Field performance of new cowpea cultivars inoculated with efficient nitrogen-fixing rhizobial strains in the Brazilian Semiarid

Rita de Cássia Nunes Marinho[(1)], Rafaela Simão Abrahão Nóbrega[(2)], Jerri Édson Zilli[(3)], Gustavo Ribeiro Xavier[(3)], Carlos Antônio Fernandes Santos[(4)], Saulo de Tarso Aidar[(4)], Lindete Míria Vieira Martins[(5)] and Paulo Ivan Fernandes Júnior[(4)]

[(1)]Universidade Federal do Piauí, Campus Professora Cinobelina Elvas, Rodovia BR-135, Km 03, Planalto Horizonte, CEP 64900-000 Bom Jesus, PI, Brazil. E-mail: cassia_nmarinho@hotmail.com [(2)]Universidade Federal do Recôncavo da Bahia, Centro de Ciências Agrárias, Ambientais e Biológicas, Rua Rui Barbosa, nº 710, Centro, CEP 44380-000 Cruz das Almas, BA, Brazil. E-mail: rafaela.nobrega@gmail.com [(3)]Embrapa Agrobiologia, BR 465, Km 7, CEP 23890-000 Seropédica, RJ, Brazil. E-mail: jerri.zilli@embrapa.br, gustavo.xavier@embrapa.br [(4)]Embrapa Semiárido, BR 428, Km 152, Caixa Postal 23, CEP 56302-970 Petrolina, PE, Brazil. E-mail: carlos-fernandes.santos@embrapa.br, saulo.aidar@embrapa.br, paulo.ivan@embrapa.br [(5)]Universidade do Estado da Bahia, Departamento de Tecnologia e Ciência Sociais, Avenida Edgard Chastinet Guimarães, s/nº, São Geraldo, CEP 41150-000 Juazeiro, BA, Brazil. E-mail: lmvmartins@uneb.br

Abstract – The objective of this work was to evaluate the contribution of efficient nitrogen-fixing rhizobial strains to grain yield of new cowpea cultivars, indicated for cultivation in the Brazilian Semiarid region, in the sub-medium of the São Francisco River Valley. Two experiments were set up at the irrigated perimeters of Mandacaru (Juazeiro, state of Bahia) and Bebedouro (Petrolina, state of Pernambuco). The treatments consisted of single inoculation of five rhizobial strains – BR 3267, BR 3262, INPA 03-11B, UFLA 03-84 (*Bradyrhizobium* sp.), and BR 3299^T (*Microvirga vignae*) –, besides a treatment with nitrogen and a control without inoculation or N application. The following cowpea cultivars were evaluated: BRS Pujante, BRS Tapaihum, BRS Carijó, and BRS Acauã. A randomized complete block design, with four replicates, was used. Inoculated plants showed similar grain yield to the one observed with plants fertilized with 80 kg ha^{-1} N. The cultivars BRS Tapaihum and BRS Pujante stood out in grain yield and protein contents when inoculated, showing their potential for cultivation in the sub-medium of the São Francisco River Valley.

Index terms: *Bradyrhizobium*, *Microvirga vignae*, *Vigna unguiculata*, biological nitrogen fixation, inoculant.

Desempenho em campo de novas cultivares de feijão-caupi inoculadas com estirpes de rizóbio eficientes na fixação de nitrogênio no Semiárido brasileiro

