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Effect of different doses of nitrogen and inoculation with *Azospirillum brasilense* on the productive characteristics of maize

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KEYWORDS

Zea mays L.

Inoculation

Nitrogen fertilizer

Yield

ABSTRACT

This study assessed the effects of different nitrogen doses on maize crops, with and without the inoculation of *Azospirillum brasilense*. The experiment was carried out during the 2020/2021 harvest season in the administrative department of Concepción, district of Horqueta, Paraguay, at the coordinates of 23°14'31.7" S and 56°53'05.9" W. The experiment followed a randomized complete block design (RCBD) arranged in a factorial design (4 X 2). Factor A included nitrogen doses (0, 40, 80, and 120 kg ha^{-1}), while factor B corresponded to the bacterial inoculation (with and without *A. brasilense*). The experiment was performed in three replications; each experimental unit (EU) was 22.5 m^2. The study evaluated the following characteristics: plant height, cob insertion height, cob length and diameter, weight of 1000 kernels, and kernel yield. The data were analyzed using analysis of variance (ANOVA), and the averages were compared using Tukey's test at a 5% probability of error. Regression analysis was also carried out during the study. The experiment results demonstrated that increased nitrogen levels positively affected the measured characteristics, fitting a quadratic model, except for cob insertion height. Inoculation with *A. brasilense* significantly increased corn growth and productivity. The interaction of both factors produced a significant increase in cob length. Based on the experiment results, applying 104.30 kg ha^{-1} of N in combination with *A. brasilense* inoculation is recommended for improved maize production.

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1 Introduction

Maize, known as corn (*Zea mays* L.), is the second most important crop worldwide, and in 2022, global maize harvesting reached a record 1137 million tons (Erenstein et al. 2022). In Paraguay, maize is typically planted during the summer-fall season. As per estimates for the 2023 harvest, maize production in Paraguay is projected to be around 5 million tons, with an average yield of 5.8 tons per hectare (CAPECO 2023). Maize is an essential crop that contributes to food security. It is a staple food in many parts of the world and is also used as feed for animal production (Wang and Hu 2021). However, maize cultivation and production can be affected by various biotic and abiotic factors (Nigussie et al. 2021; Széles et al. 2023). In Paraguay, the most significant abiotic factor is the low fertility of subtropical soils. This, coupled with a scarcity of nitrogen-fixing microorganisms, gradually reduces the productivity of crops like maize (Teklewold et al. 2013).

Nitrogen is a macronutrient that is crucial in plant growth and development. It is essential for the production of organic plant compounds and chlorophyll. However, excessive nitrogen can lead to increased production costs and unnecessary loss of crop inputs through leaching (Picazevicz et al. 2017; Larramendi et al. 2023). Therefore, it is important to ensure that nitrogen is available in the required quantities for optimal plant growth without causing harm to the environment.

To achieve high yields, maize crops require nitrogen to be available throughout the entire vegetative phase. To ensure the provision of this element, management practices can be integrated into the farming of cereal crops (Zainab et al. 2021), such as the use of mineral fertilizers (Davies et al. 2020) and the incorporation of nitrogen-fixing microorganisms, especially the bacterium *A. brasilense*. This bacteria fulfils numerous functions, including producing phytohormones promoting root growth. In turn, this bacterium raises the capacity of plants to absorb nutrients and water (Cassán and Díaz-Zorita 2016; Niranjan et al. 2024). Furthermore, using this bacterium makes nitrogen available through biological fixation (Pedrinho et al. 2010; Dartora et al. 2013) and biocontrol of plant pathogens. These factors directly enhance root growth, thus increasing crop productivity (Milléo and Cristófoli 2016; Cadore et al. 2016). A study by Dartora et al. (2016) and Di Salvo et al. (2018) found that combining nitrogen fertilizer and seed inoculation with microorganisms increased maize growth and yield. Ferreira et al. (2013) found that maize grain yield increased by 29% when using *A. brasilense* and nitrogen compared to using nitrogen fertilization alone. In contrast, Lana et al. (2012) reported that *A. brasilense* inoculation without applying nitrogen significantly increased maize yields by 15%. In view of the above, this study aimed to evaluate the effect of different doses of nitrogen, with and without bacterial inoculation of *A. brasilense*, on maize crops.

