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Efficiency of use of symbiotic bacteria in maize culture

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Abstract: Although Brazil has an effective participation in the world maize market, grain productivity is still considered low. In this context, new technologies are essential for increasing the productivity of this crop, mainly linked to soil fertility. The use of growth-promoting bacteria presents itself as a new technology, which aims to reduce dependence on chemical fertilizers, promoting greater sustainability in grain production. The experiment was carried out in the municipalities of Santa Barbara do Sul- RS, Palmeira- PR, Santo Antônio de Posse- SP, Maracaju- MS, Diamantino- MT, Primavera do Leste- MT, Tangará da Serra- MT, Chapadão do Céu- GO, Uberlândia- MG and Lavras- MG. The experimental design was randomized blocks, with treatments differing between environments, totaling 75 treatments. 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 (x^2). Subsequently, the stratified *BLUP* model was used to specifically recommend treatments adapted to a given region. The use of *Azospirillum brasiliense* and *Pseudomonas fluorencens* via aerial spraying, in the sowing furrow and seed treatment, using 50% and 75% of the fertilizer dose demonstrated satisfactory results for maize grain productivity.

Keywords: *Azospirillum brasiliense; Pseudomonas fluorencens;* fertility; *Zea mays* L.; productivity.

Introduction

Maize (*Zea mays* L.) is an important cereal of the grass family (Poaceae). Its cultivation is mainly for human and animal food with various applications, being a component of a multitude of products ranging from the manufacture of feed to the cosmetics industry (Silva *et al*., 2020). Brazil is the third largest producer in the world, behind only the United States of America and China. According to data from the National Supply Company, the area cultivated with this cereal in the 2023/2024 harvest was 21.07 million cultivated hectares, with a production of 117.6 thousand tons of grains (CONAB, 2024).

Although Brazil has an effective participation in the world maize market, grain productivity is still considered low. In this sense, there is room to maximize productivity through some challenges that need to be overcome, such as managing insect pests, diseases and improving fertility and soil quality (Contini *et al*., 2019). In this context, new technologies are essential for increasing the productivity of this crop, mainly linked to soil fertility.

Improving soil fertility is essential, since maize has a high nutritional demand and many Brazilian soils are poor in the availability of some nutrients, requiring high amounts of fertilizers (Resende *et al*., 2019; Pinheiro *et al*., 2020). Studies by Silva *et al*. (2016), evaluating the rate of nutrient absorption by maize, observed a nutrient export rate for grains of 14 kg of N, 4.2 kg of P and 3.5 kg of K per ton of grains produced. In this way, the use of growth-promoting microorganisms appears as an alternative to minimize the use of synthetic fertilizers, based on a nutritional balance of the plant, advocating the development of new technologies that increase the efficiency of fertilizers.

The use of growth-promoting bacteria presents itself as a new technology, which aims to reduce dependence on chemical fertilizers, promoting greater sustainability in grain production. The use of these bacteria can be carried out via seed treatment, inoculation in the sowing furrow or foliar application. Among the benefits of these bacteria are the increased availability of nutrients, mainly through the solubilization of phosphorus (P), and the synthesis and release of phytohormones (such as indoleacetic acid - IAA) and volatile organic compounds, which stimulate root growth and shoot plant (Bhattacharyya *et al*., 2017; Ferreira *et al*., 2019; Zohaib *et al*., 2019; Faria *et al*., 2022).

Furthermore, inoculation with *Pseudomonas fluorescens* results in increased wheat productivity (Naiman *et al*., 2009) and maximizes maize germination, growth and productivity (Gholami *et al*., 2009). Despite all the positive reports on the use of *P. fluorescens* as a growth promoter, little is known about its action and viability in Brazilian conditions, where there are few commercial inoculants containing the species available. So far, only *Azospirillum brasilense* is successfully used in Brazil and will promote the growth and productivity of field-grown maize and wheat (HUNGRIA *et al*., 2010), growth and biomass

production by *Brachiaria* spp. in degraded pastures (HUNGRIA *et al.*, 2016) and nodulation and productivity when coinoculated with rhizobia in *Glycine max* and *Phaseolus vulgaris L.* (Hungria *et al.*, 2013).
In this way, growth-promoting bacteria present themselves as a promising technology to promote more productive, profitable and sustainable agriculture. The use of these bacteria can contribute to increasing the productivity of maize crops, reducing production costs with greater sustainability. The objective was to evaluate the influence of using the bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* and method of application on maize grain productivity.

