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Use of growth-promoting bacteria to maximize soybean yield

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Abstract: The objective of this work was to evaluate the agronomic efficiency and viability of inoculants based on *Paenibacillus azotofixans* [CCTB10], *Bacillus subtilis* [CCTB04], Bacillus licheniformis [CCTB07] and *Bacillus circulans* [CCTB15], as growth promoters in soybean crops and their effects on the nutritional aspects of the crop when applied via sowing furrow. The experiment was conducted in five environments, Chapadão do Céu-GO, Chapadão do Sul-MS, Lavras-MG, Palmeira-PR, Maracaju-MS. The experimental design used was randomized blocks organized in a factorial scheme, with thirteen treatments referring to inoculants, and two modes of application (seed treatment and via sowing furrow), arranged in six replications. At 60 days after sowing, the shoot fresh weight (SFW, g) was measured in five plants per experimental unit. Nutritional analyzes on the leaves were carried out with the culture at R1 stage, with 15 leaves being sampled per experimental unit. Furthermore, the thousand grain weigh (TGW, g) was measured. The use of *Paenibacillus azotofixans*+ *Bacillus circulans*+ *Bacillus licheniformis*+ *Bacillus subtilis*, promotes increases in the shoot dry weight, thousand grain weight, grain yield, as well as in the potassium content in the grain. It can be used in seed treatment and in-furrow application.

Keywords: *Glycine max*; microorganisms; sustainability; N- nitrogen; P- phosphor; K- potassium.

Introduction

Soybeans (*Glycine max* L.) have high economic and social importance due to their multiple purposes and applications. According to data from the National Supply Company, the 2023/2024 Brazilian soybean harvest has an estimated production of 160 million tons of grains, in a cultivated area of 45 million hectares (Conab, 2023). Soybean productivity is determined by a set of factors such as phytosanitary and nutritional management, soil and climate conditions and genetic makeup (Peter et al., 2023). In this scenario, new investments are necessary to develop new technologies and management that provide greater sustainability and cost reduction for the national, state and regional soybean production chain (Pradebon et al., 2023).

The cost of fertilizers is responsible for around 20% of the total cost of soybean crops (Pavão e Voese, 2020). Nitrogen, potassium and phosphorus are the nutrients most required by the crop, with a demand of 65, 40 and 8 kg for 1000 kg of grain, respectively (Hungria et al., 2007; Caires e Fonseca, 2000). Thus, the use of growth-promoting microorganisms appears as an alternative to reduce the use of synthetic fertilizers, based on a nutritional balance of the plant, in addition to greater biological activity of the soil.

Rhizobacteria, for example, are bacteria that inhabit the rhizosphere of plants, promoting plant growth and, in return, benefit from exudates from the roots. These bacteria promote the recovery of nutrients from the soil and are extremely important for fertility (Rezende et al., 2021). Growth-promoting bacteria or rhizobacteria (RPCP) colonize the roots and their function is to promote the absorption of nutrients, mainly phosphorus, by solubilizing phosphates and assisting in nitrogen fixation (Kloepper e Schroth, 1981).

In the inoculant scenario, three *Bacillus* species are considered important as plant growth promoters: *B. subtilis*, *B. licheniformis* and *B. circulans*. Among the species of bacteria with the function of growth promoters, the genus *Bacillus* is mentioned, whose main function is to solubilize phosphate (Ratz et al., 2017). Another genus that presents growth-promoting characteristics is *Paenibacillus*, through nitrogen fixation, production of phytohormones and siderophores, and metabolites that combat fungi (Qian et al., 2012). *P. azotofixans* is a bacterium with the ability to perform nitrogen fixation, having the nifH gene that encodes the Fe nitrogenase protein, the enzyme responsible for this fixation (Liu et al., 2019; Aquino et al., 2021).

Understanding the efficiency of microorganisms for plant development allows us to minimize the use of agricultural inputs, which promotes more productive, profitable and sustainable agriculture. Given this, the objective of this work was to evaluate the agronomic efficiency and viability of using inoculants based on *Paenibacillus azotofixans* [CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] and *Bacillus circulans* [CCTB15], as growth promoters in soybean crops and their effects on the nutritional aspects of the crop when applied via sowing furrow.

Results and Discussion

According to the mean air temperature, in the 2022/2023 agricultural harvest, it was noticed that the lowest air temperatures occurred in the month of November, with temperatures below 18ºC in Chapadão do Céu-GO, Chapadão do Sul-MS, Maracaju-MS and Palmeira-PR. According to Alsajri et al. (2020) the ideal air temperature for soybeans is between 25ºC and 33ºC, an air temperature range that can be observed in all environments evaluated.

Soybeans are made up of approximately 90% water, considered fundamental for carrying out physiological and biochemical processes, determining factors for the development of the crop (Zanon et al., 2018). It was observed that the lowest volumes of precipitation occurred in the months of October and November, as well as in the month of March, in the environments of Chapadão do Sul- MS, Chapadão do Céu- GO and Maracaju- MS, but this did not appear as a factor limiting for the development of culture.

The variance components were estimated using restricted maximum likelihood (Table 2), and the Deviance analysis showed significance ($p<0.01$) for the interactions environment x shoot fresh weight (SFW), environment x thousand grain weight (TGW), environment x grain yield (GY), environment x phosphorus in the leaf, environment x nitrogen in the leaf, environment x potassium in the leaf, environment x phosphorus in the grains, environment x nitrogen in the grains and environment x potassium in the grains.

Therefore, the heritability presented for the variables shoot fresh weight, grain yield, thousand grain weight, nitrogen in the leaf, phosphorus in the leaf, potassium in the leaf, nitrogen in the grains, phosphorus in the SFW grains and potassium in the grains was considered low with values between 0.01 and 0.07. This indicates that the environment has an influence of more than 93% on the expression of these characteristics. The analysis for the measured variables indicated values between 0.14 and 0.83 of the grain yield expression comes from this interaction, between the treatments and the study environments, that is, values considered inconstant.

Regarding average heritability (H^2mg), this is estimated when using averages as an evaluation or selection unit (Maia et al., 2009). Thus, average heritability values were found, with a magnitude between 13.09% and 54.09%, that is, these effects may come from the genotype, regardless of the environment and treatment in which it would be exposed, the variables thousand grain weight, nitrogen, phosphorus and potassium in the leaf did not present values for average heritability. According to Storck et al. (2010), accuracy is an indication of experimental precision of genotype competition tests, that is, it depends on the proportion between the genetic and experimental coefficients of variation, a value considered high was observed only for potassium in the grain, the other variables presented values intermediaries.

