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THE *Bradyrhizobium uaiense* STRAIN UFLA 03-164^T ENHANCED YIELD PERFORMANCE OF COWPEA IN SOILS WITH LOW OR HIGH PHOSPHORUS CONTENT

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Abstract

The objective of this study was to evaluate the agronomic efficiency of rhizobia strains already approved or in the selection stage for cowpea, in the Recôncavo da Bahia region, Brazil. Two field experiments were performed in the municipalities of Cruz das Almas and Maragogipe, Bahia, Brazil. A randomized block design was used with seven nitrogen sources and four replicates. Nitrogen sources consisted of the strains UFRB FA51B1, UFRB BA72C2-1, UFLA 03-164^T, UFLA 03-84, INPA 03-11B, and two controls without inoculation, one with mineral nitrogen and another without. All the strains nodulated cowpea. The efficiency of the strains was determined by the number of nodules, nodule dry matter, total dry matter, grain yield, nitrogen accumulation in shoots and grain, and relative efficiency. The strain UFLA 03-164^T can be recommended for biomass production, green manure, and promotion of grain yields in both soils. In Maragogipe, the UFLA 03-84 and INPA 03-11B strains can be recommended for biomass production, green manure, and promotion of grain yields. The UFLA 03-164^T strain showed great potential to promote growth and grain yield in the two municipalities studied. The strain INPA 03-11B can be recommended for Maragogipe soil. UFLA 03-84 can also be recommended to increase grain yield in Cruz das Almas. The return on investment based on the value-cost ratio were positive for UFLA 03-164T and UFLA 03-84 strains, showing that these strains are economically feasible and can replace the fertilization with mineral nitrogen in the cultivation of cowpea.

Keywords: Biological nitrogen fixation. Green manure. *Vigna unguiculata (L.) Walp.*

1. Introduction

Several procedures are being implemented in order to reduce microclimatic changes emerging from agricultural anthropic activities in Brazil. A number of public institutions have concentrated efforts on studying the process of biological nitrogen fixation (BNF) and on selecting strains with potential for agricultural systems. In addition, public policies such as the Low-Carbon Agriculture Plan (*Plano de Agricultura de Baixo Carbono* - ABC Plan) have been created to implement technologies that may strengthen farm productivity by reducing production costs and encouraging sustainable farming practices. One approach proposed in the ABC Plan consists in the maximization of BNF, with implementation of over 5.5 million hectares of agricultural lands, replacing the use of nitrogen fertilizers (MAPA 2013).

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important source of protein for human consumption in tropical and sub-tropical regions of the world, cultivated mainly by small farmers and in poor soils, particularly soils deficient in nitrogen and phosphorus (Almeida et al. 2010; Freire Filho et al. 2011).

This legume species is able to nodulate with various species of rhizobia already established in the soil or with native bacteria from the rhizobia group (Costa et al. 2011; Farias et al. 2016a). However, this technology, able to improve the socioeconomic conditions of local communities through the optimization of farm productivity and reduction of environmental impacts, is not widely disseminated among farmers, despite the significant body of research results obtained from different regions in Brazil (Lacerda et al. 2004; Almeida et al. 2010; Costa et al. 2011; Gualter et al. 2011; Ferreira et al. 2013; Martins et al. 2013; Costa et al. 2014; Soares et al. 2014; Farias et al. 2016a; Farias et al. 2016b).

The efficiency of diazotrophic bacteria is subordinated to the characteristics of plant cultivars, the geographical region, soil attributes, crop density, and the competitive ability of the diazotrophic bacteria itself (Borges et al. 2012; Farias et al. 2016a). Thus, due to growing interest in increasing BNF, the identification of efficient and competitive bacterial strains that are well adapted to the soil and climatic conditions of a given geographical region is essential when aiming at nitrogen contribution through BNF.

Thus, it is essential to perform experiments in different geographical regions to check the efficiency of inoculant strains that have already been approved and of native rhizobia (Costa et al. 2014). Furthermore, soil inoculation studies are opportune since they allow determination of the advantages of inoculation when efficient native strains are not available (Jaramillo et al. 2013). Although inoculation of nitrogen-fixing bacteria on cowpea is important, it has not been studied in the Recôncavo da Bahia region. Thus, the objective of this study was to evaluate the efficiency of rhizobia strains already approved for cowpea or in the selection stage, within the Recôncavo da Bahia region, Brazil.

