Ciência Agrícola, Rio Largo, v. 20, n. 1, p. 19-24, 2022

APPLICATION OF NITROGEN AND *BRADYRHIZOBIUM* SP. INOCULATION IN SOYBEAN (*Glycine max* L.) CROPS IN THE NORTHERN REGION OF RIO GRANDE DO SUL

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ABSTRACT: This study aimed to evaluate the yield components, the morpho-agronomical parameters, and the final yield of the soybean crop, after the use of inoculant and nitrogen fertilization in isolation or in the combined form. Four treatments were used: treatment 1 - control without any application; treatment 2 - application of liquid inoculant based on *Bradyrhizobium* bacterium moments before sowing; treatment 3 - application of 46 kg of nitrogen per hectare in the phenological stage of R3; treatment 4 - application of liquid inoculant based on *Bradyrhizobium* bacterium moments before sowing and application of 46 kg of nitrogen per hectare in the phenological stage of R3. Regarding the parameters evaluated, it was observed that treatments 2, 3 and 4 allow greater plant height, greater number of pods/plant, higher SMP and higher productivity. In addition, it was found that the association between *Bradyrhizobium* and mineral nitrogen caused significant increase in the number of pods per plant compared to the other treatments. Another important data observed was that the use of nitrogen fertilization may be more efficient than inoculation, but it may increase the production cost disproportionately to the productive response. Therefore, it was concluded that inoculation is the most viable alternative for the supply of N to soybean crop.

KEYWORDS: Nitrogen fertilization. Biological nitrogen fixation. Inoculant.

APLICAÇÃO DE NITROGÊNIO E INOCULAÇÃO DE *BRADYRHIZOBIUM* SP. NA CULTURA DA SOJA (*Glycine max* L.) NA REGIÃO NORTE DO RIO GRANDE DO SUL

RESUMO: Objetivou-se avaliar a altura das plantas, os componentes de rendimento e o rendimento final da cultura da soja, após a utilização de inoculante e de fertilizante nitrogenado de maneira isolada ou conjunta. Para isso, foram utilizados quatro tratamentos: Tratamento 1: testemunha, sem inoculante e sem fertilizante; Tratamento 2: aplicação de inoculante líquido a base da bactéria *Bradyrhizobium* momentos antes da semeadura; Tratamento 3: aplicação de 46 kg de nitrogênio por hectare no estádio fenológico de R3; e Tratamento 4: aplicação de inoculante líquido a base da bactéria *Bradyrhizobium* momentos antes da semeadura + aplicação de 46 kg de nitrogênio por hectare no estádio fenológico de R3. Em relação aos parâmetros avaliados, observamos que os tratamentos 2, 3 e 4 permitiram maior estatura de planta, maior número de pods/planta, maior PMS e maior produtividade. Além disso, constatou-se que a associação entre *Bradyrhizobium* e nitrogênio mineral propiciou um aumento significativo no número de pods por planta comparado aos demais tratamentos. Ressalta-se que a utilização de fertilizantes nitrogenados pode ser mais eficiente do que a inoculação, porém pode elevar o custo da lavoura de maneira não proporcional à resposta produtiva. Dessa forma, conclui-se que a inoculação é a alternativa mais viável no suprimento da demanda de N na cultura da soja.

PALAVRAS CHAVE: Fertilizantes Nitrogenados, Fixação Biológica de Nitrogênio, Inoculante.

INTRODUCTION

Soybean crop, *Glycine max* L., is of significant economic importance for Brazil, being considered the main agricultural commodity, representing more than 50% of the total grains produced. The total soybean production has been increasing year after year, with productivity gains and increase/expansion of the cultivated area (Conab, 2020a). Its estimated production for the 2020/2021 harvest is 141.2 million tons, with cultivation area reaching 39.91 million hectares (Conab, 2020b).

