__Original Research Article__

Nitrogen fertilization and inoculation of seeds with *Rhizobium tropici* on the agronomic performance of common beans

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ABSTRACT

Aims: This study aimed to evaluate the influence of nitrogen fertilization and inoculation of seeds with *Rhizobium tropici* on the agronomic performance of common bean (*Phaseolus vulgaris* L.).
Study design: The experimental design consisted of a randomized block in a 4x2 factorial layout, with 4 replicates, the first factor referring to inoculant doses (0, 50, 100 and 200 mL for each 25 kg of seed), while the second refers to nitrogen fertilization (0 and 40 kg ha of N).
Place and Duration of Study: The study was conducted to field in a no-till system area, in the southwestern region of Paraná, Brazil. The soil is classified as a Purple Latosol, with a clayey texture.
Methodology: The adopted spacing was 0.45 m between rows, and the seeding density used was 12 seeds per furrow meter. The inoculants NITRO 1000 *Rhizobium tropici* SEMIA 4077 and SEMIA 4088 were applied, varied according to the treatments. The cultivar used was IPR – Tangará.
Results: As for grain yield, it was observed that nitrogen fertilization did not contribute to the increase in grain yield, and higher productivity was obtained in the absence of N. This was probably due to the high content of organic matter present in the soil.
Conclusion: Nitrogen fertilization at sowing and seed inoculation with *Rhizobium tropici* did not influence the plant population and the 1000-grain mass. In the absence of nitrogen fertilization at sowing, pods with longer length and higher grain yield were obtained. Inoculation of the seeds with *Rhizobium tropici* exerts a positive influence on plant height, number of nodes of the main stem, number of pods per plant, and number of beans per pod, the dose recommended by the manufacturer (100 mL) being efficient, with the possibility of applying a dose of 50 mL, to satisfactory results.

Keywords: Phaseolus vulgaris L., Biological Nitrogen Fixation, Inoculation, IPR-Tangará

1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is grown in more than 100 countries, although over 60% of world production is takes place in only five countries, Brazil being the largest producer and consumer in the world. The area cultivated with beans in Brazil as of the 2017/18 harvest was estimated at 3.2 million hectares, 0.5% higher than in the 2016/17 harvest. The mean productivity is projected at 1,043 kg ha^{-1}, 2.4% less than in the last harvest. Considering the sown area and expected productivity, bean production in the 2017/18 harvest is expected to reach 3.35 million metric tons, 1.9% less than in the previous harvest [1].

A number of factors interfere in the yield of common bean, including nitrogen fertilization management. Inadequate nitrogen fertilization is a factor that often determines the failure of common bean crops. While a few producers apply excessive doses of N, others apply insufficient amounts of this element, limiting crop yield even if other factors of production are optimized [2]. Furthermore, it is necessary to correctly manage the N, as it is a nutrient that can easily be lost through the leaching, volatilization or denitrification processes [3].

N is the nutrient required in greatest quantity by beans [4] and although there is a recommendation for the use of nitrogen fertilizer for common bean crops, research results suggest that it is possible for this crop to benefit from biological nitrogen fixation (BNF), it may contribute to increased crop yield and reduce or replace the use of nitrogen fertilizers [5], thereby decreasing production costs and the environmental impact of the use of chemical fertilizers. It should be noted that the high dependence on fertilizers by crops, for example, causes an increase in the energy costs for conversion of atmospheric nitrogen, in addition to the extraction process of the other elements, such as phosphorus and potassium [6]. Moreover, excess nutrients applied in conventional agriculture may cause environmental problems. Environmental costs of all nitrogen losses in Europe have been estimated at €70-320 billion per year, which outweighs the direct economic benefits of nitrogen in agriculture [7].

