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# *AZOSPIRILLUM BRASILENSE* INOCULATION IMPROVES THE MORPHOPHYSIOLOGICAL ASPECTS OF MAIZE IN SOILS WITH HIGH AND LOW NITROGEN CONTENTS

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**ABSTRACT** – This research evaluated the physiology, root and shoot morphology, and biomass in maize plants inoculated by *Azospirillum brasilense* at two nitrogen doses in the soil. The experiment was carried out in a greenhouse using a completely randomized design. A dose that guaranteed N120 grain yield (120 kg ha$^{-1}$) and another that reduced N20 availability by 86 % (20 kg ha$^{-1}$) was stipulated. The inoculant was a mixture of two homologous *A. brasilense* strains, Az1 (CMS 7+1626) and Az2 (CMS 11+1626). The treatments consisted of a combination of nitrogen doses and inoculation by *A. brasilense*, with four replications. Root morphology, nitrate and arginase reductase, pigment content, height, and leaf area were evaluated. Inoculation by *A. brasilense* Az1 and Az2 increases root volume and specific length. Nitrate reductase (251 %) and arginase (9 %) activities in the soil were higher with *A. brasilense*, as well as chlorophylls (Chlorophyll *a* 23 %), height, and leaf area (63%) at 20 kg ha$^{-1}$ nitrogen in the soil. At 120 kg ha$^{-1}$ nitrogen, *A. brasilense* also increases specific root length (28 %) and nitrate reductase activity (Az1 22 %; Az2 95 %), resulting in greater maize height and leaf area (Az1 11 %). *A. brasilense* favors exploring the soil profile to absorb water and nutrients. Inoculation improves the morphophysiology of maize in low soil nitrogen but does not reduce the use of nitrogen fertilizers in this crop. Inoculation by *A. brasilense* is an efficient, ecological, and low-cost technology that contributes to the sustainability of maize production.
**Keywords:** Nitrate reductase, WinRhizo, Leaf area, Chlorophylls, Plant height.

**RESUMO** - A pesquisa avaliou a fisiologia, morfologia radicular e parte aérea e biomassa em plantas de milho inoculadas por *A. brasilense* em duas doses de nitrogênio no solo. O experimento foi conduzido em casa de vegetação. Foi estipulada uma dose que garantiu a produtividade de grãos de N120 (120 kg ha$^{-1}$) e outra que reduziu em 100% a disponibilidade de N20 (20 kg ha$^{-1}$). O inoculante utilizado foi uma mistura de duas estirpes de *A. brasilense*, Az1 (CMS 7+1626) e Az2 (CMS 11+1626). Os tratamentos consistiram de combinação de doses de nitrogênio e inoculação de *A. brasilense*, com quatro repetições. Foram avaliados a morfologia radicular, redutase do nitrato e arginase, teor de pigmentos, altura e área foliar. A inoculação por *A. brasilense* Az1 e Az2 aumenta o volume e o comprimento específico das raízes. As atividades de redutase do nitrato (251 %) e arginase (9%) no solo foram maiores com *A. brasilense*, assim como clorofilas (Clorofila *a* 23 %), altura e área foliar (63 %) em 20 kg ha$^{-1}$ de nitrogênio no solo. Na dose de 120 kg ha$^{-1}$ de nitrogênio, *A. brasilense* também aumentou o comprimento específico da raiz (28 %) e a atividade da redutase do nitrato (Az1 22 %; Az2 95 %), resultando em maior altura e área foliar (Az1 11 %) do milho. *A. brasilense* favorece a exploração do perfil do solo para absorção de água e nutrientes. A inoculação melhora a morfofisiologia do milho em condições de baixo nitrogênio no solo, mas não reduz o uso de fertilizantes nitrogenados. A inoculação por *A. brasilense* é uma tecnologia eficiente, ecológica e de baixo custo que contribui para sustentabilidade do milho.
**Palavras-chave:** Redutase do Nitrato, WhinRhizo, Área foliar, Clorofila, Altura de plantas.

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Maize (*Zea mays* L.) stands out as one of the most important crops in agribusiness. This cereal is an essential staple food for low-income populations in many countries, in addition to being widely used in various processed foods such as oil, beverages, and starch (Hungria et al., 2022). It is also used as an energy source for animal production. In Brazil, the cultivation area of this cereal is approximately 20.38 million hectares (CONAB, 2024), making it the world's third-largest maize producer (110,963.7 million tons) (Hungria et al., 2022; Carvalho et al., 2022; CONAB, 2024) although, considering the current genetic potential of maize plants, the average grain yield in Brazil still shows considerably low-performance levels (Petean et al., 2019; Bigolin and Talamini, 2024).

On average, nitrogen fertilization in maize is 100-150 kg ha$^{-1}$ in tropical regions (Breda et al., 2019). Furthermore, it is the most exported nutrient in this crop, with approximately 75% of the nitrogen (N) being translocated to the grain (Neumann et al., 2005). However, high N fertilization to meet the needs of this crop can significantly increase its production cost, which is only sometimes favorable for farmers.

Plant growth-promoting rhizobacteria (PGPR) have been studied with a constant focus on reducing production costs, increasing yield, and promoting plant and environmental protection by reducing the supply of chemical fertilizers (Vendruscolo & Lima, 2021). Thus, there is interest in optimizing the positive effects of the association between rhizobacteria and large crops. *Azospirillum brasilense* is a rhizobacterium that has been studied in association with Poaceae, such as maize, to reduce the use of nitrogen fertilizers. Biological nitrogen fixation and the synthesis of plant hormones by *A. brasilense* (Fukami et al., 2018; Cassán et al., 2020) increase crop yield (Vendruscolo & Lima, 2021). Furthermore, with growing concerns about the emission of greenhouse gases in agriculture, the feasibility of replacing nitrogen fertilizers with sustainable strategies acquires notable importance (Hungria et al., 2022).