The genotypic correlation between genotype x environment performance (RGE) makes it possible to classify the incident interaction as simple or complex. Low correlations were observed for shoot fresh weight, thousand grain weight, nitrogen in the leaf, nitrogen, phosphorus and potassium in grains presented values considered low. According to Cargnin et al. (2006), low correlation indicates that superior treatment in a given environment may not present the same performance in another location. High values were observed for potassium and phosphorus in the leaf. The residual coefficient of variation (CVr) refers to the experimental error, where low values are noted for all variables indicating the precision of the experiment. In relation to the coefficient of variation of the proportion between genotypic and residual coefficient of variation (CVratio), for the measured variables, it was low.

The variance decomposition analysis represents the degree of influence of the treatments on the expression of each variable (Figure 1). It was observed that for the expression of shoot fresh weight (SFW), 29.18% was due to the treatment x environment interaction and 2.29 was the contribution of the treatment. For the potassium content in the leaf (K_F) and thousand grain weight (TGW) the contribution of the interaction was 60.93% and 44.98% respectively. The grain yield (GY) was 7.31% influenced by the treatment and 41.08% was the effect of the treatment x environment interaction. Greater contributions from the interaction between environment and treatment (GEN:ENV) were evidenced for the phosphorus content in the leaf (P_F), with 83.72%. The contents of phosphorus in the grain (P_G), potassium in the grain (K_G) and nitrogen in the grain (N_G) showed a contribution from the interaction of 43.51%, 14.15% and 49.03% respectively, the effects of the treatments were from 1.72 to 6.45% for these variables. These values reveal a great contribution of the environment in the expression of the variables (Loro et al., 2022; Schimitd et al., 2023).

Through BLUP, it is possible to identify treatments with predictable behavior in relation to shoot fresh weight and that are responsive to environmental variations. The behavior of treatments can be observed in each environment studied. In Chapadão do Céu-GO and Palmeira-PR, all treatments performed below the selection range (13.12 g). In Lavras-MG, only treatments with application of Biofree via seed treatment combined with 300 kg ha^{-1} of natural fertilizer (T12), *Paenibacillus azotofixans* [CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] and *Bacillus circulans* [CCTB15] 500 mL ha^{-1} via sowing furrow combined with 300 kg ha^{-1} of mineral fertilizers (T9) and only the use of fertilizer showed a superior response for shoot dry weight (Figure 2). In Maracaju-MS, performance was found to be below the selection range (13.12 g) for treatments T2, T4, T5, T7, T9, T11, T12, T13, the other treatments were superior. In Palmeira-PR, all treatments were above the selection range. Strains of the *Bacillus* genus stimulate plant growth and development through phytohormones (gibberellins,

Table 1. Description of treatments used on soybeans.

Treatment	Application mode	Fertilizing	Fertilization Dose (kg ha[-1])	Inoculant (I.A)	Dose mL 100 Kg sementes [-1]/ mL ha[-1]	Commercial Product
1	Seed Treatment	Absence		Absence		
2	Seed Treatment	100% of NPK	300	Absence		
3	Seed Treatment	75% NPK	225	Absence		
4	Seed Treatment	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	100	BTP 0167-20
5	Seed Treatment	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	100	BTP 0167-20
6	Seed Treatment	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	300	BTP 0167-20
7	Seed Treatment	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	300	BTP 0167-20
8	Seed Treatment	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	500	BTP 0167-20
9	Seed Treatment	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	500	BTP 0167-20
10	Seed Treatment	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	1000	BTP 0167-20
11	Seed Treatment	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	1000	BTP 0167-20
12	Seed Treatment	100% NPK	300	*Pseudomonas fluorescens* CCTB03 *+Azospirillum brasiliense* Ab-V6	300	Biofree
13	Seed Treatment	75% NPK	225	*Pseudomonas fluorescens* CCTB03 *+Azospirillum brasiliense* Ab-V6	300	Biofree
14	Seeding Furrow	Absence		Absence		
15	Seeding Furrow	100% of NPK	300	Absence		
16	Seeding Furrow	75% NPK	225	Absence		
17	Seeding Furrow	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	100	BTP 0167-20
18	Seeding Furrow	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	100	BTP 0167-20
19	Seeding Furrow	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	300	BTP 0167-20
20	Seeding Furrow	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	300	BTP 0167-20
21	Seeding Furrow	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	500	BTP 0167-20
22	Seeding Furrow	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	500	BTP 0167-20
23	Seeding Furrow	100% NPK	300	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	1000	BTP 0167-20
24	Seeding Furrow	75% NPK	225	*Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] *and Bacillus circulans* [CCTB15]	1000	BTP 0167-20
25	Seeding Furrow	100% NPK	300	*Pseudomonas fluorescens* CCTB03 *+Azospirillum brasiliense* Ab-V6	300	Biofree
26	Seeding Furrow	75% NPK	225	*Pseudomonas fluorescens* CCTB03 *+Azospirillum brasiliense* Ab-V6	300	Biofree

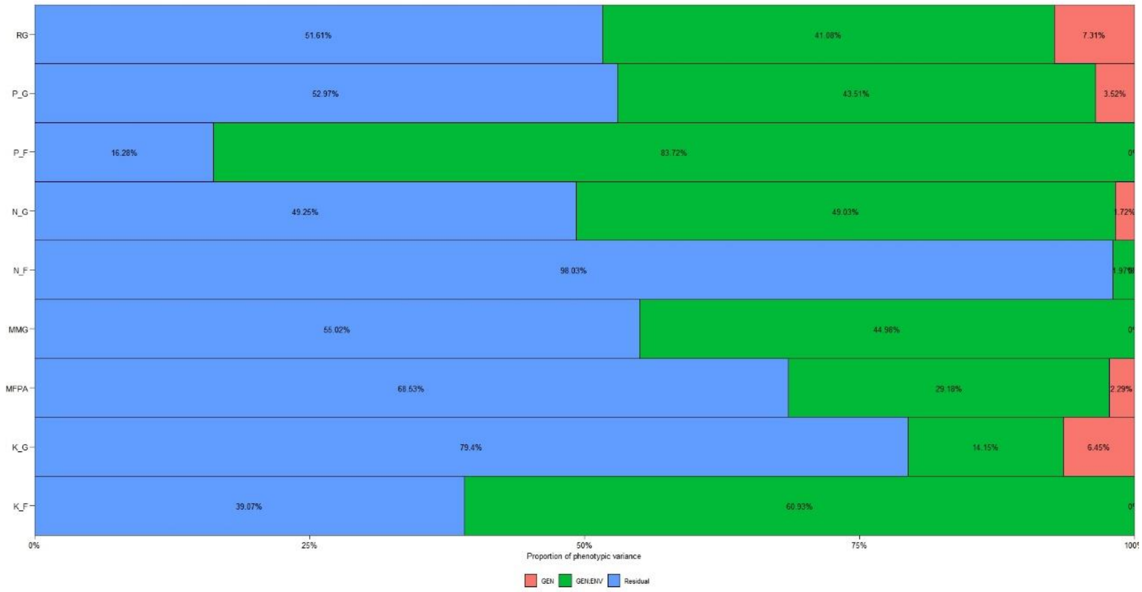

Fig 1. Decomposition of the variance of the variables, shoot fresh weight (SFW), nitrogen (N_F), phosphorus (P_F) and potassium (K_P) contents in leaves, thousand grain weught (TGW), grain yield (GY) and contents nitrogen (N_G), phosphorus (P_G) and potassium (K_G) in the grains.