The practice of seed inoculation with rhizobia represents a low-cost alternative to increase the yield of common bean, in addition to avoiding the contamination of water resources by nitrogen fertilizer and reducing greenhouse gas emissions. It is proven to be a cheap technology, simple to use, and with good economic return. Being a legume, the common bean presents conditions to benefit from symbiotic association with *Rhizobium*, which contributes specifically to N savings. Nevertheless, according to Rabelo et al. [8],there is still a need for improvements in order to develop an inoculation that fully replaces nitrogen fertilization in common beans, as is the case with soybeans. FBN contributes positively to the development of this culture, but not to a sufficient degree to dispense N applications.

This study aimed to evaluate the influence of nitrogen fertilization and inoculation of seeds with *Rhizobium tropici* on the agronomic performance of common beans.

2. MATERIAL AND METHODS

The experiment was conducted in an area under a no-till system, located on the São Pedro da Bandeira line, in the municipality of Dois Vizinhos, Paraná, Brazil. The municipality is located in the Southwest region of the State, between the geographic coordinates 25°44’ S and 53°03’ W, with an altitude of 509 m.

The climate of the region according to the Köppen classification subtropical hot humid (Cfa), with a mean annual rainfall of 2,044 mm, well distributed throughout the year. The mean daytime temperature is 19.6°C and the mean nighttime temperature is 15.2°C [9].The mean rainfall and temperature in the months of the experiment are shown in Table 1.

Table 1. Rainfall (mm), maximum temperature (MAX), minimum temperature (MIN), and mean (MDA) in °C, in the months of execution of the experiment.

Month	Precipitation (mm)	Temperature MAX	MIN	MDA
November	112	31.4	21.4	26.4
December	266	32.03	24.09	28
January	200	32.96	22.23	27.5

Data obtained at the property where the experiment was conducted.

The soil is classified as a Purple Latosol, with a clayey texture [10]. The area is characterized by presenting a slightly uneven topography, with a level curve and no erosion. Prior to the sowing of beans, the area had been sown with an oat crop. Prior to the bean sowing, a soil sample was collected at a 0-20 cm depth. The results obtained were: pH $CaCl_2$ 6,7, P 40.05 mg dm^{-3}, organic matter 38.28 g dm^{-3}, Ca, Mg, K, Al and H+Al, respectivy, 8.18, 3.33, 0.78, 0.0, 2.37 cmolc dm^{-3} and V 83.83%.

A Semeato Personale seed drill was used for sowing the beans. The adopted spacing was 0.45 m between rows, and the seeding density used was 12 seeds per furrow meter. The seeds were treated with thiamethoxan 350 g L^{-1} at the dose 0.7 L of the commercial product for 100 kg of seed. Following that, the inoculants NITRO 1000 *Rhizobium tropici* SEMIA 4077 and SEMIA 4088 were applied. According to the manufacturer the product concentration is 3.0 x 10^9 viable cells per ml g^{-1}. The inoculant dose, as well as the nitrogen fertilization, varied according to the treatments. For phosphate and potassium fertilization, 40 kg ha^{-1} was used. The fertilizer was applied to the side and below the seed.

The cultivar used was IPR – Tangará. This cultivar belongs to the Carioca group and presents an indeterminate growth habit, erect plants with long vines, a mean cycle of 87 days from emergence to harvest, and mean productive potential of 3,326 kg ha^{-1}. It has resistance to common mosaic, *Curtobacterium* wilt, *Fusarium* wilt and rust, mild resistance to powdery mildew and angular leaf spot, and susceptibility to anthracnose and common bacterial blight. It presents intermediate tolerance to high temperatures and droughts occurring during the reproductive phase. The seeds have a light beige coat with light brown stripes.

