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Yield of Cowpea Genotypes Inoculated With *Bradyrhizobium* and *Azospirillum brasilense* in Association With Phosphate Fertilization in Amazonian Soil

E. O. Araújo[1], M. Mauad[2], H. C. Tadeu[3] & H. A. Lima Filho[4]

[1] Federal Institute of Education, Science and Technology, Rodonia State, Campus Cacoal, Brazil

[2] Faculty of Agricultural Sciences, Federal University of Grande Dourados, Dourados, Brazil

[3] Federal University of Amazonas, Manaus, Brazil

[4] Federal Institute of Education, Science and Technology Goiano, Brazil

Correspondence: E. O. Araújo, Federal Institute of Education, Science and Technology Rodonia State, Cacoal, Brazil. E-mail: erica.araujo@ifro.edu.br

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Abstract

The use of inoculants containing N_2-fixing bacteria increases every year in cowpea crop in the North and Northeast regions of Brazil. In this context, this study aimed to evaluate the effects of inoculation with *Bradyrhizobium sp*. and *Azospirillum brasilense* in association with phosphate fertilization on the vegetative development, nodulation and yield of cowpea genotypes grown in Southern Rondônia state, Brazil. The experiment was conducted from February to May 2018, in the municipality of Colorado do Oeste, RO. The experimental design was randomized blocks in 4 × 3 × 2 factorial scheme, corresponding to four dose 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 three replicates. Cowpea seeds were inoculated with the commercial inoculant TotalNitro Cowpea with strains of *Bradyrhizobium sp.* (Semia 6462 and Semia 6463); co-inoculation was performed using the commercial product, containing a combination of strains of *Azospirillum brasilense*, both in liquid formulation. Co-inoculation (*Bradyrhizobium sp.*+ A*zospirillum brasilense*) did not influence the production components of cowpea plants. Inoculation with *Bradyrhizobium sp*. associated with phosphate fertilization of 120 kg ha^{-1} of P_2O_5 was sufficient to promote maximum growth and development of cowpea plants in Amazonian soil. The genotype White had higher capacity of nodulation, dry matter production and grain yield. Inoculation of *Bradyrhizobium sp.* increased N and P contents in the leaves. The genotype Butter was superior to the genotype White with respect to the accumulation of P, K, Ca and Mg in the leaves.

Keywords: *Vigna unguiculata,* phosphorus, rhizobacteria, symbiosis

1. Introduction

With origin in Africa, the cowpea crop (*Vigna unguiculata* (L.) Walp.) is extremely rustic, tolerant to high temperatures and drought, and with good conditions for adaptation and expansion of exploited areas. In Brazil, it is estimated that 1.442 million hectares of cowpea are cultivated, with mean yield of approximately 496 kg ha^{-1} (CONAB, 2018), which allows the crop to appear among the main leguminous species cultivated in the country, with predominance in the North and Northeast regions (CONAB, 2018). In addition, in some regions of the country this crop has become an important option for cultivation in the second season, in succession to traditional crops, such as corn and soybean, especially due to the low cost of production and adaptation to the rainfall regime (Zilli et al., 2011).

The utilization of biological inputs in replacement of industrialized chemical inputs has been increasingly frequent in agriculture, and biological nitrogen fixation (BNF) has proved to be indispensable for the sustainability of the Brazilian agriculture (Hungria et al., 2007), being considered as an important strategy to increase cowpea yield (Melo & Zilli, 2009). In the Amazonian region, studies have shown positive results for the increase in grain yield with inoculation of bacteria of the genus *Bradyrhizobium* in cowpea seeds (Araujo et al., 2018; Araujo et al., 2017; Rocha, 2016; Melo & Zilli, 2009), reducing production costs and increasing the producer's income.

Cowpea can produce nodules and establish symbiosis with several species of bacteria, such as the genus *Azospirillum* sp. Such low specificity of the crop regarding the microsymbiont proves to be limiting to the technological exploitation of BNF, since nodulating bacteria established in the soil, besides being competitive and in high number, exhibit variable efficiency in BNF (Hara & Oliveira, 2007). In addition to these peculiarities inherent to the microsymbiont, there are also reports that certain cowpea genotypes have higher capacity for nodulation and efficiency in BNF.

