Contribution of rhizobial stains to the development and yield of new cowpea cultivars in the sub-medium part of São Francisco River Valley

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)] e Paulo Ivan Fernandes Júnior[(4)].

[(1)] Universidade Federal do Piauí (UFPI), Rodovia Municipal Bom Jesus - Viana, km 01 Planalto Horizonte 64900-000 – Bom Jesus, PI. E-mail: cassia_nmarinho@hotmail.com; [(2)] Universidade Federal do Recôncavo da Bahia (UFRB), Centro de Ciências Agrárias, Ambientais e Biológicas. Rua Rui Barbosa, n.710 Centro 44380-000 - Cruz das Almas, BA. E-mail: rafaela.nobrega@gmail.com; [(3)]Embrapa Agrobiologia.- BR 465, km 7 CEP 23890-000-Seropédica, RJ–Brasil. E-mail: jerri.zilli@embrapa.br, gustavo.xavier@embrapa.br; [(4)]Embrapa Semiárido, BR 428, km 152, CP 23, 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, Av. Edgard Chastinet, Juazeiro, BA, Brazil. E-mail: lmvmartins@uneb.br

ABSTRACT: The aim of this study was to evaluate the contribution of inoculation with rhizobial strains to the development and grain yield of new cowpea cultivars for the Brazilian Semi-arid. Two experiments were set up at the Irrigated Perimeter Mandacaru in Juazeiro, BA, and the Irrigated Perimeter Bebedouro in Petrolina, PE. The treatments consisted of five rhizobial strains, a nitrogen (N) supplied and a control without inoculation nor N aplication. The genotypes evaluated were the BRS Pujante, BRS Tapaihum, BRS Carijó and BRS Acauã. The experimental designs were in randomized blocks with four replicates. The experimental procedures, the size of the plots, as well as the spacing that was used is recommended by Agricultural Ministry. The inoculation treatments and the cowpea genotype influenced the nodulation, the accumulation of nitrogen in the shoots, the grain yield and the grains protein content. The inoculated plants showed a grain yield similar to the plants that received 80 kg ha^{-1} of N. The varieties BRS Tapaihum and BRS Pujante stood out because of the increased protein content in the grains and productivity respectively. The new varieties that were developed for the semi-arid region responded to the inoculation with rhizobial strains.

Index terms: *Vigna unguiculata,* Inoculant, Biological nitrogen fixation.

Introduction

The cowpea (*Vigna unguiculata* L. Walp) represents a crop of great importance in the Brazilian semi-arid region, especially for small farmers that have limitation to get new technologies. The cowpea, which is cultivated especially for the production of dry and green grains for human consumption, is considered an important source of protein, carbohydrates, fibers, vitamins and minerals for the population from the Brazilian semiarid region (FREIRE FILHO et al., 2005).

This crop shows widespread edapho-climatic adaptation, however, besides its relevance, the productiviity are expressively low, especially for the northeastern region (MARTINS et al., 2003; FREIRE FILHO et al., 2005). Environmental factors such as hydric and thermal stresses of the semi-arid region, as well as the few technological resources that are employed in its cultivation may contribute to the low productivity in the semi-arid region (FERNANDES JÚNIOR and REIS, 2008; FERNANDES JÚNIOR et al., 2012). Some low expensive technologies like inoculants containing selected rhizobia with efficiency and competitivity could contribute to increase the cowpea production in Brazilian northeast. Among the low-cost technology that is already available on the market, the use of inoculants containing selected efficient rhizobia strains can be stood out. The utilization of rhizobial inoculants has been widely recognized for reducing the dependency of farmers on nitrogenized fertilizers of industrial origin, which, apart from reducing production costs, also diminish the environmental impact of the agricultural production (MOREIRA e SIQUEIRA, 2006; ARAÚJO et al., 2012).

At the moment there are four rhizobial strains authorized by the Brazilian Agricultural Ministry for the production of commercial inoculants to cowpea in Brazil (BRASIL, 2011). However, the inoculation practices of cowpea crops are not widely spread among producers. Over the years, the rather insignificant response to inoculation in terms of productivity increase resulted in a low diffusion of inoculation technology, mainly among producers of the semi-arid region. However, over the past few years research results have shown that the use of rhizobial strains of agronomic efficiency can increase the productivity of cowpea in the region (ALMEIDA et al., 2010; COSTA et al., 2011; FERNANDES JÚNIOR et al; 2012; FERREIRA et al., 2013; FREITAS et al., 2013; ALCÂNTARA et al., 2014).

Among the factors that can influence the efficiency of cowpea inoculation, edaphoclimatic factors such as native rhizobia populations, soil fertility and humidity have received a lot of attention and have been studied over the years (FERNANDES JÚNIOR and REIS, 2008). Over the past few years, research has been carried out with the objective of evaluating the interaction between the genotypes of the macro- and microsymbionts and differentiated responses among varieties have been found (ALCÂNTARA et al., 2014). This way, the evaluation of genotypes in relation to the response to inoculation of rhizobial strains can indicate the utilization of these inoculants as a part of the technical recommendations of the cowpea genotypes.