The experimental design consisted of a randomized block, with a 4x2 factorial layout, with four replications. The first factor refers to four levels of seed treatment with different doses of inoculant (0 mL, 50 mL, 100 mL, and 200 mL for every 25 kg of seed). The recommended dose according to the manufacturer is 100 mL for 25 kg of seed. The second factor refers to two levels of nitrogen fertilization (0 and 40 kg ha^{-1} of N at sowing). The treatments were as follows: T_1 = 0 kg ha^{-1} N and 0 mL inoculant; T_2 = 0 kg ha^{-1} N and 50 mL inoculant; T_3 = 0 kg ha^{-1} N and 100 mL inoculant; T_4 = 0 kg ha^{-1} N and 200 mL inoculant; T_5 = 40 kg ha^{-1} N and 0 mL inoculant; T_6 = 40 kg ha^{-1} N and 50 mL inoculant; T_7 = 40 kg ha^{-1} N and 100 mL inoculant; and T_8 = 40 kg ha^{-1} N and 200 mL inoculant.

Each experimental plot had 5 m of length composed of 5 lines and spacing of 0.45 m, totaling an area of 11.25 m^2. The three central lines were considered as usable area of the plot, excluding 0.5 m from each end of the line, totaling 4.5 m^2.

During the development of the crop, two manual weeding treatments were performed, at 10 and 25 days after sowing. Phytotoxic treatments (125 mL ha^{-1}) were also prepared aiming at the control of pests such as whitefly, white mite, leafhoppers, Cucurbit beetle and thrips with thiamethoxam (125 mL ha^{-1}) and diseases such as angular leaf spot, anthracnose and rust with fentin hydroxide (700 mL ha^{-1}), these comprising pests and diseases of major importance, which may cause greater damage to the common bean in the southwestern region of Paraná.

The evaluations were carried out at the end of the crop cycle. The following variables were evaluated: plant population, plant height, number of main stem nodes, number of pods per plant, number of grains per pod, mean pod length, 1000-grain mass (13% moisture), and productivity.

To determine the plant population, the number of plants was counted in 2 m of the usable plot. Plant height was determined by averaging 20 plants harvested from each usable plot, measured from the plant neck to the end of the main stem. The number of nodes of the main stem, number of pods per plant, number of beans per pod and the mean pod length were determined by averaging 20 plants randomly collected from each usable plot. The 1000-grain mass was determined by averaging 2 samples of each usable plot, each sample having 250 grains, then weighed in a semi-analytical balance and then calculated the moisture content corrected to 13%, estimating the 1000-grain mass. Grain productivity was determined throughout the plot area, correcting for moisture content of 13% and, subsequently, estimated productivity in kg ha^{-1}.

3. RESULTS AND DISCUSSION

There was no significant effect of the interaction between the inoculant doses and nitrogen fertilization for the variables of plant population, mean pod length, one 1000-grain mass, and productivity. Thus, the results are presented independently. For these variables, there was also no significant effect of inoculant doses. A significant difference was observed for nitrogen fertilization only for the variables of mean pod length and grain yield (Table 2).

Nitrogen application at sowing had no effect on the plant population (Table 2). This can be explained by the fact that the seed contains the nutrients necessary for the establishment of the seedling, such as in the Fabaceae (beans, soybean), in which the endosperm is partially or fully absorbed during the development of the seed in favor of the cotyledons, which assumes the function of tissue [11]. Additionally, the temperature, which had an average value of 26.4℃ (Table 1) may have contributed to the homogeneity of the booth, it being noted that the optimal temperature for bean germination is around 28℃, and rainfall occurred shortly after sowing (Table 1) [12].

Table 2. Population of plants per hectare, mean pod length (cm), 1000-grain mass (g) and yield (kg ha^{-1}) of common beans as a function of nitrogen fertilization.

	Plant population (ha)	Mean pod length (cm)	1000-grain mass (g)	Yield (kg ha^{-1})
Without N	239.777 a	9.12 a	284.31 a	1,716.6 a
With N	237.333 a	8.88 b	287.37 a	1,686.6 b
CV	3.7	2.31	2.33	1.05

Mean values followed by the same lowercase letter in the columns do not differ significantly from each other by the F test (P<0.05).

