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Response of the common bean to liquid fertilizer and *Rhizobium tropici* inoculation

Resposta do feijoeiro comum a fertilizante líquido e inoculação com *Rhizobium tropici*

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Highlights:
Synergism between liquid fertilizer and biological nitrogen fixation.
Liquid fertilizer applied at 6 cm improves crop management.
Increased grain yield in inoculated common bean.

Abstract

The common bean production system is majorly based on the use of granular fertilizers to provide nutrients for the crop. Studies on the use of liquid fertilization at an appropriated depth and, seed inoculation with *Rhizobium tropici* can provide significant increases in the grain yield of the common bean. The objective of this study was to determine the growth and productivity of common bean as affected by N-P formulations, application depths and the inoculation with *R. tropici*. Field experiments were carried out in 2015 and 2016 cropping years using a complete block design, in a 2x2x2 factorial arrangement, with four replicates. The treatments involved the combination of N-P formulation (granular and liquid), two application depths of the N-P formulation (6 and 12 cm) and with or without rhizobia inoculant. The plant density (PD), number of pods (NP), number of grains (NG), mass of 100 grains (M100) and grain yield (GY) were determined. The granular and liquid N-P formulations provided similar results for PD, NP, NG, and GY of common bean. Similarly, the application depth of the N-P formulations did not affect GY. Inoculation of the seed with *R. tropici* stimulated NP and NG, increasing GY. Growth and productivity parameters were equally affected by the type of formulation and application depth; however, GY was greater with rhizobial inoculant. Thus, the liquid N-P formulation, applied at 6 cm depth, associated with rhizobial inoculant can improve the crop management providing better control of application uniformity, minimal soil mobilization, less fuel consumption, and increased grain yield.

Key words: Application depth. Cerrado. *Phaseolus vulgaris.* Rhizobia.

Resumo

O sistema de produção do feijoeiro comum baseia-se principalmente no uso de fertilizantes granulares para fornecer nutrientes para a cultura. Estudos sobre o uso de adubação líquida em profundidade apropriada e a inoculação de sementes com *Rhizobium tropici* podem proporcionar aumentos significativos no rendimento de grãos do feijoeiro comum. O objetivo deste estudo foi determinar o

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crescimento e a produtividade do feijoeiro afetado pelas formulações de N-P, profundidade de aplicação e inoculação com rizóbio. Os experimentos foram conduzidos nos anos agrícolas de 2015 e 2016. O delineamento experimental foi o de blocos completos, em esquema fatorial 2x2x2, com quatro repetições. Os tratamentos envolveram a combinação da formulação N-P (sólida e líquida), duas profundidades de aplicação da formulação N-P (6 e 12 cm) e com ou sem inoculante (*Rhizobium* tropici). A densidade de plantas (DP), número de vagens (NV), número de grãos (NG), massa de 100 grãos (M100) e produção de grãos (PG) foram determinados. As formulações de N-P sólida e líquida forneceram resultados semelhantes para DP, NV, NG e PG do feijão comum. Da mesma forma, a profundidade de aplicação das formulações de N-P não afetou a PG. A inoculação da semente com rizóbio estimulou o NV e NG, aumentando a PG. Os parâmetros de crescimento e produtividade foram igualmente afetados pelo tipo de formulação e profundidade de aplicação; contudo, a PG foi maior com a inoculação. Assim, a formulação N-P líquida, aplicada a 6 cm de profundidade, associada ao inoculante, pode melhorar o manejo do feijoeiro comum, proporcionando melhor controle da uniformidade da aplicação, menor revolvimento do solo, menor consumo de combustível e maior produção de grãos.

Palavras-chave: Cerrado. *Phaseolus vulgaris*. Profundidade de aplicação.