Despite several promising results with inoculation by *A. brasilense* in maize, response assumptions were based on a minimal number of assays in the field and the greenhouse (Barbosa et al., 2022). Another factor is the interaction of new and different *A. brasilense* strains with a wide variety of maize genotypes may present uncertain responses in a country with great diversity, such as Brazil, with different soil fertility profiles. Moreover, the physiological and morphological parameters could be better explored in crops. Consequently, new studies are always necessary to optimize the amount of nitrogen used in a sustainable system associated with *A. brasilense* in this crop.

Therefore, the hypothesis of this research was that maize inoculated with *A. brasilense* reduces the use of nitrogen fertilizers under irrigated conditions. The objective was to evaluate the physiology, root and shoot morphology and biomass in maize plants inoculated by *A. brasilense* in different soil nitrogen applications.

## Material and Methods

### Plant and microbiological material, growth conditions and experimental design

The experiment was conducted in a greenhouse at Embrapa Milho e Sorgo, in Sete Lagoas - MG, Brazil, located at geographic coordinates 19º28' S, 44º15'08'' W, and average altitude of 732 m. The average temperatures recorded during the evaluation period were a maximum of 31.2ºC and a minimum of 12.91ºC. Air relative humidity ranged from 30% to 72%. A drought-sensitive single hybrid BRS 1040, developed by the maize breeding program of Embrapa Milho and Sorgo, was used.

The inoculant used was obtained from the mixture of two homologous *Azospirillum brasilense* strains, at a proportion 1:1, Az1 (CMS 7+1626) and Az2 (CMS 11+1626), belonging to the collection of diazotrophic bacteria of the Laboratory of Soil Microbiology and Biochemistry of Embrapa Milho e Sorgo (Marques et al., 2021). The strains were isolated from the rhizosphere soil of maize plants and the stem of sorghum plants.

The homologous strains were cultivated in liquid trypticase soy broth for 72 hours at 29ºC under constant stirring. After this period, the suspension each strain was centrifuged at 6500 rpm for 15 min. The precipitate was resuspended in saline solution (0.85% NaCl) and adjusted to an optical density equal to 1.0 at 500 nm absorbance, which is equivalent to approximately $10^8$ viable cells.dc $m^{-3}$ (Marques et al., 2021). The amount used for each inoculant (Az1 and Az2) was 150 mL for 60000 maize seeds. The seeds were inoculated using ground charcoal and cassava starch gum for adhesion; sowing was then carried out (Marques et al., 2021).

The doses of N in this study were based on Coelho (2006) and Breda et al. (2019), who used, for agriculture with high technology, doses of 100 to 160 kg $ha^{-1}$ of N for high maize yields. Therefore, a dose was stipulated that would guarantee a good yield of N120 grains (120 kg $ha^{-1}$) and another that would reduce N availability by 86 % (20 kg $ha^{-1}$), called N20, for maize plants.

Sowing was carried out in 20-kg plastic pots containing Oxisol. Five seeds were planted per pot, and after germination, thinning was performed, leaving two plants per pot. Top dressing fertilizations were performed according to soil analysis and treatments (Table 1), using the formulated products 08-28-16 NPK, FTE BR12, and urea, following the recommendation for maize in the State of Minas Gerais (Ribeiro, 1999). All treatments were maintained at field capacity (FC) (soil water tension of -18 kPa) throughout the experiment.

The experimental design was completely randomized (CRD), consisting of *A. brasilense* (Az1 and Az2) and without inoculant, in combination with two doses of nitrogen (N20 and N120), with four replications. The nitrogen source used in this experiment was urea.

**Table 1.** Chemical attributes of the soil used for maize cultivation. Sete Lagoas - MG. Brazil.

| O.M. | pH | P (Mehlich) | K | Ca | Mg | Al | SB* | V* | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| dag $kg^{-1}$ | $H_2O$ | ----- mg $dm^{-3}$ ----- | | ------- cmolc $dm^{-3}$ ------- | | | | % | ----------mg $dm^{-3}$-- ------- | | | |
| 2.94 | 6.2 | 16.76 | 70.0 | 7.91 | 1.8 | 0.03 | 9.92 | 61.0 | 1.7 | 29.0 | 20.7 | 0.9 |

### Root morphology aspects

For the analysis of root system morphology, the image analysis system WinRhizo Pro 2007[a] (Regent Instruments, Sainte-Foy, QC, Canada), coupled with a professional scanner (Epson, Expression 10000 XL, Epson America, Inc., USA) was used, equipped with an additional light unit (TPU). The procedures for the obtention of the images were performed according to Marques et al. (2019) when the maize plants reached the phenological reproductive stage R1. The following characteristics were determined: length (cm), surface area ($cm^2$), mean diameter (mm) and root volume ($cm^3$). The software also analyzed length, surface area, and root volume by diameter classes (0 to 4.5 millimeters).

Subsequently, the roots were stored in paper bags and transported to an oven with forced air circulation at 65 °C until constant mass was obtained. Another attribute involving morphological and dry matter data was verified - specific root length (SRL: root length/root dry matter, cm $g^{-1}$). After the constant weight of shoots and roots, the total dry matter of maize plants was calculated at the end of the experiment.

