Nativa, Sinop, v. 7, n. 4, p. 336-342, jul./ago. 2019.
Pesquisas Agrárias e Ambientais
DOI: http://dx.doi.org/10.31413/nativa.v7i4.6606
http://www.ufmt.br/nativa

ISSN: 2318-7670

Response of cowpea to inoculation with strains of *Bradyrhizobium* spp. and to nitrogen fertilizer under protected cultivation

Arlete da Silva BANDEIRA[1*], Paulo Araquém Ramos CAIRO[1], Ramon Correia de VASCONCELOS[1], Adriana Dias CARDOSO[1], Manoel Nelson de CASTRO FILHO[1], Maria Caroline Aguiar AMARAL[1], Sávio de Oliveira RIBEIRO[1]

[1]Universidade Estadual do Sudoeste da Bahia, UESB, Vitória da Conquista, BA, Brasil.
E-mail: arletebandeira@yahoo.com.br

Received in May/2018; Accepted in March/2019.

ABSTRACT: Inoculating cowpea seeds with atmospheric nitrogen-fixing bacteria may be a more sustainable management alternative. The objective of this study was to assess the effects of inoculation and N fertilizer rates on characteristics of cowpea grown in greenhouse. A randomized complete block design was used. Treatments were laid out in a 4 x 4 factorial experiment consisting of four N sources: three inoculant strains (BR 3262, BR 3267 and BR 3299) and a non-inoculated control without mineral N supply; and four N fertilizer rates (0, 20, 40 and 60 kg ha^{-1}). The following characteristics were evaluated: plant height, shoot dry weight, root dry weight, total dry weight, number of nodules, nodule dry weight, and relative efficiency of treatments. For the treatment inoculated with strain BR 3267, shoot dry weight, root dry weight and total dry weight increased with increasing N rate up to 30 kg ha^{-1}. However, nodulation was inhibited with increasing N rate. The symbiotic efficiency of strain 3262 was similar to that of the control and lower than the remaining strains, indicating that the indigenous population of rhizobia, by itself, is able to meet the plant's N demand.

***Keywords*:** *Bradyrhizobium*; biological nitrogen fixation; mineral nutrition; *Vigna unguiculata* (L.) Walp.

Resposta do feijão-caupi a inoculação com estirpes de *Bradyrhizobium* e adubação nitrogenada em cultivo protegido

RESUMO: A inoculação de sementes de feijão-caupi com bactérias fixadoras de nitrogênio atmosférico pode ser uma alternativa de manejo mais sustentável. Neste estudo, objetivou-se avaliar os efeitos da inoculação e doses de N sobre características do feijão-caupi, em cultivo protegido. Utilizou-se o delineamento experimental em blocos ao acaso, arranjados em esquema fatorial 4 x 4, com quatro fontes de N, caracterizadas por três estirpes (BR 3262, BR 3267 e BR 3299) e uma testemunha sem inoculação e sem N mineral, e quatro doses de adubação nitrogenada (0, 20, 40 e 60 kg ha^{-1}). As características avaliadas foram: altura de plantas, massas secas da parte aérea, da raiz e de toda a planta, número de nódulos, massa seca de nódulos e eficiência relativa dos tratamentos. No tratamento inoculado com a estirpe BR 3267, a massa seca da parte aérea, raiz e de toda a planta aumentaram, com doses crescentes de N até 30 kg ha^{-1}. Entretanto, a nodulação foi inibida, com o aumento da dose de N. A eficiência simbiótica da estirpe BR 3262 foi semelhante à da testemunha, sendo as demais inferiores, indicando que a população nativa de rizóbios, por si só, é capaz de suprir a demanda da planta.

Palavras-chave: *Bradyrhizobium*; fixação biológica de nitrogênio; nutrição mineral; *Vigna unguiculata* (L.) Walp.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a grain legume that has high protein content in its grains and is a major food component of low-income populations, mainly in the North and Northeast regions of Brazil. In these regions, cowpea is grown especially by smallholder, family-based farms where traditional practices are still used.

On the other hand, in the Central-West region, large-scale, intensive farming predominates. Compared with other crops, the genetic potential of cowpea is still poorly explored, which is one of the causes of its low yields, especially in the Northeast, where average yields do not exceed 311 kg ha^{-1} (CONAB, 2018). The low use of technologies aimed at the exploitation of the crop also contributes to this low productivity.

Efficient rhizobium inoculants and nitrogen fertilizers are some of the main technologies that can be used in the cultivation of legumes, such as cowpea, so as to improve agronomic characteristics. Cowpea can benefit from biological nitrogen fixation (BNF), which reduces production costs by decreasing mineral fertilizer input, in addition to enabling the sustainability of its cultivation.

The symbiotic process occurs between the legume and bacteria, mainly in species such as *Bradyrhizobium japonicum* and *B. elkanii*, known as rhizobia. The establishment of this symbiotic association is highly specific (HUNGRIA, 1994).

Several studies have shown over the years that some strains of rhizobium can increase crop yield potential by effective symbiosis and high agronomic efficiency, thereby replacing, partially or wholly, nitrogen fertilizers (COSTA et al., 2011; ALCÂNTARA et al., 2014; COSTA et al., 2014; FARIAS et al., 2016). However, positive responses are not always observed regarding the associative efficiency of inoculant strains since these may compete with indigenous rhizobia for nodule sites and N_2 fixation (KANEKO et al., 2010; FREITAS et al., 2012).

The increase or reduction of nodulation may be linked to the association and competition between indigenous rhizobia and inoculant strains. It depends on the occupation capacity of nodules, the adaptation of inoculant strains to different environments, and the genetic characteristics of the host plant (XAVIER et al., 2006).

