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NITROGEN FERTILIZATION AND RHIZOBIUM STRAINS EFFICIENT IN THE DEVELOPMENT AND PRODUCTIVITY OF COWPEA

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ABSTRACT

The objective was to evaluate the effect of inoculation with rhizobia associated with nitrogen fertilization on the growth and productivity of cowpea cultivars. The experiment was carried out using two native isolates and the BR3262 strain (recommended for cowpea) plus nitrogen fertilization (30, 60 and 90 kg N ha^{-1}) and a control without fertilization and inoculation, and two cultivars of cowpea (BRS Guariba and Novaera). The experimental design was in randomized blocks, with four replications, in a triple factorial scheme (4 x 4 x 2). At flowering, the following parameters were evaluated: Plant height, stem diameter, shoot dry weight, SPAD Index, and number of nodules. At harvest, data on yield components were collected: Number of pods per plant, pod length, number of seeds per pod, mass of 100 grains and yield. Inoculation with rhizobia strains favors the reduction of nitrogen fertilization, resulting in increases in plant growth and yield. The native isolate UESB R5 resulted in the highest final profit and reduced costs with nitrogen fertilization for both cultivars.

KEYWORDS: Biological fixation of nitrogen, *Vigna unguiculata* (L.), Grain yield.

ADUBAÇÃO NITROGENADA E ESTIRPES DE RIZÓBIOS EFICIENTES NO DESENVOLVIMENTO E PRODUTIVIDADE DO FEIJÃO-CAUPI

RESUMO

Objetivou-se avaliar o efeito da inoculação com rizóbios associado à adubação nitrogenada sobre o crescimento e produtividade de cultivares de feijão-caupi. Foram testados dois isolados nativos e a estirpe BR3262, acrescidos de adubação nitrogenada (30, 60 e 90 kg ha-1de N) e um controle sem adubação e inoculação e duas cultivares de feijão-caupi (BRS Guariba e Novaera). O delineamento experimental empregado foi em blocos casualizados, com quatro repetições, em esquema fatorial triplo (4 x 4 x 2). No florescimento, foram avaliados os seguintes parâmetros: Altura de plantas, diâmetro do colmo, massa seca de parte aérea, Índice SPAD e número de nódulos. Na colheita foram coletados dados dos componentes de produção: Número de vagens por planta, comprimento de vagem, número de sementes por vagem, massa de 100 grãos e produtividade. A inoculação com as estirpes de rizóbios favorecem a redução da adubação nitrogenada, obtendo maior destaque o isolado nativo UESB R5.

PALAVRAS-CHAVE: Fixação Biológica de Nitrogênio, Vigna unguiculata (L.), Rendimento de grãos.

1. INTRODUÇÃO

Cowpea is an economically important species in Brazil. The cultivated area in the 2012/2013 season was around 3.06 million hectares, contributing to the productivity of 2.98 tons of grains (CONAB, 2013).

The crop has a worldwide distribution, mainly in tropical regions, due to its similarity to its place of origin, Africa. Similar soil and climate conditions make the northeast region of the country the main area for cowpea cultivation.

However, the average yield of cowpea is lower in northeastern Brazil than in other regions where growers employ newer technology and cultivars with better genetic potential, resulting in harvests with an average yield of 386 kg ha^{-1} (CONAB, 2017).

Low yields may be due to the type of crop system, such as less efficient technology, use of traditional cultivars with low yield potential, and low natural fertility and organic matter content in soil.

Nitrogen is one of the macronutrients most required by cowpea, requiring an amount greater than 100 kg ha^{-1} for improved growth (FREIRE FILHO; LIMA; RIBEIRO, 2005).

Due to the high cost of N fertilizers, losses in soil, and contribution to environmental pollution, it is essential to seek alternatives aiming at improving N use efficiency in soil, coupled with breeding N-efficient cowpea genotypes. It is necessary to study and apply technologies that aim for greater yields while reducing costs and environmental impacts.

Crop yields can be increased using inoculants with diazotrophic bacteria efficient in biological nitrogen fixation (BNF). These microorganisms not only reduce input costs by providing assimilable N to plants, but also bring direct and indirect benefits to the crop.

In recent years, studies have been carried out to evaluate the effect of rhizobia inoculation on cowpea, aiming for greater grain production with partial or total replacement of N fertilization (MARTINS et al., 2003; XAVIER et al., 2007; BRITO et al., 2010; CHAGAS JÚNIOR et al., 2010; CAVALCANTE et al., 2017). The effort has enabled the development of four strains of *Bradyrhizobium* recommended for the production of inoculants for cowpea grown in Brazil: UFLA3-84 (SEMIA 6461), BR 3267 (SEMIA 6462), INPA3-11B (SEMIA 6463) and BR 3262 (SEMIA 6464) (ZILLI et al., 2008).

A challenge is that cowpea easily forms nodules with several species of bacteria from the rhizobium group, such as: *Azorhizobium, Burkholderia, Bradyrhizobium, Mesorhizobium, Rhizobium, Sinorhizobium*, among others (NEVES & RUMJANEK, 1997; WILLEMS, 2006; ZILLI et al., 2006; ZHANG et al., 2007; MOREIRA, 2008).

Therefore, the exploitation of BNF is impaired by many factors, including high competition with native isolates in soil, crop health specific adaptation to edaphoclimatic conditions, and the low efficiency of certain cowpea genotypes in establishing symbiotic relationships. These factors justify the present study aiming at the selection of cowpea cultivars and strains that result in greater response to BFN, with less use of nitrogen fertilizer.

Therefore, the objective of this work was to evaluate the effect of inoculation with rhizobia associated with nitrogen fertilizer doses on the growth and yield of cowpea cultivars.

2. Methodology

From March to June 2018, a field experiment was conducted in the experimental area of the State University of Southwest Bahia (UESB), located in the municipality of Vitória da Conquista, Bahia state, Brazil, located at 928 m altitude, 14°51' S and 40°50' W. According to the Köppen classification, the regional climate is classified as high-altitude tropical (Cwa), with an average annual precipitation of 733.9 mm (SOUZA et al, 2010). The soil in the area was classified as Cambisol Haplic dystrophic Tb, with textural class clay- sandy loam.

Samples were taken for chemical and physical analyses of soil, at a depth of 0-20 cm. The results are: pH (H_2O) = 5.5; Al = 0.1 $cmol_c\ dm^{-3}$; Ca = 1.8 $cmol_c\ dm^{-3}$; Mg = 1.0 $cmol_c\ dm^{-3}$; K = 0.46 $cmol_c\ dm^{-3}$ and P = 16 mg dm^{-3}. Soil was plowed once and harrowed twice, then furrows were at 0.50 m apart. Sowing fertilization was carried out according to the recommendations of ALVAREZ V. & RIBEIRO, 1999.

The total area of the experiment was 98 m long x 20 m wide. The total size of the plot was 5 mx 2.5 m, with a spacing between rows of 0.5 m. The two central rows were the observational units, except the two plants on each end of the rows as borders.

