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Full Length Article

Rhizobia and Azospirilla Co-Inoculation Boosts Growth and Productivity of Common Bean

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Abstract

The rhizobia and azospirilla combined use is an alternative to N-fertilizers, besides to guarantee high grain yields for the crops. In this work we evaluated the effect of on seed rhizobia and on aerial part azospirilla co-inoculation on some selected production parameters of common bean. A random block design with four replicates was used for the field experiments. The evaluated treatments were: 1- AC- Absolute control; 2- NfT- Nitrogen fertilization; 3- Rt; 4- Rt + Ab1l; 5- Rt + Ab2l; 6- Rt + Ab3l; 7- Rt + Ab4l and 8- RP- Registered product. *Rhizobium tropici* (Rt) was applied using 2 doses ha^{-1} and, *Azospirillum brasilense* (Ab) was applied using 1, 2, 3 or 4 doses ha^{-1} at V_2/V_3 phenological stage, while the RP treatment received two 2 ha^{-1} of *R. tropici* and 3 doses ha^{-1} of *A. brasilense* at V_2/V_3 phenological stage. Nodulation (nodule number – NN, nodule dry weight – NDW); growth (shoot dry weight – SDW, root dry weight - RDW) and, productive (number of pods – NP; number of grains - NG, and grain yield - GY) parameters were evaluated. The Rt+Ab3l co-inoculation provided an increase in NN, MNS, NV and NG, in addition to presenting the highest GY stability, producing about 3439 kg ha^{-1} as an average from the five locations, which represents an increase of 100, 93, 74 and 83 kg ha^{-1} in comparison to TN, PR, Rt, and TA, respectively.

Key words: Biological nitrogen fixation; Plant-bacteria interaction; Symbiosis; Inoculation; Co-inoculation

Introduction

Nitrogen fertilizers are crucial for the development and attainment of high yields in the common bean crop (Lacerda *et al.* 2019). However, the excessive use of nitrogen fertilizers is quite common among producers, who apply doses higher than 120 kg ha^{-1} of N (Peres *et al*. 2018). Under these conditions, nitrogen fertilizers pose risks to the environment, mainly through the emission of greenhouse gases, in addition to the high costs and losses through leaching and volatilization (Souza and Ferreira 2017).

Co-inoculation is a viable alternative to replacing nitrogen fertilization, characterized by the associated use of plant growth-promoting microorganisms, exhibiting different mechanisms of action, such as the association of the bacteria *Rhizobium tropici* and *Azospirillum brasilense*. Co-inoculation results in a synergistic effect, which provides a greater stimulus compared to the effect of each mechanism of action alone (Hungria *et al.* 2013). Azospirillum bacteria are strongly related to the production of phytohormones, such as indole acetic acid (Masciarelli *et al.* 2013), gibberellins (Lenin and Jayanthi 2012), cytokinins and ethylene (Strzelczyk *et al.* 1994), providing better plant development, greater absorption of nutrients, increased tolerance to water deficit (Huergo *et al.* 2008), due to the increase in the exploited soil area triggered by the greater root development (Puente *et al.* 2018).

The co-inoculation is an environmentally friendly option, which can partially or totally replace the use of N-fertilizers inputs. This fact was highlighted in the study conducted by Bettiol *et al.* (2021), where co-inoculation increased the grain yield by 187.75 kg ha^{-1} in camparison with the nitrogen fertilization, thus demonstrating the ability of co-inoculation to replace nitrogen fertilizers in common bean cultivation.

In general, seed inoculation is the most used form of application of plant growth-promoting rhizobacteria (PGPR). However, considering the possibilities of inoculation via seed, furrow, or foliar, there are different technical recommendations for carrying out co-inoculation. In those where *R. tropici* is applied *via* seed and *A. brasilense* via spraying the plants, the best results were observed with the highest concentration of *A. brasilense* (Souza and Ferreira 2017). These authors stated that the application of three doses of *A. brasilense* via foliar resulted in production increases equivalent to 278 and 560 kg ha^{-1} in

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comparison to treatments with nitrogen fertilization and inoculation only with *R. tropici*, respectively. Azospirillum foliar spraying is an alternative that allows microorganisms to interact with the phyllosphere, altering the dynamics and composition of plant hormones (Gonzalez-Lopez *et al.* 1991; Filipini *et al.* 2021). The use of PGPR's foliar spraying increased the common bean production (Souza and Ferreira 2017), corn biomass production (Portugal *et al.* 2016; Filipini *et al.* 2021) and improved soybean nodulation (Puente *et al.* 2018; Filipini *et al.* 2021).

Knowing the benefits of *A. brasilense* as a plant growth promoter and its help in N fixation improvement when associated with *R. tropici*, it is essential to study different forms and doses of Azospirilla application in field experiments. Thus, this work aimed to determine the effect of the co-inoculation of rhizobia and different doses of azospiilla applied *via* foliar spraying over nodulation, growth and grain production parameters of the common bean cropped under field conditions in commercial farms.

Materials and Methods

Location and experimental areas detailing

Five field experiments were performed in three different cropping seasons (winter 2018, water 2018–2019, winter 2019, and water 2019–2020) in different areas, to evaluate the efficiency of the treatments in different soil and climatic conditions, cultural treatments, and technological levels of each experimental area. Detailed information is presented in Table 1.

As defined by the Köppen classification, the experimental areas show an Aw climate, tropical savanna, megathermal. The average annual temperature ranges from 20.5°C to 23°C and average annual rainfall from 1465 to 1600 mm. The climatic conditions during the periods of the experiments are presented in Fig. 1.