### Soil nitrate reductase and arginase

The soil nitrate reductase activity and arginase activity were analyzed when maize plants were in the phenological reproductive stage R1. Nitrate reductase activity (NRA) was determined according to Su et al. (1996). The enzyme arginase, which is involved in the nitrogen dynamics of the soil, was analyzed. Arginase activity (arginine hydrolysis rate) was determined using the method proposed by Alef and Kleiner (1986), where 1 g of soil was weighed, added with 0.25 mL of reagent 1 (0.05 g of arginine in 25 mL of distilled water) and incubated for 2 hours at 37º C. Subsequently, reagent 2 (1M KCl) was added and stirred for 30 minutes. The samples were centrifuged; a 100-µL aliquot of the samples was then transferred, added with reagent 3 (sodium salicylate, sodium citrate, sodium tartrate, and sodium nitroprusside), and incubated for 15 minutes. Reagent 4 (NaOH and 5% sodium hypochlorite) was added, incubated for 1 hour, and read in a spectrophotometer at 660 nm. The calculation is based on substrate degradation.

### Pigment content

Pigment content was analyzed on the first leaf above the ear when the plants reached the phenological reproductive stage – R1.


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To determine chlorophylls, a, b, and carotenoids, the middle third of the leaf was collected in four plants/replications. Subsequently, 0.1 g of leaf tissue was fragmented into pieces of approximately 3 mm and immersed in 20 mL of 80% acetone (v/v) for 24 hours in an environment protected from light. After this period, the parameters were determined according to Linchtenthanler and Buschmann (2001).

**Biometric aspects and leaf area**

When maize plants reached the phenological reproductive stage R1, their height was measured with a measuring tape. The total leaf area (LA) was measured using a leaf area reader (LI-3100C, Licor, Nebraska, USA), at stage R1. The shoots were placed in paper bags and submitted to drying in an oven with forced air circulation at 65ºC for 72 hours to obtain the dry matter weight.

**Data analysis**

For the statistical analysis of the results, analysis of variance (ANOVA) and the Scott Knott test were used, at 0.05% significance ($p \leq 0.05$), in the Sisvar software, version 5.6 (Universidade Federal de Lavras, Lavras, Brazil).

## Results

**Root morphological attributes**

Inoculation of maize plants by *A. brasilense* with Az1 and Az2 significantly improved the root morphological attributes in soils with low and high N. In root morphology, root length, and surface area were higher in the maize of treatments N120 and Az2+N120 ($p \leq 0.05$) (Figure 1ab). In terms of mean diameter and root volume, plants from treatment N120 were superior when compared to the diameter of plants cultivated in the other treatments ($p \leq 0.05$) (Figure 1cd). Total dry matter (TDM) was higher in maize plants inoculated in Az1+N20 and Az2+N20 compared to control N20 ($p \leq 0.05$) (Figure 1e). On the other hand, there was no significant difference between treatments at high N in the soil (N120, Az1+N120, and Az2+N120) for TDM ($p \leq 0.05$) (Figure 1e).

Specific root length (SRL) was higher in maize plants in treatments with Az1+N20 and Az2+N20 inoculation compared to non-inoculated plants at 20 kg $ha^{-1}$ N ($p \leq 0.05$) (Figure 1f). At high N (120 kg $ha^{-1}$), plants from treatment Az2+N120 showed a higher SRL ($p \leq 0.05$) (Figure 1f). In root morphology, it was possible to observe greater root volume and specific root length (SRL) in maize plants inoculated with *A. brasilense* at 20 kg $ha^{-1}$ N. On average, there was an increase of 30% with Az1 in root volume with Az1 and Az2 28% in SRL, and 14% in total dry matter compared to the non-inoculated plants in the low N in the soil (20 kg $ha^{-1}$). The same pattern was observed at 120 kg $ha^{-1}$ N for SRL in inoculated maize plants.

In diameter classes, roots classified as very fine had greater root length (Figure 2), while maize plants had thicker diameters greater than 0.2 mm (Figure 2) in surface area and root volume. Moreover, for excellent roots, maize showed greater length, surface area, and root volume in the plants


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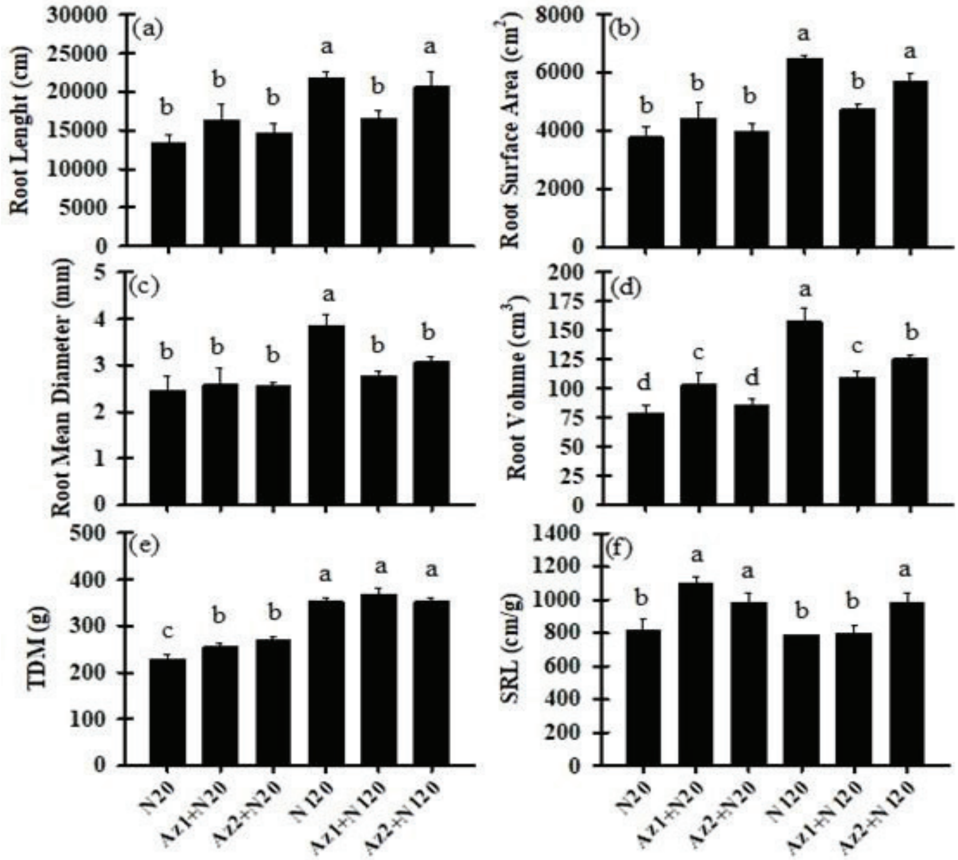


