

*Full Length Research Paper*

## **Agronomic performance of soybean treated with *Bacillus amyloliquefaciens***

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The development of practices that maintain or increases soybean yield can increase the profitability of this crop. In this context, the use of microorganism-based products in crops has been extensively studied. Among the species, *Bacillus amyloliquefaciens* has shown significant potential for agronomic use due to its ability to control phytoparasitic microorganisms and its effects in promoting plant growth. The aim of this study is to evaluate the effects of *B. amyloliquefaciens* application on soybean. Fields experiments were conducted at four sites. The experimental design used was randomized block design, with six treatments and four replications. The treatments consisted of seed treatment with *B. amyloliquefaciens* strain MBI600 (Integral II SC)-based product at 2.5, 5, 10, 15 and 20 mL c.p. 100 kg<sup>-1</sup> of seeds, plus a control without treatment. The variables evaluated were plant stand, phytotoxicity, plant height, root and shoot dry mass, number of nodules and crop yield. Increasing doses of the *B. amyloliquefaciens*-based product promote an increase in all variables related to crop development. All doses of the *B. amyloliquefaciens*-based product provided an increase in soybean yield compared to the control. The dose that produces the maximum agronomic efficiency was 15 mL c.p. 100 kg<sup>-1</sup> of seeds.

**Key words:** Biological control, *Bacillus subtilis*, *Glycine max*, growth promoters, seed treatment.

### **INTRODUCTION**

In recent years Brazil has been facing severe economic crisis (Filho, 2017). However, the agricultural sector has contributed to a far less gloomy situation than might otherwise be occurring. Soybean is the main crop

produced in Brazilian agriculture, and forecasts show Brazil becoming the world's largest producer of this oilseed in upcoming seasons (USP, 2018).

The mean domestic soybean yield is 3,185 kg ha<sup>-1</sup>

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according to provisional data from the 2017/2018 season (CONAB, 2018). When analyzing data among seasons or within the same season in different Brazilian states, it is common to observe large fluctuations in mean yield. This is due to several factors, including those that cannot be controlled by man, such as climate, to those that can be managed, such as adequate plant nutrition and health control.

In this context, it is important to develop technologies and management practices that maintain productivity levels of soybean or that can even promote higher yields, as these will enable farmers to obtain higher profits when growing this crop. One of the primary lines of research that has grown considerably in the agricultural scenario in recent years is related to the use of biological control products. The factors that have contributed to this growth include the potential for reducing the use of pesticides, the provision of a more specific spectrum of control of phytoparasitic pests and microorganisms, and greater selectivity for natural enemies (Dreistadt, 2014).

In addition to the benefits related to microorganism control provided by biological products (Lobna and Zawam, 2010; Alfonzo et al., 2012), recent research has shown that several of these agents act as promoters of plant growth, favoring the development of plants to which they are applied (Wang et al., 2012; Szilagyi-Zecchin et al., 2015). In the scientific literature, beneficial effects on plant development have already been reported for bacteria, which are called plant-growth-promoting rhizobacteria (PGPR) (Araújo, 2008). Among the species most studied for this purpose are those of the genus *Bacillus* sp, which are characterized by cosmopolitan habits—their natural habitat is the soil, and they are commonly found associated with the rhizosphere and endophytic environment of plants (Meng et al., 2012).

The species *Bacillus amyloliquefaciens* has been extensively studied and has phylogenetic affinity with *B. subtilis*; for a long time it was even considered its subspecies. Studies developed with *B. amyloliquefaciens* have associated the use of this bacterium with increased plant defense, showing positive results in the control of nematodes and other microorganisms. In addition, there are reports that, when used in different crop species, the benefit is not only restricted to the control of diseases but also promotes plant growth, as observed in such diverse crops as rice, eucalyptus, wheat and tomato, among others (Ng et al., 2012; Paz et al., 2012; Kasim et al., 2013; Szilagyi-Zecchin et al., 2015).