Introduction

The common bean (*Phaseolus vulgaris* L.) has great economic importance for Brazil. The cultivated area in the 2018/2019 harvest was about 3.14 million hectares, resulting in grain production of 3.06 million tons (Compania Nacional de Abastecimento [CONAB], 2019). The common bean is grown in three cropping seasons and, despite its importance, advanced technology is poorly used on the crop management, mainly in the first and second cropping seasons, resulting in productivity level ranging from 877 to 1,088 kg ha^{-1} (CONAB, 2019). The third cropping season is characterized as the most advanced technologically (Nascente, Kluthcouski, Crusciol, Cobucci, & Oliveira, 2012), with an average yield of 2,631 kg ha^{-1} in the Center-South region in the 2018/2019 harvest. Even in the third cropping season, technological adjustments are needed to improve grain yield of the common bean. Some aspects related to the plant nutrition can be improved, such as the fertilization management (Melém, Brito, Fonseca, Fonseca, & Aguiar, 2011) and the use of seed inoculation aiming at biological nitrogen fixation-BNF (Brito, Muraoka, & Silva, 2011).

The fertilization management of the common bean in Brazil is based on the use of conventional inputs, such as granulated fertilizers, which demand great logistics for their use. Notably, instead of using BNF, the nitrogen fertilization of the common bean is based on the use of N-fertilizers, which have high prices and risks of environmental impact (Siqueira, Piccoli, Costa, Cerri, & Bernoux, 2011; Bortolotto et al., 2012; Rodrigues, Mello, Conceição, Souza, & Silva, 2017). Currently, common bean producers are looking for inputs and technologies that make easier the crop management and also reduce environmental impacts and production costs.

Cutting edge technologies are always on the focus of the common bean farmers. Thus, liquid fertilizers are increasingly gaining space in the market. Its use is spreading in Brazil and attracting the attention of the largest companies of the agribusiness. Liquid fertilizers can increase the efficiency of nutrients use due to some factors, such as easier handling, better distribution in the soil and greater agronomic efficiency through the use of combined sources (Desenvolvimento Rural, 2020). However, there are few scientific studies stating its effectiveness. Thus, more experimental data are needed to provide information to technicians and farmers on the use of this technology.

Another factor growing in importance is the seed inoculation with *Rhizobium tropici* before common bean sowing. The use of *R. tropici* in the common bean can provide significant increases in grain yield, besides reducing the production cost by reducing the need for N-fertilization. According to

A. P. Oliveira, Silva, Arruda, Nascimento and Alves (2003), the common bean requires the application of a certain amount of nitrogen until the nodulation is fully established. Brito et al. (2011) stated that this dose should provide good plant development, but not harm nodulation. In this sense, it is necessary to know the cultivar and the development conditions of the culture to define the appropriate nitrogen dose that does not affect the BNF. According to Brito et al. (2011), common beans need a starting dose of 40 kg ha^{-1} of N, combined with the seed inoculation with *R. tropici*, to obtain economically acceptable productivity.

Thus, agronomic techniques such as the use of liquid fertilization at the sowing time and seed inoculation with *R. tropici* can provide significant increases in the grain yield of the common bean. However, published works combining these technologies are rare in literature. Thus, the objective of this study was to determine the effect of liquid and granular formulations of N-P fertilizer, applied at different depths and the seed inoculation with *R. tropici* on the agronomical performance of the common bean, cultivated in the Cerrado conditions.

Material and Methods

Two field experiments were conducted in two consective cropping years (2015 and 2016) at Embrapa Arroz e Feijão, located in the municipality of Santo Antônio de Goiás, GO, under the geographical coordinates 16º28’00”S and 49º17’00”W, and 823 m above sea level. According to Köppen’s classification the climate of the region is classified as Aw, tropical savanna (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013), with a dry season from May to September (autumn/winter) and rainy season from October to April (spring/summer). The average annual rainfall is between 1,500 to 1,700 mm. The average annual temperature is 22.7 °C, varying from 14.2 °C to 34.8 °C.

