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Viability and agronomic efficiency of using *Bacillus licheniformis* to complement nitrogen fertilization and pre-treatment and storage of mayze seeds

Viabilidade e eficiência agronômica do uso de Bacillus licheniformis na complementação da adubação nitrogenada e no pré-tratamento e armazenamento de sementes de milho

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ABSTRACT

The objective of the study was to evaluate the agronomic efficiency and viability of using the bacteria Bacillus licheniformis to complement nitrogen fertilization in top dressing and in the pre-treatment and storage of maze seeds. The study was conducted in the municipalities of Candói- PR and Lapa-PR (phase 1) and in the municipalities of Serranópolis do Iguaçu–PR and São Miguel do Iguaçu (phase 2). Inoculation with Bacillus licheniformis presents agronomic efficiency similar to nitrogen control and inoculation with Azospirillum brasilense during the harvest period. Inoculation with Bacillus licheniformis 90 days before sowing is capable of reducing the application of N-fertilizer by 25% without compromising the yield of maze grains in the second harvest period.

Keywords: Microorganisms; Productivity; *Zea mays*.

RESUMO

O objetivo do estudo foi avaliar a eficiência agronômica e a viabilidade do uso da bactéria Bacillus licheniformis na complementação da adubação nitrogenada em cobertura e no pré-tratamento e armazenamento de sementes de labirinto. O estudo foi realizado nos municípios de Candói-PR e Lapa-PR (fase 1) e nos municípios de Serranópolis do Iguaçu-PR e São Miguel do Iguaçu (fase 2). A inoculação com Bacillus licheniformis apresenta eficiência agronômica semelhante ao controle de nitrogênio e à inoculação com Azospirillum brasilense no período de colheita. A inoculação com Bacillus licheniformis 90 dias antes da semeadura é capaz de reduzir em 25% a aplicação de N-fertilizante sem comprometer o rendimento de grãos labirinto na segunda colheita.

Palavras-chave: Microrganismos; Produtividade; *Zea mays*.

INTRODUCTION

Among the main cereals cultivated in the world are wheat (*Triticum aestivum* L.), maze (*Zea mays* L.) and rice (*Oryza sativa*) (ERENSTEIN *et al*., 2022). Maze has wide genetic variability, which allows it to be cultivated in tropical, subtropical and temperate regions. This leads to a high demand for external inputs due to the great diversity of cultivation systems (SOUSA *et al*., 2021). Its food potential makes the culture a fundamental ingredient for human and animal consumption (AGUIAR *et al*., 2022), which has as its main constituents 72% starch, 10% protein and 4% fat (NUSS and TANUMIHARDIJO, 2010).

Even though the maze crop is highly responsive to nutritional management, the demand for fertilizers is high in order to obtain high productivity (RANUM *et al*., 2014), with nitrogen being the main restrictive nutrient for grain yield (NAKATANI, GATO and SANDINI, 2024). It is a constituent of a large part of important biomolecules such as ATP, NADH, NADPH, chlorophyll, proteins and several enzymes, which directly influence the growth and development of the plant (ARENHARDT *et al*., 2017; PEREIRA *et al*., 2018). However, nitrogen applications under conditions of low nutrient use efficiency can lead to serious environmental problems (MÓRING *et al*., 2021). In the past, the extensive use of chemical fertilizers ended up promoting soil acidification and, as a consequence, a reduction in nutrient availability, (GUO *et al*., 2010) in addition to the possibility of a relationship with the imbalance of soil microbiota (ZHONG *et al*., 2010) al., 2010).

New technologies are developed every year with the aim of maximizing crop productivity, reducing environmental impact and promoting sustainability with high returns for farmers (SOUSA *et al*., 2021). In this context, plant growth-promoting rhizobacteria (PGPR) are a group of microorganisms with recognized potential for beneficial responses in different plant species (MORENO *et al*., 2021). In particular, endophytic growth-promoting bacteria (PGP) can promote plant growth by mechanisms that include nitrogen fixation (SUN *et al*., 2022), improvements in nutrient absorption (KUSHWAHA *et al*., 2022), production of growth-promoting compounds (KUMAR *et al*., 2019) and increased stress tolerance (AZIZ *et al*., 2022). Among these, those of the Bacillus genus stand out, whose benefits have been demonstrated in several crops such as soybeans (CHAGAS JUNIOR *et al*., 2022), beans (ARAUJO *et al*., 2010) and maze (AGUIAR *et al*., 2022).

Bacillus strains have heat tolerance and chemical-resistant spores, increasing their adaptation to field applications and commercial formulations of long-lasting products (ROCHA *et al*., 2019). However, there are gaps in information regarding the applicability of bacteria in complementing external inputs used in maze cultivation. Based on the above, the objective of the study was to evaluate the agronomic efficiency and viability of using the bacteria *Bacillus licheniformis* to complement nitrogen fertilization in top dressing and in the pre-treatment and storage of maze seeds.

MATERIAL AND METHODS

The study was conducted in experiments during the harvest period (Phase 1), in the municipalities of Candói – PR (51º 48' 00” W, 25º 31' 57” S, altitude of 920 m) and Lapa – PR (49º 39' 8” W, 25º 50' 52” S, altitude of 855 m), and in the second harvest period (Phase 2), in the municipalities of Serranópolis do Iguaçu – PR (53º 58' 38” W, 25º 23' 08” S, altitude of 325 meters) and São Miguel do Iguaçu – PR (54º 12' 55” W, 25º 22' 28” S, altitude of 321 meters). According to the Köppen climate classification, the climate in Candói – PR and Lapa – PR is type Cfb (temperate, with mild summer), while in Serranópolis do Iguaçu – PR and São Miguel do Iguaçu – PR the climate is type Cfa (humid subtropical) (SETZER, 1966). The physical-chemical attributes of the soil are described in Table 1.

Table 1. Physico-chemical attributes of the soil in the experimental area in the environments of Serranópolis do Iguaçu – PR and São Miguel do Iguaçu – PR.

Attributes	Candói – PR	Lapa – PR	Serranópolis do Iguaçu – PR	São Miguel do Iguaçu – PR
Clay (g kg^{-1})	550	220	710	690
Silt (g kg^{-1})	290	310	190	150
Sand (g kg^{-1})	160	470	100	160
Textural Class	Clayey	Mean	Clayey	Clayey
pH (CaCl)	5.35	5.42	5.29	6.56
pH (SMP)	6.33	6.65	6.1	6.8
O.M. (g dm^{-3})	26.51	21.75	20.08	27.7
P - Mehlich (mg dm^{-3})	3.22	4.89	7.52	8.7
K (cmol dm^{-3})	0.46	0.12	0.62	0.63
Ca (cmol dm^{-3})	4.56	4.06	6.13	8.1
Mg (cmol dm^{-3})	1.95	1.35	2.23	3.16
Al (cmol dm^{-3})	0	0	0	0
H+Al (cmol dm^{-3})	3.88	3.06	4.51	2.74
SB (cmol dm^{-3})	6.97	5.63	8.98	11.89
CTC- pH 7.0 (cmol dm^{-3})	10.85	8.69	13.59	14.63

Source: Authors, 2024.