However, the benefits of the ecological processes performed by plant growth-promoting bacteria (PGPB) have contributed to achieving sustainability in the agricultural sector. Co-inoculation between rhizobium and PGPB can lead to increased nodulation and fixation of atmospheric N_2 and also to improvements in plant growth and development. However, the efficiency of N_2 fixation depends on the availability of P, due to its participation in the symbiotic process (Burity et al., 2000). Several studies have already observed the effect of phosphate fertilization on the growth and nodulation of cowpea plants (Araujo et al., 2018; Araujo et al., 2017; Coutinho et al., 2014; Araujo et al., 2012; Silva et al., 2010a; Silva et al., 2010b), but information on the response of different genotypes is scarce in the literature. Thus, further research is necessary, for each species, especially with respect to the peculiarities related to growth promotion by *Azospirillum brasilense* combined with *Bradyrhizobium sp*., since alterations in biometric and production components can be an indication of the effect of the association between diazotrophic bacteria.

Given the above, the present study aimed to evaluate the effects of inoculation with *Bradyrhizobium sp.* and *Azospirillum brasilense* in association with phosphate fertilization on the vegetative development, nodulation and yield of cowpea genotypes grown in southern Rondônia state, Brazil.

2. Materials and Methods

The experiment was carried out from February to May 2018 in the experimental area of the Federal Institute of Education, Science and Technology of Rondônia, municipality of Colorado do Oeste, RO, Brazil, at geographic coordinates 13°06′23.51″ S and 60°30′37.65″ W, 412 m, in Red Yellow Latosol. The climate according to Köppen's classification is Awa, 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).

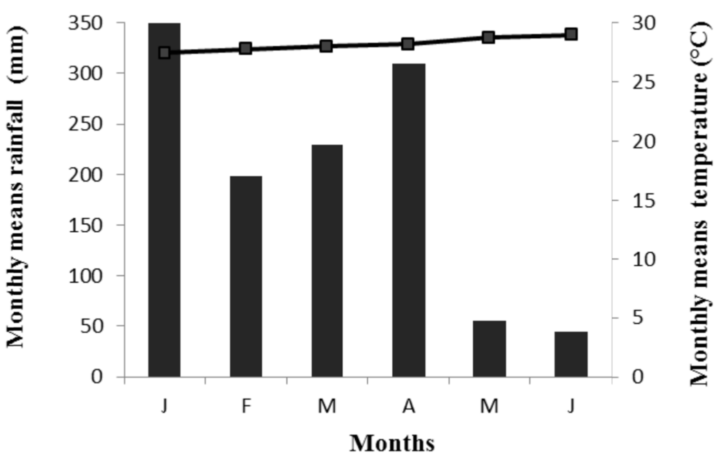

Figure 1. Mean monthly rainfall (mm) and temperature (°C), recorded at the meteorological station of the National Institute of Meteorology from February to May in the agricultural year of 2018

The chemical analysis of the soil sample collected in the 0-20 cm layer, carried out before the experiment, showed the following chemical and granulometric characteristics: O.M.: 35.8 g dm^{-3}; pH ($CaCl_2$): 5.1; P: 1 mg dm^{-3}; K: 2 mmolc dm^{-3}; Ca: 94 mmolc dm^{-3}; Mg: 13 mmolc dm^{-3}; Al: 1 mmolc dm^{-3}; H+Al: 29 mmolc dm^{-3}; SB: 109 mmolc dm^{-3}; CEC: 138 mmolc dm^{-3}, base saturation: 79%, 576 g dm^{-3} of clay, 178 g dm^{-3} of sand 246 g dm^{-3} of silt.

The experimental design was randomized blocks in a 4 × 3 × 2 factorial scheme, corresponding to four doses of phosphorus; absence, inoculation and co-inoculation with rhizobacteria; and two local cowpea genotypes (White and Butter), with three replicates. The P doses were 0, 80, 120 and 160 kg ha^{-1} of P_2O_5, applied in the planting furrow. The source of P_2O_5 used was single superphosphate (21% P_2O_5). The cowpea genotypes used exhibit highly upright habit, indeterminate growth and do not tend to entwine on the stake.

Basal fertilization consisted of nitrogen and potassium, 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 TotalNitro cowpea (concentration of 2×10^9 CFU/mL) with strains of *Bradyrhizobium* sp. (Semia 6462 and Semia 6463). The rate used was 100 mL of the liquid inoculant for 50 kg of seeds; for co-inoculation, a commercial product was used, containing a combination of strains of *Azospirillum brasilense*. The applied rate was 150 mL for every 50 kg of seeds. Both inoculants were used in liquid formulation, produced and donated by the company Total Biotecnologia.

