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MORPHOPHYSIOLOGICAL AND PHOTOSYNTHETIC RESPONSES OF MAIZE GENOTYPES TO APPLICATION METHODS AND RATES OF *Azospirillum brasilense*

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ABSTRACT - Brazil is the third largest maize producing country in the world, despite having soils that are often acids and with low nutrient reserves. However, the high cost of fertilizers generated the need for developing alternative technologies to reduce production costs. This work aimed to evaluate the effect of application methods and rates of *Azospirillum brasilense* on morphophysiological and photosynthetic parameters of maize genotypes. A randomized block experimental design was used, in a 4×4×2 factorial arrangement, with three replications. The parameters evaluated were chlorophyll contents; the fresh and dry weight of plant shoot components; root dry weight; culm diameter; root volume; leaf area; plant height; the number of developed leaves; initial, maximum, variable, and final fluorescence; and photochemical efficiency. Applying *A. brasilense* at rates of up to 200 mL ha^{-1} increased the total chlorophyll contents of leaves by 15.4% compared to the plants without inoculation. The maize variety SCS 156 presented higher shoot biomass production, regardless of inoculation, application method, or rate used; the varieties SCS 154 and SCS 155 presented significant increases in this parameter with the application of *A. brasilense* rates of up to 200 mL ha^{-1}. Regarding the inoculant application methods, the variety SCS 155 presented better development when inoculated in the shoots. In contrast, the varieties SCS 155 and SCS 156 and the hybrid VIP 3 presented better development when inoculated in the soil at planting.

Keywords: *Zea mays* L., diazotrophic bacteria, biological nitrogen fixation, phytohormones, biomass production.

RESPOSTAS MORFOFISIOLÓGICAS E FOTOSSINTÉTICAS DE MATERIAIS GENÉTICOS DE MILHO A FORMAS DE APLICAÇÃO E DOSES DE *Azospirillum brasiliense*

RESUMO - O Brasil é o terceiro maior produtor mundial de milho, apesar de possuir solos frequentemente ácidos e com baixa reserva de nutrientes. Contudo, o elevado custo dos fertilizantes gerou a necessidade de desenvolvimento de tecnologias alternativas para reduzir os custos de produção. O trabalho objetivou avaliar o efeito de doses e formas de aplicação de *Azospirillum brasilense* nos parâmetros morfofisiológicos e fotossintéticos em genótipos de milho. Utilizou-se o delineamento experimental em blocos casualizados, em esquema fatorial 4x4x2, com três repetições. Os parâmetros avaliados foram teor de clorofila; massa fresca e seca dos componentes da parte aérea da planta, massa seca de raiz, diâmetro médio do colmo; volume de raiz; área foliar; altura da planta; número de folhas desenvolvidas; fluorescência inicial, máxima, variável e terminal; e eficiência fotoquímica. *A. brasilense* aplicado em doses de até 200 mL ha^{-1} incrementou o teor de clorofila total das folhas em 15,4% quando comparado com as plantas sem inoculação. Para a variedade SCS 156 independentes da inoculação, forma de aplicação ou dose utilizada, houve maior produção de fitomassa pela parte aérea da planta, enquanto para as variedades SCS 154 e SCS 155, incremento significativo nestes parâmetros ocorreram com a aplicação de doses de até 200 mL ha^{-1}. Quanto ao modo de aplicação do inoculante, para a variedade SCS 155, maior desenvolvimento das plantas ocorreu quando a inoculação é realizada via parte aérea, já para as variedades SCS 155 e SCS 156, bem como para o híbrido VIP 3, quando realizada via semente no momento do plantio.

Palavras-chave: *Zea mays* L., bactéria diazotrófica, fixação biológica de nitrogênio, fitohormônios, produção de biomassa.

