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Bradyrhizobium japonicum and phosphorus fertilization on *Vigna unguiculata* (L.) walp.

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ABSTRACT

This study aimed to estimate the association of P_2O_5 doses with and without rhizobia inoculation to maximize the agronomic performance and yield of cowpea on an Oxisol in the Cerrado region of Tocantins. The experiments were carried out in two periods during the 2014/2015 growing season at the Experimental Farm of Gurupi belonging to the Federal University of Tocantins, using the variety BRS Novaera. Inoculation was carried out with a standard strain recommended for cowpea. The experimental designs were randomized in blocks in a 2×6 factorial arrangement, with the first factor consisting of the inoculation (presence and absence) and the second factor consisting of six phosphorus doses (0, 30, 60, 90, 120, and 150 kg ha^{-1} of P_2O_5), totaling 12 treatments with four replications. Total chlorophyll, leaf P content, flowering, hundred-grain weight, and yield were evaluated. Phosphorus fertilization had a positive influence on the total chlorophyll, flowering, and leaf P content of cowpea on the Oxisol in the Cerrado region of Tocantins. The application of 113.42 kg ha^{-1} of P_2O_5 provided a yield of 1,124 kg ha^{-1} in the first planting period, corresponding to an 83% increase. However, phosphorus fertilization and inoculation increased grain yield in the second period, with the maximum yield reaching 145.94 kg ha^{-1}, obtained with the maximum effect dose of 123.04 kg ha^{-1} of P_2O_5, with a yield gain of 257.3%.

Keywords: Cowpea, Doses P_2O_5, Rhizobia, Productivity.

Bradyrhizobium japonicum and phosphate fertilization in *Vigna unguiculata* (l.) walp.

RESUMO

Objetivou-se estimar a combinação de doses de P_2O_5 com e sem inoculação de rizóbio, para maximizar o desempenho agronômico e a produtividade do feijão-caupi em um Latossolo da região do cerrado Tocantinense. Os experimentos foram conduzidos em duas épocas, na safra do ano agrícola 2014/15 na Fazenda Experimental de Gurupi pertencente à Universidade Federal do Tocantins, com a variedade BRS Novaera. A inoculação foi com estirpe padrão recomendada para a cultura do feijão-caupi. Os delineamentos experimentais foram blocos casualizados, em arranjo fatorial (2 x 6) sendo o primeiro fator (presença e ausência inoculação) e o segundo fator seis doses de fósforo (0, 30, 60, 90, 120 e 150 kg ha^{-1} de P_2O_5), totalizando 12 tratamentos, com quatro repetições. As características avaliadas foram clorofila total, teor foliar de P, florescimento, massa de cem grãos e produtividade. A adubação fosfatada teve influência positiva nas variáveis: clorofila total, florescimento e teor foliar de P de feijão-caupi em Latossolo, no cerrado do Tocantins. Na primeira época de plantio, a aplicação de 113,42 kg ha^{-1} de P_2O_5 proporcionou produtividade de 1.124 kg ha^{-1}, valor correspondente à 83% de acréscimo. Na segunda época, a adubação com fósforo e a inoculação aumentou a produtividade de grãos, sendo a máxima produtividade 145,94 kg ha^{-1} obtida com a dose de máximo efeito de 123,04 kg ha^{-1} de P_2O_5, com ganho de produtividade de 257,3%.

Palavras-chave: Feijão-caupi, Doses P_2O_5, Rizóbio, Produtividade.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) stands out for its socioeconomic importance for low-income families living in the North and Northeast regions of Brazil. It is a high nutritional value food due to its high protein content, surpassing the common bean (*Phaseolus vulgaris*), in addition to generating employment and income (Fernandes et al., 2013).

The Brazilian production of cowpea in 2019/2020 reached approximately 718 thousand tons, accounting for 20.8% of the total bean production, and occupied an area of 387.5 thousand hectares, with a mean yield of 550 kg ha^{-1} (CONAB, 2020).

Bezerra et al. (2014) studied cowpea growth characteristics under the residual effect of phosphorus fertilization and found a requirement for phosphorus levels higher than 160 kg ha^{-1} to achieve maximum growth.

However, Cerrado soils are naturally deficient in phosphorus and have high acidity, but these characteristics are not necessarily factors that prevent bean cultivation and the increase in grain yield when using phosphorus-efficient cultivars, adequate phosphorus fertilization, and soil acidity correction (Fidelis et al., 2017).

Thus, phosphorus fertilization has been studied with great emphasis because this nutrient participates in various physiological processes in plants and helps in the nodulation, consequently optimizing the process of biological nitrogen fixation (BNF) (Silva et al., 2019).

Araújo (2019) evaluated the effects of inoculation with *Bradyrhizobium* sp. and *Azospirillum brasilense* in association with phosphorus fertilization on the morphological and production components of cowpea and observed significant effects of the interaction between bacteria and phosphorus dose.

Considering the importance of phosphorus fertilization and biological nitrogen fixation on the cowpea crop, which has shown good acceptance of low-cost technologies, it is necessary to develop studies to reach efficiency levels equal to or close to what occur in the case of soybean and the elaboration of a recommendation for phosphorus fertilization for this region.

In this sense, this study aimed to estimate the association of P_2O_5 doses with and without rhizobia inoculation to maximize the agronomic performance and yield of cowpea on an Oxisol in the Cerrado region of Tocantins.

2. Material and Methods

The experiments were conducted in two planting seasons at the Experimental Farm of Gurupi of the Federal University of Tocantins (UFT), located in Gurupi, State of Tocantins, Brazil, at the geographic coordinates 11°43′ S and 49°15′ W and an altitude of 300 m, on a soil classified as an Oxisol (Santos et al., 2018). Planting in two seasons was carried out to observe the possible repetition of the effects of treatments at the same planting site. The local climate is a humid tropical climate with a small water deficit (B1wA′a′) according to the Thornthwaite classification, with mean temperatures of 26.14 and 26.25 °C, relative humidity values of 81.08%, and precipitations of 164.88 and 148.8 mm in the first and second experiment in the 2014/2015 agricultural year, respectively, according to the UFT weather station on the campus of Gurupi, with precipitation data provided by INMET.