2 Materials and Methods

The experiment occurred in Horqueta, Department of Concepción, Paraguay, during the 2020/2021 harvest season at coordinates 23°14'31.7"S and 56°53'05.9"W.

The climate in the region has an average temperature of 26°C, with maximum highs of 45°C in summer and minimum lows of 4°C in winter, with low levels of frost. The average annual rainfall during the study time is 1,400 mm. Figure 1 (DMH 2021) shows the average rainfall and temperature levels recorded throughout the experiment. The soil in the experimental area belongs to the Alfisol classification with the subgroup Mollic Paleudalf (López et

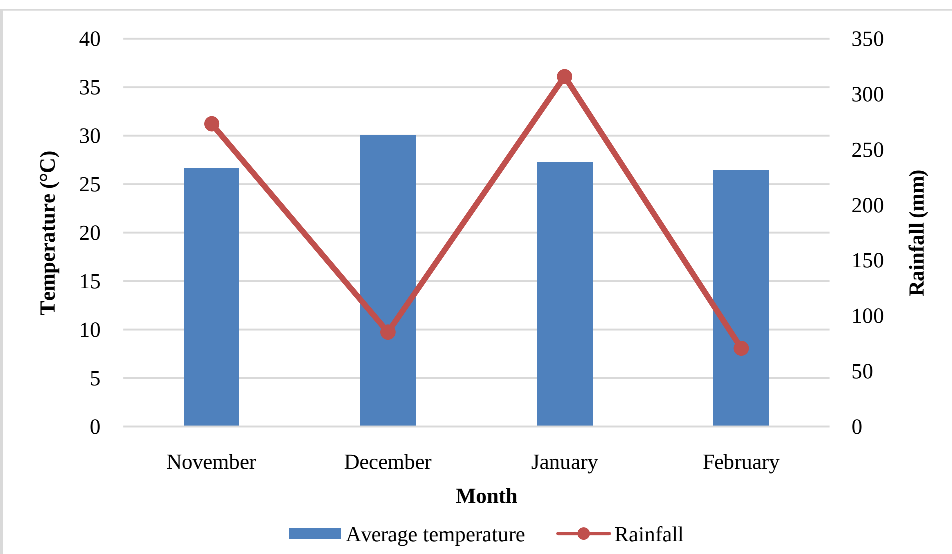


Figure 1 Average rainfall and temperature data for November to February 2020/2021

Table 1 Description of the formulated Treatments

Factor A (Nitrogen dose)	Factor B (Bacterial inoculation)	Combination
0 kg ha^{-1}	With *A. brasilense*	0 kg ha^{-1} + With *A. brasilense*
40 kg ha^{-1}		40 kg ha^{-1} + With *A. brasilense*
80 kg ha^{-1}		80 kg ha^{-1} + With *A. brasilense*
120 kg ha^{-1}		120 kg ha^{-1} + With *A. brasilense*
0 kg ha^{-1}	Without *A. brasilense*	0 kg ha^{-1} + Without *A. brasilense*
40 kg ha^{-1}		40 kg ha^{-1} + Without *A. brasilense*
80 kg ha^{-1}		80 kg ha^{-1} + Without *A. brasilense*
120 kg ha^{-1}		120 kg ha^{-1} + Without *A. brasilense*