2. Material and Methods

One field experiment was performed in the municipality of Cruz das Almas, BA, and another in the municipality of Maragogipe, BA, Brazil. The climate of the region was classified as Aw, tropical hot and humid (Köppen and Geiger 1928), with defined rainy and dry seasons and average annual rainfall of 1170 mm, being March to August the rainiest period of the year and September to February the driest. The mean rainfall registered during the experiment was of 740 mm, registered at the Inmet meteorological station in the municipality of Cruz das Almas.

The soil from both municipalities, without any history of inoculation, was classified as a *Latossolo amarelo* (Oxisol), with medium texture. In Cruz das Almas, the sampling area was previously planted with castor bean (*Ricinus communis* L.) under conventional tillage. In Maragogipe, poultry manure was used as organic fertilizer, and management of the area included crop rotation and intercropping with maize (*Zea mays* L.) and cassava (*Manihot sculenta* Crantz).

The first experiment was performed in the experimental farm at the Universidade Federal do Recôncavo da Bahia, Cruz das Almas Campus (12°40’19’’S and 39°06’22’’W), from July to October 2016. The chemical attributes, evaluated in the 0-0.2 m layer were as follow: pH ($CaCl_2$): 4.7; pH (H_2O): 6.0; P - Mehlich 1 (mg dm^{-3}): 9.3; K (mg dm^{-3}): 68.9; Ca (cmol$_c$ dm^{-3}): 1.0; Mg (cmol$_c$ dm^{-3}): 0.7; Al (cmol$_c$ dm^{-3}): 0.2; +Al (cmol$_c$ dm^{-3}): 7.9; SB (cmol$_c$ dm^{-3}): 1.9; CEC (cmol$_c$ dm^{-3}): 9.8; V (%): 19.4; m (%): 9.52; Cu (mg dm^{-3}): 0.65; Fe (mg dm^{-3}): 28.8; Mn (mg dm^{-3}): 12.95; Zn (mg dm^{-3}): 1.8; B (mg dm^{-3}): 0.28; S (mg dm^{-3}): 8.4; and organic matter (g dm^{-3}): 17.7.

The second experiment was performed in a family farming area, in the community of Oitizeiro, municipality of Maragogipe-BA (12°46’42’’S and 38°55’10’’W), from August to November 2016. The chemical attributes, evaluated in the 0-0.2 m layer were as folow: pH ($CaCl_2$): 4.4; pH (H_2O): 5.7; P - Mehlich 1 (mg dm^{-3}): 55.2; K (mg dm^{-3}): 86.1; Ca (cmol$_c$ dm^{-3}): 1.7; Mg (cmol$_c$ dm^{-3}): 0.5; Al (cmol$_c$ dm^{-3}): 0.2; H+Al (cmol$_c$ dm^{-3}):

9.3; SB ($cmol_c$ dm^{-3}): 2.4; CEC ($cmol_c$ dm^{-3}): 11.7; V (%): 20.5; m (%): 7.69%; Cu (mg dm^{-3}): 1.6; Fe (mg dm^{-3}): 49.05; Mn (mg dm^{-3}): 9.1; Zn (mg dm^{-3}): 5.05; B (mg dm^{-3}): 0.57; S (mg dm^{-3}): 10.4; and organic matter (g dm^{-3}): 14.4.

The experiments were set up in a randomized block design with seven nitrogen sources and four replicates, using the cowpea cultivar EPACE 10. The nitrogen sources consisted of five inoculant strains and two controls without inoculation, one with application of 70 kg N ha^{-1}, as recommended by Freire Filho et al. (2007), in which the N source was urea, and one without any N source. The strains tested were two new rhizobia strains, UFRB FA51B1 and UFRB BA72C2-1, which had exceptional performance in a previous greenhouse trial; two strains already approved and certified by the Brazilian Ministry of Agriculture as references of cowpea inoculants (MAPA, 2011), UFLA 03-84 - SEMIA 6461 (*Bradyrhizobium viridifuturi*) (Costa et al. 2019) and INPA 03-11B - SEMIA 6462 (*B. elkanni*), (Guimarães et al. 2015); and one strain from a selection process under progress, UFLA 03-164^T, originating from bauxite mining soils and recently described as a new species denominated *Bradyrhizobium uaiense* (Michel et al. 2020). The last three strains described originated from the culture collection of the Soil Microbiology Laboratory at the Universidade Federal de Lavras, MG, Brazil.

