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SOIL AND PLANT NUTRITION - Article

Symbiotic and agronomic efficiency of new cowpea rhizobia from Brazilian Semi-Arid

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ABSTRACT: Cowpea is a very important crop to Brazilian Semi-Arid mainly small family-based farmers. Rhizobia inoculation is a practice, easy to use, and cheap technology that increases cowpea productivity. The aim of this study was to evaluate the efficiency of two new rhizobia isolates in greenhouse and field as well as classify them taxonomically. To bacterial identification the 16S rRNA gene of ESA 17 and ESA 18 isolates were sequenced. The greenhouse test was conducted with pots containing 3 L of soil and the bacterial isolates evaluated were ESA 17, ESA 18, BR 3267 or BR 3262 strains. A field experiment was implemented on a Vertisol in Juazeiro, Bahia State, to evaluate the cowpea growth and productivity. In this experiment, the peat-based inoculants with ESA 17, ESA 18, BR 3267 or UFLA 3-84 were used in 2 cowpea cultivars. Both bacteria were identified as *Bradyrhizobium*, but related to different species. ESA 17 was related to *B. japonicum* and ESA 18 was closer to *B. pachyrhizi*. At greenhouse, both isolates increased cowpea nitrogen content in the shoots due to the presence of very efficient nodules. In the field, the isolate ESA 18 inoculated at BRS Pujante cultivar induced higher production than observed for the absolute control, and for BR 17 Gurguéia cultivar, the ESA 17 and BR 3267 stood out both by inducing high production and grain protein content. The results indicate that both isolates can be evaluated in network experiments aiming at official recommendation for new bacteria to cowpea inoculant in Brazil.

Key words: *Bradyrhizobium*, inoculant, biological nitrogen fixation, strain selection, São Francisco River Valley.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a very important crop to the agricultural systems in the Brazilian northeastern, mainly those family-based farming systems. The cowpea genotypes, generally, can be produced in soils with low fertility, under low water availability, and with high temperatures (Santos et al. 2008), characteristics that predominate in the Brazilian Semi-Arid region.

In the northeastern, the area cropped with Brazilian cowpea is very large, with more than 1.2 million of hectares annually. Despite its importance, the productivity of cowpea in the Brazilian northeastern is very low and achieves only 328 kg·ha^{-1}, far below 850 and 1,200 kg·ha^{-1} found in the Brazilian northern and mid-western regions, respectively (Freire Filho 2011).

Several attempts have been carried out aiming to increase the cowpea production in the Brazilian northeastern based on improved fertilization strategies (Martins et al. 2013), improvement of water use efficiency and irrigation strategies (Ramos et al. 2014), and development of new plant genotypes (Santos et al. 2008; Santos 2011; Freire Filho 2011). In addition to this research, over the years, efforts have been applied on the constant selection of new rhizobial isolates to cowpea (Martins et al. 2003; Fernandes Júnior et al. 2012).

Rhizobia is a group of nitrogen-fixing bacteria able to establish root and/or stem association with legume plants (Moreira and Siqueira 2006). In agriculture, this association is exploited by application of biotechnological products (inoculants) containing selected bacteria. The constant efforts applied in the selection of efficient rhizobial bacteria for cowpea inoculants lead, up to now, to 4 high-efficiency bacteria, and the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) allows the commercialization of these bacteria in the inoculant formulations in Brazilian Market.

Investments to the selection of new bacteria efficient at the northeast region in field conditions have been carried out (Martins et al. 2003; Fernandes Júnior et al. 2012; Ferreira et al. 2013; Marinho et al. 2014). Studies conducted in the northeastern soils indicated a great diversity of cowpea rhizobia (Zilli et al. 2004; Leite et al. 2009; Radl et al. 2014) with promising bacteria to be used as inoculants (Fernandes Júnior et al. 2012; Ferreira et al. 2013; Marinho et al. 2014). To exemplify the potential of bacterial isolates from the soils of the northeastern region, the strain BR 3267 (*Bradyrhizobium yuanmingense*), which is the main bacteria used in cowpea inoculants in Brazil, was isolated from the soils of Petrolina (PE) in the semi-arid region of Brazil, in the lower mid São Francisco River Valley (Martins et al. 2003). In spite of the high diversity of indigenous bacteria in this particular region and its potential as a source of agronomically-efficient bacteria, the study published 14 years ago by Martins et al. (2003) was the last attempt to select bacteria to cowpea regarding its field performance from the lower mid São Francisco River Valley.