Moreover, the selection of genotypes with recognized agronomic efficiency and responsivity to inoculation may indicate promising materials for improvement the breeding programs in Brazil (RUMJANEK et al., 2005; ALCÂNTARA et al., 2014). In the last few years some cowpea cultivars has been developed for the Brazilian Semi-arid region but the response of those plant genotypes to the inoculation of rhizobia were not determined. Among the newly developed cultivars, the "Mulato" type BRS Pujante, was already studied regarding its responses to inoculation ad showed highly responsive (CHAGAS JÚNIOR et al., 2010). The "Canapu" type BRS Acauã, the "Fradinho" type BRS Carijó and the black coated BRS Tapaihum were developed for conditions of the Semi-arid and show a short cycle, elevated levels of minerals and micronutrients (SANTOS et al., 2008; SANTOS, 2011). In spite of its technological features up to now those genotypes were not evaluated regarding the association with rhizobia.

Therefore, the evaluation of the new cowpea varieties inoculated with rhizobial strains is important to indicate the response of the plant genotypes and to determinate which isolate, should be used for inoculation under field conditions. The objective of this study was to evaluate the interaction between four new cowpea cultivars adapted to the semi-arid region of Brazil and nitrogen-fixing bacteria with recognized agronomic efficiency under field conditions in the sub-medium part of São Francisco River Valley.

Methods and Materials

Two experiments were conducted under field conditions. In both assays the inoculation treatments consisted of five strains of symbiotic diazotrophic bacteria: BR3267 (SEMIA 6462), BR3262 (SEMIA 6464), INPA03-11B (SEMIA 6463) and UFLA3-84 (SEMIA 6461) that belongs to *Bradyrhizobium* sp., indicated for inoculant production for cowpea in Brazil (BRASIL, 2011), apart from the strain BR3299 that were recently classified as the new specie *Microvirga vignae* (RADL et al., 2014). Besides the inoculation treatment, a control without inoculation and without N fertilization was used and another was used without inoculation and with N fertilization in the form of urea equivalent to 80 kg ha^{-1} of N, which the application was splitted in two times. The cowpea cultivars BRS Pujante, BRS Tapaihum, BRS Acauã and BRS Carijó were evaluated in the present study.

The experiments were implemented in a factorial of 7 (inoculation treatments and controls) x 4 (cultivars), setting a total of 28 treatments. The experimental design was in randomized blocks with four replications, totaling 112 plots. The experimental plots measured 12 m^2 and consisted of eight lines of plants, 3 m long. The adopted spacing was 0,5 m between the rows and 0,25 m between the plants in accordance with the recommendations of the Ministry of Agriculture, Livestock and Supply (MAPA) and the agronomical recommendations of the varieties.

The first experiment was carried out in the period of June to August 2012, in an area of the Irrigated Perimeter Mandacaru, at the Mandacaru Experimental Field (MEF) (09º24'S 40º26'W), of Embrapa Semiárido, in Juazeiro, BA. The soil of the experiment area is classified as a Hydromorphic Vertisol with a clay texture. The second experiment were conducted during the period from December 2012 to February 2013 at the Bebedouro Experimental Field (BEF) (09º09'S 40º22'W) of Embrapa Semiárido, located at the Bebedouro Irrigated Perimeter in Petrolina, PE. The soil from experimental area is classified as a Yellow Distrophyc Oxisol with a sandy loam texture. The soils of both experimental areas were prepared with a plowing and a harrowing. In both experiments a soil sample was used for chemical analysis in agreement with Claessen (1997).

The soil at the MEF showed the following chemical characteristics: pH= 6,8; P= 44,62 $mg.dm^3$; K= 0,36 $mg.dm^3$; Ca= 20,4 $cmolc.dm^{-3}$; Mg^{2+}= 5,6 $cmolc.dm^{-3}$; Al^{3+}= 0,05 $cmolc.dm^{-3}$; $H+Al^{3+}$= 4,62 $cmolc.dm^{-3}$; S= 26,45 $cmolc.dm^{-3}$; CTC= 31,07 $cmolc.dm^{-3}$; V= 85 %; organic matter = 7,2 $g.kg^{-1}$. The soil of the area used at the BEF showed the following chemical characteristics: pH= 6,3; P= 11,92 $mg.dm^3$; K= 0,33 $mg.dm^3$; Ca= 2,0 $cmolc.dm^{-3}$; Mg^{2+}= 0,4 cmolc.dm^{-3}; Al^{3+}= 0,05 cmolc.dm^{-3}; $H+Al^{3+}$= 0,66 cmolc.dm^{-3}; S= 2,78 cmolc.dm^{-3}; CTC=3,44 cmolc.dm^{-3}; V= 81 %; organic matter: 6,3 g.kg^{-1}. Basal fertilizations were carried out with 20 kg of P_2O_5 ha^{-1} with simple superphosphate, 20 kg of K_2O in the form of potassium chloride. The experiment carried out at the BEF was further fertilized with 20 kg of MgO ha^{-1} in the form of magnesium sulfate. During the planting of the experiments, soil samples were collected in the two planting areas to determine the rhizobial populations in the soil, which was carried out by the most probable number (MPN) method with infection in cowpea plants (BRS Pujante) in accordance with Hungria and Araújo (1994).

To prepare the inoculant, the bacteria were cultivated in YM liquid medium (VINCENT, 1970) under constant stirring for 7 days. After growth, 10 mL of the culture broth of each bacteria was inoculated individually in bags containing 30 g of sterilized peat, reaching the cell concentration of 10^9 viable cells per gram of inoculant. The inoculation in the proportion of 40 g of inoculant per 1 kg of seeds was carried out in plastic bags where the seeds, the inoculant and a sugar solution (sucrose 10% w/v) were mixed handy. The inoculated seeds were dried in the shadow and the sowing was done manually, immediately after inoculation.

