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Agronomic effectiveness of rhizobia strains on cowpea in two consecutive years

Gustavo Ribeiro Xavier[1], Norma Gouvêa Runjanek[1], Carolina Etienne de Rosália e Silva Santos[2], Ana Dolores Santiago de Freitas[2], Vinicius Santos Gomes da Silva[2]*, Aleksandro Ferreira da Silva[2], Juscélia da Silva Ferreira[2], Newton Pereira Stamford[2], Lindete Míria Vieira Martins[3], Jakson Leite[4], Luiz Balbino Morgado[5], Rosa Maria Cardoso Mota de Alcantara[6]

[1]Embrapa Agrobiologia, Rodovia BR-465, Km 7. 23891-000, Seropédica, Rio de Janeiro, Brazil
[2]Departamento de Agronomia, Universidade Federal Rural de Pernambuco. 52171-900, Recife, Pernambuco, Brazil
[3]Departamento de Tecnologia e Ciências Sociais Universidade do Estado da Bahia.Av. Edgard Chastinet, S/N, São Geraldo, 48900-000, Juazeiro, Bahia, Brazil
[4]Universidade Federal de Alagoas, *Campus* Arapiraca, Av. Manoel Severino Barbosa, Bom Sucesso, 57309-005, Arapiraca, Alagoas, Brazil
[5]Embrapa Semiárido, Rodovia BR-428, Km 152, 56302-970, Petrolina, Pernambuco, Brazil
[6]Embrapa Meio Norte, Av. Duque de Caxias, nº 5.650, 64006-220, Teresina, Piauí, Brazil

***Corresponding author: vinicius.agro2008.1@gmail.com**

Abstract

Cowpea (*Vigna unguiculata* L Walp) is a legume of great social and economic importance in tropical regions. The plant is tolerant to soil and climatic adversity, while it has high nutritional value with high protein, minerals and vitamins contents. The cowpea legume has a very high ability to fix N_2 which is important because it can reduce the costs of soluble mineral fertilizers and may contribute to sustainable agriculture, preserving the environment and natural resources. In order to observe the influence of Rhizobia the agronomic effectiveness of cowpea strains was tested in two consecutive years compared with the mineral N fertilization. The experiments were carried out in different soils of the Brazilian Northeast (semiarid and rain forest region) which measured the effectiveness of rhizobia on grain yield. The results revealed the effectiveness of strain BR 3299 in the experiment that significantly increased the grain yield (3 times when 50 kg ha^{-1} of N, and 2 times when 80 kg ha^{-1} N were used). The Rhizobia treatment promoted the grain yield (up to 1,600 kg ha^{-1}), compared to application of 80 kg ha^{-1} of N. In general, all applied strains promoted the Brazilian Northeast average cowpea yield (300-400 kg ha^{-1}).

Keywords: BNF, inoculants, residual power, sustainable agriculture, *Vigna unguiculata*.
Abbreviation: BR_Brazil; Conab_Conselho Nacional de Abastecimento in Brazil; FAO_ Food and Agriculture Organization of the United Nations; INPA_Intituto Nacional de Pesquisa Agronomica; UFLA_Universidade Federal de Lavras.

Introduction

The cowpea (*Vigna unguiculata* L. Walp) is an annual legume of great social and economic importance, widely distributed in tropical regions of the Asian, African and American continents and is one of the major important source of protein for low-income populations (Moray et al., 2014; Xiong et al., 2016.). The production system is predominantly carried out by small and medium farmers that use genotypes for grain production. Currently, about 6 million tons of cowpea beans annually produced in 10 million hectares (FAO, 2010). The Brazilian Northeast is the region with highest cowpea production which corresponds to about 90% of the country yield obtained in the cultivated areas, which are predominantly performed for subsistence farming and characterized by low-technology system. The Brazilian soils for cowpea production are deficient in nutrients in general, especially N, and therefore, have low yield response (Conab, 2008, Saboya et al., 2013).

