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### ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

## SOIL CORRECTION AND Azospirillum brasilense: STRATEGIES IN THE CULTIVATION OF MAIZE GENOTYPES IN SEMI-ARID REGIONS

# Corrección de suelos y *Azospirillum brasilense*: estrategias en el cultivo de genotipos de maíz en regiones semiáridas

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#### **ABSTRACT**

The replacement of Caatinga by extensive agriculture has led to soil degradation in maize (*Zea mays* L.) growing areas. The objective of this study was to evaluate the effects of soil correction in association with *Azospirillum brasiliense* to replace nitrogen (N) fertilizer on the vegetative growth of two maize genotypes cultivated on degraded soil. The soil was collected in an area undergoing desertification in Irauçuba, Ceará state, Brazil. A 2x2x5 factorial experiment was carried out in blocks: two soil fertility levels (corrected and uncorrected soil in terms of fertility), two maize genotypes (BRS Caimbé and BRS Gorutuba), and five nitrogen sources (control, inoculation with *A. brasilense*, inoculation with *A. brasilense* + 50 kg ha<sup>-1</sup> of N, 50 kg ha<sup>-1</sup> of N, and 100 kg ha<sup>-1</sup> of N). BRS Gorutuba genotype showed greater adaptability to the growing conditions evaluated. However, macronutrient concentration did not affect maize dry matter yield. The response of maize inoculated with *A. brasilense* suggests the inoculation efficacy, supported by the N accumulation and the effect on plant growth. The dry matter yield in *A. brasilense* inoculated plants was equivalent to using N fertilizer. Therefore, the inoculation of BRS Gorutuba maize grown on degraded soil with *A. brasilense* is a promising alternative for farmers in semi-arid regions.

Keywords: inoculation of non-legumes, nutrients accumulated, plant growth promoting bacteria, Zea mays L.

#### **RESUMEN**

La sustitución de la Caatinga por la agricultura extensiva ha provocado la degradación del suelo en zonas donde se cultiva maíz (*Zea mays* L.). El objetivo de este estudio fue evaluar los efectos de la corrección del suelo en asociación con *Azospirillum brasiliense* para reemplazar el fertilizante nitrogenado (N) en el crecimiento vegetativo de dos genotipos de maíz cultivados en suelo degradado. El suelo se recolectó en un área en proceso de desertificación en Irauçuba, Ceará, Brasil. Se realizó un experimento factorial 2x2x5 en bloques: dos niveles de fertilidad del suelo (suelo corregido y no corregido en términos de fertilidad), dos genotipos de maíz (BRS



Caimbé y BRS Gorutuba) y cinco fuentes de nitrógeno (testigo, inoculación con *A. brasilense*, inoculación con *A. brasilense* + 50 kg ha<sup>-1</sup> de N, 50 kg ha<sup>-1</sup> de N y 100 kg ha<sup>-1</sup> de N). El genotipo BRS Gorutuba mostró mayor adaptabilidad a las condiciones de crecimiento evaluadas. Sin embargo, la concentración de macronutrientes no afectó el rendimiento de materia seca del maíz. La respuesta del maíz inoculado con *A. brasiliense* sugiere la eficacia de la inoculación, que se soporta en la acumulación de N y el efecto sobre el crecimiento de la planta. El rendimiento de materia seca de las plantas inoculadas fue equivalente al uso de fertilizante nitrogenado. Por lo tanto, la inoculación de maíz BRS Gorutuba cultivado en suelo degradado con *A. brasilense* es una alternativa promisoria para los agricultores en regiones semiáridas.

Palabras clave: inoculación de no leguminosas, nutrientes acumulados, bacterias promotoras del crecimiento vegetal, Zea mays L.

#### INTRODUCTION

Maize (*Zea mays* L. 1895) plays a significant role in the food security in semi-arid regions of Brazil, being a staple food for local populations and fodder for animal husbandry (Martins et al., 2019). In the Northeast region, where the semi-arid region predominates, the maize planted area is 3177 thousand ha. However, the average yield is 3370 kg ha<sup>-1</sup>, which is 36 % lower than the Brazilian national average (Companhia Nacional de Abastecimento [CONAB], 2022). Low grain productivity in semi-arid regions is explained by the difficulty in maintaining or increasing yields under such conditions, which is further worsened by the susceptibility of the land to desertification (Aquino et al., 2018).