The weed control in the experimental areas was done manually, according to necessity. The control of incidences of plagues and diseases was carried out with the application of products recommended for the crops in the region. The cultures were irrigated with two distinct systems. For the experiment implemented at the MEF, a furrow irrigation system was used, applying a blade that was calculated for approximately 5,5 mm of evapotranspiration. For the experiment implemented at the BEF, an irrigation system of 16” hoses was used with drippers spaced at 0,5 m with a flow of 1,6 L.h^{-1}. During the execution of the experiments at the MEF and at the BEF, average monthly precipitation was between 1,4 and 27,6 mm respectively and a daily average temperature of 24°C to 28°C respectively.

At the first evaluation during flowering (45 days after the emergence of the plants), ten plants were collected from one linear meter of the second row of each plot. The 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 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 were then weighed. The following characteristics were determined: dry mass of the shoots (DMS), dry mass of the nodules (DMN) and nitrogen accumulated in the shoots (NAS) by menas of the semimicro Kjeldahl method (LIAO, 1981).

At the second evaluation, during harvesting, the beans were gathered from the usefull area (4 m^2 central to each parcel). All the gains were threshed for weighing and the grain yield (GY) was calculated. The grain protein content (GPC) was determined in accordance with Williams (1984). The data were submitted for analysis of variance using the statistical analysis system SISVAR 4.2 (FERREIRA, 2008). To compare the means, Student's t-test with a 10% probability was used. All data were transformed by the square root of X + 1.0.

Results and Discussion

Rhizobial population of the experimental areas of the MEF estimated were of $2{,}8.10^3$ and $4{,}3.10^3$ rhizobial cells per g of soil for MEF and BEF, respectively. The established populations in the test areas were high and factors such as irrigation and the particular characteristics of the soils may contributed for the establishment (RUMJANEK et al., 2005). In soils that contain established native rhizobia populations, the efficiency of the inoculation may be compromised because the introduced rhizobia needs to compete with the indigenous isolates for the nodulation sites (ZILLI et al., 2013).

In the at the Mandacaru Experimental Field the nodules number (NN), for the variety BRS Pujante, was influenced by the inoculated strain (Table 1). All inoculation treatments with means for the NN variable reached levels that were superior to the treatment that received 80 kg ha^{-1} of N. Among the strains the BR 3262; BR 3299; INPA 03-11B and UFLA 03-84 stood out once the plants inoculated with then showed NN superior to what was observed in the absolute control treatment. The low quantity of nodules that was observed in the nitrogenized treatment indicates that the addition of nitrogen inhibited the formation of nodules. Silva et al. (2012) found a similar result when evaluating the effect of rhizobia inoculation in a BRS Pujante cultivar and reported that a control treatment with addition of N showed lower NN in absolute values when compared to the control treatment.

In the varieties BRS Tapaihum, BRS Carijó and BRS Acauã, the inoculation treatments did not differed from the control treatments regarding the NN, which indicates that there is a different response to inoculation among the evaluated varieties. Chagas Júnior et al. (2010), when evaluating the effect of inoculation with five strains in cowpea cultivars observed that the cultivar BRS Pujante nodulated abundantly with all strains evaluated, which was higher than the nodulation rates observed in the nitrogen fertilized and control control treatment as well.

For the nodule dry mass (NDM), the variety BRS Pujante showed a statistical difference among the inoculation treatments while the other varieties showed equal averages. For this variety, the inoculation with strain BR 3262 resulted in an increased NDM when compared to the control treatments and was equal to the inoculation treatments with strains BR 3299; UFLA 03-84 and INPA 03-11B. The other cowpea genotypes did not show differences among the treatments for the NDM variable.

Regarding the shoot dry mass (SDM), no significant differences between the inoculated and the control treatments was observed. However, for the nitrogen accumulation in the shoots (NAS), the cultivars BRS Pujante inoculated with BR 3267 and BRS Carijó inoculated with BR 3262 showed significant increase of N accumulated compared to the absolute control treatment and the N fertilized treatment, respectively. For the cultivar BRS Tapaihum and BRS Acauã, treatments did not differed among each other. Apart from nodulation, the efficiency of the nodules may be influenced by the plant genotype and the efficiency of the bacteria that are present in the nodules is dependent on the genotype of the macrosymbiont (ALCÂNTARA et al., 2014). In these way, the different accumulation of nitrogen in the shoots indicates that the genotypes BRS Carijó and BRS Pujante presents differential responses to inoculation.

In the second experiment that was carried out at the Bebedouro Experimental Field, different means among the treatments for the variable NN were observed for the genotypes BRS Pujante, BRS Carijó and BRS Acauã (Table 2). The genotype BRS Pujante inoculated with BR 3267 and BR 3262 showed higher number of nodules compared to the other inoculated treatments and the non-inoculated treatment. In this trail as well as in the first one, the fertilization with N inhibited the formation of nodules and the strains BR 3267 and BR 3262 resulted in an increased nodule mass in relation to the other treatments.

In the genotype BRS Carijó, the strains BR 3267 and BR 3262 stood out in terms of NN and NDM, respectively, which were statistically superior to the absolute control. On the other hand, for BRS Acauã, only the treatment inoculated with the strain BR 3262 differed from the treatment with addition of nitrogen, being equal to the other treatments. The variety BRS Taipahum did not show any significant differences for NN and NDM in none of the evaluated treatments. In relation to the DMS, the strain BR 3299 resulted in an increased mass in the four cultivars that were examined.

