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Effect of *Azospirillum brasilense* in gas exchanges and production of soybean

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Abstract

The present study aimed to verify the photosynthetic responses and yield of soybean cultivars in relation to foliar inoculation with *Azospirillum brasilense* at different sowing dates. For this, three field experiments were carried out at the Federal University of Tocantins, Palmas campus, Brazil. Consecutive seasons were carried out on three sowing dates, November 15th, 2019, December 2nd, 2019 and December 20th, 2019. The experiment was conducted in a randomized block design with four replicates, in a 2 × 5 factorial scheme, represented by two soybean cultivars (M9144RR and TMG1188RR) and five doses of *Azospirillum brasilense* (0, 100, 200, 300 and 400 mL), applied *via* foliar at the stage of growth V5. Stomatal conductance rate (*gs*), internal CO_2 concentration (*iC*), transpiration rate (*E*), net photosynthetic rate (*A*) and grain yield were evaluated. The cultivars showed different responses to sowing dates and to doses of *A. brasilense* in all evaluated parameters. The cultivar TMG1188RR showed higher yield (3,450 $kg \cdot ha^{-1}$) and *gs* (4.89 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$) at the lowest dose applied, while the cultivar M9144RR showed higher yield (3,441 $kg \cdot ha^{-1}$), *gs* (3.35 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$) and *A* (17.32 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$) at highest dose applied. The leaf inoculation with *A. brasilense* was more responsive in periods in which there were unfavorable environmental conditions for soybean cultivation and can be recommended as complementary management in these conditions.

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Introduction

Soybean (*Glycine max* (L.) Merrill) is the most cultivated grain in the State of Tocantins, being the largest producer in the North region of Brazil[1]. The climate in the region is favorable for planting during the rainy season, however, dry periods have been observed frequently, affecting soybean development through changes in physiological parameters, resulting from unfavorable weather conditions[2].

Water deficiency is responsible for causing changes from molecular to morphophysiological levels, limiting plant growth and development[3]. One of the first physiological signs in response to increased temperature and reduced soil moisture is related to stomatal conductance, where a series of coordinated responses occur, preventing and/or hindering gas exchange[4]. Thus, there is a change in the photosynthetic apparatus with effects on carbon assimilation, nitrogen fixation and water use efficiency[5].

Studies conducted by Wang et al.[6] with soybean under water deficit conditions indicated a reduction in photosynthetic activity due to stomatal closure, causing a drop in productivity. Therefore, ensuring that, even under stress conditions, the plant manages to reduce cell damage, avoiding drastic losses in productivity, is a challenge.

Some strategies, such as the use of plant growth-promoting bacteria, have shown promising results. Kasim et al.[7] observed a reduction in the effects of severe water stress on the relative water content of wheat plants inoculated with *A. brasilense*. In addition, the authors also found a decrease in reactive oxygen species and a reduction in the inhibitory effects of drought on chlorophylls a and b. Bulegon et al.[8] also observed positive effects in the removal of reactive oxygen species and in the protection of chlorophyll a in *Urochloa ruziziensis* plants inoculated with *A. brasilense*.

Studies conducted by Armanhi et al.[9] verified that the inoculation of corn plants with colonizing microorganisms optimized water use in plants under water stress, in addition to reducing leaf temperature. Naoe et al.[10] also found an increase in water use efficiency in soybean under water deficit and inoculated with *A. brasilense*.

Thus, inoculation with *A. brasilense* seems to bring benefits to crops under unfavorable environmental conditions[8,11]. In addition, in soybeans, its use associated with *Bradirhyzobium japonicum* is indicated as a management technique that increases crop productivity[12] and contributes to improving morphological characteristics of plants[13]. Another important point in studies with *A. brasilense* is in relation to the best dose and forms of application, as there is still no consensus in the literature, since the beneficial effects attributed to plants are multifactorial and difficult to understand.

Therefore, considering the frequent extreme drought and temperature events, especially in the State of Tocantins, it is necessary to seek alternatives that reduce losses in the productivity of crops. Therefore, the present study aimed to evaluate the alterations resulting from the foliar application of *Azospirillum brasilense* in the physiological parameters and in the productivity of two soybean cultivars in the Savanna Tocantinense, cultivated in three sowing dates and five different doses of inoculant.

