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The inoculation of cowpea culture with rhizobial lineage in Brazilian Cerrado Region

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Cowpea culture is spreading in the Brazilian Cerrado Region and specifically in Mato Grosso State. To evaluate the productive characteristics of cowpea inoculated with rhizobia lineages and grown in the Cerrado of Mato Grosso State, we conducted an experiment in a randomized block design with seven treatments, which consisted of five rhizobia lineages. Four lineages were previously isolated from cowpea (MT08, MT15, MT16 and MT23); one was a recommended lineage for cowpea in Brazil (BR3267). Two controls were included: one was equivalent to 75 kg N ha^{-1} nitrogen and another was without nitrogen fertilization and without inoculation of rhizobia lineage. They were arranged in six blocks totaling 42 parts of 12.5 m² each. At 40 days after sowing (d.a.s.), six plants of each part of floor area were collected to determine the variables plant height, dry mass of shoots and roots, total dry mass, number of nodules, dry mass of nodules, SPAD reading and relative efficiency of each lineage. Data were subjected to analysis of variance using the statistical analysis program SISVAR. To compare the means, it was evaluated with Tukey test at 5% probability and contrast analysis. Regarding the lineage used, the most satisfactory result for plant height was BR3267 lineage; however, there were statistical differences between treatments with nitrogen fertilization. There were significant differences between dry weight of shoots, total dry weight and dry weight of nodules. The BR3267 lineage presented better results in the dry mass of roots and for relative efficiency. The most satisfactory results for SPAD readings and number of nodules were observed in MT15 lineage. The BR3267 and MT15 lineages tested and analyzed had high symbiotic effectiveness for inoculation in cowpea.

Key words: *Vigna unguiculata*, rhizobium, nitrogen fixation.

INTRODUCTION

The cowpea (*Vigna unguiculata* (L.) Walp.) is a native legume of Africa and largely grown in the tropical regions of Africa, Asia and America, being the main source of protein, especially for low-income populations (Silva et al., 2006). This culture is highlighted in the Mid-North Brazilian as being of great socioeconomic importance as the source of vegetable protein for the population, especially the rural areas (Almeida et al., 2010).

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According to Zilli et al. (2009), Brazil is the third largest producer of cowpea; however, there is still a deficit in product offering as has been observed. The Brazilian Northeast is known for being the largest domestic producer of this grain, which is a major component of population diet, besides being an important generator of employment and income for families who cultivate the practice of family farming (Santos et al., 2008).

Despite this, its productivity is considered low due to few technological resources used in farming; there are regions in the States of Amazonas, Goiás, Mato Grosso do Sul and Mato Grosso where productivity has achieved good results using nitrogen fertilizer (Silva, 2009). According to Barbosa et al. (2010), the culture of cowpea is not only a subsistence activity and is becoming culture of high economic impact.

It is a very demanding culture in relation to nitrogen supply because most of this nutrient is exported to the grains during the plant reproductive period (Santos et al., 2003).

Furthermore, most part of this nutrient can be obtained directly from the atmosphere through association with rhizobial in a symbiotic relationship where the plant provides the metabolites to microorganisms while the bacterium in the root nodules performs the conversion of atmospheric nitrogen into ammonia (Figueiredo et al., 2008; Taiz and Zeiger, 2004).

In order to raise the productivity of cowpea, reduce production costs and increase the income of farmers, the biological nitrogen fixation (BNF) by adopting the practice of seed inoculation with efficient rhizobial lineages has been extensively explored (Zilli et al., 2009).

Thus, the aim of this study was to evaluate the productive characteristics of cowpea inoculated with rhizobia lineages and grown in the Cerrado of Mato Grosso, Brazil.

MATERIALS AND METHODS

Tillage and fertilization

The experiment was conducted from January to April of 2013 in the Federal University of Mato Grosso, Rondonópolis-MT campus, geographically located in the Southern region of the State which is situated at latitude 1° 28'15 " south and longitude 54° 38 '08" west. A heavy disk was used for soil leveling and to remove invasive plants. The soil was classified as Oxisol Red (EMBRAPA, 2009).

Soil samples were collected for chemical analysis and the results were: pH ($CaCl_2$) = 5.0, P = 3.7 and K = 60 mg dm^{-3} respectively. Ca = 1.2, Mg = 9.0, Al = 0.7, H = 0.0 and CTC = 6.0 $cmol_c$ dm^{-3} respectively. Organic material = 24.8 gdm^{-3}, base saturation = 44.8% and aluminum saturation = 55.2%.