Regarding to the evaluation of NAS for the variety BRS Pujante, the strain BR 3299 performed better among the inoculated treatments, but was statistically similar to the nitrogenized and absolute control. In the variety BRS Tapaihum, the strain that produced the largest increase of N was the strain BR 3262, higher than what was observed in plants plants inoculated with the strains UFLA 03-84 and INPA 03-11B and the absolute control. For the variety BRS Carijó, there were no differences among the inoculation treatments and the test controls with regard to the variable NAS. The genotype BRS Acauã inoculated with BR 3262, showed higher values to NAS than the other inoculated treatments, in spite of did not presented differences among this and the control treatments.

The similarity among the treatments without inoculation and the inoculated treatments with regard to nodulation parameters and vegetative development indicates the efficiency of the increased native rhizobial population to establish symbiosis with cowpea, a legume which is considered capable of nodulating with a large number of soil rhizobia (THIES et al., 1991; ZILLI et al, 2006; LEITE et al., 2009; JARAMILO et al., 2013). Studies that were done have been carried out in the semi-arid region of Brazil showed the capacity of the native rhizobial population to establish symbiosis and to generate a great biomass production and N fixation in cowpea (MARTINS et al., 2003; FREITAS et al., 2013; ALCÂNTARA et al., 2014), which indicates that these soils can be efficient bacteria sources for this crop.

The results for the grain yield (GY) were dependent on the inoculation treatment and on the cowpea genotype in both experiments. For the first trial, GY ranged from 1091 to 1629 kg ha^{-1} and from 817 to 1824 kg ha^{-1} in the second experiment (Table 3). The GY levels were higher than 400 kg.ha^{-1}, which is the average for the northeastern region, however, within the expected productivity levels for inoculation experiments under irrigated condition in the Brazilian semi-arid (SANTOS et al., 2008; SANTOS, 2011).

For the BRS Pujante, at the plot implemented at the MEF, association with the strains BR 3267 and UFLA 03-84 resulted in a productivity from 1496 to 1629 kg.ha^{-1}, which are averages superior to what was observed for the plants inoculated with the strains BR 3262, BR 3299 and INPA 03-11B. The non-inoculated treatments showed a productivity that is statistically equal to what was found for the strains BR 3267 and BR 3299 while at the plot that was implemented at the BEF, all inoculated strains were similar to the absolute control treatments and the N fertilized treatment, but association with the strains UFLA 03-84 and BR 3262, however, promoted an increase of 30% and 31% in grain yield, respectively. Ferreira et al. (2013) reported that the inoculation of cowpea cultivar BR 17 Gurguéia with strain BR 3262, enabled a significant grain yield, promoting an increase of 50,17% compared to untreated mineral N without inoculation and which justifies the use of this biotechnology, as well as presenting increases in production, also reduces spending, since the cost of inoculation is much smaller than in systems that adopts the use of nitrogen fertilizers.

With regard to BRS Tapaihum, in the trial that was carried out at the MEF, the inoculation treatments were similar among the control treatments. In the second trial that was carried out at the BEF, the association with the strains BR 3262, UFLA 03-84 and INPA 03-11-B resulted in a grain yield equal to the N supplied control and greater than the absolute control. Inoculation with the strain INPA 03-11B stood out, generating an increase of 89% when compared to the absolute control treatment. For BRS Carijó, in the trial at the Mandacaru Experimental Field, the inoculation of the strains BR 3262, BR 3299 and UFLA 03-84 showed means superior to those treatments that were inoculated with the strains BR3267 and INPA 03-11B and similar to the absolute control treatment. At the BEF, all evaluated treatments were equal. In terms of grain productivity for the variety BRS Acauã at the plot at Mandacaru, all treatments were statistically equal. However, at the plot at Bebedouro, the association with strains BR 3267, BR 3299 and INPA 3-11B promoted productivity increases of 88%, 83% and 73%, respectively, being superior to the non-inoculated, non-fertilized treatment, and statistically equal to the nitrogen supplied treatment.

The results found in the present study corroborate the findings of author authors who showed that inoculation with efficient rhizobia strains can promote an increase in productivity. Chagas Júnior et. al. (2010), evaluating the vegetative development and the productivity of three cowpea varieties in the Cerrado, found that inoculation with strains recommended for the crop resulted in productivity that was similar to crops fertilized with N. Costa et al. (2011), when evaluating the productivity of cowpea in soil that was representative of the Production Center in Bom Jesus, PI, found that inoculation with the strain INPA 3-11B showed results similar to the N fertilized control, reaching the yield of 1604 $kg.ha^{-1}$. Almeida et al. (2010), in a trial conducted at the municipality of Teresina, PI, using the cowpea cultivar BR 17 Gurguéia, found a grain yield of 1637 $kg.ha^{-1}$ for the treatment inoculated with the strain BR 3267 and 1823 kg ha^{-1} for the treatment inoculated with the strain BR 3262, resulting in increases of 24,6 and 38,9%, respectively, in relation to the absolute treatment.

The grain protein content of the genotype BRS Pujante in both plots was influenced by the inoculation treatments or N fertilization (Table 3). In both trials, all inoculation treatments and the N supplied controls showed protein contents in the grains (PCG) higher than those observed in the absolute control treatments especially in the case of the strain BR 3299, which, when compared to the absolute control, resulted in an increased grain protein content of 58 to 37% in the first and second crop, respectively.