Based on the soil analysis, liming was not necessary and base fertilization was carried out with 70 kg ha^{-1} of P_2O_5 in the form of single superphosphate and 20 kg ha^{-1} of K_2O, in the form of potassium chloride. N was supplied using urea divided into two applications, one at planting and the other 25 days after emergence of the plants (DAE).

The experimental design was in randomized blocks in a 4 x 4 x 2 factorial with four replications. The treatments comprised (i) three N doses in the form of urea: 30, 60 and 90 kg N ha^{-1} and a control without N fertilization; (ii) three bacteria, two native strains (UESB R1 and UESB R5), an inoculant recommended for crop, the BR 3262 strain (ZILLI et al., 1999), and a control without inoculation; and (iii) two cowpea cultivars (BRS Novaera and BRS Guariba).

The bacteria were isolated and cultured until pure material was obtained, without contaminants, and placed to grow for five days to produce the inoculant. Subsequently, the cell concentration of each bacterium was verified and adjusted to 10^9 UFC mL^{-1}, following the recommendation of the Ministry of Agriculture, Livestock and Supply (BRASIL, 2011). Before sowing, bacteria were inoculated at a rate of 1 mL of inoculant for 1,000 seeds.

At flowering, at 50 DAE, ten plants were randomly collected from each plot, within the observational area, for evaluations of plant height (PH), stem diameter (DC), number of nodules (NN), shoot dry weight (SDW), and chlorophyll index (SPAD).

The second evaluation was carried out at harvest, using ten plants from the observation area of each plot. Then, the following were evaluated: number of pods per plant (NPP), pod length (PL), number of grains per pod (NGP), mass of one hundred grains (M100G), and grain yield (GY).

To determine the SDW, a forced-air oven was used at 65ºC, until constant weight was reached. The evaluations of plant and pod length and stem diameter were measured using a ruler and caliper. And to evaluate grain production, total seeds of the observational area of each plot were weighed, in which the data were estimated for kg ha^{-1}, corrected for 13% moisture.

The data obtained were subjected to analysis of variance and grouped by the test Scott Knot and regression analysis at 5% probability using the *software* Sisvar 5.3 (FERREIRA, 2011).

3. RESULTS AND DISCUSSION

At the time of flowering, a significant effect of the joint interaction (Bacteria x Dose x cultivar) was verified on the variables H, SD, SDW, NN and SPAD (Table 1).

Table 1: Summary of the analysis of variance and coefficient of variation for the growth characteristics and symbiotic efficiency of the cowpea cultivars BRS Guariba and BRS Novaera, as a function of inoculation with native strains and *Bradyhizobium* sp. BR 3262, associated with different N doses.

		Mean squares				
S.V.	D.F.	2/ PH	SD	SDW	2/ NN	SPAD
1/ Bacteria (B)	3	378.51 *	0.397 *	193.68 *	8730.56 *	672.97 *
3/ Doses(D)	3	1266.03 *	0.185 *	567.51*	3477.64 *	1415.16 *
4/ Cultivar (C)	1	242.60 *	0.124 *	303.16 *	1.12NS –	87.93 *
B x D	9	55.53 *	0.640 *	81.14 *	431.87 *	101.62*
B x C	3	11.94 *	0.074 *	19.94 *	181.31*	61.18 *
D x C	3	26.40 *	0.268 *	19.68 *	264.97 *	93.34 *
B x D x C	9	10.25 *	0.057 *	24.40 *	112.83 *	9.16 *
Residue	96	2.54	0.0059	4.13	6.54	6.19
CV (%)	-----	4.56	7.51	5.81	6.55	3.56

*Significant at 5% probability using the F test; 1/ B: Bacteria; 2/ PH: plant height, SD: stem length; SDW: shoot dry weight; NN: number of nodules; SPAD: chlorophyll index; 3/ D: Doses: 0,30,60 and 90 kg ha^{-1}; 4/ C: Cowpea cultivars; BRS NovaEra and BRS Guariba.

We discussed only significant regression data about the bacteria. Therefore, when evaluating plant height, it appears that plant growth had a quadratic behavior for the two cultivars (Figure 1).

According to the model, the estimated maximum doses of 67 .79 and 56.87 kg ha^{-1} of N fertilizer combined with isolates UESB R1 (41.75 cm) and UESB R5 (43.09 cm), respectively, for BRS Guariba cultivar to attain the greatest height (Figure 1 A; 1 B).

At the same time, BRS Nova Era cultivar reached its maximum height (41.60 and

40.40 cm) at doses of 69.49 and 61.50 kg N ha^{-1} when associated with the isolates UESB R1 and UESB R5 (Figure 1 A; 1 B).

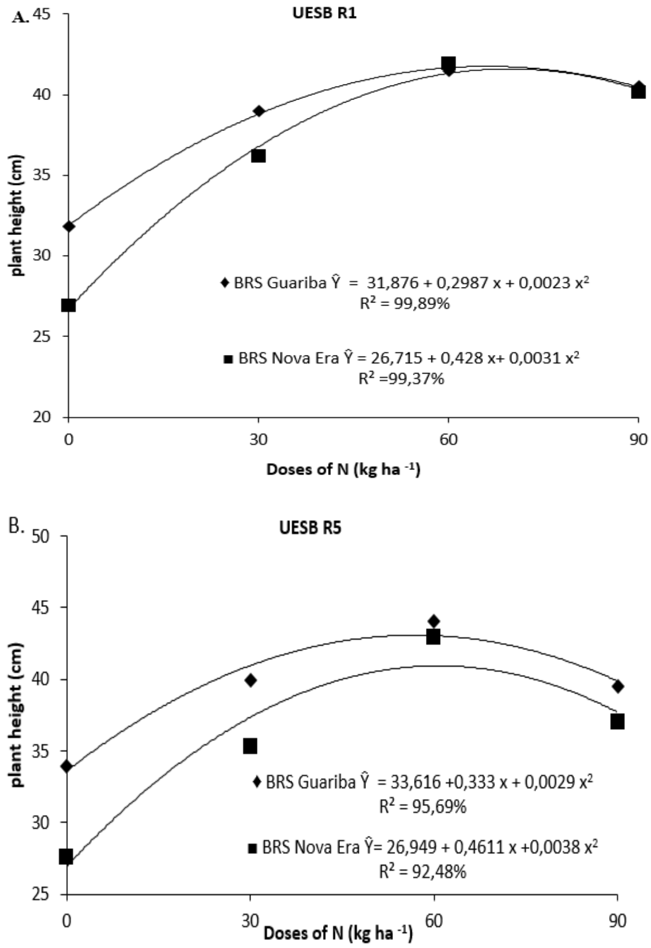

Figure 1. Plant height (PH) of cowpea cultivars BRS Novaera and BRS Guariba, at 50 DAE, under the effect of inoculation of bacterial strains UESB R1 (A) and UESB R5 (B) associated with different doses of N.

These results are in line with other studies, which verified the influence of inoculation with rhizobia on height of cowpea plants when compared to the control (RODRIGUES et al., 2012; SCHOSSLER et al., 2016).