For the chemical analysis of the soil, 10 soil subsamples were taken from each experimental area at 0–20 cm depth before sowing. The subsamples were homogenized to generate a composite sample used to determine the chemical characteristics: Soil pH; Exchangeable Ca, Mg, and Al; P, K, Cu, Zn, Fe and Mn contents and, soil organic matter according to Donagema *et al*. (2011). The soil analysis results are presented in Table 2.

Experimental planning

Commercial inoculants formulated separately with SEMIA 4077 strain of *R. tropici* and Ab-V5 strain of *A. brasilense* were used: 1) Biomax Premium turfoso Feijão, containing *R. tropici* in a concentration of 2x10^9 CFU mL^{-1} (Colony Forming unit) and 2) Biomax Premium Azum, containing *A. brasilense* in a concentration of 3x10^8 CFU mL^{-1}. Both inoculants were provided by Vittia Fertilizantes e Biológicos Ltda. These inoculants were compared with the inoculant registered for the common bean crop, provided by Stoller do Brasil Ltda, as follow: Masterfix Feijão (turfoso), containing *R. tropici* in a concentration of 2x10^9 CFU g^{-1} and Masterfix Gramíneas (liquido), containing *A. brasilense* in a concentration of 2x10^8 CFU mL^{-1}.

Eight treatments were evaluated, consisting of a inoculation only with *R. tropici* (Rt), four combinations of *R. tropici* and *A. brasilense* doses, an absolute control (AC - without co-inoculation and without N fertilization), a control N-fertilization (NfT - without co-inoculation), and the registered product (RP). In N-fertilizer treatment (NfT), 80 kg ha^{-1} of N were used, with 20 kg ha^{-1} of N applied at sowing and 60 kg ha^{-1} of N applied at the V_4 stage using urea. Complementary information about the eight treatments is given in Table 3.

Experiments were performed under field condictions on a random block design with 4 repetitions. The experiments were installed in plots composed of six four-meter-long rows, spaced by 0.45 m between rows, totaling 10.8 m² per plot.

Experimental areas management

Aiming to lead the base soil saturation to 70% and the soil pH to 5.5, limestone was applied 50 days before the experiment settlement, according to the needs of each location as shown in Table.

The fertilization with phosphorus (P_2O_5) and potassium (K_2O) was done in the sowing operation, according to the needs indicated by soil analysis. According to the results of the soil chemical analysis and the technical recommendation for the common bean crop (Carvalho and Silveira 2021), the experiments conducted in Formosa-GO/2018–19 and Cristalina-GO/2019 did not require fertilization.

In SAG-GO/2018, SAG-GO/2019 and SAG-GO/2019–20 were needed to apply 270, 262.5 and, 260 kg ha^{-1} of Triple superphosphate, respectively. However, 90 and 87.5 kg ha^{-1} of potassium chloride were applied only in SAG-GO/2018 and SAG-GO/2019, respectively.

The phytosanitary control was performed after monitoring and evaluation of possible economic damage using products registered for the common bean. For weed control, in the experiments conducted in SAG-GO/2018, SAG-GO/2019, and SAG-GO/2019–20 seven days before sowing (DBS) desiccation of the areas was done with the herbicide Paraquat - SL 200 g L^{-1} IA (2.0 L ha^{-1}). In the experiment from Formosa-GO/2018–19, ten DBS desiccation of the area was carried out with the herbicides Aurora - EC 400 IA g L^{-1} IA (50 mL ha^{-1}) and Roundup - SL 445 IA g L^{-1} (2.0 L ha^{-1}). In the experiment from Cristalina-GO/2019, five DBS desiccation of the area was accomplished with the herbicide Roundup - SL 445 IA g L^{-1} (2.0 L ha^{-1}).

Pre-emergence herbicide application was done, between 2–3 days after sowing (DAS), in SAG-GO/2018,

Table 1: Location, cropping season, Geographical coordinates, altitude, and previous crops of the experimental areas.

Location/cropping season	Geographical coordinates		Altitude	Previous crop
	Latitude (S)	Longitude (W)	m	
SAG-GO/2018	16º29`16.20"	49º17`57.80"	777	Corn
Formosa-GO/2018-19	15º44`05.64"	47º26`06.71"	996	Corn
Cristalina-GO/2019	16º56`14.70"	47º46`02.70"	905	Wheat
SAG-GO/2019	16º29`15.22"	49º17`54.74"	776	Corn
SAG-GO/2019-20	16º29`55.76"	49º17`15.54"	806	Corn

SAG- Santo Antônio de Goiás

Table 2: Chemical characteristics of the soil in the 0-0.20 m layer of the experimental areas where the field experiments were conducted

Location/cropping season	pH	Ca	Mg	Al	H+Al	SB[1]	CEC[2]	BS[3]	SOM[4]
	H_2O	mmolc dm^{-3}				cmolc dm^{-3}		%	g kg^{-1}
SAG-GO/2018	5.4	18.5	8.7	1	29	2.80	5.7	49	31.27
Formosa-GO/2018-19	5.6	45.6	12.0	0	24	6.57	9.0	73	46.93
Cristalina-GO/2019	5.8	38.5	13.6	0	22	5.69	7.9	72	51.90
SAG-GO/2019	5.8	17.0	9.8	0	24	2.87	5.3	54	28.91
SAG-GO/2019-20	5.8	18.2	9.0	0	24	3.09	5.5	56	23.00