The results available in the scientific literature indicate that the obtainment of new rhizobial isolates and the evaluation of their agronomic efficiency are important to get more efficient bacteria and reach a new technological slope regarding the technology efficiency and use by farmers. In this context, the aim of this study was to evaluate the efficiency of 2 new bacterial isolates from the lower mid São Francisco River Valley in greenhouse and field experiments, as well as to determinate their taxonomic position.

MATERIAL AND METHODS

Identification of the bacterial isolates by the 16S rRNA sequences and phylogenetic analysis

The 2 new bacterial isolates used in this study were obtained from field-grown cowpea (BRS Pujante) under a crop rotation system at the Bebedouro Experimental Field, Embrapa Semiárido, Petrolina (PE). The bacteria were isolated in YMA medium (Vincent 1970), purified, and selected due to their performance in preliminary authentication tests regarding the nodule and shoot dry weight in cowpea BRS Pujante on sterile substrate in greenhouse experimental conditions. The bacterial isolates were deposited at the Collection of Micro-Organisms with Agricultural Interests of Embrapa Semiárido (CMISA), in Petrolina.

To identificate bacterial isolates, the 16S rRNA gene of both bacteria was sequenced. The bacterial isolates were grown in YM liquid medium under constant shaking for 5 days, and the broths were used for DNA extraction using the commercial kit HiYield™ Genomic DNA Mini Kit (Real Biotech Corp, Taipei, Taiwan), according to the manufacturer's instructions. The PCRs were made with the universal primer pair 27F and 1492R (Weisburg et al. 1991), and the sequencing was carried out in a 3730*xl* genetic analyzer (Applied Biosystems, Drive Foster City, USA) at Macrogen Inc (Seoul, South Korea). The →

sequences were compared to those available at the GenBank® database by means of the Basic Local Alignment Search Tool (BLASTn; http://www.ncbi.nlm.nih.gov/Genbank). The most similar sequences and some from type strains were downloaded and used to construct the phylogenetic tree. The alignment was carried with the ClustalW algorithm, and the trees were constructed using the Neighbor-Joining method and the Jukes-Cantor model. The sequences alignments and phylogenetic analysis were made with the MEGA 6.0 software (Tamura et al. 2013). The sequences of both bacterial isolates were deposited at GenBank® under the accession numbers KU510276 and KU510277 for ESA 18 and ESA 17, respectively.

Symbiotic performance in a greenhouse conditions experiment

The greenhouse conditions experiment was conducted to evaluate the efficiency of the bacterial isolates under non-sterile conditions. This experiment was conducted at a greenhouse in Petrolina from March to April 2013. For this assay, the bacteria evaluated were the 2 new slow growing bacterial isolates ESA 17 and ESA 18 and the inoculant bacterial strains *Bradyrhizobium yuanmingense* (BR 3267) and *B. pachyrhizi* (BR 3262) in the cowpea genotype BRS Pujante. Pots with 3-L capacity were filled with non-sterilized soil samples collected at an Ultisol in Petrolina. The chemical characteristics of the soil sample were evaluated, and the following features were achieved: pH (water) = 6.3; P = 11.92 $mg{\cdot}dm^{-3}$; K = 0.33 $mg{\cdot}dm^{-3}$; Ca = 2.0 $cmol_c{\cdot}dm^{-3}$; Mg^{2+} = 0.4 $cmol_c{\cdot}dm^{-3}$; Al^{3+} = 0.0 $cmol_c{\cdot}dm^{-3}$; H + Al^{3+} = 0.66 $cmol_c{\cdot}dm^{-3}$; S = 2.78 $cmol_c{\cdot}dm^{-3}$; CTC = 3.44 $cmol_c{\cdot}dm^{-3}$; V (base saturation) = 81%, and organic matter: 6.3 $g{\cdot}kg^{-1}$. As the soil did not present limitations for this kind of experiments, no fertility corrections were made.

