Nitrogen fertilization and application of brassinosteroid in cowpea in the Amazon

Adubação nitrogenada e aplicação de brassinosteroides em feijão-caupi na Amazônia

Fertilización nitrogenada y aplicación de brasinoesteroides al caupí en la Amazonia

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ABSTRACT
Cowpea [Vigna unguiculata (L.) Walp.] is a legume of socioeconomic importance in the northern region of the country, however, its production requires high fertilizer costs, especially nitrogen fertilizers. The use of techniques with the potential to increase crop productivity, such as the application of plant regulators (brassinosteroids-BRs) and the use of nitrogen (N) are necessary. Thus, the objective of this work was to evaluate the vegetative and reproductive phases of cowpea (BR3-Tracuateua) in relation to the application of BRs, mineral nitrogen fertilization and inoculation with the bacterium Azospirillum brasilense. The research was carried out in the experimental area of the Federal Rural University of the Amazon, Campus Capitão Poço. An experimental design was used in randomized blocks, with three replications, in a 3 x 4 factorial scheme: three N supply conditions (Without N, with mineral N and with biological N) and foliar application of four doses of BRs (0, 15, 30 and 45nM). The growth and production variables were not significantly influenced by the application of BRs and the forms of N supply. However, M100 and productivity were increased by the supply of N, both mineral and biological. Therefore, inoculation with Azospirillum brasilense in cowpea cultivation becomes a viable practice, with the objective of supplying N to plants, replacing mineral nitrogen fertilization.

Keywords: Vigna unguiculata, biological nitrogen, hormone, bacteria.
RESUMO
O feijão-caupi [Vigna unguiculata (L.) Walp.] é uma leguminosa de importância socioeconômica na região Norte do país, no entanto, para sua produção tem-se um alto custo com fertilizantes, principalmente os nitrogenados. O uso de técnicas com potencial para aumentar a produtividade das culturas, como a aplicação de reguladores vegetais (brassinosteroides-BRs) e o uso de nitrogênio (N) são necessárias. Assim, o objetivo deste trabalho foi avaliar as fases vegetativa e reprodutiva do feijão-caupi (BR3-Tracuateua) em relação à aplicação de BRs, adubação nitrogenada mineral e inoculação com a bactéria Azospirillum brasilense. A pesquisa foi realizada na área experimental da Universidade Federal Rural da Amazônia, Campus Capitão Poço. Utilizou-se delineamento experimental em blocos casualizados, com três repetições, em esquema fatorial 3 x 4: três condições de fornecimento de N (Sem N, com N mineral e com N biológico) e aplicação foliar de quatro doses de BRs (0, 15, 30 e 45nM). As variáveis de crescimento e produção não foram influenciadas significativamente pela aplicação dos BRs e pelas formas de oferta de N. Porém, o M100 e a produtividade foram aumentados pelo fornecimento de N, tanto mineral quanto biológico. Portanto, a inoculação com Azospirillum brasilense no cultivo do feijão-caupi torna-se uma prática viável, com o objetivo de fornecer N às plantas, em substituição à adubação nitrogenada mineral.

Palavras-chave: Vigna unguiculata, nitrogênio biológico, hormônio, bactéria.

RESUMEN
El caupí [Vigna unguiculata (L.) Walp.] es una leguminosa de importancia socioeconómica en la región norte del país, sin embargo, su producción requiere altos costos de fertilización, especialmente de fertilizantes nitrogenados. Es necesario el uso de técnicas con potencial para incrementar la productividad del cultivo, como la aplicación de reguladores vegetales (brasinoesteroides-BRs) y el uso de nitrógeno (N). Así, el objetivo de este trabajo fue evaluar las fases vegetativa y reproductiva del caupí (BR3-Tracuateua) en relación a la aplicación de BRs, fertilización nitrogenada mineral e inoculación con la bacteria Azospirillum brasilense. La investigación se realizó en el área experimental de la Universidad Federal Rural del Amazonas, Campus Capitão Poço. Se utilizó un diseño experimental en bloques al azar, con tres repeticiones, en un esquema factorial 3 x 4: tres condiciones de suministro de N (Sin N, con N mineral y con N biológico) y aplicación foliar de cuatro dosis de BRs (0, 15, 30 y 45nM). Las variables de crecimiento y producción no se vieron significativamente influydas por la aplicación de BRs y las formas de suministro de N. Sin embargo, M100 y la productividad se incrementaron por el suministro de N, tanto mineral como biológico. Por lo tanto, la inoculación con Azospirillum brasilense en el cultivo de caupí se convierte en una práctica viable, con el objetivo de suministrar N a las plantas, sustituyendo la fertilización nitrogenada mineral.