Many stressors present in semi-arid regions affect natural and human systems. The stressors are mostly climate variations, intensified by land use changes resulting from the replacement of the Caatinga biome by croplands and extensive livestock (Marengo et al., 2018; Mutti et al., 2020). Over 10 % of the region underwent intense environmental degradation processes due to human interference in this biome (Mariano et al., 2018), leading to a high risk of desertification (Ferreira et al., 2018). Of the principal causes of desertification, overgrazing stands out because the intensive conversion of natural vegetation to pasture has led to a decrease in lands' productive potential due to soil compaction, alteration of plant species composition, increased water runoff, and decreased soil fertility (Schulz et al., 2016; Gaitán et al., 2018; Ferreira et al., 2018).

Due to the vulnerability of agroecosystems in semi-arid regions, alternatives have been sought to enhance the productivity of agricultural systems despite the low technological resources of smallholder farmers associated with degraded soils with low concentrations of essential elements and high concentrations of sodium (Zhou et al., 2019; Miranda et al., 2020; Nascimento et al., 2021a). Using soil amendments and fertilizers is a proper way of improving soil fertility and has been incorporated into maize production systems in semi-arid environments (Lamptey et al., 2019; Roohi et al., 2020). However, there is limited information about using such practices on degraded soils. Furthermore, because maize demands large amounts of nitrogen (N) to reach high productivity (DeBruin et al., 2017), this element, whose availability in degraded soils in the semi-arid region of Brazil is, on average, 1.04 g kg-1 (Sousa et al., 2012), requires greater emphasis.

Therefore, to explore the productive potential of maize, farmers are expected to use high amounts of N fertilizers, which often raises production costs (Galindo et al., 2019). Ponte-Filho et al. (2023) highlighted that maize requires 112 kg ha<sup>-1</sup> of N in semi-arid regions. Yet, as N fertilizer is susceptible to several transformations in soil, it poses a hazard to the environment (Skonieski et al., 2019). A promising alternative to improve N use efficiency by non-legume species is using inoculants containing diazotrophic bacteria, such as Azospirillum spp., capable of performing biological N<sub>2</sub> fixation (BNF) and synthesizing plant growth-promoting hormones (Hungria et al., 2016; Aguirre et al., 2020; Galindo et al., 2020). However, N supplementation using inoculants still needs more scientific investigations on maize cultivation under abiotic stress conditions resulting from soil degradation.

Plant and soil conditions control the association between microbes and plant growth promotion (Carvalho et al., 2016). The interaction of microorganisms with maize grown on degraded soils has not yet been explored, especially considering specific plant genetic pools and rhizosphere that may influence the response of the inoculated strain (Vidotti et al., 2019; Zeffa et al., 2019). Therefore, this research proposes to study the effects of soil correction (lime and fertilizer application) in association with *Azospirillum brasiliense* in total or partial replacement of conventional N fertilizer application to two maize genotypes cultivated on degraded soil.

#### MATERIALS AND METHODS

A pot trial was carried out in an open area, in full sun, in the second half of 2015 at Embrapa Goats and Sheep, located in Sobral, state of Ceará, Brazil (03°41' S and 40°20' W). According to the Köppen classification, the region's climate is BShw type, with a rainy season between January and June. The average annual temperature is 28 °C, and the average yearly precipitation is 759 mm.

Treatments were laid out in four randomized blocks, arranged in 2x2x5 factorial: two soil fertility levels (with and without soil correction); two maize genotypes (BRS Caimbé and BRS Gorutuba); and five N sources: (i) control treatment (no N fertilization or *A. brasilense* inoculation); (ii) inoculation with *A. brasilense*; (iii) inoculation with *A. brasilense* + 50 kg ha<sup>-1</sup> of N; (iv) application of 50 kg ha<sup>-1</sup> of N; and (v) application of 100 kg ha<sup>-1</sup> of N. In each block, a potted maize

