Full Length Research Paper

Nitrogen application and inoculation with *Rhizobium tropici* on common bean in the fall/winter

Antonio Carlos Rebeschini¹, Rita de Cássia Lima Mazzuchelli¹, Ademir Sergio Ferreira de Araujo² and Fabio Fernando de Araujo^{1*}

¹UNOESTE, Agricultural Science Faculty, 19067-175, Presidente Prudente, SP, Brazil.

²Federal University of Piauí, Agricultural Science Center, Teresina, PI, Brazil.

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The objective of this study was to evaluate doses of nitrogen in the cultivation of bean under field conditions, associated with seed inoculation. The study was conducted under field conditions in two traditional bean cultivation in Paraná northern (Cafeara and Florestópolis). The experiment consisted of plots with or without seed inoculation with *Rhizobium tropici* and use of five doses of nitrogen coverage (0, 20, 40, 60 and 80 kg ha⁻¹). Evaluations were made about the nodulation, N content in leaves, shoot dry matter, N uptake, number of pods per plant and grain yield. The experimental design was randomized blocks with ten treatments and four replicates. It was factorially arranged in five levels of nitrogen with and without inoculation. In this study there was no response to inoculation in the evaluation of the yield of bean in Cafeara and Florestópolis with different soil management. The nodulation of bean with *Rhizobium* is reduced by fertilization with mineral nitrogen. The system with tillage cultivation favored the development of bean during autumn-winter.

Key words: N₂ fixation, *Rhizobium* spp., *Phaseolus vulgaris*.

INTRODUCTION

With an average yield of 951 kg ha⁻¹ in 2011 Brazil produced approximately 3.5 million tons of beans (IBGE, 2012). The cultivation of this legume is accomplished in three seasons, the first being called "rainy season", the second "the dry season" and the third "season of fall / winter." The small bean producers have invested frequently in the rainy season, due to the dependence on weather conditions, while large producers invest in other crops by the increased availability of irrigation (Person, 2012).

For production of protein-rich grain bean requires an adequate supply of nitrogen (N), to care for their growth

as well as for the formation of pods and grains (Fancelli and Dourado, 2007). The availability of mineral nitrogen for plants is directly dependent on the continuous decomposition of organic matter (N mineralization), fertilization (Binotti et al., 2010) or biological N₂ fixation (BNF) made by symbiotic association with rhizobia roots (Araújo et al., 2007). The need to improve productivity of legumes as a global source of dietary protein, however, has made it vital to understand the factors that influence nitrogen fixation (Schulze, 2004).

The efficiency of BNF in bean is very variable and under favorable environmental conditions can provide up

*Corresponding author. E-mail: fabio@unoeste.br

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Table 1. Analysis of soil composition of the experimental area of Cafeara and Florestopolis.

Parameter	Units	Cafeara	Florestopolis
pH CaCl ₂		4.9	5.0
pH in SMP		6.4	6.8
Al	mmolc.dm ⁻³	0	0
Organic matter	g.dm ⁻³	16.0	11.0
Ca	mmolc.dm ⁻³	10.0	7.0
Mg	mmolc.dm ⁻³	4.0	3.0
K	mmolc.dm ⁻³	1.0	0.9
P	mg.dm ⁻³	2.0	11
SO ₄	mg.dm ⁻³	8.9	6.2
Mn	mg.dm ⁻³	80.5	15.8
Fe	mg.dm ⁻³	31.9	15.1
Cu	mg.dm ⁻³	3.7	2.5
Zn	mg.dm ⁻³	6.9	1.2
B	mg.dm ⁻³	0.32	0.29

to 80 kg ha⁻¹ of N fixed (Melo and Zilli, 2009). The application of 20 kg ha⁻¹ of nitrogen at seeding associated with rhizobia inoculation can provide input of 160 kg ha⁻¹ nitrogen (Pelegri et al., 2009). It is necessary to care for the dose of N at sowing as studies have documented the negative impact of high nitrate concentration in soil on nodulation and nodule weight (Muller and Pereira, 1995).

The species of *Rhizobium* with higher prevalence in symbiosis with bean show frequencies of 17.1% at *Rhizobium tropici*, 35.9% *Rhizobium etli*, 32.5% *Rhizobium leguminosarum*, 1.7% *Rhizobium giardinii* and 12.8% with distinct profiles of described species of bean rhizobia, emphasizing the high genetic diversity of rhizobia, including the appointment of new species (Stocco et al., 2008).