A soil chemical and particle size analysis at a depth of 0–20 cm was carried out in the experimental area approximately 30 days before planting and presented the following results: pH in $CaCl_2$ of 5.1, H+Al of 3.40 $cmol_c$ dm^{-3}, Ca of 2.2 $cmol_c$ dm^{-3}, Mg of 0.7 $cmol_c$ dm^{-3}, K of 0.10 $cmol_c$ dm^{-3}, S of 2 mg dm^{-3}, P (Mehlich I) of 0.5 mg dm^{-3}, CEC of 6.40 $cmol_c$ dm^{-3}, base saturation of 47%, organic matter of 2.4 dag kg^{-1}, clay of 285 g kg^{-1}, of silt 37 g kg^{-1}, and sand of 678 g kg^{-1}. Liming was carried out by applying 3 t ha^{-1} of dolomitic limestone with 6–10% MgO and 80–85% relative neutralizing power by the base saturation method (Ca+Mg+K). The limestone was applied to the soil and incorporated into the 0–20 cm layer.

Conventional soil tillage was carried out using a harrow (25″) and a leveling harrow (16″), with subsequent soil furrowing. The experimental unit consisted of four rows of 5 m in length, with an inter-row spacing of 0.50 m, totaling an area of 10 m^2. The data on production components were obtained from the two central rows, with a useful area of 4.0 m^2, excluding borders, formed by the external rows and half a meter from the ends of the central rows. Manual sowing practices were carried out in November 2014 and January 2015 using the cultivar BRS Novaera under the presence and absence of inoculation with the INPA 03-11B rhizobia strain, characterized as *Bradyrhizobium japonicum*, identified by the 16S sequencing, and obtained at the Laboratory of Applied Agromicrobiology and Biotechnology of the Graduate Program in Crop Production of UFT. After growth in YMA medium (yeast extract, mannitol, and agar) for five days, it was suspended in saline solution (0.2% $MgSO_4$) added to the seeds at a concentration of 10^9 cells mL^{-1} one hour before sowing and using 50 mL kg^{-1}.

The experimental designs were randomized in blocks in a 2×6 factorial arrangement, with the first factor consisting of the inoculation (presence and absence) and the second factor consisting of six phosphorus doses (0, 30, 60, 90, 120, and 150 kg ha^{-1} of P_2O_5), totaling 12 treatments with four replications in the first planting season (November 2014) and three replications in the planting season (January 2015). Moreover, 60 kg ha^{-1} K_2O with potassium chloride as a source and 20 kg ha^{-1} of N with urea as a source were added at planting.

Topdressing fertilizations were carried out at 15 and 30 days after emergence (DAE), each with 30 kg ha^{-1} of N only for treatments that did not receive inoculation and treatments with a dose of 0 kg ha^{-1} of P_2O_5.

Crop management consisted of manual weeding whenever necessary. Water supply for the crop was carried out through rainwater and/or complementary irrigation when necessary.

The emerged plants were thinned from the 12th day after sowing, leaving 10 plants per linear meter and a population of 200,000 plants ha^{-1}.

Readings of the total chlorophyll content (CHLOR) on cowpea leaves at full bloom were always carried out in the morning using a clorofiLOG® CFL 1030 chlorophyll meter. The measurement units, called Falker chlorophyll index (FCI), are products of photodiodes that emit at 635, 660, and 880 nm. The reading value assigned to the plot was represented by the mean of 5 trifoliate leaves per plot. The chlorophyll meter readings avoided sampling very old or very young leaves, that is, it was carried out using new, fully expanded leaves. After selecting the leaf to be sampled (3rd or 4th complete trifoliate leaf from the plant apex), the reading was carried out in at same place on each leaf (middle portion of the leaf).

Thirty trifoliate leaves were collected per plot to determine the leaf P content (dag kg^{-1}) (LPC) at full bloom (45 DAE). Sampling was carried out in the middle third of the plants in the useful area of the plots. These samples of plant material from the leaves were placed in paper bags, dried in a forced-air circulation oven at 65 °C, ground, weighed, and subjected to sulfuric digestion to determine the P content by colorimetry using the molybdenum blue method, with ascorbic acid as a reducing agent (Braga and Defelipo, 1974).

Flowering (FLO) was evaluated when the plants reached 50% flowering. Harvest was carried out at stage R5 (90% maturity of pods) and more than once per plot.

Subsequently, the hundred-grain weight (100W) was determined in the useful area by weighing and correcting the moisture to 13%, and the grain yield (YIELD) was determined by the grain weight of the useful area in kilograms, corrected for 13% moisture, transforming the data into kg ha^{-1}. The means of flowering, total chlorophyll, hundred-grain weight, and grain yield were transformed using square root in the first experiment. In addition, flowering and hundred-grain weight were transformed using square root and grain yield was transformed using log base 10 y in the second experiment.

The data were subjected to analysis of variance using the statistical program SISVAR (Ferreira, 2019) by the F-test. Regressions were generated using the software SigmaPlot 14.0 by adjusting to the beta values and the R^2 value.

3. Results and Discussion

The analysis of variance (Table 1) for the isolated behavior of the source of variation P doses showed that all evaluated characteristics were significant at 1% probability by the F-test. The source of variation inoculation (Table 1), separately, showed a significance at the 1% probability level by the F-test only for hundred-grain weight and grain yield.

The analysis of variance (Table 1) showed that leaf phosphorus content, hundred-grain weight, and grain yield had a significant effect on the interaction between P doses and inoculation at 1% probability by the F-test. P_2O_5 application had a significant effect on the total chlorophyll content (Figure 1A). The quadratic regression model showed that the maximum technical efficiency for total chlorophyll content (63.57 FCI) resulted from the application of 115.5 kg ha^{-1} of P_2O_5. Therefore, the increase in P_2O_5 doses leads to a positive influence relative to the total chlorophyll content.