plant was assigned to each factorial treatment. Pots were filled with 10 dm³ of soil collected from 0 to 0.20 m deep layer on an area undergoing desertification in the municipality of Irauçuba, Ceará state, Brazil (3°47' S and 39°47' W). The soil, classified as Orthic Natric Planosol (Santos et al., 2018), has been covered with native pasture for feeding goats and sheep. Soil fertility and particle size analyses of the soil samples from the area revealed the following characteristics (Teixeira et al., 2017): pH (H<sub>2</sub>O): 5.4; organic matter (OM): 5 g dm³; phosphorus (P): 8 mg dm³; potassium (K): 47 mg dm³; calcium (Ca): 16 mmol<sub>c</sub> dm³; magnesium (Mg): 7 mmol<sub>c</sub> dm³; potential acidity (H+Al): 22 mmol<sub>c</sub> dm³; cation exchange capacity (CEC): 48.2 mmol<sub>c</sub> dm³; base saturation (BS): 54 %; percent sodium saturation (PST): 4.2 %; clay: 72 g kg⁻¹; silt: 48 g kg⁻¹; sand: 880 g kg⁻¹.

Soil correction consisted of raising base saturation to 75 % by liming with calcium carbonate at a rate of 1 Mg ha<sup>-1</sup>. In addition, 80 kg ha<sup>-1</sup> of single superphosphate (18 %  $P_2O_5$ ), 40 kg ha<sup>-1</sup> of potassium chloride (58 %  $K_2O$ ), and 2 kg ha<sup>-1</sup> of Zn (FTE BR-12, 9 % Zn) were applied (Fernandes, 1993). Phosphorus was the only nutrient equally applied to each treatment, even the control treatment, because of the low P values (8 mg dm<sup>-3</sup>) measured in the initial soil analysis.

Nitrogen fertilizer was applied at planting and 35 days after germination as topdressing. As a result, the treatments inoculation with A. brasilense + 50 kg ha<sup>-1</sup> of N and 50 kg ha<sup>-1</sup> of N received 25 kg ha<sup>-1</sup> of N at planting, and 25 kg ha<sup>-1</sup> of N as topdressing. The 100 kg ha<sup>-1</sup> of N treatment received 50 kg ha<sup>-1</sup> of N at planting and 50 kg ha<sup>-1</sup> of N as topdressing. The mineral source of N was urea (45 % N). Inoculation with a commercial inoculant of the strains AbV5 and AbV6 of A. brasilense (Azototal®, TotalBio, Curitiba, Paraná, Brazil) was performed one hour before sowing, according to the manufacturer's recommendations. Seven seeds were sown per pot, and thinning was performed five days after germination to keep only one plant per pot. The pots were placed on a 1-m-tall bench. Irrigation scheduling was based on crop evapotranspiration (ETc). Reference evapotranspiration (ETo) was obtained by an INMET class 'A' pan (National Institute of Meteorology) located at Embrapa Sheep and Goats. Irrigation runtime was calculated according to Medeiros et al. (2013) using crop coefficients (kc) recommended by Santos et al. (2014) for semi-arid conditions.

At 73 days after germination, the following maize plant variables were measured: plant height (PH), from collar to tassel, using a measuring tape; stem diameter (SD), measured at 8 cm from the base of the plant with a digital caliper; the number of leaves (NL), by direct counting; leaf area (LA), calculated using a leaf area meter (LI3100–LICOR®); relative chlorophyll content (RCC), measured using a portable chlorophyll meter (Minolta SPAD 502®, Japan) on the third fully expanded leaf; and dry mass of the aerial part (DMAP), mass determined using a scale after oven-drying plant shoots at 65°C for 72 h.

Dry matter was used to assess macronutrient contents (Bataglia et al., 1983). N was determined by the Kjeldahl method. In this method, the sample is digested with concentrated sulfuric acid, and then distillation of the digested sample takes place. After distillation, the indicator boric acid sample is titrated with standardized hydrochloric acid. To determine the concentration of P, K, Ca, Mg, and S, the samples were initially digested in nitric and perchloric solution. After digestion, P and S were determined after spectrophotometer reading by colorimetry and turbidimetry, respectively. K, Ca, and Mg were quantified after reading in atomic absorption spectroscopy. The accumulation of macronutrients in the plant was calculated considering nutrient concentration and plant dry matter. In this study, the colonization of maize rhizosphere by strains of A. brasiliense was not directly evaluated. Efforts were focused only on recording the possible effects of A. brasiliense inoculation in maize grown on degraded soil under conditions of reduced supply of mineral N. Statistical analysis was performed by analysis of variance (F test) and, when significant, cluster analysis was performed (Scott-Knott, 5 % probability) using SISVAR statistical software (Ferreira, 2019).