However, BFN effects on the growth of cowpea plants are not always satisfactory (SILVA et al., 2006; FRIGO, 2013) because inoculated treatments suggest a greater expenditure of energy to produce nodules and N_2 fixation.

Furthermore, during cell divisions for colonization and development of nodules, a large expenditure of energy occurs, which is obtained from the oxidation of carbohydrates produced by shoots of the host plant (NEVES, 1981; HUNGRIA et al., 1999) and can thus reflect in shoot growth.

As for stem diameter, BRS Guariba cultivar associated with the bacteria UESB R1 (1.14 cm) and UESB R5 (1.15 cm) showed greater diameter values when fertilized with maximum doses of 50.52 and 53.68 kg N ha^{-1}, showing quadratic behavior of the data

(Figure 2 B; 2 C).

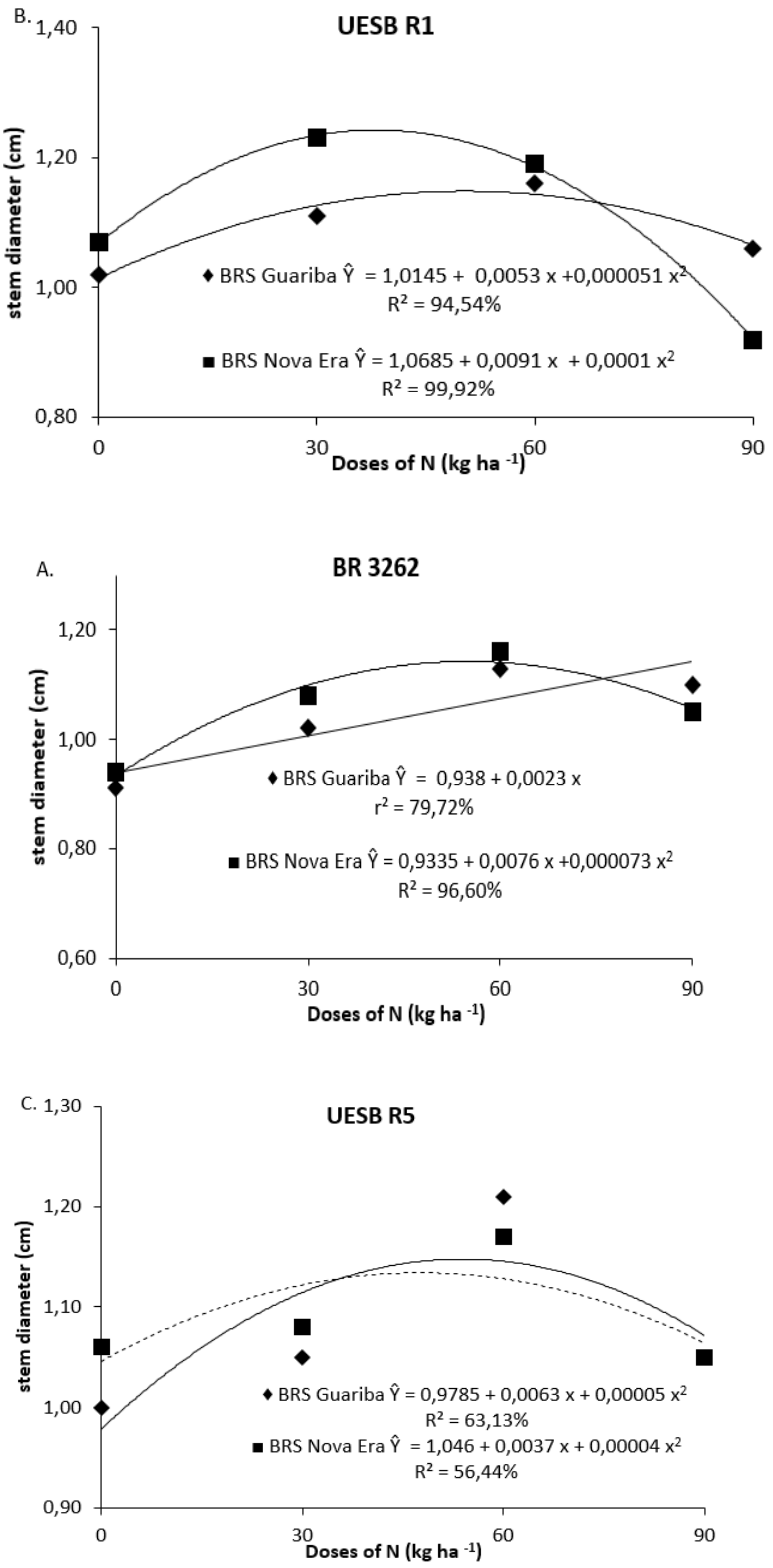

Figure 2. Stem diameter (SD) of cowpea cultivars BRS Novaera and BRS Guariba, at 50 DAE, under the effect of inoculation of bacterial strains BR 3262 (A), UESB R1 (B) and UESB R5 (C) associated with different N doses.

Conversely, BR 3262 strain had a linear increase under N fertilization, obtaining the maximum diameter value (1.10 cm) when at the maximum dose of 90 kg N ha^{-1} (Figure 2 A).

The diameter measured in BRS Novaera cultivar fitted a quadratic model for bacteria, providing maximum diameters (BR 3262 1.14 cm; UESB R1 1.24 cm; UESB R5 1.13 cm) at fertilizer doses of 54.36, 38.10 and 47.6 kg N ha^{-1}, respectively (Figure 2). These results corroborate those of other authors who reported the influence of bacteria on the stem diameter of plants (DARTORA et al., 2013; SANTOS et al., 2021).

The increases in stem diameter and plant height were more pronounced when inoculated with rhizobia. These effects are probably the result of the production of phytohormones and a greater stimulus to the increase in root hairs, which contribute to a greater contact to soil, resulting in greater water and nutrient uptake.

The use of the bacteria UESB R1 (37.39 g) and UESB R5 (37.36 g) satisfactorily increased SDW of BRS Guariba at doses of 49.70 and 62.31 kg N ha^{-1}, respectively (Figure 3). For the BRS Novaera cultivar, the maximum estimated values were (UESB R1) 42.71 and (UESB R5) 45.41 g at doses of 54.09 and 49.11 kg N ha^{-1} (Figure 3).