Leaf phosphorus content (0.37 dag kg^{-1}) increased by 61% compared to the mean value of 0.23 dag kg^{-1} of the control without inoculation and 68% compared to the inoculated control (0.22 dag kg^{-1}) (Figure 2A). Silva et al. (2019) observed that a dose of 90 kg ha^{-1} of P_2O_5 presented the best differentiation regarding the effect of strains, while 0 kg ha^{-1} of P_2O_5 had the lowest means for most strains, demonstrating the synergistic effect of phosphorus fertilization on the strains to increase the shoot dry mass. The highest estimated mean was conditioned by the strain INPA 03-11B, with a value of 1.75 g at a dose of 107 kg ha^{-1} of P_2O_5. The estimated mean for the control was 0.36 g for the same dose, and the bacteria was superior to the control treatment at approximately 5 times when using the same rhizobium strain as that used in our study, thus corroborating, in general, the data obtained in thhis study.

According to Taiz et al. (2017), as nutrient availability and absorption continue to increase, a point is reached at which further nutrient addition is no longer related to increases in growth or yield but is reflected only in increased concentrations in the tissues. The hundred-grain weight showed a significant effect at 1% probability for the applied P_2O_5 doses. The regression equation modelthat best fit the applied P_2O_5 doses was the quadratic regression in the presence of inoculation. P doses increased the variable up to the point of maximum effect, with the maximum hundred-grain weight (28.75g) estimated with the dose of 97.67 kg ha^{-1} of P_2O_5 (Figure 2B).

Table 1. Summary of the analysis of variance for total chlorophyll (CHLOR), leaf P content (LPC), flowering (FLO), hundred-grain weight (100W), and grain yield (YIELD) of cowpea submitted to phosphorus doses with and without inoculation in the first planting season (November 2014), Gurupi, State of Tocantins, Brazil.

Source of variation	DF	p ($0.01 \leq p \leq 0.05$)				
		CHLOR	LPC	FLO	100W	YIELD
P doses (D)	5	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**
Inoculation (I)	1	0.6801^{ns}	0.2858^{ns}	0.5441^{ns}	0.0042**	0.0000**
(D × I)	5	0.0974^{ns}	0.0023**	0.9948^{ns}	0.0000**	0.0000**
Block	3	0.2670	0.9213	0.0603	0.4166	0.3600
Residual	33	–	–	–	–	–
CV (%)		1.12	9.09	2.06	1.83	0.31
Mean		7.76	0.32	7.48	5.21	30.11

Not significant; ** significant at $P \leq 0.01$ and * significant at $P \leq 0.05$ by the F-test.

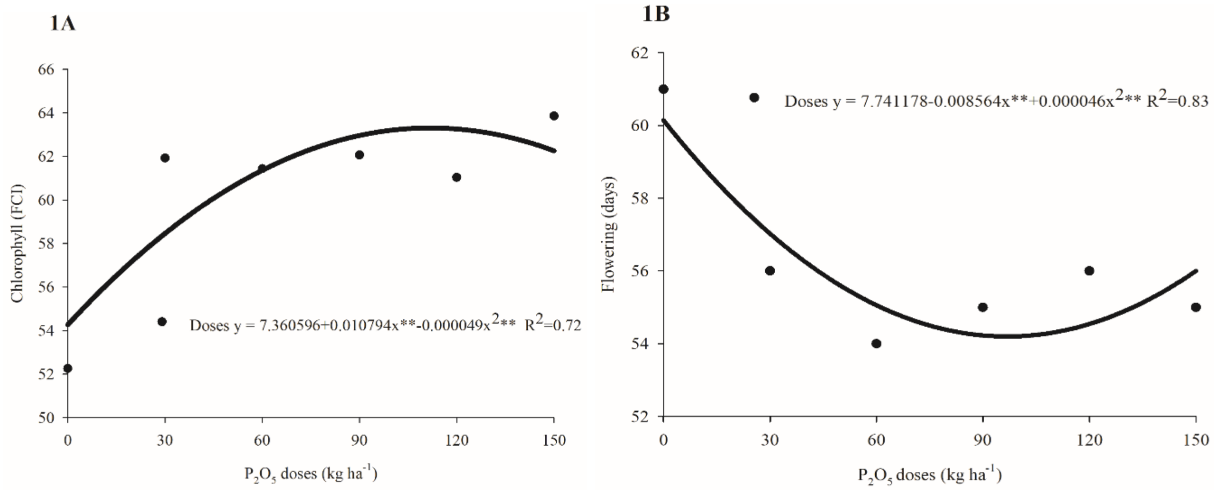

Figure 1. Regression analysis for total chlorophyll (A) and flowering (B) of BRS Novaera cowpea as a function of phosphorus doses in the first planting of the 2014/2015 growing season.

The quadratic regression model had the best fit in the absence of inoculation, with a reduction up to the dose of the minimum effect of 95.75 kg ha^{-1} of P_2O_5, resulting in a value of 26.25 g. The maximum grain weight (28.07 g) was found in the control without inoculation (0 kg ha^{-1} of P_2O_5). Santos et al. (2019) reported different results when evaluating the efficiency of rhizobium strains and phosphorus doses in cowpea cultivars. The authors found that the estimated hundred-grain weight for the maximum agronomic efficiency of 13.72 g was obtained with the dose of 101.5 kg ha^{-1} of P_2O_5 for the strain INPA 03-11B. There was also an isolated effect of the P_2O_5 dose with the maximum agronomic efficiency of 19.95 g obtained at the dose of 84.6 kg ha^{-1} for the control treatment without bacteria (BRS Sempre Verde).

