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Technical and economic viability of co-inoculation with *Azospirillum brasilense* in soybean cultivars in the Cerrado

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ABSTRACT

Biological nitrogen fixation (BNF) efficiency can be increased by co-inoculation with bradyrhizobia and *Azospirillum brasilense*, allowing even greater uptake of water and nutrients, leading to higher yields. Thus, this study aimed to evaluate the technical and economic viability of soybean in the Cerrado, according to the cultivars and co-inoculation with *Azospirillum brasilense*. The experiment was conducted in Selvíria, MS, in no-tillage system, in Oxisol, arranged in a randomized block design in a 2 x 2 factorial scheme with two cultivars ('Potência' and 'Valiosa'), with and without co-inoculation with *Azospirillum brasilense* in the seed. Co-inoculation with *A. brasilense* increases grain yield in the cultivars 'Potência' and 'Valiosa', being economically viable. However, using the cultivar 'Potência' co-inoculated led to the highest profitability.

Palavras-chave:
fixação biológica de nitrogênio
custo operacional total
Glycine max
desempenho técnico
desempenho econômico

Viabilidade técnica e econômica da coinoculação com *Azospirillum brasilense* em cultivares de soja no cerrado

RESUMO

A eficiência da fixação biológica de nitrogênio (FBN) pode ser aumentada por meio da coinoculação de bradirrizóbios e *Azospirillum brasilense*, possibilitando ainda, maior absorção de água e nutrientes, com incremento em produtividade. Sendo assim, objetivou-se estudar a viabilidade técnica e econômica da cultura da soja no Cerrado, em função de cultivares e coinoculação com *Azospirillum brasilense*. O experimento foi desenvolvido em Selvíria, MS, em sistema plantio direto, em Latossolo Vermelho distrófico, disposto em delineamento experimental de blocos casualizados em esquema fatorial 2 x 2, com duas cultivares (Potência e Valiosa), com e sem coinoculação com *Azospirillum brasilense* via semente, com seis repetições. A coinoculação com *A. brasilense* aumenta a produtividade de grãos das cultivares Potência e Valiosa, sendo economicamente viável. Entretanto, a utilização da cultivar Potência coinoculada propiciou a maior lucratividade.

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Introduction

The soybean crop occupied an area of 31.57 million hectares, with mean yield of 3,011 kg ha^{-1} in the 2014/2015 season, presenting itself as the main annual agricultural crop in planted area and marketing value (CONAB, 2015). Soybean grain is the main source of vegetal protein, an essential production component in animal feed, besides the increasing use in human diet (Ignácio et al., 2015).

Due to the high N requirement of the crop (estimated to be 80 kg of N to produce 1000 kg of soybean grains) to obtain high yields, N_2 fixation must work with maximum efficiency (Figueiredo et al., 2008; Vieira Neto et al., 2008; Zilli et al., 2008; Hungria et al., 2010; Rodrigues et al., 2012; Bulegon et al., 2016). Considering the main current and potential limitations of BNF in the soybean crop and the benefits caused in various crops by the inoculation with *Azospirillum* (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 bradyrhizobia and *Azospirillum* can enhance crop performance, in an approach that respects the current demands for agricultural, economic, social and environmental sustainability (Hungria et al., 2013).

In addition, as pondered by Hungria et al. (2013), these effects promoted by the co-inoculation with plant growth-promoting bacteria (PGPB) and rhizobia seem to be influenced by specific signals among the bacterial genotypes involved and the host plant genotypes. Thus, further research on the response of co-inoculation as a function of different genotypes is important for the development of cultivars more responsive to co-inoculation.

In this context, it becomes necessary to conduct more studies to increase BNF efficiency, associating the co-inoculation of bradyrhizobia and *Azospirillum brasilense* in different soybean genotypes under the conditions of Brazil, such as in the Cerrado region, allowing better utilization of water and nutrients, to increment soybean grain yield in a more sustainable way. However, the literature has few studies economically evidencing the effect of co-inoculation with *Azospirillum brasilense* in different cultivars.