For mean pod length, it was observed that there were no significant differences in the different doses of the inoculant (Table 2). This can be explained by the fact that this component presents high genetic heritability, being intrinsically linked to the characteristic of the cultivar [13]. Notwithstanding, a significant difference was observed for nitrogen fertilization, in which the best result was observed in the absence of N, with 9.12 cm (Table 2). This was probably due to the high content of organic matter present in the soil.

The 1000-grain mass and grain yield were also not influenced by nitrogen fertilization at sowing (Table 2), as well as by seed inoculation. As for grain yield, it was observed that nitrogen fertilization did not contribute to the increase in grain yield, and higher productivity (1,716.6 kg ha^{-1}) was obtained in the absence of N (Table 2). This was probably due to the high content of organic matter present in the soil.

It was also possible to observe that the different inoculant doses did not present significant differences. The high production levels obtained, even in the absence of inoculation, demonstrate the great capacity of competition and effectiveness in symbiotic N fixation by native strains. The yields obtained in the absence of inoculation were similar to high doses in some treatments. The non-response to seed inoculation in relation to productivity can be explained by the presence of native *Rhizobium* strain in the soil, which is usually more aggressive than the introduced strain. The presence of native strains in the soil makes it difficult to perform the introduced strain, as they compete for the sites of nodular infection, their presence in the nodulation in plants that did not receive the inoculant being evident, even in a smaller number of nodules, which may be equivalent to other treatments thanks to their greater efficiency [14].

Ferreira et al. [14] studying *Rhizobium* strains, concluded that the inoculation of efficient strains in common bean-nodulating cultivar, or their cultivation in soils with an efficient native population, may allow the non-use of nitrogen in the bean crop without affecting productivity. Rabelo et al. [8] mention that FBN contributes positively to the development of this crop, but not enough to dispense N applications.

Peres et al. [15], in three years of cultivation and using several cultivars, verified that the inoculation with rhizobia contributed to the increase in grain yield. These data show that the biological nitrogen fixation may supplement the nitrogen fertilization in the bean crop, allowing a reduction in nitrogen fertilizer rates, without losses in grain yield. Nevertheless, Matoso and Kusdra [16] did not find positive results regarding the inoculation of common bean with *R. tropici*.

There was a significant effect of the interaction between inoculant doses and nitrogen fertilization for the variables of plant height, number of main stem nodes, number of pods per plant, and number of grains per pod.

Following the interaction between nitrogen fertilization and seed inoculation, a similar behavior can be observed for plant height, number of nodes of the main stem per plant, number of pods per plant, and number of grains per pod. Nitrogen fertilization obtained the lowest mean values in all studied variables, even in inoculated treatments. In the absence of nitrogen fertilization, inoculation provided a considerable increase in some agronomic variables of importance, such as the number of pods per plant and number of grains per pod.

The height of the plants showed a significant interaction ($P<0.05$). It was noted that, in the absence of N, the values were higher, and that these values decreased when increasing the inoculant dose (Figure 1), thereby reinforcing the idea of action of the native strains in the area. The adaptation of rhizobia to the soil depends on the biotic and abiotic conditions of the environment and the wild or cultivated legume species, both in size and variability. As the cultivated area has a high content of organic matter and has been worked under a no-till system for a long time, the biotic, physical and chemical conditions of the soil are in favorable conditions for the development of the native strains.

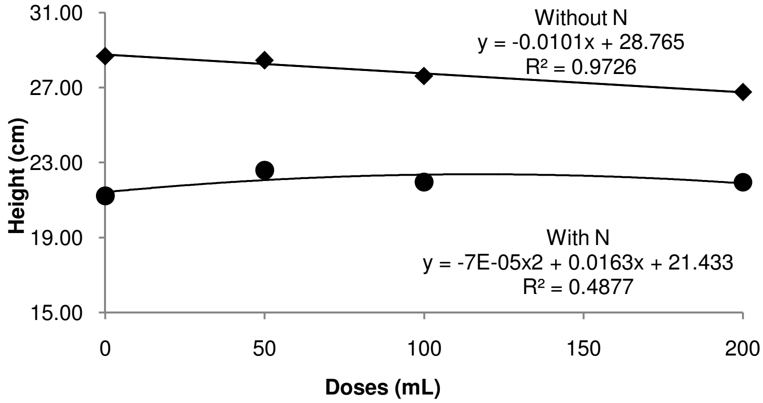

Fig 1. Plant height of cv. IPR Tangará bean as a function of inoculant doses and nitrogen fertilization.