The statistical analysis showed significant effects at the 1% probability level for grain yield among the studied treatments (Table 1). The best fit was observed for the quadratic polynomial regression in both inoculation factors (with × without) as a function of P doses. Grain yield in the presence of inoculation obtained a maximum agronomic efficiency of 1,574.65 kg ha^{-1} at the dose of maximum effect of 149.26 kg ha^{-1} of P_2O_5, with an increase of 521.3% when compared to the control treatment, whose grain yield was 302.06 kg ha^{-1}. Thus, the phosphorus doses used associated with inoculation provided maximum yield. On the other hand, the maximum grain yield in the absence of inoculation was 1,124.82 kg ha^{-1} (113.42 kg ha^{-1} of P_2O_5), with an increase of 82.8% compared to the control treatment, whose grain yield was 615.19 kg ha^{-1} (Figure 2C), showing the expressive effect of using phosphorus fertilization for this variety on an Oxisol in the Cerrado of the State of Tocantins. The trend to increase yield when applied at higher doses possibly occurred because phosphorus is a basic component of several molecules, such as ATP, DNA, and RNA, influencing plant growth and development (Taiz et al., 2017). Moreover, grain yield was much higher than the Brazilian mean of 550 kg ha^{-1} in the 2019/2020 growing season (CONAB, 2020).

P doses make symbiosis more efficient, maybe because phosphorus assists root development and nodule formation, thus increasing the sites of infection for the rhizobium, making nitrogen available to the plant, absorbing more phosphorus, improving the plant nutrition, and resulting in higher yield. (Rocha et al. 2018b).

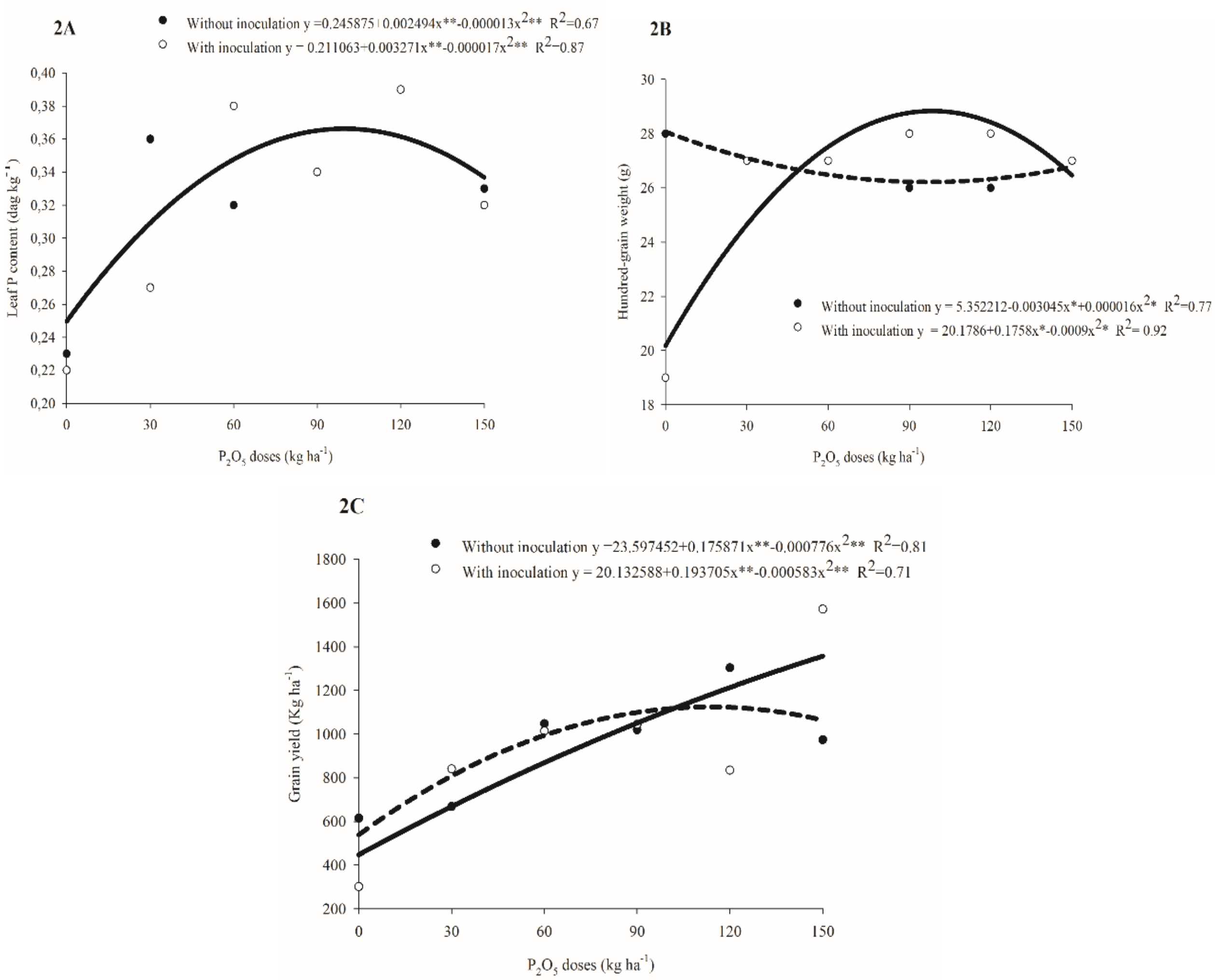

Figure 2. Regression analysis for leaf phosphorus content (A), hundred-grain weight (B), and grain yield (C) of BRS Novaera cowpea as a function of the interaction between phosphorus doses and inoculation in the first planting of the 2014/2015 growing season.

In the experiment 1, Rocha et al. (2018a) observed a linear response ($p<0.05$) for grain yield using the variety BRS Novaera for the tested P_2O_5 doses. However, these results corroborate with those found in the present study regarding the maximum grain yield (1,411 kg ha^{-1}) obtained with the estimated dose of 150 kg ha^{-1} of P_2O_5 and was very close to the yield observed in this experiment (1,574.65 kg ha^{-1}).

In the experiment 2, analysis of variance (Table 2) showed that all evaluated characteristics were significant at 1% probability by the F-test for the isolated behavior of the source of variation P doses. The source of variation inoculation (Table 2), separately, showed significance at the 1% probability level by the F-test for almost all characteristics, except for leaf phosphorus content.