Materials and methods

Location and characterization of experimental area

The study was conducted at the experimental fields of the Federal University of Tocantins, municipality of Palmas, TO, Brazil (10°10' S and 48°21' W, at 216 m of altitude), from November 2019 to March 2020. Consecutive seasons were carried out on three sowing sessions, November 15th, 2019 (S1); December 2nd, 2019 (S2) and December 20th, 2019 (S3). Figure 1 presents the climatic data observed throughout both experiments.

The soil of the experimental area was classified as Oxisol, with loamy sand texture, showing a pH of 4.9, available concentrations of P and K of 3.00 and 26.00 mg·dm^{-3}, respectively; Ca and Mg concentrations of 1.5 and 0.7 cmolc·dm^{-3}, respectively, and base saturation equal to 54.44%. The mean soil bulk density was 1.55 kg·dm^{-3}, with sand, silt and clay percentage of 82, 13 and 5 dag·kg^{-1}, respectively.

The cultivars M9144RR (Monsoy Ltda) and TMG1188RR (Tropical Genetic Improvement) were used, with maturation grade 9.1 and 8.8, respectively, both with determined growth habit and demanding in terms of fertility.

Installation and conduction of experiments

At each sowing date, the experiments were conducted in a randomized block design, consisting of five treatments and four replications. The treatments were arranged in a 2 × 5 factorial scheme, represented by the two cultivars (M9144RR and TMG1188RR) and five doses of *A. brasilense* (0, 100, 200, 300 and 400 mL·ha^{-1}), applied *via* foliar.

At the time of sowing, the seeds were inoculated with strains of *B. japonicum* Semia 5079 and Semia 5080 (5.0 × 10^9 CFU·mL^{-1}) at a dose of 60 g/50 kg of seeds. When the plants reached the stage of growth V5 (Fifth fully developed trifoliate leaf), the foliar application of the treatments was realized with *A. brasilense* strains AbV5 and AbV6 (2.0 × 108 CFU·mL^{-1}) at doses 0, 100, 200, 300 and 400 mL·ha^{-1}.

The experimental plot consisted of four 5-m-long rows at spacing of 0.50 m between rows and 1.0 m between treatments, with a density of 15 plants per linear meter.

Parameter measurements

To measure net photosynthesis rate [(*A*) µmol CO_2 m^{2}·s^{-1}], stomatal conductance [(*gs*) µmol CO_2 m^{2}·s^{-1}], transpiration [(*E*) mmol H_2O m^{2}·s^{-1})] and internal CO_2 concentration [(*iC*) µmol CO_2 m^{2}·s^{-1}], was used an infrared gas analyzer (IRGA; Li-COR CO_2/H_2O Gas analyzer 6400, Lincoln, NE, USA). Measurements were always performed in the morning, between 8:00AM and 10:00AM, on fully expanded leaves, at R1 and R5 stages.

Grain yield per hectare (GY) was calculated considering the observation area of the plot of 3 m^2, eliminating the border effect.

Statistical analysis

The data were subjected to individual analysis of variance and, subsequently, to a joint analysis when the homogeneity of variances was verified. Means of yield were grouped by the test of Scott & Knott[14] at $p \leq 0.05$. After that, the doses of *A. brasilense* were subjected to regression analysis, using first-, second-, and third-degree polynomial models. The statistical programs SISVAR 5.0[15] and OriginPro (2015) were used.

Results and discussion

Table 1 presents the summary of the joint analysis of variance for physiological parameters and grain yield of two soybean cultivars, submitted to different doses of *A. brasilense*, at three different sowing dates.

Isolated effects of sowing dates (S), cultivars (C) and doses of *A. brasilense* (D) were observed in all evaluated physiological parameters and grain yield. In addition, there was interaction between all sources of variation studied.