Location/cropping season	P	K	Cu	Zn	Fe	Mn
			mg dm^{-3}			
SAG-GO/2018	14.9	33	1.0	4.0	31.0	5.8
Formosa-GO/2018-19	40.2	281	0.5	6.3	15.9	21.6
Cristalina-GO/2019	45.7	187	2.1	8.1	32.7	22.2
SAG-GO/2019	11.1	75	1.3	5.2	33.8	10.4
SAG-GO/2019-20	24.9	145	1.3	5.2	11.6	40.8

[1]SB = sum of bases; [2]CEC = cation exchange capacity; [3]BS = bases saturation ((K + Ca + Mg)/Tcec) × 100, where Tcec = K + Ca + Mg + total acidity at pH 7.0 (H + Al); [4]SOM = Soil organic matter; [5]SAG- Santo Antônio de Goiás

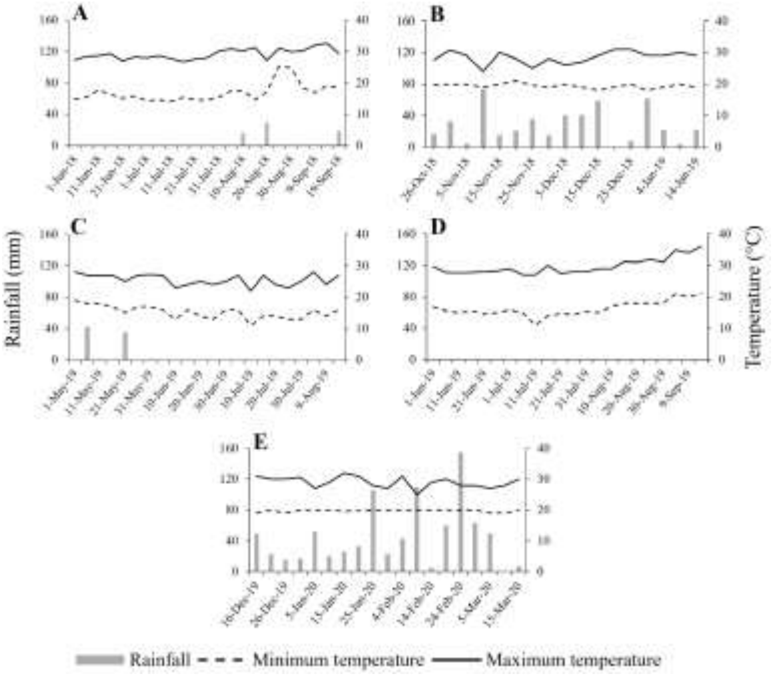


Fig. 1: Rainfall, maximum and minimum mean temperatures during the experimental periods. SAG-GO/2018 (**A**), Formosa/2018-19 (**B**), Cristalina/2019 (**C**), SAG-GO/2019 (**D**) and SAG-GO/2019-20 (**E**)

SAG-GO/2019, and SAG-GO/2019–20, using Gramoxone - SL 200 IA g L^{-1} (2.0 L ha^{-1}). Pre-emergence application was also performed in Formosa-GO/2018–19 and Cristalina-GO/2019, using Gramocil - SC 200 g L^{-1} IA (2.0 L ha^{-1}). In SAG-GO/2018, SAG-GO/2018–19, SAG-GO/2019 and Formosa-GO/2018–19, post-emergence herbicide application was done, between 20 to 30 days after emergence (DAE), using Flex - SL 250 g L^{-1} IA (1.0 L ha^{-1}) and Fusilade - EW 250 g L^{-1} IA (0.75 L ha^{-1}).

Table 3: Description of the treatment composition for the field experiments

Treatments[1]	Amount of individual treatment components				
	Nitrogen Fertilizer	*R. tropici*		*A. brasilense*	
		Biomax Premium Turfoso Feijão	Masterfix Feijão (turfoso)	Biomax Azum	Masterfix Gramíneas
AC	-	-	-	-	-
NfT	80 kg ha^{-1}	-	-	-	-
Rt	-	2 doses ha^{-1}	-	-	-
Rt + Ab1l	-	2 doses ha^{-1}	-	1 dose ha^{-1}	-
Rt + Ab2l	-	2 doses ha^{-1}	-	2 doses ha^{-1}	-
Rt + Ab3l	-	2 doses ha^{-1}	-	3 doses ha^{-1}	-
Rt + Ab4l	-	2 doses ha^{-1}	-	4 doses ha^{-1}	-
RP	-	-	2 doses ha^{-1}	-	3 doses ha^{-1}

[1] AC = without co-inoculation and without N-fertilizer; NfT = 80 kg N ha^{-1} (20 kg N ha^{-1} applied at sowing and 60 kg N ha^{-1} applied at V_4 stage; Rt = seed inoculation with *R. tropici* (2.4x10^{7} cells seed^{-1}); Ab = spraying inoculation with *A. brasilense* in different concentrations (1l- 0.8×10^5 cells plant^{-1}; 2l- 1.6×10^5 cells plant^{-1}; 3l- 2.4×10^5 cells plant^{-1}; and 4l- 3.2×10^5 cells plant^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4x10^{7} cells seed^{-1} and leaf inoculation with *A. brasilense*- 2.4×10^5 cells seed^{-1})

The experiments conducted in SAG-GO/2018 and SAG-GO/2018–19 witnessed the occurrence of *Bemisia tabaci*, requiring 2 applications of the insecticide Engeo Pleno - ZC 141 g L^{-1} IA (125 mL ha^{-1}). In the experiment from Formosa-GO/2018–19 the insecticides Actara - WG 250 g kg^{-1} IA (200 g ha^{-1}), Benevia - OD 100 g L^{-1} IA (500 mL ha^{-1}), and Acephate - SP 750 g kg^{-1} IA (200 g ha^{-1}) were used for the control of *B. tabaci* with 3 applications in preventive and curative control. For the control of *Etiella zinckenella*, the biological Bt insecticide (*Bacillus thuringiensis*) was used in the experiment from Formosa-GO/2018–19.