The lack of response to inoculation of bean is usually due to the inability of strains inoculated to compete with native species for soil infection sites in roots (Ferreira et al., 2011). The bean can respond similarly with and without inoculation, indicating soils with high native communities of rhizobia in the soil (Pelegri et al., 2009). There is a variability in responses to both bean rhizobial inoculation and to nitrogen fertilization, mainly influenced by the levels of soil fertility and other techniques used in production systems, especially the use of irrigated systems (Pelegri et al., 2009). The efficiency of nitrogen absorption is enhanced in these cultivation systems, with this reducing production costs (Sant'ana et al., 2011).

In many regions of the world where common beans are grown, nitrogen fixation is limited by unfavorable soil conditions and temperature and water stress (Kabahuma, 2013). The tilling season and soil management contribute decisively to obtain high yields in dry beans. Bean plants grown in no-tillage has provided better conditions for nitrogen use, possibly because of increased soil water retention which may buffer temperature (Romanini Junior et al., 2007). In this context the hypothesis of this study is

that the climatic conditions and soil water availability, found in the growing season of the third season (autumn-winter), are crucial for the evaluation of BNF. This work aimed at the effect of rhizobia inoculation and application of nitrogen in cover on bean cultivation in two distinct areas, the autumn-winter season.

MATERIALS AND METHODS

Experimental site

The study was conducted in two areas of traditional cultivation of bean in northern Paraná, Brazil in 2010. One area is located at Cafeara (22° 46'22"S; 51° 43'18"W) altitude of 377 m. The other at Florestopolis (22° 52'25"S; 51° 22'55"W) altitude 400 m. The soil in both areas is classified as ultisol, emphasizing that the soil Cafeara is under no-tillage (NT). The soil fertility analysis are presented in Table 1. The experiment was conducted in the fall / winter season, from April to August 2010.

Biological material

In the experiment was used bean genotype Carioca (IPR 139), indeterminate growth. The inoculum used in the study was commercial inoculant in peat formulation (Turfal, Curitiba, PR) containing *R. tropici*, strain SEMIA 4088, the concentration of 1.0 x 10⁹ viable cells per gram of product. The dosage used was that recommended by the manufacturer of 100 g per 50 kg of seed. The product was added to seeds with use of sugar adhesive solution plus 10% (weight) immediately prior to sowing.

Experimental design

The experiment consisted of plots with or without inoculation of *R. tropici* seeds and use of five N rates (0, 20, 40, 60 and 80 kg ha⁻¹) in cover effected at 20 and 40 days after plant emergency. A completely randomized block with ten treatments arranged factorially with four replications was used. The experimental unit consisted of plots with six bean lines (5 m) spaced at 0.45 m

Figure 1. Rainfall accumulation in 2010 in the experimental areas in Cafeara and Florestopolis.

between rows and a total area of 13.5 m² per plot.

Cultivation

The plants was conducted in the field under natural conditions of climate and soil. Based on the analysis of soil fertility was effective at sowing fertilization with 250 kg ha⁻¹ of NPK fertilizer (00-20-20), and afterwards we made fertilization coverage with urea (45% N) according to pre-established treatments. The seeds were sown in April. The production system and plant management such as control of pests, diseases and weeds were conducted in accordance with the detected events and technical recommendations for culture in the region.

Number and weight of nodules, foliar N and biomass

For evaluation of nodulation and dry matter of the plant were collected 10 plants per plot at the stage of full flowering (R6). To determine concentration of nitrogen leaves were collected from the middle third of 30 plants per plot. To count the number of pods per plant were collected ten plants per plot at maturity stage (R9). The analysis and quantification of shoot dry weight and dry weight of nodules were performed in the laboratory after drying the material on forced ventilation oven (60-70°C) to constant weight. The analysis of leaf total N were performed according to methodology (Malavolta et al., 1997).

Evaluation of yield

For the determination of final yield, the plants were collected from two central rows of each plot, ignoring 0.5 m of each line at the beginning and end, that after threshing and grain moisture correction to 13% was calculated grain yield per hectare.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by F test

($P \leq 0.05$ and $P \leq 0.01$) and when found statistical differences were applied to regression analysis doses nitrogen using the statistical software SISVAR (Ferreira, 2000).

RESULTS AND DISCUSSION

Climatic conditions, monitored here as water availability during the experiment period (Figure 1), showed in Cafeara precipitation total of 140 mm, below the crop necessity because there are reports that at 75 days cycle development bean has one evapotranspiration 220 mm (Silveira and Stone, 1979). In Florestopolis, there was total precipitation of 108 mm. With respect to temperature variation in both locations, there were minimum temperature of 12°C and maximum 25°C, during the period of the experiment.