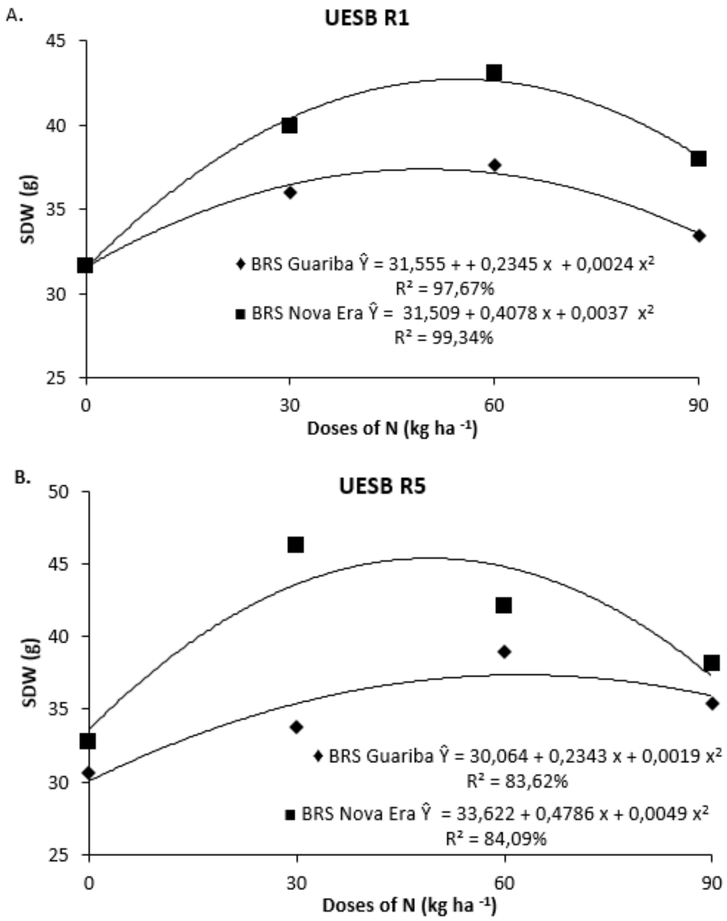

Figure 3. Shoot dry weight (SDW) of cowpea cultivars BRS Novaera and BRS Guariba, at 50 DAE, under the effect of inoculation with bacterial strains UESB R1 (A) and UESB R5 (B) and different N doses.

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Similar results of increased SDW were observed in other studies evaluating the agronomic performance of inoculated plants in association with N fertilization (QUADROS et al., 2009; MARTINS et al., 2013, DARTORA et al., 2013).

Furthermore, both cultivars inoculated with the strains were more efficient than control plants, with maximum increases in relative efficiency of 155.27% forUESB R5 associated with BRS Novaera at a dose of 30 kg ha^{-1} and 144.99% of plant biomass from BRS Guariba when using UESB R1, without N fertilization.

Thus, the results of increase in SDW in the present study, in treatments with absence of N and fertilization with 30 kg N ha^{-1} indicate the potential that the association of cowpea with rhizobia can completely replace mineral N fertilization and that, if soil and plant conditions are adequate for full symbiosis, starter fertilization is not even necessary. Similarly, Brito et al., (2011) confirmed that the greatest increases in shoot dry weight was observed when in the absence or lower N fertilizer application.

Regarding the number of nodules (Figure 4), as N doses increased, the number of nodules of both cultivars was reduced.

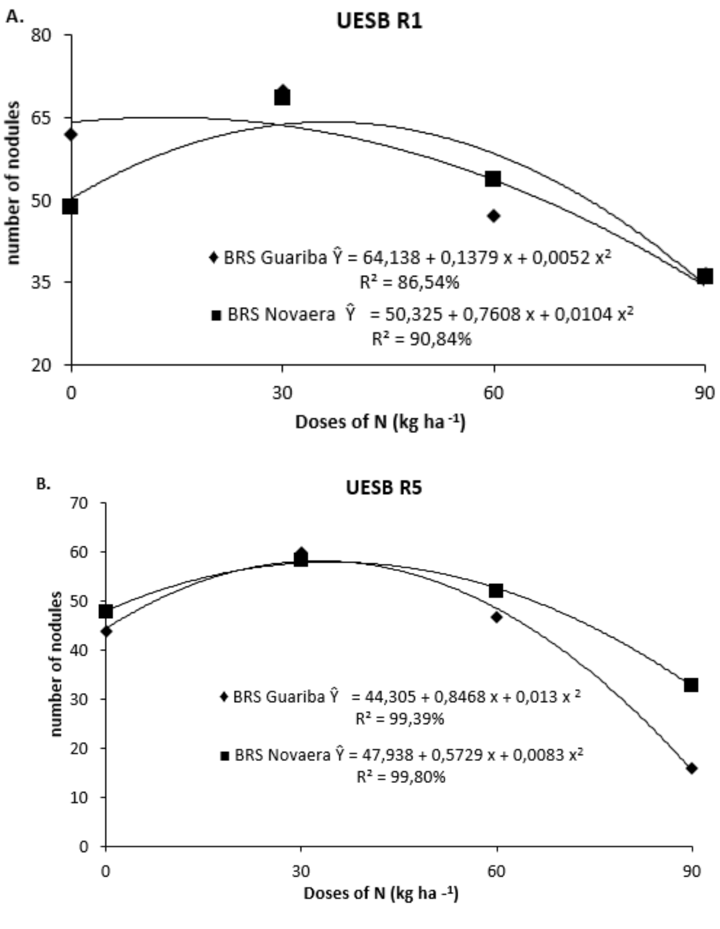


Figure 4. Number of nodules (NN) of cowpea cultivars BRS Novaera and BRS Guariba at 50 DAE under the effect of inoculation with bacterial strains UESB R1 (A) and UESB R5 (B) and different N doses.

The NN values were higher for cultivar BRS Guariba when associated with the isolates UESB R1 (67 n.plant^{-1}) and UESB R5 (58 n.plant^{-1}), with maximum rates of 13.24 and 58.01 kg N ha^{-1}. Similarly, the cultivar BRS Novaera showed a higher number of NN (UESB R1 64 n.plant^{-1} ; UESB R5 58 n.plant^{-1}) when subjected to N fertilization of 36.51 and 34.66 kg N ha^{-1} (Figure 4).

The number of nodules as affected by bacteria inoculation was higher than that of the cowpea cultivars studied by Calvacante et al., (2017). It is notable that the success of nodulation by bacteria is dependent on the symbiotic selectivity of the genotypes, which is directly influenced by the effectiveness of the rhizobia strains and how promiscuous they are.

Evaluating and comparing the nodular efficiency of different native strains of different cowpea cultivars under field conditions is quite difficult due to the variability of biotic and abiotic factors affecting these microorganisms.

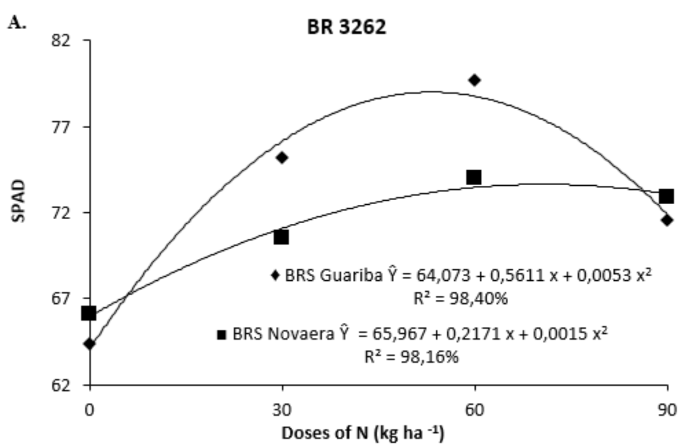
However, the evaluation of the number of nodules parameters are a criterium for evaluating symbiosis between rhizobia and cultivars, including established evaluation protocols (Network of laboratories for recommendation, standardization, and dissemination of microbiological inoculant technology of agricultural interest - RELARE) for selection of strains (BRITO et al., 2011).