**Figure 1**. Length (a); surface area (b); mean diameter (c); root volume (d); total dry matter (TDM) (e); specific root length (SRL) (f) of maize plants. Means followed by the same letter do not differ by the Scott Knott test at 5% probability (p≤0.05). Each value indicates the mean of the treatment ± SE.

of treatments N120 and Az2+N120 (p≤0.05) (Figure 2). In fine roots (>0.05<0.2 mm), it was possible to observe the action of inoculant Az1+N20 with the increase in length, surface area, and root volume of maize plants when compared to the other treatments at low N (20 kg ha$^{-1}$) (Figure 2). Root length in thick roots (> 0.2 mm) was higher in plants from treatments with high N in the soil (120 kg ha$^{-1}$) (p≤0.05) (Figure 2a). The surface area was greater with Az1+N20 inoculation in the soil with 20 kg ha$^{-1}$ N in the > 0.2 mm diameter class (Figure 2b). Treatments N120 and Az2+N120 provided an increase in root volume in the same classification in soils with 120 kg ha$^{-1}$ N (p≤0.05) (Figure 2bc).

**Soil nitrate reductase and arginase**

Nitrate reductase activity (NRA) increased with inoculation by *A. brasilense* (Az1 and Az2) at low N in the soil (N20) when compared to the


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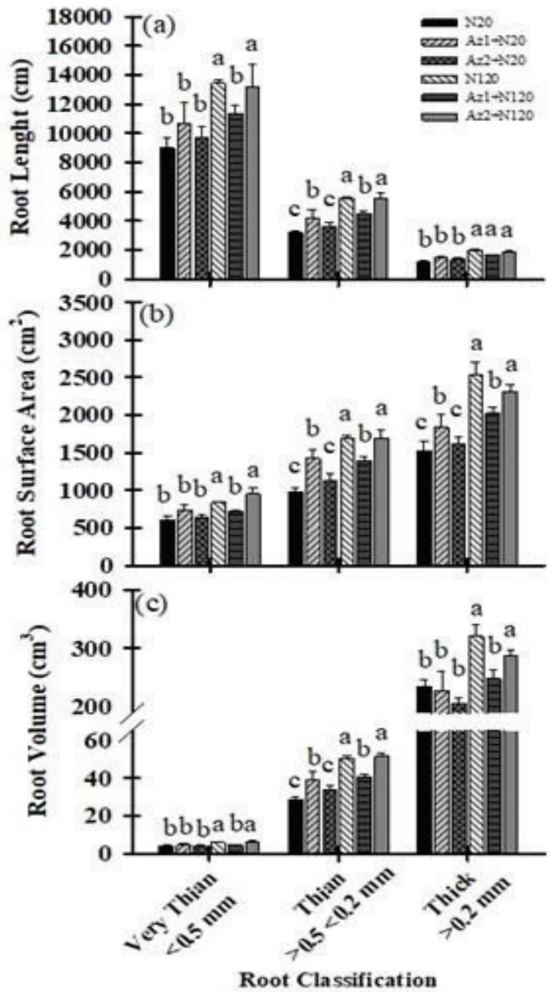


**Figure 2.** (a) Length, (b) surface area, and (c) root volume of maize plants organized in diameter classes. Means followed by the same letter do not differ by the Scott Knott test at 5% probability (p≤0.05). Each value indicates the mean of the treatment ± SE.

treatment without inoculation at the same N dose (Figure 3a). The same pattern was observed in the high N in the soil (120 kg $ha^{-1}$) with inoculation, reflecting higher NRA activity in maize plants (p≤0.05) (Figure 3a). Arginase activity was higher in the soil inoculated by *A. brasilense* in treatments Az1+N20 and Az2+N20 than in the soils of the other treatments (p≤0.05) (Figure 3b).