Based on the results available in the literature, we hypothesized that *B. amyloliquefaciens* may positively influence soybean development. However, it is necessary to determine how the agronomic performance of this important crop is affected by different doses of the bacterium in order to determine the concentration that can generate the greatest benefits in soybean. Therefore, this work sought to evaluate the effects of the application of different doses of a *B. amyloliquefaciens*-based

product on soybean development and yield.

## MATERIALS AND METHODS

The study was conducted at four sites to evaluate the potential use of a *B. amyloliquefaciens*-based product on the treatment of soybean seeds and its potential effects as a growth promoter. The experiments were conducted in the municipalities of Francisco Alves (Paraná State-PR), latitude 25°08'9.65"S, longitude 53°52'36.72"W and 321 m altitude; Terra Roxa (PR), 24°17'15.87"S, 54°02'31.11"W and 332 m altitude; Maringá (PR), 23°23'48.33"S, 51°56'58.20"W and 501 m altitude; and Ourizona (PR), 23.27,3.7,S, 52.15,28.99,W and 367 m altitude. All experiments were carried out from January to June 2015.

For all experimental sites, Cfa - humid mesothermal – was the predominant climate according to the Köppen classification, which is characterized by warm summers and infrequent frosts with a tendency towards rainfall accumulation in the summer months and with no well-defined dry season. Mean air temperatures in the warmer months are higher than 22°C (below 13°C in the colder months) and the mean annual precipitation is between 1,600 and 1,800 mm (IAPAR, 2014). Figure 1 shows precipitation data for the time period during the experiments.

Prior to the initiation of the experiments, soil samples (0 to 20 cm layer) were collected in the experimental areas, and the soil physical and chemical characteristics, together with the ultimate crop data, are presented in Table 1. To ensure there were no negative effects on soybean development caused by pests and diseases, or losses due to weed interference, the phytosanitary treatments recommended by EMBRAPA (2010) were performed in all experimental areas.

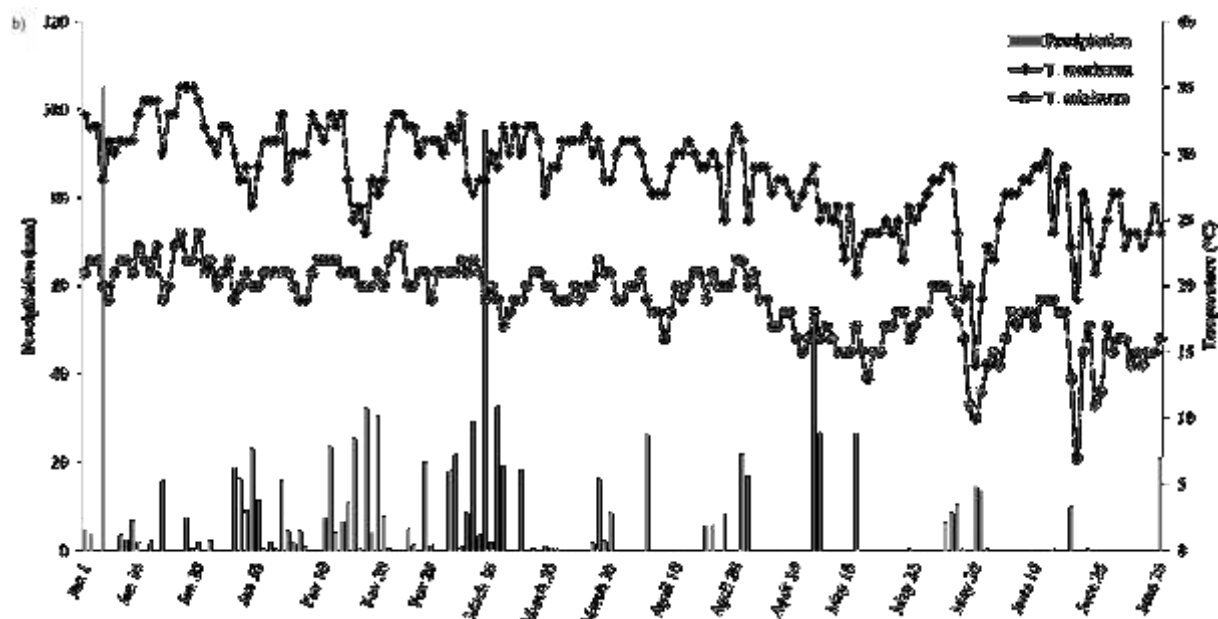
In all experiments, the experimental design adopted was a randomized block design (RBD), with six treatments and four replicates. The treatments consisted of application of five doses of a *B. amyloliquefaciens*-based product, plus one control without product, to seeds (Table 2). The formulation used was isolated from *B. amyloliquefaciens* strain MBI600 (II Integral SC) and provided by BASF (BASF Corporation Agricultural Products Research Triangle Park, USA; <http://agro.basf.com>).