Studies on the performance of strains of nitrogen-fixing bacteria, carried out in a range of soil conditions and in environments with different concentrations of N_2, are important since these strains can interfere with the nodulation process on host plant.

The objective of this study was to assess the effect of inoculation with previously selected strains, in association with nitrogen fertilization, on characteristics of cowpea, cv. BRS Novaera grown under protected cultivation.

2. MATERIAL AND METHODS

The experiment was conducted at the State University of Southwestern Bahia (UESB), campus Vitória da Conquista, Bahia state, Brazil, in a polypropylene mesh-covered environment. Seed inoculation and evaluations were carried out in laboratory between Nov 2015 and Jan 2016. The species used was cowpea, cv. BRS Novaera. Vitória da Conquista is located in the micro-region of Planalto de Conquista, southwestern Bahia, at 874.81 m altitude andS14° 53' and W40° 48'. The average annual rainfall is 733.9 mm and the local climate is tropical of altitude, Cwb, according to the Köppen classification.

During the experiment, internal temperatures fluctuated from 14.8 ° (minimum) to 29.7 ° C (maximum).

The soil used as substrate was collected in an area with no previous land-use background, classified as Typical Dystrophic Yellow Latosol, with clay-sandy loam texture. It was disintegrated, sieved, homogenized, mixed with washed sand (2:1) and put in 7-dm^3 plastic pots. The results of the soil chemical analysis from samples taken from 0 to 0.20 m deep, prior to setting up the experiment, were: pH in water = 6.3; P = 11.0 mg dm^{-3} (Mehlich-1 Extractor); K^+ = 0.4 $cmolcdm^{-3}$ (Mehlich-1 Extractor); Ca^{2+}= 2.6 $cmolcdm^{-3}$ (KCl 1 mol L^{-1} extractor); Mg^{2+}= 1.1 $cmolcdm^{-3}$ (KCl 1 mol L^{-1} extractor); Al^{3+}= 0.0 $cmolcdm^{-3}$ (KCl 1 mol L^{-1} extractor); H^+ = 2.0 $cmolcdm^{-3}$ (SMP buffer solution, pH 7.5 to 7.6).

Phosphorus and potassium fertilizers were applied to all treatments at a rate of 315 mg of P_2O_5per pot (equivalent to 90 kg ha^{-1}), as single superphosphate, and 70 mg of K_2O per pot (equivalent to 20 kg ha^{-1}), as potassium chloride. These rates were based on results of the soil analysis and fertilization recommendations for Minas Gerais state, (CHAGAS et al., 1999).

A randomized complete block design with five replications was used. The treatments were arranged in a 4 x 4 factorial: four N sources (three rhizobia strains, BR 3262, BR 3267, BR 3299 and seeds without inoculation) and four N rates (0, 20, 40 and 60 kg ha^{-1}). The treatment with neither seed inoculation nor N fertilization was the control, totaling 80 experimental units.

The strains BR 3262 (SEMIA 6464), BR 3267 (SEMIA 6462) and BR 3299 are classified as *Bradyrhizobium elkanni*, *B. japonicum* and *B.* ssp, respectively.

Peat-based inoculant strains were supplied by Embrapa Agrobiology with a rhizobial concentration of about 35.60 x 10^9 for strain BR 3262; 0.76 x 10^9 for strain BR 3267; and 8.80 x 10^9 for strain BR 3299.

Seeds were inoculated in laboratory, before sowing, at a ratio of 500 g of inoculant for each 50 kg of seeds. 300 mL of 10% sugar solution (p: v) was added to the mix, so the inoculant strains could stick better to the seeds.

N fertilization was performed with urea (45% N), 50% at sowing and 50% as top dressing (25 days after emergence). Treatments were: 20 kg N ha^{-1} at sowing, 40 kg N ha^{-1} (20 kg ha^{-1} at sowing and 20 kg ha^{-1}as top dressing) and 60 kg N ha^{-1} (30 kg ha^{-1} at sowing and 30 kg ha^{-1}as top dressing). The amounts of urea for each treatment corresponding to 20, 40 and 60 kg N ha^{-1} were 70, 140 and 210 mg of urea per pot, respectively.

Three seeds per pot were used. Seedlings were thinned seven days after emergence, leaving one seedling per pot. Over the conduction of the experiment, daily irrigation was performed to maintain soil moisture near the field capacity (CASAROLI; VAN LIER, 2008). Weed control and phytosanitary treatments were done whenever needed.

At 35 days after emergence (beginning of flowering), the following characteristics were evaluated: plant height (cm), shoot dry weight (g), root dry weight (g), total dry weight (g), number of nodules (nodules $plant^{-1}$) and nodule dry weight per plant (mg $plant^{-1}$). The relative efficiency of the treatments was determined by the ratio of shoot dry weight of inoculated plants to the shoot dry weight of N-fertilized plants, according to the following formula proposed by Bergensen et al. (1971): RE = (inoculated SDW / N-fertilized SDW) x 100, where: RE = relative efficiency; inoculated SDW = shoot dry weight of inoculated plant; and N-fertilized SDW = shoot dry weight of N-fertilized plants.

Shoots were separated from roots at the stem base. Roots were rinsed on sieves and the nodules were detached, counted and dried on absorbent paper.

To determine dry weight production, the whole plant (nodules, shoot and roots) was oven-dried at 65-70 ºC for 72 h to constant weight and then weighed on as cale with accuracy of 0.001g.