With respect to use of inoculant only in the number of pods in Cafeara experiment showed positive effect with $P < 0.01$ (Table 2), no significant response was obtained in the experiment Florestopolis (Table 3). Souza et al. (2011) also found that seed inoculation only increased the number of pods per plant in the absence of N. Was found significative F values in most parameters in the analysis of the effect of N rates. The bean yield ranged from 1883 to 2037 kg.ha⁻¹ in Cafeara and 525 to 705 kg.ha⁻¹ Florestopolis. The values obtained in Cafeara were well above the average of 951 kg.ha⁻¹ in Brazil and 705 kg.ha⁻¹ in the Paraná State (Conab, 2011).

There was a decrease in nodulation on the roots of bean, with regression adjusted a linear ($P < 0.05$), when increase the dose of mineral nitrogen in Cafeara (Figure 2A). In Florestopolis no significant adjustment for nodulation between treatments with doses of N, in the absence and presence of inoculation (Figure 2B). In

Table 2. Mean values of nodulation, pod number, plant dry matter, leaf N and yield of beans in doses of inoculant and nitrogen. Cafeara – PR.

Treatments	Nodulation (number plant ⁻¹)	Nodules weight (mg.plant ⁻¹)	Nº of pods per plant	N foliar (g.kg ⁻¹)	Yield (kg.ha ⁻¹)
Inoculation					
Yes	20.6	43.4	9.32	37.5	1996.9
No	24.4	53.1	8.38	36.9	1983.8
F	3.09	3.64	7.59**	0.65	0.073
N doses (Kg.ha ⁻¹)					
0	33.7 ^a	57.6 ^{ab}	7.46 ^c	34.9 ^b	1932
20	25.5 ^{ab}	63.2 ^a	8.45 ^{bc}	36.7 ^{ab}	2001
40	19.6 ^b	46.2 ^{ab}	8.91 ^{abc}	37.0 ^{ab}	1949
60	16.2 ^b	35.3 ^b	9.03 ^{ab}	38.1 ^{ab}	2046
80	17.4 ^b	38.9 ^b	10.4 ^a	39.2 ^a	2021
F	9.13**	4.43**	7.86**	3.61*	0.79
CV	30.6	33.1	8.85	6.42	7.66

* and ** significant at the 5% and 1% probability according to the F test.

Table 3. Mean values of nodulation, pod number, plant dry matter, leaf N and yield of beans in doses of inoculant and nitrogen. Florestopolis – PR.

Treatments	Nodulation (number plant ⁻¹)	Nodules weight (mg.plant ⁻¹)	Nº of pods per plant	N foliar (g.Kg ⁻¹)	Yield (Kg.ha ⁻¹)
Inoculation					
Yes	36.9	45.8	3.50	37.1	612
No	30.3	44.7	3.78	37.6	624
F	2.24	0.011	1.05	1.53	0.185
N doses(Kg.ha ⁻¹)					
0	46.3	79.4 ^a	3.21	37.2 ^{ab}	509 ^b
20	26.6	43.2 ^b	3.68	36.5 ^b	642 ^a
40	34.3	34.9 ^b	3.58	36.5 ^b	658 ^a
60	33.9	38.4 ^b	4.01	38.1 ^a	656 ^a
80	26.9	29.2 ^b	3.71	38.6 ^a	624 ^{ab}
F	2.54	7.34**	0.85	3.75*	
CV	42.1	46.4	24.3	3.65	14.1

* and ** significant at the 5% and 1% probability according to the F test.

general, there was good nodulation of bean plants in the absence of inoculation, in two locations measured.

Pelegri et al. (2009) observed that nodulation in bean plants that were not inoculated was similar to inoculated, mainly due to population of native rhizobia in the soil. Similarly, Hungria et al. (2003) observed no significant differences in the number of nodules of bean plants inoculated with rhizobia and that received N fertilization (30 kg ha⁻¹ at sowing + 30 kg ha⁻¹ in coverage) when comparing nodulation provided by the native population soil. The inhibitory effect of N on nodulation is probably plant mediated; however, differences in tolerance to nitrate and ammonium have also been found between rhizobial isolates when investigated in nodulated systems

(Nelsen, 1987).

There was significant adjustment ($P < 0.05$) for increasing the number of pods when increased doses of N and inoculated with *Rhizobium* in Cafeara (Figure 3A). In Florestopolis the adjustment quadratic was significant ($P < 0.05$) for doses of N without inoculation (Figure 3B). In the analysis of the dry mass of the shoot, there was a significant adjustment quadratic ($P < 0.05$) with increased dry matter at the highest levels of mineral N in uninoculated treatments in Cafeara experiment (Figure 4). In Florestopolis the regression analysis was significant ($P < 0.01$), showing maximum production at the dose of 40 kg N per hectare, when using inoculation. Responses of bean to nitrogen fertilization have been variable in dry

Figure 2. Nodulation after inoculation of bean seeds and applied dosages of nitrogen in (A) Cafeara and (B) Florestopolis. * significant differences ($P < 0.05$).