Results and Discussion

Through analysis of Biplot main components, it is possible to observe that the environments Tangará da Serra- MT and Diamantino- MT, showed greater affinity for the minimum temperature and average air temperature, with averages of 25ºC and minimums of 17ºC. Lavras-MG had a greater affinity for precipitation, with an average of 5.68 mm daily. The maximum air temperature (Figure 1), was observed greater affinity for Primavera do Leste- MT, Maracaja- MS and Santa Barbara do Sul- RS, where the maximums reached close to 36ºC. Temperatures are higher than the upper base temperature (35ºC) (Renato et al., 2013), thus stopping the development of the genotype, resulting in a decrease in the plant's physiological processes.
The descriptive analysis, stratified by phenological stage and maize cultivation environments (Figure 2), showed that in the vegetative emergence (VE) stage, the mean temperatures remained within the optimum range for the crop in all environments, with Diamantino- MT and Tangará da Serra- MT with the highest mean temperatures (25.7ºC to 29.2ºC). At the V4 stage, there was a predominance of lower mean temperatures with values between 14.7 and 22ºC in all environments with the exception of Diamantino- MT. In the V6 stage, there was a predominance of temperatures above 25ºC, unlike the reproductive period (R1 and R6), where the means were concentrated between 22 and 23.9ºC.
The maximum air temperatures (figure 2b), at the emergency stage, were higher frequencies inferred for temperatures above 31ºC, with the exception of Santa Barbara do Sul-RS and Palmeira-PR, which were environments with milder temperatures. In the other stages of crop development, maximum temperatures were within the optimum range, only in Santa Barbara do Sul-RS, in R6 temperatures above 31ºC were observed. Temperatures above the optimum range for maize (>32ºC), the photosynthesis process is reduced, stomatal closure occurs as the rate of water loss to the atmosphere is greater than the water absorbed by the roots in the soil. This minimizes CO_2 assimilation, while respiration rates increase, decreasing biomass accumulation (Roig-Oliver *et al.*, 2021).
Air temperature has a great influence on the growth and development of maize, as according to Laudien *et al.* (2020), events with higher minimum temperatures (18ºC) and greater rainfall promote greater productivity of maize grains in tropical climates. Therefore, it was observed that the minimum temperatures were very close to this range for most of the cultivation period, not being a limiting factor for crop productivity (Figure 2c). From accumulated precipitation, it was inferred that all locations, with the exception of Santa Barbara do Sul-RS and Palmeira-PR, presented satisfactory precipitation volumes. Several negative responses occur in water deficits that precede pollination, such as grain abscission, even if water is available at the time of pollination. Water deficit is one of the main reasons related to meteorological conditions for the low productivity of maize grains (Laudien *et al.*, 2020), a fact that justifies the lower grain productivity in Santa Barbara do Sul-RS and Palmeira- PR.
The variance components were estimated using restricted maximum likelihood (Table 2), and the *Deviance* analysis showed significance ($p<0.10$) of probability for the effects of environments in relation to grain productivity. According to Loro *et al.* (2022), this indicates that there is different behavior of treatments depending on the location. These results reveal the possibility of recommending different treatments for all environments.
In Chapadão do Céu- GO, Palmeira- PR, Tangará da Serra- MT, Lavras- MG and Santo Antonio de Posse- SP, high genotypic variance was observed, with values above 0.54 and residual variance below 0.45 (Table 3). This indicates a high contribution of treatments to maize grain productivity and a low contribution of the environment to the expression of this characteristic. However, in other locations, high values of residual variance and low contribution of genotypic variance were inferred. Thus, there was a great contribution from the environment in Diamantino- MT, Maracaju- MS, Primavera do Leste- MT, Santa Barbara do Sul- RS and Uberlândia- MG.
Therefore, it can be stated according to the *REML* analysis (Table 2), that the heritability (H^2) for the grain productivity variable was considered low to moderate, this indicates that the environment has a high influence on the expression of this character in, in Diamantino- MT, Maracaju- MS, Primavera do Leste- MT, Santa Barbara do Sul- RS and Uberlândia- MG. Studies by Resende (2002) consider low heritability with values alternating between 0.01 and 0.15 and moderate between 0.16 and 0.49 for grain productivity.
In relation to the average heritability of genotypes (H^2mg), this is used when using averages as an evaluation or selection unit (Maia *et al.*, 2009). In other words, this value shows how much of the percentage of phenotypic variation comes from a genetic nature (Pupin *et al.*, 2015). High values were measured in the environments of Chapadão do Céu- GO, Lavras- MG, Maracaju- MS, Palmeira- PR, Primavera do Leste- MT, Santo Antônio de Posse- SP and Tangará da Serra- MT. Pradebon *et al.* (2023), when evaluating soybean grain productivity, they observed values of 0.56, thus attributing most of the variation in grain productivity to genetic effects.
Accuracy is a parameter used to infer the experimental precision of competition tests. Within the *REML* analysis (Table 3), values greater than 0.7 were observed for the locations Chapadão do Céu- GO, Lavras- MG, Maracaju- MS, Palmeira- PR, Primavera do Leste- MT, Santo Antônio de Posse- SP and Tangará da Serra- MT. Cargnelutti Filho *et al.* (2012) and

Table 1. Description of the treatments used.