Genetic improvement constantly seeks to adapt soybeans to different planting locations and increase crop productivity (Embrapa, 2011). This increase in productivity is associated, among other factors, with the increase in nutritional requirements. The launch of new cultivars with high yields has raised doubts about the need to use nitrogen fertilization to complement nutrition (Barranqueiro and Dalchiavon, 2017; Moreno et al., 2018).

Nitrogen (N) is one of the main components of proteins, one of the most important and abundant biomolecules for plants. This element comes from the soil and is converted into mineral through existing organic matter. Other sources of N are obtained from inorganic forms, such as ammonium and ammonia (Tisdale et al., 2005). The levels of N present in plants are between 3 to 5% of the total dry matter, and some symptoms of the lack of this nutrient in plants include reduction in plant height, number of flowers and fruits, and also chlorosis of photosynthetic tissues (Faquin, 2005).

Soybean obtains most of the N it needs for development through biological nitrogen fixation (BNF), a process that occurs through the symbiotic association of plant roots with bacteria of the genus *Bradyrhizobium* (Vitti and Trevisan, 2000). Since pods, which include soybeans, have the ability to fix atmospheric N through BNF, it is important to stimulate this process through the use of inoculants containing *Bradyrhizobium*, as this provides increase in soybean yield. Inoculation with *Bradyrhizobium* is able to ensure the supply of N necessary for the crop, even at high productivity levels (Nogueira, 2019).

This biological N fixation by leguminous plants can supply mineral fertilization, depending on the species and cultivation system, however, studies report that fixation can drop rapidly when the soybean crop reaches the R5 growth stage (Zilli et al., 2008). The great demand of soybeans for N should be highlighted, where more than 80 kg of the nutrient are needed for each ton of grain produced (Seixas et al., 2020).

In studies on the influence of nitrogen fertilization in relation to yield components and morphological characters of soybean, it was observed that increasing the N dose to a certain concentration increases productivity and the number of pods per plant (Petter et al., 2012), as well as the mass of one thousand grains (Marcon et al., 2017). In the plant height characteristic, it was observed that there was no variation with different nitrogen sources (Bahry et al., 2013; Marcon et al., 2017). On the other hand, other studies have reported that the use of nitrogen fertilization can reduce the BNF efficiency (Aratani et al. 2008; Kaschuk et al., 2016; Zuffo et al., 2019).

In the search for better answers about nitrogen application and inoculation of soybean with nitrogen-fixing bacteria, the aim of this study was to evaluate plant height, yield components and final soybean yield, after the use of inoculant containing *Bradyrhizobium* and nitrogen fertilization, alone or in association.

MATERIAL AND METHODS

This work was carried out in an experimental field located in the municipality of Coxilha, Rio Grande do Sul, with the following geographic coordinates 28° 10' 21'' S and 52° 18' 37'' W, in the 2018/2019 agricultural year. The predominant soil in the area is the red Latosol, the climate is the subtropical type, with rigorous winter and high temperatures in the summer. The average annual temperature is 17.5 C° and the average annual rainfall is 1,787.8 mm (SOMAR).

The area where the experiment was implemented had cultural remains of black oat, which was harvested to fractionate the straw and facilitate the sowing operation. Test sowing was carried out in the first week of December 2018, and seeds were inoculated on the same date. The implantation was carried out with specific seeder, where each plot was composed of four rows, five meters in length each, spaced 50 centimeters, and with width of one meter between each plot, totaling 20 m^2 for each plot. The design used was randomized blocks (RBD), composed of four treatments (Table 1) and four replicates, totaling 16 plots/experimental units.

Table 1. Experimental treatments evaluated in the study. Coxilha-RS, 2019.

Treatments	Inoculant [1]	Nitrogen [2]
Treat. 1	0 ml/ha^{-1}	0 kg/ha^{-1}
Treat. 2	100 ml/ha^{-1}	0 kg/ha^{-1}
Treat. 3	0 ml/ha^{-1}	46 kg/ha^{-1}
Treat. 4	100 ml/ha^{-1}	46 kg/ha^{-1}

[1]The inoculant used had concentration of 5x109 CFU.mL^{-1} and was applied on the day of sowing.

