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Agronomic and Economic Efficiency of Common-Bean Inoculation with Rhizobia and Mineral Nitrogen Fertilization

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ABSTRACT: Management of biological nitrogen fixation in common bean still requires improvement. The objective of this study was to verify the compatibility of nitrogen fertilization with biological N_2 fixation to increase common bean yield and profitability. Four field experiments were performed in four municipalities of Minas Gerais, Brazil, during the 2009 and 2010 winter crop season. The 2009 crop experiments were set up under a no-till system and the 2010 crop was conducted using conventional tillage. A randomized block experimental design was used with four replications and seven treatments combining application rates of mineral N (at sowing and/or topdressing) and seed inoculation with *Rhizobium tropici* strain CIAT899. Inoculation with 20 kg ha^{-1} N-urea at sowing and seed inoculation does not interfere with nodule dry matter and promotes yield comparable to that observed with 80 kg ha^{-1} N-urea with economic profitability in both no-till and conventional tillage systems. These results show the possibility of economic savings with respect to N fertilizers, but also a significant ecological contribution by avoiding problems associated with misuse of these fertilizers, such as eutrophication of waterways and denitrification.

Keywords: *Phaseolus vulgaris*, nitrogen fixation, no-till, conventional tillage.

INTRODUCTION

Dry/edible beans are among the crops most consumed throughout the world (Broughton et al., 2003). Brazil is the largest global producer and consumer of common bean (*Phaseolus vulgaris* L.), which is the main source of protein and an important source of carbohydrates for the population, especially farmers, who produce it as a subsistence crop. Small farmers are primarily responsible for the domestic supply of this crop so important to Brazil; however, because of advances in mechanization and irrigation, there are now large producers in the market, especially for the winter crop. However, the national average yield is still low, approximately 1,058 kg ha^{-1} (Conab, 2015).

Common bean requires high levels of N, and the low availability of this nutrient in the soil, coupled with the short cycle and shallow roots of the plant, contribute to its low yield.

One N source is decomposition of soil organic matter, originating either naturally or from previous cropping. However, organic matter storage in tropical soils is low and is not sufficient for crop requirements; thus, management practices are a more effective option. Arf et al. (1999) observed that treatments containing the crop residue of velvet bean and lablab (2,407 and 2,149 kg ha^{-1}, respectively) showed higher grain yield values compared to treatment with maize crop residue (1,189 kg ha^{-1}). Mineral fertilization (Andrade et al., 2001; Araújo et al., 2007) and biological N_2 fixation (Raposeiras et al., 2006; Soares et al., 2006; Ferreira et al., 2009) are solutions for N-deficiency in plants. However, inoculation is not always satisfactory, and the addition of mineral N may be required (Raposeiras et al., 2006; Pelegrin et al., 2009; Kaneko et al., 2010).

There are a few studies related to combined mineral N fertilization and inoculation with N_2-fixing bacteria in common bean, and most of them are in greenhouse conditions. According to a study conducted by Tsai et al. (1993), small application rates of N (15 mg kg^{-1}) at the beginning of the bean cycle, with average levels of fertility complementation in 3 kg pots of soil improve conditions for nodule formation. Brito et al. (2011), in experiments using N_{15} in pots containing 5 kg of soil, showed that with a starting application rate of 15 mg kg^{-1} of mineral N, biological N_2 fixation was able to meet plant nutritional requirements. According to Franco et al. (1979), topdressing with low application rates of mineral N fertilizer should be performed 25 to 30 days after sowing for improved biological nitrogen fixation (BNF). Therefore, technical and economic evaluation of research data are quite relevant in terms of maximization of fertilizer use efficiency, based on theoretical yield and cost (Freire et al., 2011).

Considering the diversity of climate and soil conditions, we hypothesized that variations commonly found in field results regarding the effects of inoculation can be reduced by joint application of inoculation and mineral N fertilization. The objective of this study was to verify the compatibility of nitrogen fertilization with biological N_2 fixation to increase common-bean yield, and to improve the economic viability of the common bean crop in Oxisols of different textures in the state of Minas Gerais, Brazil.

MATERIALS AND METHODS

Four experiments were conducted in the field (Table 1); two of them were performed on the winter/spring 2009 crop in the municipalities of Lavras (Universidade Federal de Lavras – UFLA, Departamento de Biologia/DBI) and Ijaci [Fundação de Apoio ao Ensino Pesquisa e Extensão de Ijaci (Education, Research and Extension Support Foundation) /Faepe]. The climate of Lavras and Ijaci, according to the Köppen classification system, is Cwa type with a humid temperate climate, a dry winter, and a hot and rainy summer. The chemical and physical properties of the soil at 0.00-0.20 m, as well as the textural class and soil classification are shown in table 2. Two other experiments were

Table 1. Sites, date, geographic coordinates, soil preparation, technological level and irrigation system used in the experiments