TT	Application mode	Inoculants	Treatment	Fertilizer Dose	Formulation	Inoculant Dose
T1	Pulverization	Biofree	100%	190 Kg	Triple superphosphate + 120 Ammonium sulfate + 80 KCL	0.3
T2	Pulverization	Biofree	100%	250 Kg	NPK 10-15-15 + 200 Kg Urea	0.3
T3	Pulverization	Biofree	50%	300 Kg	NPK 04-14-08 + 300 Kg Urea	0.3
T4	Furrow	Biofree	100%	360 Kg	NPK 04-14-08 + 160 Kg Urea	0.3
T5	Pulverization	Biofree	100%	400 Kg	NPK 08-24-12 + 100 Kg Urea	0.3
T6	Pulverization	Absence	100%	190 Kg	Triple superphosphate + 120 Ammonium sulfate + 80 KCL	
T7	Pulverization	Absence	100%	190 Kg	Triple superphosphate + 120 Ammonium sulfate + 80 KCL	
T8	Pulverization	Absence	100%	250 Kg	NPK 10-15-15 + 200 Kg Urea	
T9	Pulverization	Absence	100%	250 Kg	NPK 10-15-15 + 200 Kg Urea	
T10	Pulverization	Absence	100%	300 Kg	NPK 04-14-08 + 300 Kg Urea	
T11	Pulverization	Absence	100%	300 Kg	NPK 04-14-08 + 300 Kg Urea	
T12	Pulverization	Absence	100%	360 Kg	NPK 04-14-08 + 100 Kg Urea	
T13	Pulverization	Absence	100%	360 Kg	NPK 04-14-08 + 160 Kg Urea	
T14	Pulverization	Absence	100%	400 Kg	NPK 08-24-12 + 100 Kg Urea	
T15	Pulverization	Absence	100%	400 Kg	NPK 08-24-12 + 100 Kg Urea	
T16	Pulverization	Absence	50%	125 Kg	NPK 10-15-15 + 100Kg Urea	
T17	Pulverization	Absence	50%	150 Kg	NPK 04-14-08 + 150 Kg Urea	
T18	Pulverization	Absence	50%	180 Kg	NPK 04-14-08 + 80 Kg Urea	
T19	Pulverization	Absence	50%	200 Kg	NPK 08-24-12 + 50 Kg Urea	
T20	Pulverization	Absence	50%	80 Kg	Triple superphosphate + 60 Ammonium sulfate +40 KCL	
T21	Pulverization	Absence	50%	125 Kg	NPK 10-15-15 + 100 Kg Urea	
T22	Pulverization	Absence	50%	125 Kg	NPK 10-15-15 + 100 Kg Urea	
T23	Pulverization	Absence	50%	150 Kg	NPK 04-14-08 + 150 Kg Urea	
T24	Pulverization	Absence	50%	150 Kg	NPK 04-14-08 + 150 Kg Urea	
T25	Pulverization	Absence	50%	180 Kg	NPK 04-14-08 + 80 Kg Urea	
T26	Pulverization	Absence	50%	180 Kg	NPK 04-14-08 + 80 Kg Urea	
T27	Pulverization	Absence	50%	200 Kg	NPK 08-24-12 + 50 Kg Urea	
T28	Pulverization	Absence	50%	200 Kg	NPK 08-24-12 + 50 Kg Urea	
T29	Pulverization	Absence	50%	80 Kg	Triple superphosphate + 60 kg Ammonium sulfate +40 kg KCL	
T30	Pulverization	Absence	50%	80 Kg	Triple superphosphate + 60 kg Ammonium sulfate +40 KCL	
T31	Furrow	0	0%	0	0	0
T32	Furrow	Biofree	100%	250 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	0.3
T33	Furrow	Biofree	100%	250 Kg	NPK 10-15-15 + 200 Kg Urea	0.3
T34	Furrow	Biofree	100%	350 Kg	NPK 10-15-15	0.3
T35	Furrow	Biofree	100%	360 Kg	NPK 04-14-08 + 160 Kg Urea	0.3
T36	Furrow	Biofree	100%	400 Kg	NPK 04-30-15 + 200 Kg Urea + 100 KCL	0.3
T37	Furrow	Absence	100%	250 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	
T38	Furrow	Absence	100%	250 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	
T39	Furrow	Absence	100%	250 Kg	NPK 10-15-15 + 200 Kg Urea	
T40	Furrow	Absence	100%	250 Kg	NPK 04-14-08 + 160 Kg Urea	
T41	Furrow	Absence	100%	350 Kg	NPK 10-15-15	
T42	Furrow	Absence	100%	350 Kg	NPK 10-15-15	
T43	Furrow	Absence	100%	360 Kg	NPK 04-14-08 + 160 Kg Urea	
T44	Furrow	Absence	100%	360 Kg	NPK 04-14-08 + 160 Kg Urea	
T45	Furrow	Absence	100%	400 Kg	NPK 4-30-15 + 200 Kg Urea + 100 KCL	
T46	Furrow	Absence	100%	400 Kg	NPK 04-30-15 + 200 Kg Urea + 100 KCL	
T47	Furrow	Biofree	50%	125 Kg	NPK 0-15-15 + 100 Kg Urea	0.3
T48	Furrow	Biofree	50%	180 Kg	NPK 04-14-08 + 80 Kg Urea	0.3
T49	Furrow	Biofree	50%	200 Kg	NPK 04-30-15 + 100 Kg Urea + 50 KCL	0.3