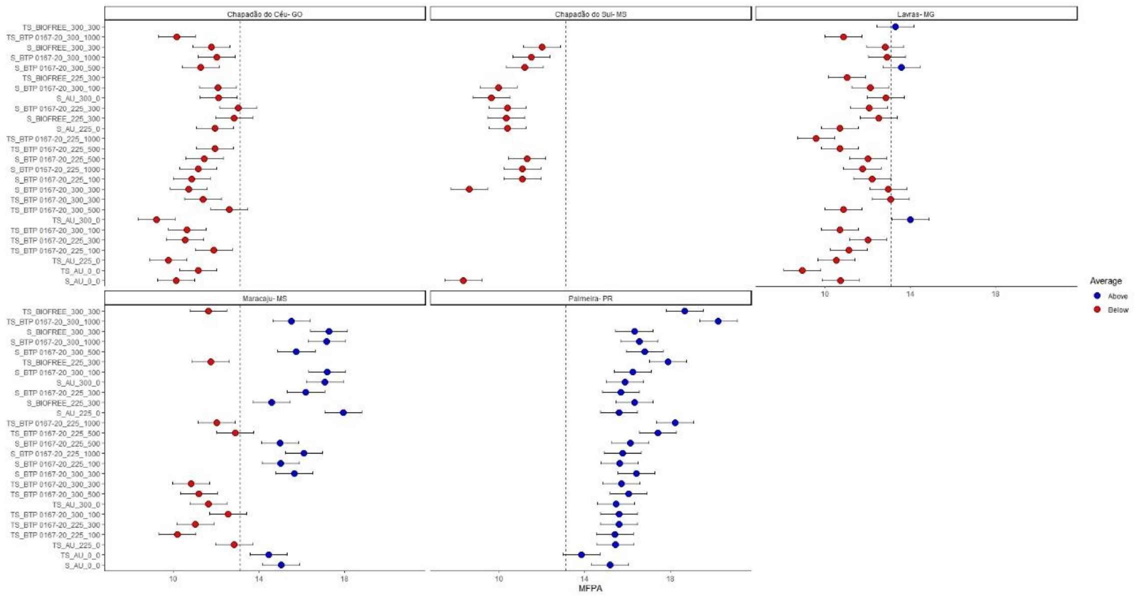

Fig 2. Estimates of the components of the average shoot dry weight per specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

ethylene, auxins and cytokines), in addition to carrying out nutrient mineralization (Hashem et al., 2019).

The nitrogen content in the leaves, there was no difference between the treatments, where all were above the selection range, with values 48.42 g.kg^{-1} (Figure 3). The highest phosphorus contents in the leaf in Lavras-MG were evident for treatments T11, T12 and T13, with values of 7.37, 7.29 and 7.03 g.kg^{-1} (Figure 4). In Maracaju-MS, inferior results were observed for treatments T24, T25 and T26. In Palmeira-PR, all treatments performed below the selection point. The increase in phosphorus (P) accumulation was observed by Zucareli et al. (2018) through inoculation with *B. subtilis* in bean plants, demonstrating the efficiency of this genus of bacteria.

Potassium levels in the leaf (Figure 5) in Lavras-MG were lower only in the absence treatments (T1) and in the use of a combination of *Paenibacillus azotofixans*[CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] and *Bacillus circulans* [CCTB15] (500 mL ha^{-1} via sowing furrow) with 225 kg ha^{-1} of fertilizer (T24). In Maracaju-MS, lower performance was observed in the exclusive use of 300 kg ha^{-1} of fertilizer without inoculation (T3) and in the absence of fertilization and inoculation (T1).

Beneduzi and Passaglia (2011) found, in cherry plants treated with bacteria of the genera Bacillus and Pseudomonas, significant increases in the levels of phosphorus and potassium in the leaves. In turn, studies conducted by Stepien et al. (2022) showed increases in nitrogen, phosphorus and potassium contents in the soil when mineral fertilizers were combined with *P. azotofixans*, *B. megaterium* and *B. subtilis*. These results possibly contributed to increasing the availability of these nutrients in the soil, reflected in the leaf content of the plants.

In the analysis of the thousand grain weight (Figure 6) in Chapadão do Céu-GO and Chapadão do Sul-MS, all treatments demonstrated positive responses above the selection point. However, in Chapadão do Céu-GO, treatments T22, T23 and T26, applied via sowing furrow,

Table 2. Estimates of variance components for shoot dry weight, grain yield, thousand-grain weight, leaf nitrogen, leaf phosphorus, leaf potassium, grain nitrogen, grain phosphorus, grain potassium, in different environments.

VAR	MODEL	LOGLINK	AIC	LRT	PR>(CHISQ)
SFW	GEN	-1680.315004	3424.630009	0.434422244	0.509826961
SFW	GEN:ENV	-1718.287483	3500.574966	76.37937934	2.34083E-18
GY	GEN	-5026.480409	10116.96082	2.670086977	0.102250435
GY	GEN:ENV	-5109.185724	10282.37145	168.0807181	1.94254E-38
TGW	GEN	-2807.441178	5678.882356	-9.27685E-11	1
TGW	GEN:ENV	-2908.22656	5880.45312	201.570764	9.48555E-46
N_F	GEN	-1461.13839	2962.27678	-9.09495E-13	1
N_F	GEN:ENV	-1461.344026	2962.688052	0.411271776	0.521324707
P_F	GEN	-473.5871374	987.1742747	-3.41061E-13	1
P_F	GEN:ENV	-715.5396877	1471.079375	483.9051006	3.02E-107
K_F	GEN	-1015.43807	2070.876141	-3.38878E-09	1
K_F	GEN:ENV	-1138.970577	2317.941154	247.0650137	1.1332E-55
N_G	GEN	-833.5689657	1695.137931	0.013780658	0.906550177
N_G	GEN:ENV	-855.9470092	1739.894018	44.76986765	2.21607E-11
P_G	GEN	-239.124461	506.248922	0.085848248	0.769523133
P_G	GEN:ENV	-259.8125854	547.6251708	41.46209706	1.20179E-10
K_G	GEN	-264.7399996	557.4799992	0.291467052	0.589281939
K_G	GEN:ENV	-266.1374583	560.2749166	3.086384454	0.078950051

