomass, nodulation, agronomic characteristics, plant population and soybean yield under field conditions. Considering the main characteristics evaluated in the different field experiments with the doses tested, there were positive results for doses above 200 mL. The inoculation of *Bacillus subtilis* Bs10 proved to be more efficient compared to the commercial product in promoting plant growth and productivity in soybean. *Bacillus subtilis* Bs10 bio formulate should be recommended as plant growth promoting inoculant in soybean.

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