Palabras clave: Vigna unguiculata, nitrógeno biológico, hormona, bactéria.

1 INTRODUCTION

Belonging to the Fabaceae family, cowpea [Vigna unguiculata (L.) Walp.] is a crop that has great economic, social and nutritional importance, being widely used in human and animal
nutrition (Mendonça et al., 2015; Boukar et al., 2016), so it is necessary to improve its productivity.

A technique that has been studied in several crops to increase productivity and improve their quality is the use of plant regulators, due to their controlling effects on plant development, as it has been shown to be important in increasing crop productivity (Santos et al., 2017), such as brassinosteroids. Also, nitrogen (N) plays an important role in the growth and production of cowpea, being the macronutrient that plants demand most in their metabolism (Santana et al., 2020). Cowpea is a plant with a high demand for N and, therefore, the crop has high production costs (Silva et al., 2019).

In this way, the supply of N becomes essential for plant cultivation, which is mainly done via mineral fertilization. On the other hand, cultivating cowpea with N-fixing bacteria also guarantees the supply of N, reducing production costs due to less use of mineral nitrogen fertilizers (Costa et al., 2021), such as Azospirillum spp. These bacteria produce phytohormones capable of stimulating the growth of the root system of different plant species (Lopes et al., 2021) and, thus, can increase the absorption of water and nutrients with possible impacts on crop production.

In this context, and given the scarcity of regional literature on the subject, there is a need to develop research that will prove the potential beneficial effects of the application of BRs and the inoculation of seeds with N-fixing bacteria. For this reason, the present work aimed to evaluate the growth and production of cowpea in relation to the application of the phytohormone BRs, mineral nitrogen fertilization and inoculation with Azospirillum brasilense.

2 MATERIAL AND METHODS

2.1 CHARACTERIZATION OF THE STUDY SITE

The research was carried out between September and November 2022, through an experiment conducted in the experimental area of the Universidade Federal Rural da Amazônia (UFRA) – Campus Capitão Poço (CCP), located in the Microregion of Guamá (1°44'5.87" S and 47°3'28.38" W). The region's climate, according to the Köppen classification, is Am type with annual precipitation around 2,500 mm and a brief dry season between September and November (monthly precipitation around 60 mm), average annual temperature of 26 °C and relative air humidity between 75 and 89%, in the months with the lowest and highest rainfall, respectively.
(Schwart, 2007). To characterize the soil in the experimental area, 20 simple soil samples were randomly collected (0 to 20 cm) using a Dutch auger to form a composite sample, which was sent to the Labominas® laboratory (Manhuaçu-MG) for chemical and physics (Table 1).

<table>
<thead>
<tr>
<th>pH (water)</th>
<th>SOM %</th>
<th>P mg dm³</th>
<th>K</th>
<th>Ca²⁺ cmol dm³</th>
<th>Mg²⁺</th>
<th>Al³⁺</th>
<th>H⁺Al %</th>
<th>m</th>
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<td>19</td>
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<table>
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<th>Sand</th>
<th>silt</th>
<th>Clay</th>
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<td>590</td>
<td>105</td>
<td>305</td>
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</table>

SOM- (Soil organic matter) - dichromate extraction; P e K- extraction with Mehlich-1; Ca²⁺, Mg²⁺ e Al³⁺- KCl extraction (1.0 mol L⁻¹); H⁺Al- extraction with Ca acetate at pH 7; m- Al saturation; V- base saturation; Clay, silt and sand- stirring and separation with NaOH (0,1 mol L⁻¹) and determination with a densimeter.