The analysis of variance (Table 2) showed that only leaf phosphorus content and hundred-grain weight did not present a significant effect of the interaction between P doses and inoculation at 1% probability by the F-test. The maximum leaf phosphorus content was 0.39 dag kg^{-1}, obtained with the estimated maximum effect dose of 135.30 kg ha^{-1} of P_2O_5 (Figure 3A). As expected, the lowest value was observed at a dose of 0 kg ha^{-1} of P_2O_5 (0.24 dag kg^{-1}), with an increase of 62.5% compared to the control treatment, showing that the absence of phosphorus is a limiting factor to leaf phosphorus content. The linear polynomial model presented the best fit to the behavior of the variable leaf phosphorus content as a function of P doses.

Silva et al. (2014) evaluated the residual effect of phosphorus fertilization in three successive cowpea cultivations on an Entisol and found the highest values for leaf P content in the first cultivation (4.43 g kg^{-1}). On the contrary, the third cultivation presented the lowest leaf P concentration (1.56 g kg^{-1}). The reason for these reductions was probably due to a higher absorption by cowpea as a function of higher P contents in the soil during the first and second cultivations, that is, 41 and 49 mg dm^{-1}, respectively. The hundred-grain weight (Figure 3B) presented a quadratic response to P_2O_5 application, with a minimum technical efficiency dose of 64.46 kg ha^{-1} of P_2O_5, showing a value of 14.65 g.

Table 2. Summary of analysis of variance for total chlorophyll (CHLOR), leaf P content (LPC), flowering (FLO), hundred-grain weight (100W), and grain yield (YIELD) of cowpea submitted to five phosphorus doses with and without inoculation in the second planting season, Gurupi, State of Tocantins, Brazil, 2015.

Source of variation	DF	p (0,01≤ p ≤0,05)				
		CHLOR	LPC	FLO	100W	YIELD
P doses (D)	5	0.0000**	0.0005**	0.0000**	0.0000**	0.0000**
Inoculation (I)	1	0.0000**	0.3081^{ns}	0.0000**	0.0003**	0.0000**
(D × I)	5	0.0000**	0.7541^{ns}	0.0000**	0.1628^{ns}	0.0000**
Block	2	0.9802	0.3140	0.0106	0.0635	0.1289
Residual	22	–	–	–	–	–
CV (%)		1.32	21.95	0.58	2.13	0.34
Mean		48.85	0.32	7.23	3.92	2.03

Not significant; ** significant at P ≤ 0.01 and * significant at P ≤ 0.05 by the F-test.

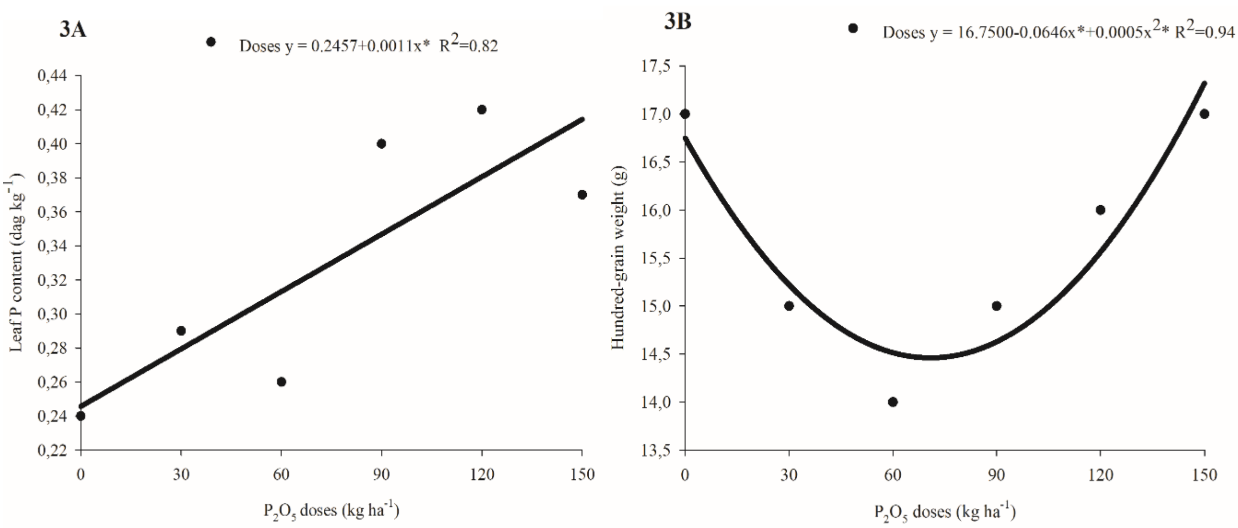

Figure 3. Regression analysis for leaf P content (A) and hundred-grain weight (B) of BRS Novaera cowpea as a function of phosphorus doses in the second planting of the 2014/2015 growing season.

Table 3. Mean of hundred-grain weight (100W) for the isolated effect of the variable inoculation.

Inoculation	100W (g)*
Without	3.98 a
With	3.86 b

Means followed by the same uppercase letter in the column do not differ statistically from each other at the 5% probability level by the F-test. *Mean transformed into the square root.

Our results corroborate with those found by Chagas Junior et al. (2010), who evaluated the agronomic efficiency using the shoot, root, and nodule biomass accumulation, number and biomass of nodules, relative efficiency, and yield of three cowpea cultivars under field conditions. Authors also observed the occurrence of spontaneous nodulation and positive results of inoculation with rhizobium strains under field conditions.

The data regarding chlorophyll content of cowpea plants (Figure 4A) grown in the presence of inoculation fit the linear regression model, with a value of 45.8 FCI on average. Maximum technical efficiency was observed with the dose of 144.57 kg ha^{-1} of P_2O_5 (47.74 FCI), with an increase of 15.9% relative to the control (0 kg ha^{-1} P_2O_5). On the other hand, plants grown in the absence of inoculation presented data fit to the quadratic regression model, with an increase in estimation from 48.29 to 54.06 FCI when increasing the effect of level from 0 to 74 .25 kg ha^{-1} P_2O_5, corresponding to an increase of 11.9% in the leaf chlorophyll content of cowpea.