Data were subjected to analysis of variance ($p \leq 0.05$). Tukey's test was used to compare the effect of N sources and the relative efficiency of these treatments as a function of N rates, as well as the number of nodules and nodule dry weight. N rates were fitted to polynomial regression up to 3rd degree. Statistical analysis was performed using the software SISVAR 5.4 (FERREIRA, 2014).

3. RESULTS

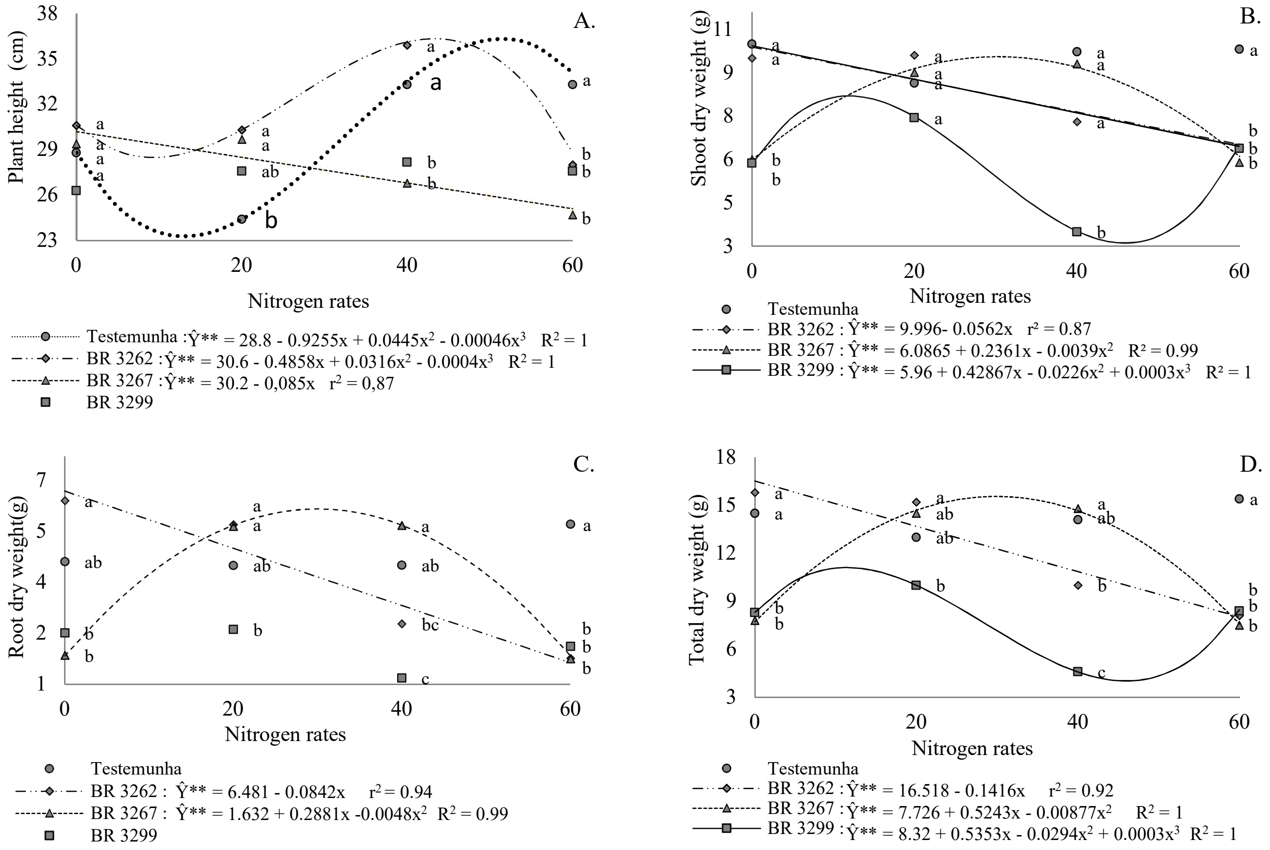
There was a significant interaction between N sources and N rates only for plant height (PH), shoot dry weight (SDW), root dry weight (DRW) and total dry weight (TDW) (Table 1). As for the number of nodules (NNOD) and nodule dry weight (NODDW), they were affected by the main effect of the factors. PH, SDW, RDW and TDW data were fitted to linear, quadratic and cubic models. For the quadratic models, maximum N rate was calculated, while for cubic models, maximum and minimum N rates were calculated (Figure 1).

Table 1. Summary of analysis of variance and coefficient of variation (CV) for plant height (PH), shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), number of nodules (NNOD) and nodule dry weight (NODDW) of cowpea, cv. BRS Novaera, subjected to N sources (F) and N rates (D) in kg ha^{-1} and their interaction (F * D).

Tabela 1. Resumo da análise de variância e do coeficiente de variação (CV) para a altura de planta (APL), massa seca de parte aérea (MSPA), massa seca de raiz (MSR), massa seca total da planta (MST), número de nódulos (NNOD) e massa seca de nódulos (MSNOD) do feijão-caupi, cv. BRS Novaera, submetido às fontes de N (F), doses de N (D) em kg ha^{-1} e sua interação (F*D).

