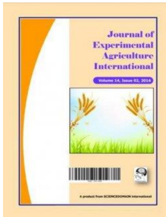


Journal of Experimental Agriculture International

23(5): 1-13, 2018; Article no.JEAI.42145
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Nutritional Status of Cowpea Plants Inoculated with *Bradyrhizobium* and *Azospirillum brasilense* in Associated with Phosphate Fertilization in Soil Amazonian

Érica de Oliveira Araújo[1*], Munir Mauad[2], Hugo César Tadeu[3], Heleno Alexandrino de Lima Filho[1], José Augusto Figueira da Silva[1] and José Avelino Cardoso[1]

[1]Federal Institute of Education, Science and Technology, Amazonas State, Brazil.
[2]Faculty of Agricultural Sciences, Federal University of Grande Dourados, Rod Dourados/Itahum, km 12, University City, 79804-970, Dourados, MS, Brazil.
[3]Federal University of Amazonas, Av. General Rodrigo Octávio, 6200, 69080-900, Manaus, AM, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author EOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MM and HCT managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2018/42145
Editor(s):
(1) Lanzhuang Chen, Professor, Laboratory of Plant Biotechnology, Faculty of Environment and Horticulture, Minami Kyushu University, Miyazaki, Japan.
Reviewers:
(1) Clint Magill, Texas A&M University, USA.
(2) V. Vasanthabharathi, Annamalai University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/24992

Original Research Article

Received 22nd March 2018
Accepted 30th May 2018
Published 5th June 2018

ABSTRACT

Biological nitrogen fixation (BNF) plays an important role in cowpea cultivation. Thus, the present study aimed to evaluate the effects of inoculation and co-inoculation of rhizobacteria associated with phosphate fertilization on biometric parameters and absorption and use of nutrients by different cowpea genotypes. The experimental design was randomized blocks in a 4x3x2 factorial scheme,

*Corresponding author: E-mail: ericabb25@hotmail.com;

corresponding to four rates of phosphorus (0, 80, 120 and 160 kg ha^{-1} of P_2O_5), absence, inoculation and co-inoculation with rhizobacteria; two cowpea genotypes (White and Butter), with four replicates. The results revealed variability between the cowpea genotypes with respect to efficiency and response to phosphorus application. Cowpea plants inoculated with *Bradyrhizobium sp*. showed increments of approximately 22.66%, 23.58%, 55.31%, 47.95%, 65.66% and 20%, respectively, in N contents in the roots, leaves, shoots and in the plant, N absorption efficiency and N use efficiency. Inoculation of *Bradyrhizobium sp.* associated with 120 and 160 kg ha^{-1} of P_2O_5 increases plant height and P and S contents in the shoots of cowpea plants. Increased P rates lead to increment in dry matter production and N, P and K contents in cowpea plants. Coinoculation of *Bradyrhizobium sp.* and A*zospirillum brasilense* did not positively influence any of the variables studied over the effects of *Bradyrhizobium sp.* alone. Cowpea has potential to be used as green fertilizer after a short period of cultivation, due to the accumulation of nutrients in its leaves and shoots.

Keywords: Vigna unguiculata; phosphorus; rhizobacteria; symbiosis; nutritional status.

1. INTRODUCTION

The area planted with cowpea (*Vigna unguiculata* (L.) Walp) in the world is 10.4 million hectares and is mainly located in tropical and subtropical regions of America, Asia and Africa. The global production of cowpea grains is about 5.5 million tons, and Nigeria is the largest producer in the world [1]. In Brazil, cowpea cultivation is estimated to be approximately 1.442 million hectares, with mean yield of about 496 kg ha^{-1} [2], which allows the crop to be among the main leguminous crops cultivated in the country, predominantly in the North and Northeast regions, which grew 1.192 million hectares of cowpea in the 2017/18 season [2]. Nevertheless, low yields have been observed in production areas and one of the main causes is the low availability of nutrients in the soil, particularly due to the insufficient supply of nitrogen (N) and phosphorus (P) [3]. In tropical soils, the application of N and phosphate fertilizers is essential to obtain adequate yields for most crops of economic interest [4].

The cowpea crop, through symbiosis with bacteria from the genera *Bradyrhizobium* and *Azospirillum*, can obtain N through biological fixation of N_2 (BNF), which is one of the ways to increase the yield of leguminous plants, avoiding costs of soluble N fertilizers [5]. BNF is known to be efficient in cowpea, which can reach high yields when well nodulated [6]. P is fundamental for cowpea yield because it stimulates growth, initiates the formation of nodules and influences the efficiency of rhizobium-plant symbiosis [7]. Studies conducted by [4] revealed high potential for response to phosphate fertilization by the cowpea crop in soils characterized by low P availability, significantly contributing to the increase in vegetative growth, nodulation and grain yield. [5] observed that *Bradyrhizobium sp*. inoculation increases dry matter production and N and P absorption efficiency in cowpea plants. [8] report that cowpea responded significantly to inoculation, with increments in its biometric parameters to or higher than those obtained with N fertilization. N_2 fixation efficiency depends on P availability, due to its participation in the symbioti[c process [9].

Obtaining higher efficiency in N use has been the goal of both capitalized and low-input agriculture. In this sense, for high productivity of cowpea beans, in addition to the use of phosphate fertilization and selecting efficient genotypes, the use of symbiotic of rhizobacteria can enable greater economic returns, inoculation witch rhizobacteria thaus become a fundamental alternative in the substitution in whole or in part of nitrogenous fertilizers and better use of available phosphorus. And, although the application of N_2-fixing bacteria in cowpea is promising, scarce work on use of phosphate fertilization in association with rhizobacteria for culture in the northern region.

Given the above, the present study aimed to evaluate the effects of inoculation and co-inoculation of rhizobacteria associated with phosphate fertilization on biometric parameters and absorption and use of nutrients by different cowpea genotypes.

2. MATERIALS AND METHODS

The experiment was carried out from October 2017 to January 2018 in the experimental area of the Federal Institute of Education, Science and Technology of Amazonas, Campus of Lábrea, municipality of Lábrea, AM, Brazil, at geographic coordinates 7° 15' 18.02'' S and 64° 46' 58'' W, with altitude of 65 m, in a dystrophic Red Yellow Latosol. According to Köppen's classification, the climate of the region is Am, hot and humid tropical with two well-defined seasons. Mean data of temperature and rainfall along the experiment were obtained from the database of the National Institute of Meteorology (INMET).