Site[1]	Date	Geographic coordinates, altitude	ST[2]	TL[3]	IS[4]
Lavras (DBI)	June/2009 winter/spring crop	21° 14′ S, 45° 00′ W, 920 m	NTS	NT4	Yes
Ijaci (Faepe)	June/2009 winter/spring crop	21° 10′ S, 55° 30′ W, 832 m	NTS	NT4	Yes
Lavras (DAG)	June/2010 winter/spring crop	18° 34′ S, 46° 31′ W, 833 m	CTS	NT3	Yes
Patos de Minas (Epamig)	June/2010 winter/spring crop	20° 00′ S, 45° 58′ W, 706 m	CTS	NT3	Yes

[1] DBI (Universidade Federal de Lavras, Departamento de Biologia); Faepe (Fundação de Apoio ao Ensino Pesquisa e Extensão de Ijaci); DAG (Universidade Federal de Lavras, Departamento de Agricultura); Epamig (Empresa de Pesquisa Agropecuária de Minas Gerais – Fazenda Sertãozinho). [2] ST: Soil Tillage: (NTS: No-till system; CTS: Conventional tillage system). [3] TL: Technological level (Ribeiro et al., 1999); NT1: liming, fertilization, seed collected, 220,000 to 240,000 plants ha^{-1}, weeding up to 30 days after emergence; NT2: liming, fertilization, supervised seeds, 220,000 to 240,000 plants ha^{-1}, phytosanitary control, seed treatment; NT3: NT2, herbicides, irrigation; NT4: NT3 with higher fertilizer application rates. [4] IS: Irrigation System.

Table 2. Chemical and physical properties of soils samples (0.00-0.20 m layer), textural class, and soil classification

Property	Site			
	DBI	Faepe	Epamig	DAG
pH(H_2O)	5.8 G	6.0 G	6.0 G	6.0 G
P (Mehlich-1) (mg dm^{-3})	29.9 VG	12.8 G	35.7 VG	3.1 VL
K (Mehlich-1) (mg dm^{-3})	67 M	97 G	75 G	51 M
Ca^{2+} ($cmol_c$ dm^3)	3.3 G	2.6 G	1.8 M	1.9 M
Mg^{2+} ($cmol_c$ dm^3)	1. 3 G	1.2 G	0.8 M	0.5 M
Al^{3+} ($cmol_c$ dm^3)	0.0 VL	0.0 VL	0.1 VL	0.0 VL
H+Al ($cmol_c$ dm^3)	2.9 M	2.9 M	5.0 M	2.1 L
SB ($cmol_c$ dm^3)	4.8 G	4.1 G	2.8 M	2.5 M
t ($cmol_c$ dm^3)	4.8 G	4.4 M	7.8 G	4.6 M
T ($cmol_c$ dm^3)	7.7 M	7.0 M	2.9 L	2.5 L
m (%)	0.0 VL	0.0 VL	3.5 VL	0.0 VL
V (%)	62.2 G	58.3 M	35.6 L	55.0 M
OM (dag kg^{-1})	2.6 M	2.1 M	4.4 G	2.9 M
Zn (mg dm^{-3})	11.1 H	6.8 H	6.9 H	1.5 M
Fe (mg dm^{-3})	25.7 M	27.8 M	32.7 G	75.2 VG
Mn (mg dm^{-3})	29.5 H	14.7 H	96.3 H	12.0 H
Cu (mg dm^{-3})	4.8 H	1.2 M	10.9 H	1.4 G
B (mg dm^{-3})	0.3 L	0.3 L	0.3 L	0.1 VL
S (mg dm^{-3})	16.0	13.8	77.2	11.8
Sand (g kg^{-1})	110	460	470	390
Silt (g kg^{-1})	290	190	350	70
Clay (g kg^{-1})	600	350	180	540
Textural class	Clayey	Medium texture	Sandy Loam	Clayey
Soil classification[1]	LVd	LVd	LVe	LVd

VL (Very low), L (Low), M (Medium), G (Good), VG (Very Good) and A (High), according to Ribeiro et al. (1999). [1] *Latossolo Vermelho Distroférrico* (LVd) (Oxisol according Soil taxonomy); *Latossolo Vermelho Eutroférrico* (LVe) (Embrapa, 1999) (Oxisol according Soil taxonomy).

conducted on the winter/spring 2010 crop: in Lavras (Universidade Federal de Lavras, Departamento de Agricultura/DAG) and Patos de Minas, in the Alto Paranaíba region on the Sertãozinho farm of the Empresa de Pesquisa Agropecuária de Minas Gerais (Agricultural Research Company – Minas Gerais/Epamig). The Patos de Minas climate, according to the Köppen classification system, is Cwa type with a humid high-altitude tropical climate with a hot dry summer and cold winter.

Rhizobia inoculation had not previously been used for the bean crop in any of the areas. Preceding crops were corn in DBI, common bean in DAG and Faepe, and wheat in Epamig. In DBI and Faepe, desiccation of the remaining foliage was performed with glyphosate (Roundup®) at a rate of 360 g L^{-1}. The herbicide fluazifop-p-butyl + fomesafen (Robust®) was then applied in post-emergence at 240 g L^{-1} ha^{-1} of a.i.

