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Technical and economic viability of cowpea co-inoculated with *Azospirillum brasilense* and *Bradyrhizobium* spp. and nitrogen doses

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ABSTRACT: Biological nitrogen fixation efficiency can be increased by co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*, allowing even greater uptake of water and nutrients, leading to higher yields and enabling the insertion of unusual crops, such as cowpea (*Vigna unguiculata* L. Walp.), in the agricultural production system in the Cerrado region of Brazil. Thus, this study aimed to evaluate the technical and economic viability of cowpea in the Cerrado region, as a function of N doses and co-inoculation of seeds with *Azospirillum brasilense* and *Bradyrhizobium*. The study was carried out in a no-tillage system in Selvíria, MS, Brazil. The experimental design was a randomized block design, with four repetitions, arranged in a 3 x 5 factorial scheme, corresponding to three types of inoculation (without inoculation - control, with two strains of *Bradyrhizobium* spp. SEMIA 6462 and SEMIA 6463 - the conventional inoculation of cowpea, and these two strains of *Bradyrhizobium* spp. plus *A. brasilense* strains Ab-V5 and Ab-V6); and five N doses (0, 20, 40, 80 and 160 kg ha^{-1}), as urea, applied as topdressing. The following evaluations were performed: grain yield, total operating cost, effective operating cost, gross revenue, operating profit, profitability index, equilibrium price and equilibrium yield. Co-inoculation with *A. brasilense* increases cowpea grain yield, which makes cowpea production in the Cerrado region of Brazil technically and economically viable, without the need to apply N fertilizers in topdressing.

Key words: *Vigna unguiculata*, plant growth-promoting bacteria, biological nitrogen fixation, total operating cost, famers profit

Viabilidade técnica e econômica da coinoculação com *Azospirillum brasilense* e *Bradyrhizobium* e doses de nitrogênio no feijão-caupi

RESUMO: A eficiência da fixação biológica de nitrogênio pode ser aumentada pela coinoculação com *Azospirillum brasilense* e *Bradyrhizobium*, propiciando maior absorção de água e nutrientes, refletindo em maiores produtividades e permitindo a inserção de culturas não usuais nos sistemas agrícolas na região de Cerrado, como o feijão-caupi (*Vigna unguiculata* L. Walp.). Desta forma, o objetivo com este estudo foi avaliar a viabilidade técnica e econômica da produção de feijão-caupi em região de Cerrado, em função de doses de N e da coinoculação nas sementes com *Azospirillum brasilense* e *Bradyrhizobium*. O estudo foi desenvolvido em sistema plantio direto em Selvíria, MS. O delineamento experimental foi em blocos casualizados com quarto repetições, dispostos em esquema factorial 3 x 5, correspondendo a três tipos de inoculação (sem inoculação - testemunha, com duas estirpes de *Bradyrhizobium* spp., SEMIA 6462 e SEMIA 6463 - a inoculação convencional de feijão-caupi, e estas duas estirpes de *Bradyrhizobium* sp mais *A. brasilense* estirpes Ab-V5 e Ab-V6 - a coinoculação); e cinco doses de N (0, 20, 40, 80 e 160 kg ha^{-1}, na forma de ureia, aplicada em cobertura. As seguintes avaliações foram realizadas: produtividade de grãos, custo operational total, custo operacional efetivo, receita bruta, lucro operacional, índice de lucratividade, preço de equilíbrio e produtividade de equilíbrio. A coinoculação com *A. brasilense* aumentou a produtividade de grãos do feijão-caupi, sendo uma tecnologia tecnicamente e economicamente viável, sem a necessidade de aplicação de fertilizante nitrogenado em cobertura.

Palavras-chave: *Vigna unguiculata*, bactérias promotoras de crescimento de plantas, fixação biológica de nitrogênio, custo operacional total, lucratividade do produtor

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Introduction

Due to its wide genetic variability, tolerance to unfavorable climate conditions, N_2 fixation, high yield potential plus an excellent nutritional value, cowpea (*Vigna unguiculata* L. Walp.) is a potential crop under tropical conditions (Ferreira et al., 2013; Marinho et al., 2017). In Brazil, it is estimated that about 1.5 million hectares of cowpea are cultivated, with an average yield of approximately 520 kg ha^{-1} (CONAB, 2018), well below the potential of the crop, which can reach yields greater than 2000 kg ha^{-1} (Ferreira et al., 2013; Marinho et al., 2017). One of the factors that contribute to the low average yield of this species is soil fertility management, particularly due to insufficient nitrogen supply (Costa et al., 2014; Marinho et al., 2014).