Nitrogen fertilization was applied in two equal portions, one portion at planting and other 20 days after plant emergence.

The inoculants were supplied in peat substrate, prepared with sterilized peat at a proportion of 3:1 of peat and YMA semi-solid culture medium after five days of growth, with a concentration of at least 10^9 bacterial cells. Cowpea seeds were inoculated with 500 g of inoculant per 50 kg of seed. Seeds were sown immediately after inoculation and plant spacing was 1 m between rows and 0.2 m between plants, with four seeds per plant hole.

Plants were thinned at 15 days after sowing, keeping two plants per plant hill. Plots consisted of six 4-m-length rows, for a total of 24 m^2 per plot, with an area of 8 m^2 used for data collection.

The first evaluation was performed 45 after sowing, during the blooming period. Photosynthetic pigments were determined using an electronic chlorophyll meter, then ten plants from each plot were randomly collected to determine number of nodules (NN), nodule dry matter (NDM), shoot dry matter (SDM), and efficiency in relation to the control with high concentration of mineral N (EFCN).

After weighing the shoots, they were pulverized in a Wiley mill. Samples of 50 mg were placed in small tin foils to determine nitrogen content and the C/N ratio by the dry combustion method in an Elementar Vario EL (LECO USA) analyzer. Accumulation of N in the shoots was calculated by multiplying the dry weight of the shoots (g) * (% of N)/100.

The second evaluation was performed at the beginning of maturation, 60 days after sowing. Pods from ten plants were collected at random. All pods were then air dried and threshed to evaluate the number of pods per plant (NPP), length of pods per plant (LPP), (using a ruler), and weight of pods per plant (WPP). To determine grain yield per hectare (GY), yield of the 8 m^2 data collection area of the plot (in g $plot^{-1}$) was converted to g ha^{-1}, with moisture corrected to 13% (after 50-seed samples had been dried in a forced air oven at 105°C).

After weighing the cowpea grain, it was ground to determine nitrogen content and the C/N ratio, as had been done for the shoots. The grain N accumulation (GNA) was calculated by multiplying grain weight (g) * (% of N)/100.

Analysis of variance was performed on the data and the F test at 5% probability. Means were compared by the Scott-Knott test at 5% probability using the Sisvar 5.6 statistical software (Ferreira 2011). Data of NN, nodule fresh matter (NFM), and NDM were transformed to $(X + 0.5)^{0.5}$.

The value-cost ratio (VCR) was calculated to determine return on investment when using nitrogen sources. VCR was calculated based on the equation VCR = ((YB-YC)PG)/(QB × PB) (Nziguheba et al. 2010), where YB is the grain yield from mineral fertilizer treatments, YC is the grain yield from the control treatment without the N source, PG is the price per kilogram of cowpea in the region studied, PB is the unit price of the inoculant or the fertilizer, and QB is the quantity of inoculant or fertilizer used per hectare. The exchange rate used in the study was of 1.00 US$ for 4.74 R$. Any strain with positive VCR was considered economically viable, and when superior to a limit of 3-4, the strain was considered profitable (Dittoh et al. 2012).

3. Results

The first evaluation in the field experiment was performed during blossom period. A significant effect from N sources on NN and NDM was observed in both environments (Table 1).

Table 1. Mean values of number of nodules (NN) and nodule dry matter (NDM) in cowpea cultivar EPACE 10 from field experiments performed in Cruz das Almas and Maragogipe, BA, Brazil.

Nitrogen sources	NN (number plant^{-1})	NDM (mg plant^{-1})	NN (number plant^{-1})	NDM (mg plant^{-1})
	Cruz das Almas, BA		Maragogipe, BA	
Without N	0.90 b	2.24 b	2.58 b	25.03 b
With N	0.02 c	0.71 c	0.82 c	9.38 c
UFLA 03-164[T]	4.16 a	3.47 a	2.76 b	69.59 a
UFLA 03-84	1.46 b	3.45 a	2.53 b	71.49 a
INPA 03-11B	3.25 a	2.25 b	7.72 a	70.79 a
UFRB BA72C2-1	4.30 a	2.19 b	6.17 a	23.14 b
UFRB FA51B1	1.66 b	2.32 b	2.52 b	23.86 b
CV (%)	11.15	9.78	4.28	4.36

Means followed by the same letter are not statistically different by the Scott-Knott test at 5% probability.