Soil chemical analysis in the 0-20 cm layer, carried out in the area before the experiment, resulted in the following values: O.M.: 14.2 g dm^{-3}; pH ($CaCl_2$): 5.7; P: 12 mg dm^{-3}; K: 0.8 $mmol_c$ dm^{-3}; Ca: 128 $mmol_c$ dm^{-3}; Mg: 40 $mmol_c$ dm^{-3}; Al: 50 $mmol_c$ dm^{-3}; H+Al: 168 $mmol_c$ dm^{-3}; SB: 336.8 $mmol_c$ dm^{-3}; CEC: 168.8 $mmol_c$ dm^{-3}, base saturation: 50.11%. Granulometric analysis showed 463 g dm^{-3} of clay, 423 g dm^{-3} of sand and 114 g dm^{-3} of silt. Soil correction was performed thirty days before sowing, considering soil analysis results, to increase base saturation to 60%.

The experimental design was randomized blocks in 4x3x2 factorial scheme, corresponding to four P rates; absence, inoculation and co-inoculation with rhizobacteria; and two cowpea genotypes (White and Butter), with four replicates. P rates consisted of 0, 80, 120 and 160 kg ha^{-1} of P_2O_5, applied in the planting furrow. The source of P_2O_5 was single superphosphate (21% P_2O_5). Basal fertilization consisted of N and K, using 30 kg ha^{-1} of N and 60 kg ha^{-1} of K_2O, in the form of urea (45% N) and potassium chloride (56% K_2O), respectively, incorporated to the soil.

Cowpea seeds were inoculated with the commercial inoculant Total Nitro Feijão-Caupi (concentration of 10^9 cells g^{-1}), which contains strains of *Bradyrhizobium sp.* (Semia 6462 and Semia 6463), in the peat formulation. The applied dose was 100 g of the peat inoculant for 50 kg of seeds. Co-inoculation was performed using the commercial product, containing a combination of *Azospirillum brasilense* strains in inoculant with liquid formulation. The applied dose was 150 mL for 50 kg of seeds. Co-inoculation was performed by using combinations of different microorganisms, in mixed inoculation. Both inoculants were produced and provided by the company Total Biotecnologia®.

Soil tillage consisted in harrowing (disc harrow) to 15 cm depth. Planting and fertilization furrows were manually opened using a hoe, with depths between 5 and 10 cm. Sowing was performed manually using a hand-held planter known as 'matraca', by placing four seeds per hole, leaving five plants per linear meter after thinning. Each experimental unit was composed of four 3-m-long rows spaced by 0.50 m between rows and 0.20 m between plants. The two central rows, disregarding 0.5 m on each end, were used for evaluations. At 10 and 30 days after emergence (DAE), an insecticide was applied with the active ingredients Imidacloprid 100 g/L and Beta-cyfluthrin 12.5 g/L, from the chemical groups Neonicotinoid (Imidacloprid) and Pyrethroid (Beta-cyfluthrin), recommended for the cowpea crop at dose of 750-1000 mL/ha to control *Diabrotica speciosa*.

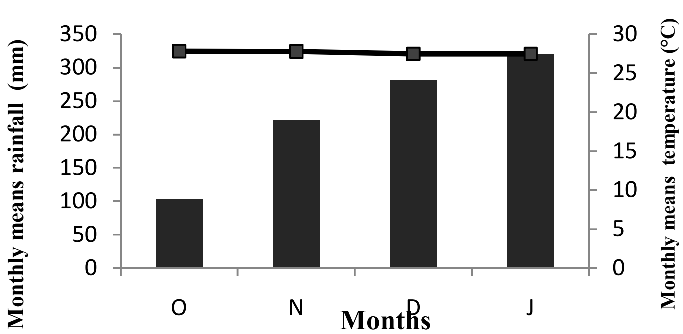


Fig. 1. Monthly means of rainfall (mm) and temperature (°C) recorded at the meteorological station of the National Institute of Meteorology from October to January in the 2017/18 agricultural year

At 45 DAE, at full flowering, 20 trifoliate leaves were collected per plot in the middle third of the plants to determine the leaf contents of N, P, K, Ca, Mg and S. All plant material collected was washed in running water and deionized water, and the samples were stored in paper bags, dried in a forced-air oven at 65ºC for 72 hours and then ground. Ground samples were subjected to sulfuric digestion and nitric-perchloric digestion, using the methodology described in [10]. In this same phenological stage, the following parameters were determined: plant height, measured from the base to the apical meristem using a tape measure; stem diameter, measured 2 cm above the base of the plant with a digital caliper; and number of leaves, obtained by counting.

At 60 DAE, shoot and root dry matter production and contents of macronutrients in shoots and roots were determined. Plant dry matter was determined by drying the samples in a forced-air oven at temperature of 65ºC for 72 hours, and the data were expressed in g/plant. After drying, the dry matter was weighed and ground in Wiley mill and the samples were subjected to sulfuric digestion and nitric-perchloric digestion to determine the contents of macronutrients, according to the methodology described in [10]. In this same period, plant roots were collected and washed in running water, and the nodules were counted.

Indices of nutrient absorption efficiency, ratio between total content of the nutrient in the plant and root dry matter, was calculated according to [11], whereas the indices of nutrient use, ratio between total dry matter produced and total accumulation of nutrient in the plant, were calculated according to [12].

The data were subjected to normality test (Shapiro Wilk) and analysis of variance using the statistical analysis program Sisvar. Effects between inoculation and co-inoculation, for each genotype, were evaluated by Scott-Knott test at 0.05 probability level. For variables with statistical significance as a function of P rates, regression analysis was used.

3. RESULTS AND DISCUSSION

The interaction between genotypes (G) and rates (D) had significant effect on plant height, number of nodules, S content in the leaves at full flowering and S content in the shoots of cowpea plants (Tables 1 and 2). On the other hand, the interaction between genotype (G) and bacteria (B) influenced the number of nodules, N content in the plant, N absorption efficiency, contents of K, Ca and Mg in the leaves at full flowering, and contents of N, P, K, Mg and S in the shoots of cowpea plants (Tables 1 and 2). The interaction between rates (D) and bacteria (B) influenced only plant height, P content in the leaves at full flowering and S content in the shoots of cowpea plants (Tables 1 and 2). The other results showed no significant effects interaction and were independently presented for each genotype, rate and bacteria. There was no significance ($p \le 0.05$) of the triple interaction of treatments for any of the parameters evaluated (Tables 1 and 2).