In Epamig and DAG, weed control was manually performed when necessary. In DAG, there was a moderate attack of *Lagria villosa* (Coleoptera), which was controlled with deltamethrin (DECIS®, 25 g L^{-1} ha^{-1} of a.i.), a pyrethroid insecticide, at 64 days post-emergence (DPE), applied as a liquid mix at 222 L ha^{-1}. In the same location, bait was used to control leafcutter ants at 7 DPE. All experimental areas were irrigated; at Faepe, a center pivot was used, and in other areas, overhead irrigation was used.

Basic fertilization followed the recommendations of Ribeiro et al. (1999) for the common bean crop. In DBI and Faepe, 400 kg ha^{-1} of the 0-28-10 formulation of N-P-K was applied. In DAG and Epamig, 70 kg ha^{-1} of P_2O_5 and 20 kg ha^{-1} of K_2O were applied as triple super phosphate and potassium chloride, respectively.

The common bean cultivar BRSMG Majestoso was used, which has a *carioca* (beige with brown stripes) grain type (30.6 g per 100 grains), a Type II/III growth habit, and an 87 days cycle; it is a cultivar officially recommended for the state of Minas Gerais (Abreu et al., 2007).

The strain used for inoculation was *Rhizobium tropici* CIAT 899 (Graham et al., 1994), approved by the Ministry of Agriculture, Livestock and Food Supply (MAPA) as an inoculant for the common bean crop. The inoculants were prepared at the soil microbiology laboratory of the Department of Soil Science – UFLA and were grown in sterilized liquid medium 79 (Fred and Waksman, 1928). After 48 h of growth, during the log phase, the material was transferred to an Erlenmeyer flask containing peat sterilized by autoclaving for 20 min. The resulting mixture (inoculant) had a 3:2 ratio (w:v) of peat: culture and was used as the base at 100 g per kg of seed. Inoculant quality was monitored through counting of colony-forming unit (CFU), given that the statutory minimum number of viable cells is approximately 10^9 CFU of *Rhizobium* per gram of inoculant at sowing.

A randomized block experimental design was used with seven treatments and four replicates at DBI and Faepe and three replicates at DAG and Epamig. The treatments were: 1 – no mineral nitrogen (N) and no seed inoculation (Control); 2 – only seed inoculation (Inoc); 3 – only 20 kg ha^{-1} of N-urea at sowing (20S); 4 – Inoculation + 20 kg ha^{-1} of N-urea at sowing (Inoc + 20S); 5 –inoculation + 20 kg ha^{-1} of N-urea at sowing + 20 kg ha^{-1} of N-urea topdressing (Inoc + 20S + 20T); 6 – inoculation + 20 kg ha^{-1} of N-urea at sowing + 40 kg ha^{-1} of N-urea at topdressing (Inoc + 20S + 40T); and 7 – inoculation + 20 kg ha^{-1} of N-urea at sowing + 60 kg ha^{-1} of N-urea at topdressing (Inoc + 20S + 60T). In the experiments conducted at DBI and Faepe, topdressing was split two times in the case of treatment 6 (20 + 20 kg ha^{-1} of N-urea) and three times for treatment 7 (20 + 20 + 20 kg ha^{-1} of N-urea), starting from the onset of the 1st trifoliate leaf pair and applied successively at intervals of 10 days. In the DAG experiment, the N topdressing in treatments 6 and 7 was split into two, and a single application was performed at Epamig.

Each experimental unit (12 m^2) consisted of six 4-m-length rows spaced at 0.5 m, and the area used for data collection corresponded to the four central rows. Immediately after inoculation, manual sowing was performed at a density of 15 seeds per meter. Rows 1 and 6 were considered borders, rows 2 and 3 were used for sampling at flowering, and rows 4 and 5 were used for harvest after maturation.

At full flowering (stage R6 of the growth cycle), 10 plants were randomly collected from each plot to assess the number of nodules (NN), nodule dry matter (NDM), shoot dry matter (SDM) and N accumulation per shoot (NAS). At harvest (stage R9), the grain yield (GY) and the primary components of production were determined, including pods per plant (PP), grains per pod (GPP), 100 grain weight (100 GW) and grain N accumulation (GNA). Grain yield (13 % moisture) was the result of the track pods of all plants in rows 4 and 5, and the components were determined at random in these 10 plants. The N content was determined by the Semimicro-Kjeldahl method, according to Sarruge and Haag (1979). Shoot N accumulation was calculated by multiplying the SDM by the N content and dividing by 100. The calculation of grain N accumulation (GNA) was performed in the same manner using grain yield and grain N content.

