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# Técnicas de inoculação na soja para a região de Tangará da Serra - MT, Brasil

**Soybean inoculation techniques for the region of Tangará da Serra - MT, Brazil**

**Técnicas de inoculación de soja para la región de Tangará da Serra - MT, Brasil**

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**Resumo**

A utilização de microrganismos inoculados em sementes vem crescendo no Brasil, pois auxiliam na fixação e utilização de nutrientes pelas plantas proporcionando aumento de

produtividade, principalmente pela redução na aplicação de adubos nitrogenados. O objetivo deste trabalho foi avaliar a influência da inoculação de sementes com o uso de bactérias do gênero *Azospirillum brasilense* e *Bradyrhizobium japonicum*, de forma isolada, co-inoculada e com inoculação de cobertura em soja, na região de Tangará da Serra – MT, Brasil. O delineamento utilizado foi em blocos casualizados com 9 tratamentos 4 repetições, sendo utilizadas bactérias do gênero *B. japonicum* e *A. brasilense* em diferentes tratamentos e épocas de aplicação, no tratamento de sementes e em aplicação via foliar. O uso do *A. brasilense* juntamente com o *B. japonicum* melhorou significativamente as características agronômicas do cultivar avaliado. Houve um incremento na produtividade com o uso do *A. brasilense* por meio da técnica de co-inoculaçao quando comparado com o uso do *Bradyrhizobium* somente. O uso da co-inoculação em V3 proporcionou maior nodulação e produtividade que a inoculação padrão realizada nas sementes, garantindo melhores resultados de nodulação e produtividade além do incremento do tamanho do sistema radicular da soja, quando aplicado a técnica da co-inoculação.
**Palavras-chave:** *Azospirillum brasilense*; *Bradyrhizobium japonicum*; Inoculação; Co-inoculação; Re-inoculação.

**Abstract**

The use of inoculated microorganisms on seeds has increased in Brazil, as they help on the fixation and use of nutrients by plants, promoting increasing yields, mainly due to the reduction in the application of nitrogen fertilizers. The aim of this study was to evaluate the influence of single inoculation and co-inoculation of *Azospirillum brasilense* and *Bradyrhizobium japonicum* bacteria on seeds and foliar inoculation on soybean, in the region of Tangará da Serra – MT, Brazil. The experimental design used was a randomized block with 9 treatments and 4 replicates. The treatments were composed by using *B. japonicum* and *A. brasilense* bacteria in different combinations and times of application on seeds and sprayed on the leaves. The use of *A. brasilense* together with *B. japonicum* significantly improved the agronomic characteristics of the evaluated soybean cultivar. There was an increase in yield with the use of *A. brasilense* through the co-inoculation technique when compared to the single use of *Bradyrhizobium*. The use of co-inoculation at V3 soybean stage promoted higher nodulation and yield than the standard inoculation performed in the seeds, ensuring better results of nodulation and yield besides increasing the size of the soybean root system, when there and co-inoculation techniques were implemented.

**Keywords:** *Azospirillum brasilense*; *Bradyrhizobium japonicum*; Inoculation; Co-inoculation; Re-inoculation.

**Resumen**

El uso de microorganismos inoculados en semillas ha estado creciendo en Brasil, ya que ayudan en la fijación y el uso de nutrientes por parte de las plantas, aumentando la productividad, principalmente al reducir la aplicación de fertilizantes nitrogenados. El objetivo de este trabajo fue evaluar la influencia de la inoculación de semillas con el uso de bacterias del género *Azospirillum brasilense* y *Bradyrhizobium japonicum*, en forma aislada, re-inoculada y con la inoculación de la cubierta de soja, en la región de Tangará da Serra - MT, Brasil. El diseño utilizado fue en bloques aleatorizados con 9 tratamientos 4 repeticiones, utilizando bacterias del género *B. japonicum* y *A. brasilense* en diferentes tratamientos y tiempos de aplicación, en tratamiento de semillas y en aplicación foliar. El uso de *A. brasilense* junto con *B. japonicum* mejoró significativamente las características agronómicas del cultivar evaluado. Hubo un aumento en la productividad con el uso de *A. brasilense* a través de la técnica de co-inoculación en comparación con el uso de *Bradyrhizobium* solamente. El uso de la co-inoculación en V3 proporcionó una mayor nodulación y productividad que la inoculación estándar realizada en las semillas, garantizando mejores resultados de nodulación y productividad además del aumento en el tamaño del sistema de raíz de soja, cuando se aplicó la técnica de co-inoculación.