#### **RESULTS**

The soil correction factor provided a significant increase in the height of maize plants; however, the stem diameter was reduced in plants grown on corrected soil. Overall, the Gorutuba maize genotype exhibited greater growth and biomass accumulation, including plant height, shoot dry mass, and relative chlorophyll content, except for the number of leaves, which was more responsive in the Caimbé genotype. As for N sources, the use of A. brasilense contributed to an increase in the relative chlorophyll content compared to the control treatment but was not sufficient to reach the RCC measured in the 100 kg ha<sup>-1</sup> of N treatment. Conversely, the leaf area in plants under the A. brasilense treatments was not statistically different from the LA in plants receiving mineral nitrogen at 100 kg of N ha<sup>-1</sup>. Likewise, the inoculation of A. brasilense increased the DMAP, similar to that observed when 100 kg ha<sup>-1</sup> of N was used (Table 1).

**Table 1.** Mean values of the biometric variables, dry matter and relative chlorophyll content measured in maize plants under the treatments soil correction, genotypes and nitrogen sources.

Treatments	PH	SD	· NL	LA	DMAP	RCC	
	(cm)	(cm)		(cm²)	(g)		
Soil Correction (SC)							
Corrected	72.15a¹	1.45b	10.1	1228.35	33.15	23.28	
Uncorrected	67.26b	1.58a	10.12	1276.26	33.13	22.94	
Significance	**	**	ns	ns	ns	ns	
Genotypes (G)							

	PH	SD	- NL	LA	DMAP		
Treatments	(cm)	(cm)		(cm <sup>2</sup> )	(g)	RCC	
BRS Caimbé	67.56b	1.52	10.76a	1292.98	31.31b	21.13b	
BRS Gorutuba	71.85a	1.51	9.45b	1211.62	34.97a	25.09a	
Significance	**	ns	**	ns	**	**	
Nitrogen Sources (NS)							
Control	64.65c	1.47	10.00	1067.19Ь	28.19Ь	16.68c	
Azospirillum	69.31c	1.47	10.50	1326.83a	32.30a	23.57Ь	
Azospirillum + 50 kg ha <sup>-1</sup> of N	77.04a	1.55	10.39	1371.66a	36.15a	24.74b	
50 kg ha <sup>-1</sup> of N	71.47b	1.52	9.68	1163.80Ь	33.44a	22.99Ь	
100 kg ha <sup>-1</sup> of N	66.04c	1.56	9.97	1332.03a	35.63a	27.55a	
Significance	**	ns	ns	**	**	**	
SC x G	**	ns	ns	ns	ns	**	
SC x NS	**	ns	ns	ns	ns	*	
G x NS	**	ns	ns	ns	ns	**	
SC x G x NS	ns	ns	ns	ns	ns	**	
CV (%)	9.7	14.4	11.3	18.9	14.7	16.0	

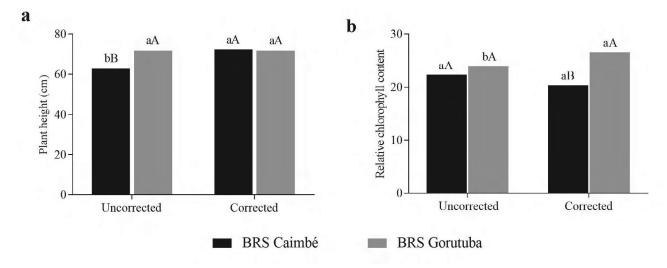
PH: plant height; SD: stem diameter; NL: number of leaves; LA: leaf area; DMAP: dry mass of the aerial part; RCC: relative chlorophyll content. CV: coefficient of variation. ns: not significant; \*: significant at 0.05 probability; \*\*: significant at 0.01 probability. ¹Means followed by the same letters within the same column do not differ according to the Scott-Knott test (*p*<0.05).

Plant height and relative chlorophyll content had an interaction response to treatments (Table 1). Regarding the interaction between genotype and soil correction, plants of the Caimbé genotype were taller when grown on corrected soil. In contrast, the Gorutuba maize genotype did not respond to soil correction. The opposite result was observed for the relative chlorophyll content (Fig. 1).