In all experiments, soybean seeds were treated with the *B. amyloliquefaciens*-based product following the method described below, using the dose established for each treatment. Initially, four samples of soybean seeds were weighed to obtain 3 kg samples, sufficient to sow the four replicates corresponding to each treatment. Subsequently, these samples were placed in plastic bags and then the dose of the biological product corresponding to each treatment was added directly into the bags containing the seeds. After this procedure, the seeds were homogenized by vigorously shaking the bag for 3 min.

The experimental units measured 5.5 m wide by 5 m long, totaling a gross area of 27.5 m<sup>2</sup>. All the response variables were measured in the useful area of the experimental units, which measured 22.5 m<sup>2</sup> after excluding the borders.

In order to evaluate the effect of the treatments on soybean development, the response variables plant stand, phytotoxicity, plant height, root and shoot dry weight, number of nodules and crop yield were evaluated. The plant stand was measured at 7 and 14 days after emergence (DAE), counting the number of seedlings in 2 linear m using the two central rows of the experimental unit for evaluation.

The toxicity of soybean plants was evaluated at 7 and 14 DAE using a percentage scale as a reference to assign the scores, in which 0% corresponded to the absence of symptoms and 100% corresponded to death of the plants. Two plant height evaluations were performed, one at 14 and one at 35 DAE. To calculate this variable, the distance from the soil level to the apical meristem of



**Figure 1.** Temperature (maximum and minimum) and precipitation (mm) during the experimental period for the regions of Francisco Alves and Terra Roxa (a) and Maringá and Ourizona (b), Paraná, Brazil (2015 season). Source: INMET - National Institute of Meteorology.

**Table 1.** Physical and chemical characteristics of the soils of the experimental areas and crop data in the experiments with the *B. amyloliquefaciens*-based product applied to soybean seeds. 2015 season.

Parameter	Francisco Alves	Ourizona	Terra Roxa	Maringá
<b>Physical and Chemical Characteristics of the Soil</b>				
pH (H <sub>2</sub> O)	4.8	7.1	6.1	6.0
CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	4.55	13.20	12.22	9.43
H <sup>+</sup> + Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	2.64	1.50	2.10	2.60
OM (mg dm <sup>-3</sup> )	10.54	2.80	2.90	2.80
Clay (g kg <sup>-1</sup> )	180	718	684	730
Silt (g kg <sup>-1</sup> )	20	225	213	218
Sand (g kg <sup>-1</sup> )	800	57	103	52
Textural class	Loamy-sandy	Very clayey	Very clayey	Very clayey
<b>Crop data</b>				
Sowing date	01/06/2015	01/29/2015	02/26/2015	03/03/2015
Variety	V-Max RR <sup>®</sup>	Monsoy 6210 IPRO <sup>®</sup>	Monsoy 6410 IPRO <sup>®</sup>	V-Max RR <sup>®</sup>
Spacing (m)	0.45	0.40	0.45	0.45
Density (seeds m <sup>-1</sup> )	18	16	17	14
Fertilization (kg ha <sup>-1</sup> )	250 (00-20-20)	280 (00-20-20)	300 (00-20-20)	250 (00-20-20)

OM, Organic matter.

the plant was measured, averaging across ten plants per experimental unit.