The genotype White was statistically superior ($p \le 0.05$) to Butter with respect to plant height, stem diameter, number of leaves, number of nodules, root dry matter, shoot dry matter, total dry matter, N absorption efficiency, N content in the roots, N content in the shoots, N content in the plant, and contents of N, P and K in the leaves at full flowering (Tables 3 and 4). Opposite results were found for the other variables, in which the contents of Ca, Mg and S in the leaves at full flowering and the contents of Ca, Mg and S in the shoots of the cowpea genotype Butter were higher than those of the genotype White (Table 4). [4] and [7] observed significant difference between cowpea genotypes with respect to plant height, leaf area, number of leaves and dry matter production, demonstrating that growth and development were different between the genotypes.

N contents in the roots, shoots and in the plant for the genotype White were higher than those for Butter, demonstrating that the former is more efficient in N absorption (Tables 3 and 4), which can be related to the higher number of viable nodules. N absorption by the cowpea genotypes ranged from 32.83 and 18.30 mg of dry weight per g of N absorbed. [3] also oserved the different responses between cowpea genotypes regarding growth and contents of nutrients in the shoots. This fact is usually attributed to the existence of nutritional behavior differentiated by genetic factors of adaptability.

Inoculation with rhizobacteria influenced the number of nodules, N contents in the roots, leaves, shoots and in the plant, N absorption efficiency, N use efficiency, K content in the leaves, and P and Ca contents in the shoots of cowpea plants (Table 5). The number of nodules in the inoculated treatment was 52.26 nodules/plant, which was more than 100% higher compared with the control and did not differ statistically from the co-inoculated treatment (*Bradyrhizobium sp.* + *Azospirillum brasilense)* (Table 5 and Fig. 2). These values for the number of nodules demonstrate a satisfactory inoculation in both cowpea genotypes, suggesting that plants with higher number and mass of nodules fix more N.

Plants inoculated with *Bradyrhizobium sp*. showed increments of about 22.66%, 23.58%, 55.31%, 47.95%, 65.66% and 20%, respectively, in the N contents in the roots, leaves, shoots and plant, N absorption efficiency and N use efficiency, not differing statistically from the co-inoculated treatment (*Bradyrhizobium sp.* + *Azospirillum brasilense)* (Table 5). Corroborating with the results found, [5] observed that inoculation with *Bradyrhizobium sp.* in the presence of humic acids influenced the number of nodules, N contents in roots and plant, and N absorption efficiency by cowpea plants cultivated in the North region of Brazil; and [13] found increased number of nodules and N content in cowpea plants cultivated under field conditions in Tanzania. Thus, it has been demonstrated that the population of rhizobia inoculated and indicated for cowpea is able to assume a symbiotic relationship to provide the N necessary for crop development, and that the inoculant was effective in the process of biological fixation because it promoted greater N fixation by roots and plant, when compared with the control treatment (not inoculated), presenting itself as a viable alternative for N fertilization in the crop.

In addition, co-inoculation (*Bradyrhizobium sp.* + *Azospirillum brasilense*) did not increase N and P accumulation in cowpea plants, not differing statistically ($p \leq 0.05$) from the treatment inoculated with *Bradyrhizobium sp.* (Table 5), which suggests that this treatment has somehow helped native rhizobia fix more N in symbiosis with the cowpea crop. Inoculation of only one bacterium in the seed promotes greater potential of the inoculum, i.e., greater amount of bacteria is added to the soil through the seed. Regarding N absorption and use efficiency, as well as K accumulation in the leaves and Ca accumulation in the shoots, the co-inoculated treatment did not differ from the control (not inoculated and not fertilized) (Table 5).

P rates had a significant ($p \leq 0.05$) increasing linea effect on plant height, stem diameter, number of leaves, shoot dry matter and total dry matter (Fig. 3A, 3B, 3C, 3E and 3F). This demonstrates that this crop is highly efficient in absorbing soil-P, originating from phosphate fertilization. Higher P availability may trigger changes in photosynthesis, due to greater capture of solar radiation and increase in the production of photoassimilates, because P acts as an ATP molecule-forming agent and, under conditions of low ATP production, plant growth is directly affected [14]. In the present study, soil P content considering the percentage of clay was equal to 12 mg dm^{-3}, which is regarded as low, justifying the increasing linear response of the production variables for shoot dry matter and total dry matter as a function of increasing P_2O_5 doses applied at sowing.

The production of viable nodules responded quadratically to the increase in P rates, with maximum production at the P_2O_5 rates of 120 kg ha^{-1}, 53.16 nodules/plant, slightly at higher rates (Fig. 3D). This occurs because P also reduces available ATP and consequently available and carbohydrates to rhizobium bacteria, thus helping the development of the nodules, up to a certain limit of fertilization [14]. Above this level of fertilization, the plant no longer gains from providing substrates for symbiotic bacteria availability of nutrients in the soil for plant development, thus is assumed to results in reducing the number of nodules produced. Thus, besides being important for nodule formation, P influences the rhizobium-plant symbiosis efficiency, hence reinforcing N fixation [15]. Similar results were reported by [5,4,7,13,16], who found significant increase in vegetative growth and number of nodules of cowpea plants in response to phosphate fertilization with the lowest result was observed in the treatment of 0 kg ha^{-1} of P_2O_5. Despite the limited amount of this nutrient in the soil, nodule development was still possible (27.33 nodules/plant) most likely because of the rhizobium capacity to solubilize non-labile phosphate from the soil and release it to the plant.

The cowpea crop has low demand for P but it has shown increased growth response when cultivated in soil with good P availability. N contents in the shoots and in the plant responded quadratically to the increase in P rates, and maximum N contents in the shoots (46.24 g kg^{-1}) and in the plant (58.23 g kg^{-1}) were obtained at the rate of 80 kg ha^{-1} (Fig. 4A and 4B). [17] found leaf N contents in cowpea ranging from 30.51 to 35.01 g kg^{-1} in the vegetative stage and from 18.05 to 22.19 g kg^{-1} in the pod filling stage, indicating that young plants exhibit higher leaf contents of this macronutrient.