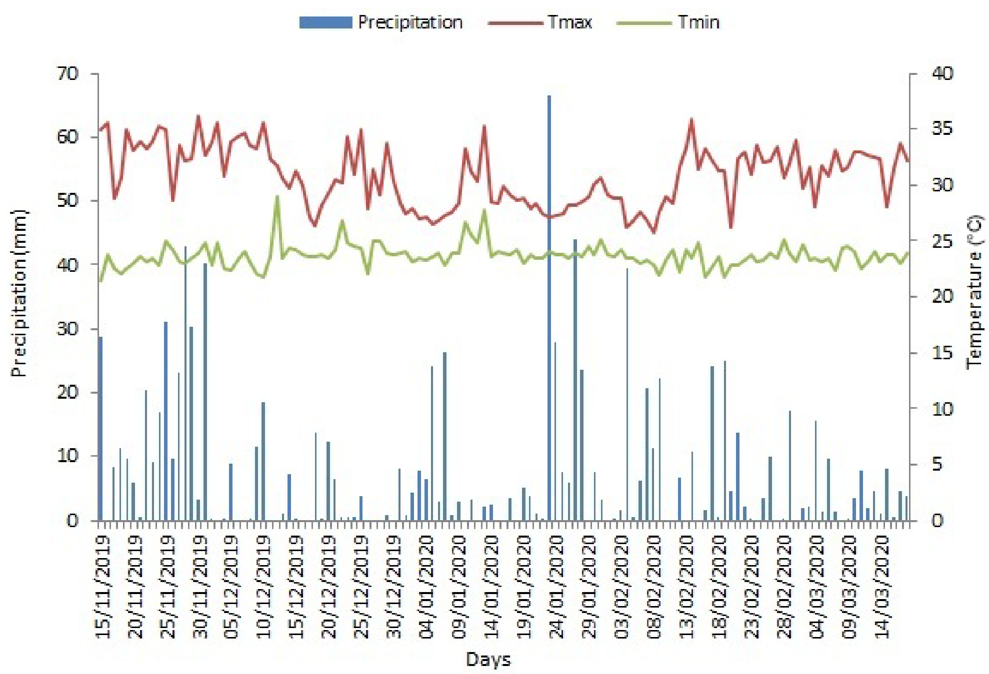

Fig. 1 Mean values of maximum (Tmax) and minimum (Tmin) temperature (°C), and precipitation (mm).

For the double interaction D × S, the significant effect showed that environmental variations influenced the plant/bacteria system, which, in turn, reflected in gas exchange. Similarly, the C × D interaction shows that different cultivars present different behavior in response to *A. brasilense*, as already observed by Hungria[16].

In addition, the triple interaction S × C × D demonstrated that, under some conditions, *A. brasilense* exerts a direct influence on the photosynthetic apparatus and benefits plants in stress situations, resulting from environmental variations on different sowing dates.

Stomatal conductance

Table 2 presents the *gs* averages of two soybean cultivars, cultivated on three sowing dates and different doses of *A. brasilense* applied *via* foliar application.

The study between sowing times showed that, for the M9144RR cultivar, the highest *gs* averages were observed at S1 and S2, at the highest applied dose, while in the TMG1188RR cultivar, the highest average was observed only at S2 and at the lowest applied dose (Table 2).

Table 1. Analysis of variance related to the characteristics: net photosynthesis rate (*A* μmol CO_2 $m^2{\cdot}s^{-1}$), stomatal conductance (*gs* μmol CO_2 $m^2{\cdot}s^{-1}$), transpiration rate (*E* mmol H_2O $m^2{\cdot}s^{-1}$), internal CO_2 concentration (*iC* μmol CO_2 $m^2{\cdot}s^{-1}$) and grain yield (GY kg·ha^{-1}) of the soybean cultivars (C), M9144RR and TMG1188RR, produced on three sowing dates (S).