For pathogen control in the experiment from SAG-GO/2019 the fungicides Difere - SC 588 g L^{-1} IA (1.5 L ha^{-1}), Fox - SC 150 g L^{-1} IA (400 mL ha^{-1}) and Amistar Top - SC 200 g L^{-1} IA (400 mL ha^{-1}) were used to control *Phaeoisariopsis griseola*, *Colletotrichum lindemuthianum* and *Erysiphe polygoni*.

Sampling and plant analysis

Sampling and evaluations to determine the nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), root dry weight (RDW), number of pods (NP), number of grains (NG) and, grain yield (GY) were performed according to Souza and Ferreira (2017).

Statistical analysis

The data obtained at the different locations were subjected to group experiment analysis. In case of significant differences between locations, the results of each location were analyzed separately. On the analysis of variance was applied the F test ($P \leq 0.05$) and, when F_c was significant, mean values of the treatments were compared by Skott-Knott test ($P \leq 0.05$). Statistical analyzes were performes using the SISVAR software (Ferreira 2019).

Results

According to the group analysis, locations showed significant differences among them. Thus, the data analysis was performed separately by location.

Nodulation assessment

For most of the experiments, the nodule number (NN) and nodule dry weight (NDW) were affected by the evaluated treatments, and response variations were observed for each location. The inoculation with only two doses of *R. tropici* (Rt) resulted in higher NN in three of the five evaluated locations (Fig. 2), followed by the co-inoculation treatments with two (Rt + Ab2l) and three (Rt + Ab3l) doses of *A. brasilense*, which resulted in higher NN values in two of the five evaluated locations (Fig. 2).

The absolute control (AC) showed higher NN values than the registered commercial product (RP) in SAG-2019 and SAG-2019/2020. The treatment with nitrogen fertilization (NfT) resulted in NN values significantly lower than the other treatments in SAG-2018, SAG-2019, and SAG-2019/20 (Fig. 2). In general, most of the inoculated and/or co-inoculated treatments presented NN values from 14 to 200 nodules plant^{-1} and NDW ranging from 17 to 190 mg plant^{-1}. In this work, although no significant differences were found in Cristalina-2019 for NN and NDW, co-inoculation with Rt+Ab1l resulted in 19 nodules plant^{-1} and NDW of 19 mg plant^{-1}, providing a grain yield of 4457 kg ha^{-1}, higher than the Rt treatment.

NDW results were similar to NN, showing significant differences between treatments in SAG-2018 and SAG-2019/2020 (Fig. 2). The Rt + Ab2l treatment resulted in higher NDW values in SAG-2018 and SAG-2019/2020. On the other hand, in SAG-2019 and SAG-2019/2020 higher NDW values were observed in the TA treatment (Fig. 2). Similar to what was observed for NN, in SAG-2019 and SAG-2020, the TA treatment resulted in higher NDW values compared to the PR treatment (Fig. 2). The TN treatment behaved differently for NDW, and in SAG-2018 and SAG-2019 it resulted in the lowest MNS values (Fig. 2).

Regarding NDW, significant effect of treatments was found at three of the five evaluated locations. High NDW values were found in treatments with co-inoculation, especially Rt + Ab2l, Rt + Ab3l, and Rt + Ab4l co-inoculations in SAG-2019/2020, where these treatments resulted in higher NDW values as compared to Rt and RP treatments (Fig. 2).

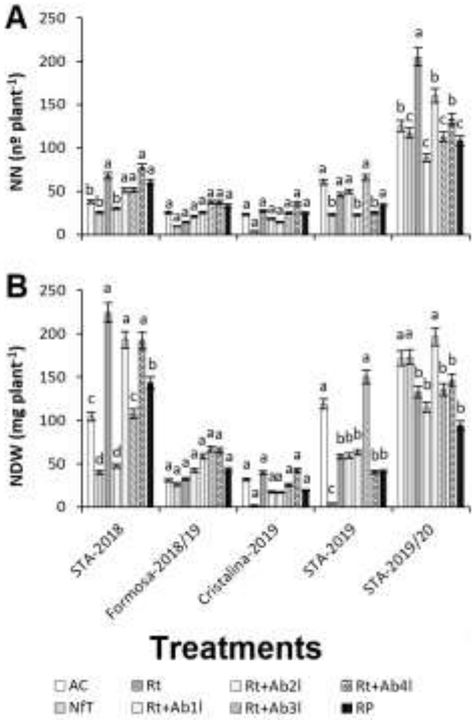


Fig. 2: Effect of different doses of co-inoculation with *R. tropici* and *A. brasilense* applied at the phenological phase V$_2$-V$_3$ on the nodule number (**A**) and nodule dry weight (**B**) of the common bean cropped in different locations and sowing seasons. STA = SAG. AC = without co-inoculation and without N-fertilizer; NfT = 80 kg N ha^{-1} (20 kg N ha^{-1} applied at sowing and 60 kg N ha^{-1} applied at V$_4$ stage; Rt = seed inoculation with *R. tropici* (2.4x10^7 cells seed^{-1}); Ab = spraying inoculation with *A. brasilense* in different concentrations (1l- 0.8×10^5 cells plant^{-1}; 2l- 1.6×10^5 cells plant^{-1}; 3l- 2.4×10^5 cells plant^{-1}; and 4l- 3.2×10^5 cells plant^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4x10^7 cells seed^{-1} and leaf inoculation with *A. brasilense*- 2.4×10^5 cells seed^{-1}). Means followed by the same letter, within each location, do not differ by the Scott-Knott test ($P < 0.05$)

The Rt+Ab2l treatment provided an increase in NDW in SAG-2019 and SAG-2019/2020, resulting in higher values than the Rt treatment (Fig. 2). The NfT treatment was one of the treatments with the lowest NN and NDW values in SAG-2018 and SAG-2019 (Fig. 2).