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Maize (*Zea mays* L.) is one of the most grown grass species in the world because its grains have high nutritive value and great carbohydrate and starch concentrations, and it is widely used as food for humans and animals. In Brazil, the total area with maize crops in the 2021/2022 crop season was 21.239 million hectares, with a production of 115.602 million Mg of grains (Acompanhamento da Safra Brasileira de Grãos, 2022).

Nitrogen (N) is one of the most absorbed nutrients by maize; most of the maize N demand is supplied through synthetic fertilizers (Coelho & Resende, 2008). Therefore, many maize growers apply N rates from 30 to 120 kg ha^{-1} to meet the crop needs (Meira et al., 2009); however, expressive N losses may occur by leaching and volatilization, depending on the climate conditions, mainly when high N rates are applied (Picazevicz, 2017).

Biological nitrogen fixation is a critical alternative to reduce the use of N fertilizers, as well as environmental contamination risks due to losses of N by leaching, as plant growth-promoting bacteria, such as *Azospirillum brasilense*, can break the triple bond of atmospheric nitrogen (N_2) and reduce it to ammonia (NH_3), which plants absorb. In addition, it stimulates the production of phytohormones (De-Bashan et al., 2010), favoring root and shoot development (Picazevicz et al., 2017). However, there are still many questions about adequate inoculation methods and rates, as well as responses of maize genotypes to *A. brasilense* (Barbosa et al., 2022).

Considering the low purchasing power of most maize growers in Brazil, the high demand for N fertilizers, and the benefits of using *A. brasilense* for maize crops, the objective of this work was to evaluate the effects of application methods and rates of *A. brasilense* on morphophysiological and photosynthetic parameters of maize plants.

Material and Methods

The experiment was conducted from October to December 2018 in a greenhouse of the Federal Institute Goiano, in Ipora, GO, Brazil (16°25'23"S, 51°08'53"W, and altitude of 610 m). According to the Koppen classification, the region presents an Aw tropical climate characterized by well-defined dry and rainy seasons. The soil was classified as a Typic Hapludox (Latossolo Vermelho; Santos et al., 2018) of clay texture (Table 1).

The experiment was conducted in a randomized block design with 3 replications in a 4×4×2 factorial arrangement consisting of four rates of an inoculant formulated with *A. brasilense* (0, 200, 400, and 800 mL ha^{-1}), four maize genotypes (hybrid Supremo Viptera 3 and the varieties SCS 154 Fortuna, SCS 155 Catarina, and SCS 156 Colorado), and two inoculation methods (inoculation in the soil at

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planting and inoculation in the shoots at seven days after emergence). The open-pollinated maize varieties SCS 154 Fortuna, SCS 155 Catarina, and SCS 156 Colorado were from the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI).

Table 1. Chemical attributes and texture of the 0-0.20 m layer of the soil collected for filling the pots.

pH	P	Al	H+Al	K	Ca	Mg	CEC	BS	OM	Sand	Silt	Clay
($CaCl_2$)	mg dm^{-3}	---------- cmolc dm^{-3} ---------						%	dag kg^{-1}	--------- g kg^{-1} ----------		
5.6	14.7	0	2.2	0.3	3.4	1.4	7.3	69.3	2.2	340	230	430

CEC = cation exchange capacity; BS = base saturation; OM = organic matter.

Considering the soil chemical analysis (Table 1), liming and soil fertilizer application were not carried out for the soil in the pots. The application of *A. brasilense* in the soil was carried out at planting. The volume of inoculant to be applied per pot was obtained considering a plant stand of 60.000 plants ha^{-1}. Therefore, the volume of inoculant for each rate tested (0.04, 0.08, and 0.16 mL) was diluted in water to complete a total volume of 12 mL of solution, applying 1.0 mL of the diluted inoculant in each pot at planting. The application of *A. brasilense* in the leaves was carried out seven days after the emergence of the plants. The volume of inoculant for each rate (0, 0.04, 0.8, and 0.16 mL) was diluted to complete 24 mL of solution; the pots were grouped according to each treatment to facilitate the application with a manual sprayer. The inoculant AzzoFix (Tradecorp, Hortolandia, Brazil) was used; it contains the strains Ab-V5 and Ab-V6 of *A. brasilense* at the concentration of 2.0 × 10^{8} UFC mL^{-1}.