Resumo – O objetivo deste trabalho foi avaliar a contribuição de estirpes de rizóbio, eficientes na fixação de nitrogênio, sobre a produção de grãos de novas cultivares de feijão-caupi, indicadas para cultivo no Semiárido brasileiro, no Submédio do Vale do Rio São Francisco. Dois experimentos foram implantados nos perímetros irrigados de Mandacaru (Juazeiro, BA) e Bebedouro (Petrolina, PE). Os tratamentos consistiram da inoculação isolada de cinco estirpes de rizóbio – BR 3267, BR 3262, INPA 03-11B, UFLA 03-84 (*Bradyrhizobium* sp.) e BR 3299^T (*Microvirga vignae*) –, além de um tratamento com nitrogênio e de um controle sem inoculação ou aplicação de N. As seguintes cultivares de feijão-caupi foram avaliadas: BRS Pujante, BRS Tapaihum, BRS Carijó e BRS Acauã. Utilizou-se o delineamento experimental de blocos ao acaso, com quatro repetições. As plantas inoculadas apresentaram produtividade de grãos similar à observada em plantas adubadas com 80 kg ha^{-1} de N. As cultivares BRS Tapaihum e BRS Pujante destacaram-se quanto à produtividade e ao teor de proteínas nos grãos, quando inoculadas, o que mostra seu potencial para cultivo na região do Submédio do Vale do São Francisco.

Termos para indexação: *Bradyrhizobium*, *Microvirga vignae*, *Vigna unguiculata*, fixação biológica de nitrogênio, inoculante.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] has great importance in the Brazilian Semiarid region, especially for small farmers. This crop is mainly cultivated for the production of dry and green beans for human consumption, and it is considered an essential source of proteins, carbohydrates, fibers, vitamins, and minerals for the populations in the Semiarid region of northeastern Brazil (Santos et al., 2008). According to Freire Filho (2011), the area cultivated with cowpea in the Northeast region achieved almost 1.3 million

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hectares and produced more than 382 thousand tons, with productivity around 330 kg ha^{-1} in 2009.

Water and thermal stresses in the Semiarid region, as well as the few technological resources available for use in cowpea cropping systems, are the main responsible for the low productivity in the region (Fernandes Júnior & Reis, 2008; Fernandes Júnior et al., 2012). Some low-cost technologies can contribute to increase cowpea production in the Brazilian Northeast. Among these, the use of inoculants containing selected efficient rhizobia strains can be highlighted. The use of rhizobial inoculants has beneficial economic and environmental impacts for leguminous crops (Moreira & Siqueira, 2006; Araújo et al., 2012). Currently, four *Bradyrhizobium* strains (BR 3267, BR 3262, INPA 03-11B, and UFLA 03-84) are authorized for use in the commercial production of cowpea inoculants in Brazil (Martins et al., 2003; Lacerda et al., 2004; Zilli et al., 2009). However, the rather insignificant productive response of cowpea genotypes to inoculation resulted in low diffusion of the technology among farmers in the Semiarid region. Nevertheless, over the past few years, researches have shown that some rhizobial strains, with high agronomic efficiency, can increase the productivity of cowpea in the region (Almeida et al., 2010; Costa et al., 2011; Fernandes Júnior et al., 2012; Freitas et al., 2012; Ferreira et al., 2013; Alcantara et al., 2014).

Despite these studies, there is still a lack of knowledge on the efficiency of these strains at the sub-medium of the São Francisco River Valley. Martins et al. (2003) studied the agronomic efficiency and the competitive ability of the strain BR 3267 and those results supported its inclusion in the list of bacteria used as cowpea inoculants. Up to now, field evaluations of the other three strains available for cowpea inoculation were not carried out in the region. Additionally, the field performance of the promising new strain BR 3299^T, isolated from cowpea grown in the state of Sergipe and recently described as the new specie *Microvirga vignae* (Radl et al., 2014), in the São Francisco River Valley, still needs to be assessed.

Moreover, the interaction between macro- and microsymbionts is an important factor that affects productive response to inoculation (Alcantara et al., 2014). The evaluation of this interaction might make it possible to more properly indicate specific inoculants, enhancing the technical recommendations for cowpea genotypes.

