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Full Length Article

Co-Inoculation with *Bacillus amyloliquefaciens* in Soybean in different Modalities of Application and under Different Edaphoclimatic Conditions

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Abstract

Co-inoculation of nitrogen-fixing microorganisms and plant growth promoting bacteria (PGPB) is a sustainable alternative to increase soybean crop yield. The objective of this study was to evaluate the effect of co-inoculation of soybean with PGPB *Bacillus amyloliquefaciens* with nitrogen-fixing bacteria (*Bradyrhizobium elkanii*), in different application modalities and under different edaphoclimatic conditions, on yield and other yield parameters. The experiment was conducted in a randomized block design with four replications in a 4 × 10 factorial arrangement: 4 sites and 10 treatments (PGPB in association with standard inoculation – in different forms of application – seed treatment or sowing furrow). The results showed that there was no interaction between site and treatment, which indicates the stability of the treatments. Compared to inoculation, co-inoculation provided an increase in productivity of 4.34 and 4.83%, respectively for seed treatment and sowing furrow. For the other variables studied, no statistical differences were observed between co-inoculation in the seed treatment or in the sowing furrow. The results demonstrated that co inoculation of nitrogen-fixing bacteria, regardless of the modality of application, is an important management option to sustainably increase soybean productivity. © 2024 Friends Science Publishers

Keywords: Growth-promoting bacteria; Nutrient solubilization; Plant hormone; Nitrogen

Introduction

Soybean cultivation is one of the main agricultural activities in Brazil. Therefore, efforts to achieve sustainable high production ceilings are always highly relevant. Some of the factors that limit crop yield are the supply of nutrients and stimuli to the growth of this crop.

According to the literature, approximately 80 kg ha^{-1} of N is needed to produce one ton of soybean grains. Although N is abundant in the atmosphere, at approximately 78%, this high level is not readily available to plants, animals, or humans (Yang *et al.* 2019). High N uptake is important for high-yielding soybean cultivars (Santachiara *et al.* 2017). Fundamentally, N uptake by soybean plants is dependent on two alternative sources of N, biological N fixation (BNF) and N uptake from the soil. The relative contribution of each N source is the result of environmental conditions, management, and genetic factors (Salvagiotti *et al.* 2008; Santachiara *et al.* 2017; Córdova *et al.* 2019).

Although BNF is the main source of N for soybean crops and can provide all the N that soybean needs (Seixas *et al.* 2020), the estimation of BNF in soybean-intensive farming systems is essential (Landriscini *et al.* 2019), since the BNF process is affected by environmental conditions such as temperature, water content, N concentration, root zone pH, plant nutritional status, including C and N substrates in roots, and genetic variation in potential nitrogen fixation capacity (Liu *et al.* 2011). Furthermore, the continuous development of soybean cultivars to obtain higher yields, and that demand a greater supply of N for the crop, in turn, implies that research must be continued to ensure the benefits of fixing N_2 in the crop supply (Hungria

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et al. 2015).

One of the potential alternatives to achieve these goals is the use of PGPB (Mariano *et al.* 2004). PGPB are epiphytic or endophytic, non-pathogenic colonizers that promote plant growth directly or indirectly. These bacteria mainly help to increase crop yield; however, they can also act as biological management agents in promoting plant health (Yang *et al.* 2009). Inoculants carrying plant growth-promoting bacteria have been increasingly used to fully or partially replace chemical fertilizers (Santos *et al.* 2019).

PGPB act in the synthesis of hormones (auxins, gibberellins and cytokinins), reduction in ethylene levels (delaying senescence), solubilization of nutrients (such as iron and phosphorus), synthesis of enzymes related to systemic resistance and substances secreted to the apoplast in the fight against fungal penetration and acting in synergy with nitrogen-fixing bacteria (Bhattacharyya and Jha 2012). It is evident that PGPB provide benefits to plants, and the most used PGPB in agriculture include species of the genera *Azospirillum*, *Enterobacter*, *Pseudomonas*, and *Bacillus* (Babu *et al.* 2015). The genus *Bacillus* comprises growth-promoting, endospore-forming, gram-positive bacteria that can be isolated from soils and plant material worldwide. *Bacillus* sp. is natural soil inhabitants that produce antibiotics, enzymes and phytohormones beneficial to plants (Mazzuchelli *et al.* 2014). They are endospore formers, which consist of resistance structures capable of increasing their survival in the presence of adverse environmental factors (Nicholson *et al.* 2000). Accordingly, they can be stored as inoculants for a longer period and have a longer time of permanence in the soil. In addition, their application is easily done via seeds, spraying or sowing furrows.