Table 1. F values, means and coefficients of variation for plant height (PH), stem diameter (SD), number of leaves (NLv), number of nodules (NN), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), nitrogen content in the roots (NR), nitrogen content in the plant (NP), nitrogen absorption efficiency (NAE) and nitrogen use efficiency (NUE) by cowpea genotypes inoculated and co-inoculated with rhizobacteria associated with phosphate fertilization. Lábrea, AM, Brazil (2018)

Source of variation	GL	Pr > F										
		PH	SD	MLv	NN	SDM	RDM	TDM	NR	NP	NAE	NUE
Genotypes (G)	1	0.00*	0.00*	0.78	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.12
Rate (D)	3	0.00*	0.00*	0.03*	0.00*	0.01*	0.20	0.03*	0.08	0.01*	0,16	0.18
Bacteria (B)	2	0.25	0.41	0.01*	0.00*	0.40	0.08	0.79	0.00*	0.00*	0.00*	0.04
G x D	3	0.00*	0.18	0.07	0.00*	0.34	0.25	0.16	0.26	0.46	0.63	0.56
G x B	2	0.49	0.74	0.12	0.00*	0.37	0.08	0.18	0.24	0.01*	0.00*	0.19
D x B	6	0.02*	0.16	0.06	0.25	0.08	0.21	0.18	0.08	0.22	0.11	0.87
G x D x B	6	0.09	0.90	0.21	0.06	0.53	0.81	0.95	0.08	0.18	0.43	0.70
Residue	72											
Average		58.03	7.66	21.93	43.09	23.41	10.00	33.41	36.43	53.20	255.68	0.65
CV (%)		18.60	17.13	20.97	23.82	20.76	21.71	28.66	11.29	22.92	26.53	26.91

** – significant by Skott and Knott at 5% probability. CV – coefficient of variation*

Table 2. F values, means and coefficients of variation for the contents of nitrogen (NL), phosphorus (PL), potassium (KL), calcium (CaL), magnesium (MgL) and sulfur (SL) in the leaves at full flowering, and contents of nitrogen (NS), phosphorus (PS), potassium (KS), calcium (CaS), magnesium (MgS) and sulfur (SS) in the shoots of cowpea genotypes inoculated and co-inoculated with rhizobacteria associated with phosphate fertilization. Lábrea, AM, Brazil (2018)

Source of variation	GL	Pr > F											
		NL	PL	KL	CaL	MgL	SL	NS	PS	KS	CaS	MgS	SS
Genotypes (G)	1	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.68	0.05	0.00*	0.02*	0.00*
Rate (D)	3	0.70	0.00*	0.02	0.00*	0.19	0.00*	0.03*	0.00*	0.46	0.79	0.97	0.31
Bacteria (B)	2	0.00*	0.00*	0.00*	0.09	0.14	0.14	0.00*	0.00*	0.64	0.00*	0.89	0.18
G x D	3	0.20	0.25	0.31	0.39	0.27	0.02*	0.55	0.43	0.70	0.32	0.31	0.04*
G x B	2	0.89	0.22	0.00*	0.04*	0.01*	0.06	0.00*	0.00*	0.01*	0.19	0.00*	0.04*
D x B	6	0.05	0.00*	0.30	0.83	0.14	0.03*	0.12	0.01*	0.88	0.95	0.64	0.27
G x D x B	6	0.51	0.70	0.66	0.32	0.75	0.45	0.08	0.60	0.62	0.36	0.99	0.59
Residue	72												
Average		25.78	2.17	18.95	15.80	4.43	2.50	41.90	2.48	20.58	10.30	4.63	3.73
CV (%)		18.10	17.58	16.28	20.55	16.39	22.72	24.95	16.64	21.75	27.44	15.88	20.45

** – significant by Skott and Knott at 5% probability. CV – coefficient of variation*

Table 3. Plant height (PH), stem diameter (SD), number of nodules (NN), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM) and nitrogen absorption efficiency (NAE) by cowpea genotypes. Lábrea, AM, Brazil (2018)

Genotypes	PH	SD	NN	SDM	RDM	TDM	NAE
	(cm)	(mm)	(un.)		(g)		(mg g^{-1})
White	67.43 a	8.39 a	77.75 a	27.75 a	15.54 a	41.30 a	32.83 a
Butter	48.62 b	6.93 b	8.47 b	21.07 b	5.46 b	26.53 b	18.30 b

Medium followed by the same letter in the column, do not differ statistically by Scott and Knott at 5% probability

Table 4. Nitrogen content in the roots (NR), nitrogen content in the shoots (NS), nitrogen content in the plant (NP), contents of nitrogen (NL), phosphorus (PL), potassium (KL), calcium (CaL), magnesium (MgL) and sulfur (SL) in the leaves at full flowering, and contents of calcium (CaS), magnesium (MgS) and sulfur (SS) in the shoots of cowpea genotypes. Lábrea, AM, Brazil (2018)

Genotypes	NR	NS	NP	NL	PL	KL
			(g kg^{-1})			
White	13.59 a	46.43 a	60.03 a	28.06 a	2.33 a	20.94 a
Butter	8.99 b	37.38 b	46.37 b	23.50 b	2.00 b	16.96 b
Genotypes	CaL	MgL	SL	CaS	MgS	SS
			(g kg^{-1})			
White	13.49 b	4.03 b	2.03 b	8.02 b	4.46 b	4.02 b
Butter	18.11 a	4.82 a	2.98 a	12.58 a	4.81 a	4.44 a

Medium followed by the same letter in the column, do not differ statistically by Scott and Knott at 5% probability