Source: Labominas Laboratory, Manhuaçu, MG.

2.2 EXPERIMENTAL DESIGN AND TREATMENTS

A randomized block design (RBD) was used, with three replications, in a 3 x 4 factorial scheme: three N supply conditions (Without N - SN, with mineral N - NM and with biological N - BN) and four doses of the phytohormone brassinosteroids - BRs (0, 15, 30 and 45 nM). Mineral N was applied via urea and the inoculation of cowpea seeds with N-fixing bacteria (Azospirillum brasilense) was considered biological N. Each experimental plot (15 m²) consisted of six sowing lines measuring 5 m each, with a spacing of 0.5 m between rows, with the two central rows being used for evaluations, excluding four plants at the ends of each row.

2.3 PLANT MATERIAL, INOCULANT AND PHYTOHORMONE

The cowpea [Vigna unguiculata (L.) Walp.] seeds used in the experiment were from the BR3-Tracuateua cultivar, acquired, with a good percentage of germination and vigor, from a reputable producer in the region. This cultivar has indeterminate growth of prostrate size, with a cycle of 65 to 70 days. The phytohormone brassinosteroids (BRs) was acquired from UFRA-Campus Belém, through the Biodiversity Studies in Higher Plants (EBPS) research group. The inoculant used was the bacteria Azospirillum brasilense strain Ab-v5 and Ab-v6, at the dose recommended by the manufacturer (1 g/ 1000 seeds), with the seeds being inoculated five days before sowing in the Irrigation Engineering-LEI laboratory at the Universidade Federal Rural da Amazônia, Campus of Capitão Poço.
2.4 SETUP AND CONDUCT OF THE EXPERIMENT

To correct the soil, liming was carried out throughout the experimental area, according to the results of the soil analysis (Table 1), aiming to increase base saturation to 50% (Cravo & Souza, 2020). Dolomitic limestone (relative power of total neutralization - RPTN= 99%) was manually applied to the soil surface and incorporated (0-20 cm) with the plow harrow. Subsequently, thirty days after liming, manual sowing of cowpea was carried out (09/10/2022). Three seeds were sown per hole at a spacing of 0.3 x 0.5 m. Thinning was carried out fifteen days after emergence, leaving only one plant, the one with the greatest vigor, per pit.

Nitrogen, phosphate and potassium fertilization were carried out according to the results of the soil analysis (Table 1) and the recommendations for the cultivation of cowpea in the region (Cravo & Souza, 2020). For the treatment with application of mineral N, 20 kg/ha of N in the form of urea (45% N) were applied as cover, 25 days after sowing (DAS), which was incorporated into the soil (5 cm) at the beginning of the day, with subsequent irrigation. For all treatments, immediately after emergence, mineral phosphate fertilizer was applied with 80 kg/ha of P₂O₅ in the form of triple superphosphate (46% P₂O₅). Also, in all treatments, potassium mineral fertilizer was applied by applying 90 kg/ha of K₂O in the form of potassium chloride (58% K₂O), dividing the dose into two equal applications. The first application of K was carried out shortly after emergence, together with phosphate fertilizer, and the second at 25 DAS, the same time as the application of mineral N. All mineral fertilizers were applied manually to each plant in the planting line at an approximate depth of 5 cm from the soil surface.

The foliar application of brassinosteroids (BRs) was carried out at two times: at stage V3, when the plants had three pairs of completely expanded trifoliate leaves, which occurred 23 DAS; and, at stage R5, which represents the beginning of the plants' reproductive stage, which ran 45 DAS. The doses of BRs (0, 15, 30 and 45 nM) were suggested by the Higher Plant Biodiversity Study Group (HPBS) and applied with the aid of backpack electric pumps, with each dose being prepared in a single pump for each treatment. Each pump was filled with 10 L of deionized water, with 1 mL of the “Tween” adjuvant being diluted for each liter of water. For doses of 15, 30 and 45 nM of BRs, 1.5 mL, 3.0 mL and 4.5 mL of the BRs solution were added to the solution of each pump, respectively. Hormone applications were always carried out in the morning, isolating the plots with a plastic sheet at their ends.
2.5 IRRIGATION, CULTURAL AND PHYTOSANITARY TREATMENTS

