International Journal of Plant Production (2023) 17:637–650
https://doi.org/10.1007/s42106-023-00259-y

RESEARCH

In Furrow Co-inoculation of Rhizobia and Azospirilla Influences the Growth and Productivity of the Common Bean

Matheus Messias[1] · Princewill Chukwuma Asobia[1,2] · Enderson Petrônio de Brito Ferreira[3]

Received: 25 December 2022 / Accepted: 17 June 2023 / Published online: 26 June 2023
© Springer Nature Switzerland AG 2023

Abstract

Besides being a significant part of the Brazilian national economy, the common bean is a legume whose grains are part of the daily diet of the population of developing nations globally. Combining N-fixing bacteria and plant phytohormone producers through the use of inoculants is an alternative to improve plant growth and increase grain yield in the common bean production system. This study aimed to evaluate the effect of co-inoculation doses with *Rhizobium tropici* and *Azospirillum brasilense*, applied in the planting groove, on nodulation, growth and productivity parameters of the common bean. Five field experiments were conducted in a randomized block design with four replications. Restrictions were formulated, namely: absolute control, without fertilization and without co-inoculation (AC); nitrogen fertilization (NfT); single inoculation of *R. tropici* (Rt); *R. tropici* + one dose of *A. brasiliense* (Rt + Ab1f); *R. tropici* + two doses of *A. brasiliense* (Rt + Ab2f); *R. tropici* + three doses of *A. brasiliense* (Rt + Ab3f); *R. tropici* + four doses of *A. brasiliense* (Rt + Ab4f) and *R. tropici* + three doses of *A. brasiliense* (RP). The number of nodules (NN), nodule dry mass (NDM), aerial dry mass (ADM), root dry mass (RDM), pod number (NP), number of grains (NG), mass of 100 grain (100G) and grain yield (GY) were taken. The Rt + Ab4f co-inoculation provided a significant increase in NN, NDM, ADM and RDM values than the Rt and AC treatments. In addition, it also presented the highest GY stability, with an average production of 3499 kg ha^{-1} from the five sites, which represents an increase of 15.4%, 14.8%, 11.0%, and 1.0% as compared to Rt, AC, RP, and NfT treatments, respectively. The results indicate the feasibility of the use of co-inoculation with *R. tropici* and *A. brasilense* in commercial production farms and for small producers in several countries of the world with similar edaphoclimatic conditions, reducing the use of nitrogen fertilizers, production costs and the prevention of negative environmental impacts.

Keywords Biological nitrogen fixation · Co-inoculation · Nitrogen fixing bacteria · Plant-bacteria interaction · Plant growth promoting bacteria

Introduction

The common bean (*Phaseolus vulgaris* L.) is a leguminous plant whose grains is part of the daily diet of the poorest populations worldwide (Ganascini et al., 2019). As it is the most important cheap source of protein and minerals for the human diet (Sampaio et al., 2016), and an important component of the Brazilian national economy. In 2020 the world common bean production was 27.5 million tons, with Brazil standing out in the last decades as one of the largest world producers of this crop, just behind Myanmar and India (FAO 2022). In Brazil, the common bean is grown in three cropping seasons distributed throughout the year, in small, medium, and large farms, with a total cultivated area of about 3 million hectares in 2019/2020 and a production of 3.15 million tons (CONAB 2020a).

Among the essential nutrients for the bean crop, N is the most absorbed, being fundamental for plant development (Gabre et al., 2020; Melo Filho et al., 2020). The primary method of supplying this nutrient to the crop is through the use of N fertilizers. However, the efficiency of N fertilization is low, around 50%, and can reach 5 to 10% in sandy

✉ Matheus Messias
messyas023@gmail.com

1 Federal University of Goiás, Graduate Program in Agronomy, Goiânia, Goiás, Brazil

2 Michael Okpara University of Agriculture, Umudike, Abia, Nigeria

3 Embrapa Rice and Beans, Santo Antônio de Goiás, Goiás, Brazil

soils, due to losses by leaching and denitrification processes (Figueredo et al., 2016; Melo Filho et al., 2020). Besides, because it is an expensive input, the use of N fertilizer increases the production costs, leading producers to look for more sustainable technologies (Ferreira et al., 2020).

Achieving high yields has been the main goal for most common bean producers, especially among medium and large ones. For these producers, the use of N fertilizers usually exceeds the recommended amount for the crop (Santi et al., 2013; Guimarães et al., 2017). The use of doses beyond 120 kg of N ha^{-1} is a usual practice, representing a cost of approximately 121 US$ ha^{-1} (Tristão et al., 2019; CONAB 2020b).

The application of commercial inoculants has been studied as an alternative to the use of N fertilizers in common bean crops, especially through co-inoculation of the crop with *Rhizobium tropici* and *Azospirillum brasilense*. The employment of this technology generates a synergistic effect, exceeding the productivity gains when bacteria were used as the single inoculation of *R. tropici* or *A. brasilense* (Melo Filho et al., 2020; Filipini et al., 2021; Messias et al., 2023). This synergistic effect produced by these bacteria occurs due to the Biological N Fixation, by *R. tropici*, and the production of phytohormones by *A. brasilense* (Hungria et al., 2015; Peres et al., 2016; Melo Filho et al., 2020), which has led to the development of studies focusing on the forms of application and the appropriate doses of each bacterium.

The results of co-inoculation in common bean have shown very promising responses regarding the reduction of production cost and increased productivity (Souza & Ferreira, 2017; Ferreira et al., 2020). Under Cerrado conditions, located at the Central part of Brazil, co-inoculation of *R. tropici* (SEMIA 4070 = CIAT899) and *A. brasilense* (Ab-V5 and Ab-V6) resulted in an increase of 560 kg ha^{-1} (26%) over inoculation with *R. tropici* (Souza & Ferreira, 2017). Under a similar conditions, in the work of Messias et al. (2023), conducting five field experiments, they observed that co-inoculation with *R. tropici* and three doses of *A. brasilense* promoted an increase of 100, 74 and 83 kg ha^{-1}, when compared to the use of nitrogen fertilizer, simple inoculation with *R. tropici* and absolute control (without fertilization and without co-inoculation), respectively. In both studies, even in the first study where co-inoculation was performed in the sowing furrow, as in the second where *A. brasilense* was applied by spraying the plants, the results demonstrated the importance of co-inoculation for the common bean. However, field studies are still needed to establish the best combination of dose and positioning of the inoculant in the sowing furrow, especially for the Cerrado region.

Therefore, the objective of this study was to evaluate the effect of co-inoculation via sowing furrow of *R. tropici* and different doses of *A. brasilense* on nodulation, growth, and productivity parameters of the common bean.

Materials and Methods

Location and Description of the Experimental Areas

Five field experiments were conducted in three different cropping seasons (winter 2018, water 2018–2019, and winter 2019) in different areas, to evaluate the efficiency of the treatments in different soil and climate conditions, production system management, and technological levels adopted at each experimental area. Detailed information is presented in Table 1.

According to the Köppen classification, the location where the experiments were conducted show an Aw climate, tropical savanna, megathermal. The rainfall regime is well defined, with a dry period from May to September and a rainy period from October to April, with an average annual temperature ranging from 20.5 °C to 23 ºC and average annual rainfall ranging from 1465 to 1600 mm. The prevailing climatic conditions during the conduction of the experiments are presented in Fig. 1.

For the chemical analysis of the soil, 10 soil subsamples were collected from each experimental area at a depth of 0–20 cm before sowing. The subsamples were homogenized to compose a composite sample used to determine the chemical characteristics according to the methodology described in the manual of soil analysis methods of EMBRAPA - CNPS (Donagema et al., 2011). Soil pH was determined in 0.01 M H2O (1:2.5; soil: solution) after stirring for 1 h. Exchangeable Ca, Mg, and Al were determined

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	
STA-GO/2018	16º29`16.2”	49º17`57.80”	777	Corn
Formosa-GO/2018-19	15º44`05.64”	47º26`06.71”	996	Corn
STA-GO/2018-19	16º30`12.21”	49º17`17.02”	811	Soybean
Cristalina-GO/2019	16º56`14.70”	47º46`02.70”	905	Wheat
STA-GO/2019	16º29`15.22”	49º17`54.74”	776	Corn