The experimental treatments consisted of the single inoculation of the 4 bacteria added by a non-inoculated and fertilized with mineral nitrogen (NH_4NO_3) at rates of 700 mg $N{\cdot}plant^{-1}$ (splitted in 2 applications) and a non-inoculated and non-nitrogen fertilization treatment. For the inoculum preparation, the bacterial isolates were grown in YM liquid medium under constant shaking for the 5 days at the end of exponential growth, reaching around 10^9 cells per milliliter. Two milliliters of the bacterial broth were inoculated at each seed at the sowing in order to make more effective the establishment of the strains in the presence of a soil with high native rhizobial populations (Marinho et al. 2014). The seeds were previously superficially disinfected with ethanol 96°GL (30 s), NaClO 2.5% (w/v) (5 min) and 8 washes in distilled autoclaved water. Four seeds were sowed at each pot and ten days after the plant emergence, the spare plants were thinned and 1 plant per pot was left. The pots received water as necessary and the experiment was conducted up to 45 days after the emergence of plants.

At the harvest the roots were separated to the shoots and were washed in current tap water to remove the soil adhered. At this point, predominant nodules position at the crown region or sparse through the roots was evaluated (Cardoso et al. 2009). The nodules were detached from the roots and counted. Separately, the shoots and nodules were put in paper bags, dried at 65 °C in air circulation chamber for 5 days and weighted. The dried shoots were milled and sieved (0.5 mm mesh) and the shoots nitrogen content was determined by the semi-micro Kjeldahl method (Liao 1981). The parameters evaluated were the nodule number, the dry weight of nodules, roots and shoots, shoot nitrogen content. The nodule efficiency, where nitrogen content of the shoots was divided to the nodule dry mass of the same plot, was calculated to infer the efficiency of each weight unity of nodules to provide nitrogen translocated to the shoots.

The experimental design adopted was the complete randomized with 5 replications. The data was evaluated by means of the variance analysis followed by the application of the Scott-Knott mean range test ($p < 0.05$). The analysis was conducted using the software Sisvar 4.2 (Ferreira 2011).

Agronomic efficiency in the field conditions

The field experiment was set up in a Vertisol at the Mandacaru Experimental Field belonging to Embrapa Semiárido, Juazeiro (BA) (lat 09°24′S; long 40°26′W) and was conducted from July to September 2013. The accumulated rainfall in the period was 12.3 mm and the temperature average was 26 °C. The soil was prepared with a plowing and a harrowing. A composite soil sample (0 – 0.2 m depth) was taken to chemical analysis and the following characteristics were achieved: pH = 6.7; P = 43.15 $mg{\cdot}dm^{-3}$; K = 0.42 $mg{\cdot}dm^{-3}$; Ca = 20.4 $cmol_c{\cdot}dm^{-3}$; Mg^{2+} = 5.6 $cmol_c{\cdot}dm^{-3}$; Al^{3+} = 0.05 $cmol_c{\cdot}dm^{-3}$; H + Al^{3+} = 4.62 $cmol_c{\cdot}dm^{-3}$; S = 26.45 $cmol_c{\cdot}dm^{-3}$; CTC = 31.07 $cmol_c{\cdot}dm^{-3}$; V (base saturation) = 83% and organic matter = 6.9 $g{\cdot}kg^{-1}$. Soil fertilization was carried out with 20 $kg{\cdot}ha^{-1}$ of P_2O_5, applying simple super-phosphate, and with 20 kg·ha^{-1} of K_2O, applying potassium chloride according the technical recommendations. Furrow irrigation used twice a week with for approximately 6 mm of evapotranspiration.

The plots measured 12 m^2 and consisted of 8 lines with 3 m length. The spacing of 0.50 m between lines and 0.25 m between plants in the same line was adopted, in accordance to the technical recommendations for the crop and the MAPA determination to the evaluation of agronomic efficiency of new rhizobia. In this experiment, the cowpea genotypes evaluated were the BRS Pujante and BR 17 Gurguéia. The inoculation treatments used were the single inoculation of the bacteria ESA 17; ESA 18; BR 3267 (*B. yuanmingense*) and UFLA 03-84 (*Bradyrhizobium* sp.).