To meet the assumptions for analysis of variance, all data were tested for variance in normality and homogeneity using R software (R Development Core Team, 2012), and the NN and GPP data were processed in $(x+0.5)^{0.5}$. The data were then subjected to analysis of variance for each crop using Sisvar software version 4.0 (Ferreira, 2011). The viability of combined analysis was measured by comparing the magnitude of the residual mean square of individual analysis as described in Banzatto and Kronka (2006). In cases of a significant treatment effect, grouping of means was conducted using the Scott-Knott test at 5 % probability.

Regression curves considering all treatments (except the Control and 20S treatment) allowed estimation of the N application rate to obtain the maximum yield expected at each site (Theory of Production). This evaluation did not consider a joint analysis of the 2009 crop experiments (DBI and Faepe) and was based on estimates of the following equations:

$$Y = a + b(N) + c(N)^2 \qquad \text{Eq. 1}$$

$$Nmax = -b/2c \qquad \text{Eq. 2}$$

where Y is common bean commercial crop yield, kg ha^{-1}; N is total amount of mineral N applied; a, b, and c are parameters of the equation: $c < 0$; Nmax is N application rate corresponding to maximum yield.

In order to estimate the Rate of Return on Invested Capital (RR), cost theory was applied by calculating the difference between the monetary amount of production (MAP) and the costs of the N applied, inoculants, and tractor services. The cost (Cost) was calculated as the sum of fertilizer and inoculant expenditures and one tractor-hour for each treatment, which represents additional hours for topdressing applications at each experimental site. Thus, Rate of Return on Invested Capital can be calculated as follows:

$$RR = MAP - (COST) \qquad \text{Eq. 3}$$

$$MAP = Py \times GY \qquad \text{Eq. 4}$$

$$COST = Pn \times N + C \qquad \text{Eq. 5}$$

$$C = T + I \qquad \text{Eq. 6}$$

where RR is the Rate of Return on Invested Capital (R$); Py is the unit price of common bean products according Agrolink (October 2009 = R$ 1.14 kg^{-1}; October 2010 = R$ 2.57 kg^{-1}), Y is the bean production (kg); Pn is the unit price of N according "Instituto de Economia Agrícola" (October 2009 = R$ 2.75 kg^{-1}; October 2010 = R$ 2.71 kg^{-1}); N is the N-urea applied (kg); and C is the inoculant costs (I = R$ 5.00 per doses) and 1 h of tractor cost (T = R$ 60.00) for topdressing of N. Values were converted to US$ considering the mean rate of ‘Banco Central do Brasil’ in the period of the experiments, i.e., June 10th to September 10th, 2009 => 1 US$ = R$ 1.885 and June 10th to September 10th, 2010 => 1 US$ = R$ 1.77.

RESULTS

Winter/spring crop 2009

In analysis of variance of the data obtained at flowering, a significant treatment effect (T) was observed on SDM and NDM, and a site effect (S) was observed on SDM, NDM, and NAS. The T × S interaction was significant only for SDM.

For the S × T interaction in relation to SDM, there was no difference among treatments at Faepe (Table 3), but at DBI, the SDM of the Control was inferior to the treatments that received N supplementation; the cultivation site did not interfere in the SDM of the Control, but among the other treatments, the highest values were obtained at DBI. Although the F-test was significant, there was no difference among treatments for NN (Table 4). With respect to NDM, the treatments that received mineral N topdressing (Inoc + 20S + 20T, Inoc + 20S + 40T and Inoc + 20S + 60T) showed the lowest means. In comparison of the sites, DBI exhibited higher average NDM and NAS (Table 4).

At maturity, the winter-spring 2009 crop all parameters were influenced by cultivation site (S), and only 100 GW was not affected by treatment (T). Only the S × T interaction, however, significantly influenced GPP.

In general, the highest PP, 100 GW, GPP, and GNA were observed at Faepe (Table 4). The 100 GW showed little variation and did not differ among the treatments. As for GY, the Control and Inoc treatments were statistically inferior to the others, followed by the 20S treatment, which represented an intermediate group. The highest yields occurred from treatments of the combined application of the inoculant and mineral N. Inoculation followed by the application of 20 kg ha^{-1} of N-urea at sowing (Inoc + 20S) did not differ statistically from inoculation plus 20 kg ha^{-1} of N-urea at sowing and 60 kg ha^{-1} of N-urea in topdressing. Regarding the GNA variable, the treatments that received mineral N fertilization proved superior to the Control and Inoc.

