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Soybean cultivars agronomic performance and yield according to doses of *Azospirillum brasilense* applied to leaves

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Abstract

Alternative methods of inoculation such as the application of the bacteria *Azospirillum brasilense* via spray in an advanced stage of the crop can increase the soybean performance. The aim of this study was to evaluate the agronomic traits and grain yield in soybean crops through *Azospirillum brasilense* doses applied by spray coverage. The experiment was conducted in randomized blocks in a 4 × 6 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and six doses of *Azospirillum brasilense* (0, 300, 400, 500, 600, 700 mL ha^{-1}) with three replications in two crop years. The characters such as plant height, shoot dry mass, chlorophyll content, leaf nitrogen content at flowering were evaluated. At harvest, the evaluated characters were plant height, height of the first pod insertion, number of pods per plant, number of grains per pod, thousand grain mass, grain yield and grain harvest index. For the conditions that this study was carried out and according to the results, the spray with different *Azospirillum brasilense* doses did not affect the agronomic variables studied and the grain yield in RR® soybean cultivars.

Keywords: agronomic traits; growth promoting bacteria; *Glycine max* (L.) Merrill.

Abbreviations: BNF_ Biological nitrogen fixation; RR_Roundup Ready; PHF _Plant height on flowering; SDM_Shoot dry mass; CC_chlorophyll content; NC_N content; PHH_Plant height at harvest; IFP_ height of the first pod insertion; NPP_Number of pods per plant; NGP_Number of grains per pod; TGM_Thousand grain mass; GY_Grain yield; GHI_ Grain harvest index. G.M.R._Group of maturity; CFU_ Colony Forming Unit; R_1_ Phenological Stage – flowering; SPAD_Chlorophyllmeter; GHI_Grain harvest index.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops in Brazil and worldwide. According to the Brazilian National Company of Food and Supply - Conab (2015), in 2014/15 crop year, the soybean area under cultivation in Brazil reached approximately 31,902,400 ha, with an average grain yield of 3011 kg ha^{-1}. From the perspective of nitrogen fertilization on soybeans, the use of *Bradyrhizobium*, which perform the biological nitrogen fixation (BNF), was one of the major driving for large-scale cultivation in Brazil (Zuffo et al., 2015a). However, when considering the current limitations (high temperatures, water stress, nutritional factors and the seed treatments with fungicides and insecticides) and potential benefits of BNF on soybeans and also the promoted benefits on different crops with the inoculation of *Azospirillum brasilense*, it can be inferred that these bacteria could increase the soybean crop performance (Hungria et al. 2007). Bacteria of the genus *Azospirillum* have the advantages of being endophytic or can penetrate plant root, presents antagonism to pathogens, are able to produce phytohormones, are not very sensitive to temperature variations and can be present in all soil and climate types (Araujo, 2008). Among the plant hormones, research has demonstrated the ability of *Azospirillum brasilense* to produce auxin, gibberellins and cytokinins under "in vitro" conditions (Masciarelli et al., 2013). It is possible to find on literature the positive effects of *Azospirillum brasiliense* use on legume crops (Hungria et al., 2013; Hungria et al. 2015), however, it is questionable if the benefits are only due to increased nodulation and nitrogen fixation, or if other indirect factors are involved. For Yadegari et al. (2008), these bacteria improve the drought resistance. The traditional inoculation of nitrogen fixing bacteria has been done on seeds, however, according to Campo et al., (2009) and Zilli et al. (2009), the success of the inoculation depends on environmental conditions and management practices before inoculation, such as seed chemical treatments with insecticides and/or fungicides. Thus, alternative methods of inoculation, as the spray bacteria application in crops in advanced stages, can provide interesting results. In addition, different doses of inoculants can be a viable strategy to determine the amount to be used in new areas without inoculation history or in areas with high soil acidity, where the recommendation is the application of double dose (Chueiri et al., 2005). Therefore, all those management practices are due to accomplish the modern requirements of agriculture, seeking the economic, social and environmental sustainability (Chaparro et al., 2012). Thus, the aim of this study was to evaluate the agronomic treatments and grain yield in soybeans according to *Azospirillum brasilense* doses applied by spray coverage.

Results and Discussion

For all evaluated variables, significant influence ($p \leq 0.01$) of the crop year was observed (Tables 2 and 3). Soares et al. (2015a) also observed the influence of this source of variation in soybean agronomic characteristics. Except for the number of grains per pod and the grains index of harvest, the cultivars promoted a significant influence ($p \leq 0.01$) on other studied variables (Tables 2 and 3). This fact was already expected due to the cultivar different genetic background, growth habit, maturation group and other atributes that promoted these kind of variation (Soares et al. 2015a; Felisberto et al. 2015).