STA- Santo Antônio de Goiás

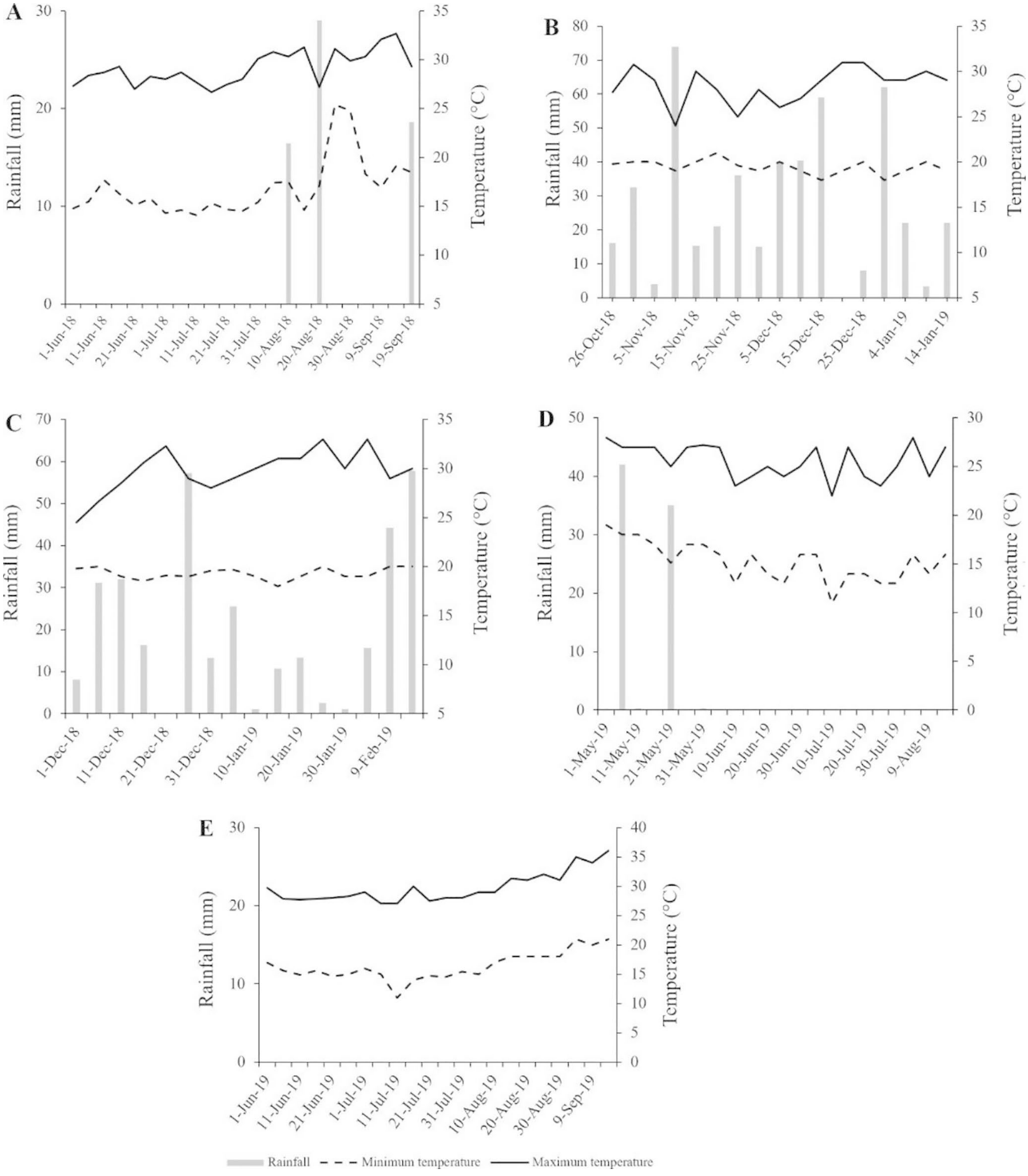

Fig. 1 Rainfall, maximum and minimum mean temperatures during the experimental periods. Santo Antônio de Goiás-GO/2018 (**A**), Formosa-GO/2018-19 (**B**), Santo Antônio de Goiás-GO/2018-19 (**C**), Cristalina-GO/2019 (**D**), and Santo Antônio de Goiás-GO/2019 (**E**)

in the extract obtained with 1 mol L^{-1} KCl (1:10; soil: solution) after stirring for 10 min. P and K contents were evaluated in the Mehlich^{-1} (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) extract (1:10; soil: solution) after stirring for 10 min. Al was determined by titration with 0.015 mol L^{-1} standardized NaOH, using bromo-azultimol as indicator. The concentrations of Ca and Mg were determined in an atomic absorption spectrophotometer, K in a flame photometer and

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}
STA-GO/2018[5]	5.4	18.5	8.7	1	29	2.80	5.7	49	31.27
Formosa-GO/2018-19	5.6	45.6	12.9	0	24	6.57	9.0	73	46.93
STA-GO/2018-19	6.0	18.3	10.8	0	18	3.22	5.0	64	27.00
Cristalina-GO/2019	5.8	38.5	13.6	0	22	5.69	7.9	72	51.90
STA-GO/2019	5.8	17.0	9.8	0	24	2.87	5.3	54	28.91

Location/cropping season	P	K	Cu	Zn	Fe	Mn
	mg dm^{-3}					
STA-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
STA-GO/2018-19	8.9	122	1.4	3.2	15.5	11.1
Cristalina-GO/2019	45.7	187	2.1	8.1	32.7	22.2
STA-GO/2019	11.1	75	1.3	5.2	33.8	10.4

[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]STA- Santo Antônio de Goiás

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 Liquido Feijão	Total Nitro Feijão	Biomax Azum	Azo-Total Max
AC	-	-	-	-	-
NfT	80 kg ha^{-1}	-	-	-	-
Rt	-	2 doses ha^{-1}	-	-	-
Rt+Ab1f	-	2 doses ha^{-1}	-	1 dose ha^{-1}	-
Rt+Ab2f	-	2 doses ha^{-1}	-	2 doses ha^{-1}	-
Rt+Ab3f	-	2 doses ha^{-1}	-	3 doses ha^{-1}	-
Rt+Ab4f	-	2 doses ha^{-1}	-	4 doses ha^{-1}	-
RP	-	-	1 dose 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 = in furrow inoculation with *R. tropici* (2.4×10^7 cells $seed^{-1}$); Ab = in furrow inoculation with *A. brasilense* in different concentrations (1f- 0.8×10^5 cells $seed^{-1}$; 2f- 1.6×10^5 cells $seed^{-1}$; 3f- 2.4×10^5 cells $seed^{-1}$; and 4f- 3.2×10^5 cells $seed^{-1}$); RP = Registered product (seed inoculation with *R. tropici*-2.4×10^7 cells $seed^{-1}$ and in furrow inoculation with *A. brasilense*- 2.4×10^5 cells $seed^{-1}$)

P by colorimetry, using the molybdenum-blue method and ascorbic acid as reducing agent. Organic matter was determined by the Walkley & Black method. The results of the soil analysis are presented in Table 2.

Inoculants, Treatments and Experimental Design

Commercial inoculants formulated separately with SEMIA 4077 strain of *R. tropici* and Ab-V5 strain of *A. brasilense* were used: (1) Biomax Premium Liquido Feijão, containing *R. tropici* in a concentration of de 2×10^9 CFU mL^{-1} (Colony Forming unit) and (2) Biomax Premium Azum, containing *A. brasilense* in a concentration of 3×10^8 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 Total BioTecnologia do Brasil Ltda, as follow: Total Nitro Feijão, containing *R. tropici* in a concentration of de 2×10^9 CFU g^{-1} and AzoTotal Max, containing *A. brasilense* in a concentration of 2×10^8 CFU mL^{-1}.

Eight treatments were evaluated, consisting of a single inoculation with *R. tropici* (Rt), four combinations of doses of *R. tropici* and *A. brasilense*, an absolute control without co-inoculation and without N fertilization (AC), a control N fertilization without co-inoculation (NfT), and the registered product (RP). Detailed description of the eight treatments is shown in Table 3.

In N-fertilizer treatment (NfT), a total of 80 kg N ha^{-1} was applied, with 20 kg N ha^{-1} applied at sowing and 60 kg N ha^{-1} applied at the V4 stage using urea. In the treatment RP (registered product), for the inoculation of the seeds with the peat inoculant, a 10% sugar solution was used to facilitate the adhesion of the inoculant on the seeds.

All field experiments were conducted in a randomized block design with 4 replications. The plots were composed of six four-meter-long rows, with a spacing of 0.45 m between rows, totaling 10.8 m² per plot. The final plant population was about 240,000 plants ha^{-1}.

Field Management

About 50 days before the implementation of the experiments, limestone was applied to increase the base saturation to 70% and soil pH to 5.5, in the locations where these parameters were below these values, as shown in Table 2.