**Physiological and biometric aspects**

The contents of chlorophyll a (Chl *a*), total chlorophyll (Total Chl), and carotenoids showed the same pattern, being higher in the leaves of maize plants kept in treatment N120, Az1+N120, and Az2+N120 (p≤0.05) (Figure 4acd). However, it is observed that inoculation by *A. brasilense* increased Chl *a* and Toral Chl of maize plants


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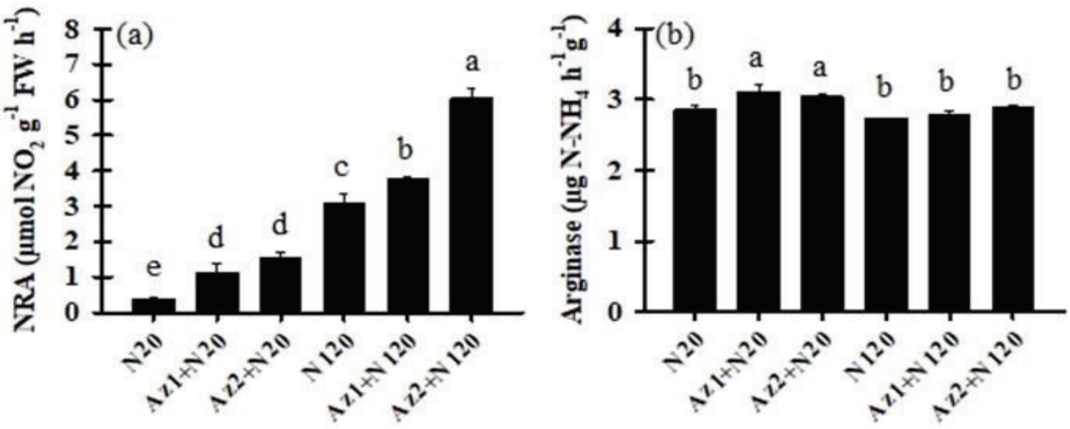


**Figure 3.** Nitrate reductase activity (NRA) (a) and arginase activity (b) of maize plants in the soil. Means followed by the same letter do not differ by the Scott Knott test at 5% probability (p≤0.05). Each value indicates the mean of the treatment ± SE.

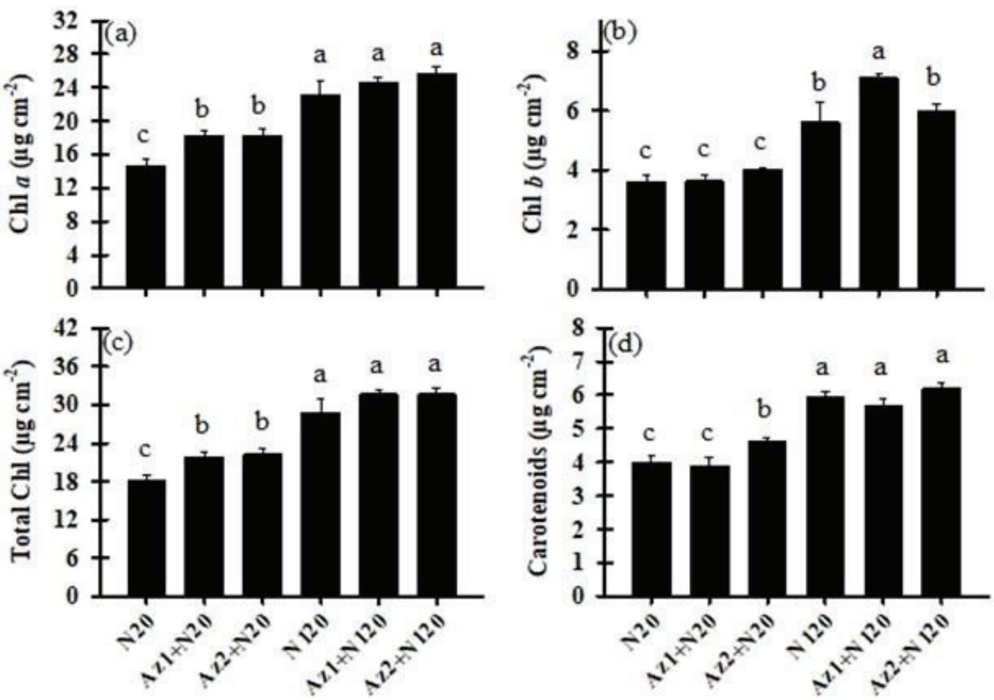


**Figure 4.** Content of chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and carotenoids (d) of maize plants. Means followed by the same letter for each treatment do not differ by the Scott Knott test at 5% probability (p≤0.05). Each value indicates the mean of the treatment ± SE.


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at low N when compared to non-inoculated plants (Figure 4ac). For the content of chlorophyll b (Chl *b*), the plants from treatment Az1+N120 showed a more significant increase in Chl *b* compared to the plants from the other treatments (p≤0.05) (Figure 4b).

The height of maize plants was more significant in the treatment using *A. brasilense* Az2+N20 than in the treatments at the same N dose (N20) (Figure 5). At high N in the soil (120 kg ha$^{-1}$), inoculants Az1+N120 and Az2+N120 increased the height of maize plants (p≤0.05) (Figure 5a). For leaf area (LA), all inoculants (Az1 and Az2) increased the LA of maize plants about the control at low N (20 kg ha$^{-1}$) (Figure 5b). The LA of plants at high N (N120) was higher with *A. brasilense* Az1+N120 (p≤0.05) (Figure 5b).

**Discussion**

The maize crop is highly demanding in N, possibly explaining the increase in morphological variables with the increase in soil N. In addition, N is an essential element found in most macromolecules and in many secondary and signal compounds, including proteins, nucleic acids, cell wall components, hormones, and vitamins (Krapp, 2015). Both *A. brasilense* strains, through their multiple mechanisms of action (N fixation and phytohormones) (Fukami et al.,2018; Cassán et al., 2020), provided this increase in maize TDM.

Increases in these root variables may indicate that the soil profile can be exploited further with inoculation. An increase in SRL is reflected in this greater exploration and acquisition of water and nutrients from the soil per unit of carbon invested (Bouma et al., 2001), showing a positive effect with Az1 and Az2.