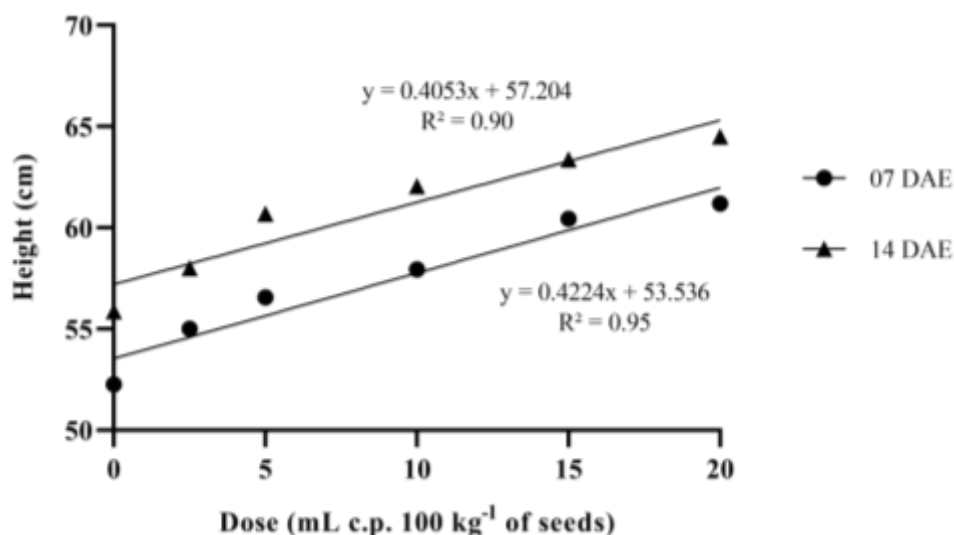
At 14 and 35 DAE, the dry weights of the roots and shoot were measured. For this evaluation, the soybean plants were carefully removed from the experimental units, separated into roots and shoots and placed in a forced-air oven for 72 h at an average

temperature of 65°C to obtain the dry weight of each material. Ten plants were used per experimental unit. The number of nodules was quantified at 14 and 35 DAE, counting the nodules present in the roots of the soybean plants. For this variable, ten plants were used per experimental unit, and the mean number of nodules for all plants sampled was used (Costa et al., 2013).

**Table 2.** Treatments and respective doses of a *B. amyloliquefaciens*-based product applied to soybean seeds. 2015 season.

Treatments	Dose	
	mL c.p. 100 kg <sup>-1</sup> of seeds	g a.i. 100 kg <sup>-1</sup> of seeds
1 Control	-	-
2 <i>B. amyloliquefaciens</i> strain MBI600 *	2.5	0.0045
3 <i>B. amyloliquefaciens</i> strain MBI600	5	0.009
4 <i>B. amyloliquefaciens</i> strain MBI600	10	0.018
5 <i>B. amyloliquefaciens</i> strain MBI600	15	0.027
6 <i>B. amyloliquefaciens</i> strain MBI600	20	0.036

\*Integral II SC (MBI 600). Concentration:  $2 \times 10^{10}$  viable spores mL<sup>-1</sup>.



**Figure 2.** Density of soybean plants at 7 and 14 DAE as a function of the application of different doses of a *B. amyloliquefaciens*-based product to soybean seeds (2015 season).

Regarding the yield, the soybean plants present in the useful area were harvested and threshed, and the moisture corrected to 13%. The production value obtained per experimental unit was converted to hectares, and the yield results were expressed in kg ha<sup>-1</sup>.