Assessment of growth parameters

For root dry weight (RDW), significant differences were observed between treatments at all five locations. In SAG-GO-2018, the highest values of RDW were found in the treatments Rt + Ab4l and TN. The highest RDW values in Formosa-2018/2019 were found in the treatments Rt + Ab3l, Rt + Ab4l, TA, and PR. While in Cristalina-2019 the highest value of RDW was observed in the Rt treatment and, in SAG-2019 for the Rt + Ab3l, Rt + Ab4l, and TA treatments. For SAG 2019/2020, the highest RDW values were found in the treatments Rt + Ab2l, Rt, PR, and Rt + Ab4l (Fig. 3).

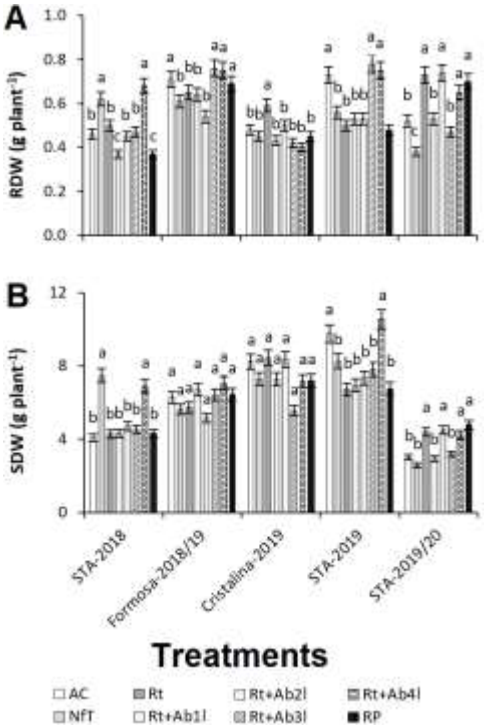


Fig. 3: Effect of different doses of co-inoculation with *R. tropici* and *A. brasilense* applied at the phenological phase V$_2$-V$_3$ on root dry weight (**A**) and shoot dry weight (**B**) of the common bean cropped in different locations and sowing seasons. STA = SAG. AC = without co-inoculation and without N-fertilizer; NfT = 80 kg N ha^{-1} (20 kg N ha^{-1} applied at sowing and 60 kg N ha^{-1} applied at V$_4$ stage; Rt = seed inoculation with *R. tropici* (2.4x10^7 cells seed^{-1}); Ab = spraying inoculation with *A. brasilense* in different concentrations (1l- 0.8×10^5 cells plant^{-1}; 2l- 1.6×10^5 cells plant^{-1}; 3l- 2.4×10^5 cells plant^{-1}; and 4l- 3.2 × 10^5 cells plant^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4x10^7 cells seed^{-1} and leaf inoculation with *A. brasilense*- 2.4×10^5 cells seed^{-1}). Means followed by the same letter, within each location, do not differ by the Scott-Knott test ($P < 0.05$)

The Rt+Ab4l treatment provided higher RDW values in three of the five locations, being higher values than the TN, PR, Rt, and TA treatments (Fig. 3).

For shoot dry weight (SDW), statistical differences were observed among treatments in four of the five evaluated locations. In SAG-2018, higher SDW values were shown by TN and Rt + Ab4l treatments. In Cristalina-2019, higher SDW values were shown by the treatments Rt, Rt + Ab2l, TA, TN, Rt + Ab1l, PR and Rt + Abl4f and, in SAG-2019 the treatments Rt + Ab4l and TA showed higher SDW values. In SAG-2019/2020, higher SDW values were found in the treatments PR, Rt + Ab2l, Rt, and Rt + Ab4l (Fig. 3).

Yielding components and grain yield evaluation

For the number of pods (NP), treatments were significantly differents in four of the five evaluated locations. In SAG-GO-2018, higher NP values were shown by TN, Rt, PR, Rt

+ Ab1l, Rt + Ab4l, and Rt + Ab2l treatments. In Cristalina-2019, higher NP values were found in the treatments TA, Rt + Ab4l, and PR, and in SAG-GO-2019 in the treatments PR, Rt + Ab4l, Rt + Ab3l, and Rt + Ab2l. For SAG-GO-2019/2020, higher NP values were observed in PR and Rt treatments (Fig. 4). The PR and Rt + Ab4l treatments significantly increased the NP in four of the five evaluated locations (SAG-GO-2018, Cristalina-2019, SAG-2019 and SAG-2019/2020), with higher values as compared to those of the treatments TN, Rt and TA (Fig. 4).

As for NP, significant differences for number of grains (NG) were also observed between treatments in four of the five evaluated locations. In SAG-GO-2018 higher NG values were shown by TN, Rt, Rt + Ab1l, and Rt+Ab4l treatments. In Formosa-2018/2019, higher NG values were observed in the Rt + Ab2l treatment. In Cristalina-2019, higher NG values were provided by the treatments Rt + Ab4l, Rt + Ab2l and TA and, in SAG-GO-2019/2020, by the treatments Rt, PR, Rt + Ab4l, TN, and Rt + Ab1l (Fig. 4). In general, the Rt + Ab4l co-inoculation treatment resulted in NG values higher than those of TN, PR, Rt, and TA treatments (Fig. 4).