Given the above, the objective was to evaluate the technical and economic viability of the soybean crop in the Cerrado, as a function of cultivars and co-inoculation of seeds with *Azospirillum brasilense.*

Material and Methods

The study was carried out in the experimental area of the Engineering Faculty of the São Paulo State University - UNESP, located in Selvíria, MS, Brazil (20° 22’ S; 51° 22’ W; 335 m). The soil of the experimental area was classified by Santos et al. (2013) as Latossolo Vermelho distrófico (Oxisol) with clay texture and has been cultivated by annual crops for more than 27 years; in the last 10 years in no-tillage system and the previous crop was corn. This area had never been under experiments with *Azospirillum brasilense*. The climate in the region is Aw, according to Köppen’s classification, characterized as humid tropical with rainy season in the summer and dry season in the winter. The climatic data recorded along the experimental period are presented in Figure 1.

Soil chemical attributes in the arable layer determined before the experiment, according to the methodology proposed by Raij et al. (2001), were: 10 mg dm^{-3} of P (resin); 5 mg dm^{-3} of $S-SO_4$; 22 g dm^{-3} of OM; 5.3 of pH ($CaCl_2$); K, Ca, Mg, H + Al = 2.4; 21.0; 18.0 and 28.0 mmol$_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 3.2; 22.0; 24.2 and 1.2 mg dm^{-3}, respectively; 0.16 mg dm^{-3} of B (hot water) and 60% of base saturation. Based on soil analysis and on the fertilization recommendation for the soybean crop (Mascarenhas & Tanaka, 1997), fertilization at sowing was performed in the sowing furrow using 80 kg ha^{-1} of P_2O_5 and 80 kg ha^{-1} of K_2O. Seeds were treated with the fungicides Carbendazim + Thiram at dose of 30 + 70 g of a.i. for every 100 kg of seeds, respectively. Inoculation with rhizobium was performed at dose of 200 mL ha^{-1} (strains: SEMIA 5019 (*Bradyrhizobium elkanii*) and SEMIA 5079 (*Bradyrhizobium japonicum*), with guarantee of 5 x 10^9 viable cells per mL), using a clean concrete mixer for incorporation, after the seeds were dried following the treatment with pesticides and one hour before sowing. The experiment was installed in December 2014

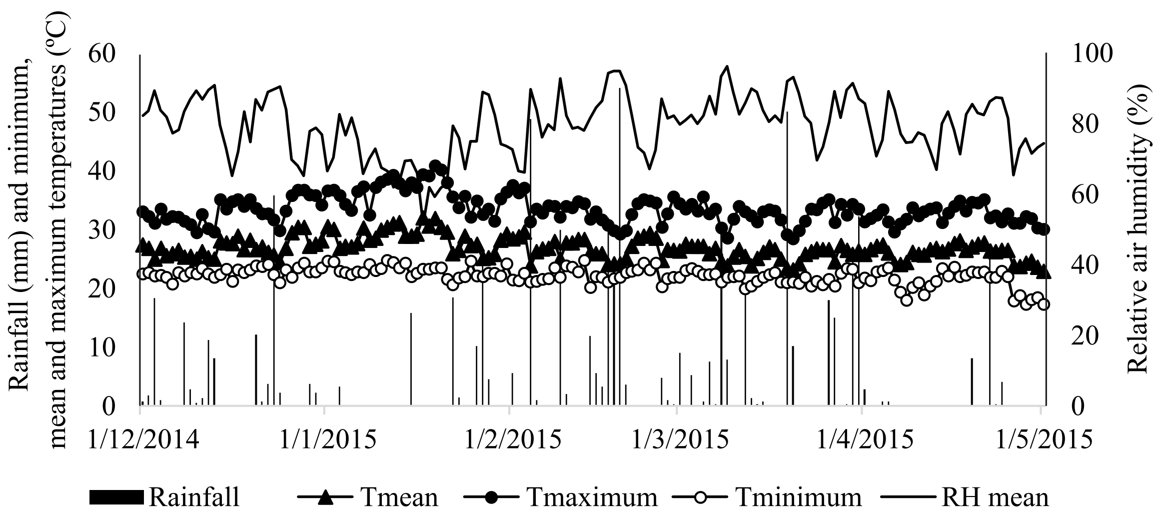

Figure 1. Rainfall, relative air humidity and maximum, mean and minimum temperatures obtained by the weather station situated on the Farm of Teaching, Research and Extension of UNESP - Ilha Solteira, located in Selvíria, MS, during the soybean cultivation from December 2014 to April 2015

and the soybean cultivars 'BMX Potência RR' and 'BRS Valiosa RR' were sown at spacing of 0.45 m between rows, using 17 and 15 seeds per meter, respectively.