		Mean Squares					
SV	DF	PH	SDW	RDW	TDW	NNOD	NODDW
N sources (F)	3	66.68**	48.37**	26.22**	145.94**	6,352.46**	64,579.79**
N rates (D)	3	37.63**	8.79*	10.92**	39.31**	2,776.94**	52,132.26**
F*D	9	47.10**	11.94**	14.33**	48.96**	670.7	14,756.80
Block	4	18.92	5.14	0.78	7.72	379.96	8,105.08
Residual	60	8.94	2.74	2.87	7.90	692.67	7,944.94
CV (%)		10.29	21.00	48.31	24.69	62.45	59.40

**Significant ($p \leq 0{,}01$) by F-test; *significant ($p \leq 0{,}05$) by F-test.



** Significant ($p \leq 0.01$), by analysis of variation of regression.

Distinct lowercase letters indicate significant differences between N sources within each N rate based on Tukey`s range test ($p \leq 0.05$).

Figure 1. Plant height (A), shoot dry mass (B), root dry mass (C) and total dry mass of the plant (D) of cowpea, cv. BRS Novaera, as a function of N sources and N rates (kg ha^{-1}).

Figura 1. Altura de plantas (A), massa seca de parte aérea (B), massa seca de raiz (C) e massa seca total da planta (D) de feijão-caupi, cv. BRS Novaera, em função das fontes e doses de N (kg ha^{-1}). Letras minúsculas distintas indicam diferenças significativas entre as fontes de N, dentro de cada dose de N, com base no teste de Tukey ($p \leq 0{,}05$)

PH was fitted to a linear model for inoculation with strain BR 3267, with a decrease in mean PH of 0.08 cm for each additional kg of N fertilizer. The responses of control and BR 3262 were fitted to a cubic model, with a decrease in PH up to 10 and 15 kg N ha^{-1} for strain BR 3262 and control when they reached the height of 28.50 and 23.38 cm, respectively. From these rates on, PH increased again up to the N rates of 45 and 50 kg ha^{-1} for the strain and the control, respectively, when they both reached 36.28 cm high. Despite the variation in PH, it was not possible to fit a model ($p \leq 0.05$) for strain BR 3299 as a function of N rates (Figure 1A).

Analyzing the interaction between N sources and N rates, the strains and control did not promote significant differences in PH in the absence of N. At N rate of 20 kg ha^{-1}, BR 3262 and BR 3267 were more efficient than the control, which may indicate greater adaptation of these strains to the environment and less competition with indigenous rhizobia. The N rate of 40 kg ha^{-1} led to higher increases in PH for the control and strain BR 3262, whereas at rate of 60 kg ha^{-1}, the control was more efficient than the strains (Figure 1A).

The increase in N rates linearly decreased SDW when strain BR 3262 was used, as a decrease of 0.05 g in SDW was recorded for each additional kg of N fertilizer, representing a33.80% loss up to the N rate of 60 kg ha^{-1}. For strain BR 3267, SDW was fitted to an increasing quadratic model, with an estimated turning point of 9.66 g at the N rate of 30 kg ha^{-1}. For strain BR 3299, there was a cubic effect, with N rates of 10 kg ha^{-1} and 45 kg ha^{-1} promoting the highest (8.25 g) and the lowest (3.23 g) SDW, respectively. In contrast to PH, inoculation with strain BR 3262 did not favor the production of SDW with increasing N rates (Figure 1B).

Regarding the effect of N sources within N rates, control treatments and inoculation with strain BR 3262 had higher SDW than the remaining inoculated treatments at N rate of 0 kgha^{-1}. At N rate of 20 kg ha^{-1}, there were no significant differences in SDW between the strains and the control, while at N rate of 40 kg ha^{-1}, strains BR 3262, BR 3267 and control had higher SDW than BR 3299. At 60 kg ha^{-1}, the control exhibited higher SDW than the inoculated treatments, indicating a deleterious effect of N at this rate on inoculated treatments. Indigenous soil bacteria may also have contributed to the reduction of efficiency of the inoculant strains at this N rate (Figure 1B).

As for RDW, there was a decreasing linear effect for strain BR 3262, and an increasing quadratic effect for strain BR 3267. For strain BR 3299, as well as in control, it was not possible to fit regression models (Figure 1C).

RDW was higher at N rate of 0 kg ha^{-1} (6.48 g plant^{-1}) and lower at N rate of 60 kg ha^{-1} (1.43 g plant^{-1}). Thus, the increase in N rates decreased 0.08 g of RDW for each additional kg of N when using strain BR 3262. As for strain BR 3267, the highest value of RDW was 5.96 g plant^{-1} at N rate of 30 kg ha^{-1}, followed by decreases at higher N rates.

As for the interaction between N sources and N rate of 0 kg ha^{-1}, the association with strain BR 3262 resulted in RDW 74.19 and 62.90% higher than the RDW obtained in the associations with the strains BR 3267 and BR 3299, respectively. At N rate of 20 kg ha^{-1}, the strains BR 3262 and BR 3267 were more effective than strain BR 3299. At N rate of 40 kg ha^{-1}, strain BR 3267 was superior to strains BR 3262 and BR 3299, while at N rate of 60 kg ha^{-1}, RDW recorded in inoculated treatments was lower than in the control, which suggests that high N rates can damage the inoculant strains (Figure 1C). The response of TDW to N rates was fitted to a decreasing linear model for the strain BR 3262; to an increasing quadratic model for the strain BR 3267; and to a cubic model for the strain BR 3299. As for the control, the data did not fit any regression model (Figure 1D).

The lower N rate in strain BR 3262 increased TDW (16.52 g), while the higher rate resulted in lower TDW (8.02 g).Therefore, the increase in N rates caused a decrease of 0.14 g in TDW for each kg of N fertilizer added to plants, representing a reduction of 51.45% up to 60 kg ha^{-1}. For strain BR 3267, the quadratic model indicated that up to 30 kg N ha^{-1}, TDW reached the maximum value of 15.57 g plant^{-1}. For strain BR 3299, the maximum and minimum TDW values were, respectively, 11.08 g at N rate of 10 kg ha^{-1} and 4.04 g at the rate of 45 kg ha^{-1} (Figure 1D).

