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# Development of cv. BRS Novaera cowpea inoculated with rhizobium recommended for pigeonpea

# Desenvolvimento do feijão caupi cv. BRS Novaera inoculado com rizóbio recomendado para feijão guandu

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#### Abstract

The cultivation of cowpea (*Vigna unguiculata*) has grown in Brazil, especially in the Cerrado of Mato Grosso, where it is produced in off-season. The association of cowpea plants with rhizobia has provided much of the nitrogen required by this culture. This study aimed at evaluating the development of cowpea inoculated with rhizobia recommended for pigeonpea (*Cajanus cajan*). The experiment was conducted at the Federal University of Mato Grosso, University Campus Rondonópolis-MT under greenhouse conditions. The experimental design was completely randomized with three treatments and four replications. The treatments were composed by commercial inoculant recommended for pigeonpea (composed by the combination of strains BR2003 and BR2801), nitrogen (without inoculation and application of 50 mg N dm<sup>-3</sup>) and a control (without inoculation and nitrogen fertilization). Plants height, SPAD index, number of pods, number of grain, number of nodules, length of pods, dry weight of nodules, dry grain, dry weight of shoot and relative efficiency of the strains were analyzed. Cowpea show significant response for inoculation in all variables, with increase in production equal or superior to the treatment with nitrogen fertilizer. These results indicate the inoculant recommended for pigeonpea has potential use for cowpea under the tested conditions.

Additional keywords: cerrado; inoculation; Vigna unguiculata.

#### Resumo

O cultivo do feijão caupi (*Vigna unguiculata*) tem crescido no Brasil, especialmente no cerrado matogrossense, onde vem sendo produzido na safrinha. A associação de plantas de feijão caupi com o rizóbio tem suprido grande parte do nitrogênio requerido por essa cultura. Assim, objetivou-se, com esse trabalho, avaliar o desenvolvimento do feijão caupi inoculado com rizóbio recomendado para o feijão guandu (*Cajanus cajan*). O experimento foi conduzido na Universidade Federal do Mato Grosso, Campus Universitário de Rondonópolis-MT, em casa de vegetação. O delineamento experimental utilizado foi inteiramente casualizado, com três tratamentos e quatro repetições. Os tratamentos foram compostos por um inoculante comercial recomendado para o feijão guandu (composto pela combinação das estirpes BR2003 e BR2801), adubação nitrogenada (sem inoculação e com aplicação de 50 mg N dm<sup>-3</sup>) e um controle (sem inoculação e sem adubação nitrogenada). As variáveis analisadas foram altura das plantas, índice SPAD, número de vagens, número de grãos, número de nódulos, comprimento de vagens, massa seca de nódulos, massa seca de grãos, massa seca da parte aérea e a eficiência relativa das estirpes. O feijão caupi respondeu significativamente à inoculação em todas as variáveis analisadas, com incrementos na produção iguais ou superiores à adubação nitrogenada. Esses resultados indicam que nas condições testadas, o inoculante recomendado para o feijão guandu apresenta potencial de uso para o feijão caupi.

Palavras-chave adicionais: cerrado; inoculação; Vigna unguiculata.

#### Introduction

In Brazil, cowpea (*Vigna unguiculata*) production is concentrated mainly in the Northeastern and Northern regions. However, it is gaining space in the Midwestern region due to the development of cultivars with characteristics that favor its cultivation,

being incorporated into productive arrangements as second crop, thus increasing the interest of producers for this culture (Freire Filho et al., 2011).

Given this scenario, it is extremely important to study new technologies that intend to increase production sustainably and without increasing costs. In this context, legumes such as cowpea have a key role in agriculture, since they have the capacity of association with nitrogen-fixing bacteria such as rhizobium, getting much of the nitrogen required for their development (Zilli et al., 2009, Guimarães et al., 2013).

The use of nitrogen fertilizers in tropical soils, besides their high cost of production, also have an additional ecological cost, considering that the loss of applied nitrogen fertilizers are of around 50%, what is mainly caused by leaching and runoff caused by rainfall and/or irrigation (Straliotto et al., 1999).

Biological nitrogen fixation (BNF) has been proved essential for the sustainability of Brazilian agriculture, given the fact that it supplies nitrogen to crops with low economic cost and reduced environmental impact, improving its agronomic efficiency (Hungria & Bohrer, 2000; Lima et al., 2011; Florentino & Moreira, 2009).