The experimental design was randomized blocks with four treatments and six replicates, arranged in a 2 x 2 factorial scheme. Treatments corresponded to two soybean cultivars ('BRS Valiosa RR' and 'BMX Potência RR'), with different types of growth and precocity, with and without inoculation with the diazotrophic bacteria *Azospirillum brasilense* at dose of 200 mL ha^{-1} (strains AbV5 and AbV6, with guarantee of 2 x 10^8 CFU mL^{-1}) in the seed, immediately before sowing, in the shade, and after seeds were completely dry following the chemical treatment. Each plot consisted of fourteen 15-m-long rows spaced by 0.45 m, totaling 94.5 m^2. The six central rows were used for evaluation, disregarding the single border, leading to a total sampling area of 40.5 m^2 per plot.

The following variables were evaluated: a) Number of pods per plant, by counting the pods of 10 plants per experimental plot, at harvest; b) 100 grain weight, determined on 0.01 g precision scale, at 13% (wet basis); and c) grain yield, determined by the harvest of plants contained in the 3 evaluated rows of each plot. After mechanical threshing, grains were quantified and the data were transformed to kg ha^{-1} at 13% (wet basis).

Economic analysis was performed using the structure based on the total operating cost (TOC) of production, used by the Institute of Agricultural Economics (IEA), according to Matsunaga & Toledo (1976), which consists in 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 costing interest rates, representing 5% of EOC (Matsunaga & Toledo, 1976), thus resulting in the total operating cost (TOC), which were extrapolated to one hectare.

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.

The analysis considered the prices paid in 2016 (AGRIANUAL, 2015) adjusted to those practiced in commercial plantations in the region of Selvíria, MS, based on the mean of the last 3 agricultural years. Simulations were made as if each treatment of the experiments represented commercial plantations. To facilitate the discussion, yield values were transformed to 60 kg sacks, because this is the basic marketing unit used by the local producers.

Results and Discussion

The interaction between cultivars and inoculation was significant for the number of pods per plant and 100 grain weight. For the number of pods, in the presence of co-inoculation with *Azospirillum brasilense*, the cultivar 'Potência' showed higher quantity of pods in comparison to non-inoculated treatments. For 100 grain weight, the cultivar 'Valiosa' exhibited higher values with and without co-inoculation with *A. brasilense* (Table 1), compared with the cultivar 'Potência'. However, both cultivars showed higher 100 grain weight when co-inoculation was performed (Table 1).

It is worth highlighting that the cultivars 'Potência' and 'Valiosa' showed increments of 25.7 and 10.2% in the number of pods and 5.7 and 1.6% in 100 grain weight, respectively, which led to higher soybean grain yield when *A. brasilense* was co-inoculated (Table 1). Grain yield increment with the co-inoculation of diazotrophic bacteria was equal to 545 kg ha^{-1}, equivalent to 11.2%, corroborating Hungria et al. (2013), who studied co-inoculation with *A. brasilense* and obtained increment of 16.1% in soybean grain yield, compared with the individual use of *Bradyrhizobium* strains.

According to Bárbaro et al. (2009), the literature reports that bacteria called PGPB (plant growth-promoting bacteria), such as *A. brasilense*, can act in the relationships between rhizobia and leguminous species, promoting increments in plant growth and grain yield, in total 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.

These effects can be due to various mechanisms, including early BNF of the nodules, increment in nodule dry matter, promotion of the occurrence of heterologous nodulation through the increase in the formation of root hairs and secondary roots, with increment 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). Furthermore, as pondered by Hungria et

Table 1. Pods per plant, 100 grain weight and grain yield of soybean as a function of cultivars and co-inoculation with *Azospirillum brasilense*, and interaction between cultivars and co-inoculation for the number of pods per plant and 100 grain weight

Co-inoculation	Pods per plant	100-grain weight (g)	Grain yield (kg ha^{-1})
With *A. brasilense*	70.00	17.13	5413 a
Without *A. brasilense*	61.63	16.35	4868 b
Cultivar			
'Potência'	64.63	15.84	5235 a
'Valiosa'	67.00	17.64	5046 a
Overall mean	65.81	16.74	5141
C.V. (%)	7.40	2.24	8.08
LSD (5%)	5.51	0.42	470