In Cruz das Almas, inoculation with the UFRB BA72C2-1, UFLA 03-164[T], and INPA 03-11B strains resulted in higher nodulation in plants. The UFRB FA51B1 and UFLA 03-84 strains were not different from the treatment without inoculation and without mineral N. A significant reduction in nodulation was observed when mineral N was applied.

In Maragogipe, the highest NN were observed when inoculating with the UFRB BA72C2-1 and INPA 03-11B strains. The UFRB FA51B1, UFLA 03-164[T], and UFLA 03-84 strains did not differ from the treatment without inoculation and without mineral N.

Regarding the variables chlorophyll *a* and *b;* total chlorophyll; SDM; shoot nitrogen accumulation (SNA); C/N ratio in the shoots and EFCN, no significant effect was observed in Cruz das Almas, except for the C/N ratio in the shoots. In Maragogipe, no significant effect was observed for chlorophyll *a*, total chlorophyll, LPP, 100SW, and C/N ratio in grain, while for all other variables, there was a significant effect among treatments.

In Cruz das Almas, the UFLA 03-84 strain and the nitrogen sources with mineral N resulted in higher indices of chlorophyll *a, b* and total chlorophyll; these N sources differed from the other N sources. Concerning chlorophyll indices in Maragogipe, there was a significant effect ($p < 0.05$) only for the index of chlorophyll *b*, brought about by the treatment with application of 70 kg of N per ha^{-1} and by the treatment without any source of N (Table 2).

In the second evaluation, during harvest, a significant effect was found for the GNA and GY variables in both environments studied. The NPP showed a significant effect ($p < 0.05$) only in Cruz das Almas, and LPP showed a significant effect only in Maragogipe. No influence from inoculation was observed on the variables WPP, 100SW, and C/N ratio in cowpea grain, since there was no significant effect between the sources of N used in both environments.

In Maragogipe, the UFLA 03-164[T] strain resulted in average values similar to the control treatment with mineral N for NPP, constituting the best nitrogen source for this variable, even exceeding the two MAPA-approved strains UFLA 03-84 and INPA 03-11B. These latter two strains were superior to the two new strains tested and to the control without inoculation and without mineral N (Table 3).

In Cruz das Almas, the treatment with mineral N stands out among all treatments by promoting higher mean values for LPP, followed by the UFLA UFLA 03-164[T] and UFLA 03-84 strains, which differed from the other inoculated strains and from the control treatment without mineral N.

Concerning GNA, in Cruz das Almas, nitrogen sources which were inoculated with the UFLA 03-164[T] and UFLA 03-84 strains and with mineral N showed higher mean values than the other nitrogen sources. In Maragogipe, the UFLA 03-164[T] and UFLA 03-84 strains also led to the best results overall, along with the INPA 03-11B strain and the control treatment with mineral N. Previous studies performed under field conditions also showed a similar effect among strains regarding grain N accumulation (Ferreira et al. 2013; Farias et al. 2016b); however, in those studies, the strains did not differ from the control with mineral N.

Table 2. Mean values of chlorophyll *a* and *b* and total chlorophyll, shoot dry matter (SDM), shoot nitrogen accumulation (SNA), C/N ratio in the shoots, and agronomic efficiency in relation to the control with high concentration of mineral N (EFCN) for the cultivar EPACE 10 from field experiments performed in Cruz das Almas and Maragogipe, BA, Brazil.