**Palabras clave:** *Azospirillum brasilense*; *Bradyrhizobium japonicum*; Inoculación; Co-inoculación; Re-inoculación.

## 1. Introduction

Soybean (*Glycine max* (L.) Merrill) production is among the most prominent agricultural activities in the global market, as it is the fourth most produced and consumed grain in the world, behind wheat, rice and maize, and the main oilseed crop cultivated (Lazzarotto & Hirakuri, 2010). One of the main factors that contribute to the success of its yield is the proper management of nutrition (Souza, 2016).

In this context, nitrogen is one of the nutrients most required by the crop, as 80 kg of N are necessary to produce 1,000 kg of grains, and nitrogen is considered crucial for crop production, because the grains are very rich in proteins, with an average protein content of 40% (Hungria et al., 2001). This element can be obtained from the soil, through the decomposition of organic matter, nitrogen fertilizers, chemical fixation of $N_2$ in electrical discharges, and also through biological fixation of $N_2$, which occurs by the symbiotic association with *Bradyrhizobium* spp. strains (Vieira Neto et al., 2008a).

From an ecological and economic point of view, this last-mentioned process is one of the most important. Currently, in Brazil, nitrogen fertilizers are not recommended for soybean crops, because the inoculation of seeds with bacteria of the genus *Bradyrhizobium* spp. provides virtually all the nitrogen required by the plant (Campos et al., 2001).

In general, Brazilian soils are originally free of $N_2$-fixing bacteria, which are capable of forming an effective symbiosis with soybean (Hungria et al., 1996). In this context, inoculations of nitrogen-fixing bacteria can play an important role in ensuring high yield at lower costs. There are other groups of beneficial microorganisms capable of promoting plant growth that are also capable of biologically fixing atmospheric nitrogen (Huergo et al., 2008), especially those belonging to the genus *Azospirillum*, which are used worldwide as inoculants in grasses (Braccini et al., 2016).

The use of bacteria of the genera *Azospirillum* and *Bradyrhizobium* with the technique of co-inoculation in legume crops substantially improves nodulation and root growth. The effect of *Azospirillum* improves the absorption of nutrients and water, besides having a beneficial hormonal effect on the plant, resulting in yield gains (Mendes et al., 2011).

Considering the current and potential limitations of biological nitrogen fixation with soybean and the benefits for various crops attributed to the inoculation with *Azospirillum brasilense*, it is inferred that co-inoculation with the two organisms and a supplementary foliar inoculation can improve the performance of root nodulation and consequently lead to gains in yield, taking an approach that respects the current demands of agricultural, economic, social and environmental sustainability (Hungria et al., 2013).

The aim of this study was to evaluate the influence of single inoculation and co-inoculation of *Azospirillum brasilense* and *Bradyrhizobium japonicum* bacteria on seed treatment and their foliar inoculation on soybean in the region of Tangará da Serra - MT, Brazil.

## 2. Material and Methods

A quantitative field research (Pereira et al., 2018), was carried out at the experimental field of the Mato Grosso State University (UNEMAT), Campus Professor Eugênio Carlos Stieler on the cropping season of 2017/2018. The area is located at the geographic coordinates

14˚ 65' 00" S, 57˚ 43' 15" W, with elevation of 440 meters, in the municipality of Tangará da Serra, Mato Grosso State, Brazil.

The region has two defined seasons, a dry season from May to September and a rainy season from October to April, with average annual values of air temperature, precipitation and relative humidity of 26.1 ºC, 1,830 mm and 70-80%, respectively (Dallacort et al., 2011). The experimental area has soil classified as clayey dystropheric Red Latosol (Oxisol) (EMBRAPA, 2017a). Its physicochemical characteristics are detailed in Table 1.