Recently, field trials have shown that *Ba. subtilis*, *Ba. pumilus* and *Ba. amyloliquefaciens* in commercial inoculants prepared from the species alone or in combination – co-inoculation, were able to improve the chemical and microbiological attributes of the soil in addition to yield parameters in agricultural crops (Venancio *et al.* 2019; Alves *et al.* 2021). Co-inoculation is the association of at least two microorganisms that contribute to various microbial processes and improve plant growth and development (Redondo-Gómez *et al.* 2021). The combined inoculation of two or more PGPB species has recently become an emerging agricultural technology, leading to high reproducibility and efficiency under field conditions (Mesa-Marín *et al.* 2019). Co-inoculation studies with rhizobia and PGPBs are becoming a frequent practice in soybean cultivation, with the aim of developing sustainable agriculture (Pérez-Montaño *et al.* 2014; Galindo *et al.* 2022).

Although promising, co-inoculation of soybeans presents high variability of results, meaning that the results depend on the interaction between the application modalities and edaphoclimatic conditions. Therefore, results that demonstrate the possibility of carrying out co-inoculation in different forms of application and without depending on edaphoclimatic conditions are mandatory for the full adoption of this management. Thus, the aim of this study was to evaluate the effect of co-inoculation of soybean with plant growth-promoting bacteria (*Bacillus amyloliquefaciens)* with nitrogen-fixing bacteria (*Bradyrhizobium elkanii*), in different application modalities and under different edaphoclimatic conditions, on the yield and other yield parameters of the crop.

Materials and Methods

Experimental details

Four trials were conducted in locations with distinct edaphoclimatic characteristics, namely: Água Doce in Santa Catarina, Candói and Guarapuava in Paraná and Sertão in Rio Grande do Sul. The information on each location is presented in Table 1. The experiment was conducted in a randomized block design with four replications in a 4 × 10 factorial arrangement: 4 locations – Água Doce, Candói, Guarapuava and Sertão and 10 treatments, which are shown in Table 2. Each experimental unit consisted of 10 rows, with row spacing of 0.45 m, and length of 6.0 m.

Crop husbandry

The cultivar used was ZEUS IPRO, marketed by the company Brasmax, at a density of 300,000 plants per hectare. The experimental areas were desiccated with glyphosate (720 g ha^{-1} of a.i.). Sowing was carried out in a no-tillage system where the sowing furrows were opened with a commercial seeder, and the sowing operation was carried out with a manual SB seeder, which consists of a drive wheel, seed box and disc plough. Base fertilization with 0 kg ha^{-1} of N, 90 kg ha^{-1} of phosphorus (P_2O_5) and potassium (K_2O) were used for all sites. During the crop cycle, agrochemicals were applied to manage pests, diseases, and weeds.

When seed inoculation was carried out, they were inoculated in the shade, moments before sowing and, when via sowing furrow, they were opened with a bag, the products were applied with a manual sprayer, with an application rate of 60 liters per hectare and immediately sealed. In all treatments, the seeds were treated with Standak Top insecticide and fungicide formulation at a dosage of 2.0 mL for 1.0 kg of seed. Inoculant with a guaranteed minimum concentration of 2 x 108 CFU/mL on the expiration date. The inoculants were: *Br. elkanii* (SEMIA 587 and SEMIA 5019), a bacterium widely used in Brazil and *Ba. amyloliquefaciens*, strain MBI 600), naturally occurring, of wild origin. The strain was isolated in the United Kingdom and is deposited at the National Collection of Industrial, Marine and Food Bacteria Ltd (NCIMB), Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA, Scotland. Accession number: NCIMB 12376.