Our results corroborate those found by Silva et al. (2016), who analyzed the variable chlorophyll content and observed a significant difference with adjustment to a linear regression model. The authors concluded that fertilization with reactive phosphate rock promoted a positive influence on the chlorophyll content of pigeon pea cv. Fava Larga on the Oxisol of the Cerrado biome.

Benício et al. (2012) found a similar result when evaluating the effect of different biofertilizers on cowpea nodulation. On the one hand, the results indicate the positive relationship of phosphorus fertilization on the action of chlorophyll molecules; otherwise, they indicate that it is not necessary to use high doses for these molecules to be expressed.

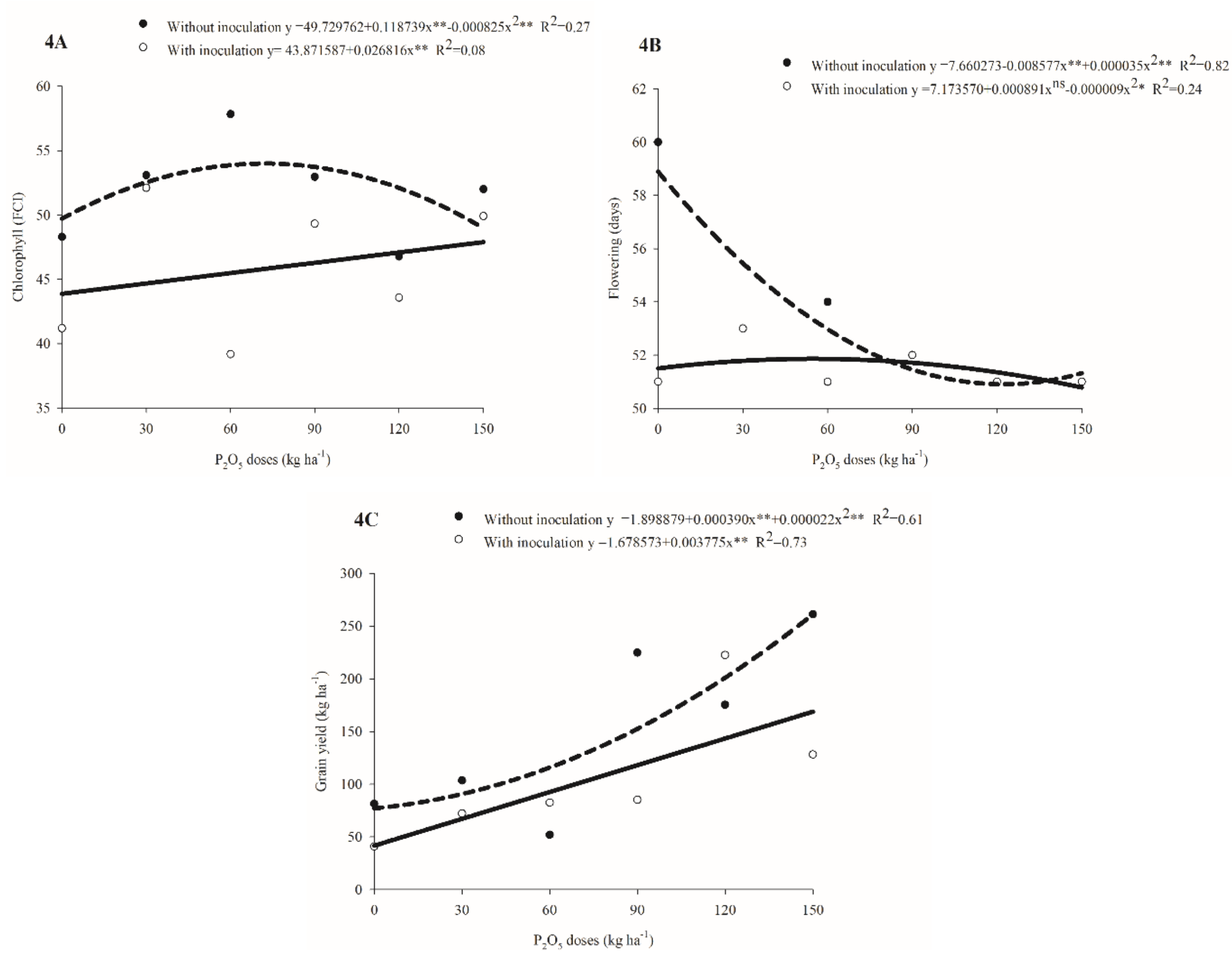

Figure 4. Regression analysis for total chlorophyll (A), flowering (B), and grain yield (C) of BRS Novaera cowpea as a function of the interaction between phosphorus doses and inoculation in the second planting of the 2014/2015 growing season.

Both control treatments of variables with and without inoculation provided lower values than the other treatments with P_2O_5 doses. Nascimento et al. (2012) studying changes in chlorophyll levels in cowpea plants grown under different nitrogen sources and found similar results.

The minimum time for the estimated flowering of plants was 50.5 days when analyzing the significant effect on flowering of cowpea plants as a function of phosphorus and inoculation doses, both being adjusted to the quadratic regression model (Figure 4B). The dose of 130.8 kg ha^{-1} of P_2O_5 promoted this minimum point (Figure 4B) in the absence of inoculation, showing a percentage time reduction of 15.8% relative to the control treatment. The dose with the highest technical efficiency was 65.5 kg ha^{-1} of P_2O_5 in the presence of inoculation, taking 52.5 days to flowering, that is, higher P doses are required to reduce the time for flowering of cowpea in the absence of inoculation, while the time for flowering can be reduced even at low P contents in the presence of inoculation.