The treatments differ in their levels of GPP, but this effect was influenced by the cultivation site (Table 3). The Inoc + 20S and Inoc + 20S + 60T treatments provided higher GPP at DBI, whereas no differences were observed at Faepe. The Control, Inoc,

Table 3. Mean values for shoot dry matter (SDM) and grains per pod (GPP) as a function of cultivation site and treatments. DBI and Faepe, 2009

Treatment	SDM		GPP	
	DBI	Faepe	DBI	Faepe
	g		unit	
Control	58.31 bA	72.73 aA	4.64 bA	4.10 aA
Inoc	134.38 aA	94.56 aB	4.59 bA	4.61 aA
20S	148.21 aA	79.23 aB	4.73 bA	4.18 aB
Inoc + 20S	175.51 aA	81.03 aB	5.68 aA	4.42 aB
Inoc + 20S + 20T	156.52 aA	85.40 aB	4.35 bA	4.56 aA
Inoc + 20S + 40T	153.00 aA	64.82 aB	4.92 bA	4.32 aB
Inoc + 20S + 60T	163.52 aA	67.33 aB	5.16 aA	4.33 aB
Mean	141.35	77.87	4.88	4.36

Means followed by the same letters, uppercase in the line and lowercase in the column, belong to the same group according to the Scott-Knott test at 5 % probability.

Table 4. Mean values for shoot dry matter (SDM), number of nodules (NN), nodule dry matter (NDM), nitrogen accumulation in shoots (NAS), pods per plant (PP), grains per pod (GPP), hundred grain weight (100 GW), grain yield (GY), and grain nitrogen accumulation (GNA), with and without inoculation of common bean, with different nitrogen fertilization rates at DBI and Faepe in 2009, DAG in 2010, and Epamig in 2010 during the flowering period and harvest

Treatment	SDM	NN	NDM	NAS	PP	GPP	100 GW	GY	GNA
	g		g	mg per plant			g	kg ha^{-1}	
DBI and Faepe (2009)									
Control	65.52	295.25	1.05 a	419.07	9.06 b	4.37	20.75	1,587.42 c	51.51b
Inoc	114.47	327.37	1.12 a	661.70	10.00 b	4.60	21.13	1,564.82 c	52.92 b
20S	113.72	300.00	1.13 a	658.78	9.23 b	4.46	21.22	1,997.11 b	62.46 a
Inoc + 20S	128.27	174.75	1.00 a	667.75	11.01 b	5.05	21.73	2,220.60 a	68.30 a
Inoc + 20S + 20T	120.96	180.25	0.42 b	785.74	11.00 b	4.45	21.41	2,487.50 a	69.64 a
Inoc + 20S + 40T	108.91	203.00	0.70 b	704.23	14.15 a	4.62	21.14	2,463.50 a	69.14 a
Inoc + 20S + 60T	115.43	98.25	0.33 b	682.38	13.03 a	4.74	21.32	2,424.27 a	68.35 a
DBI	141.35	209.10	1.03 a	868.49 a	10.18 b	4.87	20.25 b	1,790.39 b	50.48 b
Faepe	77.87	245.68	0.61 b	439.98 b	11.95 a	4.36	22.23 a	2,422.53 a	75.90 a
Mean	109.61	227.39	0.82	654.24	11.07	2.36	21.24	2,106.46	63.19
CV (%)	23.54	36.94	17.52	34.81	22.23	3.67	5.31	15.05	24.18
DAG (2010)									
Control	15.02	274.66 b	0.19 b	37.39 b	3.66	4.89 a	26.13	618.25 c	12.70
Inoc	23.22	649.00 a	0.66 a	48.45 b	5.96	3.20 b	25.40	603.78 c	8.57
20S	21.60	360.00 b	0.84 a	49.19 b	5.23	5.07 a	27.96	1,066.32 b	33.48
Inoc + 20S	28.00	432.33 a	0.87 a	49.50 b	5.00	3.99 b	25.16	1,543.21 a	37.45
Inoc + 20S + 20T	25.74	176.00 b	0.20 b	70.61 a	5.63	4.17 b	27.80	1,655.73 a	33.60
Inoc + 20S + 40T	20.14	157.00 b	0.17 b	63.62 a	5.80	4.65 a	24.03	1,697.27 a	29.09
Inoc + 20S + 60T	26.90	260.00 b	0.30 b	92.23 a	7.00	5.15 a	25.93	1,909.15 a	50.13
Mean	22.95	329.85	0.46	58.71	5.47	4.44	26.06	1,299.10	29.30
CV (%)	26.17	17.35	12.25	24.41	31.46	14.52	6.14	19.30	36.83
Epamig (2010)									
Control	21.10	260.00	0.35 a	61.26 b	5.36 b	4.16	28.90	658.45 b	17.39 b
Inoc	20.89	122.00	0.36 a	59.57 b	6.46 b	4.10	26.43	873.16 b	27.00 b
20S	26.71	87.00	0.20 b	101.29 a	7.33 b	4.33	26.90	926.63 b	22.26 b
Inoc + 20S	29.25	103.33	0.12 b	91.20 a	9.03 a	4.36	27.83	1,567.98 a	44.74 a
Inoc + 20S + 20T	22.81	52.00	0.02 b	62.72 b	7.33 b	3.91	27.76	1,449.90 a	53.99 a
Inoc + 20S + 40T	29.29	151.00	0.07 b	107.81 a	7.13 b	3.73	28.53	1,490.54 a	56.39 a
Inoc + 20S + 60T	28.69	145.00	0.06 b	108.39 a	9.46 a	4.26	29.76	1,640.28 a	53.90 a
Mean	25.53	131.47	0.17	84.61	7.44	4.12	28.01	1,229.56	39.38
CV (%)	18.80	32.10	57.82	16.28	15.98	14.00	6.14	15.46	27.04

In each column, the averages followed by the same letter belong to the same group, according to the Scott-Knott test at 5 % probability.

and Inoc + 20S + 20T treatments resulted in similar means of GPP at both cultivation sites. In relation to other treatments (20S and Inoc + 20S + 40T), GPP in DBI had higher values.