Benin *et al*. (2013), when evaluating experimental precision in irrigated rice trials and wheat positioning in different regions, it is classified as high (>0.7).

Regarding the coefficient of variation of the proportion between genotypic and residual variation coefficient (*CVratio*), for grain productivity, it presented satisfactory values only in Chapadão do Céu- GO, Lavras- MG, Palmeira- PR, Santo Antonio de Posse- SP and Tangará da Serra- MT (>1.0). According to Veconvski and Barriga (1992), the occurrence of values greater than 1 means the selection of treatments is favorable and promising.

Table 2. Description of the treatments used.

T50	Furrow	Absence	50%	125 Kg	NPK 10-15-15 + 100 Kg Urea	
T51	Furrow	Absence	50%	125 Kg	NPK 10-15-15 + 100 Kg Urea	
T52	Furrow	Absence	50%	180 Kg	NPK 04-14-08 + 80 Kg Urea	
T53	Furrow	Absence	50%	180 Kg	NPK 04-14-08 + 80 Kg Urea	
T54	Furrow	Absence	50%	200 Kg	NPK 04-30-15 + 100 Kg Urea + 50 KCL	
T55	Furrow	Absence	50%	200 Kg	NPK 04-30-15 + 100 Kg Urea + 50 KCL	
T56	Furrow	Biofree	75%	187.5 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	0.3
T57	Furrow	Biofree	75%	262 Kg	NPK 10-15-15	0.3
T58	Furrow	Absence	75%	187.5 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	
T59	Furrow	Absence	75%	187.5 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	
T60	Furrow	Absence	75%	262 Kg	NPK 10-15-150	
T61	Furrow	Absence	75%	262 Kg	NPK 10-15-15	
T62	Furrow	Absence	0%	0	0	
T63	TS	Biofree	100%	250 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	0.3
T65	TS	Biofree	100%	350 Kg	NPK 10-15-15	0.3
T66	TS	Absence	100%	250 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	
T67	TS	Absence	100%	250 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	
T68	TS	Absence	100%	350 Kg	NPK 10-15-15	
T69	TS	Absence	100%	350 Kg	NPK 10-15-15	
T70	TS	Biofree	75%	187.5 Kg	NPK 05-35-00 + 10% de Ca + 10% de S	0.3
T71	TS	Biofree	75%	262 Kg	NPK 10-15-15	0.3
T72	TS	Absence	75%	187.5 Kg	NPK 5-35-00 + 10% de Ca + 10% de S	
T73	TS	Absence	75%	187.5 Kg	NPK 5-35-00 + 10% de Ca + 10% de S	
T74	TS	Absence	75%	262 Kg	NPK 10-15-15	
T75	TS	Absence	75%	262 Kg	NPK 10-15-15	