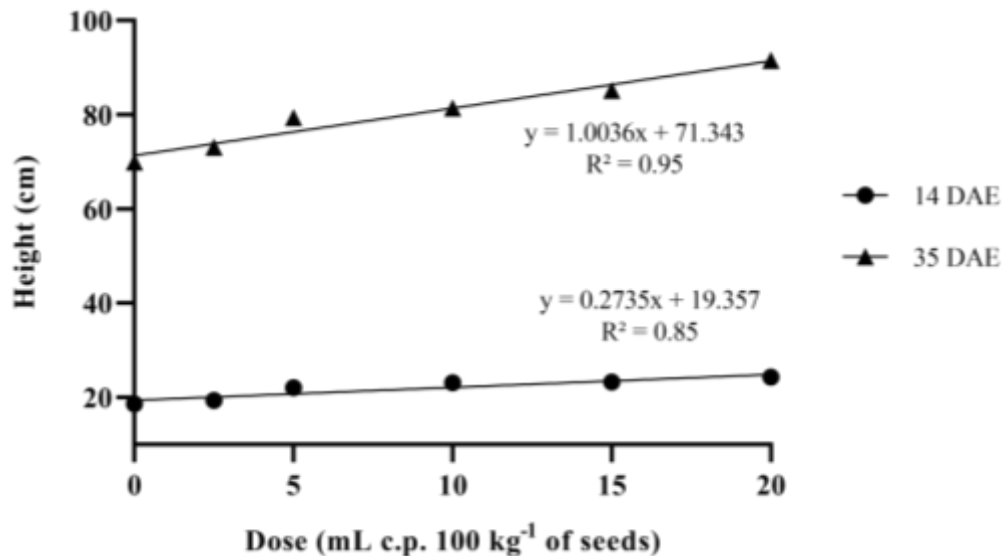
Statistical analysis was performed using the Sisvar software (Ferreira, 2011). After the end of the study period, the data were subjected to analysis of variance ( $p \leq 0.05$ ). In addition, a joint analysis of the experiments was carried out to assess whether there was any effect of the specific experimental site. When a significant effect of the doses evaluated was observed, the data were subjected to regression analysis ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

Joint analysis of the experiments showed similar behaviors for all response variables, with no effect of the experimental site observed. Therefore, the data from the different evaluation sites performed are presented together, and the means of the four experiments are shown in the graphs.

The plant population per hectare is directly related to the productive potential of the crop, as crop stand losses can lead to lower yields (Scheeren et al., 2010). In this sense, the use of products that benefit the initial development of soybean seedlings, ensuring adequate stand, is an intriguing tool for potential use in crop management. At 7 DAE of soybean, there was a positive linear effect of increasing doses of the *B. amyloliquefaciens*-based product on the plant stand variable (Figure 2). Comparing the increase in the plant stand generated by the highest dose of the *B. amyloliquefaciens*-based product to the control, in which the product was not applied to the seeds, there was a 15% increase in the overall plant population.

The behavior displayed in the first evaluation of the stand persisted until 14 DAE, with increasing dose of the *B. amyloliquefaciens*-based product again promoting an increase in the number of emerged seedlings. Based on the fitted equation, a dose of approximately 2.5 mL c.p. 100 kg<sup>-1</sup> of soybean seeds would be required to promote



**Figure 3.** Height of soybean plants at 14 and 35 DAE as a function of the application of different doses of a *B. amyloliquefaciens*-based product to soybean seeds (2015 season).

an increase of approximately one plant every 2 linear m.

The application of the *B. amyloliquefaciens*-based product to seeds did not produce toxic effects to soybean plants in any of the evaluations performed (data not shown). The evaluation of the potential for this biological product to have negative effects on the crop when applied to soybean seeds is essential; in the event of damage to plants, it is necessary to carry out new studies to evaluate if the injuries are caused by the formulation of the commercial product developed or the phytotoxic action of the bacteria.