Table 1: Description and characterization of soybean test locations in the 2021/22 harvest

Description	Location			
	Água Doce/SC	Guarapuava/PR	Candói/PR	Sertão/RS
Property	CRK	EEAB	Capão Redondo	São Gabriel
Owner	Cícero Kuntz	AgrisusBrasil	Rodolpho Botelho	Oswaldo Sandini
Previous Summer crop	Soy	Soy	Corn	Soy
Previous Winter crop	Oats	Oats	Wheat	Ryegrass
Location				
Latitude	26° 40´ 36,28"	25° 16´ 27,5"	25° 21' 33.6"	28° 03´ 14,25"
Longitude	51° 33´ 9,19"	51° 31´ 25,1"	51° 49´ 14,13"	52° 16´ 33,24"
Altitude Meters	1195	995	993	720
Climate (Köppen)	Cfb	Cfb	Cfa	Cfa
Soil Texture				
Clay (g/kg)	660	590	610	560
Silt (g/kg)	270	290	290	270
Sand (g/kg)	70	120	100	170
Textural Class	Very clayey	Clay	Very clayey	Clay
Soil Classification	Alic Tb A Humic Cambisol	Typic Dystroferric Bruno Latosol	Typic Dystroferric Bruno Latosol	Alumino-ferric Red Latosol
Soil Chemical Analysis (0 to 20 cm depth)				
pH (CaCl)	4.8	5.14	6.17	5.06
O.M. (g/dm^3)	56.4	46.55	45.72	45.94
P - Mehlich (mg/dm^3)	6.97	9.44	3.21	18.14
K (cmol/dm^3)	0.38	0.61	0.19	0.23
Ca (cmol/dm^3)	5.25	6.3	8.23	7.18
Mg (cmol/dm^3)	1.45	2.01	3.85	2.4
Al (cmol/dm^3)	0.13	0.03	0	0.05
H+Al (cmol/dm^3)	5.92	5.91	2.75	6.03
SB (cmol/dm^3)	7.08	8.92	12.27	9.81
CEC- pH 7.0 (cmol/dm^3)	12.99	14.8	15.02	16.1
Rhizobia population (NMP g^{-1})	1.4x10^6	1.1x10^6	9.5x10^5	7.6x10^5
Crop data				
Sowing date	11/20/2021	12/17/2021	11/27/2021	11/21/2021
Harvest date	04/10/2022	04/21/2022	04/03/2022	05/06/2022

Table 2: Description of the treatments applied, in all the locations studied, in the soybean crop, 2021/22 harvest

N.	Application Method	Treatment (mL per ha or 100 kg seed)		
		Gelfix[1]	Integral[2]	Extender[3]
1	Control	Absolute Control		
2	Seed	200	Control	Inoculated
3	Furrow	300		
4	Furrow	600		
5	Seed	200	10	66
6	Furrow	100	5	33
7	Furrow	300	15	198
8	Furrow	600	30	396
9	Cover	Nitrogen (200 kg per hectare)		
10	Seed	200	(5.0 mL/100 kg)[4]	

[1] Gelfix - Liquid inoculant manufactured by BASF Ltd. with a minimum guaranteed concentration of 5×10^9 CFU/mL upon expiry. The inoculant contains *Bradyrhizobium elkanii* SEMIA 587 and SEMIA 5019. Registration in MAPA Nº SP 002768-5.000018. 1,500 mL bottle.

[2] Integral - Liquid inoculant manufactured by BASF Ltd. with a minimum guaranteed concentration of 2.2×10^{10} CFU/mL upon expiry. The inoculant contains the bacterium *Bacillus amyloliquefaciens* strain MBI 600. Registration in MAPA Nº SP 002768-5.000030. 1,500 mL bottle

[3] Sucrose and bacteria-protective polymers

[4] Synthetic Hormones - Kinetin = 0.09 g/L; Gibberellic acid = 0.05 g/L; 4-indole-3-butyric acid = 0.05 g/L

Data recorded

The variables studied were: yield, 1000-grain weight, number and mass of nodules, N content in leaves and grains, aerial and root dry matter production. After physiological maturity, the four central lines of the plot were harvested, 0.50 meters were discarded from each headland, and then the material was threshed and dried; the yield (kg ha^{-1}) of grains was then determined at 13% moisture.