Practices that allow greater input of N are required to make the cowpea farming more productive and competitive. Inoculation with effective N_2-fixing rhizobia bacteria is an economic and environmentally beneficial option to promote the provision of N for plants. The symbiotic association of effective bacteria with cowpea is able to increase the grain yield and it may viable alternative to replace, partially or totally the application of mineral N fertilizers (Farias et al., 2016). However, in Brazil, the cowpea inoculation is not a common practice implemented in sustainable agriculture, mainly due to the lack of technical information for agronomic and economic viability. The main reason for this occurrence is the ability of cowpea to nodulate with a great variety of indigenous rhizobia, which often reduces the response to inoculation (Almeida et al., 2010, Chagas Junior et al., 2010). Therefore, the nodulation of cowpea with effective rhizobia may represent an important strategy to increase the interest to adopt the nitrogen fixation system.

In recent years, the evaluation of effective rhizobia on various legumes have been increased and are conducted in the Brazilian Northeast with the promising results obtained for various legumes as described by Calheiros et al. (2013, 2015) for calopo; Lima et al. (2012), for velvet beans, Torres Junior et al. (2014) and Santos et al. (2017) for peanuts, among others important species. However, few studies were carried out to evaluate the symbiotic effectiveness to increase cowpea yield by inoculation with specific strains of the Brazilian Northeast (Farias et al., 2016b).

The aim of the study was to evaluate the effectiveness of strains recommended for cowpea (cv. BR 17 Gurguéia) cultivated in soils of different Brazilian ecosystems (semiarid and rain forest) of the Northeast region, inoculated with Rhizobia strains recommended by Brazilian research Centers.

Results and discussion

Efficiency of the strains

The results of the experiments, conducted during two consecutive years at different sites showed similar responses. The results represented means of the two consecutive years and demonstrated that all used strains had been quite efficient (over 70% of efficiency) compared to plants not inoculated and without addition of mineral N fertilizer (Fig 3). The data displayed the great importance of the inoculants for cowpea culture. It was observed that the strain BR 3299 has the best results of efficiency when applied in the experiment conducted in Recife (Pernambuco state) localized in the Rain Forest region with response of up to 350% increase comparing to the others effective strains (Figure 1).

Effectiveness of the strains

The effectiveness of the strains was calculated by comparing the results obtained by the control treatment (including mineral N fertilizer of 50 and 80 kg ha^{-1} of N), respectively (Fig 2). In reference for this parameter, the rhizobia strains showed very promising result for replacement of mineral N-fertilizer in cowpea crops. The BR3299 strain exhibited the best result, demonstrating efficiency over 300% when compared to control with 50 kg ha^{-1} N, and more than 200% increase compared to control including 80kg ha^{-1} N in the experiment conducted in Recife. The others strains showed similar behavior in the different regions and others localities.

Grain yield

Gualter et al. (2011) reported the efficiency of Rhizobia strains inoculated with cowpeas in the State of Maranhão, Brazil, and observed maximum yield (679 kg ha^{-1}) for the treatments with effective strains such as INPA 03-11B. Farias et al. (2016b) also evaluated the inoculation with rhizobia strains on cowpea in the state of Maranhão and Piaui and showed that UFLA 03-84 and INPA 03-11B strains had effectiveness of 61 and 69%, respectively, compared to the treatment with application of 80 kg ha^{-1} N. The strain UFLA 03-153 was superior to the strains (UFLA 03-84 and INPA 03-11B) and showed that in the Maranhão state the highest grain yield was obtained with the inoculated treatments. In the State of Piaui, inoculation with UFLA 03-84 and INPA 03-11B, was produced yield of 797 to 1,621 kg ha^{-1}in Dom Pedro: and 923 to 1,430 kg ha^{-1}in São Luís, respectively. Marinho et al. (2014) obtained higher grain yield in the non-inoculated treatment and the results were similar to inoculation with the strains BR 3267, BR 3299T, and UFLA 03-84.

The inoculated treatments showed yield similar to the control treatment (not inoculated plants) and with application of mineral N fertilizer. However, the strains UFLA 03-84 and BR 3262 promoted an increase of 30 and 31% in grain yield, respectively, compared to the control treatment.