Regardless whether the evaluation was performed at 14 and 35 DAE, increasing doses of the *B. amyloliquefaciens*-based product showed a linear relationship with the height of the soybean plants, with this variable increasing with increasing doses applied to seeds (Figure 3). The effect of the product on soybean plant height was so dramatically evident at 35 DAE that for each 1 mL of c.p. used 100 kg<sup>-1</sup> of seeds, there was an increase of 1 cm in this variable.

Plants with more aggressive root systems have a better capacity to explore the edaphic environment, mainly in the search for water and nutrients, in addition to being more tolerant to drought periods (Pitol and Broch, 2012). At 14 DAE, the increasing doses of the *B. amyloliquefaciens*-based product in the treatment of soybean seeds showed a linear relationship with root dry weight (Figure 4). In the evaluation at 35 DAE, a similar trend was observed, with higher doses producing higher soybean root weights.

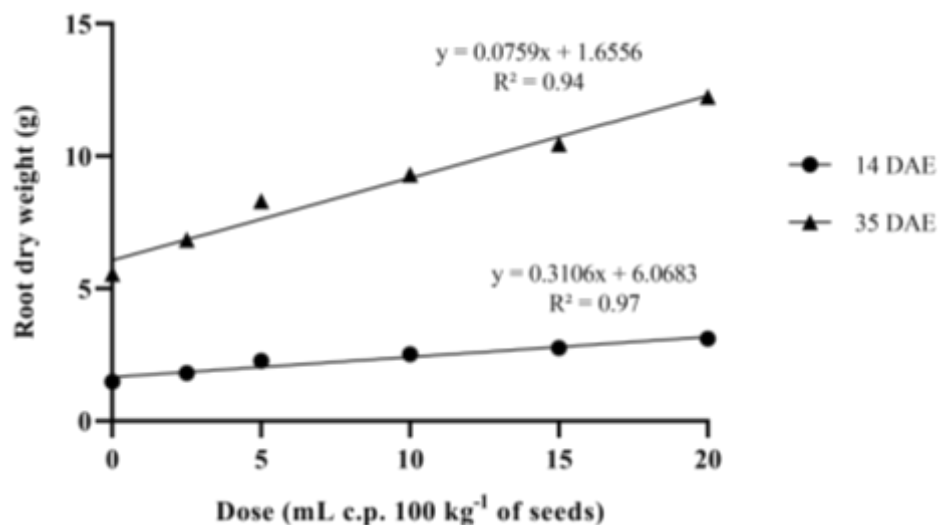
The increases promoted by the *B. amyloliquefaciens*-based biological product were not only restricted to the soybean root system; an increase was also observed in the shoots of the plants (Figure 5). The increase in shoot

dry weight was observed at both 14 and 35 DAE, with a mean increment of 5% observed relative to the control (without the product) for each mL of c.p. used in the treatment of soybean seeds.