In the first experiment, grains of the variety BRS Tapaihum, which were obtained from the treatments inoculated with the strains INPA 03-11B, BR 3299 and UFLA 03-84, resulted in a grain protein content of 26,7; 24,9 and 24,8% respectively. Comparing to the absolute control treatment, where the grains showed mean of 19,3%, the increase reaches 38,3; 29,0 and 28,5% respectively. For the same genotype, in the second experiment, the strains UFLA 03-84, BR 3262 and INPA 03-11B proportioned PCG values above to those presented in the absolute control treatment in means rates of 39,8; 37,7 and 29,8% respectively. These results indicate that inoculation of the variety BRS Taipahum, a peculiar black coated cowpea genotype, apart from not necessarily resulting to production increases, leads to more nutritious grains with improved culinary and technological qualities. The protein content in the grains in the semi-arid is a very important parameter once the production is destined for human consumption and is considered as one of the main protein sources of the nutritional diet of the rural population, mainly in the semiarid region of Brazil (FREIRE FILHO, 2005; SANTOS et al., 2008).

The results found in these experiments corroborate the findings of other authors and show that the response to inoculation is dependent on the genotype, the rhizobia strain and the environment that was studied (MELO and ZILLI, 2009; CHAGAS JÚNIOR, et. al. 2010; ALCANTARA et. al 2014). In view of the different behaviors shown by the genotypes when they are inoculated, the importance of genetic improvement of the plant to improve the supply of N via biological fixation has been emphasized, which, in this way, permits the optimization of BNF in cowpeas by using efficient cultivars that respond to inoculation (XAVIER et al., 2006; ARAÚJO et al., 2012). In this study, the evaluated genotypes, apart from developing themselves satisfactorily in the climate of the semi-arid region, were capable of associating effectively with the inoculated rhizobia, resulting in grains with an elevated protein content. The different response that were observed in the two trials indicate the importance of testing different varieties under distinct edapho-climatic conditions to get at technical recommendations of the inoculation process of each cultivar that is launched on the market.

Face of the challenge of improving the N_2 fixation capacity in cowpeas, a lot of attention has been given to the selection of the microsymbionts, understanding as the selection of the strain is the main resource for optimization of BNF (FERNANDES JÚNIOR et al., 2012). However, the success of BNF depends on the chemical signals emitted by both the plant and the bacteria partners that induce the plant infection by rhizobia and nodule development (MOREIRA and SIQUEIRA, 2006). Many breeding programs expenses low attention to this interaction while the research results demonstrate that the adequate interaction between the symbiotic partners is essential to BNF efficiency and, for this reason, should be prioritized in breeding programs for cowpea, taking the well-succeeded improvement of soybean in Brazil as an example.

Conclusions

1. All the cowpea cultivars evaluated responded to inoculation with the recommended strains in terms of grain yield. With the variety BRS Pujante stood out and showed increased levels of nodulation and productivity.

2. The genotype BRS Tapaihum stood out for showing increased grain protein content and grain yield when inoculated with the rhizobium strains INPA 03-11B, UFLA 03-84 and BR 3262.

Acknowledgments

We hereby express our gratitude to CNPq, CAPES and Embrapa for their financial and infrastructure support.

References

ALCANTARA, R.M.C.M.; XAVIER, G.R.; RUMJANEK, N.G.; ROCHA, M.M.; CARVALHO, J.S. Eficiência simbiótica de progenitores de cultivares brasileiras de feijão-caupi. **Revista Ciência Agronômica**, v.45, p.1-9, 2014. DOI: 10.1590/S1806-66902014000100001

ALMEIDA, A.L.G.; ALCÂNTARA, R.M.C.M.; NÓBREGA, R.S.A.; NÓBREGA, J.C.A.; LEITE, L.F.C.; SILVA, J.A.L. Produtividade do feijão-caupi cv BR 17 Gurguéia inoculado com bactérias diazotróficas simbióticas no Piauí. Revista **Brasileira de Ciências Agrárias**, v.5, p.364-369, 2010. DOI: 10.5039/agraria.v5i3a795

ARAÚJO, A.S.F.; LEITE, L.F.C.; IWATA, B.F.; LIRA JÚNIOR, M.A.; XAVIER, G.R.; FIGUEIREDO, M.V.B. Microbiological process in agroforestry systems. A review.

Agronomy for Sustainable Development, v. 32, p. 215–226, 2012. DOI:10.1007/s13593-011-0026-0

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº 13, de 24 de março de 2011. Aprovar as normas sobre especificações, garantias, registro, embalagem e rotulagem dos inoculantes destinados à agricultura, bem como as relações dos micro-organismos autorizados e recomendados para produção de inoculantes no Brasil, na forma dos Anexos I, II e III, desta Instrução. DOU, 25 de mar. 2011. Seção 1. p.3-7.

CLAESSEN, M.C.E. (Org.). **Manual de métodos de análise de solo.** 2.ed. rev. atual. Rio de Janeiro: Embrapa-CNPS, 1997, 212p.

COSTA, E.M.; NÓBREGA, R.S.A.; MARTINS, L.V.; AMARAL, F.H.C.; MOREIRA, F.M.S. Nodulação e produtividade de *Vigna unguiculata* (L.) Walp. por cepas de rizóbio em Bom Jesus, PI. **Revista Ciência Agronômica,** v.42, p.1-7, 2011. DOI: http://dx.doi.org/10.1590/S1806-66902011000100001

FERNANDES JÚNIOR, P.I.; REIS, V.M. **Algumas Limitações à Fixação Biológica de Nitrogênio em Leguminosas**. Seropédica: Embrapa Agrobiologia, 2008 (Série Documentos nº 252).