In both phases of the experiment, the experimental design used was randomized blocks with a 4x2 factorial scheme (four treatments and two environments), with six replications. Table 2 describes the constitution of the treatments used. The experimental units consisted of 8 lines with a spacing of 0.50 m and a length of 6.0 m. During the harvest period, sowing was carried out in the second half of September in Candói-PR and in the second half of October in Lapa – PR. The hybrid used was P30F53VYH, with a target population of 75 thousand plants ha^{-1}. In the second harvest period, sowing was carried out in the second half of February in both locations. The hybrid used was STATUS VIP3 T4F, with a target population of 60 thousand plants ha^{-1}.

In the base fertilization, 40 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O were used, without the use of nitrogen fertilizer. Phytosanitary management was carried out preventively in order to avoid biotic effects on the results of the experiment. The application of N-fertilizer in top dressing was carried out in a single application at the V4 stage of the crop, with the respective dosages for each treatment (Table 2). The inoculant was prepared with the bacteria *Bacillus licheniformis* strain CCTB 07 = CNPSo 3204. It is a pre-commercial product, which was formulated with a guarantee of $1x10^8$ UFC mL^{-1}. Inoculation with *Azospirillum brasilense* was carried out with the commercial product *Azo*Total, which contains the strains Ab-V5 (=CNPSo 2083) and Ab-V6 (=CNPSo 2084), with a guarantee of $2x10^8$ CFU mL^{-1}. The bacteria were applied via seed treatment at a dose of 150 mL for every 60 thousand seeds. The seeds inoculated with *Bacillus licheniformis* 90 DAS were stored in an air-conditioned environment at a temperature of 15 ºC, aiming to maintain high levels of seed germination and vigor. In all treatments, the seeds were treated with 35 mL of the fungicide Maxim (Thiamethoxam 350 g L^{-1}), respectively, for every 60 thousand seeds.

Table 2. Description of the treatments used in the study.

Phase 1		
Treatment	Denomination	Protocol
T1	Absolute control	No inoculation; Without application of N-fertilizer in top dressing
T2	Nitrogen Control	100% N-fertilizer at sowing base + 200 kg ha^{-1} of N-fertilizer in top dressing
T3	Inoculation 90 days before sowing	*Azospirillum brasilense* + 75% of the recommended nitrogen (120 kg ha^{-1} of N-fertilizer in top dressing).
T4	Inoculation on the day of sowing	*Bacillus licheniformis* + 75% of the recommended nitrogen (120 kg ha^{-1} of N-fertilizer in top dressing).
Phase 2		
Treatment	Denomination	Protocol
T1	Absolute control	No inoculation; Without application of N-fertilizer in top dressing
T2	Nitrogen Control	100% N-fertilizer at sowing base + 160 kg ha^{-1} of N-fertilizer in top dressing
T3	Inoculation 90 days before sowing	*Bacillus licheniformis* inoculated 90 days before sowing + 75% of the recommended nitrogen (120 kg ha^{-1} of N-fertilizer in top

		dressing).
T4	Inoculation on the day of sowing	*Bacillus licheniformis* inoculated on the day of sowing + 75% of the recommended nitrogen (120 kg ha^{-1} of N-fertilizer in top dressing).

Source: Authors, 2024.

The variables measured in the study are described below. The plant population (POP, plants ha^{-1}) was obtained by counting the number of plants in five linear meters. The ear index (EI) was determined by the ratio of ears and plants per linear meter. Plant height (PH, cm) and ear insertion height (EIH, cm) were obtained by direct measurement using a graduated ruler. The variables number of rows of grains (NGR, units), number of grains per row (NGPR, units) and number of grains per ear (NGPE, units) were determined from the average of ten ears harvested per treatment. Grain yield (GY, kg ha^{-1}) was obtained from the harvest of the four central rows of each experimental unit, with subsequent threshing, weighing and grain moisture correction to 13%. A subsample of this material was used to determine the thousand grain weight (TGW, g). The total dry mass (DM, kg ha^{-1}) was determined from sampling five plants in the useful area of the plot at stage R1, which was subjected to a forced air oven at 65 ºC for 72 hours. The nitrogen content of the plant shoots (NCP) and grains (NCG) were determined following the methodology proposed by Silva (2009), with the results expressed in g kg^{-1}. To help understand the results, satellite meteorological data on mean air temperature (in ºC) and precipitation (in mm) were obtained with the help of the NASA Power tool (NASA POWER, 2023).