Studying N sources within N rates, in non-inoculated treatments and those inoculated with strain BR 3262, TDW was higher at N rate of 0 kg ha^{-1}. At N rate of 20 kg ha^{-1}, the strain BR 3299 was lower than strain BR 3262. At N rate of 40 kg ha^{-1}, the strain BR 3267 showed higher TDW than strains BR 3262 and BR 3299, whereas for strain BR 3262, TDW was higher than BR 3299. Finally, at 60 kg ha^{-1}, TDW in the non-inoculated treatment was higher than in inoculated treatments (Figure 1D).

NNOD ranged from 24.90 to 63.95 nodulesplant^{-1}. The non-inoculated treatment was similar to that of strain BR 3262 and superior to that of strains BR 3267 and BR 3299 (61.06 and 52.07%, respectively). Concerning NODDW, the control also had a similar performance to strain BR 3262 and higher than BR 3267 and BR 3299 (44.03 and 59.29% higher, respectively). Strain BR 3262, however, performed better than BR 3299 (Table 2).

Table 2. Number of nodules (NNOD) and nodule dry weight (NODDW), per cowpea plant, cv. BRS Novaera, as a function of N sources.

Tabela 2. Número de nódulos (NNOD) e massa seca de nódulos (MSNOD), por planta de feijão-caupi, cv. BRS Novaera, em função das fontes de N.

N sources	NNOD (number plant^{-1})	NODDW (mg plant^{-1})
Strain BR 3262	49.06 ab	174.77 ab
Strain BR 3267	24.90 c	121.09 bc
Strain BR 3299	30.65 bc	88.07 c
Control	63.95 a	216.35 a

Means followed by the same letter in each column do not statistically differ from one another (Tukey, $p > 0,05$).

The effect of N rates on NNOD produced a decreasing linear effect, with a reduction of 0.37 nodules for each kg of N fertilizer added from the rate of 0 kg N ha^{-1}, which represents a loss of 49.64% in the NNOD up to N rate of 60 kg ha^{-1} (Figure 2A).

The response of the plant to N rates was fitted to a cubic model with decreasing NODDW from the N rate of 0 kg ha^{-1} (189.38 mg), up to the rate of 15 kg ha^{-1} (127.93 mg), then increasing again up to the rate of 40 kg ha^{-1} (190.52 mg). From this point on, there was a decrease in NODDW up to the rate of 60 kg ha^{-1} (81.30 mg) (Figure 2B).

The relative efficiencies of N sources, as a function of the N rates of 20, 40 and 60 kgha^{-1}, are shown in Table 3.

At N rate of 20 kg ha^{-1} (RE 20), the relative efficiency of the strain BR 3262 was higher than that of strain BR 3267 and BR 3299. For RE 40, strain BR 3262, N-fertilized control (40 kg N ha^{-1}) and the N-free control were higher than the strains BR 3267 and BR 3299. Finally, the relative efficiency of the strain BR 3262, N-fertilized control (60 kg N ha^{-1}) and N-free control were higher than the remaining strains at the rate 60 kg ha^{-1} (RE 60).

4. DISCUSSION

Concerning PH, the results indicated that the non-inoculated plants responded positively to nitrogen fertilization, which is corroborated by the findings of Melo et al. (2015), who verified that 50 kg ha^{-1}, without inoculation, is the N fertilizer rate that results in higher PH (27.8 cm) for cowpea. In the treatment inoculated with strain BR 3262, the performance of the plants was higher at the rates between 40 and 50 kgha^{-1}. However, the strain BR 3267 had a decreasing linear behavior for PH with increasing N rates. High N fertilizer rates possibly hamper BNF as a consequence of decreased nodulation, negatively affecting vegetative growth.

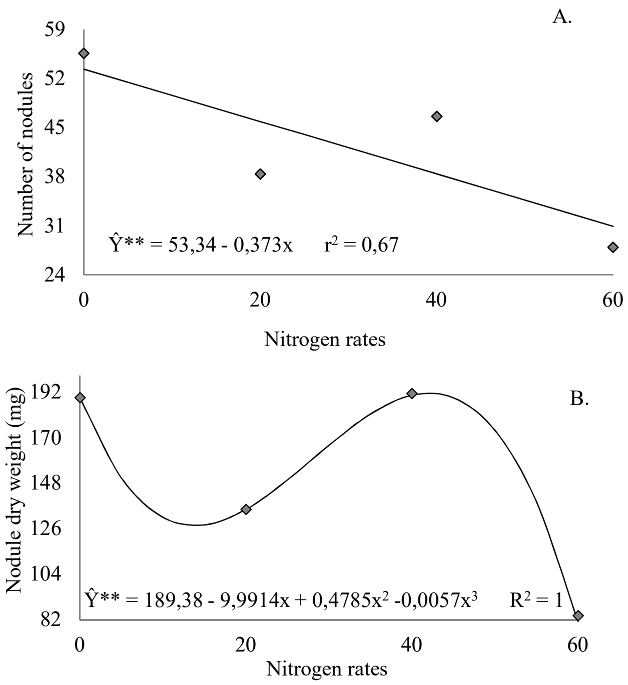


Figure 2. Number of nodules (A) and nodule dry weight (B) per cowpea plant cv. BRS Novaera as a function of N rates.
** Significant ($p \leq 0.01$), by regression analysis of variance.
Figura 2. Número de nódulos (A) e massa seca de nódulos (B), por planta de feijão-caupi, cv. BRS Novaera, em função de doses de N.
** Significativo ($p \leq 0,01$), pela análise de variância da regressão.