Techniques such as cowpea seeds inoculation with rhizobium prospect the generation of high agronomic efficiency in this culture. In addition, the use of rhizobium inoculants in grain legumes has been responsible for significant savings in the cost of agricultural production, by reducing the use of nitrogen mineral fertilizers as a benefit of the BNF process (Xavier et al., 2008).

In the state of Mato Grosso, studies conducted with inoculation techniques in cowpea through the use of rhizobium lineages are still limited or nonexistent. This study aimed to evaluate the development of cowpea inoculated with rhizobium recommended for pigeonpea and grown in Cerrado Oxisol.

#### Material and methods

The inoculation experiment in cowpea (*Vigna unguiculata*) with rhizobium inoculant recommended for pigeonpea (*Cajanus cajan*) was conducted in a greenhouse at the Federal University of Mato Grosso, Rondonópolis Campus, municipality geographically located in the southern region of the state, at latitude 16°28'15 South and longitude 54°38'08 West.

The experimental design was a completely randomized with a cultivar of cowpea (BRS Novaera), three treatments and four replications. The treatments consisted of a commercial inoculant recommended for pigeonpea (formed by the combination of BR2003 and BR2801 lineages), nitrogen (without inoculation and with the application of 50 mg N dm<sup>-3</sup>) and a control (without inoculation and nitrogen fertilization).

Fertilization was performed with phosphorus  $(P_2O_5)$  and potassium  $(K_2O)$ , with 400 and 100 mg dm<sup>-3</sup>, respectively, using superphosphate and potassium chloride as sources.

The inoculation was performed by pelleting the seeds in the proportion of 500g of inoculant per each 50 kg of seeds (Guimarães et al., 2007). Subsequently, the seeds were put to dry in the shade and then sown in pots.

Planting was done by sowing in pots with

8 dm<sup>3</sup> of soil capacity, in which five seeds were sown per vase, leaving three plants after thinning.

The evaluations were made in the R1 (beginning of flowering) and R3 (beginning of the pods maturation) growth stages, considering the following variables: plant height, SPAD index, number of nodules, nodule dry matter, number of pods, pods length, number of grains, grains dry matter, total dry matter and the relative efficiency of the lineages, using dry biomass shoot part parameter (Bergensen et al., 1971).

Data were subjected to analysis of variance by F test, and means were compared by Tukey test at 5% significance level, using the SISVAR 4.2 statistical program (Ferreira, 2008).

#### **Results and discussions**

The inoculation of cowpea seeds with rhizobium inoculant recommended for pigeonpea provided increments for the analyzed variables, with similar effects to the treatment in which plants received nitrogen fertilizer, besides setting higher development to plants that made up the control treatment.

The best performance for plant height in the R1 stage was observed in the nitrogen treatment, followed by the treatment in which plants were inoculated with the commercial inoculant. However, the results observed in the treatment with inoculation were not different from those of the control treatment. In the R3 stage, the inoculated plants showed similar development to those that received nitrogen fertilization, showing also higher height when compared to the control (Figure 1A).

These results differ from those found by Figueiredo et al. (2008), who found higher plant height in inoculated treatments. Guareschi et al. (2011) found that with the application of inoculant with *Bradyrhizobium japonicum* in adzuki beans, there was a higher plant height compared to the other treatments, except for the treatment without inoculation. This result is similar to that found in this work only by the evaluation in the R3 stage, where the control treatment showed lower height when compared to the other treatments.

Machado et al. (2008), while studying cowpea genotypes for earliness, plant architecture and grain yield, found that the average height of plants ranged from 37.2 cm to 41.33 cm. The results found by these authors differ from those found in this study, where plants height, except for the absolute control, ranged from 42.5 cm to 44.25 cm in the R3 stage.

The SPAD index provide readings that are correlated with the chlorophyll content in leaves, making it a more practical tool for the evaluation of nitrogen content in leaves, since the leaf chlorophyll concentration is positively correlated with the nitrogen content in plants and with the crop yield (Schadchina & Dmitrieva, 1995).