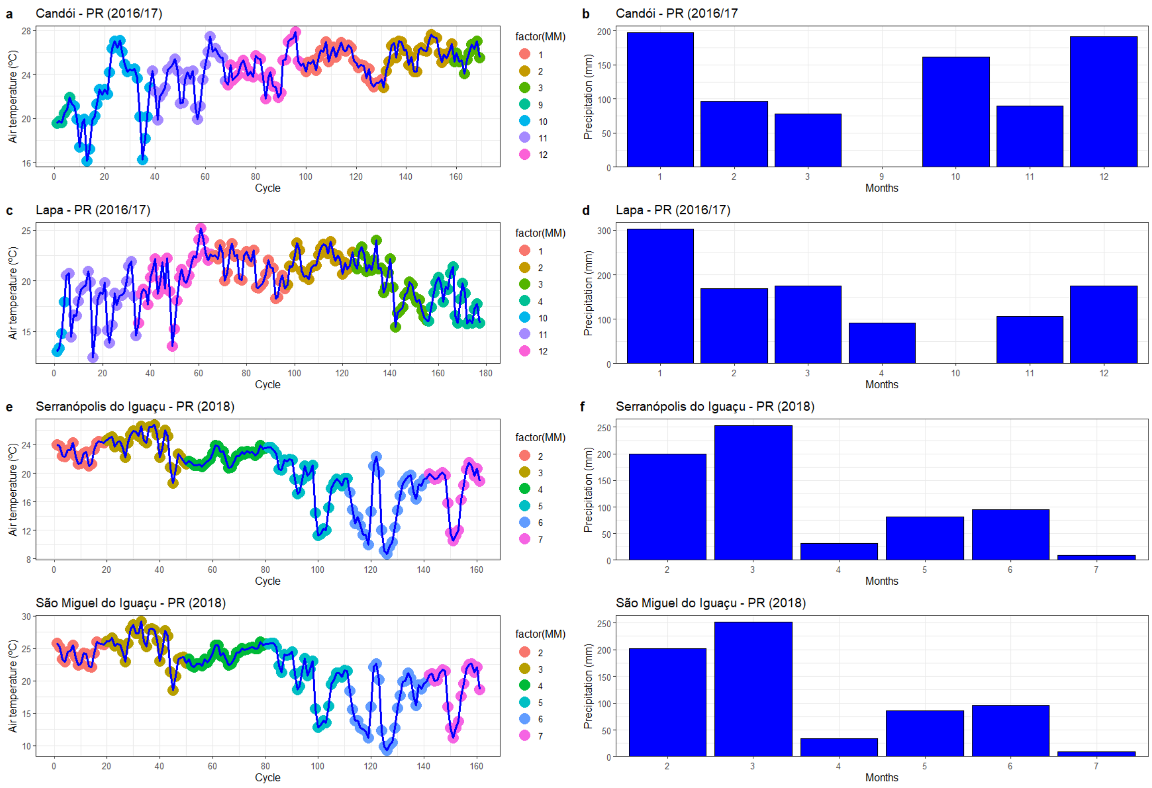
The obtained data matrix was subjected to analysis and removal of outliers. Subsequently, the data were subjected to the assumptions of the statistical model, based on tests for normality of errors, homogeneity of variances and independence of errors using the Shapiro-Wilk, Bartlett and Durbin Watson tests, respectively. With the assumptions met, analysis of variance was carried out to determine the effect of treatments and environments, as well as their interaction, on the variables, at 5% probability ($p<0.05$). In the variables on which a significant effect of the tested factors was identified, the breakdown into simple and main effects was carried out using the Tukey mean comparison test, at 5% probability ($p<0.05$). In a complementary way, the linear correlation was obtained in order to determine the linear relationships and their magnitudes between the study variables. Biplot principal component analysis was used to determine the associations between treatments and variables in addition to the contribution of each variable to the total variability of information. All analyzes were carried out with the metan version 1.18.0 (OLIVOTO and LÚCIO, 2020) and stats packages using the R software (R CORE TEAM, 2023).

RESULTS AND DISCUSSION

The productive performance of maze is influenced by several abiotic factors that occur simultaneously or in different periods of crop development (LORO *et al*., 2023), with a high influence of air temperature. The cardinal temperatures of maze consist of a lower basal temperature of 10 ºC, an optimum temperature varying between 18 and 30 ºC (KROPFF and VAN LAAR, 1993), and an upper basal temperature of 34 ºC (JONES and KINIRY, 1986). The air temperature proved to be adequate for the establishment, growth and development of the crop during the harvest period (Figure 1), remaining below the optimum only in short intervals, which favored the pollination and grain filling processes. The temperature was milder in Lapa-PR when compared to Candói-PR, especially during plant establishment. During the second harvest period, the mean air temperature varied between 20 and 24 ºC in Serranópolis do Iguaçu-PR, while the variation was 20 to 26 ºC in São Miguel do Iguaçu-PR. The peak air temperature was reached at the time of application of N-fertilizer under cover, a condition that may have contributed to reducing the efficiency of the practice. High atmospheric temperature favors losses of nitrogen fertilizer (MOTA *et al*., 2015), with a high rate of volatilization with the increase in temperature from 18 ºC to 35 ºC (TASCA, 2009).

From the accumulated volumes of precipitation, it can be seen that there was enough soil moisture to obtain good crop establishment, with values exceeding 100 mm in the harvest period and close to 200 mm in the second harvest period. On the other hand, the environmental conditions of high temperatures together with the accumulated volumes of precipitation at the time of fertilization with N-fertilizer in top dressing may have contributed to losses of the nutrient. The best use of nitrogen depends on conditions of mild temperature and adequate soil moisture, without the occurrence of high volumes of precipitation immediately after application (SCREMIN *et al*., 2020; REGINATTO *et* *al*., 2021). The period of flowering and anthesis coincided with low volumes of precipitation, which ended up favoring the pollination process. Furthermore, grain filling was favored by mild temperatures combined with the occurrence of precipitation. Thus, even with the potential for losses when applying N-fertilizer as top dressing, a good potential for grain yield was maintained.

Figure 1. Meteorological data on mean air temperature, in ºC (Figure 1a; Figure 1c) and accumulated precipitation, in mm (Figure 1b, Figure 1d) for the maze cultivation cycle in the harvest period, in the municipalities of Candói – PR and Lapa – PR, and in the second harvest period, in the environments of Serranópolis do Iguaçu – PR and São Miguel do Iguaçu – PR.



Source: Authors, 2024.

From the analysis of variance for the harvest period (Table 3), a significant effect of the interaction between treatment and environment was found for yield, total dry mass and total nitrogen in the grains. Furthermore, there was a significant effect of treatment on the variables plant height, number of rows of grains, thousand grain weight and total nitrogen in the plant. The environment was significant for the expression of the variables plant height, ear insertion height, number of rows of grains, number of grains per row, number of grains per ear and thousand grain weight. The coefficient of variation varied between 2.05 and 8.62%, considered low (less than 10%) according to the classification of Pimentel-Gomes (2022).

Table 3. Summary of the analysis of variance for the effect of four treatments (T), two environments (E) and their interaction (T x E), in the second harvest, on the variables plant population (POP, plants ha^{-1}), ear index (EI), plant height (PH, cm), ear insertion height (EIH, cm), number of grain rows (NGR, units), number of grains per row (NGPR, units), number of grains per ear (NGPE, units), grain yield (GY, kg ha^{-1}), thousand grain weight (TGW, g), dry mass (DM, kg ha^{-1}), total nitrogen in the plant (NCP, %) and total nitrogen in grains (NCG, %). SV: source of variation; DF: degrees of freedom; CV: coefficient of variation.

SV	DF	POP	EI	PH	EIH	NGR	NGPR
		MS					
T	3	13428416.57	0.006298022	284.49764*	212.86546	0.73878086*	3.141506
E	1	18605.44	0.012291997*	4496.04836*	3495.1767*	1.98890432*	62.192226*
Block	3	6589551.820	0.002777274	101.58283	54.26363	0.1021142	9.270858
T x E	3	8141493.85	0.003675837	71.38576	41.30069	0.0332253	6.051506
Residual	21	6260111.44	0.0026928432	86.68271	70.08541	0.1044775	7.867651
CV (%)	-	3.41	5.08	3.73	5.70	2.05	8.11
Mean	-	73326.58	1.022	249.4	146.82	15.78	34.58

SV	DF	NGPE	GY	TGW	MS	NCP	NCG
		MS					
T	3	2505.48	8075217.4*	827.3915*	52670940*	46.14928*	43.204050*
E	1	29588.33*	9676396.8*	13605.6387*	22130543*	8.000	22.985055*
Block	3	3306.97	882371.1	552.71790	2372946	9.410	7.823296
T x E	3	1881.08	1052024.1*	403.8485	13478230*	0.000	21.379989*
Residual	21	2214.94	243030.0	212.9618	2899047	9.170	3.025523
CV (%)	-	8.62	3.08	3.79	5.73	8.36	4.12

Mean	-	546.24	16014.84	385.3	29698.86	36.21	42.18

*: significant at 5% probability by the F test.

