Bacillus subtilis as a growth promoter inoculant on soybean plants in field

Bacillus subtilis como inoculante promotor de crescimento em plantas de soja em campo

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ABSTRACT
The use of microorganisms as a plant growth promoter such as *Bacillus subtilis* is an alternative that may provide benefits to soybean cultivation, improved development, and productivity. The objective of this work was to evaluate the biomass and productivity of soybean inoculated by *Bacillus subtilis* Bs10 cultivated in field, in Gurupi, Tocantins, in the 2015/2016 and 2016/2017 seasons. Four treatments inoculated with liquid formulation based on *Bacillus subtilis* Bs10 were carried out in different doses (100, 200, 300 and 400 ml for 50 kg of seeds) and compared to a commercial product based on *B. subtilis* (positive control) and a non-inoculated treatment (absolute control). Inoculation by *B. subtilis* Bs10 positively influenced biomass, stand maintenance and yield of soybean under field conditions in both harvests. These yields were 26.6% (200 mL) and 31.8% (300 mL) higher than the absolute control treatment for a 2015/2016 seasons and between 10.8 and 15.43% for a 2016/2017 seasons. Thus, there were significant results from the dose tested 200 mL of inoculant per 50 kg of seed. The strain of *B. subtilis* Bs10 can be recommended as plant growth promoting inoculant in soybean.

Key words: *Glycine max* (L.) Merril, inoculation, bacteria, biomass, productivity

RESUMO
O uso de microrganismos como promotor de crescimento vegetal, tal como *Bacillus subtilis* é uma alternativa que pode proporcionar benefícios para a cultura da soja, como melhor desenvolvimento e produtividade. O trabalho teve como objetivo avaliar a biomassa e produtividade da soja inoculada com *Bacillus subtilis* Bs10 cultivada em campo, em Gurupi, Tocantins, nas safras 2015/2016 e 2016/2017. Foi utilizado quatro tratamentos com doses de produto em formulação líquida a base de *Bacillus subtilis* Bs10 (100, 200, 300 e 400 mL para 50 kg de sementes) e outros dois tratamentos, um com produto comercial a base de *B. subtilis* (testemunha positivo) e uma testemunha sem inoculação (testemunha absoluta). A inoculação de *B. subtilis* Bs10 influenciou

positivamente a biomassa, a manutenção de estande e a produtividade da cultura da soja em condições de campo em Gurupi, nas duas safras. Esses rendimentos foram 26,6% (200 mL) e 31,8% (300 mL) superiores ao tratamento controle absoluto para a safra 2015/2016 e entre 10,8 e 15,43% para a safra 2016/2017. Desta forma, pode ser recomendada a dose de 200 mL do inoculante testado. A cepa de *B. subtilis* Bs10 pode ser recomendada como inoculante promotor de crescimento de plantas em soja.

Palavras-chaves: *Glycine max* (L.) Merril, inoculação, bactéria, biomassa, produtividade.

1 INTRODUCTION

An evolution in mechanization processes and sowing techniques along with agro-industry emergence, cooperatives and infrastructure in the production areas occurred with the expansion of the area of soybean (*Glycine max* (l.) Merrill) cultivation throughout the country. Soybean production is one of the economic activities that has grown the most in recent years in Brazil (Sousa et al., 2020). This can be attributed to the development and structuring of the domestic and international market by consolidating soybeans and their byproducts as important sources of vegetable protein to supply growing demands.

Bacteria of the genus *Bacillus* form a significant part of the biological products commercialized in the world. This is due to its cosmopolitan distribution, versatility in producing various substances for plant interest such as phytohormones, mineral solubilizers (Bahadir et al., 2018; Saxena et al., 2019) and the ability to control important pathogens (Shafi et al., 2017; Gabardo et al., 2020).