To obtain high yields, N_2 fixation must have maximum efficiency (Rodrigues et al., 2012; Bulegon et al., 2016; Galindo et al., 2017a; Moretti et al., 2018). A better understanding of the rhizobia-legume association under Brazilian Cerrado conditions can provide effective contribution to the N balance in both soil and plants (Chagas Junior et al., 2010a,b). Considering the main current and potential limitations of Biological Nitrogen Fixation (BNF) in cowpea and the benefits, in various crops, by inoculation with *Azospirillum brasilense* (free-living diazotrophic bacteria), especially greater root system development and, consequently, higher absorption of water and nutrients, it can be deduced that joint co-inoculation of *Bradyrhizobium* spp. and *A. brasilense* can enhance crop performance, in an approach that respects the current demands for agricultural, economic, social and environmental sustainability (Hungria et al., 2013; Galindo et al., 2018a).

In addition, Hungria et al. (2013) reported that these effects promoted by the co-inoculation with plant growth-promoting bacteria (PGPB) and *Bradyrhizobium* spp. seem to be influenced by specific signals among the bacterial genotypes involved and the host plant genotypes and species. Thus, further research on the response of co-inoculation in legume crops is important for the development of species more responsive to co-inoculation. Also, many legumes are important food for humans and animals worldwide.

In this context, it becomes necessary to conduct more studies to increase BNF efficiency, associating the co-inoculation of *Bradyrhizobium* spp. and *A. brasilense* in cowpea, allowing better utilization of water and nutrients, to increase cowpea yield in a more sustainable way. However, the literature has few economic studies of co-inoculation with *Azospirillum brasilense* in cowpea. Given the above, the objective was to evaluate the technical and economic viability of cowpea in the Cerrado region of Brazil, as a function of N doses and co-inoculation of seeds with *Azospirillum brasilense*.

Material and Methods

The study was carried out in Selvíria, MS, Brazil (20° 22' S and 51° 22' W, 335 m a.s.l.) on the Education and Research Farm of FE/UNESP. The soil of the experimental area is classified as an Oxisol, which has been cultivated with annual crops for more than 28 years, the last 12 years being under a no-tillage system with wheat, and two corn crops as previous crops. The annual average temperature was 23.5 °C, the average annual rainfall was 1,370 mm and the mean annual air relative humidity was between 70.0 and 80.0% (Figure 1).

The experimental design was a randomized block design, with four repetitions, arranged in a 3 x 5 factorial scheme, corresponding to three types of inoculation (without inoculation, with two strains of *Bradyrhizobium* spp., SEMIA 6462 and SEMIA 6463 - the conventional inoculation of cowpea, and these two strains of *Bradyrhizobium* spp. plus *Azospirillum brasilense* strains Ab-V5 and Ab-V6 - the co-inoculation); and five doses of N (0, 20, 40, 80 and 160 kg ha^{-1}, as urea) applied as topdressing, 15 days after sowing. The plots of the experiment were 5 m long with seven rows spaced by 0.45 m, and the measurements were taken in the four central rows, excluding 0.5 m from the extremities.

The herbicides glyphosate (N - (phosphonomethyl) glycine) - 1800 g ha^{-1} a.i.) and 2,4-D (Dichlorophenoxyacetic acid - 670 g 190 ha^{-1} a.i.) were used for the desiccation of the agricultural area. The granulometric analysis presented the