PARAMETERS	SFW	GY	TGW	N_F	P_F	K_F	N_G	P_G	K_G
σ^2F	10.2927	226179.1218	292.9151	34.0104	1.4553	8.1263	133.4890	0.5986	0.5855
H^2	0.0229	0.0731	0.0000	0.0000	0.0000	0.0000	0.0172	0.0352	0.0645
$GEIr^2$	0.2918	0.4108	0.4498	0.0197	0.8372	0.6093	0.4903	0.4351	0.1415
H^2mg	0.2202	0.4239	0.0000	0.0000	0.0000	0.0000	0.1309	0.2519	0.5409
Acuracy	0.4693	0.6511	0.0000	0.0000	0.0000	0.0000	0.3618	0.5019	0.7355
RGE%	0.2986	0.4432	0.4498	0.0197	0.8372	0.6093	0.4989	0.4510	0.1512
CVg	3.7020	3.1739	0.0000	0.0000	0.0000	0.0001	2.3342	2.7571	2.0934
CVr	20.2366	8.4327	8.0582	11.9249	11.4152	10.5141	12.4737	10.6894	7.3433
CV ratio	0.1829	0.3764	0.0000	0.0000	0.0000	0.0000	0.1871	0.2579	0.2851

Var: Variables; Model: Model; Log Lik: Restricted Maximum Likelihood Logarithm; AIC: Akaike Informational Criterion; LRT: Restricted Maximum Likelihood Ratio; PR: Probability by Chi-Square test: σ^2F: Phenotypic variance; H^2: broad-sense heritability $GEIr^2$: coefficient of determination of the effects of the genotype-environment interaction; H^2mg: Heritability of the genotype mean; RGE: genotypic correlation between treatment performance x environments; CVg: Genotypic coefficient of variation; CVr: Coefficient of residual variation; CV ratio: coefficient of variation of the proportion between genotypic and residual coefficient of variation.

presented the largest weights, varying between 203 and 209 grams. In Chapadão do Sul, the largest thousand grain weight was evident in the T20 treatment, registering 193.93 grams. On the other hand, in Maracaju-MS, no treatment exceeded the established selection range. In Palmeira-PR, superior performance was achieved in treatments T11, T12 and T13, applied via seed treatment. According to the Blup for soybean grain yield (Figure 7), the behavior of the treatments can be observed in each environment studied. In the Chapadão do Céu-GO environment, the inferior treatments were T4, T10, T11, T13 and T14. In Chapadão do Sul-MS superiority was found for T6, T7, T9, T12 and T13. For Lavras, it was observed that only T6 and T12 were higher than the selection point. For Maracaju-MS inferior performance occurred for T2, T3, T4, T5, T10, T13, T15, T19, T21, T22, T23 and T25. Palmeira-PR, inferiority was noticed for T1, T3, T5, T7, T9, T11, T12, T14, T15, T18 and T26. However, the highest grain yields were observed when using *P. azotofixans* + *B. megaterium* + *B. subtilis*, studies by Chagas Junior et al. (2021), found an increase in soybean grain yield, using bacteria from the *Bacillus* genus. Studies Stępień et al. (2022), showed increases in wheat grain yield with the combination of mineral fertilizer and *Paenibacillus azotofixans, Bacillus megaterium* and *Bacillus subtilis*.

The nitrogen content in the grains was higher when applied *Paenibacillus azotofixans* [CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] and *Bacillus circulans* [CCTB15] combined with mineral fertilizer with a concentration of 132.06 g kg^{-1}. However, all treatments were superior to the selection range in Lavras-MG (Figure 8). For the Palmeira-PR environment, all treatments were below the selection range. Phosphorus levels in soybean grains (Figure 9), in Lavras- MG all treatments were selected, with higher concentrations of phosphorus in the grain for T7, unlike Palmeira- PR, none were higher than the selection range. The potassium content in the grains (Figure 10), superior performance was evident for T17, T18, T19, T20, T21, T23, T26, with values greater than 9.28 $g.kg^{-1}$.

The application of bacteria that promote plant growth, facilitating the absorption of nitrogen, phosphorus and potassium, in addition to the positive influence of phytohormones, can enable crops aligned with environmental preservation without compromising productivity. This allows you to reduce the amount of chemical fertilizers used or maximize their efficiency. The use of microorganisms represents a promising strategy for more productive, profitable and sustainable agriculture. The development of new products with microorganisms can, therefore, significantly contribute to increasing productivity in soybean cultivation, reducing production costs and minimizing impacts on the environment.

Materials and Methods

The experiment was conducted in the municipalities of Chapadão do Céu-GO (18°21'25.2''S, 52°38'11.0''W and 840m altitude), Chapadão do Sul-MS (18º46'37.3"S, 52º38' 53.6"W and 810m altitude), Lavras-MG (21°12'54.7"S, 45°03'16.9"W and 902m altitude),

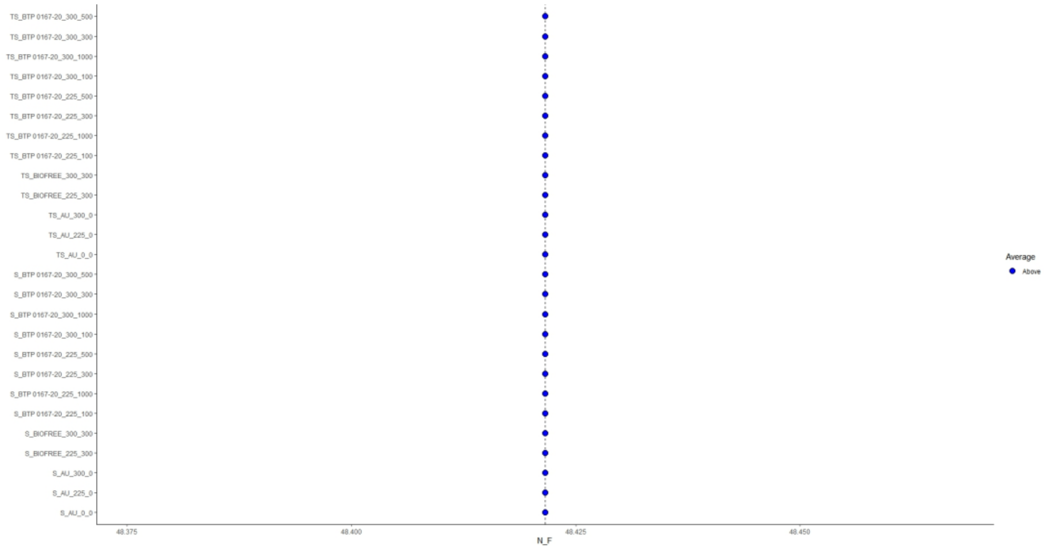

Fig 3. Estimates of the components of average nitrogen in leaves by specific BLUP.