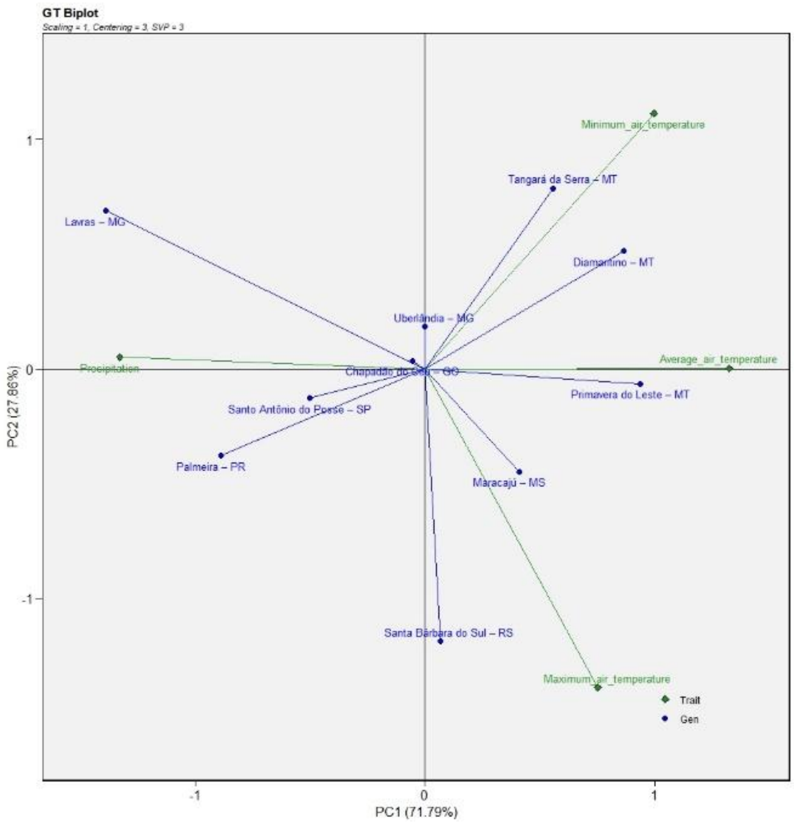

Figure 1. Biplot main components for growing environments and meteorological variables.

Multivariate *BLUP* allows the identification of superior treatments, reducing distortions induced by fluctuations in cultivation environments (Carvalho *et al.*, 2020). This result shows the usefulness of mixed models and *REML/BLUP* procedures to perform treatment selections in different locations. Thus, in Santa Barbara do Sul-RS, yields below the general average of the experiment (2500 kg ha^{-1} of grains) were observed for treatments T27 and T15 respectively (Figure 3a), that is, treatments that used only chemical fertilization. However, superior performance was observed for T14, T5, T19 and T28.