Irrigation was carried out regularly to keep the soil moisture with adequate water for the plants development over the experimental period. In addition, monoammonium phosphate (4.16 g pot ha^{-1}) was applied 25 days after planting because the plants presented symptoms of phosphorus deficiency. Control of weeds was carried out through manual removal.

Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined 40 days after planting using a portable Soil Plant Analysis Development (SPAD) chlorophyll meter (CFL1030; Falker, Porto Alegre, Brazil). The chlorophyll fluorescence emission was quantified using a portable light-modulated fluorometer (OS1-FL; Opti-Sciences, Hudson, USA) to obtain initial, maximum, variable, and final fluorescence data. The photochemical efficiency was obtained by the variable of maximum fluorescence ratio.

The morpho-physiological parameters of plant height, stem diameter, and the number of developed leaves were evaluated. The plants were withdrawn from the pots and separated into culm, leaves, and roots. Leaf area (m^2) was measured, and root volume was estimated through the water displacement method in a 1.0-liter volumetric cylinder (Basso, 1999). Culm, leaf, and total shoot fresh weights were then determined. Each plant part was placed in a paper bag and dried in a forced air circulation oven at 65 °C to determine the dry weights. The data were tabulated and subjected to analysis of variance by the F test at a 5% probability level to assess the effect of the treatments. When the effect was significant, qualitative factors were compared by Tukey's test (p <0.05), and quantitative factors were compared through linear or polynomial regression models using the statistical program SASM-Agri.

Results and Discussion

The maize varieties and application methods and rates of *A. brasilense* affected the morphophysiological parameters culm fresh weight, leaf fresh weight, total fresh weight, stem diameter, root volume, leaf area, plant height, number of developed leaves, leaf dry weight, culm dry weight, root dry weight, total shoot dry weight, and total plant dry weight (Tables 2, 3, and 4). However, regarding the photosynthetic parameters, the treatments affected only total chlorophyll contents (Figure 1).

Table 2. Root dry weight (g $plant^{-1}$) of open-pollinated maize varieties (SCS154, SCS155, and SCS156) and a maize hybrid (VIP 3).

Maize genotype	MRS[1]
SCS 154	5.08ab
SCS 155	4.92ab
SCS 156	6.90a
Hybrid Vip 3	4.34b
CV (%)	21.79

Means followed by the same letter are not different by Tukey's test at a 5% probability level. CV = coefficient of variation. [1] The data presented were transformed to $\sqrt{X}+1$. Means were presented with the original data.

Regarding the isolating effect of maize genotypes, the variety SCS 156 presented the highest root dry weight, statistically higher than that of the hybrid VIP 3 (Table 2). The root dry weight found for the variety SCS 156 was 58.9%, 35.5%, and 40.2% higher than those found for the maize hybrid and the varieties SCS 154 and SCS 155, respectively. This higher root dry weight may be connected to the vast genetic base of the variety SCS 156, which has not undergone an intense improvement process and is more rustic (hardiness) and adapted to soils with low nutrient availability. This characteristic may stimulate the production of phytohormones in environments with N deficiency, contributing to a more developed root system and, consequently, a greater volume of explored soil (Silva et al., 2021).