For the doses of *Azospirillum brasilense* source of variation, no significant effect was observed for the evaluated variables (Tables 2 and 3). The lack of benefitial effects with the use of *Azospirillum brasilense* inoculation was also verified by Gitti et al., (2012), Bassini et al. (2015), Zuffo et al. (2015a) and Zuffo et al. (2015b). The authors used the *Azospirillum* application in the seed or in the planting furrow and did not observed advantages in the use of the bacteria on soybean agronomic variables.

For the cultivar and crop year interaction, statistical differences was observed for plant dry mass, nitrogen content, height of the first pod insertion, number of pods per plant, thousand grain weight and grain yield (Tables 2 and 3). Soares et al (2015b) also verified the interaction of cultivar x crop year on the soybean, showing that the response to environmental variations was not the same for the evaluated cultivars. To other interactions, no statistical difference (Tables 2 and 3) was observed. Thus, the variation of these factors were not the same.

The higher values for shoot dry mass, chlorophyll content, plant height on harvest, number of pods per plant, number of grains per pod, grain yield and harvest index were observed during the 2013/14 crop year. This is related to climatic conditions (Figure 1) mainly during January flowering, with a higher rainfall index for the 2013/14 crop year. Except for thousand grain mass, the higher values for other characters reflected on grain yield and on higher harvest index. Although it is reported on literature that bacteria that promotes plant growth, like the ones from *Azospirillum* gender, can also promote plant growth, plant hormones production, increase the shoot and root dry mass and increase water deficit resistance (Bashan and Bashan, 2005), this benefits were not observed in this study. The efficiency lack of *Azospirillum brasilense* inoculation doses on soybean is probably due to the low capacity of competition between the native *Azospirillum diazotrophic* bacteria and soil microflora (Didonet et al., 2000). Therefore, the competition between bacteria may have inhibited the effect of *Azospirillum brasilense*. BRS 820 RR® presented grain yield of 4063 kg ha^{-1} and is the latest cultivar (G.M.R 8.2), when compared to others, evaluated in the present study. For the crop year and cultivar interaction, the cultivar BRS 820 RR® presented the lowest mean for dry mass (Figure 2A) during the 2013/14 crop year and for N content in leaf tissues (Figure 2B) during the 2014/15 crop year. For height of the first pod insertion, the cultivar Anta 82 RR® presented the lower mean during the two crop years but it is above the minimum height (10 cm) (Figure 2C). The number of pods of BRS Favorita RR® cultivar was the lowest during both evaluated crop years, but during the 2014/15 crop year it was not statistically different from Anta 82 RR® (Figure 2D). For thousand grain mass, the cultivars BRS Favorita RR® and BRS 820 RR® presented the higher mean during both evaluated crop years, but during the 2013/14 crop year it was not statistically different from BRS 780 RR® (Figure 2E). For grain yield the cultivar BRS 820 RR® presented the best yield performance for both evaluated crop years (Figure 2F). Thus, the plasticity of BRS 820 RR® cultivar was clear. According to the conditions that this study was carried out and the results, the spray with different *Azospirillum brasilense* doses did not affect the agronomic variables studied and the grain yield in RR® soybean cultivars.