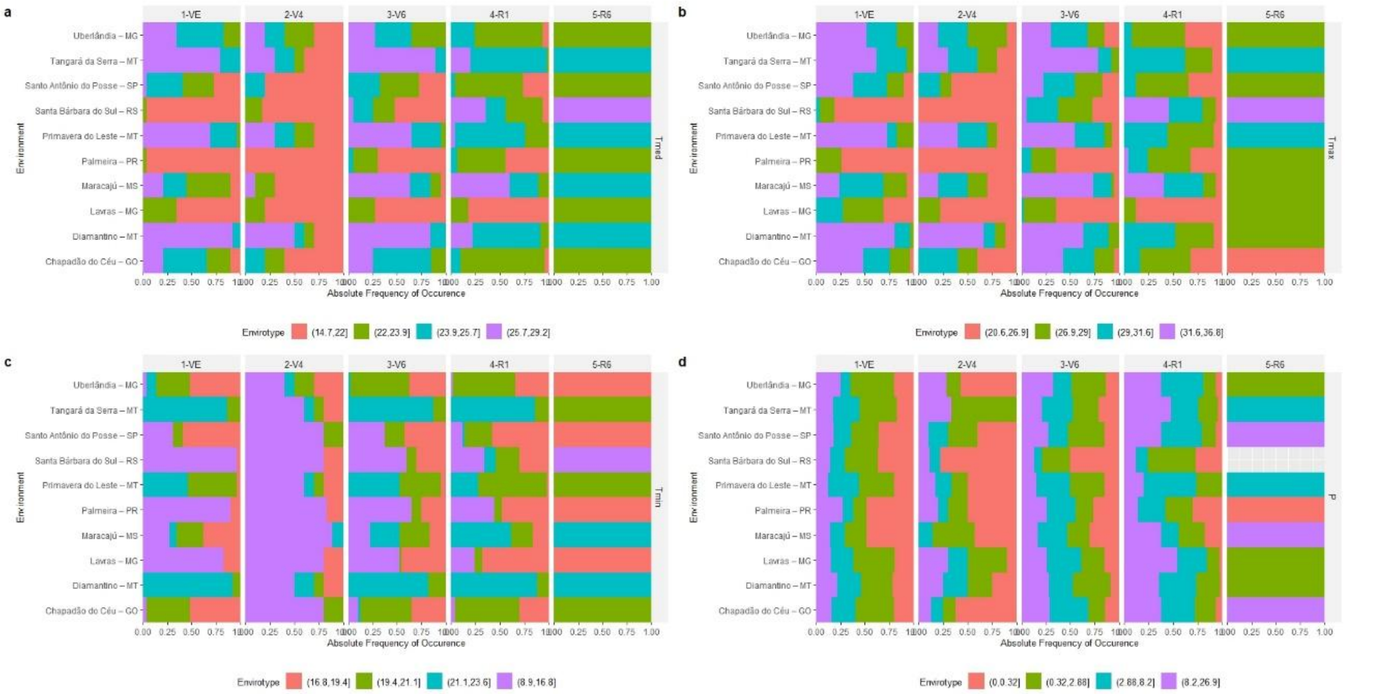

Figure 2. Typification of maize growing environments in relation to meteorological variables, Mean air temperature (Tmean, ºC), Maximum air temperature (Tmax, ºC), minimum air temperature (Tmin, ºC) and Precipitation (P, mm).

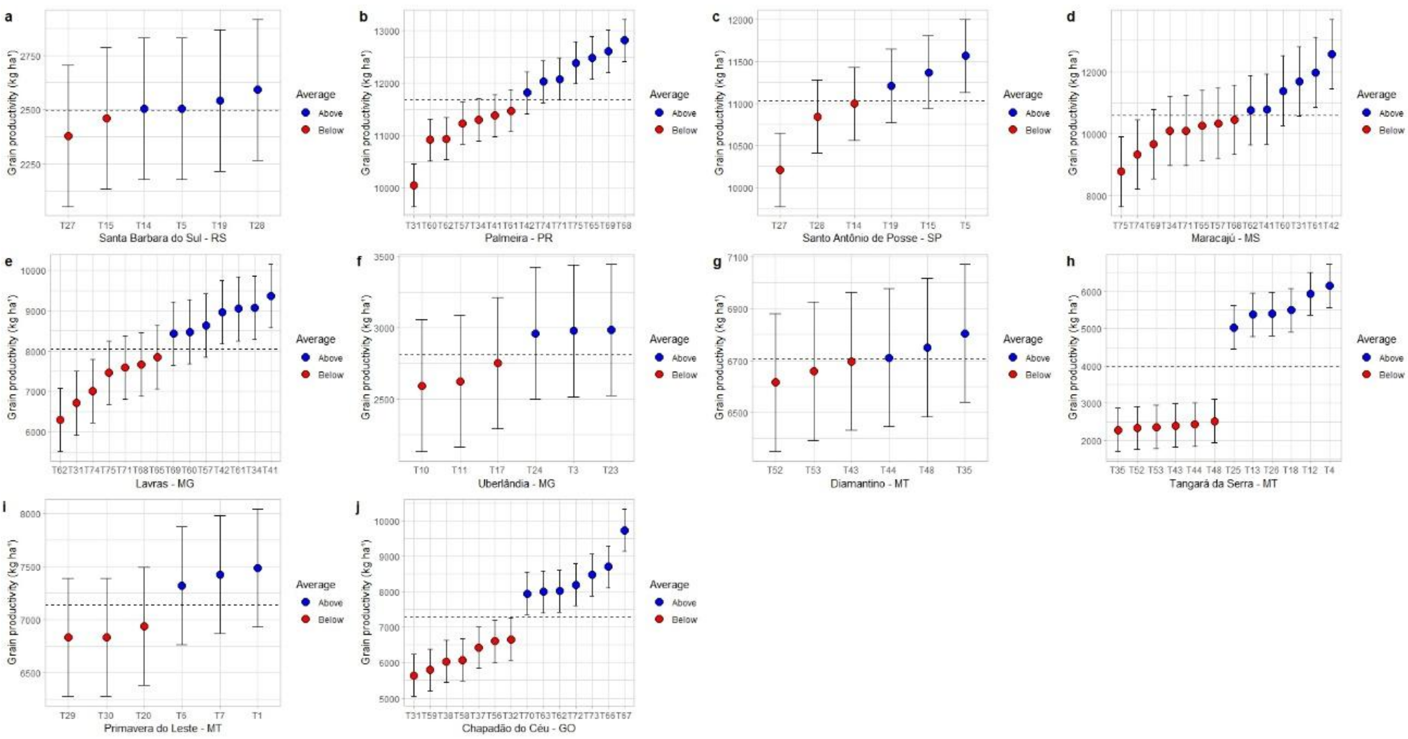

Figure 3. Estimates of components of average maize grain yields per specific BLUP measured in 75 treatments and 10 environments.