The inoculation response of new cowpea genotypes developed for the Brazilian Semiarid region has not yet been assessed. Among the newly developed cultivars, only the “Mulato” type, BRS Pujante, had its responses to inoculation studied and has been proven to be highly responsive to the practice (Chagas Junior et al., 2010). The “Canapu” type, 'BRS Acauã'; the “Fradinho” type, 'BRS Carijó'; and the black-coated 'BRS Tapaihum' have short cycle and high levels of minerals and micronutrients in grains (Santos et al., 2008; Santos, 2011); but, in spite of their favorable technological features, the efficiency of their association with rhizobia is still unknown.

The objective of this work was to evaluate the contribution of efficient nitrogen-fixing rhizobial strains to the development and grain yield of new cowpea cultivars, indicated for cultivation in the Brazilian Semiarid region, in the sub-medium of the São Francisco River Valley.

Materials and Methods

Two experiments were carried out under field conditions. In both assays, the inoculation treatments consisted of four strains of *Bradyrhizobium* sp.: BR 3267 (SEMIA 6462), BR 3262 (SEMIA 6464), INPA 03-11B (SEMIA 6463), and UFLA 03-84 (SEMIA 6461). These strains are officially indicated for cowpea inoculant production in Brazil (Brasil, 2011). Besides them, the strain BR 3299^T was also evaluated. Besides the inoculation treatments, a treatment with N fertilization and a control without inoculation and without N were assessed. The N fertilization treatment was equivalent to 80 kg ha^{-1} N, applied as urea, split in two times. The cowpea cultivars BRS Pujante, BRS Tapaihum, BRS Acauã, and BRS Carijó were evaluated. The rhizobial strains were supplied by the Johanna Döbereiner Culture Collection of Diazotrophic Bacteria, from Embrapa Agrobiologia, Seropédica, state of Rio de Janeiro. The cowpea seeds were acquired in Petrolina, state of Pernambuco, from Embrapa Produtos e Mercados.

The experiments used a factorial arrangement of five inoculation plus control treatments per four cultivars (7x4), in a total of 28 treatments. A randomized complete block design was used, with four replicates.

The experimental plots measured 12 m^2 and consisted of eight 3-m lines. A 0.50x0.25-m spacing between plants was adopted, in accordance to the agronomical recommendations for the crop.

The first experiment was carried out from June to August 2012, in the irrigated perimeter of Mandacaru, at the Mandacaru Experimental Field (MEF) (09º24'S, 40º26'W) of Embrapa Semiárido, in Juazeiro, state of Bahia. The soil of the area is classified as Vertissolo Háplico (Vertisol) (Santos et al., 2013), with a clayey texture. The second experiment was carried out from December 2012 to February 2013, at the Bebedouro Experimental Field (BEF) (09º09'S, 40º22'W), located at the Bebedouro irrigated perimeter of Embrapa Semiárido, in Petrolina, state of Pernambuco. The soil is classified as Argissolo Amarelo distrófico (Ultisol), with a sandy loam texture. The soils from both experimental areas were prepared with a plowing and a harrowing.

The soil at the MEF showed the following chemical attributes (Claessen, 1997): pH, 6.8; P, 44.62 mg dm^{-3}; K, 0.36 mg dm^{-3}; Ca^{2+}, 20.4 $cmol_c$ dm^{-3}; Mg^{2+}, 5.6 $cmol_c$ dm^{-3}; Al^{3+}, 0.05 $cmol_c$ dm^{-3}; $H+Al^{3+}$, 4.62 $cmol_c$ dm^{-3}; S, 26.45 $cmol_c$ dm^{-3}; CTC, 31.07 $cmol_c$ dm^{-3}; V, 85%; and organic matter, 7.2 g kg^{-1}. The soil at the BEF had the following chemical characteristics: pH, 6.3; P, 11.92 mg dm^{-3}; K, 0.33 mg dm^{-3}; Ca^{2+}, 2.0 $cmol_c$ dm^{-3}; Mg^{2+}, 0.4 $cmol_c$ dm^{-3}; Al^{3+}, 0.05 $cmol_c$ dm^{-3}; $H+Al^{3+}$, 0.66 $cmol_c$ dm^{-3}; S, 2.78 $cmol_c$ dm^{-3}; CTC, 3.44 $cmol_c$ dm^{-3}; V, 81%; and organic matter, 6.3 g kg^{-1}. Basal fertilizations were carried out with 20 kg ha^{-1} P_2O_5, using simple superphosphate, and with 20 kg ha^{-1} K_2O, with potassium chloride. The experiment carried out at the BEF was further fertilized with 20 kg ha^{-1} MgO, using magnesium sulfate.