Follow-up analysis

Co-inoculation	Number of pods per plant	
	'Potência'	'Valiosa'
With	69.75 aA	70,25 Aa
Without	55.50 bA	63,75 Aa
LSD (5%)	7.79	

	100-grain weight	
	'Potência'	'Valiosa'
With	16.28 aB	17,98 aA
Without	15.40 bB	17,30 bA
LSD (5%)	0.60	

Means followed by the same letter, lowercase in the column and uppercase in the row, do not differ statistically by Tukey test at 0.05 probability level al. (2013), these effects promoted by co-inoculation with PGPB and rhizobia seem to be influenced by specific signals between the involve bacterial genotypes and the plant host genotype. Thus, further research on the response of co-inoculation as a function of genotypes is important for the development of more-responsive genotypes. Hence, the importance of this study is justified, since it demonstrated technical viability of co-inoculation with *A. brasilense* for the two most cultivated soybean cultivars in the Cerrado region.

Table 2 shows the total operating cost (TOC) structure of the soybean crop, described as Control treatment, in one hectare. This TOC structure model was used in all treatments. Initial investments with soil tillage and liming were not considered in this study, since these practices were not performed, especially because the area had been under no-tillage system (NTS) for more than 10 years when the experiments were installed, contributing to the reduction of initial costs of soybean crop implementation.

The effective operating cost (EOC), composed of expenses with operations and inputs, was equal to R$ 1,679.33 ha^{-1} and the total operating cost (TOC) corresponded to R$ 1,817.87 ha^{-1}.

In the operations composing the EOC, costs with irrigation stand out, totaling 20.8% of the expenses, because to obtain high grain yields in this cultivation period, besides the characteristics of the studied region (low-altitude Cerrado), it was necessary to adopt this technology (irrigation). However, the major expenses with inputs were fertilizers and pesticides, responsible for 36.64 and 10.44% of the EOC, respectively (Table 2). This is due to the great demand for nutrients by the soybean crop, to obtain high grain yields, besides the need for desiccation, control of weeds, pests and pathogens.

In general, the expenses with mechanized operations, followed by fertilizers, were the highest ones, corresponding to 42.3 and 33.2% of the TOC, respectively (Table 2). It is worth pointing out that, with the utilization of *A. brasilense*, the percentage of expenses with fertilizers tends to increase in relation to the TOC. However, the cost with *A. brasilense* inoculation is low, representing only 1.1% of the TOC.

For TOC and soybean yield of the treatments (Table 2), the highest TOC value occurred in treatments with *A. brasilense* inoculation, regardless of the cultivar. On the other hand, the lowest TOC value corresponds to treatments only with the inoculation of *Bradyrhizobium* strains. However, it should be highlighted that the highest soybean yields were obtained when *A. brasilense* was co-inoculated (Table 1).

Table 2. Estimate of total operating cost for the soybean crop, without inoculation with *A. brasilense* in one hectare, total operating cost (TOC), grain yield, gross revenue (GR), operating profit (OP) and profitability index (PI) of soybean as a function of cultivars and co-inoculation with *A. brasilense*

Production Cost					
Description	**Specification[1]**	**Nº times**	**Coefficient**	**Unit value (R$)**	**Total value (R$)**
A - Mechanized operations					
Desiccation	MH	1.00	0.50	85.00	42.50
Mowing (triton)	MH	1.00	0.50	85.00	42.50
Sowing	MH	1.00	1.00	110.00	110.00
Spray	MH	3.00	0.60	85.00	153.00
Harvest	MH	1.00	0.60	118.00	70.80
Irrigation (pivot)	mm	1.00	140.00	2.50	350.00
Subtotal A					768.80
B - Inputs					
Fertilizer 04-20-20	T	1.00	0.40	1.510.71	604.28
Fertilizer with cobalt and molybdenum	L	1.00	0.15	73.20	10.98
Inoculant (*B. elkanii and B. japonicum*)	100 mL	1.00	2.00	10.00	20.00
Inoculant (*A. brasilense*)	100 mL	0.00	2.00	10.00	0.00
Soybean seeds	50-kg sack	1.00	1.00	100.00	100.00
Herbicide Glyphosate	L	1.00	4.00	14.51	58.04
Herbicide 2,4-D	L	1.00	1.00	13.24	13.24
Herbicide Clorimuron	kg	1.00	0.03	146.68	4.40
Fungicide for seed treatment Carbendazim	L	1.00	0.05	45.57	2.28
Insecticide for seed treatment Thiamethoxam	L	1.00	0.10	407.68	40.77
Fungicide Azoxystrobin + Cyproconazole	L	1.00	0.30	150.89	45.27
Insecticide Methomyl	L	1.00	0.50	22.54	11.27
Subtotal B					910.53
Effective operating cost (EOC)					1,679.33
Other expenses					83.97
Costing interest rate					54.58
Total operating cost (TOC)					1,817.87