The *A. brasilense* rates had an isolated effect on total chlorophyll (Figure 1). The comparison between plants without the application of *A. brasilense* and plants inoculated with 200 and 400 mL ha^{-1} showed an increase of 6% in the SPAD index; this increase was 4.4% for the rate of 800 mL ha^{-1}. According to the polynomial regression model, the rate of 400 mL ha^{-1} resulted in the highest SPAD index (45.3%), regardless of the maize genotype. Xavier et al. (2021), evaluating different maize genotypes, found SPAD index of 52% and 44% for maize plants of the hybrid Dow 2A620PW and the variety SCS 154, respectively, with the application of an *A. brasilense* rate of 200 mL ha^{-1}. Costa et al. (2015) tested inoculation with *A. brasilense* on second-crop maize and found that it promoted an increase of 4.0% in chlorophyll contents when compared to non-inoculated plants. This increase may be due to the activity of *A. brasilense*, which benefits the plants through biological N fixation (Bashan & De-Bashan, 2010; Dartora et al., 2016), and to the induction of phytohormone production, stimulating the root development of inoculated plants (Hungria, 2011). A more developed root system allows the plant to explore a greater soil volume, resulting in better conditions for absorbing water and nutrients compared to plants with a less developed root system.

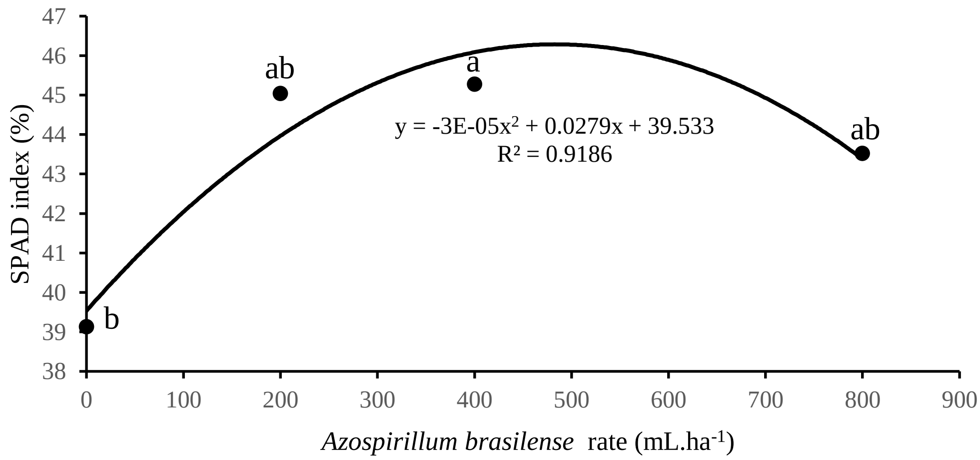

Figure 1. SPAD index in maize plants as a function of *Azospirillum brasilense* rates tested under experimental conditions.

The effect of the interaction between the factors (*A. brasilense* rate × maize genotype) was significant ($P<0.01$) for the biometric variables evaluated, except for stem diameter and total

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plant dry weight (Table 3). The inoculation with *A. brasilense* had a more promising effect on the varieties SCS 156, SCS 155, and SCS 154 than in the hybrid VIP3 regarding the shoot fresh weight (SFW), promoting higher SFW when using rates of up to 400 mL ha^{-1}. Leaf fresh weight (LFW) and culm fresh weight (CFW) presented similar results, denoting that the inoculations with *A. brasilense* at rates of up to 400 mL ha^{-1} resulted in better conditions for the development of plants of the maize genotypes SCS 154, SCS 155, and SCS 156. Regarding the effect of the rates within each maize genotype, the *A. brasilense* rates of 200 mL and 400 mL ha^{-1} resulted in statistically higher CFW for the varieties SCS 154 and SCS 155 than the rates 0 and 800 mL ha^{-1}. No difference in CFW was found for the variety SCS 156 and the hybrid VIP3, regardless of the inoculant rate. The maize varieties presented, in general, higher total biomass production in the shoot (SFW), regardless of the inoculant rate. Thus, considering the mean SFW found for the hybrid VIP3 (156.35 g $plant^{-1}$), the mean SFW found for the varieties SCS 156 (223.29 g $plant^{-1}$), SCS 155 (207.3 g $plant^{-1}$), and SCS 154 (198.89 g $plant^{-1}$) were 27.2%, 32.6%, and 42.8% higher, respectively. This better shoot development can be connected to a better interaction of these maize genotypes with *A. brasilense* and to better root development (Table 2), resulting in higher solubilization of nutrients and production of phytohormones, as auxins and gibberellins (Picazevicz, 2017).