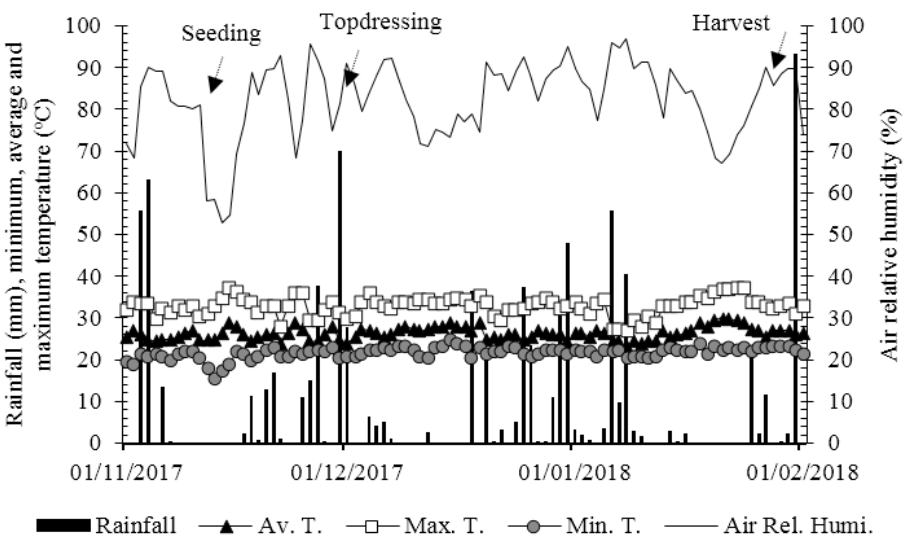


Figure 1. Rainfall, air relative humidity (Air Rel. Hum.), and maximum (Max. T.), average (Av. T.), and minimum temperature (Min. T.) obtained from the weather station located on the Education and Research Farm of FE / UNESP during the experiment (November 2017 to February 2018)

following results: 439, 471 and 90 g kg^{-1} of clay, sand and silt, respectively, at a depth of 0-0.20 m. The chemical attributes of the soil in the 0-0.20 m layer were determined prior to the cowpea experiment, according to the methodology proposed by Raij et al. (2001). The soil chemical attributes were: P (resin) = 39 mg dm^{-3}; S-SO_4 = 30 mg dm^{-3}; organic matter = 22 g dm^{-3}; pH ($CaCl_2$) = 5.1; K, Ca, Mg, H + Al and Al = 2.3; 31.0; 33.0; 34.0 and 0 $mmol_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 3.7; 25.0; 30.1 and 1.7 mg dm^{-3}, respectively; B (hot water) = 0.23 mg dm^{-3}, cation exchange capacity (pH 7.0) = 100.3 $mmol_c$ dm^{-3} and base saturation = 66.0%. Total N concentration was equal to 1.04 g kg^{-1} (Determined by the regular Kjeldahl method using a block digester, followed by diffusion with NaOH (Stevens et al., 2000).

The corn straw (previous crop) was collected at sowing to characterize and estimate the accumulation of nutrients: 77.1; 10.4; 36.0; 30.1; 16.4; 14.2 and 3.1 kg ha^{-1} of N, P, K, Ca, Mg, S and Si, respectively, and 185.5; 201.9; 1627.9; 948.5 and 740.4 g ha^{-1} of B, Cu, Fe, Mn and Zn, respectively, and C:N ratio of 38.55.

At sowing, 40 kg ha^{-1} of P_2O_5 (triple superphosphate) and 40 kg ha^{-1} of K_2O (potassium chloride) were applied by the seeder (Tatu Marchesan Ultra Flex®), distributed next to and below the seeds, for all treatments, based on the soil analysis, cowpea requirement and the crop history in the area (Andrade Jr. et al., 2002).

The inoculation of cowpea seeds with the bacterium *Bradyrhizobium* spp. strains SEMIA 6462 and SEMIA 6463 (TotalNitro - Total Biotecnologia®, guarantee of 5 x 10^9 CFU mL^{-1}) and co-inoculation with *Azospirillum brasilense* strains Ab-V5 and Ab-V6 (Azototal - Total Biotecnologia®, guarantee of 2 x 10^8 CFU mL^{-1}) were carried out at the dose of 200 mL of inoculant (liquid) per ha with the aid of a clean mixer for incorporation, one hour before sowing the crop and after treatment of the seeds with insecticide and fungicide. For seed treatment, the fungicides pyraclostrobin + thiophanate-methyl (6 g + 56 g of a.i. per 100 kg of seed) and the insecticide fipronil (62 g of a.i. per 100 kg of seed) were used.