**Table 1.** Chemical characteristics of the soil at a depth of 0.0-0.20 m from the experimental area of the State University of Mato Grosso (UNEMAT), in Tangará da Serra, Brazil, before the experiment.

| pH | $Al^{3+}$ | $H^{+}+Al^{3+}$ | $Ca^{2+}+Mg^{2+}$ | $Ca^{2+}$ | $K^{+}$ | P |
|---|---|---|---|---|---|---|
| $H_2O$ | | | $Cmol_c\ dm^{-3}$ | | | $mg\ dm^{-3}$ |
| 4.8 | 0.75 | 5.28 | 0.56 | 0.35 | 0.05 | 0.8 |
| O.M. | CEC | | V | Sand | Silt | Clay |
| $g\ dm^{-3}$ | $Cmol_c\ dm^{-3}$ | | (%) | | $g\ Kg^{-1}$ | |
| 23.4 | 5.89 | | 10.36 | 273 | 137 | 590 |

Source: PLANTE CERTO - Análises de: Solo, Calcário, Água, Nematóide, Adubo, Ração, Sal e Tecido Foliar LTDA, Várzea Grande – MT.

The levels of pH and nutrients were corrected based on the needs and interpretation of the soil analysis of the site according to Table 1, with the aid of the Manual of Soil Correction and fertilization described by Souza & Lobato (2004). Liming was performed 65 days prior to sowing, using 2.84 tons $ha^{-1}$ of dolomitic limestone filler with 103% relative power of total neutralization (PRNT). Corrective fertilization was performed in the planting furrow, using a seeder-fertilizer machine at spacing of 0.45 m, with doses of 200 kg $ha^{-1}$ of $P_2O_5$ and 100 kg $ha^{-1}$ of $K_2O$ split ($K_2O$ - 50% at sowing and 50% at the broadcasted fertilization). For the treatment with nitrogen fertilization (T2), the N dose of 200 kg $ha^{-1}$ was also split, 60% at sowing and 40% at the broadcasted fertilization. The top-dressing applications of N and $K_2O$ were performed at the soybean phenological stage V6.

The experimental design of this study was randomized blocks, with 9 experimental treatments (Table 2) and four replicates, and the plots consisted of 6 planting rows, at spacing of 0.45 m, with 15 plants per meter and length of 6 meters, totaling 16.2 $m^2$ per plot.

**Table 2.** Detailed description of the treatments used in the experiment.

| | |
|---|---|
| T1 | Without inoculant and without Nitrogen |
| T2 | 200 kg $ha^{-1}$ of nitrogen (N) |
| T3 | Inoculation of *B. japonicum* in the seeds |
| T4 | Co-inoculation of *B. japonicum and A. brasilense* in the seeds |
| T5 | Inoculation of *B. japonicum* in the seeds + foliar inoculation of *B. japonicum* |
| T6 | Inoculation of *B. japonicum* in the seeds + foliar inoculation of *A. brasilense* |
| T7 | Co-inoculation of *B. japonicum* and *A. brasilense* in the seeds + foliar inoculation of *B. japonicum* |
| T8 | Co-inoculation of *B. japonicum* and *A. brasilense* in the seeds + foliar inoculation of *A. brasilense* |
| T9 | Co-inoculation of *B. japonicum* and *A. brasilense* in the seeds + foliar co-inoculation of *B. japonicum* and *A. brasilense* |

Source: Authors.

The total area of the experiment was 583.2 m². The experimental area for the analysis and evaluations were the 4 central rows of each plot with length of 4 m (7.20 m²).

The soybean cultivar used in the experiment was TMG 2181 IPRO, which has medium cycle, determinate growth and belongs to the 8.1 maturity group. Sowing was performed on November 25, 2017, using a manual seeder (KNAPIK brand) for large grains.

The seeds were previously treated with contact and ingestion insecticide and fungicide of protective action, and then received the inoculants according to Table 2.