Materials and Methods

The experiment was performed during the 2013/14 and 2014/15 crop years, in Lavras - MG, Brazil, at the Scientific and Technological Development Center of Agriculture – Muquém Farm, located at 21°14'S latitude, 45°00'W longitude and altitude of 918m in a soil classified as Dystroferric Red Oxisol, with clayey, with the following textural values: Clay: 640 g kg^{-1}; Silt: 200 g kg^{-1}; Sand: 160 g kg^{-1}. The soil chemical analysis was realized as reccomended by EMBRAPA (1997). The chemical composition of the experimental area soil is shown on Table 1. The region climate is Cwa, according to the Köppen classification, with annual average temperature of 19.3ºC and normal annual rainfall of 1,530 mm (Dantas et al., 2007). The climatic data during the experiments were collected at the meteorological station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras-UFLA, Brazil, and are presented on Figure 1.The experiment was conducted in randomized blocks in a 4 x 6 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and six doses of *Azospirillum brasilense* (0, 300 , 400, 500, 600, 700 mL ha^{-1}) with three replications. Each plot was consisted of four sowing lines of 5 m in length, spaced with 0.50 m, and the area of each plot was 10 m^2 (5 m x 2 m)., being considered the area of the two central rows. The sowing was held in November of each crop year and the fertilization consisted of 350 kg ha^{-1} made of N-P_2O_5-K_2O (02-30-20), applied via groove. The *Bradyrhizobium japonicum* bacteria were inoculated via groove after soybean sowing. The *Bradyrhizobium japonicum* dose was 18 mL p. c. kg^{-1} of seed - strains SEMIA 5079 and 5080, containing 10.8 x 10^6 CFU/seed inoculant Nitragin Cell Tech HC® (3 x 10^9 CFU/ml). The *Azospirillum brasilense* was applied in V_3 stage (open of the second trefoil), using the Azo® inoculant (1 x 10^8 CFU/mL) with AbV_5 and AbV_6 strains. The microorganisms application was performed using a motorized backpack sprayer coupled to a bar with four spray nozzles XR 110.02, applying a water volume equivalent to 150 L ha^{-1}. The cultural trais (weeds, insects and plant diseases control) applied before and after sowing were the ones recommended for the region, when necessary. At the beginning of flowering (R_1) the following variables was determined on five plants per plot: plant height - with a millimeter ruler, and shoot dry mass - using a forced air circulation oven at 60°C for 72 hours until constant weight, with subsequent weighing of the plant residues. It was also held the collection of leaves (third trifoliate from top to bottom) then they were washed with deionized water and placed to dry in order to calculate shoot dry mass. The dried leaves were ground in a Wiley mill. Chemical analysis of nitrogen content on leaf tissue of were conducted according to the methodology described by Sarruge & Haag (1974) and the leaf chlorophyll content was carried out using a chlorophyllmeter SPAD 502 Plus®, with three measurements on different parts of the trifoliate leaves, at the leaf blade, between the third trifoliate ribs, from top to bottom. At harvest the following characteristics were obtained: plant

Table 1. Chemical composition of a typical Red Distroferric Oxisol (0-0.20m) before the experiment, during 2014/14 and 2014/15 crop years.

Crop year	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^{+}+Al^{3+}$	SB	CTC	P	K	OM	V
	H_2O	---------------------- $cmol_c\ dm^{-3}$ ----------------------						-- $mg\ dm^{-3}$ --		dag/kg^{-1}	%
2013/14	6.4	5.0	1.4	0	2.9	6.7	9.6	11.46	118	3.41	69.82
2014/15	6.2	3.80	0.80	0	0.9	4.8	5.7	20.83	92	2.23	83.52

H + Al: potential acidity; SB: sum of basis; CTC: cation Exchange capacity at pH 7.0; OM: organic matter; V: basis saturation index.

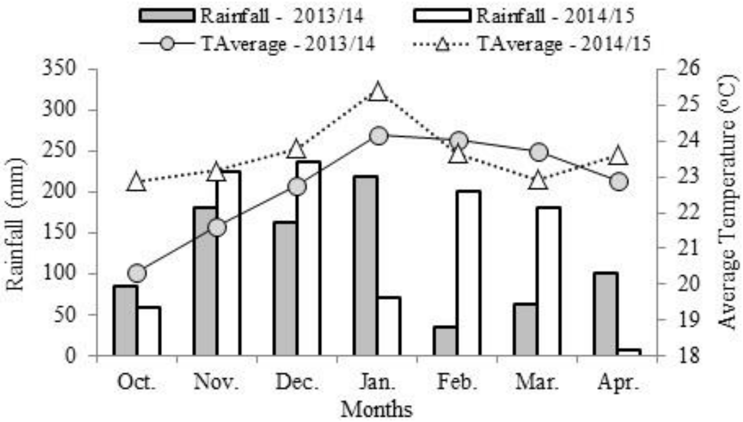

Fig 1. Means for rainfall and air temperature in Lavras, MG, Brazil, during 2013/14 and 2014/15 crop years in during the experiments conduction. Source: National Institute of Meteorology (INMET).

Table 2. Variance analysis and means for plant height on flowering (PHF), shoot dry mass (SDM), chlorophyll content (CC) and N content (NC) on leaf tissues of RR® soybean at full flowering (R_1), obtained during the experiment with doses of *Azospirillum brasiliense* applyed in V_3 stage leaves in RR® soybean cultivars, during the 2013/14 and 2014/15 crop years. Lavras, MG, Brazil.