[2]Use of 100kg.ha^{-1} of nitrogen source in the form of urea (46% of N) in topdressing in the R3 vegetative stage.

The cultivar used was Brasmax® Alvo. The plant population per hectare followed the cultivar recommendation (Brasmax Genética, 2021). For fertilization, NPK 04-23-20 formulation was used, in the amount of 300 kg.ha^{-1}, and 20 days after sowing, cast topdressing application of potassium chloride (KCl) at dose of 100 kg.ha^{-1} was performed.

The times of use of inoculant and/or nitrogen fertilization are described in Table 1. Inoculant applications were performed moments before sowing and nitrogen applications were carried out when soybean was in the R3 reproductive stage, in which N demand is greater.

During the crop cycle, some phytosanitary treatments were carried out, including the use of imidaclorid at dose of 0.400 mL/ha and fungicides based on trifloxystrobin and prothioconazole at dose of 0.400 mL/ha preventively applied to control fungal diseases. Treatments were manually applied with backpack sprayer in the same spray dosage, with volume of 165000 mL/ha.

Evaluations were carried out at the physiological maturity stage, that is, between stages R7 and R8, which took place in April 2019. The harvest took place in the two central rows of each experimental unit, totaling useful area of 10m^2. The following evaluations were carried out: a) plant height, defined as the distance (cm) from the ground level to the plant apex; b) number of pods, using the average of 40 random plants in the plot; c) number of grains per pod of 40 random plants within each experimental unit; d) grain yield in kg.ha^{-1} and; e) weight of one thousand seeds (PMS).

Results were submitted to analysis of variance, and if difference between treatments was found, the test of means comparison proposed by Tukey was performed at 5% error probability, with the aid of the Sisvar 5.6 statistical software (Ferreira, 2019).

RESULTS AND DISCUSSION

According to data obtained, the height of plants supplemented with nitrogen fertilization (Treatment 3) and with inoculant + nitrogen fertilization (Treatment 4) was greater in relation to the height of control plants (Treatment 1) and in relation to plants supplemented only with inoculant (Treatment 2); however, the height of plants in treatment 2 was greater than in Treatment 1 (Table 2). The greater height of plants in these treatments is possibly due to the fact that the soybean cultivar selected for this study has indeterminate growth habit, which may justify that the application of nitrogen fertilization has stimulated growth in height, as observed by Parente et al. (2015). In addition, bacteria such as *Bradyrhizobium* sp., produce phyto-hormones that help in plant development, which possibly showed positive response in increasing the height of inoculated plants (Manteli et al., 2019). In disagreement, Meert et al. (2020) observed that when mineral N supplementation is performed in soybeans at V3 vegetative stage, there is lower plant growth, which is probably due to the inhibition of the number of nodules per plant (Zuffo et al., 2019). It should be observed that the exaggerated growth in height can favor plant lodging, which is an undesirable condition, since lodging can lead to reduced productivity and losses related to the performance of phytosanitary management and harvest (Souza et al., 2013).

Table 2. Average of variables a) plant height; b) number of pods per plant; c) number of grains per pod and; d) weight of one thousand grains, as a function of the different treatments. Coxilha-RS, 2019.

Treatments	Height (cm)	Pods/Plant (un)	Grains/Pod (un)	PMS (grams)
Treat. 1	80.75 A	51.62 A	2.69 A	175.50 A
Treat. 2	85.50 B	56.00 B	2.69 A	181.25 B
Treat. 3	89.00 C	56.20 B	2.70 A	187.25 C
Treat. 4	91.50 C	59.79 C	2.70 A	189.00 C

Treatment 1: Control; treatment 2: 100 ml.ha^{-1} of *Bradyrhizobium* (sowing); treatment 3: 46 kg.ha^{-1}of N topdressing (R3); treatment 4: 100 ml.ha^{-1} of *Bradyrhizobium* (sowing) + 46 kg.ha^{-1} of N (R3). Means followed by different letters in the rows differ significantly by the Tukey test at 5% probability level.