Soil tillage included harrowing (disc harrow) to 15 cm depth. Planting and fertilization furrows were opened by hand using a hoe at depths between 5 and 10 cm. Sowing was manually performed using a hand-held planter known as “matraca”, by placing four seeds per hole, and five plants per linear meter were left after thinning. Each experimental unit comprised three 4-m-long rows, at spacing of 0.60 m between rows and 0.20 m between plants. The evaluated area in each plot corresponded to the central row, disregarding 0.5 m on each end. At 8 days after emergence (DAE), thinning was performed to leave only one plant/hole. At 10 and 30 DAE, insecticide with the active ingredients Imidacloprid 100 g/L and Beta-cyfluthrin 12.5 g/L, of the chemical groups Neonicotinoid (Imidacloprid) and Pyrethroid (Beta-cyfluthrin), was applied at dose of 750-1000 mL/ha to control *Diabrotica speciosa*. Weeds were controlled by manual weeding every 15 days.

At full flowering (50% of plants at flowering), 20 trifoliate leaves were collected in each plot for the determination of N, P, K, Ca, Mg and S contents in the leaves. Sampling was carried out in the middle third of the plants in the evaluated area of the plots. All plant material collected was washed in running water and deionized water, and the samples were placed in paper bags, dried in forced air circulation oven at temperature of 65 ºC for 72 h, and then ground. Ground samples were subjected to sulfuric digestion and nitric-perchloric digestion, using the methodology described in Embrapa (2009). At this same phenological stage, the following parameters were determined: plant height, measured as the distance between collar and apical meristem using a tape measure; stem diameter, measured with a digital caliper at 2 cm height from the collar; number of leaves per plant and number of viable nodules, obtained by counting. Number of pods per plant, number of grains per pod and number of grains per plant were determined based on the mean number of pods harvested in five plants of the evaluated area of each plot. Shoot, root and total dry matter and grain yield were determined in the period of physiological maturation of the crop, R5 phenological stage. Yield was determined by the weight of grains in the evaluated area of each plot in kilograms, with correction to 13% moisture content, transforming the data to kg ha^{-1}.

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

3. Results and Discussion

The results showed significant effects ($p \leq 0.05$) of the triple interaction between genotype (G) × dose (D) × bacterium (B) on plant height, stem diameter, number of leaves, number of nodules, number of pods per plant, number of grains per pod, number of grains per plant, shoot dry matter, root dry matter, total dry matter and yield. Conversely, N, P, K, Ca, Mg and S contents in the leaves at full flowering were not significantly affected by the interaction and were presented independently for each genotype, rate and bacterium (Table 1 and 2).

Table 1. F values, Mean and CV for plant height (PH), stem diameter (SD), number of leaves (NL), number of nodules (NNO), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), number of pods per plant (NP), number of grains per pod (NG), number of grains per plant (NG/PL) and yield (Y) of cowpea genotypes inoculated and co-inoculated with rhizobacteria in association with phosphate fertilization. Colorado do Oeste, RO, Brazil (2018)

Source of variation	GL	Pr > F										
		PH	SD	NL	NNO	SDM	RDM	TDM	NP	NG	NG/PL	Y
Genotype (G)	1	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.11	0.00*	0.00*
Dose (D)	3	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Bacteria (B)	2	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
G × D	3	0.00*	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.37	0.00*	0.24
G × B	2	0.00*	0.00*	0.00*	0.00*	0.23	0.01*	0.24	0.00*	0.03	0.00*	0.00*
D × B	6	0.00*	0.00*	0.00*	0.00*	0.07	0.00*	0.15	0.00*	0.00*	0.00*	0.00*
G × D × B	6	0.00*	0.04*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Residue	71											
Mean		85.20	12.13	29.75	109.63	72.54	15.46	88.00	14.47	14.73	233.48	620.25
CV (%)		3.78	6.22	5.34	4.41	10.64	6.26	8.98	4.95	4.45	5.45	8.26

Note. *: significant by Tukey test at 5% probability. CV: coefficient of variation.

Table 2. F values, Mean and CV for the contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) in the leaves of cowpea plants at full flowering inoculated and co-inoculated with rhizobacteria in association with phosphate fertilization. Colorado do Oeste, RO, Brazil (2018)

Source of variation	GL	Pr > F					
		N	P	K	Ca	Mg	S
Genotype (G)	1	0.38	0.00*	0.00*	0.00*	0.01*	0.09
Dose (D)	3	0.00*	0.00*	0.01*	0.01*	0.07	0.22
Bacteria (B)	2	0.01*	0.00*	0.54	0.62	0.74	0.00*
G × D	3	0.10	0.21	0.32	0.05	0.18	0.73
G × B	2	0.12	0.20	0.31	0.58	0.35	0.25
D × B	6	0.06	0.15	0.07	0.06	0.34	0.06
G × D × B	6	0.06*	0.15	0.08	0.18	0.54	0.98
Residue	71						
Mean		34.78	2.50	15.61	41.56	3.58	2.41
CV (%)		9.27	9.57	13.62	14.41	16.23	14.50

Note. *: significant by Tukey test at 5% probability. CV: coefficient of variation.