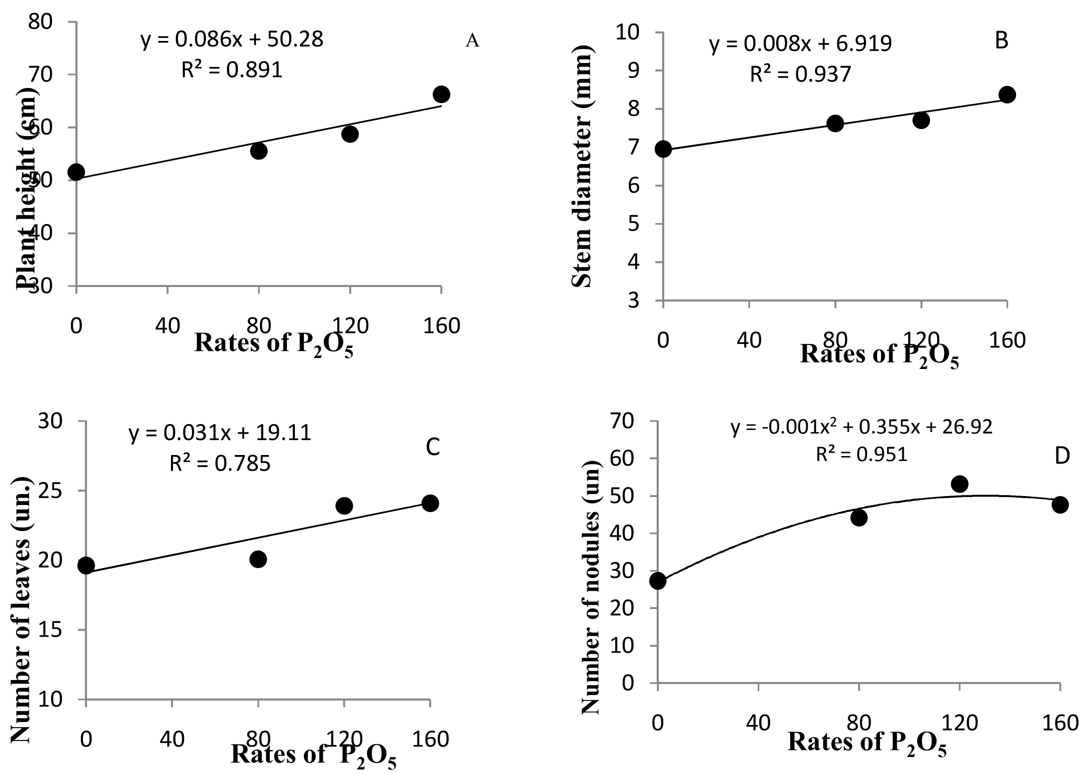
Table 5. Number of nodules (NN), nitrogen content in the roots (NR), nitrogen content in the leaves at full flowering (NL), nitrogen content in the shoots (NS), nitrogen content in the plant (NP), nitrogen absorption efficiency (NAE), nitrogen use efficiency (NUE), potassium content in the leaves at full flowering (KL), phosphorus content in the shoots (PS) and calcium content in the shoots (CaS) of cowpea plants inoculated (*Bradyrhizobium sp.)* and co-inoculated (*Bradyrhizobium sp.* + *Azospirillum brasilense).* Lábrea, AM, Brazil (2018)

Bacteria	NN	NR	NL	NS	NP
	un.			(g kg^{-1})	
Not inoculated	23.75 b	9.31 b	23.36 b	32.00 b	41.31 b
Inoculated	52.26 a	11.42 a	28.87 a	49.70 a	61.12 a
Co-inoculated	53.33 a	13.25 a	25.00 a	43.30 a	56.55 a
Bacteria	NAE	NUE	KL	PS	CaS
	mg g^{-1}			(g kg^{-1})	
Not inoculated	164.54 b	0.60 b	17.97 b	2.28 b	8.81 b
Inoculated	272.59 a	0.72 a	20.60 a	2.64 a	12.61a
Co-inoculated	177.07 b	0.58 b	18.13 b	2.48 a	9.52 b

Medium followed by the same letter in the column, do not differ statistically by Scott and Knott at 5% probability

On the other hand, P contents in the shoots and leaves and K and S contents in the leaves of cowpea plants were linearly influenced by P rates (Fig. 4C, 4D, 4E and 4F). Thus, highest P contents in the shoots and leaves and highest K and S contents in the leaves were obtained with the application of 160 kg ha^{-1} of P_2O_5, higher than the recommended rate for maintenance fertilization of cowpea in Amazonian soils. These contents are within the range considered as adequate by the literature for cowpea, which is from 2.6 to 5.0 g kg^{-1} of P, 14.4 to 17.8 g kg^{-1} of K, and from 1.5 to 2.5 g kg^{-1} of S. Similar P contents in cowpea leaves were observed by [18], who obtained maximum P content of 2.8 g kg^{-1} in broadcast application, using doses that varied from 0 to 160 kg ha^{-1} of P_2O_5. Increase of P content in cowpea leaves in response to P rates was also observed by [19], studying P doses and forms of application in Yellow Latosol in the Roraima state. Such increase in leaf P content with the application of phosphate fertilization may explain a future increase in grain production observed when the crop is until this phenological stage, considering that there are reports in the literature on the close correlation between leaf contents of nutrients and yield of crops [5].

Fig. 2. Root morphologies of cowpea plants inoculated with *Bradyrhizobium* sp. compared with the absolute control. A. Not inoculated and not fertilized; B. Inoculated with Semia 6462 and Semia 6463