Bacteria of the genus *Bacillus* can be considered plant growth-promoting rhizobacteria (PGPR), since they can colonize the rhizosphere and promote plant growth (Saxena et al., 2019; Kalan et al., 2020; Santos et al., 2020). To increase productivity, rhizobacteria are essential for nutrient recovery and have potential as biofertilizers (Mohamed et al., 2018; Saeid et al., 2018; Kalayu, 2019; Saxena et al., 2019) and may also benefit the plant during periods of stress (Pii et al., 2015). The role of *Bacillus* as PGPR has also been reported in various other crops like cotton, pepper, soybean, green gram, sunflower, and tomato (Passari et al. 2018; Zahir et al. 2018; Kalam et al., 2020).

The capacity of spore formation that allows the preparation of formulations with greater stability associated to the diversity of metabolites produced by bacteria of the genus *Bacillus* and the innocuousness to animals and humans makes these bacteria even more interesting for the development of inoculant products for agriculture (Lagerlöf et al., 2015).

The objective of achieving biomass increase and consequently productivity in soybeans with higher economic returns requires the generation of new knowledge, from the directed research. These studies seek and evaluate innovative management practices, such as the use of the *Bacillus subtilis* strain Bs10, aiming at efficiency as plant growth promoters. The objective of this work was to evaluate the biomass and productivity of soybean inoculated by *Bacillus subtilis* Bs10 cultivated in field, Gurupi, state of Tocantins, Brazil, in the 2015/2016 and 2016/2017 seasons.

2 MATERIAL AND METHODS

Two experiments were carried out with the soybean crop at the Experimental Station of the Federal University of Tocantins. The biomass and productivity evaluations were conducted at the Microbiology Laboratory. The experiments were carried out in the seasons of 2015/2016 and 2016/2017, from December to April, respectively. The experiment in the 2015/2016 seasons was installed on December 21, 2015. In the 2016/2017 seasons, it was installed on December 11, 2016. The geographical coordinates of the experimental station correspond to 11º43'45 "S and 49º04'07' 'W, with an average altitude of 280 meters. The local climatic characterization is a humid tropical climate classified as small water deficiency (B1wA'a ') cerrado vegetation or Tropical Savana according to Köppen-Geiger.

Soil samples were collected from the area of the experiments before the implementation of the experiment in the seasons of 2015/2016 and the chemical analyzes were performed presenting the following characteristics: Ca+Mg 2.55 cmol dm^{-3}; Ca 1.80 cmol dm^{-3}; Mg 0.75 cmol dm^{-3}; Al 0.00 cmol dm^{-3}; H+Al 1.54 cmol dm^{-3}; K 0.21 cmol dm^{-3}; CTC (T) 8.31 cmol dm^{-3}; SB 2.76 cmol dm^{-3}; P (Mel) 5.85 mg dm^{-3}; V 33.27%; M 0.00%; Organic matter 2.56% e 25.59 g dm^{-3}; pH $CaCl_2$ 4.80, H_2O 5.38. The granulometric characteristics were: sand 57.4%, silt 9.8% and clay 32.9% (EMBRAPA, 2011). The soil of the experimental area was classified as medium-textured dystrophic yellow red latosol (EMBRAPA, 2011). Chemical attributes of depth 0-20 cm; pH in water - Ratio 1:2.5; P and K – extractor Mehlich 1; Al^{3+}, Ca^{2+} and Mg^{2+} – Extractor KCl (1 mol L^{-1}); H + Al - Extractor SMP; SB = Sum of Exchangeable Bases; (T) = Cation exchange capacity at pH 7.0; V - Base Saturation Index; and OM = organic matter (oxidation: $Na_2Cr_2O_7$ 4N + H_2SO_4 10N).

In this first experiment the application of dolomitic filler limestone with 100% PRNT, 90 days before planting in the 2015/2016 seasons, was applied for soil correction, in the

amount of 1.4 Ton ha^{-1}. In this area sowing fertilization was carried out with the application of 400 kg ha^{-1} of formulation 5-25-15. Fertilization with nitrogen (urea) and phosphorus (simple superphosphate) was done manually in the planting lines one day before planting. Potassium (KCl) was applied to the cover at 30 days after germination at the dose of 60 kg ha^{-1}.