In the simple-effect analysis of the triple interaction, it was observed that the genotype White was statistically superior to Butter when co-inoculated and fertilized with 160 kg ha^{-1} of P. The dose of 160 kg ha^{-1} of P in the presence of *Bradyrhizobium* sp. + *Azospirillum brasilense* led to maximum height and stem diameter, statistically differing from the treatment inoculated and fertilized with 160 kg ha^{-1} of P and from the treatment fertilized with 160 kg ha^{-1} of P and without inoculation (Table 3). In the genotype White, co-inoculated and fertilized with 160 kg ha^{-1} of P, plant height and stem diameter increased by about 8.46% and 5.53%, respectively, when compared to the treatment inoculated and fertilized with 160 kg ha^{-1} of P. Conversely, in the treatment co-inoculated and without phosphate fertilization, these increments were approximately 72.59% for plant height and 30.15% for stem diameter, respectively. The positive effect of co-inoculation with *Bradyrhizobium* sp. + *Azospirillum brasilense* on the growth of cowpea plants was observed only at higher P doses in the soil.

Table 3. Plant height, stem diameter, number of leaves, number of nodules, number of pods per plant, number of grains per pod and number of grains per plant in different cowpea genotypes inoculated and co-inoculated with rhizobacteria in association with phosphate fertilization. Colorado do Oeste, RO, Brazil (2018)

Bacteria	Doses de P_2O_5 (kg ha^{-1})							
	0		80		120		160	
	Genotype		Genotype		Genotype		Genotype	
	White	Butter	White	Butter	White	Butter	White	Butter
Plant height (cm)								
NI	50.00 aDb	31.00 bCa	78.00 aCc	65.66 bBc	93,00 aBb	89,66 aAb	108,00 aAc	60,16 bBc
I	76.33 aDa	44.00 bCb	94.33 aCb	74.36 bBb	113,67 aBa	95,00 bAa	122,00 aAb	75,00 bBb
CO	76.67 aCa	62.33 bCa	113.00 aBa	87.33 bBa	113,00 aBa	96,67 bAa	132,33 aAa	93,33 bBa
Stem diameter (mm)								
NI	11.48 aCb	6.65 bCb	13.58 aBa	10.22 bAa	13.38 aBb	10.98 bAa	14.82 aAc	8.30 bBb
I	12.99 aCa	7.05 bCb	14.65 aCa	11.53 bAa	15.25 aAa	11.80 bAa	15.85 aAb	8.97 bBb
CO	12.90 aBa	9.61 bCa	13.42 aBa	11.09 bAa	14.31 aBa	12.30 bAa	16.79 aAa	11.44 bAa
Number of leaves (un.)								
NI	10.66 aCb	10.00 aCc	20.33 bBb	22.66 aBc	25.33 bAb	30.66 aAc	22.00 bAb	33.00 aAc
I	15.00 aDa	22.00 aCa	23.33 bCa	27.00 aBb	27.00 bBb	50.33 aAa	31.00 bAa	50.00 aAb
CO	16.00 aDa	24.33 aCa	20.66 bCb	50.33 aBa	32.66 bBa	54.66 aAa	37.66 bAa	55.33 aAa
Number of nodules (un.)								
NI	10.00 bCb	6.00 bDb	52.66 bBc	171.00 aBb	83.00 bAc	227.00 aAb	48.00 bBb	109.33 aCb
I	33.33 aDa	32.00 aDa	96.00 bCb	17500 aBb	111.00 bAb	274.00 aAa	94.33 bBa	168.00 aCa
CO	31.00 aDa	40.66 aDa	133.33 bBa	190.00 aBa	180.00 bAa	218.00 aAa	82.00 bCa	164.66 aCa
Number of pods per plant (un.)								
NI	0.00 aCb	0.00 aDb	8.00 bBb	16.00 aCb	11.33 bAb	22.33 aAb	12.00 bAb	20.00 aBb
I	10.00 bBa	16.00 aCa	11.66 bAa	20.00 aBa	12.66 bAa	25.00 aAa	10.33 bBa	21.00 aBb
CO	11.00 bBa	18.00 aDa	11.00 bBa	20.00 aCa	13.00 bAa	25.00 aAa	12.33 bAa	23.00 aBa
Number of grains per pod (un.)								
NI	0.00 aBb	0.00 aBb	14.66 aAb	15.66 aAb	15.00 aAb	17.00 aAa	15.66 aAa	15.00 aAa
I	15.66 aAa	16.66 aAa	16.33 aAa	17.00 aAa	17.00 aAa	16.33 aABa	16.00 aAa	16.00 aAa
CO	15.33 aBa	15.00 aABa	17.89 aAa	18.00 aAa	16.00 aBa	16.66 aAa	15.66 aBa	16.00 aAa
Number of grains per plant (un.)								
NI	0.00 aCb	0.00 aDb	117.33 bBb	250.00 aCb	169.66 bAa	379.66 aAb	188.33 bAb	310.00 aBb
I	161.00 bCa	210.00 aCa	190.33 bABa	340.00 aBa	215.33 bAa	408.00 aAa	165.33 bCa	325.00 aBa
CO	173.33 bCa	275.00 aDa	196.66 bBa	340.00 aCa	208.33 bAa	416.66 aAa	193.66 bBa	368.00 aBa