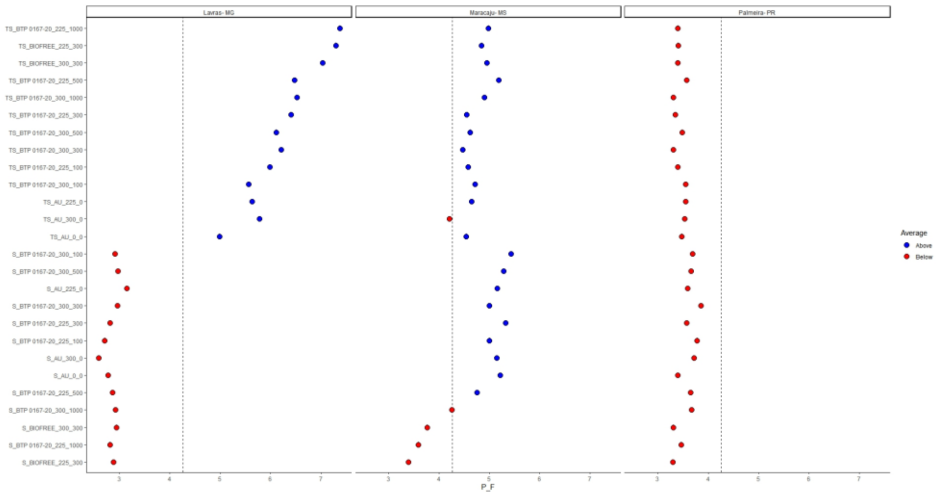

Fig 4. Estimates of the components of the average phosphorus in leaves by specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

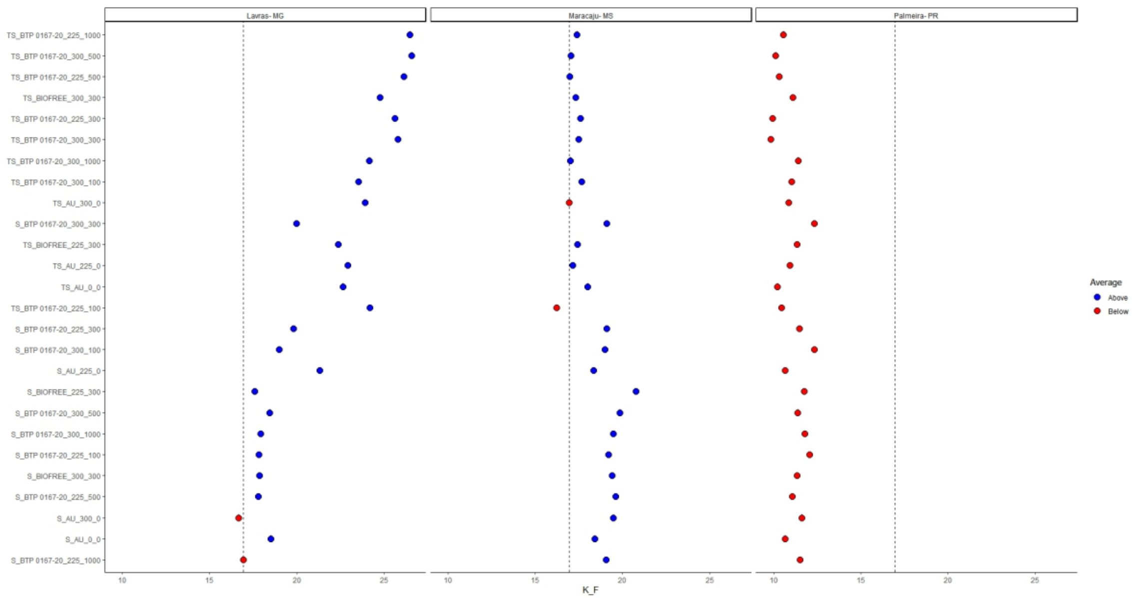

Fig 5. Estimates of the components of the average potassium in leaves by specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

Palmeira-PR (25º25'37.06"S, 50º2'53.39"W and 876m altitude) and Maracaju-MS (18°21'25.2''S, 52°38'11.0''W and 840m altitude). The experimental design used was randomized blocks organized in a factorial scheme, with thirteen treatments referring to inoculants, and two modes of application (seed treatment and via sowing furrow), arranged in six replications (Table 1). The experimental units were 12 meters long and 2 meters wide. Sowing was carried out in the first fortnight of November 2022. The soybean cultivar BMX FIBRA IPRO was used, with a sowing density of 14 seeds per linear

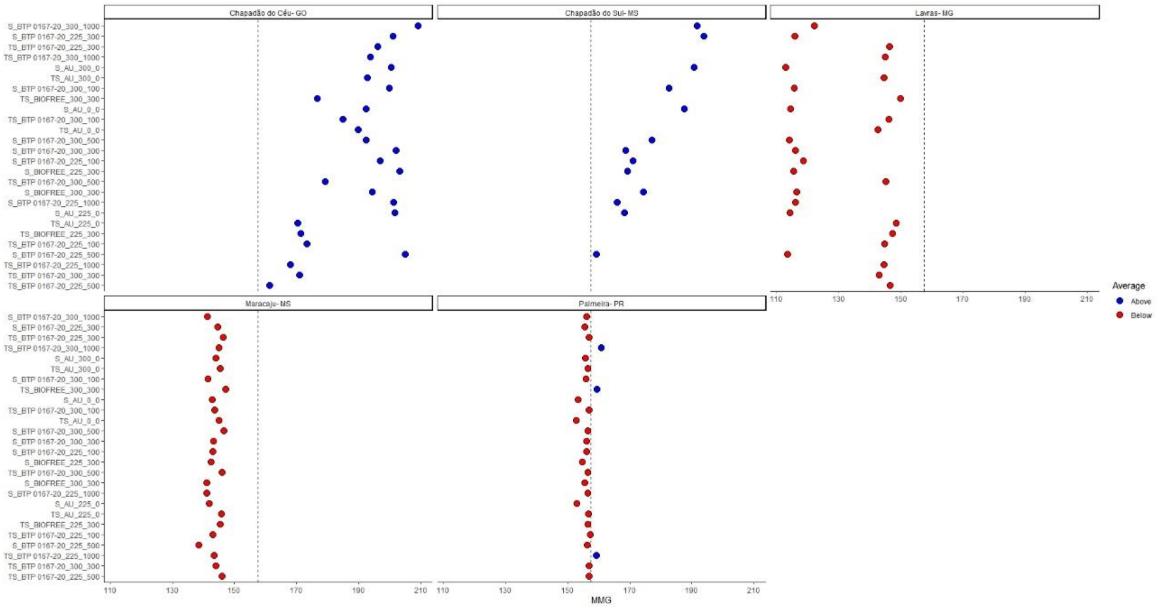

Fig 6. Estimates of the components of the average thousand soybean weight per specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