Shaharoona *et al*. (2008), who observed the effectiveness of some strains of *P. fluorescens* as a growth promoter in wheat, reducing the need for NPK fertilizer added to the soil. In the Palmeira-PR environment, a superior response for grain productivity was observed for treatments T42, T74, T71, T75, T65, T69 and T68 (Figure 3b). Studies by Sandini *et al*. (2019), report that the use of bacteria such as *Pseudomonas fluorencens* and *Azospirillum brasiliense* can reduce the need for chemical fertilizers by up to 25%, mitigating production costs, in addition to the effects related to CO_2 emissions.

Grain productivity in Santo Antônio de Posse- SP performed above average (>11.1 thousand kg ha^{-1}) for T19, T15 and T5 (Figure 3c). In Maracaju-MS, the superior treatments were T68, T62, T41, T60, T31, T61 and T42 (Figure 3d). Thus, in the latter environment, greater productivity was achieved by using 400 kg ha^{-1} of 08-24-12 NPK fertilizer, associated with growth-promoting bacteria (*Azospirillum brasiliense* and *Pseudomonas fluorescens*) via aerial spraying at a dose of 0.3 L ha^{-1}. Studies by Tang *et al*. (2020), observed greater efficiency of fertilization combined with growth-promoting bacteria. In this same study, the authors report that greater efficiency was in relation to nitrogen and phosphorus. Similar results were found by Phares *et al*. (2022) using *Bacillus spp*. and *Pseudomonas spp*., showed greater efficiency in nutrient absorption by maize.

Table 3. Estimates of variance components for grain yield depending on locations and different treatments with inoculants and fertilizers.

Environment	MODEL	LOGLINK	AIC	LRT	PR > (CHISQ)
Chapadão céu- GO	GEN	-458	925	50.9	9.96E-13
Diamantino- MT	GEN	-156	322	0.154	0.0695
Lavras	GEN	-454	917	22.0	0.00000272
Maracaju- MS	GEN	-467	943	13.3	0.000268
Palmeira- PR	GEN	-433	877	44.2	3.01e-11
Primavera do Leste-MT	GEN	-166	342	1.48	0.0223
Santa Barbara do sul-RS	GEN	-145	300	0.116	0.0734
Santo Antônio de Posse- SP	GEN	-163	337	7.03	0.00802
Tangará da Serra- MT	GEN	-590	1189	94.7	2.20e-22
Uberlândia- MG	GEN	-163	337	0.607	0.0436

Parameters	Chapadão Céu- GO	Diamantino- MT	Lavras - MG	Maracaju - MS	Palmeira- PR	Primavera do Leste- MT	Santa Barbara do Sul- RS	Santo Antônio de Posse- SP	Tangará da Serra- MT	Uberlândia- MG
Gen_var	1726288	18317	1075963	1391631	647763	163062	25927	275939	2891079	78687
Gen (%)	82.1	7.48	59.6	46.9	785	24.7	6.64	54.7	89.0	15.4
Res_var	377552	226643	729721	1574283	177658	496702	364686	228320	357767	433428
Res (%)	17.9	92.5	40.4	53.1	215	75.3	93.4	45.3	11.0	84.6
σ^2F	2103841	244961	1805685	2965914	825421	659763	390613	504260	3248847	512114
H^2	0.821	0.0748	0.596	0.469	0.785	0.247	0.0664	0.547	0.890	0.154
H^2mg	0.948	0.244	0.855	0.780	0.936	0.568	0.221	0.829	0.970	0.421
Accuracy	0.974	0.494	0.925	0.883	0.967	0.753	0.471	0.910	0.985	0.649
CVg	18.0	2.02	12.9	11.1	6.89	5.66	6.45	4.76	42.8	9.96
CVr	8.41	7.10	10.6	11.9	3.61	9.87	24.2	4.33	15.0	23.4
CV ratio	2.14	0.284	1.21	0.940	1.91	0.573	0.267	1.10	2.84	0.426

Model: Model; Log Lik: Restricted Maximum Likelihood Logarithm; AIC: Akaike Informational Criterion; LRT: Restricted Maximum Likelihood Ratio; PR: Probability by Chi-Square test (*x2*): Gen_var: Genotypic variance; GEN %: Percentage of genotypic variance; Res_var: Residual Variance; Res %: Percentage of residual variance; σ^2F: Phenotypic variance; H^2: broad sense heritability; H^2mg: Heritability of the genotype mean; RGE: genotypic correlation between genotype x environment performance; 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.