To prepare the inoculants, the bacteria were grown in YM liquid medium as described for the first experiment. The peat based inoculant was prepared by the addition of the culture broth at packs containing sterilized peat at the proportion of 1:3 (v/v) to reach the rate of 10^9 cells per gram of inoculant. The inoculants kept at 10 °C ± 2 °C prior to the experiment implementation. To inoculation, the seeds were handle mixed with the inoculants (250 g of inoculant to 10 kg of seeds) plus an aliquot of a saturated sucrose solution as adhesive. The inoculated seeds where kept to dry at free air the shadow for 20 – 30 minutes and were sowed manually soon after the inoculation.

To evaluate the nodulation and the plant development, a first harvest was conducted during flowering stage (45 days after the emergence of the plants). Ten plants were taken in the central 1 m of the second row of each plot. The whole plants were taken from soil, packed in plastic bags and transported to the laboratory, and kept at 10 °C ± 2 °C up to the processing (Fernandes Júnior et al. 2012). The roots were separated from the shoots and washed with tap water, nodules were detached and counted. The shoots and nodules were packed separately in paper bags, dried at 65 °C in a forced air chamber for seven days, and then weighed. The parameters evaluated were: number of nodules, shoot dry weight, nodule dry weight, and nitrogen accumulated in the shoots as described for the greenhouse experiment.

The second harvest was taken to evaluate the production parameters at 70 days after sowing. The pods were gathered from the useful area of each plot (4 m^2 at the center) and brought to the laboratory. Then, the grains were threshed, the humidity corrected to 13% and weighted to and the grain yield calculation. In addition, the grain protein content (GPC) was determined in accordance with Williams (1984).

The statistical analysis were made with the transformation of the data to $(X + 1)^{0.5}$ and submission to analysis of variance using the statistical analysis system Sisvar 4.2 (Ferreira 2011) and the means were compared applying the Student's t-test ($p < 0.05$).

RESULTS AND DISCUSION

Bacterial identification and phylogeny

The comparison of the 16S rRNA sequences from the 2 new bacteria with those available at the GenBank® database showed that both isolates belongs to *Bradyrhizobium* genus. In spite of belonging to the same genus, the bacteria are genetically different and the isolate ESA 17 is related to *B. japonicum* group while the isolate ESA 18 is related to *B. pachyrhizi* cluster (Figure 1).

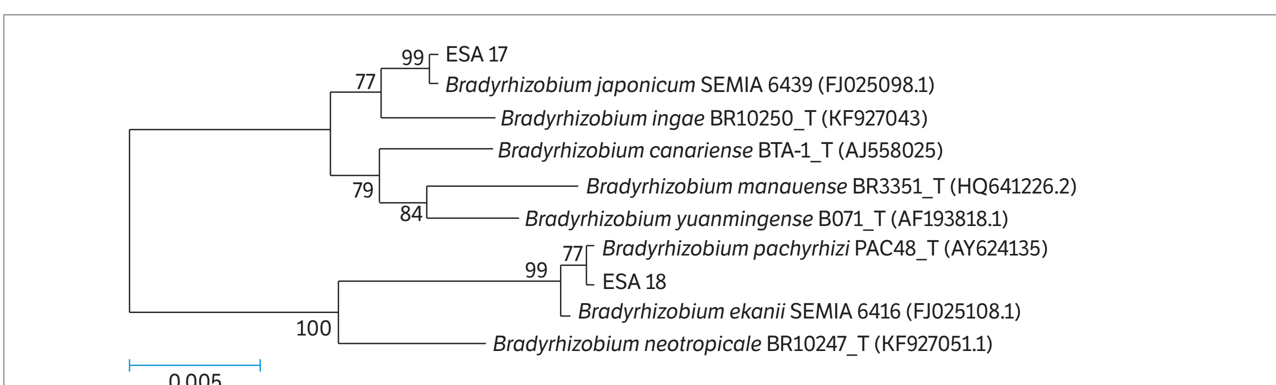

Figure 1. Neighbor-Joining phylogenetic tree based on the partial 16S rRNA gene sequence of the isolates ESA 17 and ESA 18 and other 8 *Bradyrhizobium* strains. Strains followed by "T" are type strains. Numbers in the nodes are the bootstrap values from 1,000 replications.