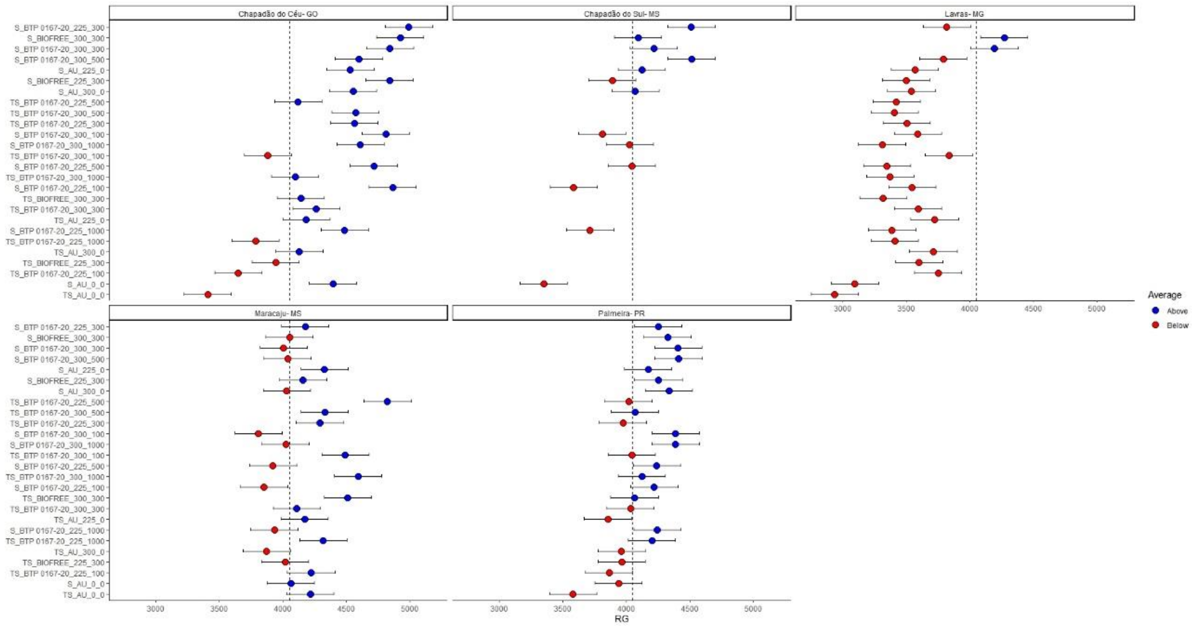

Fig 7. Estimates of the components of the average soybean grain yield by specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

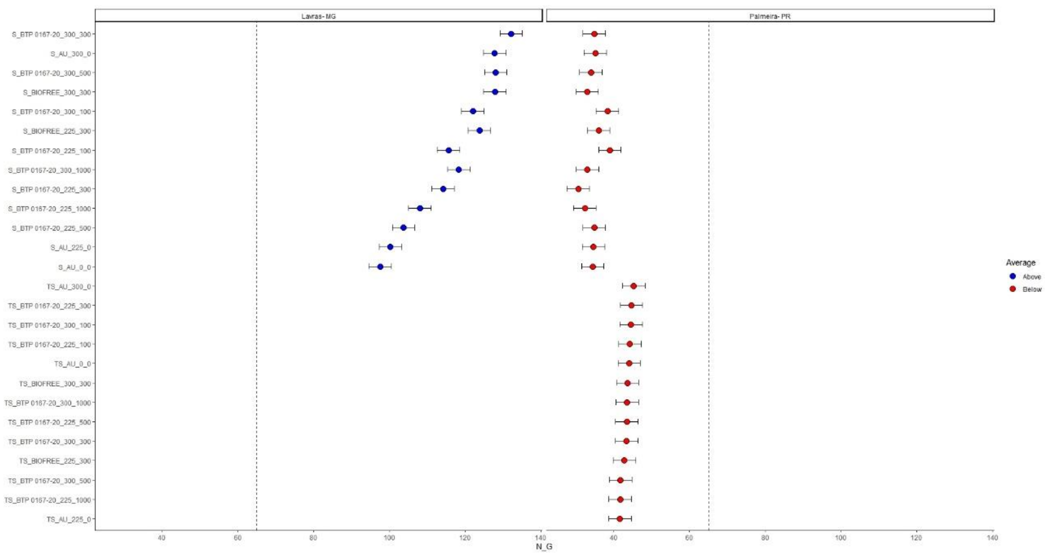

Fig 8. Estimates of the components of the average nitrogen in grains by specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

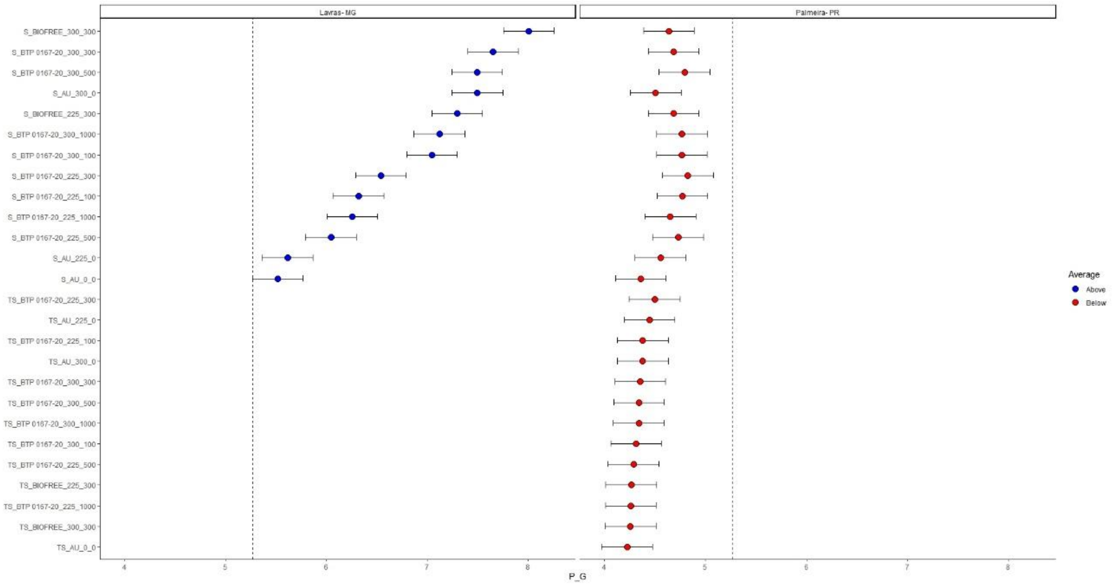

Fig 9. Estimates of the components of the average phosphorus in grains by specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