The soil in the experimental areas was classified as a Ferralsol. Before the installation of the experiments, 10 soil subsamples (0-20 cm) were taken to evaluate soil chemical properties. Chemical analyzes were performed according to the methodology proposed by Donagena, Campo, Calderano, Teixeira and Viana (2011). Before being analyzed, soil samples were dried (60 °C for 48 h) and sieved (2 mm). Soil pH was determined in 0.01 M $CaCl_2$ (1:2.5; soil/solution), after agitation for 1 h. Exchangeable Ca and Mg were determined in the extract obtained with 1 mol L^{-1} KCl (1:10; soil/solution) after agitation for 10 min. P and K contents were evaluated in the Mehlich-1 (0.05 mol L^{-1} HCl+0.0125 mol L^{-1} H_2SO_4) extract (1:10; soil/solution) after agitation for 10 min. Concentrations of Ca and Mg were determined in an atomic absorption spectrophotometer, K in a flame photometer and P by colorimetry, using the molybdenum-blue method and ascorbic acid as reducing agent. Organic matter was determined by Walkley & Black method. Soil characteristics before sowing at each site are shown in Table 1. (Table 1)

Table 1
Soil chemical attributes and native rhizobial polulation of the experimental areas in the 0-0.20 m depth layer before sowing

Cropping years	pH	Ca	Mg	P	K	SOM[a]	Rhizobial cells
	in H_2O	mmol$_c$ dm^{-3}		mg kg^{-1}		g kg^{-1}	(CFU[b] g^{-1} soil)
2015	5.7	16.7	13.4	18.1	125	26.6	2.77 x 10^4
2016	5.4	26.0	10.1	12.4	92	34.3	2.60 x 10^4

[a] SOM = soil organic matter.

[b] CFU = colony forming unit, estimated by the most probable number (MPN) method (Vincent, 1970) using common bean as trap plants.

The experiments were conducted under no-tillage system, with corn/soybeans as previous crops grown in the summer and common bean in the winter. In both cropping years, topdressing N-fertilization was carried out in the phenological stage V4 of the common bean (third fully expanded trifoliolate leaf), being applied 60 kg ha^{-1} of N, as urea.

Field experiments were addressed to a complete block design with four replicates, in a 2x2x2 factorial scheme, being the factors composed of: two N-P formulations (liquid and granular), two application depths of N-P formulations (6 and 12 cm) and two seed inoculations (with and without *R. tropici*). Before sowing the seeds were inoculated with peat inoculum, containing the commercial strain SEMIA 4077 (*Rhizobium tropici*), using 2 doses ha^{-1}, corresponding to 500 g of peat inoculum for each 50 kg of seeds. To facilitate the inoculum adhesion to the seeds, 300 mL of a 10% (w v^{-1}) sucrose solution was used for each 50 kg of seeds. Fertilizations using liquid and granular N-P formulations were made in the sowing furrow, applying 272 kg ha^{-1} of the formula 5-25-0 (N-P_2O_5-K_2O). Potassium fertilization was topdressed immediately after sowing, using 48 kg ha^{-1} of K_2O in a granular source.

The plots were 2.25 m wide (5 sowing lines) and 5 m long. The useful area consisted of the three central lines, 4 m long. The base fertilization was carried out in the sowing furrow by a fertilizer sowing machine with five sowing lines spaced 0.45 m apart. For the distribution of the liquid fertilizer, dosers with flow register were used and for the granular fertilizer, helical dosers from the machine itself. The fertilizer sowing machine was regulated to operate at a speed of 4 km h^{-1} and to distribute 15 seeds of common bean, cultivar Pérola, per meter.

The experiments were installed in the first half of June of the two cropping years, in areas with slight differences in fertility, as shown in Table 1. The pivot-central sprinkler irrigation system was used, with water management as the requirement of the crop. Phytosanitary management was carried out according to crop needs (Vieira, Gomes, & Figueira, 2006).

At the physiological maturation, in the phenological phase R9, plant density (PD) was determined in 2 m in the central row of each plot. Also, five plants were randomly collected per plot for evaluation of the number of pods per plant (NP), number of grains per pod (NG), mass of 100 grains (M100). Grain yield (GY) was determined by harvesting a useful area of 4.5 m^2 in each plot, being expressed in kg ha^{-1}, with the values corrected to 13% moisture.

The data obtained in the experiments were grouped and the analysis by experiment group was performed. When the differences were significant, the results of each cropping year were analyzed separately, however, when there was no difference between the cropping years, the data were grouped. The data were submitted to analysis of variance and when confirming a statistically significant value in the F test ($p \leq 0.05$), mean values were compared by the “t” test at 5% significance.