Under soil correction, plant height and relative chlorophyll content of maize plants increased by 25.82 and 17.52 %, respectively, when using 100 kg ha<sup>-1</sup> of N, compared to the absence of soil amendment and the control treatment (0 kg ha<sup>-1</sup> of N) (Fig. 2a-2b). However, the lack of soil correction allowed inoculated treatments to exhibit superior responses as to plant height, with emphasis on the *A. brasilense* + 50 kg ha<sup>-1</sup> of N treatment, which, with either the presence or absence of soil correction, led to taller maize plants (Fig. 2a). For the relative chlorophyll content of plants grown on uncorrected soil, any applied source resulted in higher relative chlorophyll contents than that of control plants (Fig. 2b).

As for the interaction between nitrogen sources and genotype, Gorutuba genotype had similar plant height responses to treatments with N fertilization and inoculation of microorganisms (Fig. 2c), but the application of *A. brasilense* + 50 kg ha<sup>-1</sup> of N led to increased plant height of Caimbé genotype. No significant difference was found between nitrogen fertilizer application and inoculation with *A. brasilense* for relative chlorophyll content in the Caimbé genotype. However, the Gorutuba genotype had an increased relative chlorophyll content with the application of 100 kg ha<sup>-1</sup> of N (Fig. 2d).

In uncorrected soil, there was more significant N, Mg, and S accumulation in maize plant tissues. BRS Gorotuba maize



**Figure.** 1. Mean values of plant height (a) and relative chlorophyll content (b) of maize plants as a function of the interaction between soil correction versus maize genotypes. Bars (means) with the same small letter (soil correction factor) and capital letter (maize genotypes) do not differ from each other by the Scott-Knott test (p<0.05)

genotype accumulated more N, P, and K than the Caimbé genotype. As for the N source, only the accumulation of N and S showed a significant difference. A. brasiliense + 50 kg ha<sup>-1</sup> of N and 50 kg ha<sup>-1</sup> of N resulted in a high N accumulation in plant tissues, on average 31.75, 23.60, and 6.61 % higher than that in control plants, plants inoculated with A. brasiliense, and plants fertilized with 100 kg ha<sup>-1</sup> of N, respectively. In the case of S, in addition to the treatments A. brasiliense + 50 kg ha<sup>-1</sup> of N and 50 kg ha<sup>-1</sup> of N, the treatment with 100 kg ha<sup>-1</sup> of N also resulted in a high S accumulation (Table 2).

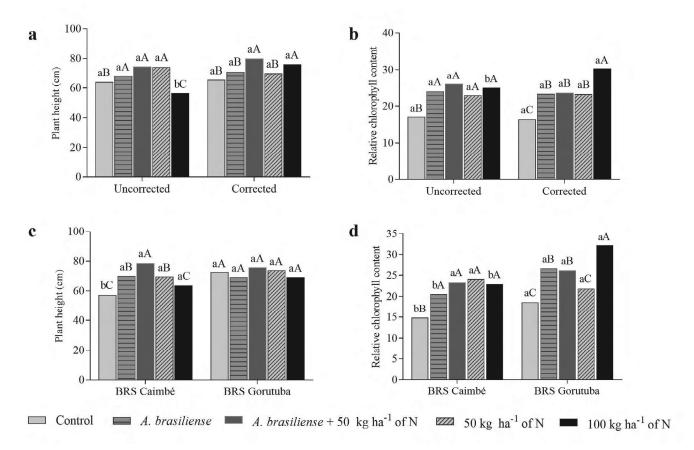
There was an interaction between maize genotypes and nitrogen sources (fertilization and inoculation) in the accumulation of N, Ca, Mg, and S (Table 2). The accumulation of N and S was high when using *Azospirillum* + 50 kg ha<sup>-1</sup> and 50 kg ha<sup>-1</sup> of N on uncorrected soil (Fig. 3a-3d). Plants fertilized with 100 kg ha<sup>-1</sup> of N accumulated 32.71 % more Ca than control plants on corrected soil (Fig. 3b). In uncorrected soil, similar to N and S accumulation, inoculation of *A. brasiliense* + 50 kg ha<sup>-1</sup> of N resulted in a 41.40 % increase in Mg accumulation (Fig. 3c).