al. 1995). Before the experiment, the soil's chemical attributes were evaluated using the methodology presented by Cardoso et al. (2009). The soil analysis showed the following results for the 0 – 20 cm soil layer: Phosphorus (4.50 mg dm^{-3}), Organic matter (11.16 g dm^{-3}), Potassium (0.16 cmol dm^{-3}), Ca (4.78 cmol dm^{-3}), Mg (0.97 cmol dm^{-3}), H+Al (2.36 cmol dm^{-3}), base saturation (5.91 cmol dm^{-3}), Cation exchange capacity (5.93 cmol dm^{-3}), pH (5.55), and volume (71.53%). The experimental design was a randomized complete block design (RCBD) arranged in a factorial design (4 X 2). Factor A corresponds to nitrogen doses (0, 40, 80, and 120 kg ha^{-1}), and factor B is bacterial inoculation (with and without *A. brasilense*) (Table 1). Each treatment had three replications. Each experimental unit (EU) measured 22.5 m^2 (5 m length by 4.5 m width).

A traditional sowing system was used for the experiment. In November 2020, the hybrid maize cultivar DKB 360 was planted manually with a spacing of 0.45 m between rows and 2.5 plants per linear meter, resulting in a population density of 55,500 plants per hectare. Before planting atrazine herbicide (2.0 kg ha^{-1}) with adjuvant (0.5 l ha^{-1}) and triflumuron insecticide (0.10 l ha^{-1}) were applied to the experimental field. Seed treatment was performed before sowing using a commercial liquid inoculant containing strains of *A. brasilense* bacteria at a concentration of 1.108 cfu.ml. Nitrogen fertilizer was applied twice, during sowing (30%) and at the V5 stage (70%). Urea was used as a nitrogen source (45% N) for all treatments. Phosphorus and potassium (57 kg ha^{-1} and 62 kg ha^{-1}, respectively) were applied during sowing using Triple Superphosphate and potassium chloride as the source of these nutrients. Weed control was carried out manually, while pest control was done chemically using insecticides such as thiodicarb (0.24 kg ha^{-1}) and triflumuron (0.10 l ha^{-1}). Disease was controlled using the fungicides tebucone + trifloxis (0.5 l ha^{-1}), which were applied using a 20 L manual sprayer.

The crop was harvested in February 2021, 115 days after sowing. A sampling area of 14.4 m^2 containing 80 plants was marked out to evaluate each experimental unit. The following variables were measured: (a) Plant height (m), measured when the plants reached physiological maturity. For this, ten plants were selected at random from the sampling area of each experimental unit and measured using a measuring tape from the base of the stem to the height of insertion of the flag leaf, (b) Cob insertion height (m), which was measured from the base at soil level to the insertion of the highest cob. This measurement was taken using a measuring tape from 10 plants from the sampling area of each experimental unit just before harvesting. (c) Cob length and diameter (cm) were measured by randomly selecting 10 cobs from each experimental unit. The length was measured using a measuring tape, and the diameter was measured at the center of the cobs using a vernier scale, (d) Weight of 1000 kernels (g), which was estimated using a scale with a precision of 0.01 g, at 13% moisture content (wet basis), and (e) Kernel yield (kg ha^{-1}), which was measured by harvesting the sampling area of each experimental unit and weighing the kernels on a digital scale.

A variance analysis (ANOVA) was carried out on the results obtained using Agrostat@ statistical software. Averages that showed significant differences were compared using the Tukey test at 5% probability. A regression analysis was conducted to identify doses providing maximum technical efficiency. The maximum fertilizer dosage was calculated using the formula $X_{max} = -b/2a$. The point of maximum productivity was calculated using the formula $Y_{max} = D/ (4a)$, where D = b2 - 4ac.

3 Results and Discussion

Table 2 shows the calculated F values and their significance. The study found that Nitrogen doses significantly affected all variables except for the height of cob insertion. Bacterial inoculation also significantly affected various variables, including plant height, height of cob insertion, weight of 1000 kernels, and maize crop yield. Additionally, the interaction between Nitrogen doses and bacterial inoculation significantly affected cob length.