Soil samples were collected during planting in order to determine rhizobial populations. This analysis was carried out with the most probable number (MPN) method, using infected cowpea plants ('BRS Pujante'), as described in Hungria & Araújo (1994).

To prepare the inoculant, the bacteria were cultivated in YM liquid medium (Vincent, 1970), under constant stirring, for five days. Then, 10 mL of the culture broth of each bacteria were inoculated individually in plastic bags containing 30 g of sterilized peat, in order to reach the cell concentration of 10^9 viable cells per gram of inoculant. The inoculation was performed with 40 g of inoculant per 1 kg of seeds, in plastic bags containing the seeds, the inoculant, and a sugar solution (sucrose 10% w/v), and then hand mixed. The inoculated seeds were shade-dried, and sowing was performed manually soon after inoculation.

Weed control was done manually, according to necessity. The control of plagues and diseases was done with products recommended for the crop in the region. For the experiment implemented at the MEF, a furrow irrigation system was used, applying a daily irrigation depth of approximately 5.5 mm. For the experiment implemented at the BEF, a dripper irrigation system was used, with drippers spaced at 0.5 m and with a 1.6 L h^{-1} water flow. During the execution of the experiments, average monthly precipitation was between 1.4 and 27.6 mm at the MEF and BEF, respectively, with a daily average temperature of 24 to 28°C.

A first evaluation of plant nodulation was performed during flowering (45 days after the emergence of the plants). Ten plants were collected in 1.0 m of the second row of each plot. These plants were cut at soil level, and the radicular system was separated and packed in plastic bags (Fernandes Júnior et al., 2012). The roots were washed, and the nodules were separated from the radicular system and counted. The aerial part and the nodules were packed in paper bags, dried at 65°C in a forced air chamber until constant weight, and then weighed. The following characteristics were determined: number of nodules (NN), shoot dry matter mass (DMS), nodule dry matter mass (DMN), and nitrogen accumulated in the shoots (NAS) using the semimicro Kjeldahl method (Liao, 1981).

A second evaluation was done during harvest. Beans were gathered from the useful plot (4 m^2, central to each plot). The grains were threshed for weighing, and grain yield (GY) was calculated. Grain protein content (GPC) was determined according to Williams (1984).

Data were subjected to analysis of variance, using the Sisvar 4.2 statistical analysis system (Ufla, Lavras, MG, Brazil). Means were compared by Student's test, at 10% probability. Previously to the analysis, data were transformed by $(x+1.0)^{0.5}$.

Results and Discussion

Rhizobial populations of the experimental areas were estimated at $2.8x10^3$ and $4.3x10^3$ cells per gram of soil, for the MEF and BEF, respectively. These populations can be considered high. In soils with established native rhizobia populations, the efficiency of the inoculation may be compromised because the introduced rhizobial strains need to compete with the indigenous isolates for nodulation sites (Zilli et al., 2013).

At the MEF, the NN in the cultivar BRS Pujante was influenced by the inoculated strain (Table 1). Inoculation treatments had higher NN than the fertilization treatment with 80 kg ha^{-1} N. BR 3262, BR 3299^T, INPA 03-11B, and UFLA 03-84 provided greater NN than the control treatment (without inoculation and N fertilization). Nodule formation inhibited by N fertilization was also observed by Silva et al. (2012), when evaluating rhizobia inoculation in the cultivar BRS Pujante.

Table 1. Response of cowpea (*Vigna unguiculta*) cultivars to the inoculation of rhizobial strains, at the experimental field of Mandacaru, Juazeiro, state of Bahia, Brazil[(1)].