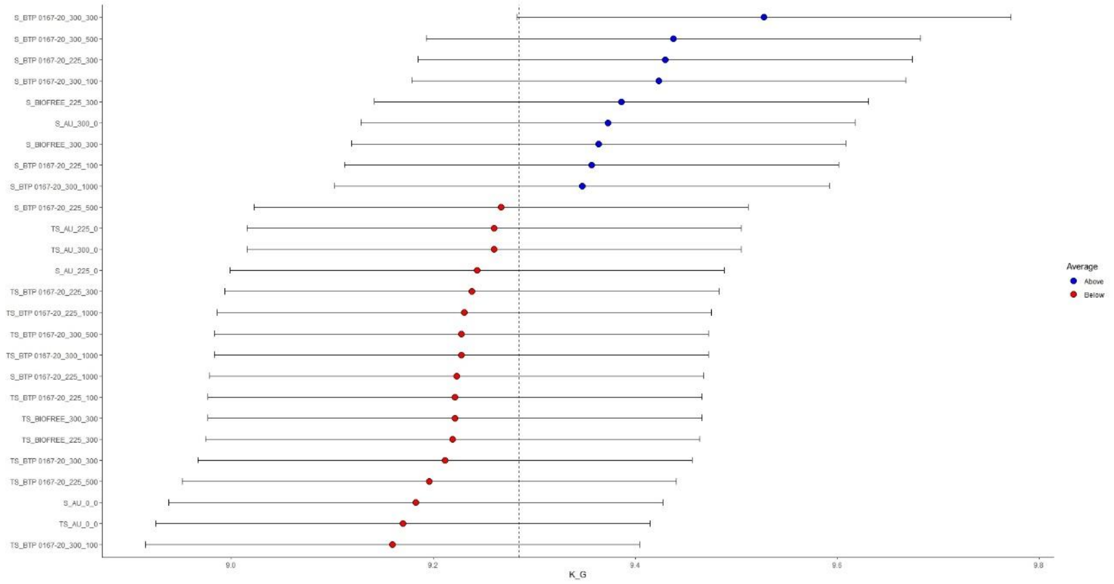

Fig 10. Estimates of the components of the average potassium in grains by specific BLUP measured in Chapadão do Céu- GO, Chapadão do Sul- MS, Lavras- MG, Palmeira- PR, Maracaju- MS and 26 treatments.

meter^{-1}. Cultural treatments were carried out preventively to minimize biotic effects on the plants in the experiment.

The inoculants BTP 0167-20 (*Paenibacillus azotofixans* [CCTB10], *Bacillus subtilis* [CCTB04], *Bacillus licheniformis* [CCTB07] and *Bacillus circulans* [CCTB15]) and Biofree (*Azospirillum brasilense* Ab-V6 and *Pseudomonas fluorescens* CCTB03) were applied via seed treatment at the time of soybean sowing. For application via seed treatment, equipment called a rotating drum was used to constantly move the seeds to obtain a homogeneous layer covering the products. The volume of syrup used was 500 ml 100 kg^{-1} of seeds.

For application in the furrow, the product was sprayed on the planting line in a spray volume of 60 L ha^{-1}, using a Camaleão Prosolos flow rate controller adapted to the seeder. In this method, the product was placed before closing the furrow, in the same sowing line where the seed was deposited. The spray tips used were a 0.62 Solid Jet nozzle with a 3 KGF Nozzle Valve pressure of 3 bar.

Fertilizations with 100% of the recommended dose of N, P and K (T2, T4, T6, T8, T10 and T12) were carried out with the application of 333 kg ha^{-1} of simple superphosphate (SSP), 103 kg ha^{-1} of potassium chloride and 13 kg ha^{-1} of urea, equivalent to 60 kg ha^{-1} of P_2O_5, 60 kg ha^{-1} of K_2O and 6 kg ha^{-1} of N. For treatments with 75% of the recommended dose of N, P and K (T3, T5, T7, T9, T11 and T13), were carried out with the application of 250 kg ha^{-1} of simple superphosphate (SSP), 78 kg ha^{-1} of potassium chloride and 10 kg ha^{-1} of urea, the equivalent of 45 kg ha^{-1} of P_2O_5, 45 kg ha^{-1} of K_2O and 4.5 kg ha^{-1} of N. At 60 days after sowing, the shoot fresh weight (SFW, g) was measured in five plants per experimental unit. Nutritional analyzes on the leaves were carried out with the culture at R1 stage, with 15 leaves being sampled per experimental unit. After collection, the samples were separated and sent to the laboratory. The nitrogen (N_F), phosphorus (P_F) and potassium (K_F) contents were expressed in g kg^{-1}. The nutrient levels in the grains were also determined in the laboratory, with data expressed in g kg^{-1}, for nitrogen (N_G), phosphorus (P_G) and potassium (K_G). Grain yield (kg ha^{-1}) was evaluated by harvesting 30 plants per experimental unit. The thousand grain weigh (TGW, g) was measured, expressed in grams, with all data corrected for 13.0% moisture.

Subsequently, the method based on Restricted Maximum Likelihood (REML) was used to estimate the variance components and genetic parameters, where significance was obtained through Deviance analysis at 5% probability using the Chi-square test. In this way, we estimated the phenotypic variance (σ^2F), broad-sense heritability (H^2), genotype mean heritability (H^2mg), coefficient of determination of the effects of the genotype-environment interaction (GEI), genotypic correlation between genotypes x environment performance (RGE), genotypic coefficient of variation (CVg), residual coefficient of variation (CVr) and coefficient of variation of the ratio between genotypic and residual coefficient of variation (CVratio). Subsequently, the stratified BLUP model was used to specifically recommend treatments adapted to a given region.