When the same inoculant doses associated with nitrogen fertilization (40 kg ha^{-1} of N) were studied, the best inoculation response was obtained, and the best result was observed when applied at half the recommended inoculant dose (50 mL). Although the mean values were lower than in treatments without N use (Figure 1). N fertilizer may cause a reduction in symbiotic efficiency but, when applied in small amounts in the bean crop, allows an increase in nodule growth and higher BNF, and very low levels of nitrate in the soil may be limiting to symbiotic activity. According to Novo et al. [17], root nodulation supplies the needs of the plants, avoiding the nitrogen fertilization, as it inhibits the formation of the nodules, affecting BNF the biomass production.

As for the number of nodes of the main stem, results similar to those already studied were observed, the best results being observed in the absence of nitrogen fertilization. In the latter, the lower dose of inoculant stands out over the others (Figure 2), followed by half of the recommended dose (50 mL), as previously discussed on the aggressiveness of the native strains in the area, with the best performance of these strains in the absence of nitrogen fertilization at sowing.

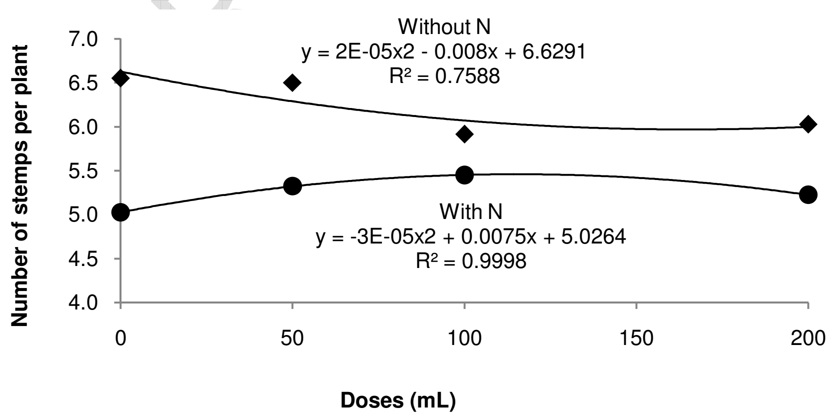

Fig 2. Number of stems per plant of the cv. IPR Tangará as a function of inoculant doses and nitrogen fertilization.

By adding 40 kg ha^{-1} of nitrogen at sowing, a 15.8% reduction was obtained in the number of nodes, achieving the best results associated with the recommended inoculant dose (100 mL) (Figure 2).

Regarding the number of pods per plant, significant differences were observed between treatments, with better results obtained in the absence of nitrogen fertilization (Figure 3). In this sense, Souza et al. [18] verified that seed inoculation only increased the number of pods per plant in the absence of N application. Nevertheless, in this variable, it is possible to observe that nitrogen fertilization with the aid of the recommended inoculant dose (100 mL) was able to reach values similar to the recommended inoculant dose without N in fertilization (Figure 3). Nevertheless, the cheapest form of fertilization, without N, with the influence of the inoculation, comprising half of the dose (50 mL), exceeded all other treatments.