Inoculation treatment	Number of nodules	Nodule dry matter	N accumulation in shoots	Dry matter of shoots
	------------------(mg per plant)		--------------	(g per plant)
BRS Pujante				
BR 3267	21bc	49.5bc	920a	23.0a
BR 3262	42a	148.6a	650bc	18.9a
BR 3299^T	36a	101.2ab	690abc	18.8a
INPA 03-11B	28ab	98.8abc	590bc	19.1a
UFLA 03-84	33ab	97.2abc	850ab	21.6a
80 kg ha^{-1} N	11d	86.5bc	500c	18.2a
Control	15cd	49.8c	680abc	19.6a
BRS Tapaihum				
BR 3267	20a	102.0a	700ab	18.9a
BR 3262	19a	95.2a	640ab	17.0a
BR 3299^T	17a	61.7a	770a	20.6a
INPA 03-11B	13a	49.8a	510b	20.0a
UFLA 03-84	16a	68.0a	750ab	21.9a
80 kg ha^{-1} N	17a	78.5a	650ab	18.6a
Control	12a	53.7a	570ab	17.7a
BRS Carijó				
BR 3267	12a	55.2a	810ab	24.0a
BR 3262	13a	68.3a	890a	26.1a
BR 3299^T	10a	45.5a	730ab	23.8a
INPA 03-11B	17a	84.1a	730ab	23.8a
UFLA 03-84	19a	64.6a	760ab	25.7a
80 kg ha^{-1} N	13a	64.7a	780ab	22.7a
Control	12a	58.0a	630b	17.9a
BRS Acauã				
BR 3267	11a	56.5a	430a	16.6a
BR 3262	12a	63.9a	460a	15.3a
BR 3299^T	9a	29.2a	550a	15.5a
INPA 03-11B	10a	43.5a	630a	17.2a
UFLA 03-84	9a	26.0a	530a	15.6a
80 kg ha^{-1} N	12a	53.3a	670a	19.6a
Control	12a	50.1a	530a	15.91a
CV (%)	27.2	32.2	6.5	24.5

[(1)]Means followed by equal letters, for a same cultivar, do not differ by Student's test, at 10% probability.

Cultivars BRS Tapaihum, BRS Carijó, and BRS Acauã did not respond to the inoculation treatments, regarding NN. Chagas Junior et al. (2010) found that the cultivar BRS Pujante nodulated abundantly with all evaluated strains, with lower nodulation rates observed in the nitrogen fertilization and in the absolute control treatments.

For DMN, BRS Pujante stood out, whereas the other cultivars showed equal means. Inoculation of the strain BR 3262 increased DMN of the cultivar BRS Pujante, when compared to the control treatment, but it did not differ from inoculation with the strains BR 3299^T, UFLA 03-84, and INPA 03-11B.

Regarding DMS, inoculated treatments did not differ from the control treatment. However, a significant increase in NAS was observed for the cultivar BRS Pujante inoculated with BR 3267, and for BRS Carijó, inoculated with BR 3262, when compared to the N fertilization and to the control treatment, respectively. Apart from nodulation, nodule efficiency may be influenced by plant genotype and by the efficiency of the strain in the nodules, which also depends on the macrosymbiont genotype (Alcantara et al., 2014). Therefore, the difference in nitrogen accumulation in shoots indicates that the genotypes BRS Carijó and BRS Pujante can benefit from inoculation.

At the BEF, NN differed according to the treatments (Table 2). Cultivar BRS Pujante, inoculated with BR 3267 and BR 3262, showed higher NN. Once again, N fertilization inhibited the formation of nodules, and the strains BR 3267 and BR 3262 provided an increase in nodule mass compared to the other treatments.