Plants of the varieties SCS 154 and SCS 155 inoculated with *A. brasilense* presented increased leaf area. These maize genotypes subjected to the inoculant rate of 400 mL ha^{-1} showed increases of 53.3% and 28.7% in leaf area, respectively, compared to control plants (without inoculation). However, the variety SCS 156 had no response to inoculation. The *A. brasilense* rates of 200 and 800 mL ha^{-1} had no significant effect on the maize genotypes. The maize hybrid VIP3 presented no apparent response to inoculation; however, at the highest rate tested (800 mL ha^{-1}), it showed an increase in leaf area compared to the control. The results corroborate those reported by Xavier et al. (2021), who found significant increases in leaf area. This beneficial effect is due to the plant-bacterium interaction and the ideal environmental conditions connected to morphophysiological changes using *A. brasilense* (Xavier et al., 2021). However, Santini et al. (2018) and Fiorini et al. (2020) found different results; they reported that using *A. brasilense* did not affect the leaf area of maize plants.

Considering plants without inoculation (control), the lowest plant height was found for the variety SCS 154, which did not differ from the genotypes SCS 155 and the hybrid VIP3. The maize hybrid had the lowest plant height at

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the inoculant rate of 800 mL ha^{-1}, similar to those found for the genotypes SCS 154 and SCS 155. The variety SCS 154 presented the highest plant height at the rate of 200 mL ha^{-1}, and no significant difference between maize genotypes was found for the rate of 400 mL ha^{-1}. Considering the effect of rates within each maize genotype, applying 200 mL ha^{-1} resulted in higher plant height for the variety SCS 154, which was statistically higher than the control. However, the variety SCS 155 had higher plant height at 800 mL ha^{-1}, with no statistical difference from those at the rates of 200 and 400 mL ha^{-1} of *A. brasilense*. The variety SCS 156 had lower plant height at the rate of 200 mL ha^{-1}, and the maize hybrid presented no significant difference for the rates tested. These are similar results to those of Picazevicz et al. (2017) and Martins et al. (2018), who found significant increases in the height of plants inoculated with *A. brasilense*. However, other studies reported that inoculation with *A. brasilense* does not increase plant height compared to plants without inoculation (Santini et al., 2018; Andrade et al., 2019; Souza et al., 2019; Silva et al., 2021). These results indicate that, in addition to the plant genotype, the efficiency of inoculation with *A. brasilense* depends on environmental and edaphoclimatic factors.

Maize varieties exhibit, in general, higher plant height than hybrids (Silva et al., 2021), and the association between genotypes and the production of phytohormones by *A. brasilense*, mainly indole-acetic acid, may result in beneficial effects that contribute to plant growth and development at initial stages (Bashan & Holguin, 1997).

The variety SCS 156 without inoculation (0 mL ha^{-1}) had statistically higher leaf dry weight (LDW), culm dry weight (CDW), total shoot dry weight (TSDW), and total plant dry weight (TPDW) as a function of inoculant rates tested, compared to the varieties SCS 154 and SCS 155. The hybrid VIP3 presented an intermediate result that was not statistically different from the varieties without an inoculant.

The maize hybrid VIP3 inoculated with *A. brasilense* at 200 and 400 mL ha^{-1} presented lower LDW, CDW, and TSDW compared to the varieties SCS 156 and SCS 154. However, no significant difference between maize genotypes was found for the highest rate tested (800 mL ha^{-1}).