Table 3. Relative efficiency (RE) of N sources as a function of the rates of 20, 40 and 60 kg N ha^{-1} in cowpea cv. BRS Novaera.
Tabela 3. Eficiência relativa (EFR) das fontes de N em função das doses 20, 40 e 60 kg N ha^{-1}, em feijão-caupi, cv. BRS Novaera.

N sources	RE 20	RE 40	RE 60
	----------------------(%)---------------------		
BR 3262	112.32 a	100.28 a	97.80 a
BR 3267	69.04 c	63.10 b	61.12 b
BR 3299	69.52 bc	62.22 b	60.54 b
Control – 20 kg ha^{-1}	100.00 abc	-	-
Control – 40 kg ha^{-1}	-	100.00 a	-
Control – 60 kg ha^{-1}	-	-	100.00 a
Control without N	105.34 ab	106.46 a	102.90 a
CV (%)	20.35	18.84	17.94

Means followed by the same letter in each column do not statistically differ from one another (Tukey, p> 0.05). CV = coefficient of variation.

The strains and the control did not significantly affect PH in the absence of N. Similarly, Valadão et al. (2009), working with inoculation of common bean seeds and nitrogen fertilization, found no significant differences between the control and the inoculated treatment in the absence of N.

In general, N rates higher than 30 kg ha^{-1} indicated a damaging effect of N on inoculated treatments. Indigenous soil bacteria may also have contributed to the reduction of efficiency of the inoculant strains. According to Kaneko et al. (2010), the presence of indigenous symbiotic bacteria in the soil can limit the development of inoculant strains, as these indigenous population can be more efficient for symbiosis with common bean.

Generally, the responses of cowpea to N fertilization and inoculation have been inconsistent as to SDW, resulting in either significant (CHAGAS JUNIOR et al., 2014; COSTA et al., 2014; FARIAS et al., 2016) or non-significant (COSTA et al., 2011) effects.

Inoculation with strain BR 3262 decreased RDW, which represents a loss of 77.93% up to the N rate of 60 kgha^{-1}. The strain BR 3262 without N fertilization had the highest shoot dry weight, root dry weight and total dry weight compared to other N sources. Nonetheless, it was negatively affected by N rates.

The values obtained for RDW are consistent with those found by Santos et al. (2014) who reported RDW of 7.57 g plant^{-1} in response to the N rate of 42.5 kg ha^{-1}for cowpea inoculated with the strain BR 3262 and grown on a Dystrophic Yellow Latosol (LAdx). There were decreases in RDW at N rates higher than 30 kg ha^{-1} for inoculated treatments, suggesting that high N rates may negatively affect the strains (Figure 1C). This response may also be associated with the lack of adaptation of the strains to local climate and soil conditions.

In this study, the use of N rates up to 30 kg ha^{-1} may favor the inoculation process of the strain BR 3267, which may have provided higher production of amino acids, proteins, enzymes and chlorophylls, as these are linked to plant growth and development. These results differ from those reported by Melo et al. (2015), who found higher TDW (21.4 g) at the N rate of 50 kgha^{-1}, without inoculation.

The findings indicate that increasing N rates had a better effect on PH than on other characteristics. However, it is still necessary to evaluate whether the use of N fertilizer is economically feasible to provide increases in PH.

In general, it is worth noting that, in the absence of N fertilization, the strain BR 3262 was the treatment that provided higher SDW, RDW and TDW. Notwithstanding, with increasing N rates, there was a linear decrease in the mean of these characteristics, indicating that N fertilization was detrimental to the efficiency of the strain and to the increase in plant dry weight.

NNOD, as a function of N sources, was corroborated by the findings reported by Alcântara et al. (2014) in cowpea inoculated with the same strains used in this study, in which nodulations varied from 35.99 to 78.86 nodulesplant^{-1}.

BR 3267 had the best performance while BR 3262 was the most efficient in the nodule formation process, indicating a better adaptation of this strain to the climate and soil conditions as well as the studied cultivar (Table 2).

The results for NNOD in this study differ from those obtained by Chagas Júnior et al. (2014), who observed greater nodulation in inoculated treatments than in non-inoculated treatments. Costa et al. (2014) evaluated two cowpea cultivars inoculated with N_2-fixing bacteria under protected cultivation and verified similar NNOD among the strains within each cultivar.

The results reported herein also diverge from those found by Cavalcante et al. (2017) and Marinho et al. (2017), in which the cowpea plants with seeds treated with strain BR 3267 showed NNOD higher than those of the control, in both field and greenhouse conditions.

Although plant nodulation was more intense in the treatment without inoculation, this fact may be related to the presence of indigenous rhizobia in the soil, as the presence of these organisms is frequent, even in non-inoculated plants (RUFINI et al. 2014). According to Xavier et al. (2006), different nodulation intensities may be a response to competition between inoculant strains and indigenous rhizobia populations, which may promote nodulation increase or reduction, depending on nodule occupancy and strain adaptation to soil and climate conditions.

The increase in N rates negatively influenced the rhizobia activity and the formation of nodules. These results are consistent with those obtained by Santos et al. (2014) and Melo et al. (2015), who observed that the addition of N to the soil contributes to the reduction of nodulation in the roots of cowpea. Parente et al. (2015) studied the nodulation in soybean plants and also verified a reduction in this process, due to the increase in N rates.