The inoculations in the seeds were performed in plastic bags using 1 kg of seed per treatment. After that, an inoculation additive for seed treatment and inoculants were added according to doses that guaranteed at least one million and two hundred thousand bacteria per seed, followed by stirring for 4 minutes for better a coating of the seeds.

The inoculants used were Brasilec TAS On-Farm® (*Bradyrhizobium japonicum*, SEMIA 5079 and SEMIA 5080) with guarantee of 6x109 CFU/mL and Grammy Crop® (*Azospirillum brasilense*, AbV5 and AbV6) with guarantee of 2x108 CFU/mL for seed treatments. Foliar application was performed using the inoculant Aladin Plus Nod+® (*Bradyrhizobium japonicum*, SEMIA 5079 and SEMIA 5080) with guarantee of 6x109 CFU/mL and Grammy Crop® (*Azospirillum brasilense*, AbV5 and AbV6), products provided by the company Forquímica Agrociência LTDA®.

Foliar inoculations at the soybean´s vegetative stage V3 were performed using a dose of liquid inoculants *A. brasilense* and *B. japonicum* that guaranteed three times the dose recommended per seed per hectare, in a volume of 150 L $ha^{-1}$. A $CO_2$-pressurized backpack sprayer, equipped with a bar with 6 fan Tip-type nozzles, under pressure of 2.5 kgf cm$^{-2}$, was used for the foliar inoculation treatments and phytosanitary applications.

Two applications of the foliar fertilizer Genium Plus were performed at the stages V3 and R1, at doses of 0.3 L ha$^{-1}$ to supply Mo, Co, Zn and S and enhance biological N fixation, besides the application plant polymers (W/W: 3.00% and W/V: 34.50 g L$^{-1}$), which enhance plant metabolism.

Inoculation and co-inoculation in the seeds were carried out in the shade, and the inoculated and co-inoculated seeds were left to dry for about 20 minutes and kept protected from the sun and excessive heat according to the method recommended by Embrapa (2017b). Phytosanitary treatments (fungicides, herbicides and insecticides) were applied according to needs of the crop previous inoculation.

Evaluations were carried out at three different stages of soybean: R1, R6 and at harvest. Number of nodules, mass of nodules and root length were evaluated at the soybean phenological stage R1, whereas number of pods, numbers of grains per pod, first pod height, plant height and number of nodes were evaluated at R6. At harvest, the evaluated parameters were yield (Y, kg ha$^{-1}$) and 1000-grain weight (1000GW, g), as described in Fipke (2015). For the evaluations at R1 and R6, 10 plants were randomly harvested between the usable area and borders of the plot, by carefully using a square-point shovel so that the root system was not affected.

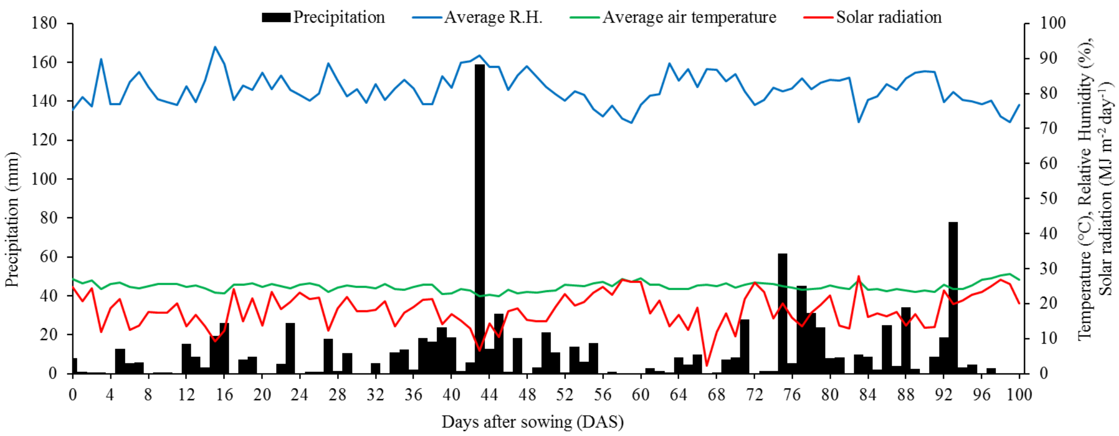
The following segment describes in detail how each evaluation was carried out according to the methodologies of Fipke (2015) and Souza (2016). Number of nodules per plant: Roots were washed, and their nodules were later removed and counted; Mass of nodules: After the nodules were removed and counted, they were weighed on a precision scale; Plant height: Determined by measuring the distance between the collar and the apex of the main stem; Root length: Determined as the distance between the plant collar and the tip of the root system; First pod height: Measured as the distance between the plant collar and the insertion of the first pod; Number of pods per plant and number of grains per pod: Determined by counting the total number of grains per plant and dividing this number by the total pods of the plant; Grain yield: All plants from the 4 central rows and 4 m away from each plot were collected, and then all the grains of each plot were mechanically threshed using a mechanical grain threshing machine, weighed, and their moisture contents were subsequently determined using a grain moisture meter device from Mini Gac, with data corrected to 13% moisture and transformed to kg ha$^{-1}$; 1000-grain weight (1000GW): The weight of 1000 grains was obtained by weighing 8 samples of 100 grains for each plot on a precision scale, following the same correction procedure described above.