Table 2 Calculated F values and significance established by the effects of the factors under study on the variables of PH, HCI, CD, CL, WK, and YIEL

Factors	PH	HCI	CD	CL	WK	YIEL
	F Test					
ND	7.22**	0.30ns	5.13*	8.32**	41.36**	85.15**
BI	29.60**	11.64**	1.91ns	1.81ns	8.45*	6.45*
Interaction NDxBI	2.09ns	0.15ns	0.41ns	4.46*	0.74ns	0.32ns

ns: not significant; (**) (*) significant at 1 and 5 % probability, PH: Plant height, HCI: Height of cob insertion, CD: Cob diameter, CL: Cob length, WK: Weight of 1000 kernels, YIEL: Yield, ND: Nitrogen dose, BI: Bacterial inoculation

Table 3 Effect of *A. brasilense* inoculation and N dose on average plant height, cob insertion height, weight of 1000 grains, and maize yield

Factor	PH (m)	HCI (m)	WK (g)	YIEL (kg ha^{-1})
Bacterial Inoculation				
WI	2.08±0.011^{a}	1.17±0.010^{a}	229.94±4.23^{a}	4774.89±254.64^{a}
WOI	1.97±0.023^{b}	1.030±026 ^{b}	222.94+5.01 ^{b}	4523.02±240.94 ^{b}
N Dose (kg ha^{-1})				
0	1.96±0.045^{b}	1.09±0.041^{ns}	205.15±3.48^{c}	3492.25±56.47^{c}
40	2.000±030^{b}	1.08±0.043	220.91±2.70^{b}	4356.78±55.51^{b}
80	2.090±026^{a}	1.13±0.050	242.54±3.13^{a}	5516.42±81.92^{a}
120	2.050±020 ^{ab}	1.11±0.031	236.07±2.22^{a}	5230.38±194.11^{a}
CV %	2.54	8.66	2.80	5.22
MSD	0.45	0.08	5.56	212.77
OA	2.03	1.10	226.17	4648.96

Letters differ from each other statistically by Tukey's test at 5%, PH: Plant height, HCI: Height of cob insertion, WK: Weight of 1000 kernels, YIEL: Yield, CV: Coefficient of variation, MSD: Minimum significant difference, OA: Overall average, WI: With inoculation, WOI: Without inoculation

During the study, the application of *A. brasilense* on maize seeds before planting resulted in greater plant height (2.08 m) and cob insertion height (1.17 m) (Table 3). This can be attributed to the bacteria's synergistic effect on the seeds, which is fundamental to the plant's growth and development (Coelho et al. 2021). However, the data obtained in this study does not coincide with the findings of Marini et al. (2015), who did not observe significant effects on plant height to combined application. Similarly, the study does not support the results of Maestrelo et al. (2014), who also did not record a positive increase in the height of cob insertion. Regarding the application of N to the maize crop, it produced a positive response concerning plant height (Figure 2) and fitted with a quadratic equation. The study observed a reduction in average height when high doses of N were applied. A maximum height of 2.09 m was achieved using 90 kg ha^{-1} of N, similar to the results observed by Marini et al. (2015). Furthermore, Morais et al. (2015) found that applying nitrogen and bacteria led to greater plant development. Gutiérrez-Peña et al. (2022) stated that nitrogen plays a vital role in several metabolite production processes that influence the growth and maintenance of leaves' photosynthetic and reproductive capacity, promoting crop growth in terms of height and development.

The application of different doses of nitrogen significantly impacted the diameter of the cobs, as shown in Figure 3. A quadratic regression model was used to analyze the data. The maximum diameter of the cobs, measuring 6.75 cm, was observed at a dose of 83.33 kg ha-1. This highlights the importance of nitrogen for plant nutrition, as it facilitates the transformation of biomass in the plant's metabolic processes. Similar results were reported by Galindo et al. (2019), who found that the combination of nitrogen fertilizer and bioinoculant resulted in a significant increase in cob diameter.