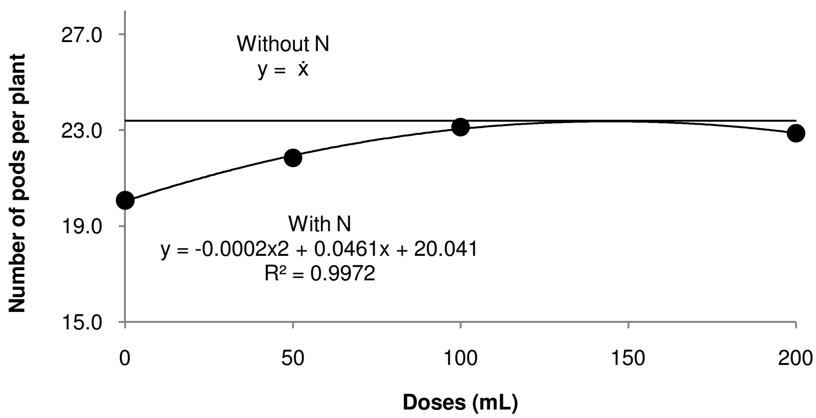

Fig 3. Number of pods per cv. IPR Tangará as a function of inoculant doses and nitrogen fertilization.

Soratto et al. [19] and Alvarez et al. [20] observed positive results for the number of pods per plant when the N doses were increased. Araújo et al. [21] observed no effect of inoculation on the number of pods per plant. The number of pods per plant depends on the cultivar being used.

The number of grains per pod, despite being a characteristic of high genetic heritability [13], and thus related to the cultivar used, showed an interaction between the treatments with the two forms of fertilization and the different doses of the inoculant. In the treatment without nitrogen fertilization, the results were higher (Figure 4), in accordance with those found by Soratto et al. [22] which also did not have significant effects on the number of grains per pod with the use of different levels of N.

With the interaction between inoculant doses, it can be observed that half of the recommended dose (50 mL) was superior compared to the others, reaching 5.7 grains per pod (Figure 4). Similar results were found by Araújo et al. [21] and Romanini Júnior et al. [23], who noted that the inoculation with rhizobia contributed to the increase in productivity. Binotti [24] did not find significant differences for the number of grains per pod, using *R. tropici* in the inoculation of the bean seeds.

In the treatment that received nitrogen fertilization, the results were lower, but half of the inoculant dose again presented superior results compared to others in this treatment, reaching 5.1 grains per pod. A different result was found in a study carried out by Farinelli et al. [25], in the state of São Paulo, Brazil, evaluating the application of N doses in the bean crop, in a no-till system and in conventional planting, finding maximum yield with the application of 185 kg ha^{-1} of N.

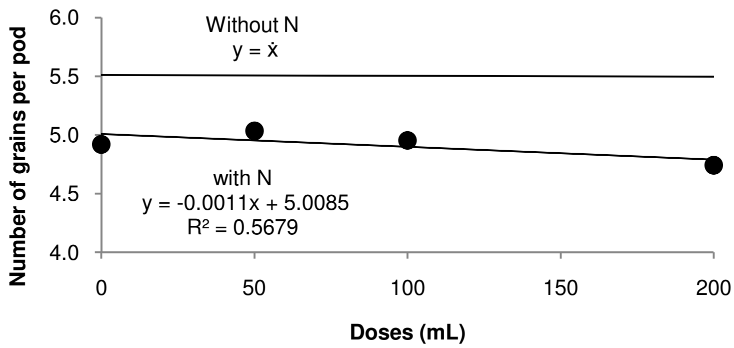

Fig 4. Number of grains per pod of cv. IPR Tangará bean as a function of inoculant doses and nitrogen fertilization.

4. CONCLUSION

Nitrogen fertilization at sowing and seed inoculation with *Rhizobium tropici* did not influence the plant population and the 1000-grain mass.

In the absence of nitrogen fertilization at sowing, pods with longer length and higher grain yield were obtained

Inoculation of the seeds with *Rhizobium tropici* exerts a positive influence on plant height, number of nodes of the main stem, number of pods per plant, and number of beans per pod, the dose recommended by the manufacturer (100 mL) being efficient, with the possibility of applying a dose of 50 mL, to satisfactory results.

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CONSENT (WHERE EVER APPLICABLE)

Not applicable.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable.

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