Results and Discussion

The evaluation of the effects of N-P formulations, the application depth of the N-P formulations and the inoculation with *Rhizobium tropici* in common bean revealed that the plant density (PD), the number of pods per plant (NP), and the grain yield (GY) did not differ between the evaluated cropping years (Table 2) and, therefore, the results obtained were analyzed together. While the number of grains (NG) and the mass of 100 grains (M100) differed between the evaluated cropping years, being analyzed separatelly (Table 2).

Table 2
Joint analysis of the experiments carried out in the 2015 and 2016 cropping years, in the presence of topdressing N-fertilization. Plant density (PD- nº m^{-1}), number of pods (NP- nº $plant^{-1}$), number of grains (NG- nº pod^{-1}), mass of 100 grains (M100- g) and grain yield (GY- kg ha^{-1}) of common bean

Cropping year (CY)	PD	NP	NG	M100	GY
2015	11.87	9.90	2.43 B	24.04 B	1.656
2016	13.00	10.88	4.25 A	25.95 A	1.831
F_{CY}	*3.9*^{ns}	*0.9*^{ns}	*160.0***	*8.69**	*5.4*^{ns}
CV(%)	12.7	25.4	15.2	8.1	22.4

*significant ($p \leq 0.05$, "F" test), **significant ($p \leq 0.01$, "F" test), ^{ns}non significant ($p \geq 0.05$, "F" test). Mean values followed by different letters within each column are statistically different ($p \leq 0.05$, teste "t").

Neither liquid or granular N-P formulation affected PD, NP and GY. Also, N-P formulation did not interact with the other factorss (Table 3). However, the application depth of the N-P formulation affected in contrasting way PD and NP, showing greater PD at 12 cm depth and, greater NP at 6 cm depth (Table 3). Also, the inoculation with *R. tropici* improved the PD, increasing the GY (Table 3). According to Bertoldo, Pelisser, Silva, Favreto and Oliveira (2015), the seed inoculation with *R. tropici* provides better plant emergence, resulting in improved PD.

Table 3
Plant density (PD- nº m^{-1}), number of pods (NP- nº $plant^{-1}$), and grain yield (GY- kg ha^{-1}) of the common bean as affected by the N-P formulation, application depth of the N-P formulation, and inoculation with *Rhizobium tropici*

Factors	PD	NP	GY
N-P formulation (F)			
Liquid	12.6	10.14	1,718.0
Granular	12.2	10.64	1,770.0
F_{F}	*1.01*^{ns}	*0.6*^{ns}	*0.29*^{ns}
Application depth (AD)			
6 cm	11.9 B	11.35 A	1,697.0
12 cm	13.0 A	9.43 B	1,791.0
F_{AD}	*8.24***	*8.44***	*0.95*^{ns}
Inoculation (I)			
With *R. tropici*	13.1 A	11.0	1,851.0 A
Without *R. tropici*	11.7 B	9.8	1,637.0 B
F_{I}	*12.05***	*3.43*^{ns}	*4.91**
Interactions			
F_{FxAD}	*1.96*^{ns}	*0.7*^{ns}	*0.08*^{ns}
F_{FxI}	*0.54*^{ns}	*0.28*^{ns}	*0.43*^{ns}
F_{ADxI}	*0.08*^{ns}	*4.69**	*8.56***
F_{FxADxI}	*0.37*^{ns}	*0.6*^{ns}	*4.73**
CV(%)	12.7	25.4	22.4

*significant ($p \leq 0.05$, "F" test), **significant ($p \leq 0.01$, "F" test), ^{ns}non significant ($p \geq 0.05$, "F" test). Mean values followed by different letters within each column are statistically different ($p \leq 0.05$, teste "t").

Since no significative diferences were observed between liquid and granular N-P formulation for PD, NP, and GY, the formulation choose should be driven by other factors. Ease of handling and application, ease of blending, uniformity of application, and blend with crop protection products are some of the advantages of liquid fertilizer over granular formulation (Korndörfer, Anderson, Mundim, & Simões, 1995; Desenvolvimento Rural, 2020). Also, compared to the granular formulation, the liquid formulation allows the application of the same concentration of nutrients in a smaller volume, allowing less energy consumption in the sowing operation and better homogeneity of the fertilizer components, which in the granular formulation are sparrows by the sower shaking (M. J. Silva, 2017). Thus, the choice of which type of formulation to use, granular or liquid, should be based on the costs, ease and convenience of application, as well as on the plant potential response.