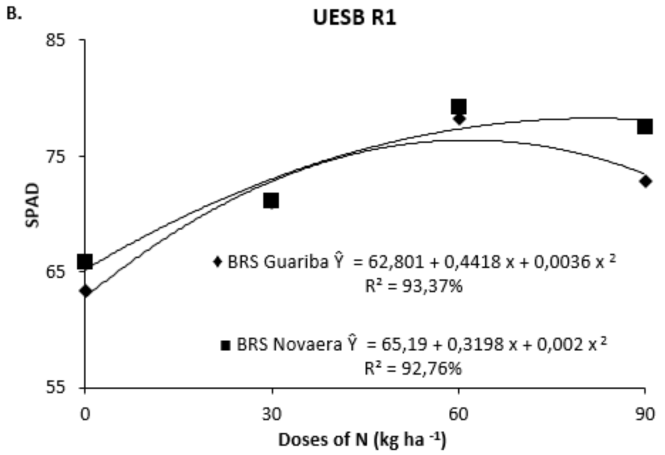
Therefore, selecting strains with a standard of effectiveness and competitive ability in the inoculation of cowpea is essential for future studies and the introduction of BFN into the crop.

Chlorophyll index readings aimed to obtain instantaneous readings, in a non-destructive way of the leaves, with the aim of capturing the chlorophyll content in the plant leaf. It is worth noting that the chlorophyll value found is directly correlated with N concentration in plant tissues, consequently, crop yields (LIMA et al, 2001; SILVEIRA et al., 2003).

A quadratic effect was observed when evaluating the results of the regression analysis for the SPAD index of leaves of cowpea cultivars.

When comparing the cultivars, BRS Guariba cultivar inoculated with the isolates BR 3262 (79%), UESB R1 (76%) and UESB R5 (84%), promoted higher SPAD rates in the presence of maximum rates of 53, 23, 61.49 and 54.26 kg N ha^{-1} fertilizer (Figure 2.5).





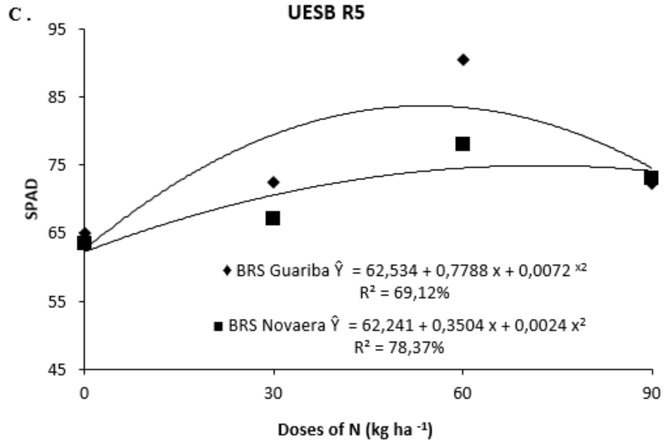


Figure 5. Estimation of the SPAD index in leaves of cowpea cultivars BRS Novaera and BRS Guariba, at 50 DAE, under the effect of inoculation of bacterial strains BR 3262 (A), UESB R1 (B) and UESB R5 (C) and different N doses.

However, for BRS Novaera to reach the maximum SPAD point (BR 3262 73%; UESB R1 78% and UESB R5 75%) it was necessary to supply N fertilizers of 70.56, 79.95 and 72.12 kg N ha^{-1} (Figure 5).

It is important to mention that the behavior of the cultivars differed from each other in terms of the chlorophyll content of the leaves. BRS Guariba demonstrated superiority and lower N requirements than BRS Novaera, even using the same bacterial strains. This suggests that the use of isolates in this cultivar provided a better symbiosis, which resulted in a greater N supply to the plant, thus increasing the chlorophyll index in the leaves.

In the second evaluation, at the time of harvest, the characteristics NPP, PL, NGP, M100G and GY were significantly influenced, for both triple effect of factors (B x D x C) and double effect for NGP (B x C and D x C) (Table 2).

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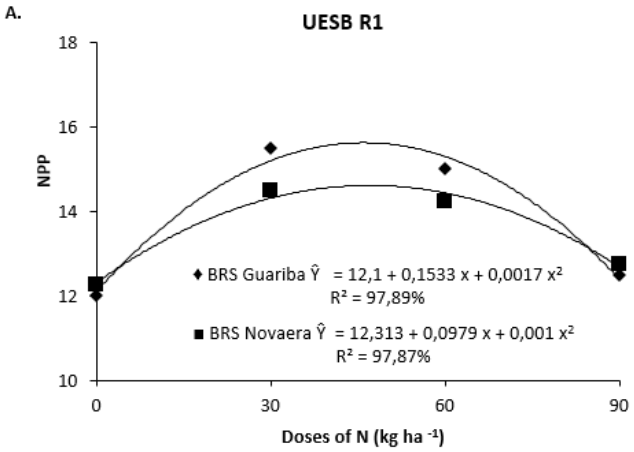
Table 2: Summary of the analysis of variance and coefficient of variation for the production variables of the cowpea cultivars BRS Guariba and BRS Novaera, depending on inoculation with native strains and *Bradyhizobium* sp. BR 3262, associated with different N doses.

		Mean squares				
S.V.	D.F.	2/NPP	PL	NGP	M100G	GY
1/ Bactéria	3	4.05 *	41.91 *	3.64 *	40.48 *	186821.30 *
3/Doses	3	17.84*	13.19 *	12.89 *	76.39 *	946521.18 *
4/Cultivar	1	318.78*	42.04 *	270.28 *	117.81 *	1505051.77 *
B x D	9	0.2256 NS	6.63 *	2.15 *	26.04 *	51264.00 *
B xC	3	0.42NS -	1.74NS -	0.80 *	3,233 NS	4186.49 *
D x C	3	0.59 NS	5.70 *	0.72 *	1.34NS -	27169 *
B x Dx C	9	0.82 *	3.63*	0.28NS -	2.98 *	20961.48 *
Residue	96	0.26	1.24	0.262	1.53	2555.33
CV (%)	-----	5.2	7.47	5.78	6.95	5.66

*Significant at 5% probability using the F test; 1/ B: Bacteria; 2/ NPP: number of pods per plant, PL: pod length, NGP: number of grains per pod; M100G: mass of one hundred grains; GY: grain yield; 3/ D: Doses; 0,30,60 and 90 kg ha^{-1}; 4/ C: Cowpea cultivars; BRS NovaEra and BRS Guariba.

Evaluating the behavior of the cultivars regarding the number of pods produced per plant, we observed that BRS Guariba was superior to BRS Novaera. In turn, to promote greater pod emission, the BRS Guariba cultivar required the application of the inoculants UESB R1 (16 pods^{-1}) and UESB R5 (16 pods^{-1}) associated with maximum doses of 45.08 and 45. 99 kg N ha^{-1} (Figure 6).