When the soil is corrected, the accumulation of nutrients follows the descending order K>N>Ca>Mg>S>P. When the

soil is not corrected, N switches place with K, and the order is N>K>Ca>Mg>S>P. The nutrient accumulation was the same for both genotypes, with the descending order K>N>Ca>Mg>S>P. For the fertilizer sources, the decreasing order of nutrient accumulation was K>N>Ca>Mg>S>P for the control, inoculation with *A. brasilense* and *A. brasilense* + 50 kg ha<sup>-1</sup> of N. However, when soluble sources (50 and 100 kg ha<sup>-1</sup> of N) were exclusively used, the order of K with N was inverted: N>K>Ca>Mg>S>P.

#### DISCUSSION

In comparison to control plants, leaf area increased following a descending order as follows: *A. brasilense* + 50 kg ha<sup>-1</sup> (28.53 %) > 100 kg ha<sup>-1</sup> (24.81 %) > *A. brasilense* (24.32 %) > 50 kg ha<sup>-1</sup> (9.05 %); for dry matter yield, gains were in the following order: *A. brasilense* + 50 kg ha<sup>-1</sup> (28.23 %) > 100 kg ha<sup>-1</sup> (26.39 %) > 50 kg ha<sup>-1</sup> (18.62 %) > *A. brasilense* (14.57 %); and for relative chlorophyll content, the descending order in relation to the control were: 100 kg ha<sup>-1</sup> (65.16 %) > *A. brasilense* + 50 kg ha<sup>-1</sup> (48.32 %) > *A. brasilense* (41.31 %) > 50 kg ha<sup>-1</sup> (37.83 %).



**Figure.** 2. Mean values of plant height (a, c) and relative chlorophyll content (b, d) of maize plants as a function of the interaction between soil correction versus nitrogen sources (a, b) and genotypes versus nitrogen sources (c, d). Bars (meas) with the same small letter (soil correction factor) and capital letter (maize genotypes) do not differ from each other by the Scott-Knott test (p<0.05).

**Table 2.** Mean values of macronutrient accumulation measured in maize plants under the treatments soil correction, genotypes and nitrogen sources.

Treatments	N	Р	K	Ca	Mg	S		
	(mg plant <sup>-1</sup> )							
Soil Correction (SC)								
Corrected	218b¹	33	265	171	112Ь	51b		
Uncorrected	297a	37	298	170	135a	71a		
Significance	* *	ns	ns	ns	*	* *		
Genotypes (G)								
BRS Caimbé	227b	29b	257Ь	163	122	57		
BRS Gorutuba	288a	41a	306a	178	125	66		
Significance	**	* *	**	ns	ns	ns		
Nitrogen Sources (NS)								
Control	201Ь	36	268	157	111	49b		
Azospirillum	225b	37	284	171	129	55b		
Azospirillum+ 50 N kg ha <sup>-1</sup>	297a	30	307	166	125	71a		
50 kg N ha <sup>-1</sup>	292a	35	283	178	130	63a		
100 kg N ha <sup>-1</sup>	275b	28	265	179	123	69a		
Significance	* *	ns	ns	ns	ns	*		
SC x G	ns	ns	ns	ns	ns	ns		
SC x NS	*	ns	ns	*	**	*		
G x NS	ns	ns	ns	ns	ns	ns		
SC x G x NS	ns	ns	ns	ns	ns	ns		
CV (%)	29.2	31.1	27.5	32.8	30.5	37.5		

N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur. CV: coefficient of variation. ns: not significant; \*: significant at 0.05 probability; \*\*: significant at 0.01 probability. 

¹Means followed by the same letters within the same column do not differ according to the Scott-Knott test (p<0.05).