Mechanical sowing (seeder Tatu Marchesan Ultra Flex®) of BRS Tumucumaque cowpea was made on November 10, 2017, by sowing 12 seeds per meter, and the emergence of seedlings occurred four days after sowing (November 14, 2017). Cowpea was irrigated using a center pivot sprinkling system, with a mean water depth of 14 mm and an irrigation interval of approximately 72 h. Nitrogen topdressing fertilization was performed between the rows 10 days after emergence (November 24, 2017). The application was done manually, distributing the fertilizer on the soil surface (without incorporation), approximately 10 cm from the rows, in order to avoid contact of the fertilizer with the plants. After topdressing fertilization, the area was irrigated by sprinkling (depth of 14 mm) at night to minimize losses by volatilization of ammonia. The herbicides clethodim (96 g ha^{-1} of a.i.) and bentazon (576 g ha^{-1} of a.i.) were applied for the control of post-emergence weeds on November 25, 2017. Insect control was performed with beta-cyfluthrin (8 g ha^{-1} a.i.) and abamectin (9 g ha^{-1} a.i.), on November 25, 2017 and December 14, 2017, respectively, and with imidacloprid + beta-cyfluthrin (9 g ha^{-1} a.i.) and deltametrin (4 g ha^{-1} a.i.) on December 1, 2017. In all insecticide applications, vegetable oil (720 g ha^{-1} of a.i.) was added as adjuvant in the insecticide mixture. Harvest was carried out on January 30, 2018 (76 days after emergence).

Grain yield was determined by harvesting the plants contained in the usable area of each plot (four central rows, excluding 0.5 m from the extremities). After harvest, the grains were quantified in 60-kg sacks ha^{-1} and corrected to 13.0% moisture (wet basis).

Data were submitted to analysis of variance (F test). When a significant result was verified by the F test ($p \leq 0.01$ and $p \leq 0.05$), the Tukey test ($p \leq 0.05$) was used for comparison of inoculation means and polynomial regression was fitted for the N doses using PROC REG procedure of SAS program (SAS Institute, 2015).

Economic analysis was performed using the structure based on the Total Operating Cost (TOC) of production, used by the Instituto de Economia Agrícola (IEA), according to Matsunaga et al. (1976), which consists of the sum of expense costs: operations, inputs (fertilizers, seeds, pesticides, etc.), labor, machinery and irrigation, called Effective Operating Cost (EOC). Besides TOC, the study considered other expenses and interest rates, representing 5.0% of EOC (Matsunaga et al., 1976), thus resulting in the total operating cost (TOC), which was extrapolated to one hectare. This methodology has been previously used in several studies on economic evaluation of crops [Kappes et al. (2015), Portugal et al. (2016), Galindo et al. (2017b) and Galindo et al. (2018b)].

The profits of the treatments were determined through profitability analyses according to Martin et al. (1998). For that, the following variables were determined: Gross Revenue (GR) (in R$), as the product between produced quantity (in number of 60-kg sacks) and mean selling price (in R$); Operating Profit (OP), as the difference between GR and TOC; Profitability Index (PI), understood as the ratio between OP and GR, in percentage, Equilibrium Price (EP), given a certain total operating production cost, as the minimum price calculated to cover this cost, considering the average yield obtained; and Equilibrium Yield (EY), given a certain total operating production cost, as the minimum yield to cover this cost, considering the average price paid to the farmer.

The analysis considered the prices practiced in commercial crops in the region of Selvíria, MS, Brazil, based on the mean of the last three agricultural years. Simulations were made as if each treatment of the experiment represented commercial crops. To facilitate the discussion, yield values were converted to 60-kg sacks, because this is the basic marketing unit used by the local farmers. The cost of the cowpea sack for the municipality of Selvíria, MS, Brazil, was R$ 70.00 per unit produced. The price paid by the farmer was R$ 1.85 kg^{-1} for urea. For the inoculum with *Bradyrhizobium* spp. and *Azospirillum brasilense*, the expenditure was approximately R$ 10.00 per dose (each inoculant), and two doses of each inoculant were used per hectare.

Results and Discussion

The interaction between N doses and inoculations was significant for cowpea grain yield (Table 1). In the absence of nitrogen fertilization, and with application of 40 and 160 kg ha^{-1}