SV	DF	Mean square				
		gs	*E*	*iC*	*A*	GY
S	2	36.0*	2.0*	394858.7*	504.7*	2667545.8*
C	1	69.0*	2.4*	6200.2*	213.9*	290306.9*
D	4	12.1*	2.4*	4320.4*	27.5*	301862.8^{ns}
R (S)	9	1.2^{ns}	0.1^{ns}	261.4^{ns}	1.2^{ns}	54749.0^{ns}
S × C	2	18.6*	3.2*	27846.9*	108.2*	543547.6*
S × D	8	8.7*	1.1*	1623.9*	12.4*	307563.6*
C × D	4	25.4*	2.2*	702.3*	3.3*	321298.1*
S × D × C	8	9.9*	0.5*	3313.1*	7.5*	507260.0*
Error	81	1.0	0.2	122.1	1.2	90896.9
Total	119					
CV (%)		16.87	6.59	4.33	6.25	12.69

*: Significant at $p \leq 0.05$ by F test; ns: Not significant. DF: Degrees of freedom; SV: Source of variation; S: sowing dates; C: cultivars; D: doses of *Azospirillum brasilense*; R: Replications.

Among the cultivars, within each sowing date, the materials behaved differently, demonstrating different responses to the imposed environmental conditions. With regards to doses, the study showed that there was only adjustment in the second sowing date, for both cultivars (Fig. 2).

For the M9144RR cultivar, the quadratic adjustment showed a higher *gs* rate (3.35 μmol CO_2 $m^2{\cdot}s^{-1}$) at the dose of 400 mL·ha^{-1} (Fig. 2a). In this case, the application of *A. brasilense* contributed to the increase in stomatal conductance.

Differently, for the TMG1188RR cultivar, the cubic adjustment showed that the highest *gs* (4.89 μmol CO_2 $m^2{\cdot}s^{-1}$) occurred at the lowest applied dose (120.52 mL·ha^{-1}), followed by the control (Fig. 2b). The difference observed between the cultivars in relation to the *gs* possibly occurred due to the plant-bacterium interaction. According to Matsumura at al.[17], inoculation response may vary according to plant genotype, bacterial strain and environmental conditions, which may be related to the genetic traits intrinsic to each soybean genotype.

The literature has reported effects on the interaction of *A. brasilense* in plants under conditions of water restriction and high temperatures[11], which could partly explain the increase in *gs* in less favorable environmental conditions for cultivar M9144RR, like those observed in E2 (Fig. 1).

Marques et al.[18] also reported an increase in *gs* in corn plants inoculated with *A. brasilense* and subjected to water

Table 2. Means of S × C × D interaction for stomatal conductance (*gs*) of two soybean cultivars, in three sowing dates and different doses of *A. brasilense*.

Sowing dates	Cultivars	*gs* (μmol CO_2 $m^2{\cdot}s^{-1}$)				
		Dose (mL·ha^{-1})				
		0	100	200	300	400
S1	M9144RR	1.70Ab	1.65Aa	1.85Aa	3.1Aa	2.45Aa
	TMG132RR	1.25Ba	1.50Ba	1.50Aa	1.55Bb	1.95Bb
S2	M9144RR	1.40Ab	1.25Ab	1.15Ab	1.55Bb	3.15Aa
	TMG132RR	3.70Aa	4.80Aa	1.80Aa	2.17Aa	2.95Aa
S3	M9144RR	1.40Aa	1.35Aa	1.55Aa	1.35Ba	1.90Ba
	TMG132RR	1.45Ba	1.45Ba	1.60Aa	1.70Ba	1.60Ba

Means between seasons, within the same cultivar, followed by the same capital letter in the column do not differ by the Scott Knott test at 5% probability. Means between cultivars, within the same season, followed by the same lowercase letter in the column do not differ by the Scott Knott test at 5% probability. * S1: sowing date 1; S2: sowing date 2; S3: sowing date 3.