Grain yield showed a linear response to phosphorus doses in the presence of inoculation (Figure 4C), similar to the statistical model obtained for leaf phosphorus content (Figure 3A). Thus, phosphorus application had a positive effect on grain yield, ranging from 40.8 kg ha^{-1} (0 kg ha^{-1} of P_2O_5) to 145.94 kg ha^{-1}, obtained with the maximum effect dose of 123.04 kg ha^{-1} of P_2O_5, indicating a yield gain of 257.3%. The adjustment in the absence of inoculation was performed to the quadratic polynomial model and maximum technical efficiency was observed with the dose of 94.58 kg ha^{-1} of P_2O_5, with an estimated maximum yield of 159.48 kg ha^{-1} of grains, demonstrating a 96% increase. The increase in yield is achieved only with a phosphorus supply at quantities compatible with the crop demand (Resende et al., 2006). Thus, applications of higher phosphorus doses are important for the maximum expression of grain yield for the cowpea crop under the studied conditions.

Plants with P and inoculation showed a favored root development and nodule formation, that is, sites of rhizobium infection, making nitrogen available to the plant through BNF, which may have affected grain yield. This same effect may have occurred for non-inoculated plants, as several types of native rhizobia that can also promote nodulation of legumes can be found in the soil. According to Soares et al. (2006), nodulating nitrogen-fixing bacteria in legumes (rhizobia) are microorganisms present and usually abundant in soils of many ecosystems. Rhizobia have high diversity and wide variability regarding symbiotic efficiency. Pereira Júnior et al. (2015) estimated the best association of recommended N and P2O5 doses, as well as the critical N and P levels in the plant for the maximum economic production of irrigated cowpea and found similar results. The authors also observed that the increase in nitrogen and phosphorus doses applied to the soil for cowpea increased the nitrogen and phosphorus contents in the leaves and the grain yield.

4. Conclusions

Phosphorus fertilization had a positive influence on the total chlorophyll, flowering, and leaf P content of cowpea on an Oxisol in the Cerrado region of Tocantins.

Phosphorus fertilization of 102.42 kg ha^{-1} of P_2O_5 alone reduced the time to flowering to 53.7 days. The association of 97.67 kg ha^{-1} of P_2O_5 and inoculation with rhizobia increased grain weight and leaf P content.

The application of 113.42 kg ha^{-1} of P_2O_5 in the first planting period provided a yield of 1,124 kg ha^{-1} of grains, corresponding to an 83% increase.The dose of 64.46 kg ha^{-1} of P_2O_5 reduced the grain weight by 13% in the second season.

The results of the second season show that phosphorus fertilization and inoculation increased grain yield, with the maximum grain yield of 145.94 kg ha^{-1} obtained with the maximum effect dose of 123.04 kg ha^{-1} of P_2O_5, with a yield gain of 257.3%.

Authors' Contribution

Weslany Silva Rocha contributed to the installation of the experiment, data collection, statistical analysis and writing of the article. Ana Paula Ribeiro Barros contributed to the implementation of the experiment and data collection. Marcelo Alves Ribeiro contributed to the implementation of the experiment and data collection.

Albert Lennon Lima Martins contributed to the article submission and translation of the article. Aloísio Freitas Chagas Júnior was the first author's co-advisor, contributed to the statistical analysis and advice on the writing and correction of the article. Manoel Mota dos Santos was the first author's advisor, contributing to the statistical analysis and advising on the writing and correction of the article.

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Bibliographic References

Araújo, E.O. 2019. *Bradyrhizobium sp.*, *Azospirillum brasilense* e adubação fosfatada na cultura do feijão-caupi cultivado em ambiente amazônico. Enciclopédia Biosfera, 16(29), 577. DOI: http://dx.doi.org/10.18677/EnciBio_2019A43.

Benício, L.P.F., Oliveira, V.A., Reis, A.F.B., Chagas Júnior, A.F., Lima, S.O. 2012. Efeitos de diferentes biofertilizantes e modos de aplicação na nodulação do feijão caupi. Revista Tropica: Ciências Agrárias e Biológicas, 6(3), 111-119. DOI: http://dx.doi.org/10.0000/rtcab.v6i3.503

Bezerra, M.A.F., Oliveira, F.A., Bezerra, F.T.C., Pereira, W.E., Silva, S.A. 2014. Cultivo de feijão-caupi em latossolos sob o efeito residual da adubação fosfatada. Revista Caatinga, 27(1), 109-115. https://periodicos.ufersa.edu.br/index.php/caatinga/article/view/2816/pdf_92. (acessado 10 de fevereiro de 2021).

Braga, J.M., Defelipo, B.V. 1974. Spectrophotometric determination of phosphorus with soil and plant extract. Revista Ceres, 21(113), 73-85. DOI:https://doi.org/10.1021/i560101a038

CONAB. COMPANHIA NACIONAL DE ABASTECIMENTO, 2020. Acompanhamento de Safra Brasileira, Safra 2019/2020. Brasília, Ministério da Agricultura, Pecuária e Abastecimento. https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos/item/download/32327_b81d579517d7bc89b15b72e841195ebf. (acessado em 02 de janeiro de 2021)

Coutinho, P.W.R., Silva, D.M.S., Saldanha, E.C.M., Okumura, R.S., Silva Júnior, M.L. 2014. Doses de fósforo na cultura do feijão-caupi na região nordeste do Estado do Pará. Revista Agro@mbiente, 8(1), 66-73. DOI: http://dx.doi.org/10.18227/1982-8470ragro.v8i1.1310

Chagas Junior, A.F., Rahmeier, W., Fidelis, R.R., Santos, G.R., Chagas, L.F.B. 2010. Eficiência agronômica de estirpes de rizóbio inoculadas em feijão-caupi no Cerrado, Gurupi-TO. Revista Ciência Agronômica. 41(4), 709-714. DOI: https://doi.org/10.1590/S1806-66902010000400027

Fernandes, A.R., Fonseca, M.R., Braz, A.M.S. 2013. Produtividade de feijão-caupi em função da calagem e fósforo. Revista Caatinga, 26(4), 54-62. https://periodicos.ufersa.edu.br/index.php/caatinga/article/view/2770/pdf_70. (acessado: 09 de dezembro de 2020).