The data of the production components were subjected to analysis of variance (ANOVA) by the F test, and the means were compared by the Scott-Knott test at 5% probability level. Data analysis was performed using the computer program SISVAR version 5.6 (Ferreira, 2011).

## 3. Results and Discussion

The daily values of precipitation and data of average air temperature, relative humidity and solar radiation for the period from sowing to harvest can be seen in Figure 1.

**Figure 1.** Precipitation, average air temperature, relative humidity and global solar radiation during the experimental period in the municipality of Tangará da Serra - MT, in the 2017/2018 harvest, between 11/25/2017 and 03/05/2018.




Source: INMET (Instituto Nacional de Meteorologia). Authors.

It is important to note that during the experimental period, the accumulated precipitation was 1058.93 mm considering the entire period of cultivation. The average air temperature, average relative humidity and average daily global solar radiation along the experimental period were 24.9 °C, 81.3% and 18.2 MJ $m^{-2}$ $day^{-1}$, respectively (Figure 1).

For growth and development, soybeans are required to have an optimal air temperature range between 20 ºC and 30 ºC, and ideal conditions of water availability between 450 to 800 mm distributed during the cycle (Farias et al., 2007). The climatic conditions of air temperature water availability and solar radiation in this study met the requirements of the crop as seen in Figure 1.

The results obtained in this study show significant differences between treatments for plant height, root length, number of nodules, mass of nodules, and first pod height (Table 3).

**Table 3.** Mean values for plant height (PH), root length (RL), number of nodules (NN), mass of nodules (MN), number of nodes (NNS) and first pod height (FPH), as a function of the treatments used in Tangará da Serra - MT, in the 2017/2018 harvest.

| Treatments | Mean squares of evaluated parameters | | | | | |
|---|---|---|---|---|---|---|
| | PH (cm) | RL (cm) | NN (nº) | MN (g) | NNS (nº) | FPH (cm) |
| T1 | 68.1e | 18.8f | 20.7e | 0.26d | 8.66$^{ns}$ | 13.2d |
| T2 | 74.2c | 20.1e | 7.0f | 0.13e | 10.66$^{ns}$ | 14.7c |
| T3 | 77.2b | 21.3d | 29.5d | 0.40c | 10.08$^{ns}$ | 13.4d |
| T4 | 71.2d | 26.6c | 30.5d | 0.33c | 9.83$^{ns}$ | 14.1c |
| T5 | 76.0b | 21.0d | 48.0b | 0.46b | 9.83$^{ns}$ | 15.4b |
| T6 | 75.6b | 28.1b | 31.2d | 0.36c | 9.75$^{ns}$ | 14.2c |
| T7 | 82.4a | 26.2c | 59.0a | 0.43b | 9.75$^{ns}$ | 16.4a |
| T8 | 76.5b | 29.6a | 40.0c | 0.36c | 9.33$^{ns}$ | 15.6b |
| T9 | 83.2a | 28.0b | 59.5a | 0.56a | 10.50$^{ns}$ | 16.8a |
| CV (%) | 1.34 | 1.81 | 7.13 | 9.12 | 9.00 | 2.74 |