Unlike the nodulation and growth parameters, for the grain yield (GY) significant differences between treatments were observed at all five locations. In SAG-2018, the treatments Rt + Ab4l, TN, PR, Rt + Ab3l, TA, and Rt provided higher GY values. In Formosa-2018/2019, higher GY values were noted in Rt + Ab1l and Rt + Ab4l treatments. In Cristalina-2019 and SAG-2019, higher values of GY were found in the treatments Rt + Ab1l and Rt + Ab3l and, in SAG-2019/2020 for the treatments Rt, PR and TA (Fig. 4).

From the GY data, an assessment of GY stability at the different locations was performed, whereby GY values were plotted, within each site and for the average of the locations (Fig. 5), ranging from light gray (worst GY) to dark gray (best GY).The treatment Rt + Ab3l presented values of GY ranging from medium to high (darker gray) in four of the five evaluated locations, followed by the treatments Rt + Ab1l and Rt + Ab4l, which presented GY values ranging from medium to high in three of the five evaluated locations. Similarly, by the average of the five locations, it was observed that the Rt + Ab3l treatment presented the best GY values (Fig. 5). By the average of the five experiments, the treatment Rt + Ab3l increased the GY in about 100, 93, 74 and 83 kg ha^{-1} as compared to the treatments TN, PR, Rt, and TA, respectively (Fig. 5).

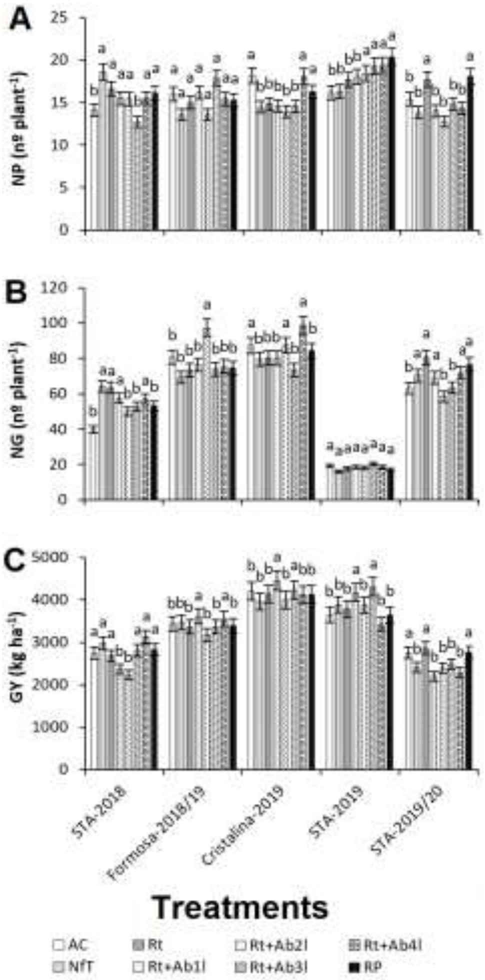
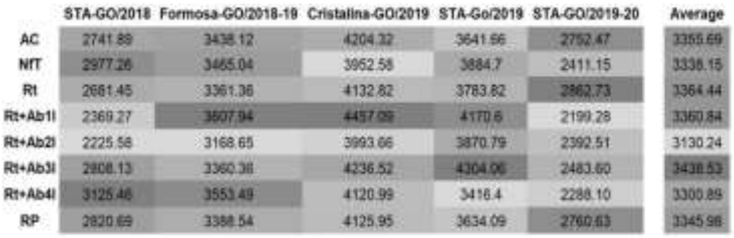


Fig. 4: Effect of different doses of co-inoculation with *R. tropici* and *A. brasilense* applied at phenological phase V_2–V_3 on number of pods (**A**), number of grains (**B**), and grain yield (**C**) of the common bean cropped in different locations and sowing seasons. STA = SAG. AC = without co-inoculation and without N-fertilizer; NfT = 80 kg N ha^{-1} (20 kg N ha^{-1} applied at sowing and 60 kg N ha^{-1} applied at V_4 stage; Rt = seed inoculation with *R. tropici* (2.4 × 10^{7} cells seed^{-1}); Ab = spraying inoculation with *A. brasilense* in different concentrations (1l- 0.8 × 10^{5} cells plant^{-1}; 2l- 1.6 × 10^{5} cells plant^{-1}; 3l- 2.4×10^{5} cells plant^{-1}; and 4l- 3.2 × 10^{5} cells plant^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4 × 10^{7} cells seed^{-1} and leaf inoculation with *A. brasilense*- 2.4 × 10^{5} cells seed^{-1}). Means followed by the same letter, within each location, do not differ by the Scott-Knott test ($P < 0.05$)

Discussion

As regards nodulation assessment is concerned, treatments with simple inoculation with *R. tropici* and co-inoculation with foliar application of different doses of *A. brasilense* positively influenced nodulation by increasing the number of nodules and dry mass of nodules plant^{-1}. This occurs due to the positive synergism of the two bacteria, in which *A. brasilense* is the first to colonize the roots of plants and prepares them for the colonization of *R. tropici*, increasing symbiotic efficiency. In this study, most inoculated and/or co-inoculated treatments, NN values ranged from 14 to 200 nodules plant^{-1}, while NDW ranged from 17 to 190 mg plant^{-1}. According to Oliveira and Santos (2011), well-nodulated plants should have 15 to 30 nodules plant^{-1}. A good indication of symbiotic efficiency, which corresponds to good nodulation, are the plants that present 100 to 200 mg of dry nodules in full bloom, are more likely to increase the fixed N contents and, consequently, present high grain