Winter/spring crop 2010 at DAG

At DAG, there was a significant effect of the treatments on all flowering variables except the SDM. The Inoc and Inoc + 20S treatments showed higher NN and NDM, while the other treatments had averages that were equivalent to each other but lower than those for Inoc and Inoc + 20S (Table 4). With respect to NDM, the 20S treatment showed values equivalent to Inoc and Inoc + 20S. When analyzing the NAS, the highest values were observed for the Inoc + 20S + 20T, Inoc + 20S + 40T and Inoc + 20S + 60T treatments.

For the winter-spring 2010 crop at DAG at maturity, only GPP and GY were influenced by the treatments. The highest GPP values were found in the Control, 20S, Inoc + 20S + 40T, and Inoc + 20S + 60T treatments, which did not differ from each other. The inoculated treatments receiving N fertilizer at sowing and/or topdressing showed similar GY values that exceeded those of the Control and Inoc, which exhibited lower yield. The 20S treatment generated an intermediate GY (Table 4).

Winter/spring 2010 crop at Epamig

At the Epamig site at flowering, there was no treatment effect on SDM and NN. However, the treatments influenced NDM and NAS. With respect to NDM (Table 4) greater values were obtained in Control and Inoc. With respect to NAS, the 20S, Inoc + 20S, Inoc + 20S + 40T, and Inoc + 20S + 60T treatments showed higher values than the other treatments.

A treatment influence was observed on PP, GY, and GNA at maturity. The PP values showed that the Inoc + 20S and Inoc + 20S + 60T treatments had greater values than the other treatments. The GY and GNA variables showed the highest average for the treatments that received both inoculation and fertilization with N (Table 4).

Rates of return on invested capital

Winter/spring 2009 crop at DBI

At the DBI site in 2009, the regression model that best fit was $\hat{y} = 1{,}259.06 + 37.88\,x - 0.37\,x^2$; R = 0.62. The maximum yield calculated by this equation was 2,228.58 kg ha^{-1}, which was obtained with 51.18 kg ha^{-1} of N-urea. The treatment with the best rate of return on invested capital (US$ 4,371.16) was Inoc + 20S + 20T (Table 5). Therefore, this rate of return was obtained with a lower N application rate (20S + 20 T = 40 kg N ha^{-1}) than that where the maximum production was obtained by the equation: 51.18 kg ha^{-1} of N-urea. This was influenced by cost increases due the application of higher amounts of N-urea (Table 5).

Winter/spring 2009 crop at Faepe

At the Faepe site in 2009, maximum production was 2,946.65 kg ha^{-1} and it was obtained with the application rate of 67.63 kg ha^{-1} of N-urea calculated from the equation $\hat{y} = 1{,}940.23 + 29.76\,x - 0.22\,x^2$; R = 0.63. However, the treatment representing highest rate of return on invested capital was Inoc + 20P + 40T, with the return of US$ 5,590.74 (Table 5), this return also came from a lower application rate than that where the maximum production obtained by the equation.

Winter/spring 2010 crop at DAG

At the DAG site in 2010, with the application rate of 66.03 kg ha^{-1} of N-urea, maximum production was obtained (1,884.75 kg ha^{-1}), calculated by the equation: $\hat{y} = 710.00 + 35.62\,x - 0.27\,x^2$; R = 0.66. Table 5 shows that treatment with the highest rate of return on invested capital – US$ 7,975.18 – was at the highest N application rate: Inoc + 20S + 60T.

Winter/spring 2010 crop at Epamig

Maximum production at Epamig in 2010 was 1,642.35 kg ha^{-1}, at an application rate of 64.34 kg ha^{-1} of N-urea. The equation representing this site was $\hat{y} = 979.95 + 20.59\,x - 0.16\,x^2$; R = 0.66. The highest rate of return on invested capital (US$ 6,923.26) was obtained in the Inoc + 20S treatment (Table 5).