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 field experiments conducted in Formosa-GO/2018-19 and Cristalina-GO/2019 did not require fertilization. In Santo Antônio de Goiás-GO/2018 270 kg ha^{-1} of Triple superphosphate and 90 kg ha^{-1} of potassium chloride were applied. In Santo Antônio de Goiás-GO/2018-19, the application of 300 kg ha^{-1} of Triple superphosphate and 100 kg ha^{-1} of potassium chloride was done. In Santo Antônio de Goiás-GO/2019 262.50 kg ha^{-1} of Triple superphosphate and 87.50 kg ha^{-1} of potassium chloride were applied.

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 Santo Antônio de Goiás-GO/2018 and Santo Antônio de Goiás-GO/2019, seven days before sowing (DBS) desiccation of the areas was done with the herbicide Paraquat - SL 200 g L^{-1} (2.0 L ha^{-1}). In the experiment conducted in Santo Antônio de Goiás-GO/2018-19, ten DBS desiccation of the area was performed with the herbicide Glyphosate - SL 480 g L^{-1} (3.0 L ha^{-1}). For the experiment conducted in Formosa-GO/2018-19, ten DBS desiccation of the area was carried out with the herbicides Aurora - EC 400 g L^{-1} (50 mL ha^{-1}) and Roundup - SL 445 g L^{-1} (2.0 L ha^{-1}). While for the experiment conducted in Cristalina-GO/2019, five DBS desiccation of the area was accomplished with the herbicide Roundup - SL 445 g L^{-1} (2.0 L ha^{-1}).

Pre-emergence herbicide application was done, between 2 and 3 days after sowing (DAS), in Santo Antônio de Goiás-GO/2018, Santo Antônio de Goiás-GO/2018-19 and Santo Antônio de Goiás-GO/2019, using Gramoxone - SL 200 g L^{-1} (2.0 L ha^{-1}). Pre-emergence herbicide application was also performed in Formosa-GO/2018-19 and Cristalina-GO/2019, using Gramocil - SC 200 g L^{-1} (2.0 L ha^{-1}). In Santo Antônio de Goiás-GO/2018, Santo Antônio de Goiás-GO/2018-19, Santo Antônio de Goiás-GO/2019 and Formosa-GO/2018-19, post-emergence herbicide application was done, between 20 and 30 days after emergence (DAE), using Flex - SL 250 g L^{-1} (1.0 L ha^{-1}) and Fusilade - EW 250 g L^{-1} (0.75 L ha^{-1}).

The experiments conducted in Santo Antônio de Goiás-GO/2018 and Santo Antônio de Goiás-GO/2018-19 witnessed the occurrence of *Bemisia tabaci*, requiring 2 applications of the insecticide Engeo Pleno - ZC 141 g L^{-1} (125 mL ha^{-1}). In the experiment conducted in Formosa-GO/2018-19 the insecticides Actara - WG 250 g kg^{-1} (200 g ha^{-1}), Benevia - OD 100 g L^{-1} IA (500 mL ha^{-1}), and Acephate - SP 750 g kg^{-1} (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 conducted in Formosa-GO/2018-19.

For pathogen control in the experiment conducted in Santo Antônio de Goiás-GO/2019 the fungicides Difere - SC 588 g L^{-1} (1.5 L ha^{-1}), Fox - SC 150 g L^{-1} (400 mL ha^{-1}) and Amistar Top - SC 200 g L^{-1} (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 number of nodules (NN), nodule dry mass (NDM), root dry mass (RDM), aerial dry mass (ADM), number of pods (NP), number of grains (NG), 100-grain mass (M100G), and grain yield (GY) were performed according to Souza and Ferreira (2017).

Statistical Analysis

The data obtained in the experiments were grouped and the analysis by experiment group was performed. When the differences were significant, the results of each cropping year were analyzed separately, however, when there was no difference between the cropping years, the data were grouped. The data were submitted to analysis of variance and when confirming a statistically significant value in the F test ($p \leq 0.05$), mean values were compared by Skott-Knott test at 5% of significance using the software SISVAR (Ferreira, 2019).

Results

The group analysis of experiments showed significant differences among growing seasons and therefore the data analysis was performed separately for each growing season.

Evaluation of Nodulation Parameters

In most experiments conducted, the number of nodules (NN) and nodule dry mass (NDM) were significantly affected by the treatments, being observed response variations for each site. The co-inoculation of two doses of *R. tropici* and two doses of *A. brasilense* (Rt + Ab2f) resulted in higher NN in three of the five locations evaluated, followed by the single inoculation of two doses of *R. tropici* (Rt), which resulted in higher NN values in two locations of the five locations evaluated (Table 4).

The co-inoculation of two doses of *R. tropici* and two doses of *A. brasilense* (Rt + Ab2f) resulted in higher NN in three of the five locations evaluated, followed by the single inoculation of two doses of *R. tropici* (Rt), which resulted in higher NN values in two locations of the five locations evaluated (Table 4).

The N-fertilizer treatment (NfT) resulted in lower NN and NDM values in four locations (Santo Antônio de Goiás-GO/2018, Formosa-GO/2018-19, Cristalina-GO/2019 and Santo Antônio de Goiás-GO/2019). The absolute control (AC) treatment, on the other hand, showed higher values for NN only in Santo Antônio de Goiás-GO/2019 and for NDM in Santo Antônio de Goiás-GO/2018, Formosa-GO/2018-19 and Santo Antônio de Goiás-GO/2019 (Table 4), showing higher values than the single inoculation with *R. tropici* (Rt) treatment. The AC treatment also showed higher nodulation results as compared to the commercial registered product (RP), with higher NN values in Formosa-Go/2018-19, Cristalina-GO/2019 and Santo Antônio de Goiás-GO/2019, and

Table 4 Effect of in-furrow co-inoculation of *R. tropici* and different doses of *A. brasilense* on number of nodules (NN - nº plant^{-1}) and nodule dry mass (NDM - mg plant^{-1}) of the common bean grown in different locations and cropping seasons

Treatments	NN		NDM		NN		NDM	
	Santo Antônio de Goiás-GO/2018				Formosa-GO/2018-19			
AC	48.50	b	117.58	b	24.00	b	47.44	b
NfT	6.75	c	3.33	c	8.75	b	16.71	b
Rt	51.00	b	115.41	b	31.50	a	24.48	b
Rt + Ab1f	46.75	b	132.16	b	24.75	b	46.74	b
Rt + Ab2f	65.25	a	105.41	b	45.00	a	87.29	a
Rt + Ab3f	48.50	b	160.00	a	29.50	a	44.16	b
Rt + Ab4f	57.75	b	180.00	a	24.75	b	57.44	b
RP	74.00	a	161.08	a	17.50	b	30.69	b
CV (%)	10.55		8.02		17.56		17.12	
	Santo Antônio de Goiás-GO/2018-19				Cristalina-GO/2019			
AC	17.00	a	39.66	a	16.75	b	26.08	a
NfT	2.50	a	7.22	a	3.25	b	2.70	a
Rt	18.00	a	32.05	a	31.25	a	45.41	a
Rt + Ab1f	16.25	a	40.33	a	21.75	b	28.93	a
Rt + Ab2f	15.50	a	29.19	a	44.25	a	52.23	a
Rt + Ab3f	13.00	a	23.10	a	23.00	b	40.17	a
Rt + Ab4f	21.50	a	57.05	a	31.75	a	24.90	a
RP	20.00	a	26.00	a	16.00	b	23.37	a
CV (%)	15.72		23.69		24.34		29.67	
	Santo Antônio de Goiás-GO/2019							
AC	73.50	a	131.06	a				
NfT	24.75	d	22.41	c				
Rt	28.25	d	59.50	c				
Rt + Ab1f	36.75	c	37.46	c				
Rt + Ab2f	38.50	c	76.31	b				
Rt + Ab3f	49.25	b	94.99	b				
Rt + Ab4f	17.25	d	34.11	c				
RP	26.00	d	42.58	c				
CV (%)	16.48		23.54					

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 = in-furrow inoculation with *R. tropici* (2.4 × 10^7 cells seed^{-1}); Ab = in-furrow inoculation with *A. brasilense* in different concentrations (1f- 0.8 × 10^5 cells seed^{-1}; 2f- 1.6 × 10^5 cells seed^{-1}; 3f- 2.4 × 10^5 cells seed^{-1}; and 4f- 3.2 × 10^5 cells seed^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4 × 10^7 cells seed^{-1} and in-furrow inoculation with *A. brasilense*- 2.4 × 10^5 cells seed^{-1}). Means followed by different letters within the same column are significantly different by the Scott-Knott test (p ≤ 0,05); CV – coefficient of variation higher NDM in Formosa-GO/2018-19 and Santo Antônio de Goiás-GO/2019 (Table 4).