Using the results of the soil chemical analysis, liming was performed in order to raise the base saturation of 60%, according to the culture needs.

The fertilization was performed according to Junior et al. (2002), applying 444 kg ha^{-1} of superphosphate, 86 kg ha^{-1} of potassium chloride and addition of micronutrients. 75 kg ha^{-1} of urea for nitrogen fertilizer was applied. This application was in two stages and next to the furrow. In the first stage, at 20 days after sowing (d.a.s.), 50% of the dose was applied. At 30 d.a.s, the second nitrogen fertilization was performed by applying the remaining dose.

Cultivar

The cultivar of cowpea used was BRS New Era, which corresponds to the lineage MNC00-553D-8-1-2-2, obtained from crosses between lineages TE97-404-1F and TE97-404-3F. This cultivar presents semi erect branches, short lateral branches and insertion of pods above the foliage. The color of the pods during physiological maturity and harvest is light yellow and may have purple pigmentation on the sides of the pods (Freire Filho et al., 2008).

Experimental design

The experimental design was a randomized complete block design with seven treatments, which consisted of five rhizobia lineages, including four previously isolated from cowpea (MT08, MT15, MT16, MT23), a rhizobia lineage recommended for cowpea in Brazil (BR3267) and two controls (one fertilized with 75 kg N ha^{-1} nitrogen and other without nitrogen fertilization and without inoculation with rhizobia lineages).

Each part consisted of an area of 12.5 m^2. The three central lines were used for floor area, excluding 0.5 m of edging. Each treatment consisted of six replicates, totaling 42 parts.

Inoculation and sowing seed

Seed inoculation was made by means of peat inoculant. The lineages were grown in liquid medium LMA (Vincent, 1970). After the growth of rhizobia lineages, 10 ml of bacterial broth was used for each 35 g of peat. Later, homogenization was done, and the inoculum was incubated at 30°C for 24 h (Guimarães et al., 2007).

Subsequently in this period, the seeds were pelleted with inoculant and made dry in the shade. And then seeding was performed with density of eight seeds per meter.

Collection of samples for analysis

At 40 d.a.s, the samples were collected, using six plants from each part of the floor area. In the samples, the followings were evaluated: SPAD reading, plant height (PH), dry mass of shoot (DMS), dry mass of root (DMR), total dry mass (TDM), number of nodules (NN), dry mass of nodules (DMN) and relative efficiency of the lineages (RE).

SPAD reading was realized using ClorofiLOG® CFL 1030 from five samples randomly chosen from each part of the floor area. Height of plants was determined using a measuring tape. The dry mass of shoot and root was determined in semi-analytical balance, after being kept in an incubator at 65°C until constant weight. The dry mass of nodules was determined through the same process however using an analytical balance.

The number of nodules was determined in the laboratory by tweeze extraction. Relative efficiency of each lineage was determined by dividing the dry mass of shoot from inoculated treatments by the nitrogen fertilizer treatment (Bergersen et al., 1971).

Data were subjected to analysis of variance using the statistical program SISVAR (Ferreira, 2008) and means were compared by Tukey test at 5% probability and contrast analysis.

RESULTS

The results concerning SPAD readings, plant height, root dry mass and number of nodules is shown in Table 1. From SPAD readings, it was observed that plants fertilized by nitrogen showed the highest values with an

Table 1. Plant height, SPAD reading, number of nodules (NN), dry mass of nodules (DMN), 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	Height (cm)	SPAD	NN*	DMN (g)*
MT 08	48.21^{b}	60.55^{ab}	6.54^{ab}	1.07^{ns}
MT 15	48.26^{b}	59.18^{ab}	9.22^{a}	1.043
MT 16	48.60^{b}	58.15^{ab}	7.43^{ab}	1.062
MT 23	48.60^{b}	59.28^{ab}	6.41^{ab}	1.084
BR3267	49.80^{ab}	57.85^{b}	7.18^{ab}	1.027
Nitrogen fertilization	54.58^{a}	63.21^{a}	4.26^{b}	1.043
Control	49.30^{b}	61.46^{ab}	3.58^{b}	1.032
CV (%)	5.54	4.44	39.05	4.6

Means followed by the same letter in the column do not differ by Tukey test at 5% probability. *Data transformed for Y +1; ns, not significant.