	STA-GO/2018	Formosa-GO/2018-19	Cristalina-GO/2019	STA-Go/2019	STA-GO/2019-20	Average
AC	2741.89	3438.12	4204.32	3641.56	2752.47	3355.69
NfT	2977.26	3465.04	3952.58	3884.7	2411.15	3338.15
Rt	2681.45	3361.36	4132.82	3783.82	2862.73	3364.44
Rt+Ab1l	2369.27	3607.94	4457.09	4170.6	2199.28	3360.84
Rt+Ab2l	2225.58	3168.65	3993.66	3870.79	2392.51	3130.24
Rt+Ab3l	2808.13	3360.38	4236.52	4304.06	2483.60	3438.53
Rt+Ab4l	3125.46	3553.49	4120.99	3416.4	2288.10	3300.89
RP	2820.69	3388.54	4125.95	3634.09	2760.63	3345.98

Fig. 5: Grain yield (GY - kg ha^{-1}) of common bean co-inoculated with *R. tropici* and *A. brasilense* cultivated in different seasons. Values highlighted in light gray (worst GY) and in dark gray (best GY). [1]AC = without co-inoculation and without N-fertilizer; NfT = 80 kg N ha^{-1} (20 kg N ha^{-1} applied at sowing and 60 kg N ha^{-1} applied at V_4 stage; Rt = seed inoculation with *R. tropici* (2.4 × 10^7 cells $seed^{-1}$); Ab = spraying inoculation with *A. brasilense* in different concentrations (1l- 0.8 × 10^5 cells $plant^{-1}$; 2l- 1.6 × 10^5 cells $plant^{-1}$; 3l- 2.4×10^5 cells $plant^{-1}$; and 4l- 3.2 × 10^5 cells $plant^{-1}$); RP = Registered product (seed inoculation with *R. tropici*-2.4 x 10^7 cells $seed^{-1}$ and leaf inoculation with *A. brasilense*- 2.4 × 10^5 cells $seed^{-1}$)

productivity (Oliveira and Santos 2011). However, in the literature there are works in which it presents high grain productivity with plants presenting low nodulation (NN and NDW). Andraus *et al.* (2016), working with the inoculation of *R. tropici* in the Estilo cultivar, reported NN of 16 nodules $plant^{-1}$ and NDW of 39 mg $plant^{-1}$, resulting in a grain yield of 3626 kg ha^{-1}. Indicating that, even with low nodulation, commercial bacteria can be efficient and competitive with native rhizobia present in the soil.

In field experiments carried out by Souza and Ferreira (2017), the inoculation of *R. tropici* (Rt) resulted in NN and NDW values of 43 nodules $plant^{-1}$ and 78 mg $plant^{-1}$, respectively, increasing grain yield in about 463 kg ha^{-1} compared to the treatment without inoculation and without fertilization, indicating high competitiveness of the SEMIA 4070 strain against native soil strains and high efficiency of biological N fixation.

In this study, the co-inoculation of *R. tropici* and *A. brasilense* provided better performance in nodulation parameters, resulting in a greater number and mass of dry nodules, corroborating the work of Souza and Ferreira (2017). Furthermore, the beneficial effect of the co-inoculation of *R. tropici* and *A. brasilense* on nodulation has been frequently observed in other crops, such as soybean (Chibeba *et al.* 2015; Steiner *et al.* 2019) and peanut (Silva *et al.* 2017; Steiner *et al.* 2019).

According to Steiner *et al.* (2019), increase in nodulation driven by co-inoculation is due to the synergistic effect produced by the two bacteria, in which A. *brasilense* qualifies the root system so that it can be inhabited by *R. tropici*, improving the nodulation rate, mainly in the root crown. Thus, beneficial results of the association of symbiotic (*R. tropici*) and non-symbiotic (*A. brasilense*) bacteria in legumes are mainly due to these rhizobacteria having ability to fix N_2, stimulate and produce growth phytohormones, improve the activity of reductase enzyme activity, solubilize soil phosphate, in addition to improving plant resistance to biotic and abiotic stresses (Chibeba *et al.* 2015; Fukami *et al.* 2018a; Steiner *et al.* 2019).

In this study, as well as several available in the literature, we found that the use of nitrogen fertilizers negatively affected plant nodulation, reducing the nodulation process (Sousa *et al.* 2020), since the application of nitrogen fertilizers has negative effects on the formation of nodules in legumes, through the inhibition of phenolic compounds in plant metabolism, especially the synthesis and release of isoflavonoids from legume roots (Steiner *et al*. 2019). A fact that the energy expenditure by the plant is high in the process of biological N fixation, unlike nitrogen fertilization, in which N is in the most accessible form, which explains the reduction of nodulation when fertilizers are applied.

The results of co-inoculation with *R. tropici* and *A. brasilense* applied via foliar route resulted in a significant increase in root growth. This increase is associated with the main mechanism of action of *A. brasilense*, the stimulation and production of phytohormones in plants. This production of phytohormones with gibberellins and auxins promotes a greater increase and development of lateral roots and root hairs, consequently increasing the volume of exploited area in the soil and improving plant performance (Chibeba *et al.* 2015; Vurukonda *et al.* 2016; Bulegon *et al.* 2017; Fukami *et al.* 2018b; Steiner *et al*. 2019). In addition, the application of A. brasilense to common bean improves the performance of plants under water stress conditions, due to the increase in the volume exploited by the roots (German *et al.* 2000). The use of foliar application of A. brasilense, as well as in common bean, in soybean significantly increased the length of the roots of the plants, in relation to the co-inoculated plants (Puente *et al.* 2018).