The treatment with absolute control (T1) was inferior to the others for grain yield in the harvest period (Table 4), regardless of the environment, with averages of 15481 kg ha^{-1} in Candói-PR and 13584 kg ha^{-1} in Lapa-PR, respectively. Treatments with nitrogen control (T2) and nitrogen fertilization supplementation with *Bacillus licheniformis* (T4) did not differ statistically. The average for grain yield using *Azospirillum brasilense* (T3) was 17018 kg ha^{-1} in Candói-PR, statistically differing from the value of 15490 kg ha^{-1} obtained in Lapa-PR. From this, it can be inferred that complementing nitrogen fertilization with PGPs is viable, without harm to grain yield and total biomass. The results corroborate the study by Sousa *et al*. (2021), which reported gains between 281.43 and 2914.53 kg ha^{-1} for inoculation with different strains of *Bacillus* in relation to the control without inoculation.

There was no difference between treatments T2, T3 and T4 for total dry mass, regardless of the cultivation environment. The T1 treatment resulted in the lowest biomass production, with 26737 kg ha^{-1} in Candói-PR and 24981 kg ha^{-1} in Lapa-PR, respectively. For total nitrogen in grains, the average of 40.0 g kg^{-1} obtained for treatment T1 in Candói-PR differed statistically from 37.4 g kg^{-1} in Lapa-PR. However, the averages for treatments T2, T3 and T4 were 45.6, 43.4 and 45.7 g kg^{-1} in Lapa-PR, higher than those obtained in Candói-PR.

Table 4. Breakdown of the interaction between environment and treatment for the variables grain yield (GY, kg ha^{-1}), total dry mass (DM, kg ha^{-1}) and total nitrogen content in grains (NCG, g kg^{-1}) during the harvest period.

GY			MS		
	Environment			Environment	
Tratamento	Candói-PR	Lapa-PR	Tratamento	Candói-PR	Lapa-PR
T1	15481 bA	13584 bB	T1	26737 bA	24981 bA
T2	16483 aA	16412 aA	T2	28743 abB	32836 aA
T3	17018 aA	15489 aB	T3	30656 aA	31780 aA
T4	16918 aA	16375 aA	T4	29332 abB	32523 aA

NCG		
	Environment	
Tratamento	Candói-PR	Lapa-PR
T1	40.0 aA	37.4 bB
T2	41.4 aB	45.6 aA
T3	42.6 aA	43.4 aA
T4	41.3 aB	45.7 aA

Means followed by the same lowercase letter in the column and capital letter in the row do not differ from each other at 5% probability using the Tukey test.

Superiority was seen in the values obtained for all variables with a significant environmental effect in Candói-PR (Table 5). In this way, values of 261.25 and 157.27 cm were obtained for plant height and ear insertion height, with 576.65 grains per ear and a thousand grain weight of 405.92 g in Candói-PR. The average values in Lapa-PR were 237.54 and 136.37 cm for plant height and ear insertion height, with 515.83 grains per ear and a thousand grain weight of 364.68 g. Treatment T3 resulted in the highest average for plant height, with 254.85 cm, while the lowest average was attributed to treatment T1, with 241.51 cm. The number of grain rows was greater when using the T2 treatment, while the inoculation treatments did not differ statistically from each other, with values of 15.53 and 15.51 for T3 and T4, respectively. The total nitrogen content in the plant was higher for treatment T4 (38.8 g kg^{-1}), with no statistical difference for treatments T2 and T3 (36.9 and 35.9 g kg^{-1}), respectively). The increases observed for agronomic traits, which include nitrogen accumulation in tissues, may be related to bacterial growth promotion mechanisms. *Bacillus licheniformis* is capable of synthesizing enzymes that act in the solubilization of chitin polymers, participating in the decomposition of organic matter, which results in the availability of nutrients for plants (SHAFI *et al*., 2017).

Table 5. Test of comparison of means for the main effects of environment and treatment on the variables ear index (EI), thousand grain weight (TGW, g), plant height (PH, cm), ear insertion height (EIH, cm), number of grain rows (NGR, units), number of grains per row (NGPR, units), number of grains per ear (NGPE, units) and total nitrogen in the plant (NCP, g kg^{-1}) in the period harvest.

Environment	EI	TGW	PH	EIH
Candói-PR	1.04 a	405.92 a	261.25 a	157.27 a
Lapa-PR	1.00 b	364.68 b	237.54 b	136.37 b
Mean	1.02	385.30	249.4	146.82

Environment	NGR	NGPR	NGPE
Candói-PR	16.03 a	35.98 a	576.65 a
Lapa-PR	15.53 b	33.19 b	515.83 b
Mean	15.78	34.58	546.24

Treatment	PH	NGR	NCP
T1	241.51 b	15.97 ab	33.10 b
T2	253.05 ab	16.10 a	36.99 ab
T3	254.85 a	15.53 bc	35.91 ab
T4	248.18 ab	15.51 c	38.84 a
Mean	249.40	15.78	36.21

Means followed by the same lowercase letter do not differ from each other using the Tukey test at 5% probability.

The summary of the analysis of variance for the second harvest period (Table 6) demonstrates that the interaction between treatment and environment was significant for total nitrogen in grains. A significant effect of the treatment was observed on the variables plant population, grain yield, thousand grain weight and total nitrogen in the plant. The environmental factor significantly influenced the behavior of the variables ear index, plant height, number of grain rows, yield, total dry mass and total nitrogen in the plant. A non-significant effect of the factors tested was found for the variables ear insertion height, number of grains per row and number of grains per ear. The values for the coefficient of variation varied between 4.23 and 14.42%, being considered low (less than 10%) to medium (between 10 and 20%) according to the classification by Pimentel-Gomes (2022).

Table 6. Summary of the analysis of variance for the effect of four treatments (T), two environments (E) and their interaction (T x E), in the second harvest, on the variables plant population (POP, plants ha-1), ear index (EI), plant height (PH, cm), ear insertion height (EIH, cm), number of grain rows (NGR, units), number of grains per row (NGPR, units), number of grains per ear (NGPE, units), grain yield (GY, kg ha-1), thousand grain weight (TGW, g), dry mass (DM, kg ha-1), total nitrogen in the plant (NCP, %) and total nitrogen in grains (NCG, %). SV: source of variation; DF: degrees of freedom; CV: coefficient of variation.