Table 2. Dry mass of shoot (DMS), total dry mass (TDM), dry mass of root (DMR) and relative efficiency (RE) of the lineages held at 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	DMS (g)	TDM (g)	DMR (g)	RE (%)
MT 08	45.76^{ns}	49.62^{ns}	3.85^{ab}	71.70^{bc}
MT 15	48.26	52.53	4.27^{ab}	81.40^{ab}
MT 16	53.10	57.27	4.16^{ab}	89.73^{ab}
MT 23	52.85	56.82	3.97^{ab}	89.03^{ab}
BR3267	57.47	62.64	5.17^{a}	94.97^{ab}
Nitrogen fertilization	63.82	68.50	5.02^{ab}	100.00^{a}
Control	47.74	51.24	3.50^{b}	47.74^{c}
CV (%)	19.21	18.92	20.45	16.96

Means followed by the same letter in the column do not differ by Tukey test at 5% probability; ns, not significant.

average of 63.2, with no statistical difference compared to MT08, MT15, MT16, MT23 lineages and control treatment; statistical difference was only observed in BR3267 lineage.

Among the inoculated treatments, the MT08 lineage is spotlighted with SPAD reading of 60.55 which is equivalent to 95.80% of SPAD reading of nitrogen fertilizer treatment. That result indicates an effective nitrogen fixation for MT08 lineage. However, the control treatment showed SPAD reading of 61.46 which may be related to the presence of native rhizobia in the tested soil.

The SPAD reading is a very important feature because it determines the plant efficiency in solar radiation absorption. The higher the solar radiation absorption efficiency is the higher the photosynthetic rates, which result in higher grain yield (Nascimento et al., 2011).

The highest average of plant height was obtained from nitrogen fertilization treatment with 54.58 cm per sample and had no statistical difference from BR 3267 lineages treatment. This result demonstrates the high efficiency of rhizobia symbiosis with cowpea. The other inoculated treatments MT08, MT15, MT16 and MT23 presented values below those observed with nitrogen fertilizer and commercial inoculant of BR3267 lineage.

The highest number of nodules was observed in MT15 treatment with average of 9.22 nodules per sample. There was no significant statistical difference compared to the other inoculated treatments. Concerning dry mass of nodules, there was no statistical significant difference between treatments and the highest quantity was observed in MT23 treatment with 1.084 g per sample; while BR3267 treatment showed less satisfactory results for this variable with 1.027 g.

It was observed that there was no statistical difference between treatments for dry mass of the shoot and the highest quantity was found for the nitrogen fertilizer treatment with an average of 63.82 g and lower result for the control treatment with 47.74 g (Table 2). The BR 3267 treatments presented 90% of result from dry mass of shoot in comparison to nitrogen fertilizer treatment, followed by MT16 (83.20%), MT23 (82.81%) and MT15 (75, 62%).

Regarding the production of total dry mass (TDM), there was no statistical difference between the treatments; however, the plants with better results were those

Table 3. Contrast results for height of the lineages held at 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	Treatment (average)							Contrast estimative
	Control	Fertilized	BR3267	MT08	MT15	MT16	MT23	
	49.30	54.58	49.80	48.21	48.26	48.60	48.60	
	Contrast coefficient							
C1	6	-1	-1	-1	-1	-1	-1	-0.376
C2		5	-1	-1	-1	-1	-1	5.878
C3			4	-1	-1	-1	-1	1.379
C4				3	-1	-1	-1	-0.273
C5					2	-1	-1	-0.348
C6						1	-1	0.000

Table 4. Contrast results for SPAD reading of the lineages held at 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	Treatment (average)							Contrast estimative
	Control	Fertilized	BR3267	MT08	MT15	MT16	MT23	
	61.46	63.21	57.85	60.55	59.18	58.15	59.28	
	Contrast coefficient							
C1	6	-1	-1	-1	-1	-1	-1	1.761
C2		5	-1	-1	-1	-1	-1	4.214
C3			4	-1	-1	-1	-1	-1.440
C4				3	-1	-1	-1	1.681
C5					2	-1	-1	0.469
C6						1	-1	-1.128

who received nitrogen fertilization treatment (Table 2). Among the inoculation treatments, the highest value for this variable was observed in BR3267 with 91% of the total dry mass compared to nitrogen fertilizer treatment; it is followed by MT16 (83%); and the MT08 lineage was the treatment that provided lowest value of total dry mass in this experiment.