The results for NDM were very similar to those for NN. However, in this study, no significant effect of treatments was observed in two of the five locations evaluated. In the other locations, Formosa-GO/2018-19, Santo Antônio de Goias-GO/2018 and, Santo Antônio de Goiás-GO/2019, higher NDM values were observed for the treatments with co-inoculation. In general, the Rt+Ab3f and Rt+Ab4f co-inoculations provided increased NDM, resulting in higher values than the Rt treatment.

Evaluation of Growth Parameters

The growth of common bean, evaluated by the determination of root dry mass (RDM) and aerial dry mass (ADM), was significantly affected by the treatments in most of the evaluated locations (Table 5).

For RDM significant differences between treatments were observed in four of the five locations. In Santo Antônio de Goiás-GO/2018 the highest RDM values were observed for the treatments RP, Rt+Ab4f, NfT, Rt, and Rt+Ab3f. In Formosa-GO/2018-19, the highest RDM values were observed in the treatments Rt+Ab2f and NfT. In Santo Antônio de Goiás-GO/2018-19 the highest RDM values were observed in the treatments Rt, NfT, and Rt+Ab4f, while the treatments Rt+Ab1f, Rt+Ab3f, Rt+Ab4f and Rt+Ab2f showed higher RDM values in Santo Antônio de Goias-GO/2019. In Cristalina-GO/2019, no significant differences were observed among treatments (Table 5).

As for ADM, the effect of the treatments was similar to that of RDM, that is, they also varied according to the

Table 5 Effect of in-furrow co-inoculation of *R. tropici* and different doses of *A. brasilense* on root dry mass (RDM - g planta^{-1}) and aerial dry mass (ADM - g plant^{-1}) of the common bean grown in different locations and cropping seasons

Treatments	RDM		ADM		RDM		ADM	
	Santo Antônio de Goiás-GO/2018				Formosa-GO/2018-19			
AC	0.44	b	4.71	c	0.62	b	5.01	b
NfT	0.67	a	9.84	a	0.76	a	6.47	a
Rt	0.62	a	5.05	c	0.63	b	5.66	b
Rt+Ab1f	0.57	b	6.77	b	0.66	b	5.80	b
Rt+Ab2f	0.55	b	4.62	c	0.86	a	6.94	a
Rt+Ab3f	0.60	a	6.55	b	0.69	b	6.71	a
Rt+Ab4f	0.67	a	7.66	b	0.51	c	3.04	c
RP	0.71	a	4.63	c	0.44	c	3.58	c
CV (%)	12.42		15.29		15.66		15.17	
	Santo Antônio de Goiás-GO/2018-19				Cristalina-GO/2019			
AC	0.81	b	6.88	b	0.56	a	5.11	b
NfT	1.05	a	8.83	a	0.49	a	7.13	a
Rt	1.06	a	6.20	b	0.48	a	6.60	b
Rt+Ab1f	0.88	b	7.25	b	0.47	a	7.88	a
Rt+Ab2f	0.81	b	6.29	b	0.42	a	5.58	b
Rt+Ab3f	0.90	b	6.13	b	0.56	a	7.69	a
Rt+Ab4f	0.97	a	6.72	b	0.48	a	7.59	a
RP	0.86	b	7.35	b	0.50	a	6.24	b
CV (%)	7.32		8.68		14.08		12.34	
	Santo Antônio de Goiás-GO/2019							
AC	0.55	b	7.37	c				
NfT	0.55	b	9.05	b				
Rt	0.50	b	7.00	c				
Rt+Ab1f	0.70	a	10.84	a				
Rt+Ab2f	0.62	a	9.11	b				
Rt+Ab3f	0.67	a	8.12	c				
Rt+Ab4f	0.65	a	8.29	c				
RP	0.55	b	7.57	c				
CV (%)	7.26		12.56					

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 = in-furrow inoculation with *R. tropici* (2.4 × 10^{7} cells seed^{-1}); Ab = in-furrow inoculation with *A. brasilense* in different concentrations (1f- 0.8 × 10^{5} cells seed^{-1}; 2f- 1.6 × 10^{5} cells seed^{-1}; 3f- 2.4 × 10^{5} cells seed^{-1}; and 4f- 3.2 × 10^{5} cells seed^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4 × 10^{7} cells seed^{-1} and in-furrow inoculation with *A. brasilense*- 2.4 × 10^{5} cells seed^{-1}). Means followed by different letters within the same column are significantly different by the Scott-Knott test ($p \leq 0,05$); CV – coefficient of variation locations. However, significant differences between treatments were observed in all five locations evaluated. For Santo Antônio de Goiás-GO/2018 and Santo Antônio de Goiás-GO/2018-19 the highest value of ADM was observed in the NfT treatment (Table 5). In Formosa-2018/2019, the highest ADM values were observed in the Rt+Ab2f, Rt+Ab3f, and NfT treatments (Table 5). While in Cristalina-GO/2019 the highest ADM values were observed in the treatments Rt+Ab1f, Rt+Ab3f, Rt+Ab4f, and NfT (Table 5), and in Santo Antônio de Goiás-GO/2019, the highest ADM value was observed in the Rt+Ab1f treatment (Table 5).

Evaluation of Yield and Productivity Parameters

The productive performance of the common bean, evaluated by determining yield components (number of pods-NP, number of grains-NG and mass of 100 grains-M100G) and grain yield (GY), was also significantly affected by the treatments, with the best treatments varying within each location (Table 6).

For NP, significant differences were observed among treatments in three of the five locations. In Santo Antônio de Goiás-GO/2018 the highest NP values were observed in the treatments NfT, Rt+Ab1f, and Rt. In Formosa-GO/2018-19 the highest NP values were observed in the treatments RP, NfT, Rt+Ab1f, Rt+Ab4f, and Rt+Ab3f and, in Santo Antônio de Goiás-GO/2019, in the treatments AC, Rt+Ab4f, NfT, RP, Rt, and Rt+Ab2f (Table 6).

Regarding NG, significant differences between treatments were also observed in four of the five locations. In Formosa-GO/2018-19, the highest NG values were observed in the treatments NfT, Rt+Ab3f, Rt+Ab4f, RP, and Rt+Ab1f. In Santo Antônio de Goiás-GO/2018-19, the highest NG values were observed in the treatments AC, RP, Rt+Ab3f, Rt+Ab1f, and Rt+Ab2f. In Cristalina-GO/2019, the highest values for NG were observed in the treatments RP, AC, and Rt+Ab2f and, in Santo Antônio de Goiás-GO/2019, for the treatments Rt+Ab4f, Rt, AC, NfT, RP, and Rt+Ab2f (Table 6).

Different from the nodulation, growth, and yield components parameters, in all five locations, a significant difference between treatments was observed for GY. In Santo Antônio de Goiás-GO/2018 the highest values for GY were observed in the treatments Rt+Ab4f, NfT, Rt+Ab3f, and Rt+Ab2f. In Formosa-GO/2018-19, the highest values of GY were observed in the treatments NfT, Rt+Ab3f, and Rt+Ab4f, and in Santo Antônio de Goiás-GO/2018-19 in the treatments Rt+Ab4f, NfT, and Rt+Ab3f. While in Cristalina-GO/2019 the highest GY values were observed in the treatments Rt+Ab4f, Rt+Ab3f, NfT, and RP and, in Santo Antônio de Goiás-GO/2019 in the treatments Rt+Ab4f and Rt+Ab2f (Table 6).

Analyzing GY separately for each site, Rt+Ab4f co-inoculation (except for Formosa-GO/2018-19), resulted in the highest GY values, outperforming the GY values of the NfT, Rt, RP, and AC treatments. Similarly, Rt+Ab3f co-inoculation outperformed the GY values of the Rt, AC and RP treatments.

From the GY data, an assessment of GY stability at the different sites was performed, whereby GY values were plotted, within each site and for the average of the sites (Fig. 2), ranging from light gray (worst GY) to dark gray (best GY).

Analyzing the pattern of GY in the five locations (Fig. 2), it is observed that the co-inoculation Rt+Ab4f showed better GY (dark brown coloration) in four of the five locations evaluated, followed by the Rt+Ab3f treatment, which showed dark brown coloration in three of the five locations evaluated. Similarly, from the average of the five locations, it can be observed that the treatments Rt+Ab4f, NfT, and Rt+Ab3f showed the best GY results, respectively. Considering the average of the five locations, Rt+Ab4f co-inoculation resulted in a GY increase of 15.4%, 14.8%, 11.0%, and 1.0% in comparison to Rt, CA, RP, and NfT treatments, respectively. In addition, co-inoculation with Rt+Ab3f resulted in an increase of 11.6%, 11.0%, and 7.3% as compared to the Rt, AC, and RP treatments, respectively (Fig. 2).