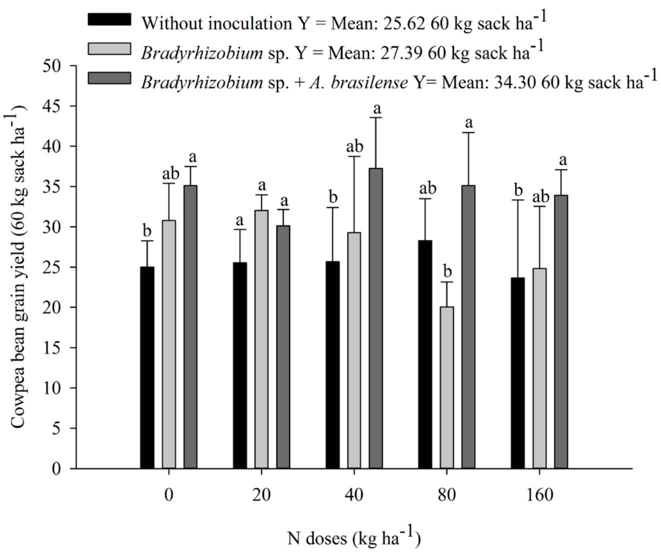
of N, the co-inoculation with *A. brasilense* resulted in higher grain yield compared to the control treatment (Figure 2), increases of 11.59 and 10.28 60-kg sacks ha^{-1}, equivalent to 45.1 and 43.5%, respectively. With application of 80 kg ha^{-1}, the co-inoculation promoted higher yield compared to conventional inoculation with *Bradyrhizobium* spp., an increase of 15 60-kg sacks ha^{-1}, equivalent to 24.1%. In the mean values, the co-inoculation with *A. brasilense* increased the yield of cowpea by 6.91 and 8.68 60-kg sacks ha^{-1}, compared to conventional inoculation and control treatment, increases equivalent to 25.2 and 33.9%, respectively (Figure 2).

According to Bárbaro et al. (2009), the literature reports that bacteria called PGPB (plant growth-promoting bacteria), such as *A. brasilense*, can act in association with rhizobia and leguminous species, promoting increments in plant growth and grain yield, in biologically fixed N, and improvements in the use of N obtained by the plant through symbiosis with rhizobia, corroborating the results observed in the present study.

Table 1. Cowpea grain yield as affected by N doses and inoculation method with *Bradyrhizobium* spp. or *Bradyrhizobium* spp. and *A. brasilense*

	Grain yield 60-kg sack ha^{-1}
N doses	
0	30.28
20	29.23
40	30.73
80	27.81
160	27.46
Inoculation	
Without	25.62
Bradyrhizobium spp.	27.39
Bradyrhizobium spp. + *A. brasilense*	34.30
LSD (0.05)	4.45
Overall Mean	29.10
CV (0.05)	19.87%
F Test	
Doses - D	0.757^{ns}
Inoculation - I	12.569^{**}
D xI	4.206^{*}

** and * Significant at p < 0.01 and 0.01 < p < 0.05, respectively; LSD - Least significant difference



The letters correspond to a significant difference at p ≤ 0.05. Error bars indicate the standard deviation of the mean (n = 4)

Figure 2. Cowpea grain yield as a function of inoculation methods with *Bradyrhizobium* spp. or *Bradyrhizobium* spp. and *A. brasilense* for each N doses

These effects can be due to various mechanisms, including early BNF of the nodules, increases in nodule dry matter, promotion of the occurrence of heterologous nodulation through the increase in the formation of root hairs and secondary roots, increase in the sites of infection, inhibition of pathogens and production of phytohormones and influences on dry matter partition among roots and shoots (Bárbaro et al., 2009; Galindo et al., 2018a). Furthermore, as observed by Hungria et al. (2013), these effects promoted by co-inoculation with PGPB and rhizobia seem to be influenced by specific signals between bacterial genotypes and the plant host genotype. Thus, further research on the response of co-inoculation as a function of genotypes and species is important for the development of more-responsive genotypes.

Although there was no adjustment to the applied N doses, there was a numerical reduction in grain yield in the absence of nitrogen fertilization and with application of the highest N doses (80 and 160 kg ha^{-1}), especially when the conventional inoculation with *Bradyrhizobium* spp. was performed, partially agreeing with Martins et al. (2013), who studied the application of N doses in topdressing (0, 35, 70, 105, 140 kg ha^{-1}) and inoculation with *Bradyrhizobium* spp. strain INPA 0311B and verified reduction in dry matter of nodules and cowpea grain yield with increasing N doses. According to Brito et al. (2009), 93% of N accumulated in cowpea comes from symbiotic fixation, which demonstrates the importance of BNF in cowpea, and the need for genetic improvement of the genotypes, associating the strains with optimal specificity to the process, making it possible to increase and popularize cowpea cultivation throughout Brazil.