Figure 1 - Height (A) and SPAD index (B) of cowpea bean plants (*Vigna unguiculata*) inoculated with rhizobium lineages recommended for pigeonpea (*Cajanus cajan*). Means followed by the same letter do not differ by Tukey test at 5% significance level.

The results obtained for the SPAD index were significant in the first reading, held at the beginning of flowering, corresponding to the R1 stage (Figure 1B). The plants that received nitrogen fertilization showed higher cumulative chlorophyll content in leaves (57.40), followed by the commercial inoculant (55.00) and the control (37.4).

In the second reading, held at the beginning of the pods maturation, R3 stage, the SPAD index was not significant because many leaves had already lost its original chlorophyll content or had even suffered abscission (Figure 1B). The results observed in the R1 stage corroborate with Vieira et al. (2010), who reported that the treatments fertilized with nitrogen gave the largest increments of nitrogen in plants. At the pod maturation phase (R3 stage), no differences were found in the chlorophyll content between treatments, which can be related to the decrease in nitrogen concentration in the vegetative fraction of the plant, translocating it to the grains. Experiments conducted by Almeida et al. (2010) with rhizobium inoculation on cowpea in the state of Piauí showed no significant difference between inoculated treatments and the control (without inoculation and without fertilization) to the nitrogen content in plants, corroborating with the results obtained in this study.

The difference between the number of cowpea pods present in the treatments was significant, and the occurrence of pods was considerably lower in the control treatment comparing to the other treatments (Figure 2A). The average number of pods value found in this study, obtained in the inoculated plants, was of 9.5, which is below the results obtained by Santos et al. (2009), who found 10.05 as the average number of pods.

Pods length was significant in all treatments, where it was observed that the commercial inoculant (average value of 12.74 cm) was statistically equal to that of the treatment that received nitrogen fertilization (13.7 cm). Only the control showed lower pods length (9.3 cm), showing similar behavior to the number of pods (Figure 2B). These results disagree with those found by Guedes et al. (2010), which under similar conditions had average values of pods length ranging from 15 cm to 18.94 cm.



Figure 2 - Number (A) and length of pods (B) of cowpea (*Vigna unguiculata*) inoculated with rhizobium lineages recommended for pigeonpea (*Cajanus cajan*). Means followed by the same letter do not differ by Tukey test at 5% significance level.

Although it is generally agreed that pods length is related to productivity, this condition is not always the most desirable, since in production systems in which the use of semi-mechanized and mechanized harvesting is advocated, pods of smaller sizes are more suitable to this type of management for being less conducive to touch the ground, thus preventing decay (Silva & Neves, 2011).

According Gerlach et al. (2013), there is a tendency to increase beans yield along with the

increase of the dose of mineral nitrogen. In this study, for the number of grains variable, the inoculated plants showed statistically equal values to the treatment that received nitrogen fertilization. In addition, it was observed that for grains dry matter the commercial inoculant was statistically equal to the plants fertilized with nitrogen, thus indicating that these bacteria recommended for pigeonpea have a high BNF potential when combined with cowpea (Figure 3, A and B).



Figure 3 - Number (A) and dry matter (B) of cowpea grains (*Vigna unguiculata*) inoculated with rhizobium strains recommended for pigeonpea (*Cajanus cajan*). Means followed by the same letter do not differ by Tukey test at 5% significance level.

These results are opposite to those found by Borges et al. (2012), who obtained the highest grain average to a vinegar cultivar in the control treatment, and corroborate with the experiment conducted by Chagas et al. (2010), who observed a positive response regarding this variable inoculation.

Xavier et al. (2008), while evaluating inoculation and nitrogen fertilization on nodulation and cowpea grain yield, concluded that productivity increases with the use of inoculation and the effect of this practice is favored when the cowpea is fertilized with a maximum of 20 kg ha<sup>-1</sup> of nitrogen.

In the study conducted by Guedes et al. (2010), evaluating the efficiency of inoculants in cowpea in the municipality of Pombal – PB, the authors found no difference between the treatment means for the number of grains per pod variable,

occurring in function to the variation between treatments for pods length. Also according to the authors, if the pods had similar length, it is common to retain this similarity as for the number of grains per pod.

In this work, no significant differences were observed for the number of nodules. However, the commercial inoculant excelled the other treatments. Moreover, in relation to the nodules dry matter, a difference was observed between treatments, with higher accumulation observed in plants treated with the commercial inoculant (Table 1).