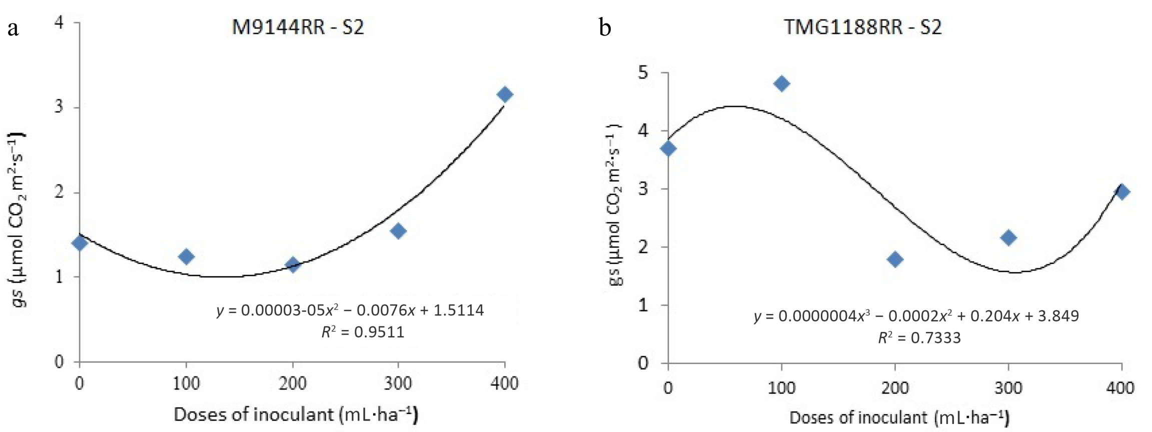

Fig. 2 Stomatal conductance (*gs*) as a function of foliar application of different doses of *A. brasilense* in soybean at vegetative stage V5, on three sowing dates.

stress. According to the authors, the increase in *gs* in inoculated plants may be related to the improvement in water use efficiency, which, in this case, may have been mediated by the bacteria. Furthermore, Tonelli et al.[19] reported that genetic characteristics and climatic conditions are also responsible for different responses between cultivars and bacteria.

On the other hand, it is necessary to consider that the increase in *gs* also entails a higher cost of water loss *via* transpiration[20]. Furthermore, Cohen et al.[21] found a reduction in stomatal conductance in *A. thaliana* plants under water deficit and inoculated with *A. brasilense*. According to the authors, this process could be related to the greater presence of ABA in the plant-bacteria system, which would benefit the plants under temporary drought conditions, as may have occurred for the cultivar TMG1188RR.

Transpiration (E)

Table 3 presents the transpiration averages of two soybean cultivars, on three sowing dates and different doses of *A. brasilense* applied *via* foliar application.

In the study between sowing dates, the cultivar M9144RR showed a distinct behavior at the lowest applied dose, in which the lowest *E* value was observed at S2 and S3. On the other hand, the TMG132RR cultivar had the lowest *E* value observed in S1 (Table 3).

Under conditions of water stress and high temperatures, such as those recorded in S2 (Fig. 1), plants may occasionally increase transpiration to reduce internal temperature. Armanhi et al.[9] found that inoculation with root-colonizing microorganisms reduced leaf temperature by up to 4 °C in maize plants subjected to thermal and water stress, which may also have occurred in the present study.

In this case, there would be a transient increase in stomatal conductance, mediated by *A. brasilense* and, consequently, an increase in transpiration.

Although the C × S × D interaction was significant for all evaluated parameters, there was no dose adjustment for *E*, indicating that there is no functional relationship between doses for this characteristic.

Internal CO_2 concentration (*iC*)

Table 4 presents the *iC* averages of two soybean cultivars, on three sowing dates and different doses of *A. brasilense* applied *via* foliar application.

Between the sowing dates, the two cultivars registered higher *iC* averages in S1, season that registered better environmental conditions (Fig. 1). Among the cultivars, there was a distinct response at S2 and S3, at the highest applied dose.

Regarding the doses, for the M9144RR cultivar, the adjustment occurred through a cubic equation, only in S3, responding with a greater accumulation of *iC* (328.65 µmol CO_2 $m^{-2}·s^{-1}$), at the highest applied dose (400 $mL·ha^{-1}$) (Fig. 3a).

Differently, for the cultivar TMG1188RR, the adjustment occurred only in S2, through a linear equation, with the highest dose providing accumulation of 247.26 mmol CO_2 $m^{-2}·s^{-1}$ of *iC*. In both cultivars, the dose of 400 $mL·ha^{-1}$ of *A. brasilense* favored the highest accumulation of *iC*. In this case of the M9144RR cultivar, this increase was accompanied by an increase in *gs* (Fig. 2a) and *E* (Table 3), which was expected under these conditions.