For the 2016/2017 seasons, soil samples were also collected from the experiment area, before the experiment was implemented, and the chemical analyzes were performed, which presented the following characteristics: Ca+Mg 2.08 cmol dm^{-3}; Ca 1.90 cmol dm^{-3}; Mg 0.70 cmol dm^{-3}; Al 0.00 cmol dm^{-3}; H+Al 1.40 cmol dm^{-3}; K 0.25 cmol dm^{-3}; CTC (T) 7.3 cmol dm^{-3}; SB 2.76 cmol dm^{-3}; P (Mel) 5.90 mg dm^{-3}; V 30.2%; M 0.00%; Organic matter 2.60% and 26.0 g dm^{-3}; pH $CaCl_2$ 5.30, H_2O 5.70, sand 55.4%, silt 9.0% and clay 35.6% (EMBRAPA, 2011). The soil of the experimental area was again classified as medium-dystrophic yellow red latosol (EMBRAPA, 2011), and the chemical attributes of 0-20 cm depth were determined in the same way.

In this second experiment (2016/2017 seasons), the application of limestone dolomitic filler with 100% PRNT was again applied at 90 days before planting, for soil correction, in the amount of 1.0 Ton ha^{-1}. In this area, sowing fertilization was carried out with the application of 400 kg ha^{-1} of formulation 5-25-15. Fertilization with nitrogen (urea) and phosphorus (simple superphosphate) was done manually in the planting lines one day before planting. Potassium (KCl) was applied to the cover at 30 days after germination at a dose of 60 kg ha^{-1}.

The preparation of the area in both experiments was done by conventional method, using a harrowing, two leveling operations to standardize the area using leveling grid and the furrow, adopting depth of furrow of 10 cm and spacing 50 cm between rows.

Different cultivars were used in the two harvests, depending on the availability of seeds, being considered the cultivars most planted in the region. In the seasons 2015/2016 the cultivar M 9144 RR was used and for the experiment in the seasons 2016/2017 the cultivar M 8349 IPRO was used.

Four treatments were used in both experiments (seasons: 2015/2016 and 2016/2017) with doses of product in liquid formulation based on *Bacillus subtilis* Bs10 (100, 200, 300 and 400 mL per 50 kg of seeds). In addition, two other treatments were performed, one with commercial product based on *B. subtilis* (positive control) and one control without inoculation (absolute control).

The liquid bioinoculant *Bacillus subtilis* Bs10 was formulated with a minimum concentration of 1 x 10^8 CFU mL^{-1}. This isolate was obtained from Cerrado soil and a preliminary identification of the morphological characteristics based on specialized bibliography was performed (Rabinovitch & Oliveira, 2015). Afterwards, the genetic characterization was performed by the sequencing of the 16S rRNA region, performed by Helixxa Genomic Services, where the Sanger technique was used. The determination of genus and bacterial species was by comparing the consensus sequence obtained against the NCBI database (2017) using the BLAST tool (Table 1).

Table 1. GenBank access codes for the *Bacillus subtilis* strain Bs10 (16S rRNA region) used in this study

Strain	Species Identification	Access GenBank	Similarity
Bs10	*Bacillus subtilis* subsp. *subtilis*	NC000964.3	99%

The positive control treatment used in the experiment was a commercial product based on *B. subtilis*, but it was recommended as a nematicide. This product was used because no product based on *B. subtilis* and/or another species belonging to the genus *Bacillus* was found in the MAPA (Brazil) register (MAPA – Ministério da Agricultura, Pecuária e Abastecimento) as a promoter of plant growth in soybean. The dose recommended by the manufacturer was used, referring to 100 mL per hectare.