Ferreira, D.F. 2019. Sisvar: a computer analysis system to fixed effects split plot type designs. Revista Brasileira de Biometria, 37(4), 529-535. DOI: https://doi.org/10.28951/rbb.v37i4.450

Fidelis, R.R., Tavares, T.C.O., Sousa, S.A., Santos, G.R., Souza, P.B. 2017. Comportamento, eficiência e resposta à adubação de fósforo em feijão comum. Tecnologia & Ciência Agropecuária, 11(5), 1-7. https://revistatca.pb.gov.br/edicoes/volume-11-2017/v-11-n-5-dezembro-2017/01-comportamento-eficiencia-e-resposta-a-adubacao-em-feijao.pdf. (acessado: 05 de novembro de 2020)

Nascimento, R., Nascimento, D.A.M., Silva, D.A., Alves, A.G. 2012. Alterações nos teores de clorofilas em plantas de feijão-caupi cultivadas sob diferentes fontes de nitrogênio. Revista Educação Agrícola Superior, 27(2), 94-96. DOI: http://dx.doi.org/10.12722/0101-756X.v27n02a03.

Nascimento, S.P., Bastos, E.A., Araújo, E.C.E., Freire Filho, F.R., Silva, E.M. 2011. Tolerância ao déficit hídrico em genótipos de feijão-caupi. Revista Brasileira de Engenharia Agrícola e Ambiental, 15(8), 853-860. DOI: https://doi.org/10.1590/S1415-43662011000800013

Pereira Júnior, E.B., Oliveira, F.H.T., Oliveira, F.T., Silva, G.F., Hafle, O.M., Silva, A.R.C. 2015. Adubação nitrogenada e fosfatada na cultura do feijão-caupi irrigado no município de Sousa - PB. Global Science Technology, 8(1), 110-121. DOI: http://dx.doi.org/10.14688/1984-3801/gst.v8n1p110-121

Resende, A.V., Neto, A.E.F., Alves, V.M.C., Muniz, J.A., Curi, N., Lago, F.J. 2006. Resposta do milho a fontes e modos de aplicação de fósforo durante três cultivos sucessivos em solo da região do cerrado. Ciência e Agrotecnologia, 30(3), 458-466. DOI: https://doi.org/10.1590/S1413-70542006000300011

Rocha, W.S., Santos, M.G., Oliveira, N.S., Barros, H.B., Santos, M.M. 2018a. Desenvolvimento do feijão-caupi em sistema de várzea submetido à doses de fósforo. Pesquisa Aplicada & Agrotecnologia, 11(3), 93-100. DOI: ttps://doi.org/10.5935/PAeT.V11.N3.09

Rocha, W.S., Santos, M.G., Sakai, T.R.P., Silva, T.A., Fidelis, R.R., Santos, M.M. 2018b. Acúmulo de biomassa em função de doses de fósforo e inoculação de rizóbio em feijão-caupi. Cultura Agronômica, 27(2), 273-286. DOI: https://doi.org/10.32929/2446-8355.2018v27n2p273-286

Santos, H.G., Jacomine, P.K.T., Anjos, L.H.C., Oliveira, V.A., Lumbreras, J.F., Coelho, M.R., Almeida, J.A., Araujo Filho, J.C., Oliveira, J.B., Cunha, T.J.F. 2018. Sistema Brasileiro de Classificação de Solos, quinta ed. Brasília, Embrapa, 356 p. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/199517/1/SiBCS-2018-ISBN-9788570358004.pdf. (acessado 22 janeiro de 2021).

Santos, M.M., Brito, V.F., Silva, E.M., Santos, M.G., Barros, H.B., Lopes, M.B.S. 2019. Produtividade de feijoeiro-caupi cultivado com estirpes rizobianas e doses de fósforo. Scientia Agraria Paranaensis, 18(3), 209-217. DOI: https://doi.org/10.18188/sap.v18i3.21812

Silva, E.M., Santos, M.M., Lopes, M.B.S., Fidelis, R.R., Rocha, W.S., Chagas Júnior, A.F. 2019. Eficiência de rizóbios sob doses de fósforo na cultura do Feijão-caupi. Revista Brasileira de Agropecuária Sustentável, 9(2), 67-77. DOI: https://doi.org/10.21206/rbas.v9i2.6415

Silva, E.M.B., Oliveira, J.R., Anicesio, E.C., Silva, T.J.A. 2016. Teor de clorofila e desenvolvimento de feijão guandu adubado com fosfato natural reativo em Latossolo do Cerrado. Revista Agrarian, 9(33), 248-253. https://ojs.ufgd.edu.br/index.php/agrarian/article/view/3965/3663. (acessado 17 março de 2020).

Silva, J.A., Oliveira, A.P., Moura, M.F., Silva,J.A.,Araújo,M.A.M. 2014. Efeito residual da adubação fosfatada em três cultivos sucessivos com feijão-caupi. Revista Caatinga, 27(4), 31-38. https://periodicos.ufersa.edu.br/index.php/caatinga/article/view/2864/pdf_168. (acessado 22 março de 2020).

Soares, A.L.L., Pereira, J.P.A.R., Ferreira, P.A.A., Do Vale, H.M.M., Lima, A.S., Andrade, M.J.B., Moreira, F.M.S. 2006. Eficiência agronômica de rizóbios selecionados e diversidade de populações nativas nodulíferas em Perdões (MG). Revista Brasileira de Ciência do Solo, 30(5), 795-802. DOI: https://doi.org/10.1590/S0100-06832006000500005

Taiz, L., Zeiger, E., Moller, I.M., Murphy, A., 2017. Fisiologia e desenvolvimento vegetal. sexta ed. Porto Alegre, Artmed.

Zucareli, C., Ramos Júnior, E.U., Barreiro, A.P., Nakagawa, J.; Cavariani, C. 2006. Adubação fosfatada, componentes de produção, produtividade e qualidade fisiológica em sementes de feijão. Revista Brasileira de Sementes, 28(1), 5-15. DOI: https://doi.org/10.1590/S0101-31222006000100002