Bradyrhizobium isolates are commonly found inside cowpea nodules in the tropics (Thies et al. 1991) and bacteria belonging to this genus can fix high amounts of N in symbiosis with cowpea (Martins et al. 2003; Lacerda et al. 2004; Rufini et al. 2014). It is also a very diverse taxonomic group and several species of *Bradyrhizobium* were isolated from cowpea nodules in Brazil (Guimarães et al. 2012; Rufini et al. 2014). As the cowpea can establish associations with a wide range of rhizobia (Thies et al. 1991; Leite et al. 2009), it is expected to obtain more than one *Bradyrhizobium* specie in the prospection of rhizobia from cowpea grown in the same field plot in a soil with a high diversity (Leite et al. 2009; Leite et al. 2017) and density (Marinho et al. 2014) of cowpea nodulating rhizobia.

Symbiotic performance in greenhouse experiment

The plants inoculated with BR 3267 showed higher nodule number for the greenhouse condition experiment, followed by the treatments inoculated with the bacteria BR 3262 and ESA 18 (Table 1). The treatment isolate ESA 17 was lower than the other inoculated in this parameter, but higher than the treatments without inoculation and associated only with the native rhizobia. Regarding the nodule dry weight, the inoculation of the BR 3267 and BR 3262 strains resulted in higher results followed by the treatments inoculated with the isolates ESA 18 and ESA 17 and the absolute control. All inoculated plants showed nodules in the crown position, indicating the success of the inoculation.

All inoculation and control treatments did not differ evaluating the shoot dry weight. Otherwise, statistic differences were achieved by comparing the averages values of the nitrogen accumulated in the shoots where all inoculated treatments stood above the values observed by the absolute control treatment. The inoculation of the *Bradyrhizobium* BR 3267, BR 3262, ESA 17, and ESA 18 induced mean increases of 76, 119, 86 and 112% in nitrogen accumulated in the shoots, respectively. The result of the greater accumulation of nitrogen in inoculated cowpea shoots, indicates that the bradyrhizobia assessed was more efficient than the native soil rhizobia regarding the BNF, and also on its ability to mineralization of soil organic nitrogen.

For the bacteria ESA 17 and ESA 18 the symbiotic efficiency can be confirmed by the estimation of the nodular efficiency. These parameters indicated that the both isolates tested can induce higher translocation of nitrogen from nodules to the shoots by the same dry weight unity of nodules. The treatments BR 3267, BR 3262 and the absolute control showed lower nodule efficiency than the observed by the other two inoculated treatments and did not differ among them.

The selection of new bacteria can indicate that the isolates evaluated can present higher efficiency than presented by the reference strains, indicating the potential of the new isolates to further inoculation evaluations (Lima et al. 2012). Costa et al. (2014) assessed the performance of 2 new bacteria (UFLA 3-164 and UFLA 3-154) comparing to the traditional inoculants to cowpea. They observed that the new isolates were able to induce the nodule formation, nodule dry weight and shoot nitrogen better than observed in some inoculant bacteria. In the other hand, the results found by those authors did not show superiority of the tested bacteria against the inoculant

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Table 1. Nodule number, shoot dry weight, nodule dry weigh, nitrogen accumulated in the shoots, and nodular efficiency in cowpea BRS Pujante inoculated with different rhizobia in pots with non-sterile soil.

Inoculation treatment	NN	SDW	NDW	NAS	Nodule efficiency	Nodulation profile
	Nodules·plant^{-1}	g·plant^{-1}	mg·plant^{-1}		mg N mg·nodule^{-1}	
BR 3267	132 a*	3.2 ns	158.0 a	91.5 b	0.5 b	Crown
BR 3262	58 b	3.2 ns	236.1 a	113.5 b	0.4 b	Crown
ESA 17	20 c	2.1 ns	93.6 b	96.6 b	1.0 a	Crown
ESA 18	50 b	3.0 ns	124.1 b	110.2 b	0.9 a	Crown
N control	0 d	2.9 ns	0 c	236.9 a	-	Sparse
Absolute control	6 d	2.8 ns	107.9 b	51.9 c	0.3 b	Sparse
CV (%)	25.6	28.2	25.8	25.2	36.4	

*Means with the same letter in the column do not differ by the Skott-Knott mean range test ($p < 0.05$). NN = Nodule number; SDW = Shoot dry weight; NDW = Nodule dry weight; NAS = Nitrogen accumulated in the shoots; ns = Non-significant; CV = Coefficient of variation.

strains regarding the nodular efficiency estimation, as the results of the present study indicated.