SV	DF	POP	EI	PH	EIH	NGR	NGPR
		MS					
T	3	26556790*	0.008018078	304.53830	131.62822	0.2411311	7.0774765
E	1	546519832	0.224891529*	4550.13561*	2707.90412	3.1789831*	1.6504548
Block	6	2833748.000	0.018525938	263.43470	149.67686	0.3250784	3.0962840
T x E	4	28367522	0.032243594	27.75903	57.74714	1.2455721	0.3379796
Residual	26	13209494	0.019417528	136.49764	78.76190	0.5186305	2.7445099
CV (%)	-	5.64	13.21	5.58	7.33	4.32	6.34
Mean	-	64425.45	1.06	209.30	121.14	16.67	26.15

SV	DF	NGPE	GY	TGW	MS	NCP	NCG
		MS					
T	3	1897.2018	1486101.47*	738.50687*	3026104.4	29.0824303*	35.235749*
E	1	684.67830	16579754.92*	329.58076	10285775.4*	80.3317356*	271.443949*
Block	6	885.77020	405004.310	12.11598	4498239.8	9.2120403	9.993656
T x E	4	1134.7492	43427.09	296.90876	328336.5	0.7277298	19.320991*
Residual	26	772.4957	319654	171.02802	1676686.3	7.9269551	3.093917
CV (%)	-	6.38	8.63	4.23	14.42	12.39	9.41
Mean	-	435.47	6552.5742	308.87	8981.5721	22.73	18.690

*: significant at 5% probability by the F test.

All treatments differed statistically from the absolute control (T1) for the variable total nitrogen in grains, in São Miguel do Iguaçu-PR, which did not occur in Serranópolis do Iguaçu-PR. Statistically higher averages were obtained in São Miguel do Iguaçu-PR, with values of 24.1 g kg^{-1} for nitrogen control (T2), 21.9 g kg^{-1} for inoculation 90 days before sowing (T3) and 21.7 g kg^{-1} for inoculation on the day of sowing (T4). Therefore, it can be inferred that the use of *Bacillus licheniformis* as a complement to N-fertilizer fertilization in top dressing was effective for the absorption and translocation of nitrogen to the grains. The higher N concentration in the plant provides a significant advantage for N storage in the stem, potential remobilization to the grains and an opportunity to increase the photosynthetic rate (DEBRUIN *et al*., 2017).

Table 7. Breakdown of the interaction between environment and treatment for the variable total nitrogen in grains (NCG, %).

Treatment	NCG	
	Environment	
	São Miguel do Iguaçu – PR	Serranópolis do Iguaçu – PR
T1	15.7 bA	15.5 aA
T2	24.1 aA	16.9 aB
T3	21.9 aA	16.0 aB
T4	21.7 aA	15.6 aB

Means followed by the same lowercase letter in the column and capital letter in the row do not differ from each other at 5% probability using the Tukey test.

There was a significant difference between the values found for the plant population (Table 8), with an average of 68312 plants ha^{-1} in Serranópolis do Iguaçu – PR and an average of 61093 plants per ha^{-1} in São Miguel do Iguaçu – PR. The ear index, also known as prolificacy, was higher in Serranópolis do Iguaçu - PR, with an average of 1.13 compared to the value of 0.97 obtained in São Miguel do Iguaçu - PR. Low levels of nitrogen in the soil can limit prolificacy, a source of reproductive plasticity for maze cultivation at low densities (DEBRUIN *et al*., 2017; PARCO *et al*., 2020), which can contribute to sustaining grain yield (ROSS *et al*., 2020). However, prolificacy indices greater than 1.2 can penalize the thousand grain weight and grain yield. Superiority was seen for plant height and, concomitantly, for ear insertion height in Serranópolis do Iguaçu – PR, with averages of 219.85 cm and 129.18 cm, respectively. A greater number of grain rows was allowed to be expressed in São Miguel do Iguaçu – PR compared to Serranópolis do Sul – PR, with averages of 16.39 and 16.98 rows, respectively.

Grain yield was lower in São Miguel do Iguaçu – PR, with an average of 5899.32 kg ha^{-1}, while in Serranópolis do Iguaçu – PR the average was 7112.32 kg ha^{-1}. There was no significant difference in the average total dry mass (DM). The percentage of total nitrogen in the plant was higher in Serranópolis do Iguaçu – PR, with 23.94 g kg^{-1}, statistically differing from the average of 21.31 g kg^{-1} obtained in São Miguel do Iguaçu – PR. The study by Bueno *et al*. (2022), conducted under similar conditions, reported that the nitrogen concentration in plants from maze seeds inoculated with *Bacillus* was 29.64 g kg^{-1}, while the absence of inoculation promoted accumulation of only 11.33 g kg^{-1}.

The T2 treatment differed statistically from the others for grain yield, with an average of 7000.58 kg ha^{-1}, while the T1 and T4 treatments were lower (6109.29 and 6294.71 kg ha^{-1}, respectively). The use of the T3 treatment allowed obtaining an average productivity of 6637.4 kg ha^{-1}, being intermediate to the others. The average grain yield obtained for the experiment was 6552.57 kg ha^{-1}, higher than that found in other studies on second-crop maze, which refers to 6261 kg ha^{-1} obtained from the use of *Azospirillum brasilense* (SMANIOTTO *et al.*, 2023) and 6476 kg ha^{-1} from the use of 300 kg ha^{-1} of N-fertilizer (ALBERT *et al.*, 2023). From this, it can be inferred that inoculation 90 days before sowing is a viable practice in reducing N-fertilizer in coverage. All treatments were superior to the T1 treatment for the thousand grain weight, which obtained an average of 294.06 g, while the values for T2, T3 and T4 were 312.03, 313.05 and 313.07 g, respectively. The T2 treatment differed statistically from the others for total nitrogen in the plant (average of 25.02 g kg^{-1}), while the averages for the inoculation treatments were 22.17 and 22.11 g kg^{-1} for T3 and T4, respectively, which did not differ statistically from T1.

Table 8. Mean comparison test for the main effects of environment (E) and treatment (T) on the variables population (POP, plants ha^{-1}), ear index (EI), plant height (PH, cm), seed ear insertion height (EIH, cm), number of grain rows (NGR, units), grain yield (GY, kg ha^{-1}), dry mass (DM, kg ha^{-1}), total nitrogen in the plant (NCP, %) and thousand grain weight (TGW, g).