According to Figure 4, the cob length was affected by a combination of bacterial inoculation and nitrogen (N) dose. The cob length was greater when *A. brasilense* bacteria was applied with doses of 0, 80, and 120 kg ha^{-1} N. However, when a 40 kg ha^{-1} N dose was used without *A. brasilense*, the cob length was the greatest. The quadratic regression model for N doses indicated

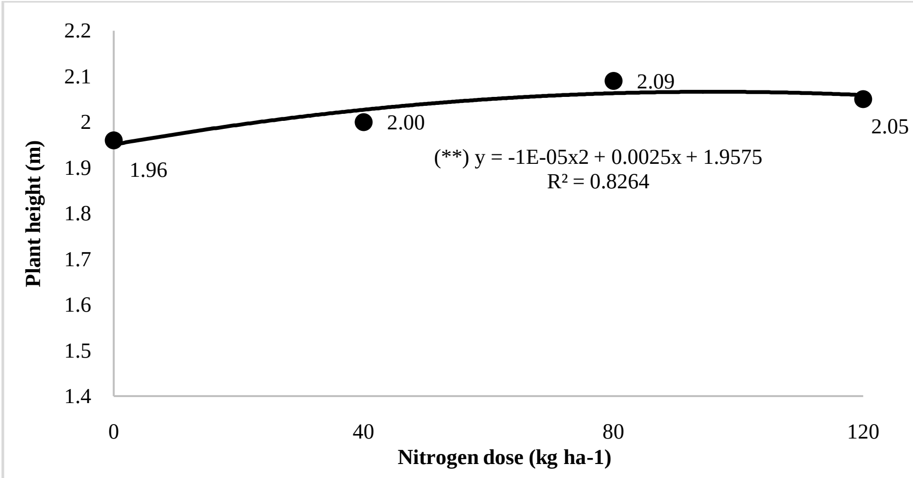


Figure 2 Regression analysis between nitrogen dose and maize plant height

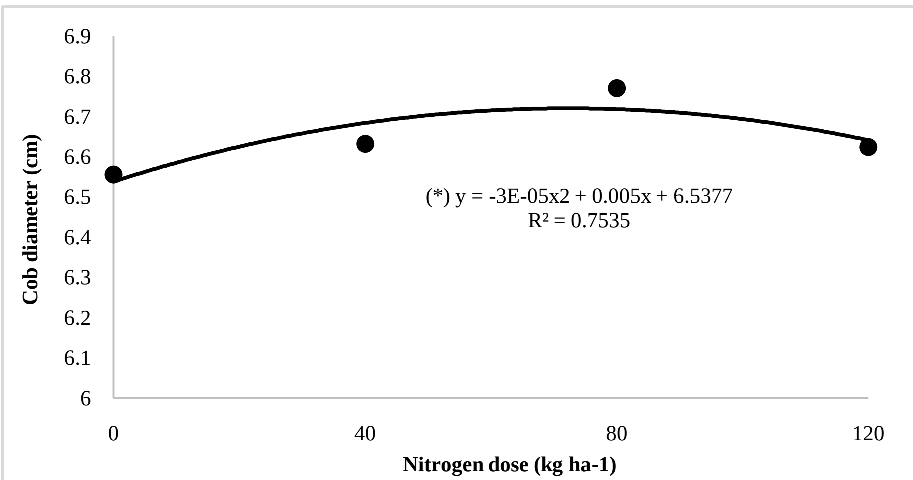


Figure 3 Regression analysis between nitrogen dose and diameter of maize cob

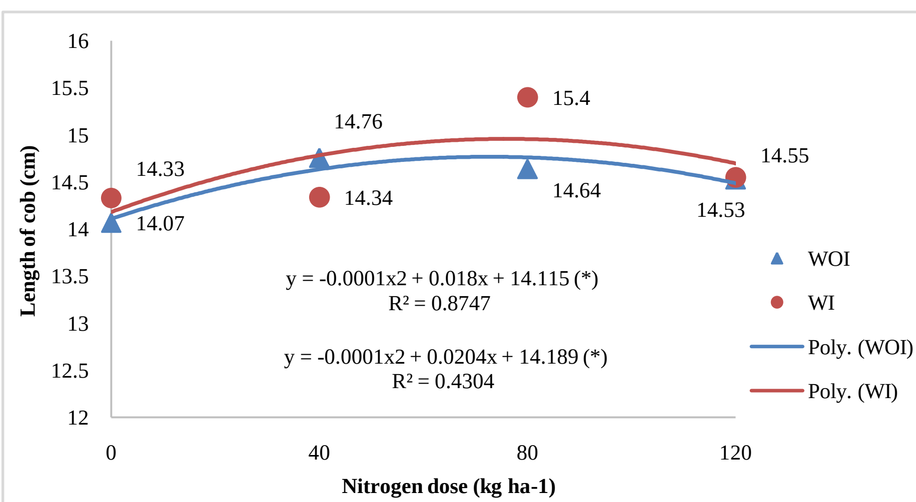
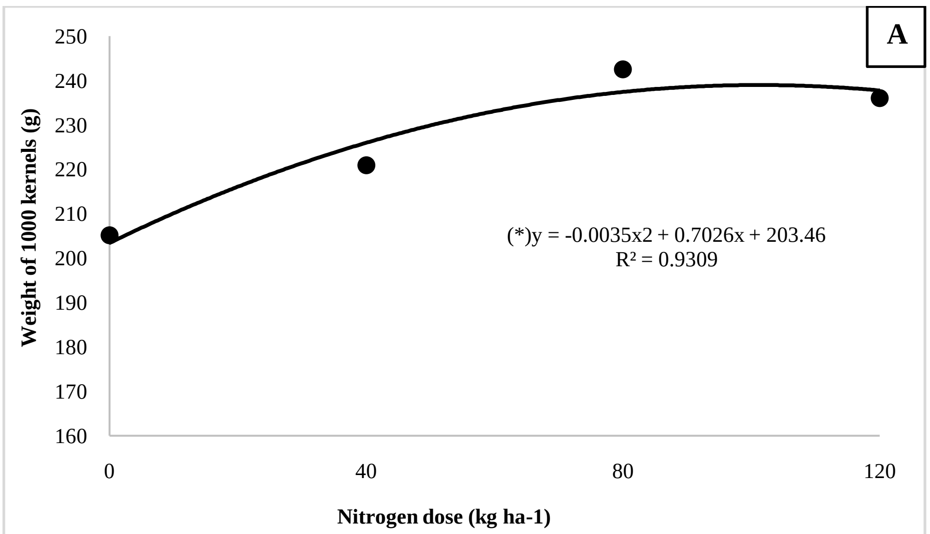


Figure 4 Regression analysis carried out on average cob length concerning inoculation and nitrogen dose, WI: With inoculation, WOI: Without inoculation, Y: cob length response as a function of X: nitrogen dose, *significant by Fisher's test at 5% probability of error.

that excessive N decreased average cob length. When *A. brasilense* bacteria was not used, the highest cob length of 15 cm was achieved with an N dose of 100 kg ha^{-1}. On the other hand, when *A. brasilense* bacteria was applied, the maximum cob length was 14.93 cm with a 90 kg ha^{-1} N dose.