An irrigation system was installed in the experimental area, due to the implementation of the crop being carried out outside the agricultural calendar indicated for cowpea in the region (September to November). Irrigation occurred once a day in the morning, always on days when there was no rain, with a maximum time of thirty minutes for each experimental plot and an average flow of 4.33 L/h in each hole. To control undesirable plants, at 38 DAS, manual weeding was carried out with a hoe. To control fungi, such as cercosporiosis (Pseudocercospora cruenta) and anthracnose (Colletotrichum lindemuthianum), at 58 DAS, the fungicide Manzate® WG was applied (10.8 L). To control pests, such as bedbugs (Nezara viridula) and cowbugs (Cerotoma arcuata tingomariana), at 48 DAS, the insecticide Decis® 25 EC was applied (10.8 L). Fungicide and insecticide applications occurred after the cowpea flowering period.

2.6 VARIABLES ANALYZED

2.6.1 Growth and biomass variables

At 55 DAS, the evaluation of the growth parameters of cowpea plants was carried out. To this end, 11 plants/plot were evaluated in the two central rows. Using a graduated ruler (cm), the plant height (PH) was measured, considering the distance between the soil surface and the insertion of the last fully expanded leaf. The collar diameter (SD) was measured (mm) with a digital caliper at 10 cm from the ground. To obtain the number of leaves (NL), a simple count of all leaves of each plant evaluated was carried out.

To estimate cowpea biomass production, also at 55 DAS, 5 plants were randomly harvested from each useful plot. At LEI (UFRA-CCP), with the help of scissors, the plants had their parts separated into root and aerial part (stem + leaves + pods/grains). After this procedure, the roots were washed in tap water to remove soil adhering to them. Then, these samples, as well as the aerial part samples, were placed in a forced air ventilation oven (65 ºC/72 hours). Subsequently, the plant material was weighed (g) on an analytical balance to obtain its dry mass production (Figure 10). The dry mass values of the root (RDM), shoot (SDM) and total - TDM (RDM+ SDM) were obtained. Also, the RDM/SDM ratio was estimated.
2.6.2 Production variables

At 79 DAS the cowpea production parameters were evaluated, measuring 5 plants/plot. The weight of pods (PW)/plant, the number of pods/plant (NPP), the height of insertion of the first pod (IHFP), the mass of 100 grains (M100) and the grain productivity of the crop were evaluated. At LEI, PV (g) and M100 (g) were obtained on an analytical balance. For PW, all pods from each plant were weighed and, for M100, 100 grains randomly separated from plants harvested from each plot were weighed. To obtain NPP values, a simple count of all pods on each plant was carried out. IHFP was determined with the aid of a ruler (cm), considering the distance between the soil surface and the end of the last floral raceme and the insertion of the first pod. Finally, the grain productivity (kg/ha) of the crop was estimated as a function of the grain mass/plant and the planting density (66,666 plants/ha), considering the crop spacing.

2.7 STATISTICAL ANALYZES

All results were subjected to the Shapiro-Wilk test to analyze the normality of residuals and the Bartlett test for homogeneity of variances. Analysis of variance (F test; p < 0.05) was performed to verify the effects of isolated factors and the interaction between them on the response variables. For the qualitative factor (N supply), the means, when significant, were compared with each other using the Tukey test at 5% probability. All statistical procedures were carried out using the Agroestat® software (Barbosa & Maldonado Junior, 2015) and the graphs were generated with the aid of the computer program Sigma Plot® v. 10.0.

3 RESULTS AND DISCUSSIONS

3.1 VEGETATIVE GROWTH OF COWPEA

At 55 days after sowing (DAS), plant height (PH), stem diameter (SD) and number of leaves (NL) per cowpea plant were not significantly influenced by doses of brassinosteroids (BRs) and by the forms of N supply (Figure 1). General averages of 40.19 cm PH (Figure 1A), 8.17 mm SD (Figure 1B) and 24.76 NL/plant (Figure 1C) of cowpea were observed. Also in the Northeast of Pará, Costa et al. (2021) observed similar results in cowpea cultivars BRS Marataoã and BRS Tapaihum in relation to the application of mineral N and inoculation with rhizobia.
Figure 1. Plant height growth - AP (A), stem diameter - DC (B) and number of leaves - NF (C) 55 days after sowing cowpea with application of doses of brassinosteroids (BRs) and N supply (No N - SN, biological N - NB and mineral N - NM). Means followed by identical letters in the bars do not show a significant difference according to the Tukey test (p > 0.05).