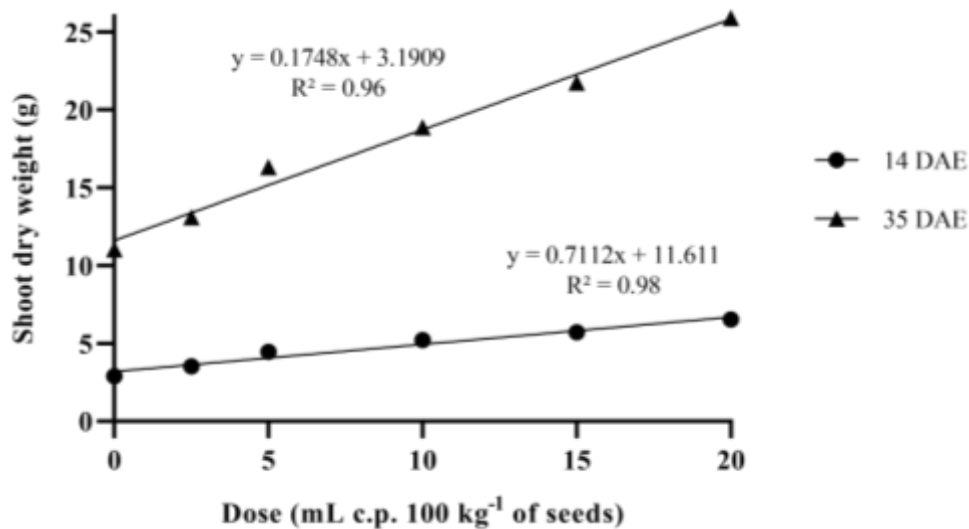
Another response variable positively influenced by the use of the *B. amyloliquefaciens*-based product (MBI 600) was the number of nodules (Figure 6). At 14 DAE, a linear relationship was observed, with an increase in the number of nodules as a function of the use of increasing doses of the biological product. This demonstrates that the use of *B. amyloliquefaciens*-based products can aid the soybean nodulation process, increasing the number of nodules and, consequently, making the biological nitrogen fixation (BNF) process more efficient (Costa et al., 2013).

At 35 DAE, there was a quadratic effect of increasing doses of the biological product on the number of nodules, with all doses within the range studied associated with increases in this variable relative to the control (Figure 6). This result indicates that there is potentially no competition among bacteria in the colonization of the rhizosphere environment of soybean plants. On the other hand, given the increase in the number of nodules of the root system of soybean plants, there is likely a positive interaction between *Bradyrhizobium japonicum* (responsible for BNF) and *B. amyloliquefaciens*. This behavior has been previously reported in soybean and common bean crops (Souza et al., 2008; Araújo et al., 2009).

All doses of the *B. amyloliquefaciens*-based biological product applied to the seeds provided an increase in soybean yield relative to the control (Figure 7). According to the fitted equation, the dose that provided maximum soybean yield was approximately 15 mL c.p. 100 kg<sup>-1</sup>



**Figure 4.** Root dry weight of soybean plants at 14 and 35 DAE as a function of the application of different doses of the *B. amyloliquefaciens*-based product to soybean seeds (2015 season).



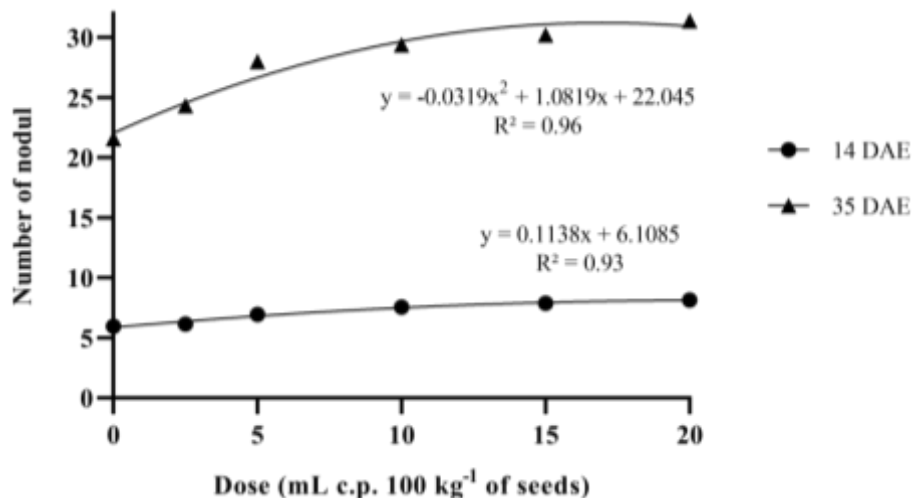
**Figure 5.** Shoot dry weights of soybean plants at 14 and 35 DAE as a function of the application of different doses of the *B. amyloliquefaciens*-based product to soybean seeds (2015 season).

seed. At this dose, the yield was close to 3,749.7 kg ha<sup>-1</sup>, an increase of approximately 16% above the control.