The bacteria inoculated in seeds when sowed in non-sterilized soils need to compete with the native soil community by the nodulation sites once the native soil bacteria are, generally, very efficient regarding their competitiveness and, generally, occupy great part of the nodule sites even with very lower cells densities than the inoculated bacteria, mainly for the promiscuous hosts such as cowpea (Thies et al. 1991; Martins et al. 2003). Higher values for the nodulation variables such as nodule number and nodule weight, as observed in the control treatments and the crown nodule position indicates the successful nodulation by the inoculated bacteria (Cardoso et al. 2009; Melo and Zilli 2009).

Although, the performance of the inoculant strains in this kind of experiment can be variable in cowpea (Melo and Zilli 2009; Costa et al. 2014), field experiments are necessary to assure the real efficiency of the new bacterial isolates. The bacteria tested in this experiment were able to induce nodulation (nodule number, weight, and position) as well as nitrogen accumulation in the shoots satisfactory compared to the control treatments and can be selected for further field evaluations.

Agronomic efficiency in the field conditions

In the field experiment two cowpea genotypes were tested. For BR 17 Gurguéia, differences were not found among the treatments analyzing the nodule number and nodule dry weight per plant, while to BRS Pujante the plants inoculated with BR 3267 presented higher nodulation than the observed for the absolute control in both parameters (Table 2). The shoot dry weight of BR 17 Gurguéia genotype showed to be significantly higher at the N control treatment, comparing to all other treatments. BRS Pujante had the lowest mean to SDW at the treatment with nitrogen fertilization, been lower than the values verified at the treatments inoculated with BR 3267 and BR 3262.

All inoculation treatments showed the same performance regarding the nitrogen accumulation in the shoots for BR 17 Gurguéia. However, only the treatments ESA 17, ESA 18 and N control were higher than the absolute control. To BRS Pujante the inoculation of BR 3267 provided more nitrogen accumulation in the shoots than the absolute control.

The evaluation of yield showed that BR 17 Gurguéia inoculated with BR 3267 and ESA 17 stood out and achieved statistically higher grain productivity at rates of 47 and 31% above the produced by the absolute control. For this cultivar,

Table 2. Nodule number, shoot dry weigh, nodule dry weigh, nitrogen accumulated in the shoots, grain yield, and grain protein content in cowpea BRS Pujante and BR 17 Gurguéia inoculated with different rhizobia in a Vertisol under field conditions.

Inoculation treatment	NN	SDW	NDW	NAS	GY	GPC
	Nodules·plant^{-1}	g·plant^{-1}	mg·plant^{-1}		kg·ha^{-1}	%
BR 17 Gurguéia						
UFLA 03-84	16 ns	14.5 ab	22.3 ns	860 ab	1,513 bc	24.0 a
BR 3267	12 ns	15.3 ab	23.7 ns	840 ab	2,007 a	24.0 a
ESA 17	14 ns	9.7 b	27.5 ns	1,020 a	1,787 ab	22.8 ab
ESA 18	10 ns	7.4 b	25.0 ns	920 a	1,477 bc	23.2 ab
N control	8 ns	34.0 a	28.2 ns	1,040 a	1,401 bc	22.6 ab
Absolute control	11 ns	16.0 ab	21.7 ns	650 b	1,369 c	21.0 b
BRS Pujante						
UFLA 03-84	25 ab	49.0 ab	17.9 b	560 b	1,542 ab	23.1 ab
BR 3267	28 a	56.3 a	26.0 a	850 a	1,359 b	21.3 b
ESA 17	16 b	29.7 abc	19.3 ab	660 ab	1,614 ab	23.8 ab
ESA 18	20 ab	28.3 bc	23.0 ab	800 ab	1,892 a	24.4 a
N control	15 b	21.2 c	24.4 ab	740 ab	1,656 ab	23.2 ab
Absolute control	18 b	33.9 abc	18.6 b	560 b	1,480 b	17.6 c
CV (%)	26.6	35.6	25.1	26.3	21.3	10.3

*Means with the same letter (in the column) do not differ by the Student's mean range test ($p < 0.05$). NN = Nodule number; SDW = Shoot dry weight; NDW = Nodule dry weight; NAS = Nitrogen accumulated in the shoots; GY = Grain yield; GPC = Grain protein content; ns = Non-significant; CV = Coefficient of variation.

the inoculation of BR 3267 and UFLA 03-84 resulted in grains with higher protein contents comparing to the absolute control. A different pattern was observed for BRS Pujante once, the best results for the grain productivity was achieved for the plants inoculated with ESA 18 that reached increase of 28%, compared to the absolute control. The protein content of BRS Pujante grains indicated that all inoculated and the nitrogen-supplied treatments were above the observed for the absolute control.