Means followed by different lowercase letters in the same column differ by the Scott-Knott test at 5% probability level. Treatments: T1: No inoculant and without Nitrogen, T2: 200 kg ha$^{-1}$ of N, T3: Inoculation of *B. japonicum* in seeds, T4: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds, T5: Inoculation of *B. japonicum* in seeds + foliar inoculation of *B. japonicum*, T6: Inoculation of *B. japonicum* in seeds + foliar inoculation of *A. brasilense*, T7: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds + foliar inoculation of *B. japonicum*, T8: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds + foliar inoculation of *A. brasilense*, T9: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds + foliar co-inoculation with *B. japonicum* and *A. brasilense*. CV = Coefficient of variation. Source: Authors.

As for plant height, the treatments that promoted the highest plans were the T7 and T9. The benefits observed for the plant height are related to different types of secondary compounds produced by the bacteria including exopolysaccharides, plant hormones and lipo-chitooligosaccharides. The *Azospirillum* ssp. bacteria has the capability to synthetize growing promoters in which the hormones Indole acetic acid and gibberellins have the most important roll (Reis Junior et al., 2008; Marks et al., 2013).

This compounds production certainly altered the plant physiology altering its metabolism promoting higher growth of the whole plant and at the same time promoting a better plant nutrition and water absorption. The average number of nodules per plant, in treatments involving inoculation only, as well as, co-inoculation with *A. brasilense* were higher than 29.5 (T3), corroborating the data obtained by Câmara (2000). The same author reveals that plants with 20 to 30 nodules at flowering have sufficient conditions to obtain high levels of fixed nitrogen and, consequently, high grain yield.

When the numbers of nodules (NN) were analyzed, the treatments used in this experiment followed the same results observed for the PH. The average number of nodules per plant were higher in treatments T7 and T9. When comparing these treatments only with inoculation via seed T3, it was observed an increase on the number of nodules in the order of 100% (Table 3). As the number of nodules increases, greater biological nitrogen fixation and, therefore, greater plant development and higher yields were obtained (Table 3).

As *B. japonicum* was re-inoculated through the leaves, according to Table 3, there was an even greater increase in the number of nodules and mass of nodules. as in the means obtained in treatments 9, 7 and 5, which were equal to 59.5, 59 and 48 nodules per plant and to 1.7, 1.3 and 1.4 grams of nodules every three plants, respectively. Such increment was also obtained using *A. brasilense* applied on the leaves, which led to higher values of root system size in treatments 8, 6 and 9, with means of 29.6, 28.1 and 28 cm of root system, respectively. This increase in root system size can help the plant withstand adverse situations such as prolonged drought, as well as improving nutrient absorption from deeper soil layers.

The use of *B. japonicum* together with *A. brasilense* through foliar re-inoculation technique proved to be feasible, due to the best results obtained in T9. Nitrogen fertilization (T2) promoted a reduction in nodulation parameters (NN and MN) and, therefore, these results corroborate those obtained in several other locations in Brazil (Hungria et al., 1996; Martins et al., 2003; Bárbaro et al., 2009). However, if nitrogen-containing fertilizer formulations are more economical than nitrogen-free formulations, they can be used if no more than 20 kg $ha^{-1}$ of N is applied (EMBRAPA, 2009).

The values obtained for first pod height and plant height are consistent with the values recommended by Ormond et al. (2015), i.e., 13 and 65 cm, respectively, which are optimum values to avoid losses during soybean harvest. As soybean harvest is fully mechanized, the first pod height is an important characteristic to reduce losses at harvest and, according to the treatments, these values can be considered favorable to the system (Lima, 2009).

Regardless of the form of application of the inoculant, it is known that the gains in yield resulting from inoculation in soils in the first year of cultivation are more significant than those obtained in areas previously cultivated with soybean (Campos & Gnatta, 2006; Vieira Neto et al., 2008b). Nevertheless, average gains of 4.5% in grain yield have been observed with inoculation in areas cultivated with this legume crop (EMBRAPA, 2013). This can be explained by the fact that the strains from the inoculant are fully active, while strains of the same species, or native, which are in the soil may be in a latent state.