The response of maize plants under the treatments that include inoculation with *A. brasiliense*, in terms of plant growth components, dry matter yield, and increased N accumulation, suggest the efficacy of the inoculation. This response can be explained by the bacteria's ability to promote BNF by reducing atmospheric N<sub>2</sub> to ammonia (NH<sup>+</sup><sub>3</sub>), which is quickly converted into ammonium (NH<sup>+</sup><sub>4</sub>) to be assimilated by plants in the form of glutamine (Sangoi et al., 2015). In addition, in the presence of *A. brasiliense*, hormonal biosynthesis modulation is also likely to occur, such as auxin produced by *Azospirillum*, thereby promoting plant growth through different metabolic pathways, even under semi-arid climate (Latef et al., 2020; Nascimento et al., 2021b). The use of *A. brasiliense* 

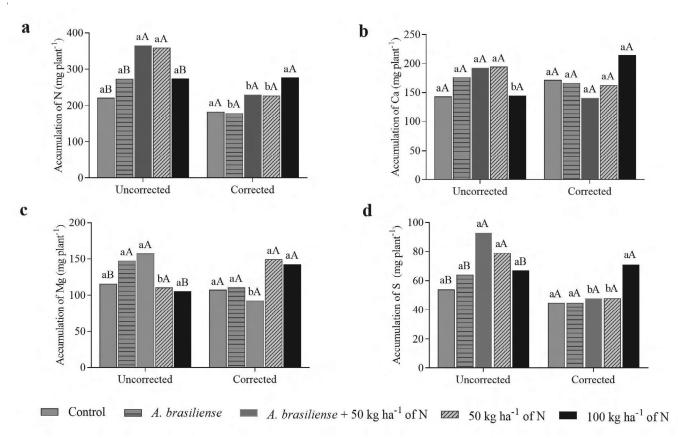
appears to have improved the absorption of nutrients, such as N and S, possibly attributed to increased soil exploration by maize roots (Shirinbayan et al., 2019). However, root development was not evaluated in this study.

Oliveira et al. (2018) reported the importance of using diazotrophic bacteria to reduce pressure on imports and to increase the potential effect of N fertilizers for sustainable agriculture. Such actions could save up to 20 kg ha<sup>-1</sup> of N. Under the soil and climate conditions evaluated in this study, the dry matter yield results suggest that the inoculation with *A. brasiliense* could reduce N demand by at least 50 kg ha<sup>-1</sup> of N.

Good performance for plant height and relative chlorophyll content in maize varieties inoculated with *A. brasilense* may be due to improved uptake and use of nitrogen by associated bacteria, which also explains previous results observed in the photosynthetic performance of maize hybrids (Cunha et al., 2016). According to Skonieski et al. (2017), mineral N supplementation and *Azospirillum* inoculation in agricultural crops can compromise nitrogenase activity and, consequently, negatively affect the amount of biologically fixed N<sub>2</sub>. However, our study shows that N supplementation in association with *A. brasilense* improved the uptake and accumulation of N, and increased the dry matter yield, which is essential for farmers who rely on forage production in the dry period.

Differences in height and relative chlorophyll content were detected between the two genotypes. However, inoculation with *A. brasilense* is an alternative to improve plant height and relative chlorophyll content compared to control plants for both genotypes. The positive association between N fertilizer and the bacteria inoculant could be explained by the genus *Azospirillum*'s use of fertilizer-derived N to assimilate carbon and reproduce more quickly, increasing the effects of the inoculation (Quadros et al., 2014). Positive responses can be obtained by inoculation with *A. brasilense*, even when cultivated with high N levels, which reiterates that the plant's responses are not only due to the fixed N<sub>2</sub>, but also to the activity of bacteria serving as plant growth promoters (Bashan et al., 2004; Okumura et al., 2013).

Such observations are well supported by our results that showed that plants inoculated with *A. brasiliense* and 50 % of the maximum N rate promote higher N accumulation than when the maximum N rate is applied. Contrary to the research carried out by Hungria et al. (2016) on *Brachiaria brizantha* (cv. Marandu) and *Brachiaria ruziziensis* (common) inoculated with *Azospirillum* and growing on degraded soil, our work showed no negative responses with N accumulation, indicating that N was not limiting, that is, biologically fixed N supply is essential to meet maize nitrogen demand. Previous studies also reported productivity increases when using *A. brasilense* in the absence of N fertilization, with increments ranging from 7.4 to 15.4 %, in edaphoclimatic conditions of Marechal Cândido Rondon, Parana state, Brazil (Lana et al., 2012).



**Figure.** 3. Mean values of macronutrients N [Nitrogen (a)], Ca [Calcium (b)], Mg. [Magnesium, (c)], and S [Sulfur, (d)] accumulated in maize plants as a function of the interaction between soil correction versus nitrogen sources. Bars (means) with the same lowercase letter (soil amendment factor) and uppercase letter (nitrogen sources) do not differ from each other according to the Scott-Knott test (p<0.05).