Factors	DF	ANOVA (MS)¹ PHF	SDM	CC	NC
		- - - - - cm - - - - -		-	g/kg
Block(B)	2	10,12	9,75	5,30	8,68
Year (Y)	1	638.40**	14351.24**	187.15**	1164.51**
Cultivar (C)	3	768.78**	178.83*	61.70**	33.45*
Dose (D)	5	3.79ns	98.01ns	8.20ns	6.11ns
Y x C	3	18.13ns	208.63*	19.27ns	30.82*
Y x D	5	18.27ns	75.19ns	3.95ns	5.91ns
C x D	15	25.07ns	48.65ns	6.39ns	9.55ns
Y x C x D	15	27.82ns	98.19ns	6.59ns	5.45ns
B x Y	2	28.73ns	161.50ns	8.16ns	2.40ns
Error	92	23.16	63.77	10.55	9.30
Mean	-	54.36	45.82	40.22	42.18
CV (%)	-	8.85	17.43	8.08	7.25
Factors	Means²				
Crop year²					
2013/14		52.26 b	55.80 a	41.36 a	39.34 b
2014/15		56.47 a	35.81 b	39.08 b	45.02 a
Azos® Doses (mL)					
0		54.60	43.25	39.45	41.81
300		54.81	44.77	40.41	42.22
400		54.60	44.74	39.70	41.93
500		53.91	48.20	41.03	43.09
600		54.38	48.23	40.22	41.70
700		53.86	45.73	40.56	42.35
Cultivars³					
Anta 82 RR®		48.72 c	44.46 b	38.78 b	41.79 b
BRS Favorita RR®		53.72 b	47.12 a	41.06 a	42.41 a
BRS 820 RR®		55.71 b	43.47 b	39.49 b	41.12 b
BRS 780 RR®		59.57 a	48.23 a	41.57 a	43.39 a

¹ ** and * significant at 1 and 5% of probability, according to F test, respectivelly. ns – not significant; MS – Mean Square; DF – degree of freedom; CV – coefficient of variation.

² means followed by the same letter are not different according to F test.

³ means followed by the same lower case in the column are not different according to Scott Knott test (1974) at 5% of probability.

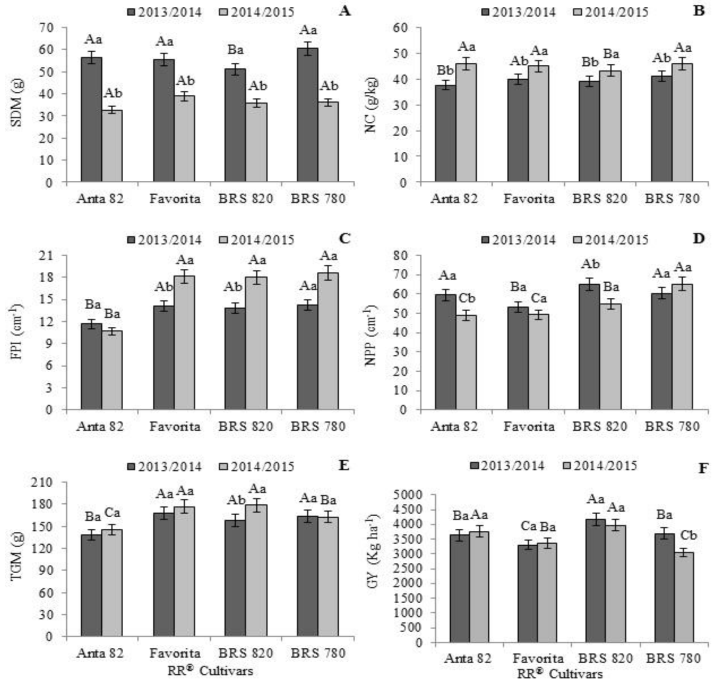

Fig 2. Shoot dry mass - SDM (A), N content in leaf tissues – NC (B), insertion of the first pod – FPI (C), number of pods per plant – NPP (D), thousand grain mass – TGM (E), grain yield – GY (F) in RR® soybean cultivars during the 2013/14 and 2014/15 crop years in Lavras, MG, Brazil. Means followed by the same capital letter in the same crop year and the same lower case for the same cultivar are not different according to Scott Knott (1974) test at 5% of probability.

Table 3. Variance analysis and mean values for plant height at harvest (PHH), insertion of the first pod (IFP), number of pods per plant (NPP), number of grains per pod (NGP), thousand grain mass (TGM), grain yield (GY) and grain harvest index (GHI) in RR® soybean at full maturation stage (R_8) obtained in the experiment with doses of *Azospirillum brasiliense* applied on V_3 stage leaves in RR® soybean cultivars during the 2013/14 and 2014/15 crop years. Lavras – MG, Brazil.