In a study on the impact of nitrogen application and *A. brasilense* inoculation on maize crops, Souza et al. (2019) found no interaction between the two factors regarding cob length. Similarly, Cadore (2014) did not observe any significant effect on cob length when using *A. brasilense* and only detected differences with varying doses of nitrogen. Reis Junior et al. (2008) suggest that a lack of response to inoculant application can often be attributed to inconsistent root colonization, inoculum survival problems, or unfavorable environmental conditions.

In Table 3, the results for the weight of 1000 grains indicate that the application of bacterial inoculant led to a significant increase in weight (229.94 g). This finding contradicts the work of Galindo et al. (2019), who recorded a negative response to inoculation of *A. brasilense* to grain weight. The weight variable of 1000 grains responded positively to applying different N doses, fitting a quadratic equation, as shown in Figure 5a. The highest average weight of 238.72 g for 1000 grains was obtained using a dose of 100.37 kg ha-1 of nitrogen. According to Bulla and Balbinot Junior (2011), increasing the dose of N applied to the maize crop led to heavier grains, and a similar effect was observed in this experiment. Repke et al. (2013) recorded similar results for N doses, fitting a quadratic distribution. However, Mota et al. (2015) and Galindo et al. (2017) recorded a positive linear equation, displaying a tendency for an increase in the weight of the 1000 grains.