Regarding the positive effect of co-inoculation with four (Rt + Ab4l) and two (Rt + Ab2l) doses of *A. brasilense* sprayed via foliar on the dry shoot mass, it is related to the synergism between *R. tropici* and *A. brasilense*, through N fixation, phytohormone production and phosphate solubilization and improved resistance to biotic and abiotic stresses, resulting in greater growth of healthier and more vigorous plants (Bulgarelli *et al.* 2013). However, in the literature it is estimated that *A. brasilense* contributes from 5 to 18% of N fixation in legumes (Bremer *et al.* 1995). In addition, co-inoculation with A. brasilense through synergism with R. tropici promotes increased N fixation and plant growth (Filipini *et al.* 2021).

A fact that there are still few studies with co-inoculation with the application of *A. brasilense* via foliar spraying, as well as, in this study the benefits of this form of application of co-inoculation with doses of *A. brasilense* promotes greater development and performance of plants, with greater dry root mass and dry shoots, increased branching, improved photosynthesis and water and nutrient absorption, resulting in higher yields (Strzelczyk *et al.* 1994; Bashan *et al.* 2004; Filipini *et al*. 2021).

Higher common bean yields are linked to increases in the number of pods, number of grains per pod or grain weight (Filipini *et al.* 2021). The N is the fundamental nutrient for most crops, being directly related to the high rates, which determine the grain weight in the filling period (Munier-Jolain *et al*. 2008; Filipini *et al*. 2021). In this study, in most of the locations considered, the co-inoculation with the foliar spraying of four doses of *A. brasilense* (Rt + Ab4l), increased the number of pods plant^{-1}, which presented the same effect and superior to the treatment with nitrogen fertilization (NfT). The results for NP obtained in our work were superior to those found in the works of Steiner *et al.* (2019) and Tocheto and Boiago (2019). According to Steiner *et al.* (2019) the explanation for the increase in the number of grains is associated with the combination of *R. tropici* and *A. brasilense*, which stimulates flower opening and pod formation, fundamental factors for achieving high productivity in common bean. Likewise, Peres *et al.* (2016) also reported that a combination of *R. tropici* and *A. brasilense* significantly influences the NP.

Furthermore, the NG values found in this study were higher than the values reported by Steiner *et al.* (2019). According to Tocheto and Boiago (2019), the co-inoculation of *R. tropici* and *A. brasilense* directly influences the number of grains and grain production of common bean. The effect may be related to the environmental conditions associated with the potential of each genetic material for co-inoculation (Ferri *et al.* 2017; Braccini *et al.* 2016; Filipini *et al.* 2021), mainly by *A. brasilense* applied *via* the leaf, which this is subject to different climate conditions in the interaction between bacteria and plants in the phyllosphere, which may affect the potential of the technique, which did not happen in this study. According to Carvalho *et al.* (2018) performance of the combination of *Rhizobium* and *Azospirillum* depends on some factors, such as the native microbiota, plant species and varieties, as well as other edaphoclimatic conditions.

Co-inoculation treatment with foliar spraying applying three doses of *A. brasilense* (Rt + Ab3l) provided greater GY than treatment with single inoculation with *R. tropici* (Rt) (Fig. 2), as well as, in studies carried out by Hungria *et al.* (2013), Souza and Ferreira (2017) and Bettiol *et al.* (2021). In the work carried out by Schossler *et al.* (2016), the co-inoculation of *R. tropici* and *A. brasilense* led to a GY of 2448.45 kg ha^{-1}, increasing the GY by 13.5% (292 kg ha^{-1}) in relation to the single inoculation with *A. brasilense* and 5.7% (131 kg ha^{-1}) for *R. tropici*. Bettiol *et al.* (2021) reported identical results in their study, in which the co-inoculation of *R. tropici* and *A. brasilense* provided a significant increase in GY of 403, 188, 392 and 363 kg ha^{-1} compared to treatments inoculated with *R. tropici*, N-fertilization with 90 kg N ha^{-1}, N-fertilization with 45 kg N ha^{-1} and control treatment without fertilization and co-inoculation, respectively.

Our work demonstrates the potential of this technology from the positive results of co-inoculation, with the combined use of *R. tropici* and foliar application of *A. brasilense*, which results in greater yield and grain production in the common bean crop. This technology can bring economic advantages and reduce environmental impacts under field conditions in commercial common bean production farms. This technology can be applied not only locally, but regionally and nationally in countries that have similar edaphlocimatic conditions for common bean cultivation, being a great alternative for increasing common bean yield in developing countries, where nitrogen fertilization is inaccessible, due to the high cost in the production system.

Conclusion

The results indicate that the co-inoculation technique with *R. tropici* applied to the seed and, associated with *A. brasilense* applied via foliar spraying, is an alternative for partial or total replacement of the use of nitrogen fertilization in the common bean crop. Furthermore, co-inoculation with two doses of *Rhizobium* and three doses of *Azospirillum* via foliar spray resulted in increased nodulation, significant increase in shoot and root biomass, increased number of pods and grains, and increased and greater stability of grain yield, when compared to nitrogen fertilization, registered product, simple inoculation with *R. tropici* and without fertilization and without co-inoculation.

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Author Contributions

MM: implemented and conducted the experiments and data collection, in addition to interpreting the statistical analysis and writing the manuscript, PCA: translated the manuscript into English, EPBF: carried out the study planning, statistical analysis and support in writing the manuscript, and all authors commented on manuscript versions. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest

Data Availability

The reported data can be made available upon requesting to the corresponding author

Ethics Approval

Not applicable in this research work

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