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Table 3. Effect of the interaction between the factors (maize genotype and *Azospirillum brasilense* rate) for culm fresh weight (CFW; g plant^{-1}), leaf fresh weight (LFW; g plant^{-1}), shoot fresh weight (SFW; g plant^{-1}), stem diameter (SD; mm plant^{-1}), leaf area (LA; m² plant^{-1}), plant height (PH; cm plant^{-1}), number of developed leaves (NDL; unit plant^{-1}), leaf dry weight (LDW; g plant^{-1}), culm dry weight (CDW; g plant^{-1}), total shoot dry weight (TSDW – g plant^{-1}), and total plant dry weight (TPDW – g plant^{-1}).

Variables	CV (%)	SCS 154				SCS 155			
		Rate of *A. brasilense* (mL ha^{-1})				Rate of *A. brasilense* (mL ha^{-1})			
		0	200	400	800	0	200	400	800
CFW	24.94	93.03Bb	151.00Aa	130.38ABab	97.41Bb	84.08Bb	134.82ABa	106.51Bab	140.37Aa
LFW	25.72	51.06Ab	78.62Aa	78.42Aab	60.85Bab	61.41Aa	84.65Aa	77.16ABa	78.38ABa
SFW	21.72	144.09Bb	229.62Aa	208.8Aa	158.26Bab	145.50Bb	219.47ABa	183.67Aab	218.75ABa
SD	9.81	12.26Bb	15.01Aa	14.78ABa	12.93Bab	13.43ABa	15.49Aa	14.38ABa	14.28ABa
LA	17.60	0.18Cb	0.30Aa	0.29Aa	0.24Bab	0.23BCb	0.30Aa	0.28Aab	0.31Aa
PH	19.26	36.33Bb	55.08Aa	50.58Aa	48.50ABab	42.50ABb	49.17ABab	50.50Aab	58.50ABa
NDL	20.28	5.17Bb	7.33Aa	6.33Aab	6.17Aab	6.17ABa	5.83ABa	6.50Aa	7.00Aa
LDW	23.47	6.73Bc	11.51Aa	10.01Aab	7.82Abc	6.98Bb	11.19Aa	9.21Aab	10.09Aab
CDW	30.82	4.96Bb	10.38Aa	6.81Ab	5.73Bb	4.72Bb	8.09ABa	6.36ABab	7.37ABab
TSDW	25.39	11.69Bb	21.88Aa	16.81Aab	13.55Ab	11.70Bb	19.28Aa	15.57Aab	17.46Aab
TPDW	28.35	15.58Bb	28.92Aa	21.60ABab	18.13Ab	16.00Ba	25.55ABa	19.96ABa	22.18Aa

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Tabela 3. Continuation...

Variables	CV (%)	SCS 156 Rate of *A. brasilense* (mL ha^{-1})				Hybrid Vip 3 Rate of *A. brasilense* (mL ha^{-1})			
		0	200	400	800	0	200	400	800
CFW	24.94	145.85Aa	140.56Aba	152.31Aa	136.35ABa	114.14ABa	99.61Ba	69.47Ba	107.52ABa
LFW	25.72	77.34Aab	74.74Aab	66.58ABb	99.33Aa	72.35Aab	62.04Aab	49.11Bb	81.32ABa
SFW	21.72	223.19Aa	215.29Aba	218.89Aa	235.69Aa	186.49ABa	161.65Bab	118.58Bb	188.84ABa
SD	9.81	14.46Aa	14.73Aa	15.05Aa	15.13Aa	14.01ABa	13.69Aa	12.67Ba	14.68ABa
LA	17.60	0.29Aa	0.28Aba	0.26Aa	0.31Aa	0.28ABa	0.24Bab	0.19Bb	0.28ABa
PH	19.26	58.67Aa	36.67Bb	57.42Aa	59.00Aa	48.00ABa	43.17ABa	43.50Aa	46.50Ba
NDL	20.28	7.17Aa	5.00Bb	6.83Aab	7.67Aa	6.50ABa	5.33Ba	6.00Aa	6.50Aa
LDW	23.47	10.80Aa	10.12Aba	10.03Aa	10.20Aa	8.62ABab	7.48Bab	5.42Bb	9.31Aa
CDW	30.82	8.93Aa	8.02Aba	8.95Aa	9.06Aa	5.89ABa	5.11Ba	3.24Ba	5.89ABa
TSDW	25.39	19.72Aa	18.14Aba	18.98Aa	19.35Aa	14.50ABab	12.59Bab	8.66Bb	15.21Aa
TPDW	28.35	26.24Aa	25.53Aba	25.93Aa	26.11Aa	19.57ABab	17.54Bab	11.01Bb	20.22Aa