For the cultivar BRS Carijó, the strains BR 3267 and BR 3262 stood out regarding NN and DMN, respectively. For 'BRS Acauã', however, only the strain BR 3262 differed from the treatment with nitrogen fertilization, but it did not differ from the other treatments. 'BRS Tapaihum' was not significantly affected by the treatments, regarding NN and DMN. The inoculation with the strain BR 3299^T increased DMS of the four studied cultivars.

Regarding NAS, cultivar BRS Pujante performed better when inoculated with the strain BR 3299T,

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but did not differ from the N fertilization and the control treatments. For 'BRS Tapaihum', the strain BR 3262 provided the highest NAS. For 'BRS Carijó', inoculation treatments did not differ from the control. For 'BRS Acauã', BR 3262 provided higher NAS than the nitrogen fertilization and the other inoculated treatments.

The similarity of nodulation parameters and vegetative development between some inoculated and non-inoculated treatments indicates that the native rhizobial population was efficient to establish symbiosis with cowpea, which is able to nodulate with a wide range of tropical soil rhizobia (Leite et al., 2009; Jaramillo et al., 2013). The capacity of the native rhizobial population to establish an efficient symbiosis with cowpea has already been observed in the Brazilian Semiarid (Martins et al., 2003; Freitas et al., 2012; Alcantara et al., 2014).

Table 2. Response of cowpea (*Vigna unguiculta*) cultivars to the inoculation of rhizobial strains, at the experimental field of Bebedouro, Petrolina, state of Pernambuco, Brazil[(1)].

Inoculation treatment	Number of nodules	Nodule dry matter	N accumulation in shoots	Dry matter of shoots
	(mg per plant)			(g per plant)
		BRS Pujante		
BR 3267	53a	170.4a	17.9c	660c
BR 3262	25b	135.5a	25.8abc	900abc
BR 3299^T	12c	55.6b	30.9a	1,220a
INPA 03-11B	14bc	51.5b	20.7bc	780bc
UFLA 03-84	13c	60.0b	28.0ab	1,120ab
80 kg ha^{-1} N	4d	13.3c	24.6abc	830abc
Control	16bc	87.6ab	22.2abc	880abc
		BRS Tapaihum		
BR 3267	5a	19.1a	27.4abc	1,070abc
BR 3262	7a	43.3a	39.9a	1,430a
BR 3299^T	5a	44.0a	31.4ab	1,140ab
INPA 03-11B	6a	42.6a	15.8c	580c
UFLA 03-84	7a	42.2a	18.6bc	780bc
80 kg ha^{-1} N	4a	13.6a	29.2ab	1,090abc
Control	6a	21.2a	24.2bc	850bc
		BRS Carijó		
BR 3267	10a	63.8ab	23.6ab	1,030a
BR 3262	8ab	70.7a	22.3ab	950a
BR 3299^T	4ab	41.8abc	32.1a	900a
INPA 03-11B	6ab	46.2abc	18.0b	720a
UFLA 03-84	4ab	27.5abc	29.4ab	1,120a
80 kg ha^{-1} N	2ab	11.7c	25.8ab	910a
Control	2.b	24.5bc	19.7ab	850a
		BRS Acauã		
BR 3267	6ab	29.3ab	21.1bc	810bc
BR 3262	7a	53.3a	37.2a	1,420a
BR 3299^T	3ab	16.9ab	35.3a	1,260ab
INPA 03-11B	3ab	14.6ab	25.4ab	1,000ab
UFLA 03-84	3ab	21.5ab	14.2c	480c
80 kg ha^{-1} N	1b	17.2b	28.2ab	850bc
Control	3ab	20.5ab	26.1abc	930abc
CV (%)	35.2	37.0	19.5	9.7

[(1)]Means followed by equal letters, for a same cultivar, do not differ by Student's test, at 10% probability.

Grain yield results suffered the interaction between inoculation treatments and cowpea genotypes, in both experiments. Grain yield ranged from 1,091 to 1,629 kg ha^{-1} in the MEF, and from 817 to 1,824 kg ha^{-1} in the BEF (Table 3). Grain yield levels were considerably higher than 330 kg ha^{-1}, which is the average for the northeastern region, but within the expected levels for inoculation experiments under irrigated conditions in the Brazilian Semiarid (Santos et al., 2008; Santos, 2011).