The lower nodulation in legumes exposed to the high availability of mineral N in the soil is a consequence of changes in the exudation pattern of organic compounds by the roots of these plants, resulting in decreased exudation of inducers. (HUNGRIA, 1994).

In the treatment without N fertilization, the plants had high values of NODDW, suggesting that when N rates do not inhibit the nodulation process, indigenous strains can meet the N demand of plants. Conversely, higher N rates (from 40 kg ha^{-1}) may reduce the NODDW, indicating deleterious effect of N on BNF process.

The results obtained herein differ from those found by Santos et al. (2014) in an experiment with cowpea subjected to nitrogen fertilization and inoculation with strain BR 3262, in which the NODDW was favored by N rates (between 40 and 62 kgha^{-1}) on all evaluated soils.

Some studies have shown the ability of indigenous rhizobia to perform nodulation with cowpea plants, in which nodulation rate varies between 20 and 90% (Costa et al., 2011; Freitas et al., 2012). In the present study, however, the results differ from those reported by Santos et al. (2014) and Farias et al. (2016), in which NNOD and NODDW, in treatment with neither inoculation nor addition of N, were lower than the treatment with strain BR 3262.

The results on RE reported here in are akin to those of Ferreira (2013), who evaluated cowpea fertilized with different N sources on two soil types. According to this author, the RE of strain BR 3262 (91.78%) did not differ from the RE of the nitrogen control (100%), in a Yellow Latosol fertilized with 300 mg N vaso^{-1}. In another study involving similar treatments with cowpea, Farias et al. (2016) verified that the control with N (80 kg ha^{-1}) had a RE similar to that of the strains evaluated, whereas the control without N and without inoculation had higher RE.

Strain BR 3262, control and treatments that received only fertilization contributed to a greater accumulation of shoot dry weight and therefore can be considered as having higher efficiency, since the RE is a parameter that indicates the occurrence of symbiotic relationship. As soils naturally have a population concentration of indigenous rhizobia capable of nodulating cowpea, these may have contributed to these results. Hence, the variability of responses obtained in this work may be associated, according to Kanekoet al. (2010) and Freitas et al. (2012), both to the density and competitive ability of indigenous rhizobia, which compete with inoculant strains for nodule sites during BNF.

3.CONCLUSIONS

Shoot dry weight, root dry weight and total plant dry weight respond efficiently to N fertilization up to the Nr ate of 30 kg ha^{-1}, with strain BR 3267.

The non-inoculated treatment without mineral N supply was similar to strain BR 3262 as to yield and nodule dry weight.

Nodulation was reduced with increasing N rates.

6. ACKNOWLEDGMENTS

To the State University of Southwestern Bahia (UESB), for the infrastructure and support to the conduction of the experiment, and to the Coordination for the Improvement of Higher Education Personnel (CAPES), for granting scholarship to the first author.

7. REFERENCES

ALCANTARA, R. M. C. M. de; XAVIER, G. R.; RUMJANEK, N. G.; ROCHA, M. M. & CARVALHO, J. dos S. Eficiência simbiótica de progenitores de cultivares brasileiras de feijão-caupi. **Revista de Ciências Agronômicas**, Fortaleza, v. 45, n. 1, p.1-9, jan./mar. 2014.

BERGENSEN, F. J.; BROCKWELL, J.; GIBSON, A. H. & SCHWINGHAMER, E. A. Studies of natural populations and mutants of Rhizobium in the improvement of legume inoculants. **Plant and Soil**, The Hague, v. 46, p. 3-16. 1971. DOI: https://dx.doi.org/10.1007/BF02661831

CASAROLI, D.; VAN LIER, Q. de J. Critérios para determinação da capacidade de vaso. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 32, n. 1, p. 59-66, jan./feb. 2008. DOI: http://dx.doi.org/10.1590/S0100-06832008000100007

CAVALCANTE, A. C. P.; CAVALCANTE, A. G.; DINIZ NETO, M. A.; MATOS, B. F.; DINIZ, B. L. M. T.; BERTINO, A. M. P. Inoculação das cultivares locais de feijão-caupi com estirpes de rizóbio. **Revista de Ciências Agrárias**, Belém,v. 60, n. 1, p. 38-44, jul. 2017.DOI: http://dx.doi.org/10.4322/rca.2170

CHAGAS JUNIOR, A. F.; OLIVEIRA, A. G.; REIS, H. B.; SANTOS, G. R.; CHAGAS, L. F. B.; MILLER, L. O. Eficiência da inoculação combinada de rizóbio e Trichoderma spp. em diferentes cultivares de feijão-caupi (*Vignaunguiculata*) no cerrado (Savana Brasileira). **Revista de Ciências Agrárias**, Lisboa, v. 37, n. 1, p. 20-28, mar. 2014.

CHAGAS, J. M.; BRAGA, J. M.; VIEIRA, C.; SALGADO, L. T.; NETO, A. J.; ARAÚJO, G. A. de A.; ANDRADE, M. J. B de.; LANA, M. R. Q.; RIBEIRO A. C. Feijão. In: RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVAREZ, V. H. **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais** - 5ª Aproximação. Viçosa, 1999. p. 274-275.

CONAB_COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira de grãos**. Safra 2017/2018 - quarto levantamento, v. 5, n. 4, p. 1-132. Disponível em: <http://www.agricultura.gov.br/noticias/safra-de-graos-podera-atingir-227-9-milhoes-de-toneladas-em-2017-2018/AcompanhamentodaSafraBrasileiradeGros4Levantamento20172018.pdf>. Acesso em: 01/05/2018.