Using a subsample of the collected material, 300 grains were counted and weighed for each plot, and the mass of one thousand grains was calculated from these values. To record number of nodules, five plants were harvested per plot at the R1/R2 stage. The plants were cut at ground level, and with a 942 cm³ volume cylinder (10 cm diameter by 12 cm in height) the roots of these plants were collected and later washed. After washing the roots, the nodules were removed and counted. Then, they were taken to a forced air ventilation oven at 65°C for 72 h and then weighed to obtain the dry mass of nodules. To evaluate the production of aerial and root dry mass, the same plants used to evaluate nodules were used. The total fresh aerial and root mass were weighed, 150 g sampled, and placed in a forced air ventilation oven at 65°C for 72 h to determine the percentage of dry mass. After this evaluation, the aerial and root dry mass per plant was estimated.

Finally, to determine the N content in the shoot, 20 leaves were collected from each plot, for which the 3rd completely expanded leaf was sampled. The grains obtained at harvest were used to evaluate the N of the grains. The leaf and grain samples were placed in a forced air ventilation oven at 65°C, until constant weight was obtained. Subsequently, the samples were ground in a Wily mill with a 1-mm diameter sieve and, after grinding, N was determined according to the methodology proposed by the Manual of Chemical Analysis of Soils, Plants and Fertilizers of Embrapa (Embrapa 2009).

Statistical analysis

The trial was statistically evaluated in a factorial arrangement. Thus, the isolated effects of the sources of local variation and treatment, as well as the interaction between these factors, were evaluated. When a significant effect of the sources of variation was observed, the Tukey test was performed at 5% significance. All analyses were performed using the Sisvar v. 5.6 software.

Results

The analysis of variance (Table 3) indicated that the source of local variation, except for the variable number of nodules, significantly influenced ($P < 0.01$) all the other variables studied. The source of treatment variation, in turn, except for the 1000-grain weight and N content in the grain, significantly influenced ($P < 0.01$) all the other variables studied. Finally, for all variables studied, there was no interaction between site and treatment.

All the sites studied were statistically different regarding yield (Table 4). The highest yield (5,137 kg ha^{-1}), as well as the highest number and weight of nodules, N content in leaves and grains, and aerial dry mass were obtained in the experiment conducted in Água Doce/SC. In addition, the lowest yield was observed in the trial conducted in Sertão/RS and this variable presented a negative relationship with TGM, since the highest TGM value was recorded in this location. The highest yield (4,833 kg ha^{-1}) was obtained with treatment 7 (co-inoculation of *Br. elkanii* and *Ba. amyloliquefaciens* applied in the sowing furrow) and differed statistically from the absolute control, inoculated control, nitrogenated control, and differed statistically from the treatments 4 (inoculation in the sowing furrow). It is also important to highlight that treatment 7 showed statistically similar yield to treatment 5 (co-inoculation of *Br. elkanii* and *Ba. amyloliquefaciens* applied in seed treatment). Treatment 7 showed a yield increase of 496 and 313 kg ha^{-1}, respectively for the absolute control and the inoculated control. Although there was no statistical difference, treatment 7 (application in the furrow) showed an increase in yield of 117 kg ha^{-1}, compared to treatment 5 (application in seed treatment). The importance of the inoculation of the soybean crop, treatments 1 and 2 – absolute control and inoculated control, was verified through the inoculation of the soybean crop, which in the average of the locations, provided an increase in yield of 183 kg ha^{-1}. It is also important to highlight that the replacement of the inoculant by N cover application showed a yield loss of 59 kg ha^{-1} (Table 5). The lowest number of nodules was 78.90 and 79.35 nodules per plant, respectively in the nitrogen control and absolute control treatments (Table 5). These treatments differed statistically from all other treatments studied. A similar behavior was observed for the mass of nodules, in which the lowest values for this variable were also observed in the nitrogen control, absolute control and synthetic hormone treatments, being statistically different from all the other treatments studied (Table 5).