In the MEF experiment, when cultivar BRS Pujante was inoculated with the strains BR 3267 and UFLA 03-84, productivity ranged from 1,496 to 1,629 kg ha^{-1}, higher than the ones observed with the strains BR 3262 and INPA 03-11B.

In the BEF, grain yield of non-inoculated treatments did not differ from the ones obtained with the strains BR 3267, BR 3299^T, and UFLA 03-84. Inoculation treatments had a similar yield to the one of the control and the N fertilization treatments. However, the strains UFLA 03-84 and BR 3262 had an increase of 30 and 31% in grain yield, respectively, compared to the control treatment. Ferreira et al. (2013) reported that the cultivar BR 17 Gurguéia, inoculated with the strain BR 3262, had a significant increase in grain yield, of 50.17%, in comparison to the untreated mineral N treatment without inoculation.

Inoculation treatments with the cultivar BRS Tapaihum had similar grain yield to that of the control treatment. At the BEF, however, the association of this cultivar with the strains BR 3262, UFLA 03-84, and INPA 03-11-B resulted in grain yield equal to the one obtained with the N-supplied control, but greater than the one of the absolute control. Inoculation with the strain INPA 03-11B stood out, increasing yield in 89%, when compared to the absolute control.

For 'BRS Carijó', the inoculation of the strains BR 3262, BR 3299^T, and UFLA 03-84 provided higher means at the MEF than the strains BR 3267 and INPA 03-11B, but similar yield to that of the absolute control. At the BEF, cultivars did not differ also. For 'BRS Acauã', grain yield at the MEF did not differ.

However, at the BEF, the association with the strains BR 3267, BR 3299^T, and INPA 03-11B increased productivity in 88, 83, and 83%, respectively, achieving higher yields than the one of the control treatment, but similar ones to that of the N fertilization treatment.

The obtained results are similar to the ones observed in regions of the Brazilian Semiarid. Costa et al. (2011) found that inoculation with INPA 03-11B promoted similar yields to that of N fertilization, in Bom Jesus, state of Piauí, with productivity of up to 1,604 kg ha^{-1}.

Table 3. Grain yield and grain protein content of the evaluated cowpea (*Vigna unguiculta*) cultivars, according to the inoculation treatments in the experimental fields of Mandacaru (MEF) and Bebedouro (BEF)[(1)].

Inoculation treatment	MEF		BEF	
	Grain yield (kg ha^{-1})	Protein (%)	Grain yield (kg ha^{-1})	Protein (%)
		BRS Punjante		
BR 3267	1,495a	24.4a	1,157b	24.0ab
BR 3262	1,219bc	24.8a	1,435ab	24.9a
BR 3299^T	1,475ab	25.4a	1,353ab	26.6a
INPA 03-11B	1,091c	21.9a	1,323ab	25.6a
UFLA 03-84	1,629a	25.9a	1,423ab	24.3a
80 kg ha^{-1} N	1,375ab	26.5a	1,824a	24.7a
Control	1,477ab	16.0b	1,088b	19.4b
		BRS Tapaihum		
BR 3267	1,340ab	21.4ab	1,346ab	23.6ab
BR 3262	1,322ab	21.8ab	1,551a	26.3a
BR 3299^T	1,198ab	24.9a	1,291ab	22.9ab
INPA 03-11B	1,087b	26.7a	1,769a	24.8a
UFLA 03-84	1,337ab	24.8a	1,589a	26.7a
80 kg ha^{-1} N	1,145ab	25.3a	1,320ab	26.1a
Control	1,379a	19.3b	934b	19.1b
		BRS Carijó		
BR 3267	1,048b	23.2ab	1,373a	23.7a
BR 3262	1,467a	21.2ab	1,570a	24.8a
BR 3299^T	1,436a	26.4a	1,370a	24.6a
INPA 03-11B	1,261ab	26.1a	1,222a	17.1b
UFLA 03-84	1,505a	25.0ab	1,197a	23.9a
80 kg ha^{-1} N	1,277ab	20.8b	1,526a	21.8a
Control	1,387a	21.4ab	1,048a	23.7a
		BRS Acauã		
BR 3267	1,210a	24.2a	1,535a	24.1a
BR 3262	1,198a	25.5a	951bc	23.2a
BR 3299^T	1,305a	24.9a	1,494a	24.3a
INPA 03-11B	1,151a	25.6a	1,494a	25.2a
UFLA 03-84	1,186a	25.0a	1,001bc	24.5a
80 kg ha^{-1} N	1,413a	24.9a	1,406ab	24.4a
Control	1,319a	20.3a	816c	26.3a
CV (%)	9.07	12.62	14.01	8.70