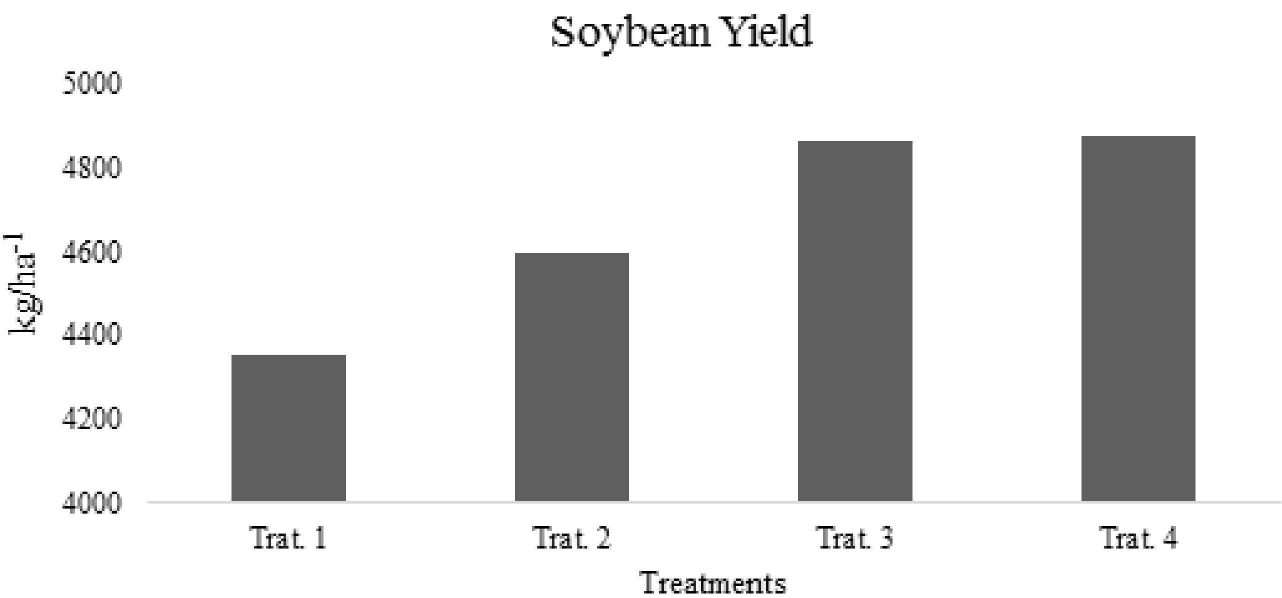
The use of inoculant + nitrogen fertilization (Treatment 4) provided greater number of pods per plant, when compared with the use of inoculant alone (Treatment 2) and with nitrogen fertilization alone (Treatment 3), which were statistically equal to each other (Table 2). The control treatment showed the lowest number of pods per plant (Table 2). This beneficial effect of the use of inoculant + nitrogen fertilization can be explained by the increase in mass that nitrogen promotes in soybean plants (Larcher, 2000). In this case, plants could have greater leaf area in the reproductive phase, maximizing photosynthesis and thus contributing to greater retention of pods in soybeans (Bahry et al., 2013). Thus, it is evident that there is a need for complementation in the form of fertilization or inoculation so that there is increase in the productive components of the crop (Embrapa, 2011).

There was no statistical difference for the grains per pod yield component (Table 2), which is in agreement with results found by Bahry et al. (2013), where from the use of nitrogen in the amide and nitric form in different reproductive stages and $N.ha^{-1}$ doses, there was no increase in the number of grains per pod. According to Thomas and Costa (2001), the number of grains per pod is the yield component that presents the least variation according to the different nitrogen doses, demonstrating uniformity in plants that compose the different treatments.