In Teresina and Juazeiro regions, where a frequent rainfall is not happened, the grain yield was higher than 1,200 kg ha^{-1} (Fig 3). The results are not statistically different from those obtained in Recife experiment, where the rainfall was more abundant and frequent. However, the yield was lower and cowpea did not express symbiotic and productive potential, although the grain yield, were up than the national average (300 kg ha^{-1}). The experiment results in Recife showed that the strain BR 3299 contributed to a yield equivalent to 1,648 kg ha^{-1} and was superior to the treatment with mineral N fertilizer (80 kg ha^{-1}) demonstrating that in this locality the best cowpea yield and effectiveness of the inoculation with recommended strains is achieved.

It is important to note that the data for bean crop yield provided by the National Company of Statistic in Agriculture included the yield for common bean's (*Phaseolus vulgaris*) and for cowpea (*Vigna unguiculata*). Recent studies show higher cowpea yield when inoculants or fertilizers are applied (Farias et al., 2016a, Farias et al., 2016b).

The BNF is able to provide the necessary N for cowpea, following of a descending order by the soil and fertilizers (Brito et al., 2009). Oliveira (2003), cultivated cowpea (cv. IPA 206), in an irrigation system in micro-region of Areias (Paraiba state) and reported the grain yield of about 3.6 t ha^{-1} when compared to application of mineral N fertilizer (56.0 kg ha^{-1} of N) to the soil and 3.4 t ha^{-1} when fertilized with 59.0 kg $ha^{-1}$of N, in foliar application.

The corresponding analysis for the treatments with and without N and inoculated with effective strains (Fig 4), showed the influence of the treatments with mineral N fertilizer (50 and 80 kg ha^{-1}) with a positive correlation between both parameters. A negative correlation was observed with all the used strains in the control treatment (not inoculated with rhizobia and without N fertilization). The INPA 03-11B, UFLA 3-84, BR 3262 and BR 3267 strains grouped in a similar way, showed a positive correlation between the inoculated treatments and a negative correlation with the treatments with and without mineral N-fertilization. The strain BR 3299 did not display a positive correlation with the other treatments (inoculants and controls).

The different groups between the strains and mineral N fertilization indicate that the application of fertilizers negatively influenced the ability of rhizobia to provide N for cowpea plants. It is known that N fixing legumes usually increase the amount of N in the soil-plant system, but when the amount of N in the soil is increased the addition of N via NFB decreases.

Materials and methods

Plant and strain characteristics

Cowpea seeds (cv. BR 17 Gurguéia) were surface-sterilized in 70% ethyl alcohol for 1 min, followed by immersion in $HgCl_2$ (1:500) for 0.5 min and washed six times with sterile water. The seeds were mixed with a sugar solution (10% w v^{-1}) in the proportion of 6 mL kg^{-1} and inoculated in accordance with Howieson (2015).

The strains effectiveness were studied in experiments conducted in the Brazilian semiarid region located in the Juazeiro (Bahia), Petrolina (Pernambuco) and in the District of Esperance (Paraiba), and the specific characteristics and

Table 1. Local, physiographic zone, coordinates and annual precipitation of the different localities.

Local	Physiographic Zone	Coordinates	Annual precipitation (mm/year)
Recife (PE)	Rain Forest	8°20´57“ S 34°56´49” W	500 a 2000, with two well defined seasons: rainy (Mar-Aug); dry (Sept-April)
Esperança (PB)	Semiarid	7°1'0.8"S 35°52'50.3"W	650 a 700, with two well defined seasons: rainy season (Feb-June); dry (April- Dec)
Petrolina (PE)	Semiarid	9º 23' 55" S 40º 30' 03" W	500 a 600, with two well defined seasons: rainy (Jan-May); dry (April-Dec)
Juazeiro (BA)	Semiarid	9º 24' 42" S 40º 29' 55" W	500 a 600, with two well defined seasons: rainy (Jan-May); dry (April- Dec)
Teresina (PI)	Semiarid	5º 05' 21" S 42º 48' 07" W	400 a 500, with two well defined seasons: rainy (Dec-May); dry (June-Nov)