Regarding the application depth of the N-P formulation, application in greater depth could provide greater root development, reflecting positively on the GY of the common bean. However, due to the common bean having a superficial root system (Vieira et al., 2006) and, since the soil presented medium to high fertility in the superficial layer (Table 1) (D. M. G. Sousa & Lobato, 2004), the demand for nutrients necessary for the full development of the crop may have been supplied by the soil. Other authors also did not find significant differences in crop development due to the fertilization depth (J. G. Silva, Kluthcouski, Di Stefano, & Aidar, 1999; J. G. Silva & Silveira, 2002; M. A. Sousa, Lima, Silva, & Andrade, 2009). Thus, applying fertilizer more superficially results in less horse-power demand and, therefore, less fuel consumption. According to Machado, Lanças, Fiorese, Fernandes and Testa (2015), superficial furrow opening operation results in less energy expenditure as compared to deeper furrow opening.

The application depth of the N-P formulations affected the NP and the GY, but the effect on the GY was dependent on the inoculation with *R. tropici* (Table 4). Interaction was observed between the inoculation with *R. tropici* and application depth factors, however, regardless of the application depth, inoculation with *R. tropici* increased GY in about 13%. In the absence of *R. tropici*, the application of N-P formulation in greater depth favored GY.

Table 4
Number of pods (NP- nº plant^{-1}) and grain yield (GY- kg ha^{-1}) as affected by the interaction between the application depth of the N-P formulation with the inoculation with *Rhizobium tropici*

Application depth	NP		GY	
	with *R. tropici*	without *R. tropici*	with *R. tropici*	without *R. tropici*
6 cm	12.68 Aa	10.02 Ab	1,945.0 Aa	1,448.0 Bb
12 cm	9.33 Ba	9.53 Aa	1,757.0 Aa	1,825.0 Aa
Average	11.01 A	9.78 A	1,851.0 A	1,636.5 B

Mean values followed by different lowercase letters in the line, and by different uppercase letters in the column, are statistically different ($p \leq 0.05$, teste "t").

In the 2016 cropping year, the interaction between inoculation with *R. tropici* and application depth of N-P formulation positively influenced NP, by which greater NP was found with the inoculation with *R. tropici* and application of N-P formulation at 6 cm depth (Table 4). Similarly, GY was also affected by this interaction, where higher GY was found under the inoculation with *R. tropici* and application of N-P formulation at 6 cm depth (Table 4). These results corroborate Moreira, Oliveira and Ferreira

(2017), who reported that inoculation with *R. tropici* provides an improvement in yield components, such as NG, reflecting in GY increasing. Additionally, as seen in table 3, the GY of common bean inoculated with *R. tropici* was about 13% higher than that not inoculated, which represents an increase in GY by 214 kg ha^{-1}. Ferreira et al. (2000) reported that the use of inoculation can provide significant increases in productivity and cost reduction, due to the decrease in the need for nitrogen application. According to Gerlach, Arf, Corsini, Silva and Coletti (2013), the application of 90 kg ha^{-1} of N represents 14% of the total operating cost. As rhizobial inoculant is a relatively inexpensive technology, this increase in productivity justifies the inoculation.

Differently of the other parameters, the effects of the treatments over NG and M100 varied between the cropping years. In both cropping years, NG and M100 were not affected by the evaluated factors neither its interactions, except the NG, which was affected in both cropping years by the interaction of the application depth of the N-P formulation and the inoculation with *R. tropici*, and by the interaction between the N-P formulation, application depth of the N-P formulation and the inoculation with *R. tropici*. Also, it was observed interaction between the N-P formulation, application depth of the N-P formulation and the inoculation with *R. tropici* for M100 in the 2016 cropping year (Table 5).