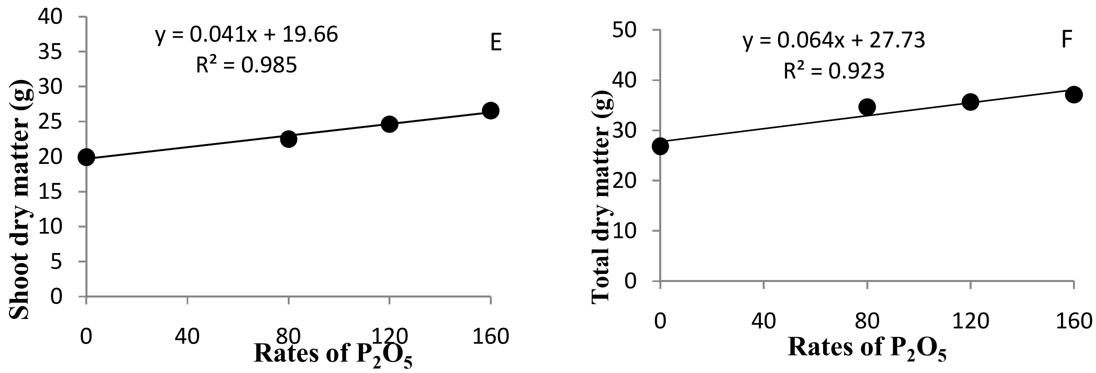
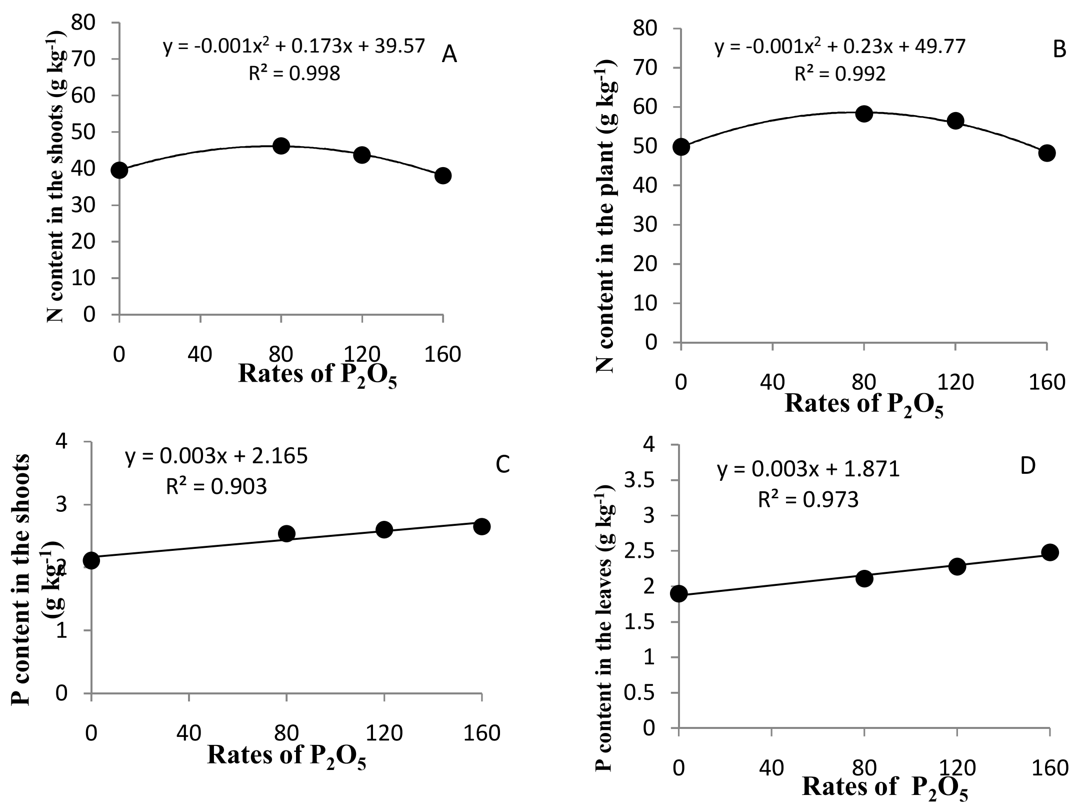
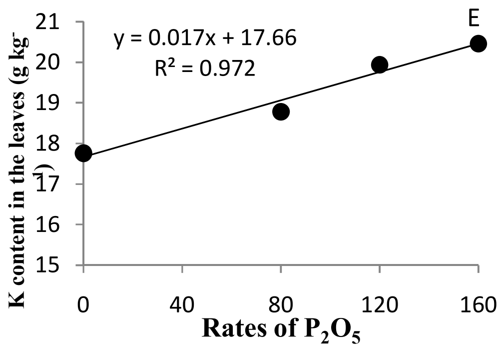


Fig. 3. Characteristic investigation of cowpea plants in response to increasing rates of phosphorus. A. Plant height; B. Stem diameter; C. Number of leaves; D. Number of nodules; E. Shoot dry matter; F. Total dry matter

There was clear interaction between cowpea genotypes (G) and P rates (D) on plant height, number of nodules and S content in the shoots. The genotype White, in the absence of phosphate fertilization, had lowest values of plant height, number of nodules and S accumulation in the shoots, statistically differing ($p \leq 0.05$) from the genotype Butter (Table 6). In addition, increasing P rates led to significant difference ($p \leq 0.05$) only for plant height and contents of P and S in the shoots of the genotype White, while not influencing the genotype Butter (Table 6). [20,21] observed a significant variation in the growth of cowpea varieties in response to P rates.





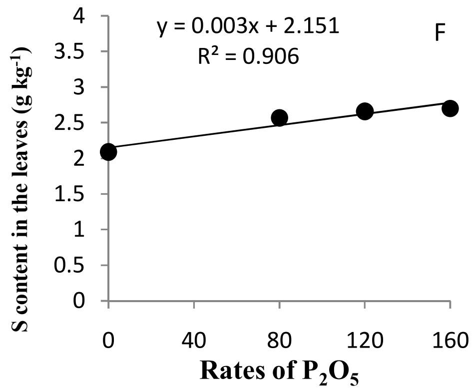


Fig. 4. Effects of several elements in different organs of cowpea plants in response to increasing rates of phosphorus. A. N content in the shoots; B. N content in the plant; C. P content in the shoots; D. P content in the leaves; E. K content in the leaves; F. S content in the leaves

Table 6. Plant height, number of nodules and sulfur content in the shoots of cowpea genotypes in response to increasing rates of phosphorus. Lábrea, AM, Brazil (2018)

Genotypes	Rates de P_2O_5 (kg ha^{-1})			
	0	80	120	160
	Plant height (cm)			
White	54.19 aC	67.67 aB	68.31 aB	79.57 aA
Butter	48.93 bA	43.40 bA	49.20 bA	52.97 bA
	Number of nodules (un.)			
White	49.66 aC	82.58 aB	95.75 aA	83.00 aB
Butter	5.00 bA	5.83 bA	10.88 bA	12.33 bA
	Sulfur content in the shoots (g kg^{-1})			
White	2.34 aB	3.13 aA	3.39 aA	3.05 aA
Butter	1.83 bA	2.01 bA	1.94 bA	2.34 bA

The lowercase letters separate averages in each column and the upper case letters separate the medium in the rows. Same letters do not differ by Skott and Knott at 5% probability

There was significant interaction between bacteria (B) and P rates (D) for plant height and contents of P and S in the shoots of cowpea plants (Table 7). Inoculation of *Bradyrhizobium sp.* associated with 160 kg ha^{-1} of P_2O_5 led to increase of about 17.75% in plant height, in comparison to the control not inoculated. However, compared with the control not fertilized, this increase was higher than 63% (Table 7). P and S contents in the shoots of cowpea plants positively responded to the inoculation of *Bradyrhizobium sp.* associated with 120 and 160 kg ha^{-1} of P_2O_5, statistically differing from the treatment not inoculated (Table 7).