Similar results in legumes were obtained by Hungria et al. (2013), who concluded that co-inoculation with *B. japonicum* + *A. brasilense* in soybean and common bean (*R. tropici* + *A. brasilense*) increased grain yield in soybean by 14.1% (compared to control - without inoculation) and 6.4% (compared to traditional inoculation with symbiotic bacteria), and in common beans by 19.6% (compared to control) and 14.7% (compared to traditional inoculation), respectively. Galindo et al. (2017a) verified an increase in the number of pods per plant, mass of 100 grains and soybean grain yield with co-inoculation with *A. brasilense*, with an increase in grain yield of 11.2% and an increase of 14.4% in operational profit. Studying co-inoculation of *B. japonicum* and *A. brasilense* associated with the use of Co and Mo in the seeds, Galindo et al. (2018a), verified an increase in grain yield (18.1%) compared to conventional inoculation, *B. japonicum* only, with an increase of 20.4% in operational profit. Souza & Ferreira (2017), observed higher common bean grain yield with co-inoculation of *R. tropici* and *A. brasilense* compared to N-fertilizer application (increase of 5%) and compared to conventional inoculation with *R. tropici* (increase of 26%).

The aforementioned positive results, together with the results obtained in the present study, highlight the importance of new studies of co-inoculation with *A. brasilense* in legumes, especially in crops such as cowpea, for which research is still scarce. The average yield obtained, regardless of the treatment

(29.10 60-kg sacks ha^{-1}) was approximately 3.3 times higher than the national average (8.7 60-kg sacks ha^{-1}, Table 1) (CONAB, 2018), elucidating the great potential of this crop in the Cerrado region of Brazil.

The Total Operating Cost (TOC) structure model, Table 2, was used in all treatments. Initial investments with soil tillage and liming were not considered in this study, since these practices were not performed, because the area had been under no-tillage system for more than 10 years when the experiment was initiated, contributing to the reduction of initial costs of crop planting.

The Effective Operating Cost (EOC), composed of expenses with operations and inputs, was equal to R$ 1,552.96 ha^{-1} and the Total Operating Cost (TOC) corresponded to R$ 1,681.08 ha^{-1}. In the EOC, the top three expenses are irrigation (14.5%), agricultural chemicals (22.7%) and seeds (21.4%) (Table 2).

In general, the highest TOC expenses were mechanized operations (24.9%), followed by agricultural chemicals (21.0 %) (Table 2). It is worth pointing out that, with the utilization of inoculation with *Bradyrhizobium* spp. and co-inoculation with *A. brasilense*, the percentage of expenses increases in relation to the TOC. However, the cost with these inoculations are low, representing only 1.2 and 2.3% of the TOC, respectively.

For TOC and cowpea yield of the treatments (Table 3), the highest TOC value occurred in treatments with *A. brasilense* co-inoculation with application of 160 kg ha^{-1} of N. On the other hand, the lowest TOC value corresponds to treatments without both inoculation and N fertilization. However, it should be highlighted that the highest cowpea yields were obtained when *A. brasilense* was co-inoculated (Table 1), especially with the application of 40 kg ha^{-1} of N and absence of nitrogen fertilization, which promoted the highest yields obtained (37.26 and 35.10 60-kg sacks ha^{-1}, respectively).

According to Bashan & Bashan (2010), this increase in grain yield due to the inoculation with bacteria of the genus *Azospirillum*, in crops of agronomic interest, results from the stimulus to plant growth by multiple mechanisms, including the synthesis of phytohormones (auxin, cytokinin and gibberellin), improvement in N nutrition, enhancement in leaf photosynthetic variables, attenuation/minimization of stress and biological control of some pathogenic agents.

It is verified that with a constant price of cowpea, the gross revenues of the treatments follow the same trend of the yields (Table 3), i.e., increments in revenue occur because of the increments in grain yield. This result is consistent with that of Galindo et al. (2017b), who claimed that yield is a primordial factor to guarantee good profitability to the producer. Also according to Galindo et al. (2017b), even in regions where the producer obtains good prices in the marketing of grains, if the yield is low, the profitability is compromised. Thus, investment in management practices, such as balanced fertilization and

Table 2. Total operating cost (TOC, R$ ha^{-1}) structure model of cowpea for the treatment control (0 kg ha^{-1} N in topdressing), and without inoculation with *Bradyrhizobium* spp. and *A. brasilense* per hectare