The highest N content in the leaf (65.15 mg kg^{-1}) was observed in the nitrogen control treatment, which differed significantly from the absolute control and inoculated control treatments, in addition to treatments 7, 8 and 6, which presented the lowest values for this variable (Table 5). The aerial part and root dry mass showed a similar behavior to that already observed for N content in the leaf, in which the highest values for these variables were 12.44 and 1.56 g plant^{-1}, respectively for the variables aerial dry mass and root dry mass, obtained with the nitrogen control treatment – for both variables this treatment differed statistically from the other treatments studied (Table 5).

Discussion

The absence of interaction between location and treatment, for all variables studied, demonstrates that all treatments behaved similarly at all locations, therefore, the results are stable. The results show that yield has a direct and positive relationship with the increase in the number and mass of nodules, N content in grains and leaves, and aerial dry mass. Similar results were obtained by Dhami and Prasad (2009), who also reported a positive relationship between yield and number of nodules. Furthermore, the fact that the yield is statistically different at all the locations studied is expected in the presence of edaphoclimatic differences.

In the mean of the treatments, the location with the highest TGM (Sertão/RS – 231.2 g) had the lowest yield (3,994 kg ha^{-1}). Thus, the compensatory effect of the soybean crop is evident, *i.e.*, in scenarios with a lower number of grains, the crop tends to increase the TGM, even if this does not represent an increase in yield.

The treatments in which the soybean crop was co-inoculated with *Ba. amyloliquefaciens* – by means of the commercial product Integral, regardless of the application modality, led to higher yields than the absolute control and the inoculated control. These results agree with Armendariz *et al.* (2019) and Dawood *et al.* (2023), who suggested that

Table 3: Summary of the analysis of variance with the mean square values for the variables yield, TGM, number of nodules and nodule mass, aerial and root dry mass, of the soybean crop from the trials carried out in the 2021/22 harvest

SV	DF	Mean Square							
		Yield	TGM	Number nodules	Mass nodules	N leaves	N grains	Aerial mass	Root Mass
Block	3	225411.87 *	524.50 ns	29.56	22366.89 ns	1.02 ns	0.68 ns	0.57 ns	0.0169 ns
Location (L)	3	9043542.98 **	4858.55 **	15816.97 ns	309958.64 **	202.70 **	136.36 **	26.51 **	0.6605 **
Treatment (T)	9	419235.72 **	325.12 ns	886.49 **	42594.33 **	9.50 **	4.05 ns	14.51 **	0.0538 **
L x T	27	19652.57 ns	387.82 ns	163.82 **	6884.33 ns	3.37 ns	6.23 ns	0.60 ns	0.0140 ns
Error	117	58213.52	404.81	154.56 ns	8433.04	3.57	3.66	0.72	0.014
CV (%)		5.22	9.14	13.51	22.6	2.98	3.17	8.11	7.83
Mean		4622	220.14	92.03	406.39	63.42	60.31	10.47	1.43

TGM – thousand grain mass; SV – source of variation; DF – degrees of freedom; CV (%) – coefficient of variation; ** significant at 1%; ns – not significant

Table 4: Yield, TGM, number of nodules, nodule mass, N content in leaf and grain and aerial and root dry mass, in the soybean crop, in the different locations and the average of the treatments, in the 2021/22 harvest

Location	Yield	TGM	Nº Nodules	Mass nodules	N leaves	N grains	Aerial mass	Root Mass
	(kg ha^{-1})	(g)	(Unit)	(mg)	(g kg^{-1})		(g)	
Água Doce	5137 a	211.7 b	117.4 a	521.6 a	65.9 a	62.4 a	11.52 a	1.48 b
Candói	4757 b	209.7 b	70.7 d	310.1 c	60.7 d	57.9 c	10.65 b	1.42 b
Guarapuava	4601 c	228.0 a	96.2 b	413.0 b	64.5 b	60.8 b	10.09 c	1.25 c
Sertão	3994 d	231.2 a	83.8 c	380.9 b	62.7 c	60.1 b	9.63 c	1.56 a
Mean	4622	220.15	92.03	406.4	63.45	60.3	10.47	1.43