Very thin and thin roots are responsible for the absorption of water and nutrients, and the growth of the root system may be evident, as well as the increase in height and LA (Figure 5). The action of *A.*

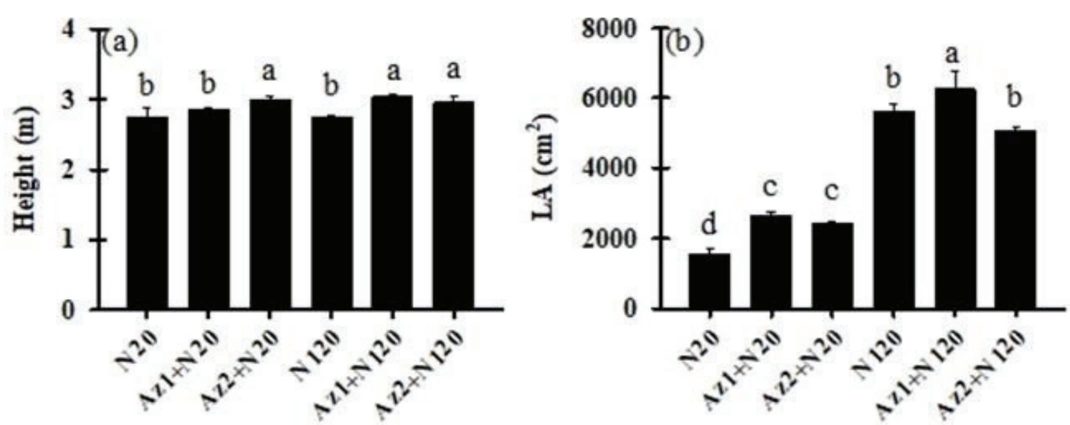


**Figure 5.** Height (a) and leaf area (b) of maize plants. Means followed by the same letter for each treatment do not differ by the Scott Knott test at 5% probability (p≤0.05). Each value indicates the mean of the treatment ± SE.


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*brasilense* on the thin roots (>0.5<0.2 mm) at 20 kg $ha^{-1}$ N indicates that inoculation favored maize plants to absorb more water and nutrients. This performance is plausible once *A. brasilense* strains CMS 07, CMS 1626, and CMS 11 (comprising inoculants Az1 and Az2) can produce indoleacetic acid (IAA) and nitrogen fixation, according to the results found by Ribeiro et al. (2022). In many circumstances, the production of phytohormones is the primary mechanism responsible for the change in root architecture and, consequently, for plant growth. Thus, the application of these inoculants (Az1 and Az2) to plants can favor root growth and development, which is reflected in greater exploration of the soil volume for more excellent capture of water and nutrients (Ullah et al., 2019) as a result, plants are hydrated and nourished.

An increase in NRA activity may indicate greater N availability ($NO_{3^-}$) for the maize crop, which is highly dependent on nitrogen. Although $NO_3^-$ was not evaluated in this study, when analyzing pigment content, height of the maize plants, LA, root morphological attributes, and total dry matter, an increase was observed in all these variables with inoculation by *A. brasilense*, indicating growth and biomass accumulation. The possible increase in $NO_3$- by Az1 and Az2 may have played a synergistic role in improving soil exploration by maize plants since $NO_3$- is a modulator of root architecture (Forde, 2014). Furthermore, nitrogen acts on several molecules with an essential role in plant growth and establishment (amino acids, proteins, nucleic acids, chlorophylls, coenzymes, phytohormones) (Ahamad et al., 2014), responsible for plant growth and development.

It is worth noting that the action of arginase is directly related to the conversion of nitrogenous compounds into ammonium for plants, also favoring growth and biomass accumulation. At the same time, the enzymatic activities of the soil can act as a bioindicator of changes in soil use and management, showing the positive effect of inoculation when compared to non-inoculated plants, both at low and high N (Marques et al., 2023). Thus, it is likely that Az1 and Az2 acted to improve nitrogen availability for maize plants, especially when observing pigment content.

The increase in chlorophylls may reflect greater photosynthesis and, consequently, more excellent carbon fixation by plants and the production of photoassimilates essential for increasing grain and dry biomass yield. Furthermore, the increase in chlorophyll b may mean an increase in the light range used in photosynthesis, allowing for higher photosynthetic rates since, when absorbing light, Chl *b* transfers energy to chlorophyll a.

This result may be associated with the greater capacity of *A. brasilense* for root absorption (Figures 1 and 2), BNF, and production of phytohormones. Furthermore, it is possible to correlate this result to the inoculation at low N (20 kg $ha^{-1}$) in the source-sink ratio of the plant, with the more excellent activity of enzymes linked to carbohydrate metabolism with the increase in total dry matter (TDM). This situation probably resulted in a stimulus for a more remarkable synthesis of chlorophyll (Figure 4), which is one of the main components of the photosynthetic apparatus.

Therefore, plants with greater heights and LA are expected to intercept more solar radiation, favoring higher photosynthetic rates due to the photosynthetic metabolism of Poaceae (C4 plants) and increased gas exchange (Marques et al., 2021).

In summary, there was likely a cascade effect with inoculation with Az1 and Az2 in the two nitrogen conditions. The most significant root system per biomass unit (SRL) provided maize plants with greater soil exploration and increased nutrient absorption due to phytohormones and FBN from *A. brasilense*. In addition, NRA and arginase activity were increased, thus making more N available for maize. Well-nourished plants can accumulate more biomass and present more chlorophylls, thus showing greater height and LA (Figures 4 and 5).