FERNANDES JÚNIOR, P.I.; SILVA JÚNIOR, E.B.; SILVA JÚNIOR, S; SANTOS, C.E.R.S.; OLIVEIRA, P.J.; RUMJANEK, N.G.; MARTINS, L.M.V.; XAVIER, G.R. Performance of polymer compositions as carrier to cowpea rhizobial inoculant formulations: survival of rhizobia in pre-inoculated seeds and field efficiency. **African Journal of Biotechnology**, v.11, p.2945-2951, 2012. DOI: 10.5897/AJB11.1885.

FERREIRA D.F. Sisvar: um programa para análises e ensino de estatística. **Revista Científica Symposium**, v. 6, p. 36-41, 2008.

FERREIRA, L.V.M.; NÓBREGA,R.S.A.; NÓBREGA, J.C.A.; AGUIAR, F.L.; MOREIRA, F.M.S.; PACHECO, L.P. Biological Nitrogen Fixation in Production of *Vigna unguiculata* (L.) Walp, Family Farming in Piauí, Brazil. **Journal of Agricultural Science**, v.5, p.153-160, 2013. DOI:10.5539/jas.v5n4p153.

FREIRE FILHO, F.R.; LIMA, J.A.A.; RIBEIRO, V.Q. **Feijão-caupi: avanços tecnológicos**. Brasília: Embrapa Informações Tecnológicas, 2005. p. 519.

FREIRE, J.R.J.; VERNETTI, F.J. A pesquisa com soja, a seleção de rizóbio e a produção de inoculantes no Brasil. **Pesquisa Agropecuária Gaúcha**, v.5, p.117-126, 1999.

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

HUNGRIA, M.; ARAÚJO, R.S. **Manual de métodos empregados em microbiologia agrícola**. Brasília, Embrapa Informações Tecnológicas. 1994, 542p.

JARAMILLO, P.M.D.; GUIMARÃES, A.A.; FLORENTINO, L.A., SILVA, K.B.; NÓBREGA, R.S.A.; MOREIRA, F.M.S. Symbiotic nitrogen-fixing bacterial populations trapped from soils under agroforestry systems in the Western Amazon. **Scientia Agricola**, v.70, n.6, p.397-404, 2013. DOI:101590/ S0103-90162013000600004

LEITE, J.; SEIDO, S.L.; PASSOS, S.R.; XAVIER, G.R.; RUMJANEK, N.G. & MARTINS, L.M.V. Biodiversity of rhizobia associated with cowpea cultivars in soils of the lower half of the São Francisco river valley. **Revista Brasileira de Ciência do Solo**, v. 33, p.1215-1226, 2009. DOI: /10.1590/S0100-06832009000500015

MARTINS, L.M.V.; XAVIER, G.R.; RANGEL, F.W.; RIBEIRO, J.R.A.; NEVES, M.C.P.; MORGADO, L.B.; RUMJANEK, N.G. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. **Biology and Fertility of Soils**, v.38, p.333-339, 2003. DOI: 10.1007/s00374-003-0668-4

MOREIRA, F.M.S.; SIQUEIRA, J.O. **Microbiologia e bioquímica do solo**. 2. ed. Atual. E ampl. Lavras: Editora UFLA, 2006, 729p.

RADL, V.; SIMÕES-ARAUJO, J.L.; LEITE, J.; PASSOS, S.R.; MARTINS, L.M.V.; XAVIER, G.R.; RUMJANEK, N.G.; BALDANI, J.I.; ZILLI, J.E. *Microvirga vignae* sp. nov., a root nodule symbiotic bacterium isolated from cowpea grown in the semi-arid of Brazil. **International Journal of Systematic and Evolutionary Microbiology**, 2014. DOI: 10.1099/ijs.0.053082-0

RUMJANEK, N.G.; MARTINS, L.M.V.; XAVIER, G.R.; NEVES, M.C.P. A Fixação biológica de nitrogênio. In: FREIRE FILHO, F.R.; LIMA, J.A.A.; RIBEIRO, V.Q. (Ed.). **Feijão-caupi: avanços tecnológicos**. Brasília: Embrapa Informação Tecnológica; Teresina: Embrapa Meio-Norte, 2005.p.280-335.

SANTOS, C.A.F. Melhoramento do feijão-caupi para temperaturas moderadas e elevadas no Vale do São Francisco. **Revista Brasileira de Geografia Física**, v. 4, p. 1151-1162, 2011.

SANTOS, C.A.F.; BARROS, G.A.A.; SANTOS, I.C.N.; FERRAZ, M.G.S.. Comportamento agronômico e qualidade culinária de grãos de linhagens de feijão-caupi avaliadas no Vale do São Francisco. **Horticultura Brasileira**, v. 26, p. 404-408, 2008. DOI: 10.1590/S0102-05362008000300023

SANTOS, C.A.F.; BARROS, G.A.A.; SANTOS, I.C.N.; FERRAZ, M.G.S.. Comportamento agronômico e qualidade tecnológica de grãos de linhagens de feijão-caupi avaliadas no Vale do São Francisco. **Horticultura Brasileira**, v. 26, p. 404-408, 2008. DOI: 10.1590/S0102-05362008000300023