The seeds were treated one day before planting with methyl thiophanate and fipronil based product, using 100 g of the product per 50 kg of seeds. One hour before planting the soybean seeds were inoculated by rhizobium (*Bradyrhizobium japonicum*, strain SEMIA 5079 and SEMIA 5080), at the time of sowing, with commercial inoculant recommended for soybeans that uses as a vehicle the peat, previously inoculated with 10^9 cells g^{-1}. The application of the inoculant was carried out in the proportion of 500 g of the inoculant per 50 kg of seed. Then, the different doses of the inoculant based on *B. subtilis* Bs10 were applied directly to the seeds and later the planting was started.

Twelve seeds per linear meter were used in the experiments resulting in a final stand of nine plants per linear meter. The experimental design was in randomized blocks with six treatments and four replications, in experimental plots of 24 m^2, nine lines with spacing of 0.5 meters by six meters in length.

Phyto-technical and phytosanitary management were carried out in the experiments. control of invasive plants at 30 days after planting at soybean V4 stage was performed

using the herbicide Roundup at the dose of 1.5 kg ha^{-1}. The control of early attacking caterpillars of soybeans was also performed using the insecticides Gamacialotrin (150 g L^{-1}) and Diflubenzuron (240 g L^{-1}) at doses of 50 mL ha^{-1} and 120 mL ha^{-1}, respectively. The control of anthracnose (*Colletotrichum truncatum*) and Asian rust (*Phakopsora pachyrhizi*) was performed in the R1 stage with the application of the fungicide based on Azoxystrobin and Ciproconazole at the dose of 500 mL ha^{-1}.

The biomass evaluations were done in two seasons, at 25 and 55 days after sowing (DAS), in the lines prior to the border for the experiments in the two harvests. The biomass evaluations were shoot dry mass (SDM), number of nodules (NN) and nodules dry mass (NDM). Ten plants of each plot were collected for each biomass analysis. The roots were washed in running water to remove impurities taking care not to lose roots and nodules with the aid of a sieve. The aerial part was separated from the roots with a cut made at the base of the stem, the nodules were removed and counted. Subsequently, the aerial part, root and nodules were placed in a paper bag and then dried in an oven at 65 °C until reaching constant weight.

The plant height, number of internodes, number of pods and number of grains per pod, in stage R8 were also determined in the lines near the useful area of the experimental plots. Ten plants per experimental plot were used, totaling 40 plants per treatment.

The grain yield was determined in the useful area of 4.5 m^2, after the physiological maturation of the plants, when approximately 80% of the pods were dried. Then, the pods were threshed manually by correcting the grain moisture to 14%. After the harvest, productivity per hectare in kg ha^{-1} and bags ha^{-1} (60 kg) were quantified.

Data from both experiments were submitted to analysis of variance and the Duncan mean test at 1 and 5% probability using the statistical program ASSISTAT version 7.6 beta.

3 RESULTS AND DISCUSSION

3.1 2015/2016 SEASON

The biomass results at 25 DAS in the 2015/2016 season showed a significant difference ($p < 0.05$) between the treatments for shoot dry mass (SDM), being higher for the treatment inoculated with doses of 200, 300 and 400 ml of *B. subtilis* Bs10. On the other hand, the evaluation at 55 DAS showed that all dosages were superior ($p < 0.05$) to the positive control and absolute control treatments, and among the dosages SDM was superior for inoculations with 200, 300 and 400 mL (Table 2). There were no significant

differences in the number of nodules (NN) at 25 DAS, but at 55 DAS the treatments at doses of 200, 300 and 400 mL were higher ($p<0.05$) than the other treatments. In the two evaluations of the nodules dry mass (NDM), treatments with 200, 300 and 400 mL doses were also superior ($p <0.05$) to the other treatments (Table 2).

Table 2. Shoot dry mass (SDM), number of nodules (NN) and nodules dry mass (NDM) at 25 and 55 days after sowing (DAS) in soybean M 9144 RR inoculated by *Bacillus subtilis* Bs10. Gurupi, TO. 2015/2016 season.