However, although inoculation by *A. brasilense* improved maize plants greatly, more inoculation was needed to supply this crop's total nitrogen demand. The reduction in N application in the soil was 100% of the recommended dose for an excellent maize grain yield (Coelho, 2006). Thus, physiological and morphological aspects were reduced compared to plants with high N availability (120 kg $ha^{-1}$). Due to the structural role N plays, its decrease in plants interferes with plant response and biomass accumulation.

In a recent study in Brazil, Hungria et al. (2022) indicated the possibility of replacing 25% of the nitrogen fertilizer by inoculation with *A. brasilense*. The same authors stated that phytohormones are produced by inoculation in this reduction in the percentage of fertilizers. As a result, root architecture is increased, thus reflecting the greater efficiency of using nitrogen fertilizers. We have to consider that this percentage (25% reduction in N fertilizer) represents, for our country, a significant economy for maize crop production.

Thus, the use of *A. brasilense* in maize production systems is viable. Inoculation has low costs and provides the farmer with improvements in the agroecosystem and the profitability of this crop, reducing environmental impacts.

## Conclusion

Inoculation by *A. brasilense* Az1 and Az2 improves the physiology, root and shoot morphology, and biomass but does not supply the total nitrogen demand for this crop. Thus, inoculation by *A. brasilense,* in this study condition, does not reduce nitrogen fertilization in maize.

Inoculants Az1 and Az2 increase nitrate reductase activity in maize in soils with low and high nitrogen availability. *A. brasilense* inoculation optimizes the morphophysiological and biochemical responses of maize in soils with high and low nitrogen.

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**References**

AHMAD, R.; WARAICH, E. A.; SHAMIM, A.; AZIZ, A. T. Does nitrogen fertilization enhance drought tolerance in sunflower? A review. **Journal of Plant Nutrition**, v. 37, n. 6, p. 942-963, 2014. DOI: https://doi.org/10.1080/01904167.2013.868480

ALEF, K.; KEINER, D. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. **Soil Biology and Biochemistry**, v. 18, n. 2, p. 233-235, 1986. DOI: https://doi.org/10.1016/0038-0717(86)90033-7

BARBOSA, J. Z.; ROBERTO, L. A.; HUNGRIA, M.; CORRÊA, R. S.; MAGRI, E.; CORREIA, T. D. Meta-analysis of maize responses to *Azospirillum brasilense* inoculation in Brazil: Benefits and lessons to improve inoculation efficiency. **Applied Soil Ecology**, v. 170, p. 104276, 2022. DOI: https://doi.org/10.1016/j.apsoil.2021.104276

BIGOLIN, T.; TALAMINI, E. Impacts of Climate Change Scenarios on the Corn and Soybean Double-Cropping System in Brazil. **Climate**, v. 12, n. 3, p. 42, 2024. DOI: https://doi.org/10.3390/cli12030042

BOUMA, T. J.; NIELSEN, K. L.; HAL, J. V.; KOUTSTAAL, B. Root system topology and diameter distribution of species from habitats differing in inundation frequency. **Journal Ecology**, v. 15, n. 3, p. 360-369, 2001. DOI: https://doi.org/10.1046/j.1365-2435.2001.00523.x

BREDA, F. A. F.; SILVA, T. R. F.; SANTOS, S. G.; ALVES, G. C.; REIS, V. M. Modulation of nitrogen metabolism of maize plants inoculated with *Azospirillum brasilense* and *Herbaspirillum seropedicae*. **Archives of Microbiology**, v. 201, p. 547-558, 2019. DOI: https://doi.org/10.1007/s00203-018-1594-z

CASSÁN, F.; CONIGLIO, A.; LÓPEZ, G.; MOLINA, R.; NIEVAS, S.; CARLAN, C. L. N.; DONADIO, F.; TORRES, D.; ROSAS, S.; PEDROSA, F. O.; SOUZA, E.; ZORITA, M. D.; BASHAN, L.; MORA, V. Everything you must know about *Azospirillum* and its impact on agriculture and beyond. **Biology and Fertility of Soils**, v. 56, p. 461-479, 2020. DOI: https://doi.org/10.1007/s00374-020-01463-y

CARVALHO, T. L. G.; BALSEMÃO-PIRES, E.; SARAIVA, R. M.; FERREIRA, P. C. G.; HEMERLY, A. S. Nitrogen signalling in plant associative and endophytic diazotrophic bacteria. **Journal of Experimental Botany**, v. 65, n. 19, p. 5631-5642, 2014. DOI: https://doi.org/10.1093/jxb/eru319

CARVALHO, I.; SILVA, J. A. G.; LORO, M. V.; SARTURI, M. V. R.; HUTRA, D. J.; PORT, E. D.; LAUTENCHLEGER, F. Canonical interrelationships in morphological characters, yield and nutritional components of corn. **Agronomy Science and Biotechnology**, v. 8, p. 1-17, 2022. DOI: https://orcid.org/0000-0001-7947-4900

CONAB. Companhia Nacional de Abastecimento. **Acompanhamento da Safra Brasileira de Grãos.** Safra 2023/2024. Brasilia, DF: [s.n.], 2022. Disponível em: <http://www.conab.gov.br>.