Environment	POP	EI	PH	EIH
Serranópolis do Iguaçu – PR	68312.76 a	1.13 a	219.85 a	129.18 a
São Miguel do Iguaçu – PR	61093.47 b	0.97 b	196.99 b	111.76 b
Mean	64425.45	1.06	209.3	121.14

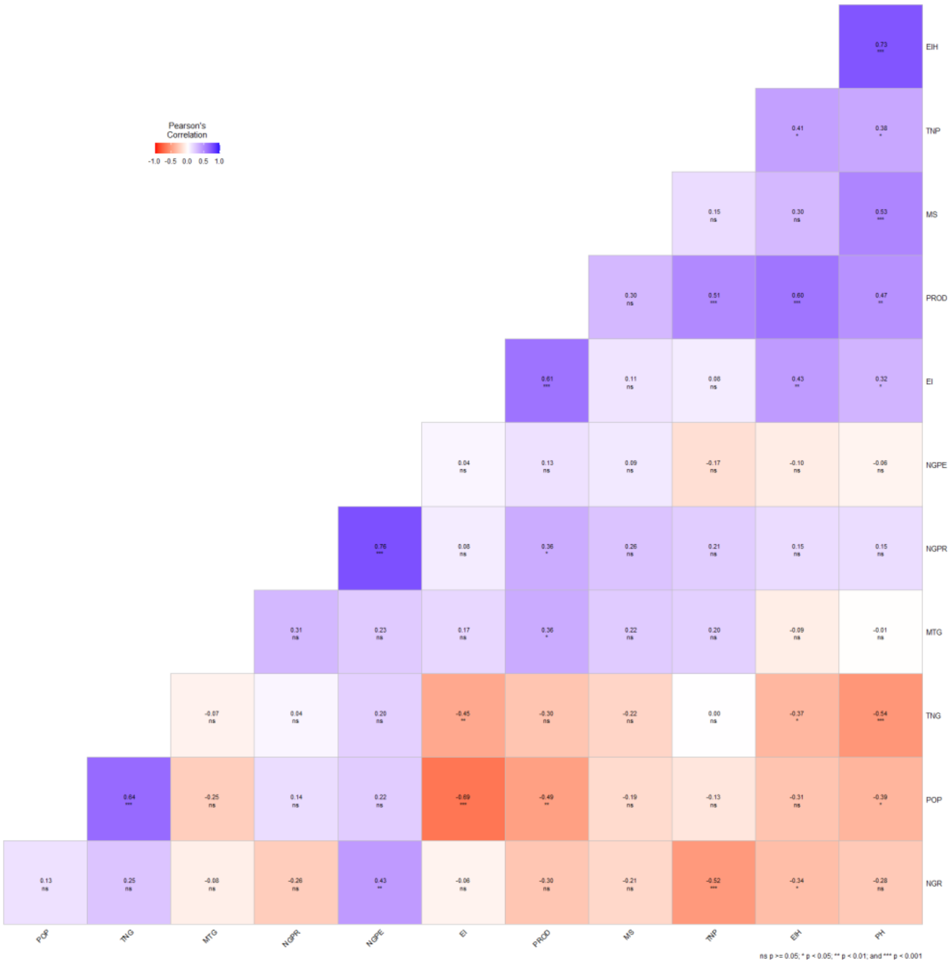
Environment	NGR	GY	MS	NCP
Serranópolis do Iguaçu – PR	16.39 b	7112.51 a	9347.85 a	23.94 a
São Miguel do Iguaçu – PR	16.98 a	5899.32 b	8554.25 a	21.31 b
Mean	16.67	6552.57	8981.57	22.73

Treatment	GY	TGW	NCP
T1	6109.29 b	294.06 b	21.04 b
T2	7000.58 a	312.03 a	25.02 a
T3	6637.94 ab	313.05 a	22.17 ab
T4	6294.71 b	313.07 a	22.11 ab
Mean	6552.57	308.87	22.73

Means followed by the same lowercase letter do not differ from each other using the Tukey test at 5% probability.

Correlation coefficients are classified according to their magnitude, where a null correlation corresponds to r=0.0, weak correlation between r=0.10 and r=0.30, medium correlation between r=0.31 and r=0.60, strong correlation between r=0.61 and r=0.90 , very strong correlation between r=0.91 and r=0.99 and perfect correlation when r=1.00 (CARVALHO *et al*., 2004). For the coefficients obtained in the study (Figure 2), a negative correlation of medium magnitude was observed between plant height and plant population and total nitrogen in grains (r=-0.39 and r=-0.54), in addition to a positive correlation of strong magnitude with ear insertion height (r=0.73) and of medium magnitude with total nitrogen in the plant, total dry mass, grain yield and ear index (r=0.38, r=0.53, r=0.47 and r=0.32). Therefore, it is logically estimated that the greater nitrogen input for plant growth negatively influenced the total nitrogen in the grains. This can be explained by the study by DeBruin *et al*. (2017), when evaluating the accumulation of N in grains in hybrids released over 80 years, which reported that the strength of the vegetative tissue to accumulate nitrogen exceeded the drain strength of the grains. There was a positive correlation between ear insertion height, total nitrogen in the plant and ear index with grain yield, with medium magnitude (r=0.60; r=0.51) and strong magnitude (r=0.61), respectively, in addition to negative correlation of strong magnitude between plant population and ear index (r=-0.69). The plant population variable strongly influenced the increase in total nitrogen in grains (r=0.64), while it presented a medium contribution to the reduction in grain yield (r=-0.49). In this way, it can be deduced that adjustments in the arrangement of maze plants can favor the accumulation of nitrogen in the grains, to the detriment of grain yield if management is carried out incorrectly.

Figure 2. Pearson's linear correlation coefficients for the variables plant population (POP, plants ha^{-1}), ear index (EI), plant height (PH, cm), ear insertion height (EIH, cm), number of grain rows (NGR, units), number of grains per row (NGPR, units), number of grains per ear (NGPE, units), grain yield (GY, kg ha^{-1}), thousand grain weight (TGW, g), dry mass (DM, kg ha^{-1}), total nitrogen in the plant (NCP, %) and total nitrogen in the grains (NCG, %).*: significant at 5% by t test.