Without *A. brasilense*					With *A. brasilense*				
TOC R$	**YIELD***	**GR (R$)**	**OP (R$)**	**PI %**	**TOC**	**YIELD***	**GR (R$)**	**OP (R$)**	**PI %**
'Potência'									
1817,87	82.11	5,090.71	3,272.84	64.29	1,839.52	92.39	5,728.07	3,888.54	67.89
'Valiosa'									
1817,87	80.16	4,969.84	3,151.97	63.42	1,839.52	88.05	5,459.00	3,619.48	66.30
Mean	81.14	5,030.28	3,212.38	63.86	Mean	90.22	5,593.54	3,752.51	67.10

*YIELD in 60-kg sack; Soybean marketing price of R$ 62.00 per sack

Regarding soybean yield, highest values were found with the cultivar 'Potência' co-inoculated with *A. brasilense*, which exhibited mean yield of approximately 92 sacks of 60 kg of grains, about 10 sacks more compared with the same cultivar without co-inoculation (Table 2). The mean yield was approximately 90 sacks in inoculated treatments and 81 sacks in non-inoculated treatments.

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 parameters, attenuation/minimization of stress and biological control of some pathogenic agents.

For the gross revenues per hectare (Table 2), obtained in the combinations of the treatments for the soybean crop, it is observed that, with a constant price of soybean, the gross revenues of the treatments follow the same trend of the yields (Table 2), i.e., increments in revenue occur because of the increments in grain yield. This result is consistent with that of Duete et al. (2009), who claimed that yield is a primordial factor to guarantee good profitability to the producer. Still according to these authors, 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, increases grain yield and the gross margin of the crops.

For the values referring to operating profit (Table 2), OP was positive for all treatments, regardless of the cultivar and co-inoculation with *Azospirillum brasilense*. Highest OP was caused by the co-inoculation with *A. brasilense* in the seeds of the cultivar 'Potência' (R$ 3,888.54). In the absence of inoculation with *A. brasilense*, which would cause reduction of costs, with possibility of increase in LO, if good yields were obtained, the soybean crop would be viable, but with a R$ 615.70 ha^{-1} lower profit, a reduction of 15.8% in the profitability. On average, treatments with co-inoculation, regardless of the cultivar, showed R$ 540.13 ha^{-1} higher profit compared with non-inoculated treatments, which is equivalent to an increment of 14.4%.

As evidenced for OP, the treatment leading to highest PI was co-inoculation with *A. brasilense* in the seeds of the cultivar 'Potência' (67.89%), which was 3.6% superior to that of non-inoculated treatment (Table 2). On average, co-inoculation caused increment of 3.24% in PI, compared with non-inoculated treatments, regardless of the cultivar, reinforcing the importance of co-inoculation with *A. brasilense* to obtain superior yields and, consequently, higher financial return.

The economic results obtained here agree with the consideration of Hungria et al. (2013). For these authors, microbial inoculants are very inexpensive and, considering only the soybean crop, Brazil is estimated to save more than US$ 7 billion per year with BNF. Therefore, the potential of co-inoculation with *A. brasilense* in the nutrition and yield of soybean is very high, especially for being a technique with low cost and investment, of easy application and use, not pollutant and, in addition, inserted in the currently desired sustainable context.

Conclusions

1. The soybean cultivars 'Potência' and 'Valiosa' co-inoculated with *Azospirillum brasilense* in the seeds showed higher number of pods per plant, 100 grain weight and grain yield.

2. Co-inoculation with *A. brasilense* was technically and economically viable for both soybean cultivars, but 'Potência' co-inoculated leads to the highest profitability.

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