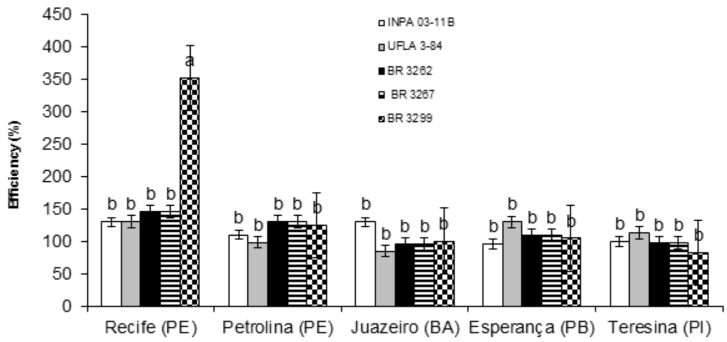

Fig 1. Efficiency of the inoculation treatments with recommended strains in the different localities.

Table 2. Chemical analysis of soils.

Locality	pH (H_2O)	Total C g kg^{-1}	Available P	available K	Ca	Mg	Al	H+Al
			mg dm^{-3}		------------ cmo$_c$ dm^{-3} --------------			
Recife (PE)	5.0	0.20	14.2	14.8	1.1	0.3	0.8	1.67
Petrolina (PE)	6.3	0.51	11.9	13.2	2.0	0.4	0.0	0.66
Juazeiro (BA)	6.8	0.42	44.6	14.4	20.4	5.6	0.0	4.62
Esperança (PB)	5.7	0.70	89.8	27.6	2.14	0.9	0.0	3.52
Teresina (PI)	5.4	0.62	37.8	20.5	2.36	1.7	0.4	2.43

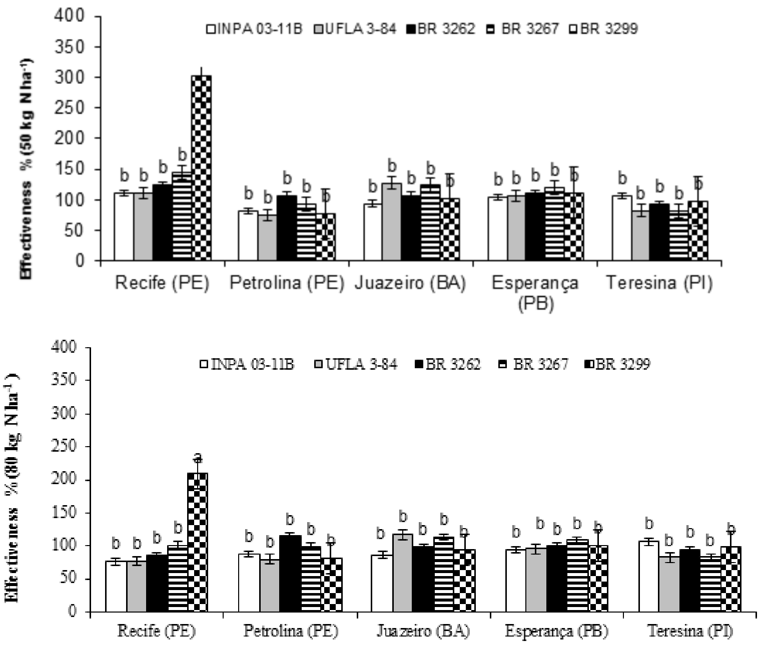

Fig 2. Effectiveness of cowpea inoculated with the recommended strains (INPA 03-11B; UFLA 3-84; BR 3262; BR 3267 and BR 3299), in the different localities, compared to control with 50 kg N ha^{-1} (A) and 80 kg N ha^{-1} (B).

Table 3. Characteristics of the Rhizobia strains used in the experiments.