Treatments	SDM (g)		NN		NDM (mg)	
	25 DAS	55 DAS	25 DAS	55 DAS	25 DAS	55 DAS
100 mL	3.3 bc	20.2 b	12 a	30 b	54 b	155 b
200 mL	3.9 a	22.8 a	14 a	37 a	65 a	170 a
300 mL	4.2 a	23.3 a	15 a	35 a	66 a	177 a
400 mL	3.7 ab	22.3 a	14 a	36 a	65 a	171 a
P. control	2.6 c	17.8 c	12 a	30 b	50 b	148 b
A. control	2.7 c	17.4 c	12 a	26 b	50 b	140 b
CV (%)	11.1	6.1	10.9	9.5	10.1	9.9

Means followed by the same lowercase letter in the columns do not differ by Duncan's test at 1 and 5% significance. DAS: Days after sowing. P. control: commercial product based on *B. subtilis*. A. control: absolute control treatment without inoculation. CV: Coefficient of variation

In the evaluations after planting, there were no significant differences for plant height and number of internodes (Table 3). The treatments with doses of 200, 300 and 400 mL were superior ($p <0.05$) in relation to the positive control and absolute control treatments for the analysis of the number of pods. In the case of the number of grains per plant, the treatments with the different dosages were superior ($p <0.05$) to the positive control and absolute control treatments (Table 3).

Table 3. Plant height, number of internodes, number of pods and number of grains per pod, in R8 soybean cv. M 9144 RR inoculated with different doses of *Bacillus subtilis* Bs10, Gurupi, TO. 2015/2016 season.

Treatments	Plant height (cm)	Number of internodes	Number of pods	Number of grains
100 mL	65.5 a	12.5 a	59.8 b	132.0 a
200 mL	64.0 a	11.8 a	74.3 a	135.3 a
300 mL	69.5 a	11.8 a	74.0 a	133.0 a
400 mL	64.3 a	12.0 a	70.3 a	118.5 a
P. control	67.5 a	11.0 a	56.8 b	108.8 b
A. control	57.3 a	10.8 a	49.0 c	91.3 c
CV (%)	7.6	8.1	12.1	8.8

Means followed by the same lowercase letter in the columns do not differ by Duncan's test at 5% significance. Mean of 10 plants per experimental plot were used. P. control: commercial product based on *B. subtilis*. A. control: absolute control treatment without inoculation. CV: Coefficient of variation

Regarding the initial and final stand for the 2015/2016 season, there was no significant difference between treatments (Table 4). However, the highest percentage of survival was for the treatment inoculated with the dose of 300 mL followed by the doses of 100 and 200 mL, these were superior ($p < 0.05$) to the other treatments, with efficiency percentage varying from 9.8 a 17.9% (Table 4).

The productivity showed a significant difference between the treatments, being higher ($p < 0.05$) for the treatments with the doses of 200 and 300 mL in a production of 2171.2 kg ha^{-1} and 2259.2 kg ha^{-1}, respectively. Thus, these productivities were 26.6% (200 mL) and 31.8% (300 mL) higher than the absolute control treatment. The other treatments with doses of 100 and 400 mL were also superior ($p < 0.05$) than the absolute control (Table 4).

Table 4. Initial stand (IS), final stand (FS), survival, efficiency and productivity of soybean cv. M 9144 RR inoculated by different doses of *Bacillus subtilis* Bs10, Gurupi, TO. 2015/2016 season.