Table 5. Grain yield (GY), monetary amount of production (MAP), cost, and rate of return on invested capital at DBI and Faepe in 2009, DAG in 2010, and Epamig in 2010 during the harvest

Treatment	GY	MAP	Cost	Rate of return on invested capital
	kg ha^{-1}	US$		
Winter/spring 2009 crop at DBI				
Control	1,433.17	3,079,74	113.10	2,966.64
Inoc	1,209.76	2,599,65	122.53	2,477.13
20S	1,725.65	3,708,25	216.70	3,491.55
Inoc + 20S	1,943.48	4,176,34	226.13	3,950.22
Inoc + 20S + 20T	2,240.21	4,813,99	442.83	4,371.16
Inoc + 20S + 40T	2,018.42	4,337,38	659.53	3,677.86
Inoc + 20S + 60T	1,962.06	4,216,27	876.23	3,340.05
Winter/spring 2009 crop at Faepe				
Control	1,741.67	3,742.67	113.10	3,629.57
Inoc	2,268.59	4,874.97	122.53	4,752.45
20S	1,919.89	4,125.65	216.70	3,908.95
Inoc + 20S	2,497.74	5,367.39	226.13	5,141.27
Inoc + 20S + 20T	2,734.80	5,876.81	442.83	5,433.99
Inoc + 20S + 40T	2,908.59	6,250.27	659.53	5,590.74
Inoc + 20S + 60T	2,886.48	6,202.76	876.23	5,326.53
Winter/spring 2010 crop at DAG				
Control	618.25	2,813.04	106.20	2,706.84
Inoc	1,066.32	4,851.76	115.05	4,736.71
20S	603.78	2,747.20	202.20	2,545.00
Inoc + 20S	1,543.21	7,021.61	211.05	6,810.56
Inoc + 20S + 20T	1,655.73	7,533.57	413.25	7,120.32
Inoc + 20S + 40T	1,697.27	7,722.58	509.25	7,213.33
Inoc + 20S + 60T	1,909.15	8,686.63	711.45	7,975.18
Winter/spring 2010 crop at Epamig				
Control	658.45	2,995.95	106.20	2,889.75
Inoc	789.24	3,591.04	115.05	3,475.99
20S	873.16	3,972.88	202.20	3,770.68
Inoc + 20S	1,567.98	7,134.31	211.05	6,923.26
Inoc + 20S + 20T	1,449.90	6,597.05	413.25	6,183.80
Inoc + 20S + 40T	1,490.54	6,781.96	509.25	6,272.71
Inoc + 20S + 60T	1,640.28	7,463.27	605.25	6,858.02

DISCUSSION

A significant effect on SDM from the treatments was only observed at DBI (Table 3). The Control in DBI was lower than in the other treatments, which shows that in the absence of N sources there is lower initial plant growth, with a reduction of more than 55 % in shoot matter compared to treatments that received only Inoc and/or mineral N. Studies of common bean related to inoculation with bacteria and mineral fertilizers have varied widely with respect to this parameter. Bassan et al. (2001), working with Pérola, and Soares et al. (2006), with a Talismã cultivar in an *Argissolo Vermelho Distrófico típico* Ultisol), found that inoculation treatments had higher SDM compared to treatments without inoculation and without mineral N. However, Pelegrin et al. (2009) studied a *Latossolo Vermelho Distroférrico* (Oxisol) in Dourados, MS and Soratto et al. (2005) and

Farinelli et al. (2006) studied a *Nitossolo Vermelho Distrófico* in Botucatu, SP, all with the cv. Pérola, and they found no differences in SDM.

Excess mineral N in the soil reduces nodulation in plants due to a lack of stimulation related to a deficiency of this nutrient (Moreira, 2006). The present study shows that NN was not affected by the treatments studied at the DBI, Faepe, and Epamig sites (Table 4). At DAG, the Inoc and Inoc + 20S treatments showed higher nodulation, indicating that inoculation was effective in forming nodules and that 20 kg ha^{-1} of N-urea at sowing favored root growth, probably increasing the points of infection in the roots (Table 4).

The inhibitory effect of mineral N on the nodule is visible in NDM (Table 4) because this variable had reduced mean values with increased N-supply in topdressing. As observed in the experiments at the DBI and Faepe sites, the treatments that received topdressing showed decreased NDM in comparison with the other treatments, and this effect was more pronounced at Faepe. Similar results were observed at the other sites, although at DAG, the Control did not differ from the treatments with N-addition in topdressing; however, at Epamig, all treatments that received mineral fertilizer showed lower values. Nodulation inhibition by high N levels is well reported in the literature (Mostasso et al., 2001; Soares et al., 2006; Ferreira et al., 2009).

Greater NAS was observed at the DBI site compared to Faepe (Table 4). N accumulation in this site may have been favored by the high organic matter content in the soil, which certainly provided N to the plants. At the DAG site, higher NAS values were achieved in the treatments that received mineral N in topdressing. However, at Epamig, the lowest N application rate in topdressing resulted in an NAS similar to what was obtained from the Control and Inoc treatments, which showed the lowest average values among those evaluated. At Epamig, the unexpected result may have come from the lower SDM value compared to the other treatments that received N because NAS is the result of multiplying SDM by the N content in the SDM.

In general, the DBI site was superior at flowering for all variables (Table 4) compared to the Faepe site (Table 5), which was not maintained at grain maturity. The results for the DBI site may also be attributed to the effect of organic matter, where the greatest N amounts were available in the vegetative phase. These N levels allowed the plant to remain in the vegetative stage for a longer period, which is not always favorable for grain productions.