Description	Unit[1]	Amount[2]	Unitary value (R$)	Total value (R$)
A. Operations				
Desiccation	1.0 HM	0.50	85.00	42.50
Hoeing (triton)	1.0 HM	0.50	85.00	42.50
Seeding	1.0 HM	1.00	110.00	110.00
Pulverization	3.0 HM	0.60	85.00	153.00
Topdressing	0.0 HM	0.50	85.00	0.00
Harvest	1.0 HM	0.60	118.00	70.80
Irrigation (pivot)	1.0 mm	90.00	2.50	225.00
Subtotal A				643.80
B - Agricultural Input				
Triple superphosphate	1.0 T	0.09	1,100.00	97.78
Potassium chloride	1.0 T	0.07	1,900.00	126.67
Urea	1.0 T	0.00	1,850.00	0.00
Bradyrhizobium spp. inoculant	1.0 Dose (100 mL)	0.00	10.00	0.00
A. brasilense inoculant	1.0 Dose (100 mL)	0.00	10.00	0.00
Cowpea BRS Tumucumaque	1.0 sc 50 kg	1.07	310.00	331.70
Herbicida glyphosate	1.0 L	4.00	14.51	58.04
Herbicida 2,4-D	1.0 L	1.00	13.24	13.24
Herbicide Clethodim	1.0 L	0.40	91.60	36.64
Herbicide Bentazon	1.0 L	1.20	66.20	79.44
Insecticide Beta-cyfluthrin	2.0 L	0.15	103.00	30.90
Insecticide Abamectin	2.0 L	0.50	23.00	23.00
Insecticide Imidacloprid + Beta-cyfluthrin	1.0 L	0.75	57.00	42.75
Insecticide Deltametrin	1.0 L	0.15	80.00	12.00
Fungicide seed treatment pyraclostrobin + thiophanate-methyl + fipronil	1.0 L	0.11	350.00	37.33
Vegetable oil adjuvant	3.0 L	0.78	8.41	19.68
Subtotal B				909.16
Effective operating costs (EOC)				1,552.96
Other expenses				77.65
Interest cost				50.47
Total operating cost (TOC)				1,681.08

[1]HM - Hour machine; sc - Sack; 2017 and 2018 average exchange rate: R$3.51 = U$1.00

[2]Amount refers to the quantity in HM of operations or the unit quantity of a given agricultural input

Table 3. Total operating cost (TOC), grain yield (YIELD), gross revenue (GR), operating profit (OP), profitability index (PI), equilibrium price (EP) and equilibrium yield (EY) of Cowpea affected by nitrogen doses with or without inoculation with *Bradyrhizobium* spp. or co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*

Doses (kg ha^{-1})	TOC (R$)	Yield (60-kg ha^{-1} sc)	GR	OP	PI (%)	EP (R$ sc^{-1})	EY (60-kg ha^{-1} sc)
			(R$)				
			Without inoculation				
0	1681.08	24.98	1,748.60	67.52	3.86	67.30	24.02
20	1816.01	25.54	1,787.80	-28.21	-1.58	71.10	25.94
40	1905.08	25.67	1,796.90	-108.18	-6.02	74.21	27.22
80	2083.10	28.29	1,980.30	-102.80	-5.19	73.63	29.76
160	2439.14	23.64	1,654.80	-784.34	-47.40	103.18	34.84
Mean	1984.88	25.62	1,793.68	-191.20	-11.27	77.89	28.36
			Bradyrhizobium spp.				
0	1702.73	30.77	2,153.90	451.17	20.95	55.34	24.32
20	1837.66	32.03	2,242.10	404.44	18.04	57.37	26.25
40	1926.73	29.27	2,048.90	122.17	5.96	65.83	27.52
80	2104.75	20.04	1,402.80	-701.95	-50.04	105.03	30.07
160	2460.79	24.84	1,738.10	-722.69	-41.58	99.11	35.15
Mean	2006.53	27.39	1,917.16	-89.37	-9.33	76.53	28.66
			Bradyrhizobium spp. and *A. brasilense*				
0	1724.38	35.10	2,457.00	732.62	29.82	49.13	24.63
20	1859.31	30.12	2,108.40	249.09	11.81	61.73	26.56
40	1948.38	37.26	2,608.20	659.82	25.30	52.29	27.83
80	2126.40	35.10	2,457.00	330.60	13.46	60.58	30.38
160	2482.44	33.92	2,374.40	-108.04	-4.55	73.19	35.46
Mean	2028.18	34.05	2,401.00	372.82	15.17	59.38	28.97

Average cowpea bean trading price R$ 70.00 per 60-kg sack according to IBRAFE (2018); 2017 and 2018 average exchange rate: R$3.51 = U$1.00

inoculation techniques, increases grain yield and the gross margin of the crops.