Nitrogen sources	Chlorophyll			SDM	SNA	C/N ratio in	EFCN
	a	*b*	Total	(g plant-1)	(mg plant-1)	shoots	(%)
			Cruz das Almas, BA				
Without N	38.34 b	20.63 b	58.97 b	2.61 c	118.70 b	10.35 a	57.24 c
With N	40.01 a	22.87 a	62.88 a	4.56 a	208.29 a	10.26 a	100.00 a
UFLA 03-164^T	38.20 b	20.77 b	58.97 b	4.86 a	211.72 a	10.73 a	106.57 a
UFLA 03-84	39.71 a	23.33 a	63.04 a	3.42 b	149.57 b	10.88 a	75.00 b
INPA 03-11B	38.09 b	20.54 b	58.63 b	2.85 c	120.38 b	10.99 a	62.50 c
UFRB BA72C2-1	38.69 b	20.11 b	58.81 b	2.81 c	125.05 b	10.56 a	61.62 c
UFRB FA51B1	38.34 b	20.26 b	58.61 b	2.58 c	112.32 b	10.81 a	56. 85 c
CV (%)	2.38	7.53	3.96	10.53	12.21	6.31	7.87
			Maragogipe, BA				
WithoutN	39.81 a	21.09 a	60.90 a	15.45 b	560.69 b	11.16 b	74.24 b
WithN	39.87 a	20.25 a	60.13 a	20.81 a	783.20 a	11.30 b	100.00 a
UFLA 03-164^T	40.10 a	19.03 b	59.13 a	20.51 a	711.27 a	11.96 a	98.55 a
UFLA 03-84	38.07 a	18.54 b	56.62 a	18.28 a	652.81 a	11.87 a	87.84 a
INPA 03-11B	39.24 a	18.22 b	57.46 a	18.83 a	728.52 a	11.05 b	90.48 a
UFRB BA72C2-1	40.04 a	18.5 b	58.64 a	15.43 b	503.68 b	12.36 a	74.14 b
UFRB FA51B1	38.88 a	18.85 b	57.73 a	15.10 b	526.68 b	12.04 a	72.56 b
CV (%)	3.03	4.53	3.06	10.87	10.87	5.61	11.03

Means followed by the same letter are not statistically different by the Scott-Knott test at 5% probability.

Table 3. Mean values for number of pods per plant (NPP), length of pods per plant (LPP), weight of pods per plant (WPP), 100 seed weight (100SW), C/N ratio, grain nitrogen accumulation (GNA), and grain yield (GY) in cowpea plants, cultivar EPACE 10, from experiments performed in Cruz das Almas and Maragogipe, BA, Brazil.

Nitrogen sources	NPP	LPP	WPP	100SW	C/N	GNA	GY
	(plant^{-1})	(cm plant^{-1})	----(g plant^{-1})----		ratio in grain	----(kg ha^{-1})----	
				Cruz das Almas, BA			
Without N	1.03 a	12.35 d	1.92 a	17.92 a	13.15 a	5.38 b	163.62 b
With N	1.42 a	15.83 a	2.35 a	19.81 a	12.66 a	7.91 a	230.37 a
UFLA 03-164^T	1.56 a	15.00 b	1.78 a	19.53 a	12.93 a	7.22 a	216.25 a
UFLA 03-84	1.50 a	13.90 c	2.15 a	19.14 a	13.13 a	7.19 a	215.37 a
INPA 03-11B	1.35 a	12.50 d	2.02 a	16.97 a	13.76 a	5.36 b	167.62 b
UFRB BA72C2-1	1.65 a	12.58 d	1.96 a	18.91 a	13.37 a	5.38 b	166.87 b
UFRB FA51B1	1.69 a	12.58 d	1.94 a	20.99 a	15.48 a	4.32 b	153.87 b
CV (%)	6.54	6.94	19.65	8.29	10.01	11.48	6.99
				Maragogipe, BA			
Without N	7.45 c	18.16 a	2.37 a	21.10 a	11.19 a	18.95 b	446.03 b
With N	11.25 a	18.97 a	2.40 a	23.69 a	11.01 a	33.00 a	761.07 a
UFLA 03-164^T	11.47 a	18.62 a	2.36 a	21.80 a	11.50 a	31.73 a	767.30 a
UFLA 03-84	10.94 b	18.89 a	2.93 a	23.08 a	11.44 a	31.31 a	747.43 a
INPA 03-11B	10.94 b	17.55 a	2.72 a	19.28 a	11.26 a	31.98 a	759.78 a
UFRB BA72C2-1	7.15 c	18.94 a	2.56 a	25.43 a	11.50 a	18.37 b	443.38 b
UFRB FA51B1	7.34 c	18.80 a	2.49 a	25.02 a	11.82 a	18.03 b	448.73 b
CV (%)	6.45	5.05	15.92	14.86	3.48	6.09	6.89

Means followed by the same letter are not statistically different by the Scott-Knott test at 5% probability.

Concerning GY, in Cruz das Almas, the UFLA 03-164^T strain had a yield similar to the MAPA-approved UFLA 03-84 strain and to the control treatment with mineral N, thus constituting the best N sources for this variable. The two new strains and the MAPA-approved INPA 03-11B strain did not differ from the treatment without inoculation and without mineral N. In Maragogipe, the UFLA 03-164^{T} strain was also prominent among the others, promoting mean values similar to the MAPA-approved strains and the control with mineral N. On the other hand, the two new strains did not differ from the treatment without mineral N and without inoculation.