Figure 3. Number of pods after inoculation in bean seed and applied dosages of nitrogen in (A) Cafeara (B) Florestopolis. * significant differences ($P < 0.05$).

matter production, observed positive effects (Carvalho et al., 2001) or no significant effects (Soratto et al., 2006). In Cafeara there was increase in the leaf N content, with significant linear adjustment for both the inoculated treatments ($P < 0.01$) and for those without inoculation ($P < 0.05$) (Figure 5A). Quadratic adjustment was found to be significant ($P < 0.05$) for doses of mineral N without inoculation in Florestopolis (Figure 5B).

In all the treatments the foliar N content was observed to be below critical level for the plants, 30 g de N kg^{-1} , in accord with Ambrosano et al. (1997), or 30 to 50 g de N kg^{-1} (Malavolta et al., 1997). These levels are found in

beans under conditions of fertility soil or native communities of rhizobia with high efficiency (Soratto et al., 2006; Farinelli et al., 2006). In conditions of poor soils in N (with low organic matter) and low populations of rhizobia symbiotically efficient the application of mineral nitrogen has provided higher foliar N than those in plants without fertilization (Carvalho et al., 2001).

Maximum values of nitrogen accumulation were found around 100 and 30 kg of N per ha, in Cafeara and Florestopolis respectively. In Brazil the average rates of BNF contribution to the beans are of the order of 60 kg N per hectare and represent 30 to 50% of total N

Figure 4. Dry mass of shoots in bean seed inoculation and after applied dosages of nitrogen in (A) Cafeara and (B) Florestópolis. * and **significant differences ($P < 0.05$) and ($P < 0.01$).

Figure 5. Leaf N content in bean seed inoculation and after applied dosages of nitrogen in (A) Cafeara and (B) Florestópolis. * and **significant differences ($P < 0.05$) and ($P < 0.01$).

accumulated by the plant (Pelegrin et al., 2009). Moura et al. (2009) concluded that the species of native soil rhizobia are as efficient as the inoculation of *R. tropici* in supplying N to the bean. The responses to nitrogen fertilization in grain production also has generated much controversy, about that Souza et al. (2011) found that yield of bean is little influenced by nitrogen fertilization. Soratto et al. (2006), in studies conducted in Brazilian Midwest, found significant responses for yield of bean, with the estimated dose for maximum response of bean in 140 kg ha^{-1} N. Farinelli et al. (2006), in Brazilian

southeast, evaluated the application of N in bean crops under no tillage and conventional tillage and found the maximum yield with the application of $185 \text{ kg N per hectare}$. There has been variability in responses in productivity to levels of mineral N, mainly influenced by the levels of soil fertility and other techniques used in production systems, especially the use of irrigation systems (Pelegrin et al., 2009).

Concerning the performance of bean production in both locations, it was observed that the experiment of Cafeara had a good grain yield, indicating that this area is two

years in no tillage. Soratto et al. (2006) also found increased yield of common bean in no-tillage, with inoculation and application of N in coverage. In Florestópolis showed low organic matter content in the soil (Table 1) and conventional soil management may have contributed to reduction in crop development especially in conditions of water stress, which may have been decisive for the low yield of bean.

The bean yield is affected by soil water availability. A deficiency of water in soil can reduce productivity in different proportions according to the different phases of the cycle, especially during flowering and early pod formation (Fancelli and Dourado, 2007).

Under the conditions of this study it was found adequate nodulation of bean and no response to inoculation of bean and supply of mineral N in two different situations, differentiated mainly by soil management. It was observed that the condition of the experiment Cafeara where good crop yield, factors N dose or inoculation was found was not used in determining this result. In this condition it can be suggested that the N mineralization of organic matter and FBN due to the presence of rhizobia provided greater supply of N to the bean. A favorable environment in the rhizosphere is vital to interaction legume-rhizobium; however, the magnitude of the stress effects and rate of inhibition of the symbiosis usually depend on the phase of growth and development, as well as the severity of the stress. For example, mild water stress reduces only the number of nodules formed on roots of soybean, while moderate and severe water stress reduces both the number and size of nodules (Williams and De Mallorca, 1984). Other factors are also related to the failure of inoculations as the competitiveness and efficiency of strains of *Rhizobium* inoculant and also by the response of bean genotype used.

Conclusions

In this study there was no response to inoculation in the evaluation of the yield of bean in Cafeara and Florestópolis with different soil management. The nodulation of bean with *Rhizobium* is reduced by fertilization with mineral nitrogen. The system with tillage cultivation favored the development of bean during autumn-winter.

Conflict of Interest

The authors have not declared any conflict of interest.

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