TGM – thousand grain mass; Means followed by the same letter do not differ statistically from each other according to the Tukey test at 5% significance

Table 5: Yield, TGM, number of nodules, nodule mass, N content in leaf and grain, and aerial and root dry mass in the soybean crop, with the different treatments and in the mean of the locations in the 2021/22 harvest

No. Tr.	Method	Treatment			Yield	TGM	Nº Nodules	Mass nodules	N leaves	N grains	Aerial mass	Root Mass
		Gelfix	Integral	Extender	(kg ha^{-1})	(g)	(Unit)	(mg)	(g kg^{-1})		(g)	
1	Control	Negative control			4337 d	220,60 ns	79,35 b	317,80 b	62,73 b	59,85 ns	8,49 c	1,34 b
2	Seed	200			4520 cd	220	96,98 a	477,13 a	62,50 b	60,48	10,11 b	1,43 ab
3	Furrow	300			4564 abcd	221	91,78 ab	416,23 ab	63,53 ab	60,28	10,54 b	1,38 b
4	Furrow	600			4543 bcd	208	97,73 a	434,68 a	63,95 ab	60,75	10,91 b	1,41 b
5	Seed	200	10	66	4716 abc	224	93,90 a	431,98 a	63,65 ab	60,45	10,31 b	1,40 b
6	Furrow	100	5	33	4714 abc	224	100,45 a	434,73 a	63,05 ab	59,83	10,53 b	1,46 ab
7	Furrow	300	15	198	4833 a	222	97,28 a	441,05 a	63,05 ab	60,7	10,47 b	1,46 ab
8	Furrow	600	30	396	4809 ab	222	90,35 ab	409,98 ab	63,03 ab	59,73	10,52 b	1,44 ab
9	Cover	Nitrogenated Control			4461 cd	220	78,90 b	325,28 b	65,15 a	61,28	12,44 a	1,56 a
10	Seed	200	(5.0 mL/100 kg) – *		4728 abc	220	93,90 a	375,45 ab	63,90 ab	59,98	10,44 b	1,43 ab
Mean					4622	220,15	92,06	406,43	63,45	60,33	10,47	1,43

TGM – thousand grain mass; Means followed by the same letter do not differ statistically from each other according to the Tukey test at 5% significance

*Synthetic hormones - (KINETIN = 0.09 g/L; GIBBERELLIC ACID = 0.05 g/L; 4-INDOLE-3-BUTYRIC ACID = 0.05 g/L)

inoculation with plant growth-promoting bacteria may be a safe and advantageous practice to improve soybean growth and yield. Co-inoculation between microorganisms provides an increase in the efficiency of biological nitrogen fixation and enables greater absorption of water and nutrients, and ultimately an increase in yield (Galindo *et al.* 2018; Alves *et al.* 2021). Co-inoculation exerts synergic action with *Bradyrhizobium* sp. in the process of biological nitrogen fixation (Hungria *et al.* 2022).

The data of this study reflects what is found in natural environments, since the promotion of plant growth by means of microorganisms is not carried out by a single bacterial isolate. Therefore, the association of compatible and synergistic microorganisms presents superior results than the isolated application of microorganisms (Fukami *et al.* 2016; Barbosa *et al.* 2022; Ngosong *et al.* 2022).

According to Pérez-Montaño *et al*. (2014), co-inoculation studies with B. japonicum and plant growth-promoting bacteria have become an increasingly frequent practice in the development of sustainable agriculture. Co-inoculation thus represents a new biotechnological tool to improve soybean yield without adding N chemical fertilizers, which contributes to current sustainability practices in agriculture (Hungria *et al.* 2015; Jardin 2015).