Treatments	IS	FS	Survival[1]	Efficiency[2]	Productivity
	25 DAS	55 DAS	(%)	(%)	(Kg ha^{-1})
100 mL	68.7 a	61.5 a	75.9 b	9.8	2055.2 b
200 mL	62.7 a	61.9 a	76.4 b	10.6	2171.2 a
300 mL	67.2 a	66.0 a	81.5 a	17.9	2259.2 a
400 mL	59.7 a	58.5 a	72.0 c	4.2	1998.4 b
P. control	61.2 a	58.5 a	72.0 c	4.2	2088.8 b
A. control	63.0 a	56.5 a	69.1 c	-	1715.6 c
CV (%)	10.1	10.2	5.3	-	6.1

Means followed by the same lowercase letter in the columns do not differ by Duncan's test at 5% significance. [1]Percentage of plant survival in relation to the expected stand of 81 plants in 4.5 m^2 (270000 plants ha^{-1}). [2] Efficiency in the use of *Bacillus subtilis* in the maintenance of the stand. DAS: Days after showing. P. control: commercial product based on *B. subtilis*. A. control: absolute control treatment without inoculation. CV: Coefficient of variation

3.2 2016/2017 SEASON

In the second experiment (2016/2017 season), biomass results at 25 DAS showed a significant difference ($p < 0.05$) between treatments for SDM, being higher for treatment with inoculation of the doses of 200 and 300 mL of *B. subtilis* Bs10, followed by treatments with 100 and 400 mL, all superior to the positive control and absolute control treatments (Table 5). In the case of biomass evaluations at 55 DAS, the treatments with the dosages of 200, 300 and 400 mL were superior to the others. The NN and MSN were higher ($p<0.01$) for treatments with 200, 300 and 400 mL dosages in both evaluations (25 and 55 DAS), followed by treatments with the 100 mL dose and the positive control compared to the absolute control (Table 5).

Table 5. Shoot dry mass SDM, number of nodules (NN) and nodules dry mass (NDM) at 25 and 55 days after showing (DAS) in soybean M 8349 ipro inoculated by *Bacillus subtilis* Bs10. Gurupi, TO. 2016/2017 season.

Treatments	SDM (g)		NN		NDM (mg)	
	25 DAS	55 DAS	25 DAS	55 DAS	25 DAS	55 DAS
100 mL	4.6 ab	23.3 bc	15 ab	32 ab	87 ab	160 b
200 mL	5.5 a	28.2 a	17 a	35 a	92 a	170 a
300 mL[1]	5.1 a	27.5 a	18 a	34 a	90 a	181 a
400 mL	6.5 ab	27.5 a	17 a	35 a	92 a	175 a
P. control	4.4 c	26.0 ab	15 ab	32 ab	85 ab	150 b
A. control	4.0 c	22.0 c	13 b	27 bc	78 bc	139 c
CV (%)	6.1	8.5	10.8	9.8	8.9	8.8

Means followed by the same lowercase letter in the columns do not differ by Duncan's test at 1 and 5% significance. DAS: Days after showing. P. control: commercial product based on *B. subtilis*. A. control: absolute control treatment without inoculation. CV: Coefficient of variation

The height characteristics of the plant showed that all the treatments with the different doses and the positive control treatment were superior to the absolute control (Table 6), where the mean for the treatment with the 300 mL dose was higher ($p < 0.05$). On the other hand, the number of internodes and number of pods were higher ($p < 0.05$) for the treatments inoculated at different doses of *B. subtilis* Bs10 in relation to the positive control and absolute control treatments. Then, the number of grains for the inoculated treatments and the positive control were higher ($p<0,05$) than the absolute control (Table 6).

Para o número de grãos por planta os tratamentos com as inoculações das diferentes doses e a testemunha positiva foram superiores ($p<0,05$) em relação a testemunha absoluta (Tabela 5).

Table 6. Plant height, number of internodes, number of pods and number of grains per pod, in R8 soybean cv. M 8349 ipro inoculated with different doses of *Bacillus subtilis* Bs10, Gurupi, TO. 2016/2017 season.