Means followed by the same uppercase letter comparing the effect of maize genotypes within each rate or lowercase letter comparing the effect of inoculant rates within each genotype are not different from each other by the Tukey's test at a 5% probability level. CV = coefficient of variation.

Considering the effect of the rates within each maize genotype, the varieties SCS 154 and SCS 155 presented positive responses to inoculation up to the rate of 200 mL ha^{-1}, with significant increases in all variables (Table 3) when compared to plants without inoculation with *A. brasilense*. However, the variety SCS 156 presented no significant response to inoculation for the variables evaluated, regardless of the inoculant rate used. The maize hybrid VIP3 presented no apparent response to inoculant rates, showing similar LFW, SFW, LA, LDW, TSDW, and TPDW to control plants (without inoculation) when using the rates of 200 and 800 mL ha^{-1}. Xavier et al. (2021), evaluating maize varieties, found that the use of 200 mL ha^{-1} of *A. brasilense* results in significant increases in total shoot dry weight of maize plants of the variety SCS 156 and the hybrid Dow 2A620PW, compared to plants without inoculation; they also found significant increases in total fresh weight, fresh weight per plant, and total dry weight per hectare for the variety SCS 156.

The different responses of the maize genotypes to inoculation may be connected to the characteristics of each genotype with *A. brasilense* and interactions with the environment where the crop is developed. Therefore, the results found for the variety SCS 156 under the absence of inoculation can be connected to this genotype's high rusticity (hardiness), as it presented high shoot dry weights, even under low nutrient availability and without inoculant. Still, the varieties SCS 154 and SCS 155 inoculated with the rate of 200 mL ha^{-1} presented a better interaction with *A. brasilense*, which favored plant development. However, this positive effect of inoculation on the maize hybrid was restricted to the highest inoculant rate (800 mL ha^{-1}). Thus, the use of inoculation with *A. brasilense*, in general, favored the development of maize plants. These results corroborate those reported by Picazevicz et al. (2017), Souza et al. (2019), Silva et al. (2021), and Xavier et al. (2021). The bacterium *A. brasilense* contributes to N availability by the biological N fixation process (Moreno et al., 2019) and stimulates the production of phytohormones, such as auxins (indoleacetic acid), gibberellins, and cytokinins. *A. brasilense* also favors the solubilization of phosphate, resulting in higher root growth and, consequently, promoting a better use and absorption of water and nutrients by plants. These factors result in better plant development and an increase in biomass production.

The effect of the interaction between maize genotypes and application methods of *A. brasilense* (Table 4) showed that the variety SCS 154 presented statistically higher CFW, CDW, LA, SDW, and TPDW than the other genotypes when the inoculant was applied in the leaves. The inoculation in the soil resulted in no

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significant differences between maize genotypes, except for LA, which was higher for the maize hybrid VIP3. The variety SCS 155 presented a better response when the application was carried out in the leaves. The varieties SCS 154 and SCS 156 presented no significant responses to the application methods, except for root volume, which was higher for the variety SCS 156 when using inoculation in the soil at planting. The application methods of *A. brasilense* had no significant effect on CDW and TPDW for the maize hybrid VIP3; however, the highest mean CFW, TPFW, and root volume were found for the inoculation in the soil at planting. This positive response to *A. brasilense* may be connected to a higher synthesis of phytohormones, which promote better growth of lateral and adventitious roots and root trichomes, consequently resulting in better absorption of water and nutrients (Cassán et al., 2020; Porto et al., 2020).