Discussion

Nodulation Parameters

It is worth noting that in all locations the NN was greater than 30 nodules per plant and, in Santo Antônio de Goiás-GO/2018, the NDM was greater than 100 mg per plant. According to Florentino et al. (2018) and Horácio et al. (2020) a well-nodulated plant should present NN values between 15 and 30 nodules per plant, or between 100 and 200 mg of NDM per plant. Therefore, a good symbiotic efficiency was observed in the experiments evaluated.

Of the five sites evaluated, in four, significant differences were observed for NN among treatments, including those without inoculation, in which the presence of nodules was also observed, indicating the presence of native strains in the soil. However, the treatments Rt+Ab2f, Rt, Rt+Ab3f, and Rt+Ab4f were among those that showed the highest NN values (Table 4). According to Brito et al. (2015), Yagi et al. (2015), Souza and Ferreira (2017), Ramires et al. (2018), Horácio et al. (2020) and Kraeski et al. (2021) inoculation using commercial product promotes beneficial and significant effects on the nodulation of the common bean, as

Table 6 Effect of in-furrow co-inoculation of *R. tropici* and different doses of *A. brasilense* on number of pods (NP – nº plant^{-1}), number of grains (NG – nº plant^{-1}), 100-grain mass (M100G - g), and grain yield (GY – kg ha^{-1}) of the common bean grown in different locations and cropping seasons

Treatments	NP		NG		M100G		GY		NP		NG		M100G		GY	
	Santo Antônio de Goiás-GO/2018								Formosa-GO/2018-19							
AC	12.20	b	43.25	a	30.75	a	3089.61	b	13.17	b	65.42	b	21.76	a	2961.67	b
NfT	14.90	a	52.15	a	29.49	a	3676.55	a	17.25	a	85.62	a	22.52	a	3816.27	a
Rt	14.50	a	54.05	a	29.93	a	3190.07	b	14.62	b	70.35	b	22.67	a	2973.55	b
Rt+Ab1f	14.80	a	52.50	a	29.74	a	3191.80	b	17.02	a	75.35	a	21.79	a	3186.53	b
Rt+Ab2f	12.80	b	47.67	a	29.44	a	3591.04	a	14.10	b	68.67	b	22.36	a	3238.67	b
Rt+Ab3f	13.30	b	53.72	a	30.05	a	3640.59	a	16.62	a	82.20	a	22.37	a	3479.78	a
Rt+Ab4f	12.80	b	43.52	a	29.91	a	3781.15	a	16.92	a	79.62	a	22.22	a	3405.49	a
RP	12.00	b	51.70	a	29.99	a	3210.01	b	15.97	a	79.42	a	22.20	a	3110.70	b
CV (%)	11.10		9.55		3.59		8.48		9.00		8.85		3.93		7.98	
	Santo Antônio de Goiás-GO/2018-19								Cristalina-GO/2019							
AC	13.92	a	49.72	a	20.77	a	1522.16	b	15.00	a	83.42	a	26.61	a	3831.10	b
NfT	11.65	a	31.47	b	20.94	a	1623.66	a	13.48	a	75.05	b	27.09	a	4163.59	a
Rt	11.37	a	33.85	b	20.07	a	1237.32	b	13.55	a	68.47	b	25.59	a	3914.35	b
Rt+Ab1f	10.55	a	42.20	a	20.83	a	1294.35	b	14.65	a	76.17	b	27.16	a	3856.64	b
Rt+Ab2f	11.92	a	41.35	a	20.15	a	1440.15	b	14.28	a	80.45	a	26.03	a	3821.48	b
Rt+Ab3f	13.75	a	43.17	a	20.91	a	1618.42	a	13.60	a	73.90	b	25.80	a	4270.36	a
Rt+Ab4f	11.90	a	34.17	b	20.50	a	1722.66	a	14.28	a	74.62	b	25.95	a	4372.72	a
RP	12.62	a	43.70	a	20.56	a	1324.10	b	16.18	a	90.05	a	26.50	a	4075.41	a
CV (%)	9.65		8.99		4.46		13.95		11.77		14.50		3.08		8.05	
	Santo Antônio de Goiás-GO/2019															
AC	18.32	a	79.62	a	27.45	b	3838.27	b								
NfT	17.50	a	78.10	a	26.76	b	4040.08	b								
Rt	16.62	a	83.45	a	27.00	b	3847.21	b								
Rt+Ab1f	13.75	b	63.25	b	28.32	a	3748.41	b								
Rt+Ab2f	16.45	a	76.12	a	26.52	b	4151.73	a								
Rt+Ab3f	15.35	b	66.02	b	28.29	a	3904.64	b								
Rt+Ab4f	17.57	a	89.95	a	26.31	b	4215.25	a								
RP	17.30	a	77.72	a	28.34	a	4042.75	b								
CV (%)	10.63		13.73		4.98		8.39									

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 = in-furrow inoculation with *R. tropici* (2.4×10^7 cells seed^{-1}); Ab = in-furrow inoculation with *A. brasilense* in different concentrations (1f- 0.8×10^5 cells seed^{-1}; 2f- 1.6×10^5 cells seed^{-1}; 3f- 2.4×10^5 cells seed^{-1}; and 4f- 3.2×10^5 cells seed^{-1}); RP = Registered product (seed inoculation with *R. tropici*-2.4×10^7 cells seed^{-1} and in-furrow inoculation with *A. brasilense*- 2.4×10^5 cells seed^{-1}). Means followed by different letters within the same column are significantly different by the Scott-Knott test ($p\leq0{,}05$); CV – coefficient of variation

	STA-GO/2018	Formosa-GO/2018-19	STA/2018-19	Cristalina-GO/2019	STA-GO/2019	Average
AC	3089.62	2961.67	1522.16	3831.10	3838.27	3048.56
NfT	3676.55	3816.27	1623.67	4163.59	4040.08	3464.03
Rt	3190.07	2973.56	1237.32	3914.36	3847.22	3032.50
Rt+Ab1f	3191.81	3186.53	1294.35	3856.64	3748.42	3055.55
Rt+Ab2f	3591.05	3238.68	1440.16	3821.49	4151.73	3248.62
Rt+Ab3f	3640.59	3479.78	1618.43	4270.36	3904.64	3382.76
Rt+Ab4f	3781.16	3405.49	1722.66	4372.72	4215.25	3499.46
RP	3210.01	3110.70	1324.11	4075.42	4042.75	3152.60

Fig. 2 Grain yield (kg ha^{-1}) of the common bean as affected by in-furrow co-inoculation of *R. tropici* and different doses of *A. brasilense*. 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 = in-furrow inoculation with *R. tropici* (2.4×10^7 cells $seed^{-1}$); Ab = in-furrow inoculation with *A. brasilense* in different concentrations (1f- 0.8×10^5 cells $seed^{-1}$; 2f- 1.6×10^5 cells $seed^{-1}$; 3f- 2.4×10^5 cells $seed^{-1}$; and 4f- 3.2×10^5 cells $seed^{-1}$); RP = Registered product (seed inoculation with *R. tropici*-2.4×10^7 cells $seed^{-1}$ and in-furrow inoculation with *A. brasilense*- 2.4×10^5 cells $seed^{-1}$); STA- Santo Antônio de Goiás-GO.

a result of the high efficiency and competitiveness of the *R. tropici* strains present in the inoculant.

The results for NDM were very similar to those for NN. However, in this study, no significant effect of treatments was observed in two of the five locations evaluated. In the other locations, Formosa-GO/2018-19, Santo Antônio de Goias-GO/2018 and, Santo Antônio de Goiás-GO/2019, higher NDM values were observed for the treatments with co-inoculation. In general, the Rt + Ab3f and Rt + Ab4f co-inoculations provided increased NDM, resulting in higher values than the Rt treatment. Similar results were reported by Messias et al. (2023) when evaluating the effect of co-inoculation with *R. tropici* and *A. brasilense* on common bean, cultivars Pérola and BRS Notável. These authors obtained higher NN and NDM with co-inoculation compared to the treatments without inoculation/fertilization, N fertilization and inoculation with *A. brasilense*.

Positive effects of co-inoculation on nodulation of common bean have been reported in literature. Souza and Ferreira (2017) reported that co-inoculation with the SEMIA 4070 strain of *R. tropici*, and with the Ab-V5 and Ab-V6 strains of *A. brasilense* resulted in higher NN in most areas evaluated, increasing NN by about 9% when compared to single inoculation with *R. tropici*. These results also corroborate those of Messias et al. (2023) who found a significant increase in the number of nodules and mass of dry nodules through the co-inoculation of *R. tropici* and three doses *A. brasilense* compared to the single inoculation with *R. tropici* and absolute control (without fertilization and without co-inoculation), promoting efficiency and competitivity to the native rhizobia present in the soil.