COSTA, E. M.; NÓBREGA, R. S. A.; MARTINS, L. V.; AMARAL, F. H. C.; MOREIRA, F. M. S. Nodulação e produtividade de *Vignaunguiculata* (L.) Walp por cepas de rizóbio em Bom Jesus, PI. **Revista Ciência Agronômica**, Fortaleza, v. 42, n. 1, p. 1-7, jan./mar.

2011. DOI: http://dx.doi.org/10.1590/S1806-66902011000100001

COSTA, E. M.; NÓBREGA, R. S. A.; SILVA, A. F. T. da; FERREIRA, L. de V. M.; NÓBREGA, J. C. A.; MOREIRA, F. M. S. Resposta de duas cultivares de feijão-caupi à inoculação com bactérias fixadoras de nitrogênio em ambiente protegido. **Revista Brasileira de Ciências Agrárias**, v. 9, n. 4, p. 489-494, 2014. DOI: http://dx.doi.org/10.5039/agraria.v9i4a3590

FARIAS, T. P.; TROCHMANN, A.; SOARES, B. L.; MOREIRA, F. M. S. Rhizobia inoculation and liming increase cowpea productivity in Maranhão State. **Acta Scientiarum Agronomy**, Maringá, v. 38, n. 3, p. 387-395, July/Sept. 2016. DOI: http://dx.doi.org/10.4025/actasciagron.v38i3.28630

FERREIRA, L. V. M. **Seleção e eficiência de estirpes de bactérias diazotróficas simbióticas para *Vignaunguiculata* (L. Walp.)**. 2013. 112f. Dissertação (Mestrado) – UniversidadeFederal do Piauí, Teresina,2013.

FERREIRA, D. F. - Sisvar: a Guide for its Bootstrap procedures in multiplecomparisons. **Ciência e Agrotecnologia**, v. 38, n. 2, p. 109-112, mar./abr., 2014. DOI: http://dx.doi.org/10.1590/S1413-70542014000200001

FREITAS, A. D. S.; SILVA, A. F.; SAMPAIO, E. V. S. B. Yield and biological nitrogen fixation of cowpea varieties in the semi-arid region of Brazil. **Biomass and Bioenergy**, Oxford, v. 45, p. 109-114, 2012. DOI: https://doi.org/10.1016/j.biombioe.2012.05.017

HUNGRIA, M. Sinais moleculares envolvidos na nodulação das leguminosas por rizóbio. **Revista Brasileira de Ciências do Solo**, Viçosa, v. 18, n. 3, p. 339-364, jun. 1994.

KANEKO, F. H.; ARF, O.; GITTI, D. C.; ARF, M. V.; FERREIRA, J. P.; BUZETTI, S. Mecanismos de abertura de sulcos, inoculação e adubação nitrogenada em feijoeiro em sistema plantio direto. **Bragantia**, Campinas, v. 69, n. 1, p. 125-133. 2010. DOI: http://dx.doi.org/10.1590/S0006-87052010000100017

MARINHO, R. de C.N.; FERREIRA, L. de V.M.; SILVA, A.F. da; MARTINS, L.M.V.; NÓBREGA, R.S.A.; FERNANDES-JÚNIOR, P.I. Symbioticandagronomicefciencyof new cowpearhizobiafromBrazilianSemi-Arid. **Bragantia**, Campinas, v. 76, n. 2, p. 273-281, apr./jun. 2017. DOI: http://dx.doi.org/10.1590/1678-4499.003

MELO, N. C.; ALMEIDA, R. F.; SILVA, V. F. A.; FERREIRA, R. L. C.; VALENTE, G.F. Análise multivariada no crescimento e nodulação de feijão-caupi com doses de nitrogênio. **Enciclopédia Biosfera**, Goiânia,v. 11, n. 21, p. 142, jun./jul. 2015.

PARENTE, T. de L.; LAZARINI, E.; CAIONI, S.; PIVETTA, R. S.; SOUZA, L. G. M. de; BOSSOLANI, J. W. Adubação nitrogenada em genótipos de soja associada à inoculação em semeadura direta no Cerrado. **Revista Brasileira de Ciências Agrárias**, Recife,v. 10, n. 2, p. 249-255, abr. 2015. DOI: http://dx.doi.org/10.5039/agraria.v10i2a5320

RUFINI, M.; SILVA, M. A. P.; FERREIRA, P. A. A.; CASSETARI, A. S.; SOARES, B. L.; ANDRADE, M. J. B.; MOREIRA, F. M. S. Symbiotic efficiency and identification of rhizobia that nodulate cowpea in a RhodicEutrudox. **Biology and Fertility of Soils**, Berlin, v. 50, n. 1, p. 115-122, jan. 2014. DOI: https://dx.doi.org/10.1007/s00374-013-0832-4

SANTOS, K. C. dos; UCHÔA, S. C. P.; MELO, V. F.; ALVES, J. M. A.; ROCHA, P. R. R.; XIMENES, C. K. dos S. Inoculação com Bradyrhizobium e adubação nitrogenada em feijão-caupi cultivado em diferentes solos. **RevistaAgro@mbiente On-line**, v. 8, n. 3, p. 306-317, set./dez., 2014. DOI: http://dx.doi.org/10.18227/1982-8470ragro.v8i3.469

XAVIER, G. R.; MARTINS, L. M. V.; RIBEIRO, J. R; de A.; RUMJANEK, N. G. Especificidade simbiótica entre rizóbios e acessos de feijão-caupi de diferentes nacionalidades. **Caatinga**, Mossoró, v. 19, n. 1, p. 25-33, jan./mar.2006.