Note. Lowercase letters separate means in each column for the factor genotype, uppercase letters separate means in the row for the factor phosphorus doses, and lowercase letters separate means in the row for the factor bacterium. Equal letters do not differ by Tukey test at 0.05 probability level. NI: no inoculation; I: inoculation with *Bradyrhizobium* sp.; CO: inoculation with *Bradyrhizobium sp* + A*zospirillum brasilense*.

Opposite results were found for the number of leaves, number of nodules, number of pods per plant and number of grains per plant. Simple-effect analysis of the triple interaction revealed that the genotype Butter was statistically ($p \leq 0.05$) superior to the genotype White when inoculated and fertilized with 120 kg ha^{-1} of P, not differing statistically from the treatment co-inoculated and fertilized with 120 kg ha^{-1} of P (Table 3). The dose of 120 kg ha^{-1} of P in the presence of *Bradyrhizobium sp.* led to increments of 64.15%, 20.70%, 11.95% and 7.46%, respectively, in the number of leaves, nodules, pods and grains of cowpea, when compared with the treatment fertilized with 120 kg ha^{-1} of P and without inoculation. When the treatment inoculated and fertilized with 120 kg ha^{-1} of P was compared with the treatment inoculated with *Bradyrhizobium sp*. and without phosphate fertilization, such increment exceeds 100% for the number of leaves and number of nodules, 56.25% for the number of pods and 94.28% for the number of grains per plant. These data revealed positive effect of inoculation with *Bradyrhizobium sp.* when associated with phosphate fertilization. It is possible to note an aid in symbiosis because, when the nutrient is applied, there is greater formation of nodules in the roots, since P is source of energy to the plant, which, when well nourished, provides carbohydrates to rhizobacteria, helping the development of nodules (Figure 2), up to a certain limit of fertilization, which allows the inference that symbiosis can be limited under very low and/or very high levels of phosphorus, as shown by the data (Table 3). In the control treatment (without inoculation and without fertilization), there was low formation of nodules in both genotypes studied, which confirms that there was low contamination and low population of nodulating bacteria established in the soil. The development of nodules, even under limited quantity of nutrient in the soil, is most likely associated with the capacity of the rhizobium to solubilize non-labile phosphate of the soil and release it to the plant. For cowpea, there is no conclusive information on the minimum number of nodules necessary to guarantee good performance in BNF. However, in the present study, both genotypes had enough capacity to form nodules to guarantee BNF when inoculated and fertilized with P.

Figure 2. Roots of the genotype Manteiga inoculated with *Bradyrhizobium* sp. (Semia 6462 and Semia 6463) and fertilized with different rates of P. (A) Inoculated; (B) Inoculated + 80 kg ha^{-1}; inoculated + 120 kg ha^{-1} (C) and (D) Inoculated + 160 kg ha^{-1}

Regarding the number of grains per pod, there was no significant difference ($p \leq 0.05$) between the cowpea genotypes at the different doses of P, whereas the for factor bacterium there was statistical difference from the control (without inoculation and without fertilization). Between the treatments inoculated and co-inoculated for both genotypes, there was also no significant difference ($p \leq 0.05$) for the production of grains per pod. It can be observed that only the inoculation with *Bradyrhizobium sp*. is sufficient to promote higher number of grains per pod, with no need for association with either phosphate fertilization or co-inoculation with rhizobacteria (Table 3), which becomes an option for family farming and/or subsistence farming considering the costs of phosphate fertilizers.