It was found that nitrogen fertilization (Treatment 3) and inoculation + nitrogen fertilization (Treatment 4) presented the highest PMS when compared to inoculation alone (Treatment 2) and control (Treatment 1) (Table 2). However, treatments 1 and 2 differed from each other, where treatment 1 had the lowest PMS (Table 2). Similar results were also found by Marcon et al. (2017), in which the use of nitrogen provided greater mass of one thousand grains regardless of source used and the time of application during the reproductive stages evaluated, indicating the benefit of the use of nitrogen for this variable.

The average grain yield of the experiment, in $kg.ha^{-1}$, is shown in Figure 1, where it can be observed that treatments 3 and 4 obtained better productive results in relation to the others. These data contrast with those obtained by Balbinot Júnior et al. (2016), who did not observe positive influence on the productivity of two cultivars, regardless of dose and time of application of the nitrogen source when evaluated in sandy soils. These contrasting results demonstrate that the success of the use of nitrogen fertilization in topdressing on soybean crop is strongly influenced by the different climatological conditions observed in each harvest.

Figure 1. Soybean grain yield in kg/ha as a function of different nitrogen sources.



Treatment 1: Control; treatment 2: 100 $ml.ha^{-1}$ of *Bradyrhizobium* (sowing); treatment 3: 46 $kg.ha^{-1}$ of N topdressing (R3); treatment 4: 100 $ml.ha^{-1}$ of *Bradyrhizobium* (sowing) + 46 $kg.ha^{-1}$ of N (R3). Means followed by different letters in the rows differ significantly by the Tukey test at 5% probability level. CV = 1.65%.

The application of nitrogen fertilization increases soybean grain yield, but there is a considerable increase in costs (Table 3). Thus, the use of a mineral source of N is an alternative when there is unsatisfactory or non-existent inoculation, or when extremely high production levels are desired, being allocated in periods of high specificity. Its use is complex and presents numerous controversies. This is due to the complexity involved in the relationship between rhizobia, plant and mineral fertilization, the relationship with soil and climate factors, management, crop stage, the additional costs of carrying out the operation and the sustainability of these mineral sources of N.

Table 3. Grain yield in kg.ha^{-1}; Bags.ha^{-1}; Practiced bag value; Total yield; Cost of applications and; Net yield of treatments used in the experiment. Coxilha-RS, 2019.

Treatments	Kg.ha^{-1}	Bags.ha^{-1}	Bag value (R$)	Total yield (R$)	Cost of applications (R$)	Net yield (R$)
Treat. 1	4351	72.5	70,00	5,075.00	-	5,075.00
Treat. 2	4595	76.6	70,00	5,362.00	11,00	5,351.00
Treat. 3	4867	81.1	70,00	5,677.00	160,00	5,517.00
Treat. 4	4876	81.2	70,00	5,684.00	171,00	5,513.00

Treatment 1: Control; treatment 2: 100 ml.ha^{-1} of *Bradyrhizobium* (sowing); treatment 3: 46 kg.ha^{-1} of N topdressing (R3); treatment 4: 100 ml.ha^{-1} of *Bradyrhizobium* (sowing) + 46 kg.ha^{-1} of N (R3).

In addition, the high productivity in treatments where there was inoculation with *Bradyrhizobium* sp. is the result of biological nitrogen fixation, a process carried out by associated bacteria, which, under appropriate conditions of temperature, pH, water availability, oxygen, soil nutrients, production of phytohormones, among others, promote nodulation, development of the root system and, consequently, plant growth (Manteli et al., 2019).

Therefore, it could be concluded that inoculation is the most sustainable alternative to meet the physiological needs of the soybean crop and thus achieve increasingly satisfactory productive results.

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