Table 4 shows the number of pods per plant, number of grains per plant, number of grains per pod, 1000-grain weight and estimated yield. As it can be verified on Table 4 all treatments tested as yield components showed significative statistical differences.

**Table 4.** Average values for number of pods per plant (NPP), number of grains per pod (NGP), number of grains per plant (NGPL), 1000-grain weight (1000GW) and estimated yield (Y) as a function of treatments used in Tangará da Serra - MT, in the 2017/2018 harvest.

| Treatments | Means of evaluated parameters | | | | |
|---|---|---|---|---|---|
| | NPP (nº) | NGP (nº) | NGPL (nº) | 1000GW (g) | Y (kg ha$^{-1}$) |
| T1 | 57.13d | 2.37c | 135.65d | 132.8b | 2483d |
| T2 | 58.91d | 2.44b | 143.46d | 135.5b | 2936c |
| T3 | 64.25c | 2.39c | 153.25c | 133.8b | 3058c |
| T4 | 67.66c | 2.34d | 158.21c | 136.1b | 3075c |
| T5 | 67.66c | 2.50a | 169.46b | 139.3a | 3519b |
| T6 | 71.66b | 2.37c | 169.80b | 141.0a | 3521b |
| T7 | 79.66a | 2.31d | 184.35a | 133.6b | 3690a |
| T8 | 72.66b | 2.40c | 174.05b | 136.1b | 3526b |
| T9 | 79.91a | 2.32d | 185.57a | 135.8b | 3701a |
| CV (%) | 2.75 | 0.76 | 4.47 | 2.54 | 3.55 |

Means followed by different lowercase letters in the same column differ by the Scott-Knott test at 5% probability level. Treatments: T1: No inoculant and without Nitrogen, T2: 200 kg ha$^{-1}$ of N, T3: Inoculation of *B. japonicum* in seeds, T4: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds, T5: Inoculation of *B. japonicum* in seeds + foliar inoculation of *B. japonicum*, T6: Inoculation of *B. japonicum* in seeds + foliar inoculation of *A. brasilense*, T7: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds + foliar inoculation of *B. japonicum*, T8: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds + foliar inoculation of *A. brasilense*, T9: Co-inoculation of *B. japonicum* and *A. brasilense* in seeds + foliar co-inoculation with *B. japonicum* and *A. brasilense*. CV = Coefficient of variation. Source: Authors.

Regarding the number of pods per plant and number of grains per plant, the treatments with the highest numbers were T9 and T7. The increase in the number of pods per plant also resulted in the significant increase in the number of grains per plant, thus leading to higher yields. The use of *B. japonicum* inoculated and reinoculated via foliar and co-inoculated with *A. brasilense* increases the number of pods by plants and the number of grains per plant.

The increase of yield resulted from these treatments (T7 and T9) may be resulted from the increment of levels of phytohormones on the plant, principally cytokinins and also because the bigger quantities of nodules which offered higher quantities of N to the plants.

Several diazotrophic bacteria are able to synthesize phytohormones. Furthermore, when a nitrogen fixing microorganism is inoculated into a rhizosphere, interactions with other microorganisms can influence its activities, production of hormones, such as auxins, cytokinins, gibberellins and ethylene (Tien et al., 1979; Cacciari et al., 1988; Bottini et al., 1989; Strzelczyk et al., 1994).

In the soybean crop, a large number of flowers are aborted during their development due to several environmental as well as physiological factors.

According to Jameson & Song (2016) hormones such as cytokinins directly affect cell differentiation as well as the growth of fruit and seed plants in addition to plant senescence. The presence and application of cytokinins can prevent floral abortion and thus increase crop production (Nonokawa et al., 2012).

The 1000GW on this work were higher for the treatments T5 and T6. However, the average numbers of pods per plant and number of grains per plant of these treatments were lower than those of T7 and T9, which resulted in lower yield when compared to these treatments.