For the production of shoot, root and total dry matter, the genotype White as statistically superior ($p \leq 0.05$) to the genotype Butter when inoculated and fertilized with 120 kg ha^{-1} of P, not differing statistically from the treatment co-inoculated and fertilized with 120 kg ha^{-1} of P. The rate of 120 kg ha^{-1} of P, in the presence of *Bradyrhizobium sp.*, led to increments of 38.07%, 58.52% and 49.34%, respectively, in shoot, root and total dry matter, compared with the treatment inoculated with *Bradyrhizobium sp*. and without phosphate fertilization (Table 4), confirming the synergism between phosphate fertilization and inoculation with rhizobacteria in cowpea plants. This can be also explained because P helps increase the growth of root hairs, which are sites of infection for bacteria. Thus, in environment with low availability of P, bacterium efficiency is limited by the lack of infection sites, a problem that is solved when adequate rates of P are applied. When the production of shoot, root and total dry matter in the treatment inoculated with *Bradyrhizobium sp*. and fertilized with 120 kg ha^{-1} of P is compared with the values in the control treatment (without inoculation and without fertilization), the increments are 89.30%, 268% and 177%, respectively.

Table 4. Shoot dry matter, root dry matter and total dry matter of different cowpea genotypes inoculated and co-inoculated with rhizobacteria in association with phosphate fertilization. Colorado do Oeste, RO, Brazil (2018)

Bacteria	Doses de P_2O_5 (kg ha^{-1})							
	0		80		120		160	
	Genotype		Genotype		Genotype		Genotype	
	White	Butter	White	Butter	White	Butter	White	Butter
Shoot dry matter (g)								
NI	50.33 aCb	34.00 bCb	70.31 aBb	57.66 bBb	87.74 aAb	64.00 bAb	64.21 aCa	65.66 aAb
I	72.97 aCa	35.66 bCa	90.08 aBa	74.66 bBa	100.75 aAa	93.33 bAa	71.77 aCa	83.33 aAa
CO	73.24 aCa	40.33 bCa	86.70 aBa	75.66 bBa	100.75 aAa	84.66 bAa	78.49 aCa	86.66 aAa
Root dry matter (g)								
NI	5.89 aCb	4.00 bCb	13.66 aBb	10.00 bBb	18.33 bAb	20.00 aAb	18.00 aAb	11.66 bBc
I	9.00 aCa	6.00 bDa	19.26 aBa	12.66 bCa	21.70 bAa	24.66 aAa	19.82 bBa	22.33 aBa
CO	8.70 aCa	6.00 bDa	20.50 aBa	13.66 bCa	22.30 bAa	26.33 aAa	20.12 aBa	15.33 bBb
Total dry matter (g)								
NI	56.23 aCb	38.00 bCb	84.00 aBb	67.66 bBb	106.07 aAb	84.00 bAb	82.21 aBb	77.33 aBb
I	81.99 aCa	41.66 bCa	109.34 aBa	87.33 bBa	122.45 aAa	118.00 bAa	91.59 aCa	105.66 bAa
CO	81.94 aCa	46.33 bCa	107.20 aBa	89.33 aBa	123.05 aAa	111.00 bAa	98.61 aCa	102.00 aAa

Note. Lowercase letters separate means in each column for the factor genotype, uppercase letters separate means in the row for the factor phosphorus doses, and lowercase letters separate means in the row for the factor bacterium. Equal letters do not differ by Tukey test at 0.05 probability level. NI: no inoculation; I: inoculation with *Bradyrhizobium* sp.; CO: inoculation with *Bradyrhizobium sp* + A*zospirillum brasilense*.

Maximum grain yield was obtained by the genotype White in the treatment inoculated with *Bradyrhizobium sp.* and fertilized with 120 kg ha^{-1} of P, which did not differ statistically ($p \leq 0.05$) from the treatment co-inoculated and fertilized with 120 kg ha^{-1} of P (Table 5). The yield of the genotype Branco inoculated and fertilized with 120 kg ha^{-1} of P was 916.85 kg ha^{-1}, whereas the yield in the treatment only inoculated with *Bradyrhizobium sp.* was 763.42 kg ha^{-1}, which represents an increase of about 20% in grain yield (Table 5). It was possible to observe that at higher P doses the association with rhizobacteria was hampered, because grain yield decreased in both cowpea genotypes evaluated. However, inoculation with *Bradyrhizobium* sp. associated with phosphate fertilization of 120 kg ha^{-1} of P was sufficient to promote maximum growth and development of cowpea plants.