If we consider that the average productivity of maize in smallholding farming systems in semi-arid regions is well below national averages due to low or no use of inputs (fertilizers), which is justified by low precipitation rates (Souza et al., 2014), an increase of around 15 % in dry matter resulted from the use of inoculant, which has a negligible cost, is a promising alternative for improving maize yields. This hypothesis is supported by the work of Arshad (2021), who found a positive correlation between maize dry mass and grain yield.

Although the BRS Caimbé genotype has specific adaptability to adverse conditions, such as low natural soil fertility (Oliveira et al., 2017), under the conditions evaluated in our study, the BRS Gorutuba genotype showed superiority for dry matter production. This can be explained by the greater adaptability of this genotype to semi-arid regions (Carvalho et al., 2010). Apparently, height increment and relative chlorophyll content influenced the higher dry mass accumulation of BRS Gorutuba maize plants. Differential growth responses between maize genotypes were also verified in previous studies (Cunha et al., 2016; Quadros et al., 2014), which suggest that the plant genotype plays an important role in inoculation by bacteria, which must be related to the rhizosphere-bacteria relationship (Quadros et al., 2014). This also explains the genotype x nitrogen source interaction, in which the use of the Gorutuba genotype resulted in increased height and relative

chlorophyll content under different N sources, including the inoculation with *Azosporillum*. On the other hand, when studying two maize genotypes together with N fertilizer application and animal grazing after maize cultivation, Brum et al. (2016) reported no distinction between maize genetic materials in relation to inoculation with *Azosporillum*. The interaction genotype x N sources clearly exemplify how bacterial inoculation is strongly affected by specific environmental conditions, thus benefiting a specific genotype under certain conditions (Skonieski et al., 2017).

It is worth noting that the macronutrients that showed a significant difference for the soil correction factor were increased in treatments without soil correction, even when there was interaction with N sources. The effect of nutrient concentration probably occurred, which is characterized when certain nutrient content is evaluated in a vegetable part with low dry mass. Similar findings previously reported by Santin et al. (2017) highlight the fact that there is no difference between the production of dry matter between plants grown on corrected and uncorrected soils, that is, despite the highest accumulation of macronutrients on uncorrected soil, this did not affect maize dry matter productivity.

The accumulation of nutrients in plants grown on uncorrected soil differs between applied N sources. Previous studies using the diazotrophic bacteria *Paraburkholderia* spp. (Alves

et al., 2016) and *Herbaspirillum* spp. (Alves et al., 2015) reported that using inoculant with nitrogen fertilization can increase the development of agricultural crops, similar to our observations with *Azospirillum*. The results observed with *A. brasilense* Ab-V5 and Ab-V6 support the recommendation of using these bacteria, already authorized for the inoculation on maize in Brazil, as an economical and conservationist agricultural practice for crop production in semi-arid regions.

#### **CONCLUSIONS**

The BRS Gorotuba maize genotype demonstrated to be more tolerant and adaptable to the edaphoclimatic conditions evaluated in this study. The inoculation of *Azospirillum brasilense* with the supplementation of 50 kg ha<sup>-1</sup> of N increases the accumulation of N in maize plants, while increasing the dry matter production similarly to that of plants fertilized with 100 kg ha<sup>-1</sup> of N. This study indicates that it is possible to reduce by 50 % the N recommendation for maize cultivation in degraded soil when *A. brasilense* is incorporated. The inoculation of maize with *A. brasilense* on degraded soil could be a sustainable and promising alternative for growers in semi-arid regions.

#### **AUTHORS PARTICIPATION**

**FMNM:** Investigation, Data Curation, Formal Analysis, Writing – Original draft. **MFA:** Investigation, Data Curation, Formal Analysis, Writing – Original draft. **RCFFP:** Conceptualization, Methodology, Supervision, Funding acquisition. **PIFJ:** Conceptualization, Methodology, Supervision, Funding acquisition. **HAFA:** Data Curation, Data visualization, Writing – Original draft, Writing – Reviewing and editing. **HAS:** Conceptualization, Methodology, Supervision, Funding acquisition, Data Curation, Writing – Reviewing and editing.

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#### **CONFLICT OF INTEREST**

The authors have no conflict of interest to declare.

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