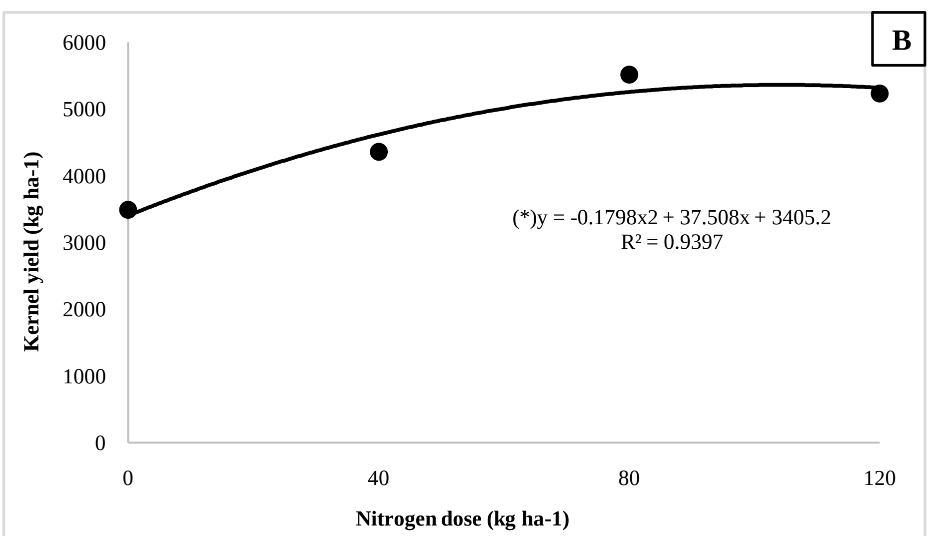


Figure 5 Regression analysis of nitrogen dose (A) weight of 1000 grains, (B) maize yield

In Table 3, the average grain yield showed a positive response to inoculation, with the highest result achieved by applying *A. brasilense* (4774.89 kg ha^{-1}). This result was more than 5.3% higher than the yield recorded without applying the bacteria. Similar results were obtained by Barbosa et al. (2022), who reported a 5.4% increase in maize grain yield when using bacterial inoculation compared to the control. The increase in yield could be attributed to greater plant development, more grains per cob, and higher grain mass, as previously reported by Oliveira et al. (2018). It is worth noting that according to Hungria (2011), the reaction of Gramineae and the plants' genetic characteristics can influence inoculation, the bacterial strains used, and environmental conditions.

A significant increase in grain yield was observed when applying nitrogen (N) to maize crops, as shown in Figure 5 B. Regression analysis revealed that the adjusted quadratic equation was substantial. The maximum yield efficiency was 5361.33 kg ha^{-1}, and the optimal dose of nitrogen fertilizer was 104.30 kg ha^{-1}. Several previous studies also support the positive effects of nitrogen fertilization. For instance, Mota et al. (2015) found that linear yield increases were obtained for nitrogen doses in maize crops, while Soratto et al. (2011) and Pereira et al. (2022) reported a quadratic increase in yield in response to increasing doses. Galindo et al. (2019) recommend using 100 kg ha^{-1} of N, in combination with the application of *A. brasilense*, to achieve the highest profitability in maize production, and similar results were obtained in this study.

Conclusion

According to the study, using nitrogen fertilizers on maize crops has a beneficial effect on factors associated with maize productivity. These were analyzed using a quadratic model. Moreover, most of these factors positively reacted to the introduction of *A. brasilense*. The research discovered that using *A. brasilense* led to a 5.3% average increase in maize yields.

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