Rhizobia strains	Brazilian ecosystem	Strains characteristics			Rhizobia species
		Growth time	Reaction	Color	
INPA 03-11B	Amazônia	Slow	Alkaline	White	*Bradyrhizobium* sp.
UFLA 3-84	Amazônia	Slow	Alkaline	White	*Bradyrhizobium* sp.
BR 3267	Caatinga	Slow	Alkaline	White	*Bradyrhizobium* sp.
BR 3262	Cerrado	Slow	Neutral	White	*Bradyrhizobium* sp.
BR 3299	Caatinga	Fast	Alkaline	White	*Microvirga vignae*

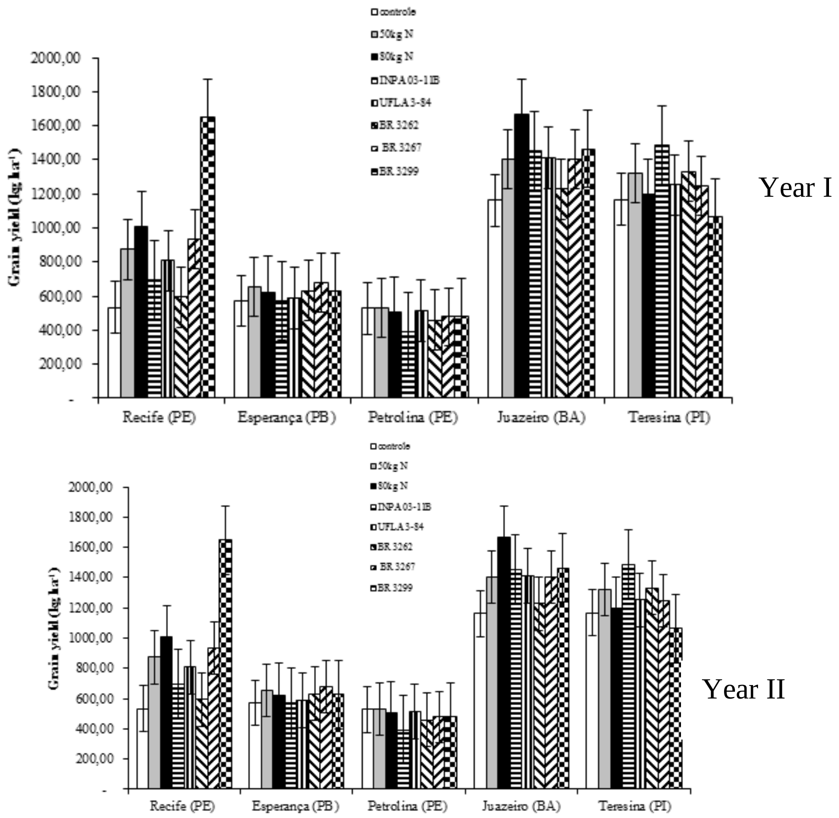

Fig 3. Cowpea grain yield (kg ha^{-1}), inoculated with recommended strains INPA 03-11B; UFLA 3-84; BR 3262; BR 3267 and BR 3299, in the different localities, in the consecutive years.

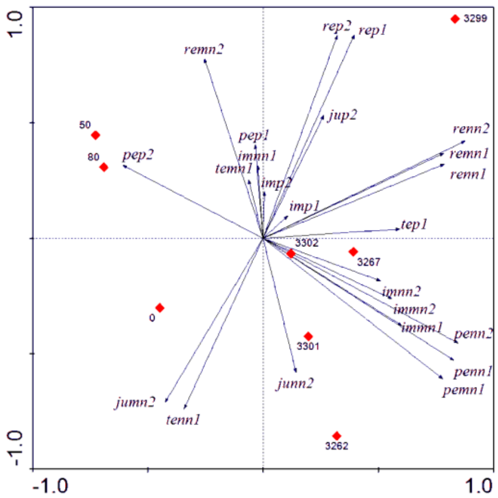

Fig 4. Multivariate analysis showing the correspondence of localities and Rhizobia strains inoculated.