In the Lavras-MG environment, superiority was inferred for T69, T60, T57, T42, T61, T34 and T41, with grain productivity greater than 8 tons of grains ha^{-1} (Figure 3e). For Uberlândia-MG, above average performance (2800 kg of grains ha^{-1}) was observed only for treatments T24, T3 and T23 (Figure 3f). Diamantino- MT, the superior treatments were T44, T48 and T35, with grain yields above 6700 kg ha^{-1} of grains (Figure 3g). Studies by Mattos *et al.* (2023), with inoculation in rice, they observed increases of 5% in the grain productivity of this cereal using *A. brasilense* + *P. fluorescens*. Tangará da Serra-MT, superiority was found for treatments T25, T13, T26, T18, T12 and T4 (Figure 3h), that is, treatments without the use of inoculants were superior.

In the Primavera do Leste-MT environment, the superior treatments were T6, T7 and T1 (Figure 3i). In Chapadão do Céu-GO, superiority was observed for T70, T63, T62, T72, T73, T66 and T67 (Figure 3j). Morais *et al.* (2016), observed maximum maize yield with fertilization of 40 kg ha^{-1} of N, combined with *Azospirillum spp*. It can, therefore, be inferred that inoculation is not efficient in increasing maize grain productivity. Hungary *et al.* (2021), observed an increase in the concentration of nitrogen, phosphorus and potassium in brachiaria, which received treatment with *A. brasiliense* and *P. fluorencens*.

The inoculation of plants with plant growth-promoting bacteria is a new sustainable technology that promotes the growth and increase in productivity of agricultural crops and can reduce the amount of mineral fertilizers used in the cultivation of large crops. Therefore, inoculants have proven to be an important tool for agriculture in general, as several studies show the efficiency of using microorganisms as growth promoters and suppressors of phytopathogens. Furthermore, there is also the possibility of improving fertilization efficiency and, in some cases, even reducing the amount of some nutrients.

Materials and Methods

The experiment was carried out in the municipalities of Santa Barbara do Sul- RS (28°21'21" S and 53°15'12" W, 511m), Palmeira- PR (21°12'54.7" S, 45°03'16.9" W, 900m), Santo Antônio de Posse - SP (22°36'24" S, 46°55'9" W, 600m), Maracaju- MS (18º46'44" S, 52º36'59" W, 786m), Diamantino- MT (14°24'11" S and 56°25'37" W, 560m), Primavera do Leste- MT (21°12'54.7" S, 45°03'16.9" W, 900m), Tangará da Serra- MT (14°37'40" S and 57°30'25" W, 427m), Chapadão do Céu- GO (18°21'25.2'' S and 52°38'11.0''W, 840m), Uberlândia- MG (18°54'41" S and 48°15'44" W, 863m) and Lavras- MG (21°12'54.7" S, 45°03'16.9" W and 900m).

The experimental design was randomized blocks, with treatments differing between environments, totaling 75 treatments (Tables 1 and 2), arranged in four replications. The experimental units were composed of five sowing lines, spaced 0.45 m apart, with a sowing density of three seeds per linear meter and five meters in length. The hybrid used was AS1633 PRO3, with sowing carried out in the first fortnight of December 2022. The evaluations took place in the useful area of each experimental unit, represented by the three central sowing lines and four meters in length.

The inoculant composed of *Azospirillum brasiliense* and *Pseudomonas fluorescens* was applied via seed treatment at the time of sowing the maize, via the sowing furrow and via the foliar route. 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 with 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 3bar. For foliar applications, a knapsack sprayer was used with constant pressure, propelled by pressurized CO_2, with a spray volume of 150 L ha$^{-1.}$

Assessments and collections

At the end of the cycle, the ears present in the useful area of each experimental unit were harvested, threshed and weighed, subsequently determining the grain productivity corrected at 13% humidity.

Statistical analysis

The statistical analysis was performed using 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 (x^2). 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 proportion between genotypic and residual coefficient of variation (*CVratio*). Subsequently, the stratified *BLUP* model was used to specifically recommend treatments adapted to a given region. To carry out the statistical analyses, the *Exp.Des.pt* (Ferreira *et al*., 2021), *metan* (Olivoto and Lucio, 2020) and *ggplot2* (Wickham, 2016) packages were used, through the R Software (R Core Team, 2024).

Conclusion

The use of *Azospirillum brasiliense* and *Pseudomonas fluorencens* via aerial spraying, in the sowing furrow and seed treatment, using 50% and 75% of the fertilizer dose demonstrated satisfactory results for maize grain productivity.

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