Source: Author (2023)

A greater physical and economic yield of cowpea was observed when mineral N was applied entirely in top dressing at 20 DAS (Cruz et al., 2022). Also, different studies with cowpea indicated that the application of mineral N did not significantly influence the SD of the plants (Duarte et al., 2021).

The sandy soil conditions (Table 1) and the rainfall that occurred during the cultivation period, in addition to irrigation, may have promoted greater N leaching and, thus, contributed to the lack of response in cowpea growth to its application in the present study. Also, urea can contribute to N losses through volatilization, when not managed properly (Sadeghian & González-Osorio, 2022).
### 3.2 COWPEA BIOMASS PRODUCTION

Regarding the variables root dry mass (RDM), shoot dry mass (SDM), RDM/SDM ratio and total dry mass (TDM), no significant effects were observed from the application of BRs and N supply (Figure 2). Romanini Júnior et al. (2007) also observed that the application of mineral N provided greater SDM of bean plants.

For the RDM variable, although without a significant effect of the BRs factors and N supply, an overall average of 2.34 g/plant was observed (Figure 2A). In the present research, the biological treatment with A. brasilense was similar to the treatment with application of mineral N in the RDM, indicating that the biological fixation of N (BFN) carried out by the bacteria was satisfactory for the development of the cowpea root system in the region.

The aforementioned author indicated that the production of soybean RDM is directly related to the growth of lateral roots, in which variations in the length of the main root do not significantly interfere with the absorption of the soil solution by the adventitious roots.

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Figure 2. Production of root dry mass - RDM (A), shoot dry mass - SDM (B), RDM/SDM ratio (C) and total dry mass - TDM (D) of cowpea with application of doses of brassinosteroids (BRs) and N supply (No N - SN, biological N - BN and mineral N - NM). Means followed by the same letters in the bars do not show a significant difference according to the Tukey test (p > 0.05).

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Source: Author (2023)
For SDM, an overall average of 22.36 g/plant was observed (Figure 2B). Okada et al. (2019), evaluating cowpea, also did not find significant results for SDM and RDM between treatments with mineral and biological N.

The RDM/SDM ratio was also not influenced by treatments with N and BRs supply, with an overall average of 0.11 (Figure 2C). A balance in the RDM/SDM ratio indicates that the parts of the plant allocated carbon in the biomass equally according to the plant's development. For the TDM of cowpea plants, there was also no significant response from the factors under study, observing an overall average of 24.72 g/plant (Figure 2D).

Mineral nitrogen fertilization increases the availability of N in the soil and, thus, its absorption by the root system is greater, resulting in an increase in the nutrient content in the aerial part of the bean, which results in greater accumulation of SDM (Soratto et al., 2017). However, in the present experiment, no response in biomass production to the supply of N (biological or mineral) was verified. Probably, as already reported, this fact occurred due to greater losses of the nutrient in the edaphoclimatic conditions of cultivation in the region, such as sandy soil (Table 1), rainfall and irrigation, in addition to the low content (1.2%) of SOM (Table 1) and application of urea as a source of N (Monteiro et al., 2012).

3.3 COWPEA PRODUCTION

Pod weight (PW), number of pods per plant (NPP) and first pod insertion height (IHFP) were not significantly influenced by the application of BRs and N supply (Figure 3). Romanini Junior (2007) also found no effect of nitrogen fertilization and inoculation with Rhizobium on the NPP of common beans.
Figure 3. Weight of pods - PW (A), number of pods per plant - NPP (B) and height of insertion of the first pod - IHFP (C) of cowpea with application of doses of brassinosteroids (BRs) and supply of N (No N-SN, biological N - NB and mineral N - NM). Means followed by identical letters in the bars do not show a significant difference according to the Tukey test (p> 0.05).