[(1)]Means followed by equal letters, for a same cultivar, do not differ by Student's test, at 10% probability.

Almeida et al. (2010) in an experiment carried out at Teresina, state of Piaui, reported a grain yield of 1,637 kg ha^{-1} for the cultivar BR 17 Gurguéia inoculated with the strain BR 3267, and of 1,823 kg ha^{-1} when inoculated with the strain BR 3262, a 24.6 and 38.9% increase, respectively, compared to that of the control treatment.

Grain protein contents in 'BRS Pujante' were influenced by the inoculation treatments and by N fertilization (Table 3). In both trials, these treatments showed higher GPC than those observed in the absolute control, especially with the strain BR 3299^T, which increased GPC in 58.8 and 37.1%, at the MEF and BEF, respectively. For 'BRS Tapaihum', higher GPC at the MEF was obtained with the strains INPA 03-11B, BR 3299T, and UFLA 03-84, with 26.7, 24.9, and 24.8%, respectively. These GPC values represented a respective increase of 38.3, 29, and 28.5% in comparison to the control treatment, which showed GPC of 19.3%. At the BEF, this genotype performed better with the strains UFLA 03-84, BR 3262, and INPA 03-11B, which increased GPC in 39.8, 37.7, and 29.8%, respectively, compared to the control. Therefore, 'BRS Tapaihum', which has a peculiar black-coated seed, despite not responding to inoculation regarding grain yield, had more nutritious grains with these treatments. For genotypes BRS Carijó end BRS Acauã, inoculation treatments had no effect on GPC, except when BRS Carijó was inoculated with INPA 03-11B, which provided lower values. In the Semiarid region, GPC is an important trait, since the production is mostly destined for human consumption and cowpea is considered one of the main protein sources for rural populations (Santos et al., 2008; Freire Filho, 2011).

The observed dependence of the inoculation treatments on genotypes, rhizobia strain, and environment is corroborated by other studies (Melo & Zilli, 2009; Alcantara et al., 2014). Therefore, plant breeding may have an important role in improving N supply for cowpea by biological fixation (Xavier et al., 2006; Araújo et al., 2012). The present study also emphasizes the importance of testing the response to inoculation of different varieties, under distinct edaphoclimatic conditions.

For cowpea, a lot of attention has been given to the selection of the microsymbionts, since the selection of the strain is the main resource for optimization of biological nitrogen fixation (BNF) (Fernandes Júnior et al., 2012). However, an adequate interaction between symbiotic partners is essential to BNF efficiency and, for this reason, it should be prioritized in breeding programs for this species, taking the well-succeeded example of soybean (*Glycine max* L.) in Brazil.

Conclusions

1. Cowpea cultivars respond differently to the inoculation of rhizobial strains, in the Brazilian Semiarid.
2. The inoculation of rhizobial strains can be beneficial to grain yield and protein contents in cowpea, mainly for the cultivars BRS Pujante and BRS Tapaihum, in the sub-medium of the São Francisco River Valley.

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