Table 5
Number of grains (NG- nº pod^{-1}) and mass of 100 grains (M100- g) of the common bean as affected by the N-P formulation, application depth of the N-P formulation, and inoculation with *Rhizobium tropici*

Factors	NG		M100	
	2015	2016	2015	2016
N-P formulation (F)				
Liquid	2.4	4.3	25.6	23.7
Granular	2.4	4.2	26.3	24.3
F_F	0.01^{ns}	0.02^{ns}	1.57^{ns}	0.42^{ns}
Application depth (AD)				
6 cm	2.5	4.2	25.4	24.5
12 cm	2.4	4.3	26.5	23.6
F_{AD}	1.11^{ns}	0.28^{ns}	3.68^{ns}	0.19^{ns}
Inoculation (I)				
With *R. tropici*	2.4	4.0	25.8	23.5
Without *R. tropici*	2.4	4.5	26.11	24.6
F_I	0.15^{ns}	3.53^{ns}	0.34^{ns}	0.13^{ns}
Interactions				
F_{FxAD}	4.06^{ns}	0.96^{ns}	0.34^{ns}	0.81^{ns}
F_{FxI}	0.92^{ns}	0.96^{ns}	3.01^{ns}	0.39^{ns}
F_{ADxI}	11.92^{**}	6.8^{*}	1.05^{ns}	0.31^{ns}
F_{FxADxI}	5.3^{*}	0.23^{ns}	5.02^{*}	0.38^{ns}
CV(%)	15.2	14.8	5.9	7.9

*significant ($p \leq 0.05$, "F" test), **significant ($p \leq 0.01$, "F" test), ^{ns}non significant ($p \geq 0.05$, "F" test). Mean values followed by different letters within the column are statistically different ($p \leq 0.05$, teste "t").

In both cropping years, it was observed interaction between the application depth of the N-P formulation and the inoculation with *R. tropici* for the NG; however, without consistency of the results, which were quite divergent across cropping years (Table 6). In 2015, greater NG was observed when the seeds were inoculated with *R. tropici* and the N-P formulation was applied at 6 cm depth, while in 2016 greater NG was observed without seeds inoculation with *R. tropici* and the N-P formulation was applied at 12 cm depth.

Table 6
Number of grains (NG- nº pod^{-1}) in the 2015 and 2016 cropping years, as afffected by the interaction between the application depth of the N-P formulation with the inoculation with *R. tropici*

Application depth	2015		2016	
	with *R. tropici*	without *R. tropici*	with *R. tropici*	without *R. tropici*
6 cm	2.7 Aa	2.2 Ab	4.3 Aa	4.1 Ba
12 cm	2.2 Bb	2.6 Aa	3.8 Ab	4.8 Aa

Mean values followed by different lowercase letters in the line, and by different uppercase letters in the column, are statistically different ($p \leq 0.05$, teste “t”).

In spite of the good results of the inoculated treatments observed in our work, BNF in common bean is still a controversial issue. Some published papers show productivity ranging from 2500 to 3500 kg ha^{-1} (Mostasso, Mostasso, Dias, Vargas, & Hungria, 2002; Pelegrin, Mercante, Otsubo, & Otsubo, 2009), while other the productivity varied from 600 to 1500 kg ha^{-1} (Raposeiras et al., 2006; Souza, Soratto, & Pagani, 2011; Valadão et al., 2009). Moreover, some authors state that the inoculation of common bean associated with a dose of N equivalent to 20 kg ha^{-1} can provide productivity equivalent to the use of 80 kg ha^{-1} of N (Soares et al., 2016) or 160 kg ha^{-1} of N (Brito et al., 2011).

Although few studies have been carried out on the use of liquid fertilizer, our results indicate that the use of liquid N-P formulation, applied at 6 cm depth, associated with the inoculation with *R. tropici*, translates into advantages for the common bean producer, providing better control of application uniformity, minimal soil mobilization, less fuel consumption, among others, with the benefit of increased grain production.

Conclusions

As compared to the granular formulation the liquid N-P formulation provides similar results for all growth and grain yield parameters of the common bean.

The application depth of the N-P formulations affects the plant density and number of pods; however, does not affect grain yield.

Inoculation of the seed with *R. tropici* stimulates the yield components, increasing grain yield.

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