For the primary yield components, there are differences among the treatments for some variables in some sites. Araújo et al. (2007) assert that these characteristics have high genetic heritability, but in some situations, no differences occur with various management types. However, improved nutritional status can increase these values. Andrade et al. (2001) assert that the mineral fertilization in topdressing resulted in a larger number of pods per plant, showing that N application at sowing additionally to topdressing, as well as inoculation additionally to N applied at topdressing resulted in higher values for PP compared to the treatments: inoculation only (Inoc) and the control (Control).

The treatments affected GY at all of the sites, and similar results were observed in GNA, except at DAG. The GY at the four sites under all treatments were higher than the national average of 1,312 kg ha^{-1} in the winter crop (Conab, 2015), except in the Control and Inoc treatments at the DAG and Epamig sites. The highest grain production occurred at the DBI and Faepe sites, which adopted a technological level of 4 (Ribeiro et al., 1999).

In general, all of the treatments that received mineral N and inoculation were in the highest group and showed a savings of 60 kg ha^{-1} of N-urea. In the 2009 experimental crop (DBI and Faepe), the Inoc + 20S treatment produced 2,220.60 kg ha^{-1} of grain, similar to the Inoc + 20S + 60T treatment, which had a yield close to 2,424.27 kg ha^{-1} (Table 4). The same treatments produced 1,543.21 and 1,909.15 kg ha^{-1} at the DAG site and 1,567.98 and 1,640.28 kg ha^{-1} at the Epamig site, respectively. These results demonstrate the beneficial effect of applying fertilizer at small application rates for better root development and subsequent promotion of infection and symbiosis establishment.

Brito et al. (2011) found a beneficial effect when applying N at small application rates at sowing of common bean, observing higher values (for N content in plants at a rate of 15 mg kg^{-1} of soil). According to Franco and Döbereiner (1968) and Tsai et al. (1993), small application rates of N at the beginning of root growth increased the infection sites and, therefore promoted greater nodule formation. Such a benefit was evident in this study, as the treatments that received Inoc + 20S had higher NDM, except at the Epamig site, where the value for the Inoc + 20S treatment was also lower than Inoc and Control.

Regarding GNA, the treatments differed at DBI, Faepe, and Epamig. At these sites, the inoculated treatments were superior to the others, and at DBI and Faepe, the 20S treatment was also superior to the Control and Inoc treatments, with greater GNA accumulation at the Faepe site. The GNA results show again that joint inoculation with 20 kg ha^{-1} of N-urea was beneficial to plants, and that increasing mineral N application by topdressing did not increase GNA. Thus, this showed the benefits of small N application rates as well as BNF. Brito et al. (2011) showed that the N supply to the plant is greater through BNF than through organic or mineral N.

According to economic analysis of production, in the winter/spring 2009 crop at DBI, winter/spring 2009 crop at Faepe, and winter/spring 2010 crop at DAG, the highest rates of return on invested capital were obtained in the treatments with the highest yields. However, in the winter/spring 2010 crop at Epamig, the highest rate of return on invested capital was obtained in the Inoc + 20S treatment, which did not have the highest yield (Table 5). When maximum yield is compared to the rate of return on invested capital, N economy was 11.18 kg ha^{-1} of N-urea in the winter/spring 2009 crop at DBI, 7.63 kg ha^{-1} of N-urea in the winter/spring 2009 crop at Faepe, and 44.33 kg ha^{-1} of N-urea in the winter/spring 2010 crop at Epamig. A similar result was found by Pelegrin et al. (2009) at Dourados, MS, Brazil, in a *Latossolo Vermelho Distroférrico* (Oxisol), as net income from a treatment of inoculation plus 20 kg ha^{-1} of N-urea did not differ from the treatment that received 160 kg ha^{-1} of N-urea. However, these authors only performed a joint inoculation with mineral N application of 20 kg ha^{-1} of N-urea in this field experiment. They did not test joint inoculation with application of N-urea above 20 kg ha^{-1}.

Our results showed that the combined inoculation with application of N-urea can contribute to greater profitability in common bean crops at all sites and that the optimal application rate of mineral N depends on the site. However, it was not possible to relate these effects to edaphic and climatic characteristics as well as to management. This can be explained because the profitability does not consider the statistical difference among treatments. The profitability just considers the absolute values of the treatments. On the other hand, treatments showed the same tendency when comparing statistically the grain yields.

CONCLUSIONS

The BRSMG Majestoso cultivar inoculated with the strain CIAT 899 and application of 20 kg ha^{-1} of N-urea at sowing shows grain yields that do not differ from treatments receiving up to 80 kg ha^{-1} of N-urea.

An increase in N application rates increases common bean vegetative growth but reduces nodulation.

Combining inoculation with application of N-urea can contribute to greater profitability in common bean crops at all sites. However, the optimal application rate depends on the site.

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