It is necessary to consider also that the genetic potential of the plant, as well as its adaptability to field conditions, influences grain yield (Mello et al., 2009).

Table 5. Yield of different cowpea genotypes inoculated and co-inoculated with rhizobacteria in association with phosphate fertilization. Colorado do Oeste, RO, Brazil (2018)

Bacteria	Doses de P_2O_5 (kg ha^{-1})							
	0		80		120		160	
	Genotype		Genotype		Genotype		Genotype	
	White	Butter	White	Butter	White	Butter	White	Butter
Yield (kg ha^{-1})								
NI	0.00 aCb	0.00 aCb	554.07 aBb	382.96 bBb	801.20 aAb	580.04 bAa	889.35 aAa	488.88 bAa
I	763.42 aCa	321.85 bCa	898.79 aBa	519.44 bAa	916.85 aAa	623.33 bAa	780.74 aCb	481.25 bBa
CO	818.51 aCa	420.13 bCa	928.70 aBa	519.44 bBa	983.79 aAa	636.57 bAa	914.53 aBa	562.22 bAa

Note. Lowercase letters separate means in each column for the factor genotype, uppercase letters separate means in the row for the factor phosphorus doses, and lowercase letters separate means in the row for the factor bacterium. Equal letters do not differ by Tukey test at 0.05 probability level. NI: no inoculation; I: inoculation with *Bradyrhizobium* sp.; CO: inoculation with *Bradyrhizobium sp* + A*zospirillum brasilense.*

Inoculation of *Bradyrhizobium sp.* led to higher N and P contents in the leaves of cowpea at full flowering, not differing statistically ($p \leq 0.05$) from the co-inoculated treatment (*Bradyrhizobium* sp. + *Azospirillum brasilense*). The mean contents of N and P in the leaves of cowpea inoculated and co-inoculated with rhizobacteria were 35.51 g kg^{-1} and 2.58 g kg^{-1}, showing increments in comparison to the control treatment (without inoculation and without fertilization) of about 6.49% and 10.94%, respectively (Figures 3A and 3C). It has been confirmed that the population of rhizobia inoculated and indicated for cowpea is able to perform symbiosis and supply the N and P required for crop development, and that the inoculant was effective in the biological fixation process because it led to higher N fixation by the plant, compared with the control treatment. Corroborating these results, Araújo et al. (2018) and Araújo et al. (2017) found increments in N and P contents in the leaves of cowpea when inoculated with strains of *Bradyrhizobium sp*., not differing statistically from the co-inoculated treatment.

Cowpea is characterized by low requirement of P but has shown greater and frequent response when grown in soil with good availability of the nutrient (Araújo et al., 2018). The contents of N, P, K and Ca in cowpea leaves responded significantly ($p \leq 0.05$) to the increment in P doses, and their maximum contents in the leaves were 37.64 g kg^{-1}, 2.74 g kg^{-1}, 16.53 g kg^{-1} and 44.01 g kg^{-1}, respectively, obtained at the dose of 120 kg ha^{-1} (Figures 3B, 3D, 3F and 3H), with a reduction at higher doses. Such increase in leaf contents of macronutrients with the application of phosphate fertilization can explain a future increase in grain production, considering that there are reports in the literature about the close correlation between leaf contents of nutrients and yield of crops (Araujo et al., 2017).

The genotype Butter was statistically superior ($p \leq 0.05$) to the genotype White for the accumulation of P, K, Ca and Mg in the leaves at full flowering (Figures 3C, 3E, 3G and 3I). This demonstrates that the genotype Butter is more efficient than White in absorbing these macronutrients under the study conditions, which can be attributed to the higher number of viable nodules. This can also be attributed to the existence of different nutritional behavior due to genetic factors of adaptability between the genotypes. Similar results were obtained by Araújo et al. (2018), evaluating the effects of inoculation and co-inoculation of rhizobacteria in association with phosphate fertilization on the absorption of macronutrients by different cowpea genotypes grown in southern Amazonas state.