SILVA, M.F.; SANTOS, C.E.R.S.; SOUSA, C.A.; ARAÚJO, R.S.L.; STAMFORD, N.P.; FIGUEIREDO, M.B.V. Nodulação e eficiência da fixação do N_2 em feijão-caupi por efeito da taxa do inóculo. **Revista Brasileira de Ciência do Solo**, v. 36, p. 1418–1425, 2012. DOI: 10.1590/S0100-06832012000500005

THIES, J.E.; BOHLOOL, B.B.; SINGLETON, P.W. Subgroups of cowpea miscellany: symbiotic specificity within *Bradyrhizobium* spp. for *Vigna unguiculata, Phaseolus lunatus, Arachis hypogaea*, and *Macroptilium atropurpureum*. **Applied and Environmental Microbiology**, v.57, p.1540-1545, 1991. DOI: PMC182982

WILLIAMS, S. (Ed.). **Official methods of analysis of the Association of Official Analytical Chemists**. 14th ed. Arlington: AOAC International, 1984.

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

ZILLI, J.E.; MARSON, L.C.; MARSON,B.F.; RUMJANEK, N.G.; XAVIER, G.R. Contribuição de estirpes de rizóbio para o desenvolvimento e produtividade de grãos de feijão-caupi em Roraima. **Acta Amazonica**, v. 39, p. 749 – 758, 2009. DOI: 10.1590/S0044-59672009000400003.

ZILLI, J.E.; PEREIRA, G.M.D.; FRANÇA JÚNIOR, I.; SILVA, K.; HUNGRIA, M.; ROUWS, J.R.C. Dinâmica de rizóbios em solo do cerrado de Roraima durante o período de estiagem. **Acta Amazonica**, v. 43, p. 153– 160, 2013. DOI: 10.1590/S0044-59672013000200004

ZILLI, J.E.; VALICHESKI, R.R.; RUMJANEK, N.G.; SIMÕES-ARAÚJO, J.L.; FREIRE FILHO, F.R.; NEVES, M.C.P. Eficiência simbiótica de estirpes de *Bradyrhizobium* isoladas de solo do Cerrado em caupi. **Pesquisa Agropecuária Brasileira**, v.41, p.811-818, 2006. DOI: 10.1590/S0100-204X2006000500013

Table 1: Number of nodules (NN), nodule dry mass (NDM), dry mass of the shoots (DMS) and nitrogen accumulation in the aerial part (NAAP) in four varieties of cowpea inoculated with rhizobial strains in a Hydromorphic Vertisoil at the Experimental Field in Mandacaru, Juazeiro, BA.

Cultivars	Inoculation treatment	NN** (nod.pl^{-1})	NDM (mg.pl^{-1})	DMS (g.pl^{-1})	NAAP (mg.pl^{-1})
BRS Pujante	BR3267	21 bc*	49,5 bc	23,0 a	920 a
	BR3262	42 a	148,6 a	18,9 a	650 bc
	BR3299	36 a	101,2 ab	18,8 a	690 abc
	INPA 03-11B	28 ab	98,8 abc	19,1 a	590 bc
	UFLA 03-84	33 ab	97,2 abc	21,6 a	850 ab
	80 kg N.ha-1	11 d	86,5 bc	18,2 a	500 b
	Test. Abs	15 cd	49,8 c	19,6 a	680 abc
BRS Tapaihum	BR3267	20 a	102,0 a	18,9 a	700 ab
	BR3262	19 a	95,2 a	17,0 a	640 ab
	BR3299	17 a	61,7 a	20,6 a	770 a
	INPA 03-11B	13 a	49,8 a	20,0 a	510 b
	UFLA 03-84	16 a	68,0 a	21,9 a	750 ab
	80 kg N.ha-1	17 a	78,5 a	18,6 a	650 ab
	Test. Abs	12 a	53,7 a	17,7 a	570 ab
BRS Carijó	BR3267	12 a	55,2 a	24,0 a	810 ab
	BR3262	13 a	68,3 a	26,1 a	890 a
	BR3299	10 a	45,5 a	23,8 a	730 ab
	INPA 03-11B	17 a	84,1 a	23,8 a	730 ab
	UFLA 03-84	19 a	64,6 a	25,7 a	760 ab
	80 kg N.ha-1	13 a	64,7 a	22,7 a	780 ab
	Test. Abs	12 a	58,0 a	17,9 a	630 b
BRS Acauã	BR3267	11 a	56,5 a	16,6 a	430 a
	BR3262	12 a	63,9 a	15,3 a	460 a
	BR3299	9 a	29,2 a	15,5 a	550 a
	INPA 03-11B	10 a	43,5 a	17,2 a	630 a
	UFLA 03-84	9 a	26,0 a	15,6 a	530 a
	80 kg N.ha-1	12 a	53,3 a	19,6 a	670 a
	Test. Abs	12 a	50,1 a	15,91 a	530 a
CV%		27,2	32,2	24,5	6,5

* Means followed by the same letter in the column and in the same variety do not differ statistically from Student's t-test (LSD) ($p < 0,1$). ** NN=nodule number; NDM = nodule dry mass; SDM=Shoot dry mass; NAS= nitrogen accumulated in the shoots.

Table 2: Number of nodules (NN), nodule dry mass (NDM), dry mass of the shoots (DMS) and nitrogen accumulation in the aerial part (NAAP) in four varieties of cowpea inoculated with rhizobial strains in a Yellow Argisoil at the Experimental Field of Bebedouro, in Petrolina, PE.