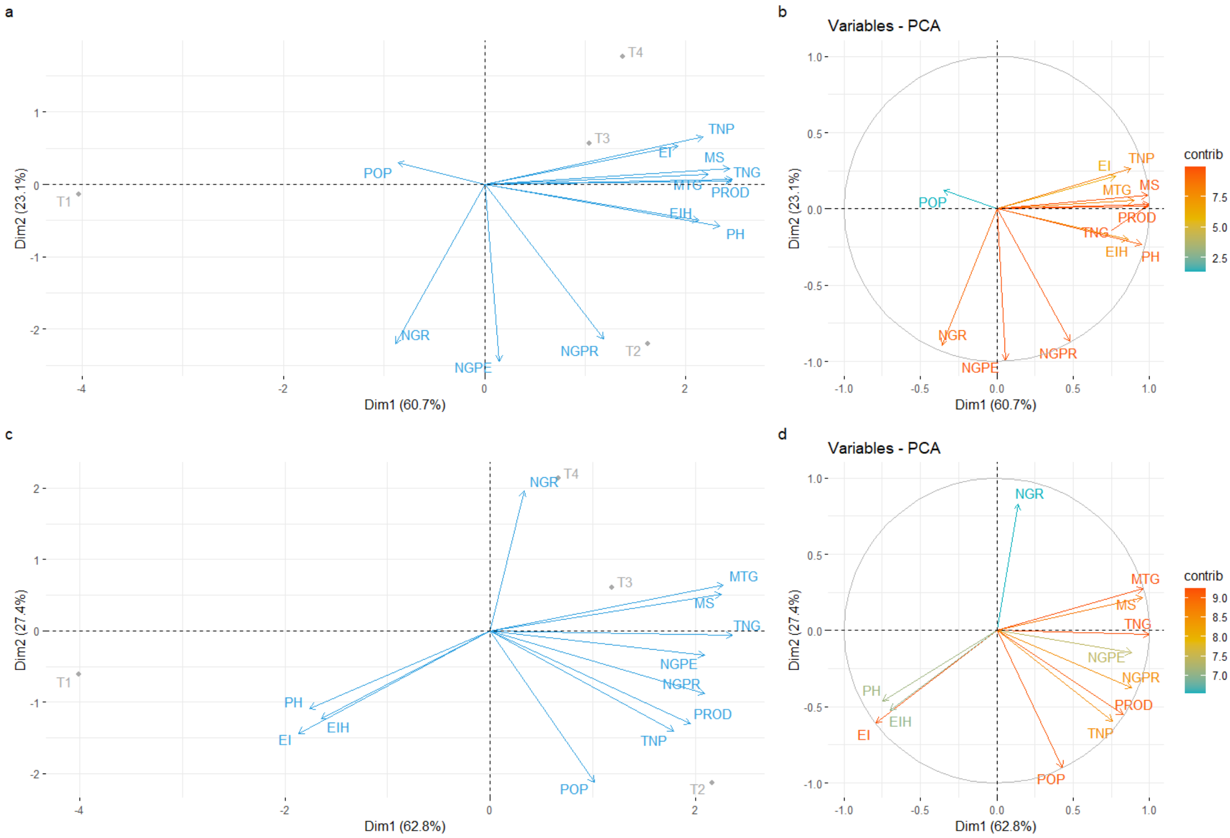


Source: Authors, 2024.

The Biplot principal component analysis for the harvest period (Figure 3a) allowed explaining 83.8% of the information variability (Dim1-60.7%; Dim2-23.1%), sufficient to define the main associations between the treatments and the variables measured. Treatments T1 and T4 did not show affinity with any of the variables. The influence of the T2 treatment on the number of grains per row was noted, in addition to the influence of the T3 treatment on the ear index, total nitrogen in the plant and total dry mass. The variables total nitrogen in grains and grain yield demonstrated the greatest importance in the explainability of the results, with a contribution greater than 7.5%. Plant population was the least important variable, with a contribution of less than 2.5%.

The Biplot principal component analysis for the second harvest period (Figure 3c) allowed explaining 84.2% of the information variability (Dim1 – 62.8%; Dim2 – 21.4%). The arrangement of treatments was similar to that estimated for the harvest period. There was an affinity between the T4 treatment and the variable number of rows of grains, while there was a similarity between the T3 treatment and the characters of thousand grain weight, total dry mass and total nitrogen in the grains. According to Katsenios *et al.* (2022), the bacteria *B. mojavensis, B. licheniformis* and *B. amyloliquefaciens* promoted the greatest accumulation of biomass per plant at 84 days after sowing, with values of 315.6, 305.7 and 298.3 g, respectively, the which also increased the values obtained for the thousand grain weight. The contribution of the characters of thousand grain weight, total nitrogen in grains, grain yield, plant population and ear index was greater than 9.0%, being those with greater explainability regarding the results obtained. The characters number of grains per ear, plant height and ear insertion height contributed with explainability between 7.0% and 7.5%, while the contribution of the character number of grain rows was less than 7.0%.

Figure 3. Principal Component Analysis (PCA) Biplot and contribution of variables for the harvest period (Figures a and b) and for the second harvest period (Figures c and d) for four treatments in the variables plant population (POP), ear index (EI), plant height (PH), ear insertion height (EIH), number of grain rows (NGR), number of grains per row (NGPR), number of grains per ear (NGPE), grain yield (GY), thousand grain weight (TGW), dry mass (DM), total plant nitrogen (NCP) and total grain nitrogen (NCG).



Source: Authors, 2024.

The ability of rhizobacteria to promote gains in the volume of the root system, especially in the case of crops with a high nutrient absorption capacity, such as corn, allows a high agronomic efficiency of nitrogen use, which is the focus of this study. In this way, highlighting the agronomic efficiency and viability of the practice.

CONCLUSION

Inoculation with *Bacillus licheniformis* presents agronomic efficiency similar to nitrogen control and inoculation with *Azospirillum brasilense* during the harvest period.

Inoculation with *Bacillus licheniformis* 90 days before sowing is capable of reducing the application of N-fertilizer by 25% without compromising the yield of maze grains in the second harvest period.

REFERENCES

AGUIAR, F. R. *et al*. Maize crop response to different levels of mineral and organomineral fertilization associated with plant growth promoting bacteria (PGPBs). Brazilian Journal of Development, v. 8, n. 11, p. 75406-75426, 2022.

ALBERT, A. M. *et al*. Nitrogen management in second-crop maize in Southwestern Goiás. Agricultural Sciences, v. 47, p. 1-12, 2023.

ARAÚJO, A. S. F. *et al*. Rhizobium and Bacillus subtilis coinoculation in cowpea and leucaena: effect on nodulation, N2 fixation and plant growth. Rural Science, Santa Maria, v. 40, n. 1, p. 1-4.

ARENHARDT, E. G. *et al*. Technical and agronomic efficiency of oat cultivars as a function of nitrogen availability. Jaboticabal Scientific, v. 45, n. 3, p. 257-270, 2017.