Factors	GL	ANOVA (MS)¹						
		PHH	IFP	NPP	NGP	TGM	GY	GHI
		- - - - - cm - - - - -		- - - - - unity - - -		- - - g - - -	Kg ha^{-1}	-
Block (B)	2	1.19	7.67	33.90	0.20	723.85	258974.97	0.0043
Year (Y)	1	750.76**	307.12**	902.00**	1.71**	2967.07**	892422.78**	0.4722**
Cultivar (C)	3	588.85**	225.03**	978.58**	0.07^{ns}	6660.63**	4149758.20**	0.0026^{ns}
Dose (D)	5	26.58^{ns}	0.86^{ns}	12.70^{ns}	0.04^{ns}	343.30^{ns}	170814.04^{ns}	0.0011^{ns}
Y x C	3	61.60^{ns}	60.67**	458.11**	0.14^{ns}	694.40*	1073466.54**	0.0002^{ns}
Y x D	5	27.96^{ns}	2.14^{ns}	74.07^{ns}	0.02^{ns}	67.80^{ns}	39668.14^{ns}	0.0002^{ns}
C x D	15	26.31^{ns}	3.15^{ns}	48.04^{ns}	0.05^{ns}	224.27^{ns}	135165.25^{ns}	0.0018^{ns}
Y x C x D	15	311.20^{ns}	4.38^{ns}	68.13^{ns}	0.18^{ns}	286.48^{ns}	181375.82^{ns}	0.0002^{ns}
B x Y	2	40.55^{ns}	2.21^{ns}	87.54^{ns}	0.09^{ns}	0.74^{ns}	171575.42^{ns}	0.00002^{ns}
Error	92	32.41	4.22	72.73	0.08	185.87	120523.19	0.0012
Mean	-	70.01	14.91	57.04	1.99	161.20	3614.92	0.45
CV (%)	-	8.13	13.78	14.95	14.66	8.46	9.60	7.92
Factors	Means²							
Crop year²								
2013/14		72.30 a	13.42 b	59.55 a	2.10 a	156.66 b	3693.65 a	0.50 a
2014/15		67.73 b	16.37 a	54.54 b	1.88 b	165.74 a	3536.20 b	0.39 b
Azos® Doses (mL)								
0		68.62	14.79	57.04	1.94	162.65	3593.76	0.45
300		69.78	14.98	57.25	2.04	154.82	3528.22	0.46
400		69.10	14.92	56.28	2.01	158.97	3601.64	0.44
500		71.32	14.94	56.30	1.93	161.59	3546.00	0.44
600		70.98	14.63	58.24	2.01	164.02	3758.08	0.45
700		70.29	15.19	57.09	1.99	165.15	3661.84	0.45
Cultivars³								
Anta 82 RR®		67.82 b	11.17 b	54.19 b	2.01 a	141.59 c	3691.24 b	0.46 a
BRS Favorita RR®		65.41 b	16.12 a	51.35 b	1.92 a	172.15 a	3335.57 c	0.44 a
BRS 820 RR®		73.41 a	15.95 a	59.83 a	1.99 a	168.09 a	4063.82 a	0.45 a
BRS 780 RR®		73.41 a	16.40 a	62.80 a	2.02 a	162.97 b	3369.07 c	0.44 a

¹ ** and * significant at 1 and 5% of probability, according to F test, respectively. ^{ns} – not significant; MS – Mean Square; DF – degree of freedom; CV – coefficient of variation. ² means followed by the same letter are not different according to F test. ³ means followed by the same lower case in the column are not different according to Scott Knott test (1974) at 5% of probability.

height and height of the first pod insertion - with the aid of a millimeter ruler. Later, five plants per pod were collected in order to assess the number of pods per plant and number of grains per pod - manually counted; the one thousand grain mass - according to the methodology described in Brasil (2009); the grain yield - standardized for grain moisture of 13% in Kg/ha^{-1}. It was also determined the grain harvest index (GHI) using the formula GHI = grain yield / grain yield + straw. The individual and joint variance analyses were performed adopting the statistical model and similar analysis procedure provided by Ramalho et al. (2012). The means were aggregated by Scott-Knott test (1974) and the statistical analysis was performed using the statistical package SISVAR® (Ferreira, 2011).

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