Treatments	Plant height (cm)	Number of internodes	Number of pods	Number of grains
100 mL	67.5 b	18.8 a	135.3 a	255.0 a
200 mL	71.5 b	17.5 a	133.8 a	249.3 a
300 mL	75.8 a	17.5 a	137.5 a	261.8 a
400 mL	70.5 b	17.3 a	140.0 a	261.0 a
P. control	67.5 b	15.5 b	113.5 b	246.5 a
A. control	57.8 c	15.3 b	101.8 c	208.0 b
CV (%)	4.9	3.7	4.9	6.8

Means followed by the same lowercase letter in the columns do not differ by Duncan's test at 5% significance. Mean of 10 plants per experimental plot were used. P. control: commercial product based on *B. subtilis*. A. control: absolute control treatment without inoculation. CV: Coefficient of variation

The initial stand, final stand and survival did not differ significantly between treatments (Table 7). The inoculation efficiency of *B. subtilis* Bs10 was higher for treatment with 400 mL inoculation with 10% followed by inoculation of 300 mL with 6.0%. The productivity in the treatments with the different doses of *B. subtilis* UFT-Bs10 and the positive control were higher ($p < 0.01$) than the absolute control treatment. The treatments with different doses of *B. subtilis* Bs10 showed productivity means ranging from 3323 to 3466 kg ha^{-1}, representing an increase between 10.8 and 15.43% relative to the absolute control (Table 7).

Table 7. Initial stand (IS), final stand (FS), survival, efficiency, and productivity of soybean cv. M 8349 ipro inoculated by different doses of *Bacillus subtilis* Bs10, Gurupi, TO. 2016/2017 season.

Treatments	IS 25 DAS	FS 55 DAS	Survival[1] (%)	Efficiency[2] (%)	Productivity (Kg ha^{-1})
100 mL	81.0 a	79.5 a	98.2 a	5.4	3427 a
200 mL	78.5 a	76.5 a	94.4 a	1.3	3466 a
300 mL	83.0 a	80.0 a	98.8 a	6.0	3410 a
400 mL	84.0 a	83.0 a	102.5 a	10	3323 a
P. control	83.0 a	80.0 a	98.8 a	6.0	3220 a
A. control	78.0 a	75.5 a	93.2 a	-	3003 b
CV (%)	11.8	13.1	11.1	-	7.6

Means followed by the same lowercase letter in the columns do not differ by Duncan's test at 5% significance. [1]Percentage of plant survival in relation to the expected stand of 81 plants in 4.5 m^2 (270000 plants ha^{-1}). [2] Efficiency in the use of *Bacillus subtilis* in the maintenance of the stand. DAS: Days after showing. P. control: commercial product based on *B. subtilis*. A. control: absolute control treatment without inoculation. CV: Coefficient of variation

In this study, both experiments presented superior results for one or more analyzed variables when compared to the absolute control (without inoculation). These results were based on field experiments related to plant growth promotion and soybean yield provided by the *Bacillus subtilis* Bs10 based inoculant. This may be linked to the ability of some microorganisms to stimulate plant growth through two pathways: a direct pathway through the production of substances that will be harnessed by plants like indole acetic acid (IAA) (Cendale et al., 2017) and phosphate solubilization (Saeid et al., 2018), and another indirect pathway through the antagonistic effect on plant pathogens (Saxena et al., 2019).

According to García-Lopes & Delgado (2016), the *B. subtilis* QST 713 isolate increased the phosphorus absorption of cucumber plants by 40% not only by the ability to solubilize different nutrient sources, but also by producing organic compounds capable of competing with the phosphorus adsorption sites. This makes the plant more available, exerts an acidifying effect on the rhizosphere as well as favors the absorption of phosphorus. Thus, positive results in biomass enhancement in field experiments by inoculation of *B. subtilis* Bs10 may be related to the ability of *B. subtilis* to be efficient in IAA production and phosphate solubilization.

These results can be compared to the studies by Tahir et al. (2017) where the growth promotion was mediated by the volatiles produced by *B. subtilis isolate* (SYST2), by increasing the rate of photosynthesis and regulating phytohormone production, as well as altering the expression of genes related to the production of auxins, gibberilins, expansin, cytokinin and ethylene.