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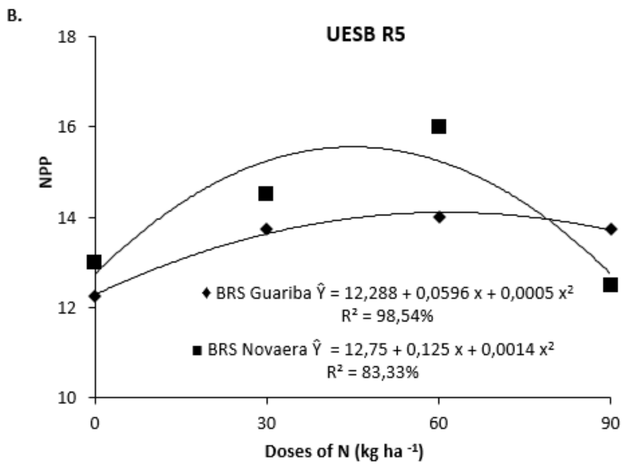


Figure 6. Number of pods per plant (NPP) in cowpea cultivars BRS Novaera and BRS Guariba at harvest, under the effect of inoculation with bacterial strains UESB R1 (A) and UESB R5 (B) and different N doses.

However, for BRS Novera to express its maximum potential, it was necessary to apply doses of 46.98 and 44.99 kg N ha^{-1} combined with the inoculation of the bacteria UESB R1 and UESB R5, to produce 14 and 15 pods, respectively (Figure 6).

The production of pods per plant in this study is similar to those found by Silva et al., (2011), who obtained average results of less than 20 pods per cowpea plant when inoculation was used together with N fertilizer.

Schossler et al., (2016) also obtained positive responses with inoculation of rhizobia in cowpea, which showed higher average number of pods when compared to the control without inoculation.

The number of pods is a trait that can be used as a criterion when breeding cowpea genotypes with yield potential (OLIVEIRA et al., 2003; SOUZA et al., 2007). However, this statement goes against the results found in this work because the cultivar BRS Novaera, although with a lower average NPP (16 pods plant^{-1}) when compared to BRS Guariba, showed greater grain yield.

However, it is worth noting that the size of the seeds is different, with BRS Novaera having larger grains, which may have contributed to higher values in grain weight, as well as it can be inferred that this cultivar stood out for having developed a better symbiosis with the isolates, which favors the best performance for grain size.

For pod length, it can be inferred that the use of UESB R1 in the BRS Guariba cultivar and UESB R5 in the BRS Novaera cultivar resulted in a linear increase in pod length with increasing N fertilization, obtaining the maximum length point of 15.15 and 16.70 cm, at the maximum dose of 90 kg N ha^{-1} (Figure 7).

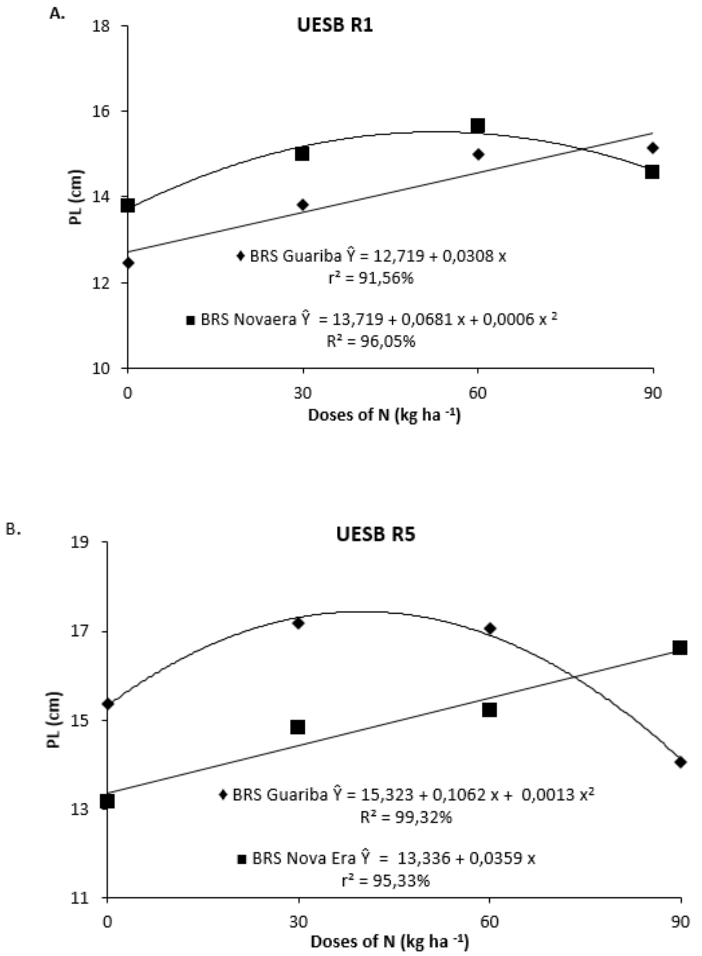


Figure 7. Pod length (PL) per plant in cowpea cultivars BRS Novaera and BRS Guariba, at harvest, under the effect of inoculation of bacteria strains UESB R1 (A) and UESB R5 (B) and different N doses.

However, when exchanging bacteria between cultivars, it was observed that the PL data were fitted to the quadratic polynomial model. The isolates UESB R1 in the BRS Novaera cultivar and UESB R5 in the BRS guariba cultivar expressed their maximum length potential of 17.44 and 15.51 cm, at the estimated doses of 39.92 and 52.83 kg N ha^{-1} (Figure 7).

Contrary to what was reported in this work, Calvacante et al., (2017) studied cowpea inoculated with the BR 3267 strain and reported no response in pod growth.

Although the pod length is peculiar to each cultivar, cultivars responded differently according to inoculation and fertilization. It is relevant to infer that the BR 3262 strain did not influence PL, highlighting the native isolates, especially UESB R1, which provided the greatest increase in size.

Additionally, native soil bacteria showed their efficiency in BFN when compared to commercially used strains, and this success can be attributed to their symbiotic capacity with cowpea cultivars, as well as being more competitive and adapting to environmental conditions. Thus, they were able to express their potential in promoting plant growth through phytohormone production.

The number of grains per pod is a trait that is directly linked to the genotype of the cultivars; however, it was verified in this work that the use of bacteria associated with N doses promoted a variation in the quantity of this variable.

When evaluating the response of bacteria to the doses, it was observed that the estimated maximum doses of 55.6, 49.07 and 57.51 kg N ha^{-1} allowed the production of 7, 9 and 7 seeds per pod, respectively, when associated with the bacteria BR 3262, UESB R1 and UESB R5 (Figure 8).