Regarding P rates, significant difference ($p \leq 0.05$) was observed only in the inoculated treatment + 0 kg ha^{-1} of P_2O_5 and not inoculated treatment + 0 kg ha^{-1} of P_2O_5 (Table 7). It should be highlighted that inoculation of *Bradyrhizobium sp.* in the presence of P_2O_5 rates of 80, 120 and 160 kg ha^{-1} proved to be efficient for the plant-bacteria system when compared with co-inoculation and with control (not fertilized and not inoculated). Thus, the effect of inoculation was higher when associated with P rates, with significant difference between treatments co-inoculated (*Bradyrhizobium sp.* + A*zospirillum brasilense*) and not inoculated.

The interaction between genotype (G) and bacteria (B) influenced the number of nodules, N contents in the shoots and in the plant, N absorption efficiency, contents of P, K and Mg in the shoots and contents of K and Ca in the leaves of cowpea at full flowering (Table 8). The genotype White was superior to the genotype Butter with respect to nodule production, N contents in the shoots and in the plant, and P content in the shoots when inoculated with the strain of *Bradyrhizobium sp.*, statistically differing ($p \leq 0.05$) from the co-inoculated treatment

(*Bradyrhizobium sp.* + A*zospirillum brasilense)* and from the control (not inoculated and not fertilized) with respect to nodule production and contents of N and P in the shoots (Table 8). There are reports that certain cowpea genotypes have higher capacity of nodulation and BNF efficiency, which indicates the possibility of optimizing the responses related to BNF by using increasingly more efficient genotypes. Opposite results were obtained for N absorption efficiency, K and Mg contents in the shoots and K and Ca contents in the leaves at full flowering, in which the genotype Butter was superior to the genotype White for all above-mentioned variables when inoculated with the strain of *Bradyrhizobium sp.*, statistically differing from the control (not inoculated and not fertilized) (Table 8). No significant difference (p≥0.05) was found between the co-inoculated treatment (*Bradyrhizobium sp.* + A*zospirillum brasilense*) and the control (not fertilized and not inoculated) for the genotypes White and Butter with respect to N contents in the shoots and in the plant, P, K and Mg contents in the shoots and Ca content in the leaves at full flowering (Table 8).

Table 7. Plant height, P content in the shoots and S content in the shoots of cowpea plants inoculated (*Bradyrhizobium sp.)* and co-inoculated (*Bradyrhizobium sp.* + Az*ospirillum brasilense*) associated with phosphate fertilization. Lábrea, AM, Brazil (2018)

Bacteria	Rates de P_2O_5 (kg ha^{-1})			
	0	80	120	160
	Plant height (cm)			
Not inoculated	44.75 bC	57.32 bB	56.71 bB	62.22 bA
Inoculated	57.52 aC	61.62 aB	61.55 aB	73.27 aA
Co-inoculated	48.17 bB	50.71 bB	57.81 bB	63.32 bA
	P content in the shoots (g kg^{-1})			
Not inoculated	1.85 aB	2.54 bA	2.50 bA	2.52 bA
Inoculated	2.33 aA	2.80 aA	3.00 aA	2.92 aA
Co-inoculated	2.09 aA	2.28 bA	2.38 bA	2.34 bA
	S content in the shoots (g kg^{-1})			
Not inoculated	1.93 aB	2.37 aB	2.31 bA	2.30 bA
Inoculated	2.19 aB	2.84 aA	2.93 aA	3.15 aA
Co-inoculated	2.22 aA	2.50 aA	2.75 aA	2.71 aA

The lowercase letters separate averages in each column and the upper case letters separate the medium in the rows. Same letters do not differ by Skott and Knott at 5% probability

Table 8. Number of nodules (NN), nitrogen content in the shoots (NS), nitrogen content in the plant (NP), nitrogen absorption efficiency (NAE), phosphorus content in the shoots (PS), potassium content in the shoots (KS), magnesium content in the shoots (MgS), potassium content in the leaves (KL) and calcium content in the leaves (CaL) of cowpea plants inoculated (*Bradyrhizobium sp.)* and co-inoculated (*Bradyrhizobium sp.* + Az*ospirillum brasilense).* Lábrea, AM, Brazil (2018)

Bacteria	Genotypes		Genotypes		Genotypes	
	White	Butter	White	Butter	White	Butter
	NN (un.)		NS (g kg^{-1})		NP (g kg^{-1})	
Not inoculated	44.68 cA	5.23 bB	41.94 bA	37.67bA	57.25 aA	32.21 bB
Inoculated	99.29 aA	5.81bB	52.43 aA	46.96 aB	63.02 aA	54.97 aA
Co-inoculated	88.60 bA	5.08 bB	41.71 bA	41.29 aA	57.67 aA	50.84 aA
	NAE (mg g^{-1})		PS (g kg^{-1})		KS (g kg^{-1})	
Not inoculated	150.47 bB	314.90 bA	2.39 bA	2.34 aA	19.02 bB	21.05 bA
Inoculated	219.43 aB	408.49 aA	2.94 aA	2.38 aB	21.92 aB	23.19 aA
Co-inoculated	218.07 aB	324.99 bA	2.44 bA	2.13 aA	18.02 bB	20.28 bA
	MgS (g kg^{-1})		KL (g kg^{-1})		CaL (g kg^{-1})	
Not inoculated	3.96 bB	4.44 bA	15.55 bB	18.48 bA	11.95 bB	15.14 bA
Inoculated	4.15 aB	5.29 aA	18.09 aB	23.10 aA	15.00 aB	19.23 aA
Co-inoculated	3.98 bB	4.71 bA	17.46 aB	21.11 aA	13.62 bB	15.64 bA

The lowercase letters separate averages in each column and the upper case letters separate the medium in the rows. Same letters do not differ by Skott and Knott at 5% probability

The lack of significant effect for the use of *Azospirillum brasilense* on the parameters evaluated in the present study can be attributed to the efficiency of the bacterium *Bradyrhizobium sp.* when compared with the genus *Azospirillum*. For the inoculation with bacteria from the genus *Azospirillum* to be effective, these bacteria must be able to compete with native diazotrophic bacteria, because cowpea is a host plant that can be easily nodulated by the soil-native rhizobium, which could hamper plant nodulation by more efficient strains introduced by inoculation. In addition, in inoculum quality, the inoculation process is of fundamental importance to reach a high number of viable bacteria. Thus, there might have been competition between bacteria from the genus *Bradyrhizobium* or even of native species, avoiding the beneficial effect of *Azospirillum brasilense* on growth, development, nodulation and nutritional status of the cowpea crop under the studied conditions.