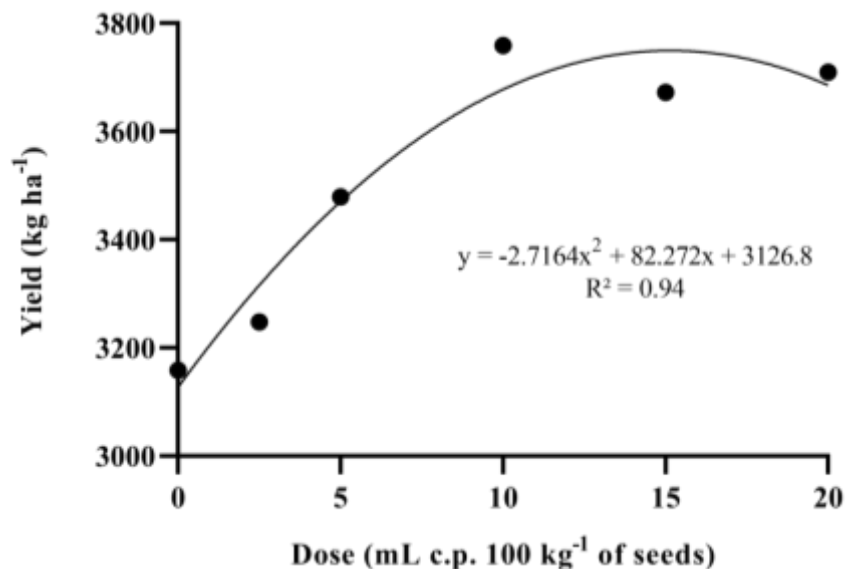
The increase in soybean yield promoted by the use of the *B. amyloliquefaciens*-based product on seeds can be explained by several factors, such as the increase in plant emergence, the increase in the number of nodules and greater development of the roots and shoots of the plants. A previous study also reported higher grain yields due to the use of rhizobacteria in soybean crops (Araújo and Hungria, 1999). Additionally, Kim et al. (2017) found

that the use of *B. amyloliquefaciens* in soybean crops provided tolerance to salt stress, demonstrating that the use of this bacterium can bring additional advantages in regions with salinized soils.

The results of the present study demonstrate that the advantages associated with the use of *B. amyloliquefaciens* (MBI 600) are not limited to those related to the biological control of other phytoparasitic microorganisms (Araújo et al., 2002), which in and of itself already represents a substantial benefit to soybean



**Figure 6.** Number of nodules in soybean plants at 14 and 35 DAE as a function of the application of different doses of the *B. amyloliquefaciens*-based product to soybean seeds (2015 season).



**Figure 7.** Soybean grain yield as a function of the application of different doses of the *B. amyloliquefaciens*-based product to soybean seeds. 2015 season.

management, but further includes the potential to generate higher crop yields. However, to ensure that the use of *B. amyloliquefaciens* (MBI 600) in soybean is successful, it is necessary to carry out additional studies to evaluate the selectivity of the phytosanitary products used in seed treatment procedures for this rhizobacteria.

## Conclusions

Under the conditions in which this study was conducted,

the following can be concluded:

- i) The use of increasing doses of a *B. amyloliquefaciens* (MBI 600)-based product, through seed treatment, promotes increases in plant stand, height, root and shoot dry weight and number of nodules per plant;
- ii) The benefits observed due to the use of the *B. amyloliquefaciens* (MBI 600)-based product resulted in an increase in soybean yield; and
- iii) All doses of the *B. amyloliquefaciens*-based product provided greater soybean yields compared to the control.

The dose that generated the maximum agronomic efficacy was approximately 15 mL c.p. 100 kg<sup>-1</sup> seed.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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