Barzotto (2015) observed in his study that soybean plants under conditions of nutritional deficiency abort flowers and reduce the number of pods and grains per plant and, when some type of nutritional replacement is performed, their pods will produce larger grains due to the reduced number, but their yield will still be reduced.

The inoculation and re-inoculation with *B. japonicum* (T5) also promoted a higher number of grains per pod. Even having a higher 1000W and NGP, that treatment was inferior when compared with T7 and T9 in yield. The importance of interaction of the two microorganisms can be observed on this work.

Meert et al. (2018) also found an increase in the number of grains per pod when they used the microorganisms *A. brasilense* and *B. japonicum* with an average of 2.77 grains per pod. On the other hand, in the work of Bulegon et al. (2016) the number of grains per pod were not altered by the treatments, thus showing that differences between cultivars, doses of N studied and other experimental factors can vary responses to inoculation and co-inoculation of microorganisms.

Regarding the efficiency of the treatments, Fipke (2015) corroborates the data of the present study for higher yields, demonstrating that associations of biological agents are efficient for soybean crop, since it positively responds to the association of the microorganisms *B. japonicum* and *A. brasilense*.

The use of *A. brasilense* was more efficient when applied on the leaves, as seen in the comparison of T4 with T6, T4 being the application via seed treatment and T6 being the foliar application. T1 obtained the lowest yields, since it did not receive any microorganisms through application in the treatment of seeds and leaves and no nitrogen supplementation.

Thus, it was found that the association of *B. japonicum* + *A. brasilense* in seed treatment and/or foliar applications may contribute to increasing the yield of soybean crop in terms of both yield and root size, whether applied via seeds or sprayed on the crop. It is worth remembering that the use of bacteria in seed treatment together with foliar re-inoculation led to better results.

Although T5, T6 and T8 were statistically equal in terms of yield according to the means comparison test, the treatments T9 (application of *B. japonicum* + *A. brasilense* at sowing and at the vegetative stage V3) stood out with one of the highest averages of yield and of the other characteristics evaluated. This treatment had higher averages than the others for both yield and almost all agronomic characteristics evaluated, hence showing viability in the use of bacteria re-inoculation in soybean crops.

Although inoculation is a frequent activity used by farmers, there may be failures in the nodulation of plants in the field, especially when it comes to areas of first cultivation of soybean, which may affect grain yield. However, the effective establishment of the symbiosis between plants and elite strains of bacteria promoting nitrogen fixation and growth is critical for high soybean yields.

For this, it is essential to use inoculants within the expiration date, with a minimum amount of viable cells, perform sowing with adequate water availability to ensure seed germination and seedling emergence, and also treat the seeds with fungicides that are compatible with bacteria, as well as other techniques suggested by research (Braccini et al., 2016).

The results of this study are corroborating with the aforementioned authors.

New technologies are being integrated in to the daily routines of soybean growers around the world. It can be observed in this study, that the use of inoculation and co-inoculation of the microorganisms *B. japonicum* and *A. brasilense* used correctly increased the yield of the soybean crop which can bring benefits for growers.

## 4. Final Considerations

The use of *Azospirillum brasilense* together with *Bradyrhizobium japonicum* significantly improved the agronomic characteristics of soybeans. There was an increase in yield with the use of *A. brasilense* through the co-inoculation technique when compared to the use of *B. japonicum*, demonstrating that there may be an indirect increment of yield, mainly because there is a significant increase in the root system.

The use of the co-inoculation technique with *B. japonicum* strains (SEMIA 5079 and 5080) recommended for the soybean crop, associated with *A. brasilense* strains in combination (AbV5 and AbV6), ensure higher yields.

The use of re-inoculation at V3 promoted higher nodulation and yield than the standard inoculation performed on the seeds. Thus, post-emergence co-inoculation ensures better results of nodulation and yield besides increasing the size of the root system for soybean crop, when the co-inoculation technique is applied. Therefore, the association of *B. japonicum* + *A. brasilense* at sowing and re-inoculation at the vegetative stage V3 proved to be efficient as an alternative nutritional management for soybean crop.

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