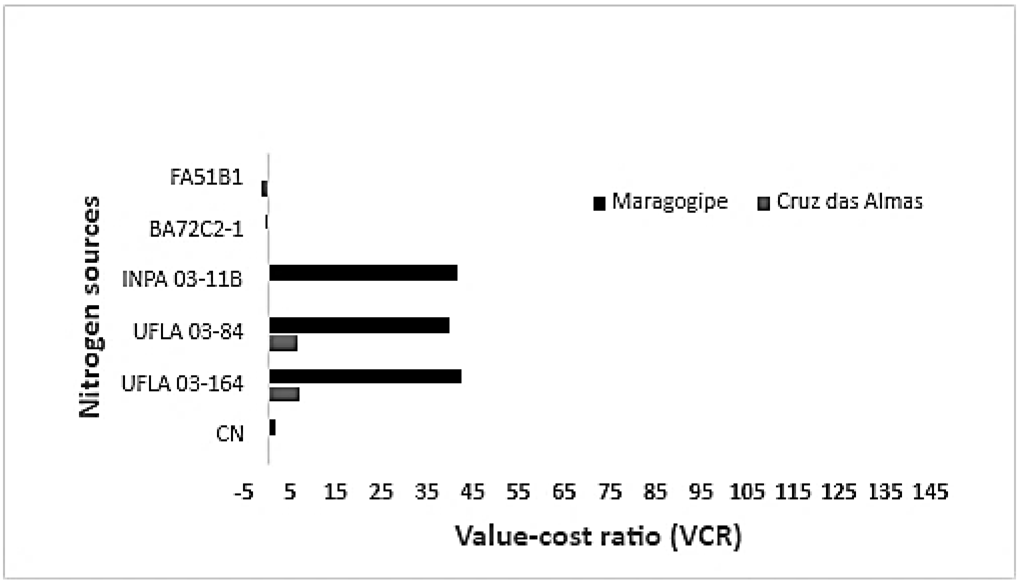

Figure 1. Value-cost ratio (VCR) for mineral fertilization and bacterial inoculants in cowpea (*Vigna unguiculata* L. Walp) plants, cultivar EPACE 10, in the municipalities of Cruz das Almas and Maragogipe, Bahia, Brazil.

4. Discussion

Nodulation observed in the treatment without any source of N reveals the presence of native bacteria able to nodulate cowpea, since these soils received no previous inoculation. Previous studies on agronomic efficiency of rhizobia strains in cowpea also showed the occurrence of nodulation in treatments without inoculation, as a result of the presence of native rhizobia populations (Almeida et al. 2010; Costa et al. 2011; Farias et al. 2016a). Native rhizobia show unstable results in relation to environmental changes, not being sensitive to the increase in the quality of the environment (Oliveira et al. 2020).

The higher mean values of NN and NDM in the treatment without any source of N in soils in Maragogipe compared to soils in Cruz das Almas may be related to the previous planting of cassava in Maragogipe, since a high density of diazotrophic bacteria in the soil, attributed to stimulus from this crop, was recorded (Jesus et al. 2005).

Although diazotrophic bacteria have broad nodulation capacity in cowpea, positive responses are not always observed since inoculation depends on biotic factors and on abiotic factors, such as the population density of native rhizobia and the ability of introduced strains to compete with them (Costa et al. 2014; Farias et al. 2016a).

Concerning NDM, the highest mean values were obtained with the UFLA 03-164^{T} and UFLA 03-84 strains in Cruz das Almas. In Maragogipe, the UFLA 03-164^{T} strain also performed well, as well as the UFLA 03-84 and INPA 03-11B strains. Previous studies performed in the Northeast region in Brazil also showed the efficiency of the UFLA 03-164^{T} strain to nodulate cowpea plants, with a similar or higher performance than the UFLA 03-84 and INPA 03-11B strains, which are currently approved as inoculants for cowpea (Costa et al. 2011; Farias et al. 2016a).

In both environments, application of 70 kg of N per ha^{-1} final dosage had a negative effect on nodulation, leading to a lower number of nodules and nodule dry matter compared with other treatments. Martins et al. (2013) found decreasing linear nodulation responses in cowpea plants as the dosages of mineral N increased, confirming the inhibitory role of this N source on nodulation.