For PW, an overall average of 21.82 g/cowpea plant was observed (Figure 3A). The authors recorded an average PW of 21.12 g/plant and reported that in the absence of urea application, inoculation is a viable alternative. Possibly, the PW of cowpea plants in the present study did not have a significant effect of BRs and N supply as it was the first year of cowpea cultivation in the experimental area, since the microbiota was still adapting to the conditions of plant cultivation.

The NPP was not influenced by the application of the phytohormone and the form of N supply, with an average value of 10.31 pods/plant being observed (Figure 3B). According to Rodrigues et al. (2016), a study with inoculation with A. brasilense indicated that there was no significant effect on the NPP of common beans, however, when associated with an irrigation depth of 75%, there was an increase in the variable (average of 10.45 pods/plant).
For IHFP, although there was no significant effect of treatments, an average of 9.78 cm was observed (Figure 3C). Carvalho without significant effect. Mundim et al. (2018) found no influence of coinoculations with Bradyrhizobium sp. and A. brasilense in the NPP and IHFP variables of soybean plants. On the other hand,

### 3.4 COWPEA PRODUCTIVITY

Both for the variable mass of 100 grains (M100) and for grain productivity, it was observed that there were no significant differences between the doses of BRs, however there was a significant effect of the N supply (biological or mineral) to the cowpea plants. (Figures 4A and 4B). Cowpea showed a higher M100 value when cultivated with the application of mineral N or with inoculation with A. brasilense, compared to the condition without N supply. Ferreira et al. (2021) also did not observe an effect from the application of mineral N in two common bean cultivars. Rocha et al. (2021) found that the application of A. brasilense to black beans also did not provide a significant effect on M100.

![Figure 4](image.png)

**Figure 4.** Mass of one hundred grains - M100 (A) and grain productivity (B) of cowpea with application of doses of brassinosteroids (BRs) and N supply (No N - SN, biological N - NB and mineral N - NM). Means followed by identical letters in the bars do not show a significant difference according to the Tukey test (p> 0.05).

Foliar application of the phytohormone BRs did not provide an increase in the M100 of cowpea, presenting an average value of 29.48 g (Figure 4A). For cowpea grain productivity, it was observed that there was a significant effect between the forms of N supply, but not the application of BRs (Figure 4B). Plants without N supply showed lower productivity (989 kg/ha)
compared to plants inoculated with *A. brasilense* (1098 kg/ha) and those fertilized with mineral N (1340 kg/ha). *A. brasilense*, similarly to the application of mineral N (Figure 4B). The cowpea productivity values recorded in the present study are considered high, when compared to the averages of Capitão Poço (800 kg/ha), Pará (769 kg/ha) and Brazil (763 kg/ha) (IBGE, 2021). Factors such as rainfall, irrigation, cultivar (BR3-Tracuateua), growing season and fertilization and liming practices may have contributed to the higher crop productivity rates under the experiment's growing conditions.

Also evaluating inoculation of cowpea cultivars with Bradyrhizobium sp. and supply of mineral N in the edaphoclimatic conditions of Capitão Poço, Costa et al. (2021) observed that the absence of soil management practices, such as fertilization and liming, reduced the crop's grain productivity. Additionally, in the second year of cultivation of BRS Marataoã and BRS Tapaihum, the aforementioned authors reported average yields of 1,177 and 1,270 kg/ha, respectively, in the condition without N supply and when inoculating the seeds with rhizobia. Correcting soil acidity through liming increases the availability of nutrients to plants, as well as the efficiency of fertilizers, and thus creates ideal conditions for crop development (Caires & Joris, 2016).

4 CONCLUSIONS

1. The inoculation of seeds with *Azospirillum brasilense*, the supply of mineral nitrogen and the application of the phytohormone Brassinosteroides, do not provide greater growth, biomass production and reproductive development of the cowpea cultivar BR3-Tracuateua plants.

2. The inoculation of seeds with *Azospirillum brasilense* and the supply of mineral nitrogen increase the grain productivity of the crop.

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