FORDE, B. G. Nitrogen signalling pathways shaping root system architecture: an update. **Current Opinion in Plant Biology**, v. 21, p. 30-36, 2014. DOI: https://doi.org/10.1016/j.pbi.2014.06.004

FUKAMI, J.; CEREZINI, P.; HUNGRIA, M. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. **AMB Express**, v. 1, p. 73, 2018. DOI: https://doi.org/10.1186/s13568-018-0608-1

HUNGRIA, M.; BARBOSA, J. Z.; RONDINA, A. B. L.; NOGUEIRA, M. A. Improving maize sustainability with partial replacement of N fertilizers by inoculation with *Azospirillum brasilense*. **Agronomy Journal**, v. 114, n. 5, p. 2969-2980, 2022. DOI: https://doi.org/10.1002/agj2.21150

LICHTENTHALER, H. K.; BUSCHMANN, C. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *In:* WROLSTAD, R. E. (Ed.). **Current protocols in food analytical chemist GY**. New York: John Wiley and Sons, F4.3.1–F4.3.8, 2001.

KHAN, N.; BANO, A.; SHAHID, M. A.; NASIM, W.; ALI BABAR, M. D. Interaction between PGPR and PGR for water conservation and plant growth attributes under drought condition. **Biologia**, v. 73, p. 1083-1098, 2018. DOI: https://doi.org/10.2478/s11756-018-0127-1

KRAPP, A. Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. **Current Opinion in Plant Biology**, v. 25, p. 115-122, 2015. DOI: https://doi.org/10.1016/j.pbi.2015.05.010

MARQUES, D. M.; SILVA, A. B.; MANTOVANI, J. R.; MAGALHÃES, P. C.; SOUZA, T. C. Root morphology and leaf gas exchange in *Peltophorum dubium* (Spreng.) Taub. (Caesalpinioideae) exposed to copper-induced toxicity. **South African Journal of Botany**, v. 121, p. 186-192, 2019. DOI: https://doi.org/10.1016/j.sajb.2018.11.007

MARQUES, D. M.; MAGALHÃES, P. C. MARRIEL, I. E.; GOMES JUNIOR, C. C.; SILVA, A. B.; SOUZA, T. C. Gas Exchange, Root Morphology and Nutrients in Maize Plants Inoculated with *Azospirillum brasilense* Cultivated Under Two Water Conditions. **Brazilian Archives of Biology and Technology**, v. 64, p. e21190580, 2021. DOI: https://doi.org/10.1590/1678-4324-2021190580

MARQUES, D. M.; MAGALHÃES, P. C.; MARRIEL, I. E.; GOMES JÚNIOR, C. C.; SILVA, A. B.; SILVA, N. T. A.; SOUZA, T. C. *Azospirillum brasilense* Reduces the Effects of Water Stress and Increases Maize Yield in Irrigated Areas with High Soil Nitrogen Doses. **Journal of Plant Growth Regulation**, v. 42, n. 7, p. 4263-4274, 2023. DOI: https://doi.org/10.1007/s00344-022-10889-7

NEUMANN, M.; SANDINI, I. E.; LUSTOSA, S. B. C.; OST, P. R.; ROMANO, M. A.; FALBO, M. K.; PANSERA, E. R. Rendimentos e componentes de produção da planta de milho (*Zea mays* L.) para silagem, em função de níveis de adubação nitrogenada em cobertura. **Revista Brasileira de Milho e Sorgo**, v. 4, n. 03, 2005. DOI: https://doi.org/10.18512/1980-6477/rbms.v4n03p%25p

PETEAN, C. C.; TEIXEIRA FILHO, M.; GALINDO, F. S.; BUZETTI, S.; MALMONGE, J. A.; MALMONGE, L. F. Polímeros orgânicos com ureia dissolvida e doses de nitrogênio no milho. **Revista de Ciências Agrárias - Amazonian Journal of Agricultural and Environmental Sciences**, v. 62, p. 1-9, 2019. DOI: http://dx.doi.org/10.22491/rca.2019.2761

RIBEIRO, A. C. **Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5ª Aproximação**. Ed 1 Comissão de Fertilidade do Solo do Estado de Minas Gerais. 1999, 359 p.

RIBEIRO, V. P.; GOMES, E. A.; SOUSA, S. M.; PAULA LANA, U. G.; COELHO, A. M.; MARRIEL, I. E.; OLIVEIRA-PAIVA, C. A. Coinoculation with tropical strains of *Azospirillum* and *Bacillus* is more efficient than single inoculation for improving plant growth and nutrient uptake in maize. **Archives of Microbiology**, v. 204, n. 2, p. 143, 2022. DOI: https://doi.org/10.1007/s00203-022-02759-3

SANTOS, M. S.; NOGUEIRA, M. A.; HUNGRIA, M. Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: Lessons that farmers are receptive to adopt new microbial inoculants. **Revista Brasileira de Ciência do Solo**, v. 45, p. e0200128, 2021. DOI: https://doi.org/10.36783/18069657rbcs20200128

SU, W.; HUBER, S. C.; CRAWFORD, N. M. Identification in vitro of a post-translational regulatory site in the hinge 1 region of *Arabidopsis* nitrate reductase. **The Plant Cell**, 8, n. 3, p. 519-527, 1996. DOI: https://doi.org/10.1105/tpc.8.3.519

ULLAH, A.; AKBAR, A.; LUO, Q.; KHAN, A. H.; MANGHWAR, H.; SHABAN, M.; YANG, X. Microbiome diversity in cotton rhizosphere under normal and drought conditions. **Microbial Ecology**, v. 77, p. 429-439, 2019. DOI: https://doi.org/10.1007/s00248-018-1260-7

VENDRUSCOLO, E. P.; DE LIMA, S. F. The *Azospirillum* genus and the cultivation of vegetables. A review. **Biotechnology, Agronomy, Society and Environment**, v. 25, n. 4, p. 236-246, 2021. DOI: https://doi.org/10.25518/1780-4507.19175