The influence of the inoculation treatments on the vegetative development of cowpea genotypes under field conditions is also dependent of plant genotype (Alcântara et al. 2014) also in the sub-medium part of São Francisco River Valley (Martins et al. 2003; Marinho et al. 2014). Some genotypes can be clearly positively influenced by the bacterial inoculation, while, for other genetic materials, this influence is not so clear. Marinho et al. (2014) evaluated the field performance of 4 inoculated plant genotypes for the same region of the present study. Regarding to the cultivars, BRS Pujante and BRS Tapaihum showed a better interaction with the inoculated bacteria, while, to BRS Carijó and BRS Acauã, this interaction was not so clear.

The association between cowpea and rhizobia in field conditions can increase the plant nitrogen nutrition and increase the nitrogen content in the grains after the translocation in the senescent plant (Freitas et al. 2012; Marinho et al. 2014). For BRS Pujante, the inoculation of the two new *Bradyrhizobium* and the nitrogen fertilization induced the positioning on the highest statistical ranking at the both parameters of productivity and to the NAS. Marinho et al. (2014) also observed that the BRS Pujante can present positive interactions with some bacteria inoculated, in despite to other strains.

This capacity is often dependent of the plant cultivar and the strain inoculated. The inoculation of the cowpea BR 17 Gurguéia, or the supplementation with nitrogen fertilizer resulted in higher amounts of nitrogen accumulated in the shoots and also at the nitrogen in the grains. For this genotype, the inoculation of the bacteria BR 3267 and ESA 17 also induced higher grain production compared to the non-inoculated control treatment. Ferreira et al. (2013) observed that this genotype was able to associate effectively with new bacteria obtained from Minas Gerais State in a field under bauxite mining (Melloni et al. 2006) belonging to *Bradyrhizobium* (UFLA 3-164) and *Burkholderia* (UFLA 3-154) in a field experiment in Itaueira, Piauí State.

Other study evaluated the performance of newly isolated bacteria from Minas Gerais State at field conditions in Bom Jesus, Piauí State. The rhizobia UFLA 3-155 had the same performance than inoculant strains and the nitrogen fertilized treatment at the parameter grain productivity (Costa et al. 2011). Fernandes Júnior et al. (2012) tested new bacteria belonging to *Rhizobium* from soils of Recife, Pernambuco State. Their results showed that the new bacteria tested provided satisfactory yield compared to the inoculant strains and the control treatments, as well as we observed in the present study.

Recently, Marinho et al. (2014) evaluated the efficiency of the inoculant bacteria and the new strain of *Microvirga vignae* at Petrolina (Pernambuco State) and Juazeiro (Bahia State). The results indicated that the performance of the *M. vignae* (isolated from Sergipe State soil) and the inoculant strains were, in average, the same in both counties. This result is in agreement to those found in the present research, where good performance was achieved by the new *Bradyrhizobium* bacteria.

In spite of some attempts to evaluate the diversity of cowpea bacteria from Semi-Arid region (Leite et al. 2009; Radl et al. 2014), few studies had evaluated performance of autoctonous bacteria to at the field conditions to the region aiming the selection of new isolates. The results found by Martins et al. (2003) in the São Francisco River Valley supported the BR 3267 strain to be officially recommended for cowpea in Brazil. Those results were obtained more than fourteen years ago, and the evaluation of newer bacteria and plant genotypes can indicate more efficient symbiotic partnerships to the region.

CONCLUSION

The results found in the present study support the indication of both *Bradyrhizobium* ESA 17 and ESA 18 to be tested in network experiments aiming the official recommendation to cowpea inoculation in Brazilian Semi-Arid. The results also assure the soils from the lower mid of São Francisco River Valley as a source of efficient *Bradyrhizobium* to cowpea.

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