For root dry mass variable, the treatment that stood out was the BR3267commercial lineage but did not differ significantly from the other treatments except the control. The production of the treatment inoculated with BR3267 lineages was superior to treatment with nitrogen fertilizer in approximately 3%. Regarding other inoculation treatments the MT15 lineage showed higher result with 83% of production observed in the commercial inoculant BR3267, followed by the lineages MT16, MT23 and MT08. All inoculation treatments had higher dry mass production of roots compared to control.

For the relative efficiency of lineages, the treatment showing the best result was BR3267 with 94.97% efficiency compared to nitrogen fertilizer. The MT16 and MT23 lineages were almost the most efficient of the BR3267 lineage (Table 2).

For contrast analysis, they were divided into six studies as follows: C1: control vs all treatment; C2: fertilized vs all treatment; C3: Br3267 vs (MT 08 + MT15 +MT 16 +MT 23); C4: MT 08 vs (MT15 +MT 16 +MT 23); C5: MT15vs (MT 16 +MT 23); C6: MT 16vs MT 23.

The cowpea height presented statistical difference for C2 ($P < 0.01$); it shows that N was more efficient in the development of the cultivar at 40 days compared to treatments using commercial inoculant as treatments inoculated with native lineage of MT 08, MT 14, MT 16 e MT 23 (Table 3). The SPAD reading presented significant difference for contrast 2 (C2), with $P < 0.01$ (Table 4). This demonstrates that treatment that uses chemical fertilizers based on nitrogen promoted more satisfactory results offering better conditions and nutritional support for the development of cowpea plants. Regarding the contrasts between inoculated treatments, there was no statistically significant difference.

The number of nodules variable presented statistically significant difference for the contrasts C1 and C5, both with $P < 0.05$. The results obtained for C1 showed the effectiveness of soil native rhizobia and its potential in relation to the BNF. Results of C5 demonstrate the effectiveness of rhizobia isolated from samples of bait plants grown in cerrado soil (MT 08, MT 14, MT 16 and

Table 5. Contrast results for number of nodules of the lineages held at 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	Treatment (average)							Contrast estimative
	Control	**Fertilized**	**BR3267**	**MT08**	**MT15**	**MT16**	**MT23**	
	3.58	**4.26**	**7.18**	**6.54**	**9.22**	**7.43**	**6.41**	
	Contrast coefficient							
C1	6	-1	-1	-1	-1	-1	-1	-41.250
C2		5	-1	-1	-1	-1	-1	-34.900
C3			4	-1	-1	-1	-1	-10.500
C4				3	-1	-1	-1	-10.444
C5					2	-1	-1	44.833
C6						1	-1	18.666

Table 6. Contrast results for dry mass of shoot of the lineages held at 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	Treatment (average)							Contrast estimative
	Control	**Fertilized**	**BR3267**	**MT08**	**MT15**	**MT16**	**MT23**	
	3.4	**5.02**	**5.17**	**3.85**	**4.27**	**4.16**	**3.97**	
	Contrast Coefficient							
C1	6	-1	-1	-1	-1	-1	-1	-0.908
C2		5	-1	-1	-1	-1	-1	0.733
C3			4	-1	-1	-1	-1	1.110
C4				3	-1	-1	-1	-0.277
C5					2	-1	-1	0.204
C6						1	-1	0.198

Table 7. Contrast results for relative efficiency of the lineages held at 40 d.a.s. in cowpea plants grown in the Cerrado Oxisol.

Treatment	Treatment (average)							Contrast estimative
	Control	**Fertilized**	**BR3267**	**MT08**	**MT15**	**MT16**	**MT23**	
	47.74	**100.00**	**94.97**	**71.70**	**81.40**	**89.73**	**89.03**	
	Contrast coefficient							
C1	6	-1	-1	-1	-1	-1	-1	-34.034
C2		5	-1	-1	-1	-1	-1	-21.549
C3			4	-1	-1	-1	-1	12.007
C4				3	-1	-1	-1	-15.021
C5					2	-1	-1	-7.989
C6						1	-1	0.695

MT 23), which for this variable presented better values than the commercial inoculant BR3267 (Table 5).

The dry mass of the roots of cowpea presented difference for C3 (P <0.01) and contrast C1 (P <0.05) (Table 6). The results obtained in C3 prove the effectiveness of the biological potential commercial inoculant BR3267; it has highest average when analyzing this variable and establishing the most effective treatment. The contrast C1 showed satisfactory results but below those verified in C3.