For the values referring to operating profit (Table 3), higher N doses lower the OP, except for 40 kg ha^{-1}. In the absence of inoculation, the OP was positive only in the absence of nitrogen fertilization, whereas when the conventional inoculation with *Bradyrhizobium* spp. was carried out, the applications of 20 and 40 kg ha^{-1} of N promoted positive profitability, and with the co-inoculation with *A. brasilense*, the applications of 20, 40 and 80 kg ha^{-1} promoted positive profitability, which shows that the relationship between the benefit of conventional inoculation and co-inoculation on cowpea grain yield in relation to the cost of these technologies is positive, generating profitability.

The highest OP was verified by the co-inoculation with *A. brasilense* in the absence of nitrogen fertilization (R$ 732.62). In the absence of inoculation with *Bradyrhizobium* spp. and co-inoculation with *A. brasilense*, which would cause reduction of costs, with possibility of increase in OP, if good yields were obtained, the cowpea crop would be viable without N fertilization, but with reductions of R$ 383.65 and R$ 665.10 ha^{-1} in the profit, which are equivalent to 85.0 and 91.0% in the profitability, compared to the conventional inoculation and co-inoculation, respectively.

The co-inoculation propitiated R$ 281.45 ha^{-1} higher profit compared with conventional inoculation in the absence of N fertilization, which is equivalent to an increment of 62.4%, which demonstrates the benefit of this technique from the economic point of view. As evidenced for OP, the treatment leading to highest PI was co-inoculation with *A. brasilense* in the seeds without N fertilization (29.82%), which was 42.3 and 72.5% superior to that of the treatments inoculated with *Bradyrhizobium* spp. and not inoculated, respectively (Table 3), reinforcing the importance of co-inoculation with *A. brasilense* to obtain superior yields and, consequently, higher financial return.

Regarding the equilibrium price, that is, the price that must be paid for the 60-kg sack of cowpea so that there is no financial loss to the farmer, the values follow the trend of TOC, that is, the higher the TOC as a function of treatments, the higher the price paid to the farmer in order to avoid losses of production. Thus, with the increase of N doses applied and with the use of co-inoculation, the equilibrium price is higher (Table 3).

The equilibrium yield, that is, the grain yield that must be obtained so that the farmers have no financial loss, considering the price paid in 60-kg sacks of cowpea and the production cost of the crop, follows the inverse trend of the OP and PI. The higher the cost with a given treatment, the higher the equilibrium yield; consequently, the higher EY was obtained with nitrogen fertilization (Table 3).

The economic results obtained agree with Hungria et al. (2013), who concluded that microbial inoculants are very inexpensive and can save billions of dollars per year with BNF. Therefore, the potential of co-inoculation with *A. brasilense* in the development and yield of cowpea is very high, especially for being a technique with low cost and investment, of easy application and use. Besides the following cowpea characteristics: genetic variability; tolerance to adverse climatic conditions; N_2 fixation potential; high yield potential; excellent nutritional value and short cycle, being earlier than the common bean (cycle varying from 65 to 75 days from sowing to harvest, on average). Cowpea has been studied and gained prominence in scientific research (Chagas Junior et al., 2010a,b; Marinho et al., 2017), being a crop with potential for expansion in several agricultural areas worldwide, such as the Brazilian Cerrado.

The results of the present study indicated that there is an opportunity to reduce N fertilizer inputs while increasing cowpea yields by co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*. These are important considerations in Brazil, where resources are limited and there is a need to improve agricultural yield and the economic and environmental sustainability, elucidating the great potential of this crop in the Cerrado of Brazil.

Conclusions

1. Co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense* increases cowpea grain yield, even when associated with N doses applied in topdressing.

2. The co-inoculation technique with these bacteria provides greater technical and economic viability with cowpea, being a recommended technology, without the need to apply N fertilizers in topdressing.

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