Species of *Bacillus* are reported by the production of signaling molecules to the expression of genes that may be related to growth promotion, as well as by the direct production of phytohormones as auxin, citocinina and giberilinas (Park et al., 2017; Kalam et al., 2020).

Saharan & Nehra (2011) observed that *Bacillus* species contributed to the improvement of different root parameters such as rooting, root length and dry matter content, they also concluded that inoculation by IAA-producing isolates increased the absorption of some nutrients, promoting sweet-potato growth and increased rooting of eucalyptus seedlings.

A factor by which the tested isolates may have acted to increase biomass is the availability and solubilization of nutrients such as phosphorus and nitrogen (Saeid et al., 2018; Saxena et al., 2019). In addition, Araújo et al. (2012) reported that the simple

inoculation of *B. subtilis* (PRBS-1) in cowpea BRS Guariba cultivar provided the greatest increase in plant growth, higher N fixation and did not affect nodulation at 40 and 55 days after sowing. In relation to the number of nodules and dry mass of the nodules, inoculation of *B. subtilis* in the two field experiments did not negatively interfere with nodulation (Tables 2 and 5).

According to Filho et al. (2010), the rapid development of the seedling conditions it to reach the adult stage more quickly, remaining less time in the field, which favors the escape of pathogens present in the soil and the external environment, also, greater resistance to adverse abiotic conditions due to being nutritionally balanced.

Despite the technological advances, this complex interaction is still poorly understood (Hardoim et al., 2015) and this explains the lack of reliable commercial products for this purpose.

Enhanced photosynthetic activity is also reported as shown by increase in gamma-aminobutyric acid metabolites, glucose, fructose, and alanine in leaves of inoculated plants (Vinci et al., 2018).

Lobo et al. (2019) and Diaz et al. (2019) also found that *B. subtilis* 290 significantly increased plant growth promotion as well as nitrogen concentration in shoots and roots in maize and cotton. Plant growth promoting bacteria have the ability to promote biological nitrogen fixation, which could be reported by Mehmood et al. (2018), who emphasized that *B. subtilis* bacteria were able to increase around 20-30% in nitrogen concentrations in grassy plants, among them, maize. Breedt et al. (2017), inoculating strains of phosphate solubilizing microorganisms in corn, including *Bacillus*, covering increases from 24% to 34% in grain yield.

Szilagyi-Zecchin et al. (2015), evaluating the growth of tomato seedlings inoculated with the bacterium *Bacillus amyloliquefaciens* subsp. plantarum FZB42, producer of indole compounds and siderophores, found increases in the levels of a, b, and total chlorophyll and in the growth of tomato seedlings of 'Santa Clara' and 'Cherry' cultivars

According to Tavanti et al. (2020), the inoculation of *Bacillus subtilis* Pant001 and QST713 strains promotes yield increments in both soybean cultivars tested, besides improving seed quality due to the increase in seedling emergence percentage and seed vigor. The use of *Bacillus subtilis* Pant001 strain at the dose of 3 mL kg^{-1} leads to better response for the soybean cultivar M7110, while the Pant001 and QST713 strains at dose of 2 mL kg^{-1} favor the cultivar Desafio, increasing seed yield and the amount of storage proteins of both cultivars.

The success of the use of *B. subtilis* in plant growth is related to the biological characteristics of this microorganism, which expresses facilities for maintaining its viability in bioformulates and potential for increasing productivity as well as reducing disease. Future works to understand and clarify the beneficial mechanisms like growth promotion and biocontrol are the objective of several research.

4 CONCLUSIONS

Inoculation by *Bacillus subtilis* Bs10 positively influenced biomass, stand maintenance and yield of soybean under field conditions in Gurupi, in the 2015/2106 and 2016/2017 crops. Finally, there were positive results from the dose of 200 mL to 50 kg of seed, so the dose of 200 mL of the inoculant tested could be recommended.

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