For relative efficiency variable, the results were significant for C2 (P <0.01) and C4 (P <0.05) (Table 7). This result demonstrates the effectiveness of native cerrado lineages (MT 08, MT 14, MT 16 and MT 23) and proves that they are efficient in biological fixation in symbiosis with cowpea under the evaluated conditions.

DISCUSSION

The cowpea culture can get N through symbiosis with bacteria of the genus Rhizobium in FBN process. This process is one way to increase the productivity of this legume (Franco et al., 2002). According to Rumjanek et al. (2005), FBN is efficient for this culture that can achieve high levels of productivity when the crop produces good nodulation. In this experiment, five rhizobia lineages were tested to evaluate the productive characteristics of the culture. However, the supply of nitrogen by biological fixation was not satisfactory for all inoculate lineage tested.

Study performed by Santana et al. (2010) to cultivate beans in the same soil type in Cerrado of Goiás State found the highest values of SPAD (47.0) for the treatment that received high doses of mineral nitrogen (ranging from 60 to 120 kg ha^{-1}*)*. These values are below those observed in this study where the fertilized soil showed a value of 63.21 for the same variable. These results demonstrate that the rate of mineral nitrogen applied on the soil in the experimented region is sufficient for obtaining acceptable results for SPAD index.

Chagas Junior et al. (2010) working with cowpea inoculated in the Cerrado of Tocantins State found greater crop production in the treatment with BR 3262 lineage.

In the actual experiment, the SPAD reading was a variable that achieved statistical significant difference when compared with the treatment fertilized with mineral nitrogen, inoculated treatments and the control treatment; higher values were obtained for the treatment fertilized with mineral nitrogen. This result may be related to the fact that mineral nitrogen is more readily absorbed by plants. In general, in the early development of the plant there is a preference for the absorption of nitrogen in the ammonium form. With the development of the vegetative phase, absorption of nitrogen as nitrate increases (Brown et al. 1983a, b; Blackmer, 2000).

Almeida et al. (2010) observed cowpea grown in Piauí State. This increased the number of nodules in treatments with lineages BR3267, which was also used as inoculum in this experiment. For this study, the highest number of nodules was obtained in treatment with the MT15 lineages; however, it did not differ statistically from the BR3267 and other inoculated treatments.

The results of this study are similar to results obtained by Chagas Junior et al. (2010) who planted the same variety of cowpea and obtained inoculant BR3267 as better treatment. In this study, the MT15 inoculant produced the highest number of nodules but there was no statistically significant difference relative to BR3267 inoculant.

The distribution of dry mass in the plant is a variable that allows one to discuss a process poorly studied which is the translocation of assimilates and that in many cases facilitates the understanding of plant response in terms of productivity (Benincasa, 2003). In addition, Gualter et al. (2011) affirm that nitrogen-fixing bacteria may contribute significantly to higher N to the plant and consequently lead to an increase of plant dry mass.

The results obtained by the dry mass of shoot in this experiment are similar to that of Almeida et al. (2010) who also found no differences in the dry mass of shoots in cowpea when comparing the fertilized ground, inoculants and control treatment.

The results for total dry mass of cowpea showed no statistically significant difference between treatments with the most satisfactory production for the treatment fertilized with N. This result was expected due to the availability of this nutrient confirming the results reported by Chagas Junior et al. (2010).

The relative efficiency of the lineages revealed that inoculation is an efficient practice for the process of biological nitrogen fixation and may even totally or partially replace nitrogen fertilization on cowpea. Nascimento et al. (2010) found positive results for the inoculum BR3267 while planting cowpea in Pernambuco State. The average percentage of the inoculum did not show statistical significant difference compared to the fertilized treatment, demonstrating efficiency in agricultural production.

Gualter et al. (2011) and Chagas Junior et al. (2010) obtained better results as to the relative efficiency of inoculated treatment. In general, the treatments that received inoculation of rhizobia lineage were promising for the supply of nitrogen through BNF in cowpea plants. This confirms the importance of this association and is also less aggressive and harmful to the agriculture environment.

The productivity of cowpea in this study was not presented because the analyses were performed 40 d.a.s. at the end of the experiment.

Conclusion

The rhizobia lineages that best express the potential of BNF in association with cowpea grown in the Cerrado Oxisol are MT15 and BR3267.

Conflict of Interests

The authors have not declared any conflict of interests.

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