Published data have shown that the co-inoculation of microorganisms has the potential for a sustainable increase in yield. When compared to single inoculations, the co-inoculation of two or more bacterial species showed a beneficial relationship with the growth and accumulation of nitrogen not only in soybean crops, but also in corn, rice, and wheat (Vargas-Díaz *et al.* 2019; Nascimento *et al.* 2021; Galindo *et al.* 2022).

The microorganisms studied can positively impact crop yield directly, indirectly, or even due to a combination of the two (Fukami *et al.* 2018a. b; Marinković *et al.* 2018; Mustafa *et al.* 2019). Although yielding positive results, it is difficult to pinpoint exactly what were the real effects of the PGPB that positively impacted soybean yields, as described previously (Hungria *et al.* 2015; Pacentchuk *et al.* 2020). The results of this study agree with Marra (2012). According to this author, inoculation with microorganisms that can contribute to the greater availability of nutrients to plants, or the management of their microbial populations, has been suggested to reduce the use of mineral fertilizers. According to Turan *et al*. (2012), some strains of *Bacillus* can act on sources of inorganic P, making them readily available to plants. Similar results in which microorganisms promote plant growth and facilitate the intake of nutrients, including P, were observed by Tabassum *et al.* (2017); Ferreira *et al*. (2019); Majeed *et al*. (2023).

Results showed that the different species belonging to the genus *Bacillus* have several mechanisms to promote plant growth. *Ba. amyloliquefaciens* comprises several strains that promote plant growth. In 2011, Borriss with his co-authors created the division "subspecies *plantarum*" to include all plant-associated strains of *Ba. amyloliquefaciens* (Borriss *et al.* 2011). Chromatographic studies of the metabolites of *Ba. amyloliquefaciens* indicate the synthesis of the auxin IAA (indoleacetic acid) as the main substance responsible for promoting plant growth (Idris *et al.* 2007; Fukami *et al*. 2018a, b). These phytohormones result in an impressive improvement in root growth and architecture (Barbosa *et al.* 2022), which means an increase in the uptake of water and nutrients, as well as an improvement in the efficiency of nitrogen absorption (Cerezini *et al.* 2016; Galindo *et al.* 2022).

Besides the direct production of auxins, *Ba. amyloliquefaciens* can promote an indirect hormonal action in the plant, through signaling molecules that alter the synthesis of endogenous phytohormones in the plant, such as cytokinins and auxins (Asari *et al.* 2017). Some strains of *B. amyloliquefaciens* also could promote plant growth through the production of phytases (enzymes), which degrade the phytate present in soil organic matter, making phosphate available to plants (Idriss *et al.* 2002; Makarewicz *et al.* 2006). The absence of a significant effect on aerial and root dry mass with the use of *B. amyloliquefaciens* together with *Br. elkanii*, when compared to the inoculated control, did not influence soybean yield. Similar results were reported by Oliveira *et al*. (2019).

There was no difference in yield between the forms of application, seed treatment or sowing furrow. This demonstrates that the application in the sowing furrow has the same technical feasibility as the use of microorganisms only in seed treatment. These findings are essential for the practical use of these treatments at the field level, as it allows the producer to choose between two different forms of application.

Conclusion

There was no interaction between location and treatment, which demonstrates the stability of the treatments and can be used in a wide range of edaphoclimatic conditions. Co-inoculation between *B. amyloliquefaciens* in association with *Br. elkanii* increased productivity by 5% when compared to only with *Br. elkanii*. There was no difference in yield between the forms of application, seed treatment or sowing furrow. Therefore, co-inoculation between *Ba. amyloliquefaciens* in association with *Br. elkanii*, regardless of the modality of application and edaphoclimatic conditions, proved to be a viable management and capable of increasing the productivity of soybean crops in a sustainable way.

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Author Contributions

RBB, LDD, and MCT planned the experiments; IES, FP, and AHS conducted the field experiments; IES and LSM interpreted the results; IES, FP, and AHS wrote the manuscript; and IES and FP statistically analyzed the data and created the illustrations.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

The data presented in this study will be available upon request to the corresponding author.

Ethics Approval

Not applicable to this article.

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