According to Jesus et al. (2018) and Horácio et al. (2020) this effect may occur due to the increased production of flavonoids, a substance responsible for attracting rhizobia to the roots of common bean (Okon et al., 2015; Puente et al., 2019; Vicario et al., 2015; Pastor-Bueis et al., 2021), which occurs when *Rhizobium* and *Azospirillum* are used in association. In addition, *Azospirillum* has strong microaerophilic binding to the rhizospheric niche of legume roots and has faster migration than *Rhizobium*. Thus, they can occupy the rhizosphere of legumes, preconditioning the roots for *Rhizobium* to colonize (Horácio et al., 2020; Pastor-Bueis et al., 2021; Rezende et al., 2021; Mortinho et al., 2022).

N fertilization significantly reduced the NN and NDM of the common bean (Table 4). In the presence of N fertilizer, the stimulus to the nodulation process is reduced, since the N in the fertilizer is available. In addition, the energy demand for BNF to occur is high, since nodules require carbon as an energy source for the bacteria and to provide carbon compounds for the assimilation of the produced ammonium (Yagi et al., 2015; Horácio et al., 2020). The results of this study corroborate with those of Florentino et al. (2018) who reported that when there is N in the soil at high concentrations, it is taken up at the expense of N from BNF.

Growth Parameters

The treatments Rt + Ab4f and NfT provided higher values of RDM in three of the evaluated locations (Table 5). These results demonstrated the marked effect of co-inoculation on root growth promotion, which has been attributed to the presence of *A. brasilense* (Jesus et al., 2018; Jalal et al., 2021; Filipini et al., 2021). These results corroborate the studies of Souza and Ferreira (2017), Horácio et al. (2020) and Filipini et al. (2021) which demonstrated that the co-inoculation with *R. tropici* and *A. brasilense* results in greater RDM of common bean plants. According to Horácio et al. (2020) and Filipini et al. (2021), the increase in MDR results from the stimulus provided by *A. brasilense* in root hair production, and this bacterium is especially a producer of auxin (Masciarelli et al., 2013; Fukami et al., 2018; Horácio et al., 2020; Filipini et al., 2021), a hormone associated with root system growth (Dartora et al., 2013; Bettiol et al., 2020; Horácio et al., 2020). Also, increased cell division in meristems is followed by increased auxin-responsive gene expression (Zamioudis et al., 2013; Bettiol et al., 2020).

Other works such as those by Souza and Ferreira (2017) and Messias et al. (2023) report the benefits of the association of *R. tropici* and *A. brasilense* on the physiology and performance of common bean. As previously described, the authors report that in the root system *A. brasilense* acts through the production of phytohormones such as gibberellins and auxins that improve the development of lateral roots and root hairs, consequently increasing the volume of area exploited in the soil and efficient performance of plants (Fukami et al., 2018; Messias et al., 2023). The increase in dry shoot mass, according to Messias et al. (2023) is related to the synergism between *R. tropici* and *A. brasilense*, through N fixation, production of phytohormones and phosphate solubilization and functional resistance of plants to biotic and abiotic stresses and, resulting in greater growth of healthier plants and vigorous (Bulgarelli et al., 2013).

According to Steiner et al. (2020), in a study of common bean in resistance to drought, the authors observed that under different stress conditions, plants co-inoculated with *R. tropici* and *A. brasilense* produced 96, 43 and 30% more leaf area, root volume and total dry matter than non-inoculated plants, respectively. Silva et al. (2019), also observed the effect on soybeans of the co-inoculation of *Bradyrhizobium japonicum* and *A. brasilense* resulted in higher dry matter production of the aerial part under non-stressful and stressful conditions. The results of this study confirm those found by Silva et al. (2019), Steiner et al. (2020) and Messias et al. (2023), on the improvement and increase in the development and performance of plants co-inoculated with rhizobia and azospilla.

Yield and Productivity Parameters

The treatments Rt + Ab1f and Rt + Ab3f presented ADM values statically equal to those of the NfT treatment and higher than the AC and Rt treatments (Table 5). The higher ADM values observed in the co-inoculated treatments (Rt + Ab1f and Rt + Ab3f), can be partially explained due to the ability of *A. brasilense* to promote increased root growth, increasing the plant's ability to absorb nutrients and water, which results in greater carbon fixation, increased photosynthetic rates and, consequently, accumulation of biomass (Bettiol et al., 2020; Horácio et al., 2020).

In general, the co-inoculation Rt + Ab4f showed NP value equal to that of the NfT treatment and higher than the Rt and AC treatments, corroborating the results found by Peres et al. (2016), Schossler et al. (2016) and Barbosa et al. (2020). These authors attributed the higher NP values in co-inoculated treatments to the joint action of *R. tropici* and *A. brasilense*, which improve the development of the root system and the aerial part of the plants, increasing the number of floral buds and, consequently, the NP.

Co-inoculation with RP resulted in higher NG values, and these values were higher than the NfT, Rt, and AC treatments. According to Hungria et al. (2013), Souza and Ferreira (2017), Filipini et al. (2021) and Galindo et al. (2021) co-inoculation significantly influences yield and yield components, such as the number of pods and grain yield. Although there are reports that co-inoculation does not influence NG (Melo Filho et al., 2020; Mortinho et al., 2022).

A significant difference between treatments was also observed for M100G. However, of the five locations evaluated, only in one location, Santo Antônio de Goiás-GO/2019, the treatments RP, Rt + Ab1f, and Rt + Ab3f provided M100G as compared to the other treatments (Table 6). The observation of the effect of treatments in only one of the locations evaluated denotes the low response of this variable to the treatments applied. This fact is known in the literature, where there are reports that M100G is little influenced, both by co-inoculation (Melo Filho et al., 2020; Galindo et al., 2021; Mortinho et al., 2022) and by treatments with different doses of N (Melo Filho et al., 2020).

Considering the average of the five locations, Rt + Ab4f co-inoculation promoted a GY increase of 15.4%, 14.8%, 11.0%, and 1.0% as compared to Rt, CA, RP, and NfT treatments, respectively. In addition, co-inoculation with Rt + Ab3f resulted in an increase of 11.6%, 11.0%, and 7.3% as compared to the Rt, AC, and RP treatments, respectively (Fig. 2).

Similar results to those found in this work were reported by Hungria et al. (2013) in which co-inoculation with *R. tropici* and *A. brasilense* in common bean resulted in a 19.6% increase (285 kg ha^{-1}) as compared to the non-inoculated treatment and 187 kg ha^{-1} as compared to single inoculation with *R. tropici*. These same authors also reported that single inoculation with *R. tropici* provided a yield gain of 98 kg ha^{-1} (8.3%) as compared to the non-inoculated control treatment.

Based on the results of this study, co-inoculation has great potential for the total replacement of fertilizers used in the culture of common bean, resulting in great benefits. According to Hungria et al. (2013), about 70% of the N fertilizers used in Brazil are imported, generating a very high production cost. Moreover, the environmental impacts arising from the use of N fertilizers should be considered, since the use of 100 kg of N fertilizer results in the emission of approximately 950 kg of CO_2 equivalent (Hungria et al., 2013; Souza & Ferreira, 2017). According to Souza and Ferreira (2017), the replacement of N fertilizer by co-inoculation in the total area cultivated in Brazil with common bean would result in the mitigation of about 0.7 Mt CO_2 equivalent. Considering that the co-inoculation that showed the best results (Rt+Ab4f) allows the application of both microorganisms during the sowing operation, representing a great operational advantage for the producer. Therefore, the results of this study reaffirm the viability of co-inoculation with *R. tropici* and *A. brasilense* in the common bean crop, resulting in greater practicality of application, economic and environmental gains, and maintaining the high productivity of the common bean.

It is noteworthy that the common bean is a legume widely consumed in the world for contributing about 65% and 32% of the protein and energy intake, respectively, mainly in East Africa and Latin America (Turan et al., 2019). In this study, we bring significant results for increasing the production of common bean in tropical regions and, therefore, can be easily adapted to countries like Myanmar and India that are world leaders in common bean production. These results, in addition to contributing to increased productivity, can contribute to the reduction of environmental impacts arising from the use of N fertilizers.