The establishment and survival capacity of lineages when inoculated in plants which are associated can determine the efficiency in nodulation. Thus, there is still no conclusive information on the minimum number of nodules required to ensure a good performance of BNF for the cowpea, which already exists for the soybean crop, in which it 15-20

nodules in the crown of the main root is recognized as sufficient (Hungria & Bohrer, 2000).

Table 1 – Number of nodules (NN), dry matter of nodules (DMN), dry matter of shoots (DMS) and relative efficiency (RF) in cowpea plants inoculated with the rhizobium recommended for pigeon pea.

Treatments	NN	DMN (g vase <sup>-1</sup> )	DMS (g vase <sup>-1</sup> )	RF (%)
Commercial inoculant	14.70 a <sup>*</sup>	0.21 a <sup>*</sup>	22.60 a	99.00 a
Nitrogen Fertilization	11.10 a	0.10 ab	22.80 a	100.00 a
Control	9.80 a	0.02 b	8.30 b	36.40 b
CV (%)	22.51	3.26	17.13	14.11

Means followed by the same letter do not differ by Tukey test at 0.05 probability. \* Transformed values by square root of (Y + 1.0)

Another important observation in this study is related to nodulation in plants that received nitrogen fertilization and to the control, both of which have similar nodules to those of inoculated plants. But in terms of agronomic efficiency, in the variables analyzed above, the commercial inoculant proved to be superior, indicating competition and establishment in the soil capacity (Guimarães et al., 2013).

For the shoot part dry matter variable a difference of the treatments compared to the control was observed, with an increase of more than 100% (Table 1).

It was noted a great loss of leaf matter at the end of the culture cycle, which was due to leaf senescence and translocation of photo-assimilated to stems and grains (Borges et al., 2012). This behavior occurs because the cowpea can respond to various environmental stimuli, changing its dry matter partitioning throughout the cycle, as a physiologic response to guarantee grain yield (Praxedes et al., 2009, Silva et al., 2009).

In plants inoculated with nitrogen-fixing bacteria, high plants dry matter values have been related to higher nitrogen fixation capacity by more efficient rhizobia (Lima et al., 2011; Florentino & Moreira, 2009), as well as indicative of high crop yields (Zilli et al., 2009; Ferreira et al., 2009).

The relative efficiency of the lineages is a parameter that serves as an indicator of the nitrogen that is fixed and used by the culture. In the present study, it was observed that the commercial inoculant for pigeon pea, when applied in cowpea cultivation, have a relative efficiency of 99%, clashing the control treatment, with efficiency of only 36% (Table 1).

The rhizobium potential of fixing nitrogen is of fundamental importance for high biomass production, which can be related to the effective capacity in the supply of nitrogen by the tested lineages, which can be demonstrated by the positive correlation between the relative efficiency and the shoot dry mass production (Chagas Júnior et al., 2010).

Similarly, it was demonstrated that the relative efficiency can be high in plants which were not inoculated with nitrogen-fixing bacteria nor fertilized with nitrogen fertilizers. This effect may be related to the cowpea ability to nodule with different rhizobia groups, thus proving the capacity of the native population to establish symbiosis with cowpea (Lima et al., 2011).

In general, the results of the treatments whose plants were inoculated with rhizobium lineages proved to be superior to the control and similar to the treatment that received nitrogen fertilization recommended for this culture, corroborating with Guedes et al. (2010), who also found differences in the inoculated treatments when compared to the absolute control. Other studies have showed the positive effect of the use of rhizobium lineages on the productivity of cowpea (Lacerda et al., 2004, Lima et al., 2005, Soares et al., 2006, Zilli et al., 2009, Zilli et al., 2011).

Further studies on the cowpea-rhizobium interaction should be performed, particularly with experiments in the Mato Grosso Cerrado region under field conditions, so that it can be established, in addition to the inoculant used in this work, which autochthonous lineages have potential for inoculants targeted to the cowpea grown in Mato Grosso.

# Conclusions

Seeds inoculation of cowpea with rhizobium inoculant recommended for pigeonpea resulted in yield of grains equivalent to the plants fertilized with mineral nitrogen.

The rhizobium inoculant recommended for pigeonpea has the potential to be used in cowpea.

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