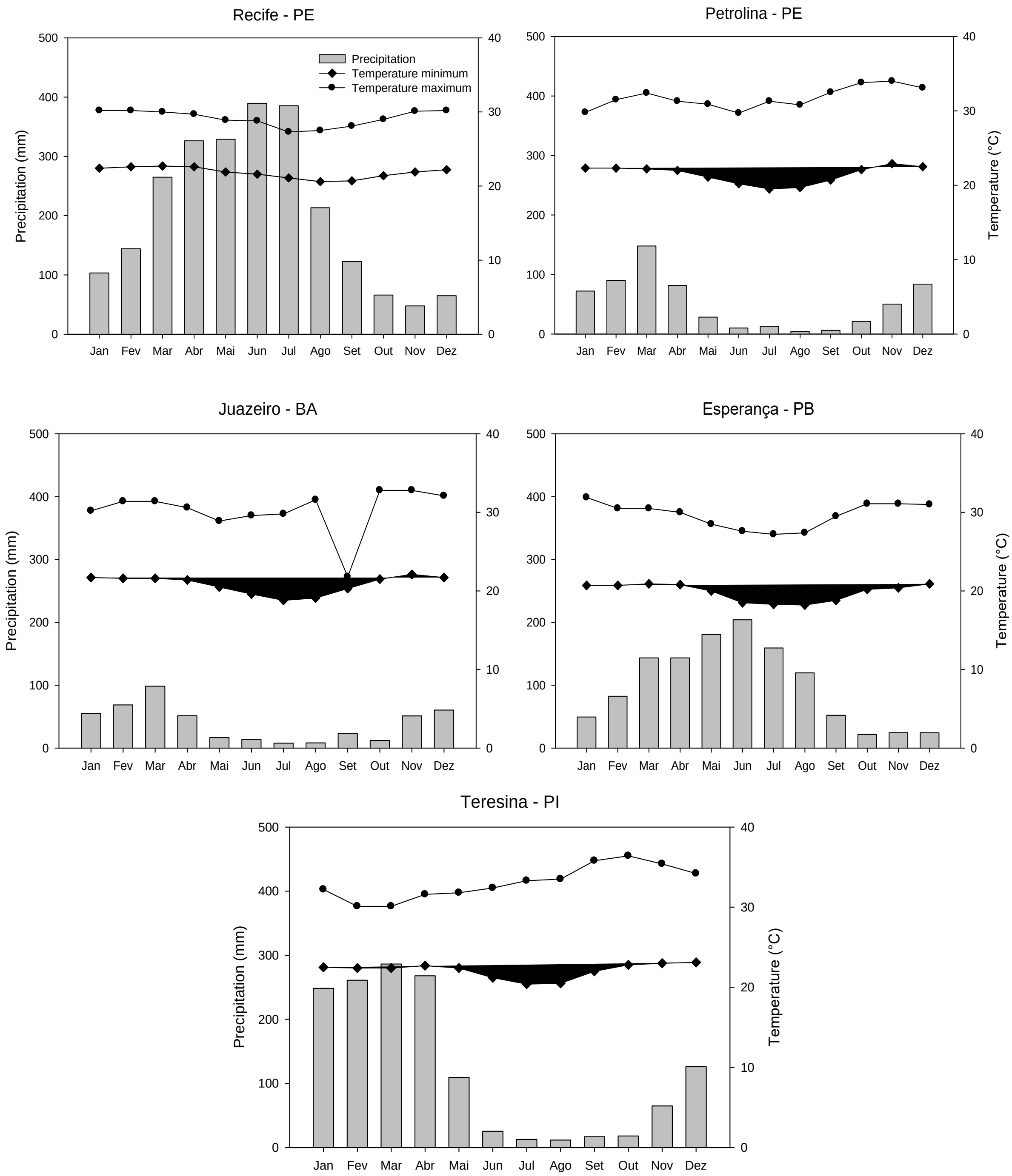

Fig 5. Annual precipitation with temperature (minimum and maximum) of the different localities.

climate of the Rain Forest Zone (Recife, Pernambuco) and (Teresina, Piaui), are described in Fig 5.

Site and soil description

The study was conducted throughout interstate interaction groups for two consecutive years. The followed institutions localized in the cowpea producing areas of the Brazilian Northeast have participated in the integrate project: (Universidade Federal Rural de Pernambuco, Universidade Federal de Pernambuco, Universidade do Estado da Bahia, Universidade Estadual da Paraiba and Embrapa Meio Norte), coordinated by the Embrapa Agrobiologia (Seropédica, Rio de Janeiro).