Conclusions

Experimental studies on the effects of the use of rhizobium co-inoculation and azospirilla on common bean culture are still necessary, mainly carried out in tropical regions. The use of nitrogen fertilizers in tropical soils have negative points such as losses and low efficiency of use by plants and increasing environmental impacts, mainly, GHG emissions. On the other hand, the use of microorganisms to provide N and promote plant growth is an alternative to reduce environmental impacts. The results of this work indicate that the co-inoculation technique with *Rhizobium tropici* and *Azospirillum brasilense* applied via planting groove, is an alternative for partial or total substitution of the use of nitrogen fertilizers in the common bean production system. Furthermore, using co-inoculation with two doses of *Rhizobium tropici* and four doses of *Azospirillum brasilense* via planting groove promotes a significant increase in nodulation, shoot and root biomass, and greater stability of grain yield and yield of about 3499 kg ha^{-1}, resulting in an increase of of 15.4%, 14.8%, 11.0%, and 1.0% as compared to single inoculation with *Rhizobium tropici*, without fertilization and without co-inoculation, of the registered product, and nitrogen fertilizer treatments, respectively. These results are highly relevant, and can consolidate the use of co-inoculation in bean crop, since four of the five experiments were conducted in commercial areas cultivated with common beans and all four experiments showed a consistent response of the common bean to the co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense*.

Acknowledgements This work was financed by the Brazilian Agricultural Research Corporation (EMBRAPA, project 02.13.08.002.00) and INCT-Plant Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility (CNPq 465133/2014-4, Fundação Araucária-STI 043/2019, CAPES). Authors gratefully acknowledge Research Productivity fellowship from National Council for Scientific and Technological Development (CNPq) awarded to E.P.B. Ferreira (grant number 313827/2020-6).

Author Contributions M. Messias implemented and conducted the experiments and data collection, in addition to interpreting the statistical analysis and writing of the manuscript. The translation of the manuscript into English was done by P.C. Asobia. Study planning, statistical analysis and support in writing the document was done by E.P.B. Ferreira and, all authors commented on manuscript versions. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare there are no conflicts of interest.

References

Barbosa, C. K. R., Reis, J. N., Brigante, G. P., & Junior, K. S. F. (2020). Nitrogen fertilization, inoculation and co-inoculation in commom bean culture. *Caderno de Ciências Agrárias*, *12*, 1–6. https://doi.org/10.35699/2447-6218.2020.19307

Bettiol, J. V. T., Filla, V. A., Leal, F. T., Coelho, A. P., Meirelles, F. C., Lemos, L. B., & Bossolani, J. W. (2020). Sustainable production of common beans: inoculation, co-inoculation and mineral fertilization in early-cycle cultivars. *Journal of Plant Nutrition*, *44*, 16–28. https://doi.org/10.1080/01904167.2020.1822403

Brito, L. F., Pacheco, R. S., Souza Filho, B. F., Ferreira, E. P. B., Straliotto, R., & Araújo, A. P. (2015). Response of Common Bean to *Rhizobium* Inoculation and Supplemental Mineral Nitrogen in two brazilian biomes. *Revista Brasileira de Ciencia do Solo*, *39*, 981–992. https://doi.org/10.1590/01000683rbcs20140322

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Themaat, E. V. L. V., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review Of Plant Biology*, *64*, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Carvalho, M. C. S., Silveira, P. M., & Fertilizing (2021). In: Silveira PM. (ed.). Knowledge three: Common bean. Brasília: Embrapa. Available at https://www.embrapa.br/agencia-de-informacao-tecnologica/cultivos/feijao/producao/adubacao. Accessed 11 November 2022 (in Portuguese).

Conab - National Supply Company - Companhia nacional de abastecimento (2020b). Agricultural prices. Prices of inputs. Brasília: Conab. Available at file:///C:/Users/123456/Downloads/relatorio.pdf. Accessed 01 August 2020 (in Portuguese).

Conab - National Supply Company - Companhia nacional de abastecimento (2020a). Agricultural monitoring. Brasília: Conab. Available at file:///C:/Users/123456/Downloads/GraosZjulhoZ-completoZ2020.pdf. Accessed 01 August 2020 (in Portuguese).

Dartora, J., Guimarães, V. F., Marini, D., & Sander, G. (2013). Nitrogen fertilization associated to inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* in the maize. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *17*, 1023–1029. https://doi.org/10.1590/S1415-43662013001000001

Donagema, G. K., Campos, D. V. B., Calderano, S. B., Teixeira, W. G., & Viana, J. H. M. (2011). Manual de métodos de análise do solo. Rio de Janeiro: *Embrapa Solo*.

Fao – Food and agriculture organization of the United (2022). Statistic Division. Roma: Fao. Available at: https://www.fao.org/faostat/en/#data/QCL/visualize. Accessed 01 August 2022 (in Portuguese).

Ferreira, D. F. (2019). Sisvar: A computer analysis system to fixed effects splitplot type designs. *Revista Brasileira de Biometria*, *37*, 529–535. https://doi.org/10.28951/rbb.v37i4.450

Ferreira, E. P. B., Silva, O. F., & Wander, A. E. (2020). Economics of rhizobia and azospirilla co-inoculation in irrigated common bean in commercial and family farming. *Pesquisa Agropecuária Brasileira*, *55*, 1–9. https://doi.org/10.1590/S1678-3921.pab2020.v55.01532

Figueredo, M. A. D., Oliveira, D. P., Soares, B. L., Morais, A. R. D., Moreira, F. M. D. S., & Andrade, M. J. B. D. (2016). Nitrogen and molybdenum fertilization and inoculation of common bean with *Rhizobium* spp. in two oxisols. *Acta Scientiarum Agronomy*, *38*, 85–92. https://doi.org/10.4025/actasciagron.v38i1.26661

Filipini, L. D., Pilatti, F. K., Meyer, E., Ventura, B. S., Lourenzi, C. R., & Lovato, P. E. (2021). Application of *Azospirillum* on seeds and leaves, associated with *Rhizobium* inoculation, increases growth and yield of common bean. *Archives of Microbiology*, *203*, 1033–1038. https://doi.org/10.1007/s00203-020-02092-7

Florentino, L. A., Junior, K. S., Mario Filho, V. P., Oliveira, T., Souza, F. R., & Silva, A. B. (2018). Inoculation and application of different doses of nitrogen in bean crop. *Revista de Ciências Agrárias*, *41*, 963–970. https://doi.org/10.19084/RCA17001

Fukami, K., Cerezini, P., & Hungria, M. (2018). *Azospirillum*: Benefits that go far beyond biological nitrogen fixation. *AMB Express*, *8*, 1–12. https://doi.org/10.1186/s13568-018-0608-1

Gabre, V. V., Venâncio, W. S., Moraes, B. A., Furmam, F. D. G., Galvão, C. W., Gonçalves, D. R. P., & Etto, R. M. (2020). Multiple effect of different plant growth promoting microorganisms on Beans (*Phaseolus vulgaris* L.) Crop. *Brazilian Archives of Biology and Technology*, *63*, 1–12. https://doi.org/10.1590/1678-4324-solo-2020190493

Galindo, F. S., Silva, E. C., Pagliari, P. H., Fernandes, G. C., Rodrigues, W. L., Biagini, A. L. C., Baratella, E. B., Silva Júnior, C. A. S., Moretti Neto, M. J., Silva, V. M., Muraoka, T., & Teixeira Filho, M. C. M. (2021). Nitrogen recovery from fertilizer and use efficiency response to *Bradyrhizobium* sp. and *Azospirillum brasilense* combined with N rates in cowpea-wheat crop sequence. *Applied Soil Ecology*, *157*, 1–13. https://doi.org/10.1016/j.apsoil.2020.103764

Ganascini, D., Laureth, J. C. U., Mendes, I. S., Tokura, L. K., Sutil, E. L., Villa, B. D., & Coelho, S. E. M. (2019). Analysis of the production chain of bean culture in Brazil. *Journal of Agricultural Science*, *11*, 256–267. https://doi.org/10.5539/jas.v11n7p256

Guimarães, R. A. M., Braz, A. J. B. P., Simon, G. A., Ferreira, C. J. B., Braz, G. B. P., & Silveira, P. M. (2017). Response of bean crops to nitrogen fertilization in different phenological stages. *Global Science and Technology*, *10*, 1–13. file:///C:/Users/nobody/Downloads/871-Texto%20do%20artigo-5598-1-10-20170405.pdf.