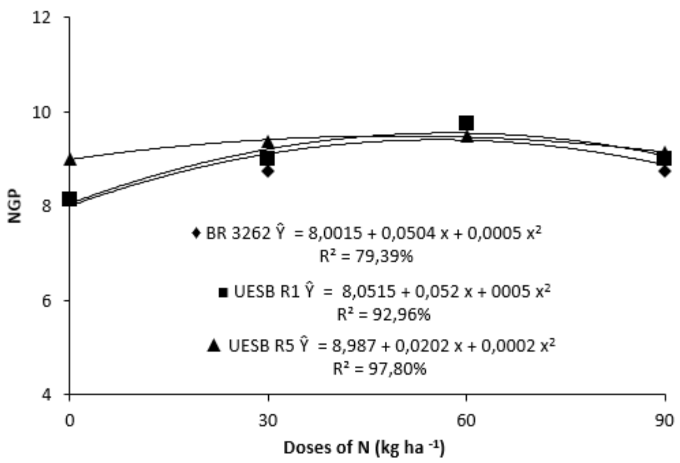


Figure 8. Number of grains per pod (NGP) in cowpea cultivars BRS Novaera and BRS Guariba, at harvest, under the effect of inoculation with bacteria strains BR 3262, UESB R1 and UESB R5 and different N doses.

Likewise, evaluating the effect of inoculation on grain yield of the cultivars, there was a significant difference between the treatments, in which all were superior to the control (Table 3).

Table 3: Number of grains per pod (NGP) of BRS Novaera and Guariba cultivars, depending on seed inoculation with rhizobia strains

BACTERIA/CULTIVARS	BRS Novaera	BRS Guariba
	NGP	
No inoculation	10.12 B	6.75B
BR 3262	10.18 B	7.50A
UESB R1	10.37A	7.56A
UESB R5	10.62A	7.87A
Means	10.32	7.42
CV (%)	5.78%	

*Means followed by the same letter in the columns do not differ from one another based on the Tukey test at 5% of probability.

Therefore, the interaction of N fertilization and BFN increase N efficiency, thereby contributing to increases in yield components. Yadegari (2014) reported a similar outcome by demonstrating an increase in the production of bean pods and grains when inoculated by rhizobia.

Contrary to what was exposed in this work, Calvacante et al., (2017) did not obtain an increase in the number of grains per pod using inoculation, explaining that this parameter is inherent to the crop genotype.

Among the cowpea yield components, the mass of one hundred grains (M100G) was affected by the interaction of the factors together, with quadratic responses of the data being observed as a function of N fertilization and inoculation (Figure 9).

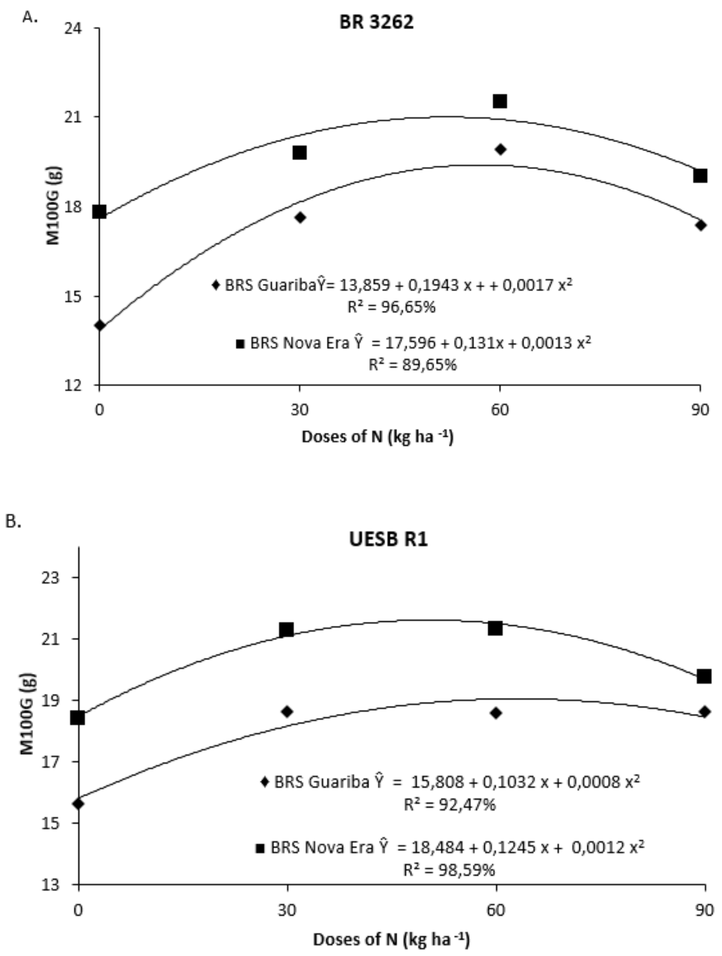


Figure 9. Mass of one hundred grains (M100G) of cowpea cultivars BRS Novaera and BRS Guariba, at harvest, under the effect of inoculation with bacterial strains BR 3262 (A), UESB R1 (B) and different N doses.

BRS Guariba cultivar showed its maximum point of 19.06 and 19.40 g of M100G, at doses of 62.99 and 57 kg N ha^{-1} associated with the bacteria BR 3262 and UESB R1, respectively (Figure 9).

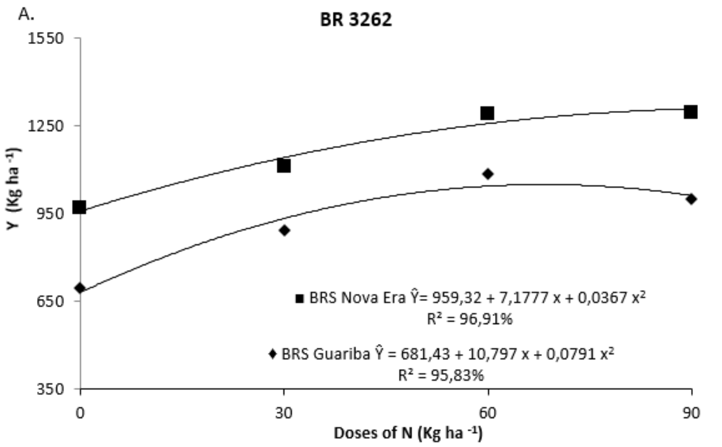
In contrast, the cultivar BRS Novaera reached its maximum M100G of 21.64 and 21.01 g, when subjected to doses of 50.62 and 52.07 kg N ha^{-1}, with the bacteria BR 3262 and UESB R1 (Figure 9).

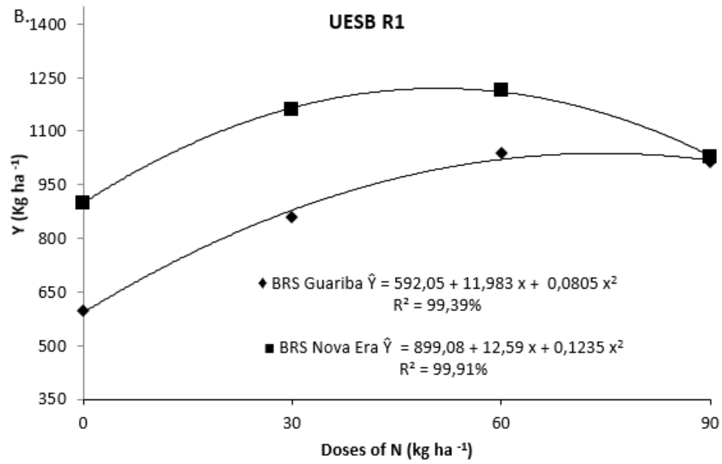
These results differed from other studies, in which the use of inoculation did not influence the weight of one hundred grains of cowpea cultivars (Silva et al, 2011; Valadão et al., 2009).