The semiarid region is characterized by high temperatures with very intense rainy season but in short period (4 to 5 months), and sometimes with no rain during the dry season. In this region, the agricultural practice is possible through irrigation techniques, as observed in the Fruit Agricultural Polo of Petrolina (PE) and Juazeiro (BA), where the largest centers of irrigated fruit production in the Brazilian Northeast resides. The Rain Forest Zone is close to the coastal tablelands, where the rains are very abundant and better distributed throughout the year, with medium and high temperatures (Table 1).

In the different localities, three months before the conduction of the experiments, soil samples collected at 0-30 cm depth and submitted to the chemical analysis (Table 2). When necessary, the soil acidity was neutralized with liming (dolomitic lime) applied to increase the base saturation to 60%.

Experimental conditions

In the field experiment the treatment with fertilizer addition were added at the recommended rate for cowpea (IPA, 2008) and with the double of recommended rate, applied at the planting date, 15 days after planting. During the experimental period the soil moisture was maintained near field holding capacity by daily application of water.

The plants were cultivated in lines and the seeds planted manually, after the seeds inoculation with the respective treatments. At planting date, the plants were fertilized with P and K applying 20 kg ha^{-1} of K_2O and 60 kg ha^{-1} of P_2O_5, based on soil analysis performed prior to the experimental conduction. The phosphorus and potassium sources were triple superphosphate and potassium chloride, respectively.

Experimental design

The experiments followed the same experimental design in randomized block with 8 treatments that consisted of peat inoculant with strains: 1) INPA 3-11B; 2) UFLA 3-84; 3) BR 3262; 4) BR 3267 and 5) BR 3299, whose characteristics are shown in Table 3. Control treatments were included and corresponded to treatments non-inoculated with rhizobia: 6) without N fertilizer; 7) with mineral N fertilization (urea 40 kg ha^{-1}); 8) with N fertilization (urea 80 kg ha^{-1}), with N applied in two times (40 kg ha^{-1} at planting date, and 40 kg ha^{-1}, 15 days after planting).

In different regions, the experiments performed in 32 plots. Each plot contained four rows with 6m long each, spaced 1.0 m between rows. Four seeds per hole were planted and two weeks after emergence two seedlings were maintained. The useful area for each plot (10m^2) corresponded to the two central rows, dispensing 0.5 m of the row sides (5m × 2m). During plant development in the different localities, the experiments were maintained free from weeds and controlled insects and diseases.

Plant determinations

50 days after emergence (DAE), ten plants of the useful area were randomly harvested and the shoots dried at 65-70 °C for 72 h to obtain the dry matter yield, and the data used to determine the agronomic efficiency and effectiveness of the rhizobia strains. The efficiency was calculated by the ratio of the total N in shoot dry biomass of the inoculated treatment to the total N in shoot dry biomass of the control treatment (non-inoculated and without mineral N fertilization). The effectiveness was calculated using the ratio of the total N in shoot dry biomass of the inoculated treatments to the total N in shoot dry biomass of the treatment non-inoculated with mineral N addition). For each treatment, the plants of the useful area were harvested and the grain yield (humidity adjusted to 13%), shoot dry weight (DW) dried at 65-70 °C for 72 h, and total N in the shoots were determined according to the Embrapa (2009).

Statistical analysis

The results of efficiency and effectiveness of the strains and the grain yield were subjected to the analysis of variance and the F test. The significance of means was compared by the Tukey test ($p \leq 0.05$), using the software Sisvar, version 4.0 (Ferreira, 2008). Then a multivariate analysis using the software Statistica 8.0 was conducted.

Conclusion

The cowpea grain yield obtained in this experiment was superior to the national average (300 kg ha^{-1}) and the tested strains showed high efficiency and effectiveness in N_2 fixation in all experiment localities, regardless of environment classification and precipitation. The strain BR 3299 applied in Recife showed the highest efficiency and effectiveness. The strains showed high positive correlation between them, and a negative correlation with the N – fertilizer treatments.

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