The responses of the maize genotypes to inoculation with *A. brasilense* were generally different. The variety SCS 155 presented better responses of most variables when using inoculation in the leaves. In contrast, the maize varieties SCS 156 and SCS 154 and the hybrid VIP 3 showed to be more promising when using inoculation in the soil at planting. Several studies report positive effects of inoculation with *A. brasilense* on the development of maize plants (Santini et al., 2018; Andrade et al., 2019; Fiorini et al., 2020). The present study evidenced the benefits of inoculation with *A. brasilense* and the need for information on the interaction between *A. brasilense* and maize genotypes. Thus, this is a technology focused on more sustainable agricultural systems (Porto et al., 2020) that may become an alternative to reduce production costs, use of chemical fertilizers, and, mainly, negative environmental impacts by indiscriminate use of fertilizers in agriculture (Mumbach et al., 2017; Porto et al., 2020).

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Table 4. Effect of the interaction between the factors (maize genotype and application method of *Azospirillum brasilense*) for culm fresh weight (CFW; g plant^{-1}) and shoot fresh weight (SFW; g plant^{-1}), root volume (RV; cm³ plant^{-1}), leaf area (LA; m² plant^{-1}), culm dry weight (CDW; g plant^{-1}), total shoot dry weight (TSDW; g plant^{-1}), and total plant dry weight (TPDW; g plant^{-1}).

Variables	CV[1] (%)	SCS 154		SCS 155	
		Application methods of *A. brasilense*		Application methods of *A. brasilense*	
		Inoculation	Leaf application	Inoculation	Leaf application
CFW	24.94	108.99Aa	109.56Ba	118.92Ab	144.07Aa
SFW	21.72	170.93Ba	178.60ABa	188.63ABb	224.39Aa
RV	28.70	79.17Aa	81.00ABa	84.92Aa	103.75Aa
LA	17.60	0.24Ba	0.24Ba	0.25Bb	0.31Aa
CDW	30.82	6.04Aa	6.20Ba	6.58Ab	9.21Aa
TSDW	25.39	14.41Aa	14.40Ba	15.64Ab	20.30Aa
TPDW	28.35	19.59Aa	19.11Ba	21.44Ab	27.33Aa

Variables	CV[1] (%)	SCS 156		Hybrid Vip 3	
		Application methods of *A. brasilense*		Application methods of *A. brasilense*	
		Inoculation	Leaf application	Inoculation	Leaf application
CFW	24.94	124.00Aa	105.34Ba	132.81Aa	108.02Bb
SFW	21.72	193.76ABa	171.21Ba	217.49Aa	183.28ABb
RV	28.70	89.17Aa	61.83Bb	97.25Aa	71.92Bb
LA	17.60	0.27ABa	0.24Ba	0.30Aa	0.27Aba
CDW	30.82	7.16Aa	5.52Ba	7.67Aa	6.35Ba
TSDW	25.39	16.43Aa	13.58Ba	17.43Aa	15.35Ba
TPDW	28.35	21.81Aa	17.43Ba	23.51Aa	19.82Ba

Means followed by the same uppercase letter comparing the effect of maize genotypes within each application method, or lowercase letters comparing the effect of application method within each genotype, are not different from each other by the Tukey's test at a 5% probability level. CV = coefficient of variation.

Conclusions

The maize genotypes presented different responses to the application methods and rates of *Azospirillum brasilense*, regardless of the maize genotype, resulting in increased chlorophyll contents.

Maize plants of the variety SCS 156 presented high biomass shoot production, regardless of the application method or rate of *A. brasilense* used. The varieties SCS 154 and SCS 155 presented better plant development using the inoculant rate of 200 mL ha^{-1}.

The variety SCS 155 presented better responses of plant morphophysiological parameters subjected to the inoculation method in the leaves, and the hybrid VIP 3 and the varieties SCS 154 and SCS 156 presented better plant development when subjected to inoculation in the soil at planting.

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