4. CONCLUSIONS

There is variability between the cowpea genotypes regarding efficiency and response to phosphorus application. The genotype White was superior with respect to growth and development, and more efficient in N absorption by roots, leaves, shoots and plant.

Cowpea plants inoculated with *Bradyrhizobium sp*. showed increments of approximately 22.66%, 23.58%, 55.31%, 47.95%, 65.66% and 20%, respectively, in N contents in the roots, leaves, shoots and in the plant, N absorption efficiency and N use efficiency.

Inoculation of *Bradyrhizobium sp.* associated with 120 and 160 kg ha^{-1} of P_2O_5 increases plant height and P and S contents in the shoots of cowpea plants.

Increasing P rates lead to increment in dry matter production and N, P and K contents in cowpea plants.

Coinoculation of *Bradyrhizobium sp.* and Az*ospirillum brasilense* did not positively influence any of the variables studied over the effects of *Bradyrhizobium sp.* alone.

Cowpea has potential to be used as green fertilizer in short period of cultivation, due to the accumulation of nutrients in its leaves and shoots.

ACKNOWLEDGEMENT

The authors are grateful to the Foundation of Agrarian Studies Luiz de Queiroz- FEALQ and Federal University of Dourados for making resources and opportunity to first author to attend the postdoc.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Food and Agriculture Organization - FAO FAOSTAT. Crops. Cow peas, dry. Available:http://faostat3.fao.org/home/index.html#DOWNLOAD (Acesso em: 08 ago. 2017)
2. Conab. Monitoring the Brazilian grain Harvest 2017/2018: Fifth Survey, February/2018, National Supply Company. Brasília, CONAB; 2018.
3. Araujo EO, Santos EF, Camacho MA. Nutritional efficiency of cowpea varieties in the absorption of phosphorus. Agron. Colombiana. 2012;30:419-424.
4. Karikari B, Arkorful E, Addy S. Growth, nodulation and yield response of cowpea to phosphorus fertilizer application in Ghana. Jour. of Agronomy. 2015;14:234-240.
5. Araujo EO, Gerola JG, Ferreira CRG, Matte LC. Effect of rates and sources of soluble phosphorus on the behavior of cowpea plants inoculated with rhizobacteria from soils of the north region of Brazil. African Jour. Microb. Research. 2017;11:1544-1550.
6. Rumjanek NG, Martins LMV, Xavier GR, Neves MCP. Biological nitrogen fixation. In: Freire Filho FR, Lima JAA, Ribeiro VQ. (Eds.) Cowpea: Technological Advances. Brasília: Embrapa; 2005.
7. Nkaa FA, Nwokeocha OW, Ihuoma O. Effect of phosphorus fertilizer on growth and yield of cowpea (*Vigna unguiculata*). IOSR Jour. of Pharmacy and Biol. Scie. 2014;9:74-82.
8. Guimarães SL, Cardinal MS, Silva EMB, Polizel AC. Development of cv. BRS Novaera cowpea inoculated with rhizobium recommended for pigeonpea. Científica. 2015;43:149–155.
9. Burity HA, Lyra MCCP, Souza ES. Effectiveness of inoculation with rhizobium

and fungi micorrízicos arbusculares in thrush seedlings subjected to different levels of phosphorus. Brazilian Pes. Agrop. 2000;35:801-807.
10. Empresa Brasileira de Pesquisa Agropecuária. Manual of chemical analysis of soils, plants and fertilizers. 2. Ed, Brasilia. Embrapa Information Technology; 2009.
11. Swider JM, Chyan Y, Freiji FG. Genotypic differences in nitrate uptake and utilization efficiency in pumpkin hybrids. Jour. of Plant Nutrition. 1994;17:1687-1699.
12. Siddiqi MY, Glass ADM. Utilization index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. Jour. of Plant Nutrition. 1981;4: 289-302.
13. Nyoki D, Patrick A, Ndakidemi R. Effects of phosphorus and *Bradyrhizobium japonicum* on growth and chlorophyll content of cowpea (*Vigna unguiculata* (L) Walp). American Jour. of Exper. Agric. 2014;40:1120-1136.
14. Rocha WS. Inoculation and rates of phosphorus in cowpea in the South of the State of Tocantins. Gurupi: UFT. Dissertation (master)-postgraduate course in agronomy, area of Concentration in Vegetable Production, Federal University of Tocantins, Gurupi. 2016;56.
15. Haruna IM, Aliyu L. Yield and economic returns of sesame (*Sesamum indicum* L.) as influenced by poultry manure, nitrogen and phosphorus at Samaru, Nigeria. Elixir Agric. 2011;39:4884-4887.
16. Nyoki D, Patrick A, Ndakidemi R. Economic benefits of *Bradyrhizobium japonicum* inoculation and phosphorus supplementation in cowpea (*Vigna unguiculata* (L.) Walp) grown in northern Tanzania. American J. of Research Comm. 2013;1:173-189.
17. Taffouo VD, Ngwene G, Akoa A, Franken P. Influence of phosphorus application and arbuscular mycorhizal inoculation on growth, foliar nitrogen mobilization, and phosphorus partitioning in cowpea plants. Mycorrhiza. 2014;24:363-368.
18. Sousa Rosal CJ. Rates of phosphorus and zinc on culture of cowpea. Jaboticabal: UNESP. Thesis (Doctoral)-Postgraduate Course in Agronomy, Area of Concentration in Vegetable Production, Universidade State Paulista, Faculty of Agronomy and Veterinary Sciences, Jaboticabal. 2013;76.
19. Silva AJJ, Uchôa SCP, Alves JMA, Lima ACS, Santos CSV, Oliveira JMF, Melo VF. Response of cowpea to the doses and application forms of phosphorus in Yellow Latosol of the State of Roraima. Acta Amaz. 2010;40:31-36.
20. Mawo YM, Mohammed IB, Garko MS. Effect of phosphorus levels on growth yield and development of cowpea (*Vigna unguiculata* (L.) Walp) varieties. Inter. Jour. of Scien. Engineering and Applied Scie. 2016;2:302-312.
21. Ndor E, Dauda N, Abimuku E, Azagatu D, Anzaku H. Effect of phosphorus fertilizer and spacing on growth, nodulation count and yield of cowpea (*Vigna unguiculata* (L.) Walp) in southern guinea savannah of Nigeria. Asian Jour. Agric. Scie. 2012;4: 254-257.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24992