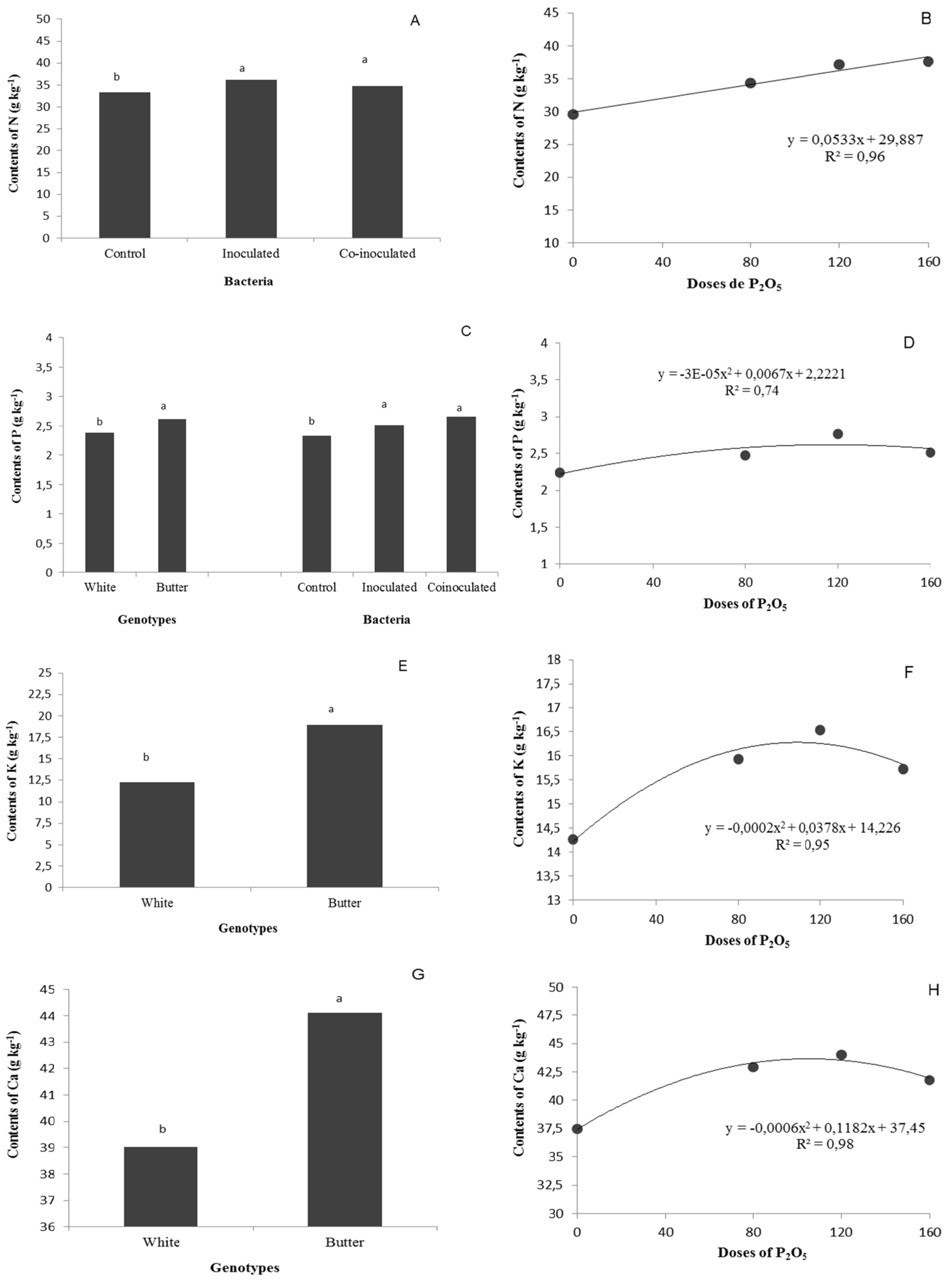

Figure 3. Contents of N, P, K, Ca, Mg and S in the leaves of different cowpea genotypes in response to inoculation and co-inoculation with rhizobacteria in association with phosphate fertilization. Medium followed by the same letter, do not differ statistically between themselves by Tukey a 5% probability

4. Conclusions

Co-inoculation (*Bradyrhizobium* sp. + A*zospirillum brasilense*) did not influence the production components of cowpea plants.

Inoculation of *Bradyrhizobium sp*. associated with phosphate fertilization of 120 kg ha^{-1} of P_2O_5 was sufficient to promote maximum growth and development of cowpea plants in Amazonian soil.

The genotype Branco showed higher capacity of nodulation, dry matter production and grain yield.

Inoculation of *Bradyrhizobium sp*. increased N and P contents in the leaves.

The genotype Butter was superior to the genotype White with respect to the accumulation of P, K, Ca and Mg in the leaves.

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