In the present study, low mean nodulation was found in both environments compared to the values found in other studies in the Northeast region of Brazil (Almeida et al. 2010; Costa et al. 2011; Ferreira et al.

2013; Costa et al. 2014; Farias et al. 2016a). Some of these authors specify 15 units (nodules) per plant as indicative of efficient symbiosis. However, the highest mean values for NN were 4.30 units per plant in Cruz das Almas and 7.72 units per plant in Maragogipe (Table 2). Different results of efficiency of strains may be attributed to the effect of environmental soil factors, survival ability of the strains, different plant cultivars, and regional rainfall standards (Borges et al. 2012; Farias et al. 2016a).

Despite the low nodulation means found in both environments, the soil from Cruz das Almas showed even lower values for NN and NFM than the soil from Maragogipe. Interestingly, the soil from Cruz das Almas has lower fertility, as confirmed through chemical characterization. These results reinforce the thesis that low soil fertility may have limited the development of symbiosis (Farias et al. 2016b).

Different management systems used in the soils studied may have affected cowpea development and yield. Management practices affect soil physical, chemical, and biological characteristics, with consequences on plant growth and yield (Pereira et al. 2015).

Amaral et al. (2013) found that fertilization with phosphorus increased shoot biomass and grain yield in cowpea inoculated with rhizobia. According to the parameters proposed in the Recommendations for Use of Fertilizers and Amendments by the Soil Fertility Commission of Minas Gerais state (CFSEMG, 1999), available phosphorus content is considered very low in the case of the Cruz das Almas soil, whereas the P content of the Maragogipe soil is classified as very good. This shows that the availability of this nutrient leads to mass gain of the shoots and grain yield, since SDM and GY were higher in the soil from Maragogipe

The UFLA 03-164^T strain was notable among the inoculant strains and similar to the nitrogen sources with mineral N for SDM, SNA, and EFCN in Cruz das Almas. In Maragogipe, the UFLA 03-84, INPA 03-11B, and UFLA 03-164^T strains showed the highest mean values for SDM, SNA, and EFCN. Costa et al. (2014) found a similar effect from those strains when inoculated on cowpea in the southeast of the state of Piauí. Farias et al. (2016b) obtained mean values of SNA similar to the values obtained in Cruz das Almas when inoculating cowpea with the UFLA 03-164^T strain. Mean values of SNA obtained through inoculation with rhizobia strains in the soil from Maragogipe were superior to the values obtained in various studies in the Northeast region of Brazil (Almeida et al. 2010; Costa et al. 2014; Farias et al. 2016b). Almeida et al. (2010) also found NPP and LPP values similar to the values observed in Maragogipe.

Farias et al. (2016a) evaluated the efficiency of symbiosis of a rhizobia strain in cowpea in the state of Maranhão and found that the UFLA 03-164^T strain brought about higher values of SDM and EFCN than the MAPA-approved UFLA 03-84 and INPA 03-11B strains. These authors emphasize the ability of this species to adapt to regional conditions and its high symbiotic efficiency. Other studies showed a similar result for this strain compared to other strains approved for cowpea by MAPA (Costa et al. 2011; Ferreira et al. 2013).

Gains in SDM have an additional advantage over other N sources, besides the use of cowpea for human consumption cowpea may be used as a green manure crop to supply nitrogen to the soil (Costa et al. 2011; Farias et al. 2016a). In tests accomplished in a greenhouse, Silva et al. (2019) observed the strain INPA 03-11B conditioned greater cowpea biomass. . Almeida et al. (2010) likewise found no significant difference ($p < 0.05$) for 100SW between control treatments with or without mineral N and the MAPA-approved UFLA 03-84 and INPA 03-11B strains in the state of Piauí, Brazil. The use of inoculants with the INPA 03-11B and UFLA 03-84 strains is advantageous especially because they ensure nitrogen supply to the crop and because of their low cost.

The GY showed that inoculation was efficient in both soils since it resulted in mean values equal to the control treatment with mineral N and superior to the control without inoculation and without mineral N, confirming that the strains have the potential as inoculants in these soils.