In general, both cultivars maintained their average mass standards of one hundred specific grains. However, it is important to highlight that the use of bacteria reduced N fertilization required by the crop, favoring seed mass in even when compared with the treatment with a higher N dose.

Thus, the growth of the aerial and root parts of the plant is favored due to the fact that N is directly linked to the constitution of proteins, enzymes, coenzymes, nucleic acids, phytochromes and photosynthetic pigments that consequently promote the increase in the plant's photosynthetic area and synthesis of photoassimilates that resulted in greater grain filling and productivity.

For grain yield of cowpea cultivars, the data were fit to a quadratic model ($p \leq 0.05$) as a function of N fertilization and inoculation (Figure 10).





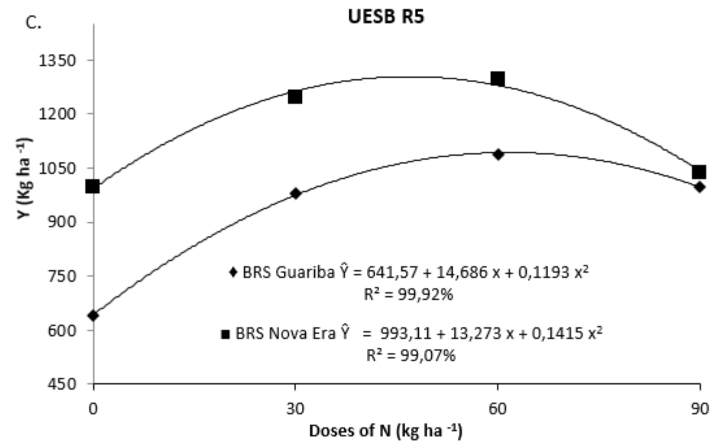


Figure 10. Grain yield (Y) of cowpea cultivars BRS Novaera and BRS Guariba, at harvest, under the effect of inoculation with bacterial strains BR 3262 (A), UESB R1 (B) and UESB R5 (C) and different N doses.

Analyzing the BRS Guariba cultivar, grain yields were highest when using the bacteria BR 3262 (1049.73 kg ha^{-1}), UESB R1 (1038.04 kg ha^{-1}) and UESB R5 (1093.31 kg ha^{-1}) associated with the addition of N fertilizer estimated at 68.22, 74.43 and 61.50 kg ha^{-1}, respectively (Figure 10).

On the other hand, when evaluating the response of the BRS Novaera cultivar to inoculation combined with N fertilization, it can be indicated that to obtain the maximum expression of grain yield, N doses of 90,50.95 and 46.89 kg ha^{-1} combined with the strains BR 3262, UESB R1 and UESB R5 are necessary to produce 1310, 1219 and 1305 kg ha^{-1} of grains (Figure 10).

Grain yield results were higher than the national average of 500 kg ha^{-1} (CRAVO & SOUZA, 2007). Likewise, work carried out also obtained satisfactory responses with inoculation on grain yield (ZILLI et al., 2009; MARTINS et al., 2013) obtaining similar yield values for BRS Guariba (GUALTER et al., 2007) and higher for Novaera (CHAGAS JÚNIOR et al., 2010). This divergence in cultivar yield responses is related to the amount of nutrients available in soil, as well as the symbiotic efficiency of the inoculant strains used in cowpea cultivation.

It is worth highlighting treatments using only inoculation as a N source promoted maximum increases in grain yield of 39.13% (BR3262) for Guariba cultivar and of 65.76% (UESB R5) for BRS Novaera, when compared to control. Demonstrating, once again, how advantageous it is to use inoculants as another source that will guarantee N supply to the crop.

Furthermore, the native strain UESB R5 promoted production of 1247.22 kg ha^{-1} at a dose of 30 kg N ha^{-1}, being higher than grain yield at the highest N dose in the control. These results corroborate the statement by actors Brito et al., (2011), who indicate the use of inoculation in cowpea production can partially or completely replace

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N fertilization.

The results indicate the efficiency of the native isolates in terms of BFN when compared to the control treatment, and it is also worth highlighting that the native isolate UESB R5 obtained superior production at the lowest N doses, losing only to BR 3262 (commercial inoculant) in the treatment without N fertilizer in Guariba.

According to the productivity obtained in each treatment, the relative efficiency of each strain over the control, the number of bags produced per kg/ of N and the final profit from grain production were estimated (Table 4).

Table 4: Final profit obtained from cowpea productivity per kilograms of nitrogen used to grow BRS Guariba (cv1) and BRS Novaera (cv2) after inoculation with rhizobacteria.

Bacteria	RE (%)		Bags produced per kg/N		Final profit (R$)	
	cv1	cv2	cv1	cv2	cv1	cv2
Control	-	-	16.78/90kg	20.62/90kg	1425.5	1847.9
BR3262	37.44	4	17/ 68.22kg	22/90kg	1551.42	1999.7
UESB R1	24.57	73.08	17.3/74.43kg	20.21/ 50.95kg	1555.42	1985.17
UESB R5	61.48	102.4	18.22/61.5kg	21.75/46.89kg	1717	2173.53

*RE: relative efficiency of the isolates in relation to the control.

* Price per kg of urea: R$ 4.67.

* Price of bag of cowpeas: R$ 110.00

Analyzing the relative efficiency of the strains, it was observed that UESB R5 was superior in both cultivars, resulting in a greater quantity of bags produced using lower doses of N fertilizer.

When evaluating N doses required by the cultivars to express their greater yield, it was observed that the use of BRS Novaera was higher than BRS Guariba when subjected to intermediate N doses.

However, the higher production in cowpea per kg of N for BRS Novaera cultivar (21 kg/46.89 kg of N) may be inherent to the plant’s genetic factors, since BRS Guariba has smaller grains.

On the other hand, BRS guariba produces a greater quantity of grains per plant pod, which can also contribute to the increase in bag weight. Thus, the highest yield achieved in this work was the result of the selection and effectiveness of the plant and bacteria, which in perfect symbiosis, resulted in greater grain yield.

In summary, the study of the bacteria UESB R5 associated with the BRS Novaera cultivar is plausible because the inoculation of this isolate is economically viable. This provided greater productivity, which resulted in greater final profit, reducing costs for the grower in relation to the use of N fertilizer.

4. CONCLUSIONS

Native cowpea strains promote a greater increase in the crop's growth characteristics when subjected to intermediate and lower doses of nitrogen fertilizer.

The native strains isolated from cowpea were superior in the characteristics evaluated when compared to the BR 3262 strain recommended for cowpea cultivation.

The highest productivity for cowpea was obtained using the cultivar BRS Novaera (R$ 2173.53) inoculated with the native strain UESB R5 associated with a dose of 46.9 kg ha^{-1}.

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