Horácio, E. H., Zucareli, C., Gavilanes, F. Z., Yunes, J. S., Sanzovo, A. W. S., & Andrade, D. S. (2020). Co-inoculation of rhizobia, azospirilla and cyanobacteria for increasing common bean production. *Semina: Ciências Agrárias*, *41*, 2015–2028. https://doi.org/10.5433/1679-0359.2020v41n5Supl1p2015

Hungria, M., Nogueira, M. A., & Araujo, R. S. (2013). Co-inoculation of soybeans and common beans with rhizobia and azospirilla: Strategies to improve sustainability. *Biology Fertility of Soils*, *49*, 791–801. https://doi.org/10.1007/s00374-012-0771-5

Hungria, M., Nogueira, M. A., & Araujo, R. S. (2015). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. *American Journal of Plant Sciences*, *6*, 811–817. https://doi.org/10.4236/ajps.2015.66087

Jalal, A., Galindo, F. S., Boleta, E. H. M., Oliveira, C. E. S., Reis, A. R., Nogueira, T. A. R., Neto, M. J. M., Mortinho, E. C., Fernandes, G. C., & Filho, M. T. C. M. (2021). Common Bean Yield and Zinc Use Efficiency in Association with diazotrophic Bacteria co-inoculations. *Agronomy*, *11*, 1–20. https://doi.org/10.3390/agronomy11050959

Jesus, E. D. C., Leite, R. D. A., Bastos, R. D. A., Aragão, O. O. D. S., & Araújo, A. P. (2018). Co-inoculation of *Bradyrhizobium* stimulates the symbiosis efficiency of *Rhizobium* with common bean. *Plant and Soil*, *425*, 201–215. https://doi.org/10.1007/s11104-017-3541-1

Kraeski, M. J., Lopes, A. S., Júnior, E. D. F., Pacheco, A., Centurião, M. A., Arevalo, A. C. M., & Medeiros, R. D. (2021). Manejo da irrigação, inoculação e nitrogênio no feijoeiro de inverno. *Research Society and Development*, *10*, 1–11. https://doi.org/10.33448/rsd-v10i8.17437

Masciarelli, O., Urbani, L., Reinoso, H., & Luna, V. (2013). Alternative mechanism for the evaluation of indole-3-acetic acid (IAA) production by *Azospirillum brasilense* strains and its effects on the germination and growth of maize seedlings. *Journal of Microbiology*, *51*, 590–597. https://doi.org/10.1007/s12275-013-3136-3

Melo Filho, L. C., Henrique, I. G., Arf, O., Oliveira, D. J. S. F., Lima, A. A., Macedo, M. A. A., Mendes, J. P., & Oliveira, R. B. L. (2020). Performance of beans after inoculation with *azospirillum brasilense* and *rhizobium tropici*, and nitrogen and molybic fertilizations under amazonian conditions. *Semina: Ciências Agrárias*, *41*, 1177–1188. https://doi.org/10.5433/1679-0359.2020v41n4p1177

Messias, M., Asobia, P. C., & Ferreira, E. P. D. B. (2023). Rhizobia and azospirilla co-inoculation boosts growth and productivity of common bean. *Intl J Agric Biol*, *29*, 65–73. https://doi.org/10.17957/IJAB/15.2004

Mortinho, E. S., Jalal, A., Oliveira, C. E. S., Fernandes, G. C., Pereira, N. C. M., Rosa, P. A. L., Nascimento, V., Sá, M. E., & Teixeira Filho, M. C. M. (2022). Co-inoculations with Plant Growth-Promoting Bacteria in the Common Bean to increase efficiency of NPK fertilization. *Agronomy*, *12*, 1–15. https://doi.org/10.3390/agronomy12061325

Okon, Y., Labandera-Gonzales, C., Lage, M., & Lage, P. (2015). *Agronomic applications of Azospirillum and other PGPR. Biological Nitrogen fixation* (pp. 925–936). Hoboken, NJ, USA: Wiley Blackwell.

Pastor-Bueis, R., Jimenez-Gomez, A., Barquero, M., Mateos, P. F., & Gonzalez-Andres, F. (2021). Yield response of common bean to co-inoculation with *Rhizobium* and *Pseudomonas* endophytes and microscopic evidence of different colonized spaces inside the nodule. *European Journal of Agronomy*, *122*, 1–13. https://doi.org/10.1016/j.eja.2020.126187

Peres, A. R., Rodrigues, R. A. F., ARF, O., Portugal, J. R., & Corsini, D. C. D. C. (2016). Co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense* in common beans grown under two irrigation depths. *Revista Ceres*, *63*, 198–207. https://doi.org/10.1590/0034-737X201663020011

Puente, M. L., Zawoznik, M., Sabando, M. L., Perez, G., Gualpa, J. L., Carletti, S. M., & Cassan, F. D. (2019). Improvement of soybean grain nutritional quality under foliar inoculation with *Azospirillum brasilense* strain Az39. *Symbiosis*, *77*, 41–47. https://doi.org/10.1007/s13199-018-0568-x

Ramires, R. V., Brasil, M. S., Lima, S. F., Simon, C. A., Alvarez, R. D. C. F., & Contardi, L. M. (2018). Inoculação com rizóbio associado ao manejo da adubação nitrogenada em feijão comum. *Colloquium Agrariae*, *14*, 49–57. https://doi.org/10.5747/ca.2018.v14.n1.a189

Rezende, C. C., Nascente, A. S., Silva, M. A., Frasca, L. L. D. M., Pires, R. A. C., Filippi, M. C. C., Lanna, A. C., & Silva, J. F. A. (2021). Physiological and agronomic performance of common bean treated with multifunctional microorganisms. *Revista Brasileira de Ciências Agrárias*, *16*, 1–9. https://doi.org/10.5039/agraria.v16i4a838

Sampaio, F. B., Knupp, A. M., Fernandes, E. P., Didonet, C. C. G. M., & Ferreira, E. P. B. (2016). Morphophysiological characterization of rhizobia isolated from wild genotypes of the common bean. *Bioscience Journal*, *32*, 1502–1511. https://seer.ufu.br/index.php/biosciencejournal/article/view/33084/19348

Santi, A. L., Basso, C. J., Lamego, F. P., Flora, L. P. D., Amado, T. J. C., & Herubin, M. R. (2013). Time and partition of nitrogen fertilization side-dressed applied on bean crop, black and carioca commercial group, in direct sowing. *Ciência Rural*, *43*, 816–822. 0.1590/S0103-84782013005000045.

Schossler, J. H., Meert, L., Rizzardi, D. A., & Michalovicz, L. (2016). Yield components and grain yield of common bean submitted to the inoculation and co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense* strains. *Scientia Agrária*, *17*, 10–15. http://revistas.ufpr.br/./29368

Silva, E. R., Zoz, J., Oliveira, C. E. S., Zuffo, A. M., Steiner, F., Zoz, T., & Vendruscolo, E. P. (2019). Can co-inoculation of *Bradyrhizobium* and *Azospirillum* alleviate adverse effects of drought stress on soybean (*Glycine max* L. Merrill)? *Archives of Microbiology*, *201*, 325–335. https://doi.org/10.1007/s00203-018-01617-5

Souza, J. E. B., & Ferreira, E. P. B. (2017). Improving sustainability of common bean production systems by co-inoculating rhizobia and azospirilla. *Agriculture Ecosystems and Environment*, *237*, 250–257. https://doi.org/10.1016/j.agee.2016.12.040

Steiner, F., Silva Oliveira, C. E., Zoz, T., Zuffo, A. M., & Freitas, R. S. (2020). Co-Inoculation of Common Bean with *Rhizobium* and *Azospirillum* enhance the Drought Tolerance. *Russian Journal of Plant Physiology*, *67*, 923–932. https://doi.org/10.1134/S1021443720050167

Tristão, F. H. L., Assis, M. W. D., Mingotte, F. L. C., Calor, P. S., Souza, J. R., Morello, O. F., & Lemos, L. B. (2019). Inoculation with *Rhizobium tropici* associated with nitrogen fertilization in topdressing on agronomic performance of winter common bean. *Science and Technology Innovation in Agronomy*, *3*, 191–201.

Turan, V., Schröder, P., Bilen, S., Insam, H., & Juárez, M. F. D. (2019). Co-inoculation efect of *Rhizobium* and *Achillea millefolium* L. oil extracts on growth of common bean (*Phaseolus vulgaris* L.) and soil microbial-chemical properties. *Scientific Reports*, *9*(1), 1–10. https://doi.org/10.1038/s41598-019-51587-x

Vicario, J. C., Gallarato, L. A., Paulucci, N. S., Perrig, D. S., Bueno, M. A., & Dardanelli, M. S. (2015). In Springer (Ed.), *Co-inoculation of Legumes with Azospirillum and Symbiotic Rhizobia* (pp. 411–418). Switzerland: Springer International Publishing. Handbook for *Azospirillum*: technical Issues and Protocols.

Yagi, R., Andrade, D. S., Waureck, A., & Gomes, J. C. (2015). Nodulações e Produtividades de Grãos de Feijoeiros diante da Adubação Nitrogenada ou da Inoculação com *Rhizobium freirei*. *Revista Brasileira de Ciência do Solo*, *39*, 1661–1670. https://doi.org/10.1590/01000683rbcs20140342

Zamioudis, C., Mastranesti, P., Dhonukshe, P., Bliou, I., & Pieterse, C. M. (2013). Unraveling root developmental programs initiated by beneficial Pseudomonas bacteria. *Plant Physiology*, *162*, 304–318. https://doi.org/10.1104/pp.112.212597

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.