Cultivars	Inoculation treatment	NN** (nod.pl^{-1})	NDM (mg.pl^{-1})	DMS (g.pl^{-1})	NAS (mg.pl^{-1})
BRS Pujante	BR3267	53 a*	170,4 a	17,9 bc	660 c
	BR3262	25 b	135,5 a	25,8 abc	900 abc
	BR3299	12 c	55,6 b	30,9 a	1220 a
	INPA 03-11B	14 bc	51,5 b	20,7 c	780 bc
	UFLA 03-84	13 c	60,0 b	28,0 ab	1120 ab
	80 kg N.ha-1	4 d	13,3 c	24,6 abc	830 abc
	Test. abs	16 bc	87,6 ab	22,2 abc	880 abc
BRS Tapaihum	BR3267	5 a	19,1 a	27,4 abc	1070 abc
	BR3262	7 a	43,3 a	39,9 a	1430 a
	BR3299	5 a	44,0 a	31,4 ab	1140 ab
	INPA 03-11B	6 a	42,6 a	15,8 c	580 c
	UFLA 03-84	7 a	42,2 a	18,6 bc	780 bc
	80 kg N.ha-1	4 a	13,6 a	29,2 ab	1090 abc
	Test. abs	6 a	21,2 a	24,2 bc	850 bc
BRS Carijó	BR3267	10 a	63,8 ab	23,6 ab	1030 a
	BR3262	8 ab	70,7 a	22,3 ab	950 a
	BR3299	4 ab	41,8 abc	32,1 a	900 a
	INPA 03-11B	6 ab	46,2 abc	18,0 b	720 a
	UFLA 03-84	4 ab	27,5 abc	29,4 ab	1120 a
	80 kg N.ha-1	2 ab	11,7 c	25,8 ab	910 a
	Test. abs	2 b	24,5 bc	19,7 ab	850 a
BRS Acauã	BR3267	6 ab	29,3 ab	21,1 bc	810 bc
	BR3262	7 a	53,3 a	37,2 a	1420 c
	BR3299	3 ab	16,9 ab	35,3 a	1260 ab
	INPA 03-11B	3 ab	14,6 ab	25,4 ab	1000 ab
	UFLA 03-84	3 ab	21,5 ab	14,2 c	480 c
	80 kg N.ha-1	1 b	17,2 b	28,2 ab	850 bc
	Test. abs	3 ab	20,5 ab	26,1 abc	930 abc
CV%		35,2	37,00	19,5	9,7

* Means followed by the same letter in the column and in the same variety do not differ statistically from Student's t-test (LSD) ($p< 0,1$). ** NN=nodule number; NDM = nodule dry mass; SDM=Shoot dry mass; NAS= nitrogen accumulated in the shoots.

Table 3: Grain yield (GY) and grain protein content (GPC) in four varieties of cowpea inoculated with rhizobial strains in two field experiments in the sub-medium part of São Francisco River Valley.

Cultivars	Inoculation treatment	Mandacaru Experimental Field		Bebedouro Experimental Field	
		GY** ($kg.ha^{-1}$)	GPC** (%)	GY ($kg.ha^{-1}$)	GPC (%)
BRS Pujante	BR3267	1495 a*	24,4 a	1157 b	24,0 ab
	BR3262	1219 bc	24,8 a	1435 ab	24,9 a
	BR3299	1475 ab	25,4 a	1353 ab	26,6 a
	INPA 03-11B	1091 c	21,9 a	1323 ab	25,6 a
	UFLA 03-84	1629 a	25,9 a	1423 ab	24,3 a
	80 kg N.ha-1	1375 ab	26,5 a	1824 a	24,7 a
	Test. abs	1477 ab	16,0 b	1088 b	19,4 b
BRS Tapaihum	BR3267	1340 ab	21,4 ab	1346 ab	23,6 ab
	BR3262	1322 ab	21,8 ab	1551 a	26,3 a
	BR3299	1198 ab	24,9 a	1291 ab	22,9 ab
	INPA 03-11B	1087 b	26,7 a	1769 a	24,8 a
	UFLA 03-84	1337 ab	24,8 a	1589 a	26,7 a
	80 kg N.ha-1	1145 ab	25,3 a	1320 ab	26,1 a
	Test. abs	1379 a	19,3 b	934 b	19,1 b
BRS Carijó	BR3267	1048 b	23,2 ab	1373 a	23,7 a
	BR3262	1467 a	21,2 ab	1570 a	24,8 a
	BR3299	1436 a	26,4 a	1370 a	24,6 a
	INPA 03-11B	1261 ab	26,1 a	1222 a	17,1 b
	UFLA 03-84	1505 a	25,0 ab	1197 a	23,9 a
	80 kg $N.ha^{-1}$	1277 ab	20,8 b	1526 a	21,8 a
	Test. abs	1387 a	21,4 ab	1048 a	23,7 a
BRS Acauã	BR3267	1210 a	24,2 a	1535 a	24,1 a
	BR3262	1198 a	25,5 a	951 bc	23,2 a
	BR3299	1305 a	24,9 a	1494 a	24,3 a
	INPA 03-11B	1151 a	25,6 a	1494 a	25,2 a
	UFLA 03-84	1186 a	25,0 a	1001 bc	24,5 a
	80 kg $N.ha^{-1}$	1413 a	24,9 a	1406 ab	24,4 a
	Test. abs	1319 a	20,3 a	816 c	26,3 a
CV%		9,07	12,62	14,01	8,70

* Means followed by the same letter in the column and in the same variety do not differ statistically from Student's t-test (LSD) (p< 0,1). ** GY= grain yield; GPC= grain protein content.