AZIZ, U. *et al*. Toward a molecular understanding of rhizosphere, phyllosphere, and spermosphere interactions in plant growth and stress response. Crit. Rev. Plant Sci., v. 40, p. 479–500, 2022. doi: 10.1080/07352689.2022.2031728

BUENO, C. B. *et al*. Effects of Chemical Fertilization and Microbial Inoculum on Bacillus subtilis Colonization in Soybean and Maize Plants. Front. Microbiol., v. 13, p. 901157, 2022. doi: 10.3389/fmicb.2022.901157.

CHAGAS JUNIOR, A. F. *et al*. Bacillus subtilis as an inoculant that promotes plant growth in soybeans. Diversitas Journal, v. 7, n. 1, p. 1-16, 2022.

DeBRUIN, J. L. *et al*. Grain Yield and Nitrogen Accumulation in Maize Hybrids Released during 1934 to 2013 in the US Midwest. Crop Science, v. 57, p. 1431-1446, 2017. doi: 10.2135/cropsci2016.08.0704.

ERENSTEIN, O. *et al*. Global maize production, consumption and trade: trends and R&D implications. Food Sec., v. 14, p. 1295–1319, 2022. doi: 10.1007/s12571-022-01288-7.

JONES, C. A.; KINIRY, J. R. CERES-Maize: A simulation model of maize growth and development. Texas, USA: A&M University Press, College Station, 1986. p. 194

KATSENIOS, N. *et al*. Assessment of plant growth promoting bacteria strains on growth, yield and quality of sweet maze. Scientific Reports, v. 12, 11598, p. 1-13, 2022.

KROPFF, M. J.; VAN LAAR, H. H. Modelling crop-weed interactions. CAB International: Wallingford, UK, 1993.

KUMAR, A. *et al*. Plant growth-promoting rhizobacteria: strategies to improve abiotic stresses under sustainable agriculture. J. Plant Nutr., v. 42, p. 1402–1415, 2019. doi: 10.1080/01904167.2019.1616757

KUSHWAHA, A. S. *et al*. Impact of arsenic on phosphate solubilization, acquisition and poly-phosphate accumulation in endophytic fungus Serendipita indica. Microbiol. Res., v. 259, p. 127014, 2022. doi: 10.1016/j.micres.2022.127014.

LORO, M. V. *et al*. Maize genetic breeding for tolerance to abiotic stress with focus on sustainable use of environmental resources. Agronomy Science and Biotechnology, v. 10, p. 1-12, 2024. doi: 10.33158/ASB.r199.v10.2024.

MORENO, A. L. *et al*. Rhizobacteria inoculation in maize associated with nitrogen and zinc fertilization in sowing. Brazilian Journal of Agricultural and Environment Engineering, v. 25, n. 2, p. 96-100, 2021.

MÓRING, A. *et al*. Nitrogen Challenges and Opportunities for Agricultural and Environmental Science in India. Front. Sustain. Food Syst., v. 5, p. 505347, 2021. doi: 10.3389/fsufs.2021.505347.

MOTA, M. R. *et al*. Stabilized nitrogen sources as an alternative for increasing grain yield and nitrogen use efficiency by maize. Brazilian Journal of Soil Science, v. 39, n. 2, p. 512-522, 2015.

NAKATANI, A. S. *et al*. Use of different Bacillus spp. plant growth promoters associated with nitrogen fertilization in maze crops. Latin American Economy Observatory Journal, Curitiba, v. 22, n. 3, p. 01-20, 2024.

NASA POWER. Prediction of Worldwide Energy Resource Applied Science Program. Available in: https://power.larc.nasa.gov/docs/. Access: July 4th, 2023.

NUSS, E.T.; TANUMIHARDJO, S.A. Maize: a paramount staple crop in the context of global nutrition. Compr. Rev. Food Sci. Food Saf., v. 9, p. 417–436, 2010.

OLIVOTO, T.; LÚCIO, A. D. metan: An R package for multi-environment trial analysis. Methods in Ecology and Evolution, v. 11, n. 6, p. 783-789, 2020. doi: 10.1111/2041-210X.13384.

PARCO, M. *et al*. Prolificacy and nitrogen internal efficiency in maize crops. Field Crops Research, v. 256, p. 107912, 2020.

PEREIRA, L. M. *et al*. Oxidative stress measurements can indicate the best dose and period of nitrogen fertilizer in white oat crop. International Journal of Development Research, v. 8, n. 1, p. 18468-18474, 2018.

PIMENTEL-GOMES, F. Experimental Statistics Course. FEALQ: Piracicaba, 15. ed., 2022. p. 451

R CORE TEAM. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2023.

RANUM, P. *et al*. Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences, v. 1312, p. 105-112, 2014.

REGINATTO, D.C. *et al*. Nitrogen management at sowing and topdressing with the time of supply in the main biotype of oats grown in southern Brazil. Australian Journal of Crop Science, v. 15, n. 04, p. 524-530, 2021.

ROCHA, I. *et al*. Seed coating: a tool for delivering beneficial microbes to agricultural crops. Front Plant Sci, v. 10, p. 1357, 2019. doi: 10.3389/fpls.2019.01357.

ROSS, F. *et al*. Maize prolificacy: a source of reproductive plasticity that contributes to yield stability when plant population varies in drought-prone environments. Field Crops Research, v. 247, p. 107699, 2020.

SCREMIN, O. B. *et al*. Artificial intelligence by artificial neural networks to simulate oat (Avena sativa L.) grain yield through the growing cycle. Journal of Agricultural Studies, v. 8, n. 4, p. 1-19, 2020.

SETZER, J. Climate and Ecological Atlas of the State of São Paulo. Paraná-Uruguay Bay Interstate Commission, 1966. p. 61

SHAFI, J.; TIAN, H.; JI, M. *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnology and Biotechnological Equipment, v. 31, n. August, p. 446-459, 2017.

SILVA, F. C. Manual of chemical analyzes of soils, plants and fertilizers. Brasília: Embrapa Technological Information, 2. ed., 2009. p. 627

SMANIOTTO, A. O. *et al*. Agronomic performance of second crop maize grown under different methods of inoculation with Azospirillum brasilense. Soil and Plant Nutrition, v. 70, n. 6, p. 1-7, 2023.

SOUSA, S. M. *et al*. Tropical Bacillus strains inoculation enhances maize root surface area, dry weight, nutrient uptake and grain yield. Journal of Plant Growth Regulation, v. 40, p. 867-877, 2021.

SUN, S. *et al*. Evidence of endophytic nitrogen fixation as a potential mechanism supporting colonization of non-nodulating pioneer plants on a glacial foreland. Biol. Fertil. Soils, v. 58, p. 527–539, 2022. doi: 10.1007/s00374-022-01640-1

TASCA, F. A. Volatilization of ammonia from the application of two sources of nitrogen, in the laboratory [dissertation]. Lages: State University of Santa Catarina, 2009.