The meteorological information on the mean temperature (Tmean, °C), minimum (Tmin, °C) and maximum air temperature (Tmax, °C), precipitation (Prec, mm), were expressed with the aim of better understanding the results obtained (nasa power, 2023). To carry out the statistical analyses, the Exp.Des.pt (Ferreira et al., 2021), metan (Olivoto; Lucio, 2020) and ggplot2 (Wickham, 2016) packages were used, through the R Software (R Core Team, 2023).

Conclusion

The use of *Paenibacillus azotofixans*+ *Bacillus circulans*+ *Bacillus licheniformis*+ *Bacillus subtilis* (BTP 0167-20), promotes increases in the shoot dry weight, thousand grain weight, grain yield, as well as in the potassium content in the grain. It can be used in seed treatment and in-furrow application. : Special thanks to the company Biotrop solutions in biological technology for funding the research.

References

Alsajri FA, Wijewardana C, Irby JT, Ballaloui N, Krutz LJ, Dourado B, Gao W, Reddy R (2020) Developing functional relationships between temperature and soybean yield and seed quality. Agronomy Journal. 112(1): 194-204.

Aquino JPA, Antunes JEL, Bonifácio A, Rocha SMB, Amorim MR, Alcantara Neto F, Araújo ASF (2021) Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum. Theoretical and Experimental Plant Physiology. 33: 249-260.

Beneduzi A, Passaglia LMP (2011) Genetic and phenotypic diversity of plant growth promoting bacilli. Bacteria in Agrobiology: Plant Growth Responses. 1-20.

Caires EF, Fonseca AF (2000) Absorption of nutrients by soybeans grown in the direct planting system due to surface liming. Bragantia. 59(2): 213-220.

Cargnin A, Souza MA, Carneiro PCS, Sofiatti V (2006) Interaction Between Genotypes and Environments and Implications for Gains from Selection in Wheat. Brazilian Agricultural Research. 41: 987-993.

Chagas Junior AF, Chagas LFB, Martins ALL, Colonia BSO, Souza MC, Braga Junior GM (2021) Efficiency of Bacillus subtilis Bs10 as a plant growth promoting inoculant in soybean crop under field conditions. Research, Society and Development. 10(14): e441101422141-e441101422141.

Conab - National Supply Company. Grains: grains harvest 2023/24 3rd research. Available in: <https://www.conab.gov.br/component/k2/item/download/36193_fcdad01edb7eb9aff3b211ee0d841a61>. Access in: Dec, 15th, 2023.

Ferreira EB, Cavalcanti PP, Nogueira DA (2021). _EXPDES.PT: Experimental Designs Package (Portuguese). R Package Version 1.2.2 <https://cran.r-project.org/package=expdes.pt>

Hashem A, Tabassum B, Abd Allah EF (2019) *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi Journal of Biological Sciences. 26: 1291–1297.

Kloepper JW, Schroth MN (1980) Plant, growth-promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopatholog. 71 (6): 642-644.

Liu X, Li YQ, Guan G, Chen S (2019) *Paenibacillus* strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. Peer J. 7: e7445.

Loro MV, Carvalho IR, Sfalcin IC, Pradebon LC (2022) Decomposition of white oat phenotypic variability by environmental covariates. Brazilian Journal of Agriculture-Revista de Agricultura. 97(3): 279-302.

Maia MCC, Resende MDV, Paiva JR, Cavalcanti JJV, Barros LMB (2009) Simultaneous selection for production, adaptability and genotypic stability in cashew clones, via mixed models. Tropical Agricultural Research. 39: 43-50.

Meert L, Müller MML, Genú AM, Espíndola JS, Aragão GN, Figueiredo AST (2020) Different inoculants, forms of inoculation and their effects on the agronomic characteristics of soybean crops. Research, Society and Development. 9(10): e2969108499-e2969108499.

Nasa Poweer: about the power project. About the power project. 2021. Available In: https://power.larc.nasa.gov/. Accsses in: Dec. 13th 2023.

Olivoto T, Lúcio AD (2020) "metan: An R package for multi-environment trial analysis." _Methods in Ecology and Evolution. 11(6): 783-789.

Pradebon LC, Carvalho IR, Loro MV, Port ED, Bonfada B, Sfalcin IC, Santos IJ, Bruinsma GMW, Silva JÁ, Alban AA, Challiol MA (2023) Soybean adaptability and stability analyzes to the organic system through AMMI, GGE Biplot and mixed models methodologies. Ciência Rural. 53(9): e20220262.

Pavão JA, Voese SB (2020) Analysis of costs and productivity of conventional and gm soy in Brazil in 2017 and 2018. Singular. Social and Humanities. 1(3).

Peter M, Dallmann MM, Pedó T, Aumonde TZ, Barbosa BS, Medeiros LB, Pimentel JR (2023) Soybean ecophysiology in response to different sowing times and cropping densities. Research, Society and Development. 12(5): e24212541765-e24212541765.

Qian CD, Liu TZ, Zhou SL, Ding R, Zhao WP, Li O, Wu XC (2012) Identification and functional analysis of gene cluster involvement in biosynthesis of the cyclic lipopeptide antibiotic pelgipeptin produced by *Paenibacillus elgii*. BMC Microbiol. 12:197.

Ratz RJ, Palácio SM, Quinõr FRE, Vicentino RC, Michelim HJ, Ritcher LB, Pimentel JR (2017) Biotechnological potential of plant growth-promoting rhizobacteria in

corn and soybean cultivation. Engevista. 19(4): 890-905.
Rezende CC, Silva MA, Frasca LLM, Faria DR, Filippi MCC, Lanna AC, Nascente AS (2021) Multifunctional microorganisms: use in agriculture. Research, Society and Development. 10(2): e50810212725.3
R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available in: https://www.R-project.org.LHÕESASASS DE TADAS
Schmidt AL, Carvalho IR, Silva JAG, Lângaro NC, Oliveira AC, Pradebon LC, Loro MV, Roza JP D, Bruinsma GMW (2023) Decomposition of phenotypic variation of white oats by meteorological and geographic covariables. Agronomy Journal. 115(5): 2239-2259.
Stępień A, Wojtkowiak K, Kolankowska E (2022) Effect of commercial microbial preparations containing *paenibacillus azotofixans*, *bacillus megaterium* and *Bacillus subtilis* on the yield and photosynthesis of winter wheat and the nitrogen and phosphorus content in the soil. Applied Sciences. 12(24): 12541.
Storck L, Cargnelutti AF, Lúcio ADC, Rubin SAL (2010) Assessment of experimental precision in competition trials of soybean cultivars. Science and Agrotechnology. 34: 572-578.
Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.