Previous authors observed similar grain yield in UFLA 03-164^T strain as well as in UFLA 03-84 and INPA 03-11B strains (Costa et al. 2011; Ferreira et al. 2013; Farias et al. 2016b), while others found that UFLA 03-164^T strain exceeded the yield results from some of the currently approved strains (Costa et al. 2014; Farias et al. 2016b). This fact shows the efficiency and adaptability of this strain under different edaphic and climatic conditions.

The GY observed in the Maragogipe soil for UFLA 03-164^T was higher than that observed by Ferreira et al. (2013) in the state of Piauí, also under a family farm environment without the use of fertilizers. According to Fonseca et al. (2010), P availability increases crop yields, specifically cowpea yields. Therefore, the GY values obtained in Cruz das Almas may have been affected by the low P availability in that soil.

In Maragogipe, the UFLA 03-164^T strain, the MAPA-approved UFLA 03-84 and INPA 03-11B strains and the treatment with mineral N, led to mean yield values higher than the Brazilian average of 466 kg ha^{-1} (CONAB 2017). The new UFRB BA72C2-1 and UFRB FA51B1 strains and the treatment without N led to mean grain yield values close to the Brazilian average.

SDM, EFCN, and GY showed the potential of the UFLA 03-164^T strain and the UFLA 03-84 and INPA 03-11B strains to produce biomass and grain in both soils studied. The UFLA 03-164^T strain showed excellent results for increments in shoot dry matter, higher efficiency compared to the control with N, and higher grain yield. Previous studies performed under field conditions also showed a similar effect among strains regarding grain N accumulation (Ferreira et al. 2013; Farias et al. 2016b); however, in those studies, the strains did not differ from the control with mineral N. Therefore, based on the results of the present study, this strain, as well as the UFLA 03-84 and INPA 03-11B strains (already approved for inoculation), may be used to replace mineral fertilization. Strains INPA 03-11B and UFLA 03-164^T, show a more stable behavior, regardless of the cultivation environment. The UFLA 03-164^T strain, in the selection stage, has great potential to be authorized as an inoculant for cowpea, promoting growth and crop production similar to mineral fertilization (Oliveira et al. 2020).

The return on investment based on the value-cost ratio were positive for UFLA 03-164^T and UFLA 03-84 strains, showing that these strains are economically feasible. The INPA 03-11B strain, approved for cowpea by MAPA, shows positive value-cost ratio in the municipality of Maragogipe. The UFLA 03-164^T strain proved to be profitable for cowpea in both the Cruz das Almas and Maragogipe municipalities. In contrast, the use of mineral fertilization was not economically viable (Figure 1).

The net returns for diazotrophic bacterial inoculants in cowpea were calculated using the VCR. In order to consider a determined technology as desirable for farmers, it must be at a VCR limit of 3 to 4 (Dittoh et al. 2012). According to this calculation, two strains are advantageous in Cruz das Almas, with an estimated return of 392.70 US$ per hectare for UFLA 03-164^T and 387.22 US$ per hectare for UFLA 03-84. This value was obtained by multiplying the increase in cowpea yield per hectare due to inoculation with the diazotrophic bacterial inoculants by 1.89 US$ (average price for one kg of grain on the regional market) and subtracting the value of 14.35 US$ (corresponding to the cost of the inoculant). These strains can replace the mineral fertilizer, which provides a similar estimated return of around 435.40 US$, but has a cost of 360.89 US$. In Maragogipe, the UFLA 03-164^T, UFLA 03-84, and INPA 03-11B strains showed a VCR higher than 4, exhibiting advantageous with a return of 1430.37 US$, 1392.82 US$, and 952.46 US$, respectively, result similar than those using mineral fertilizer, which renders 1414.13 US$. The divergence between VCR results from these two municipalities may be attributed to environmental variability, such as seasonal rains and differences in soil fertility. In both environments, the VCR for the mineral fertilizer was under the 3-4 threshold, compared to the strains considered profitable, due to its high cost.

The present study confirms once again the potential of diazotrophic bacterial strains to supply all the nitrogen required for cowpea development, without the need for mineral fertilizers, which are not only expensive, but may also contaminate the environment when inadequately used.

5. Conclusions

The UFLA 03-164^T strain can be recommended for biomass production, green manure and for increasing grain yields in both soils. In Maragogipe, UFLA 03-84 and INPA 03-11B can likewise be recommended for biomass production, as green manure and for increasing grain yields. UFLA 03-84 can be recommended for increasing grain yield in Cruz das Almas.

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