However, in the TMG1188RR cultivar, the linear increase was accompanied by a reduction in *gs* (Fig. 2b). Wei et al.[22]observed that in situations of low water availability, an increase in the internal carbon concentration in the substomatal chamber may occur, partially explaining the results obtained for this cultivar.

Net photosynthesis rate (*A*)

Table 5 presents the *A* averages of two soybean cultivars, in three sowing dates and different doses of *A. brasilense* applied *via* foliar application.

In general, between the sowing dates, the two cultivars had the highest rates of *A* in S1, as well as in *iC* (Table 4). However, they differed among themselves within each season, demonstrating their different behavior to the effects of inoculation in face of environmental variations resulting from sowing dates.

Regarding the doses, for the cultivar M9144RR, the adjustment occurred only in S2, through a cubic equation (Fig. 4a), with an *A* of 17.32 mmol CO_2 $m^{-2}·s^{-1}$, at a dose of 400 $mL·ha^{-1}$, which was also observed for *gs* (Fig. 2a) and *iC* (Fig. 3a).

For the cultivar TMG1188RR, equation adjustment was obtained on the first and second sowing dates, also by means of cubic equation. In both seasons, the highest applied dose of *A. brasilense* provided a photosynthetic rate of 23.00 CO_2 $m^{-2}·s^{-1}$ and 21.39 CO_2 $m^{-2}·s^{-1}$ (Fig. 4b & c), respectively. On the other hand, in this same condition there was a reduction in *gs* (Fig. 2b) and an increase in *iC* (Fig. 3b).

Table 3. Means of S × C × D interaction for transpiration (*E*) of two soybean cultivars, on three sowing dates and different doses of *A. brasilense*.

Sowing dates	Cultivar	E (mmol H_2O $m^{-2}·s^{-1}$)				
		Dose $mL·ha^{-1}$				
		0	100	200	300	400
S1	M9144RR	7.60Aa	7.35Aa	7.00Aa	7.10Aa	7.30Aa
	TMG132RR	7.15Aa	6.95Ba	6.90Ba	6.00Aa	7.00Aa
S2	M9144RR	7.00Aa	6.25Bb	6.80Aa	7.60Aa	7.50Aa
	TMG132RR	7.25Aa	7.30Aa	6.65Ba	6.80Aa	7.25Aa
S3	M9144RR	7.25Aa	6.95Ba	7.05Aa	7.10Aa	7.50Aa
	TMG132RR	7.00Aa	7.75Aa	7.25Aa	6.90Aa	7.55Aa

Means between seasons, within the same cultivar, followed by the same capital letter in the column do not differ by the Scott Knott test at 5% probability. Means between cultivars, within the same season, followed by the same lowercase letter in the column do not differ by the Scott Knott test at 5% probability. * S1: sowing date 1; S2: sowing date 2; S3: sowing date 3.

Table 4. Means of S × C × D interaction for internal CO_2 concentration (*iC*) of two soybean cultivars, on three sowing dates and different doses of *A. brasilense*.

Sowing dates	Cultivars	*iC* (µmol CO_2 $m^{-2}·s^{-1}$)				
		Dose $mL·ha^{-1}$				
		0	100	200	300	400
S1	M9144RR	331.00Aa	338.50Aa	329.00Aa	343.00Aa	347.00Aa
	TMG132RR	332.50Aa	344.00Aa	327.50Aa	336.50Aa	328.50Aa
S2	M9144RR	202.50Ba	198.50Ba	193.00Ba	198.50Bb	195.00Bb
	TMG132RR	202.50Ba	201.50Ba	219.00Ba	234.50Ba	247.50Ba
S3	M9144RR	211.50Ba	222.50Ba	234.00Ba	247.00Ba	305.00Aa
	TMG132RR	190.50Ba	196.00Ba	194.00Ba	192.00Ba	196.00Bb

Means between seasons, within the same cultivar, followed by the same capital letter in the column do not differ by the Scott Knott test at 5% probability. Means between cultivars, within the same season, followed by the same lowercase letter in the column do not differ by the Scott Knott test at 5% probability. * S1: sowing date 1; S2: sowing date 2; S3: sowing date 3.

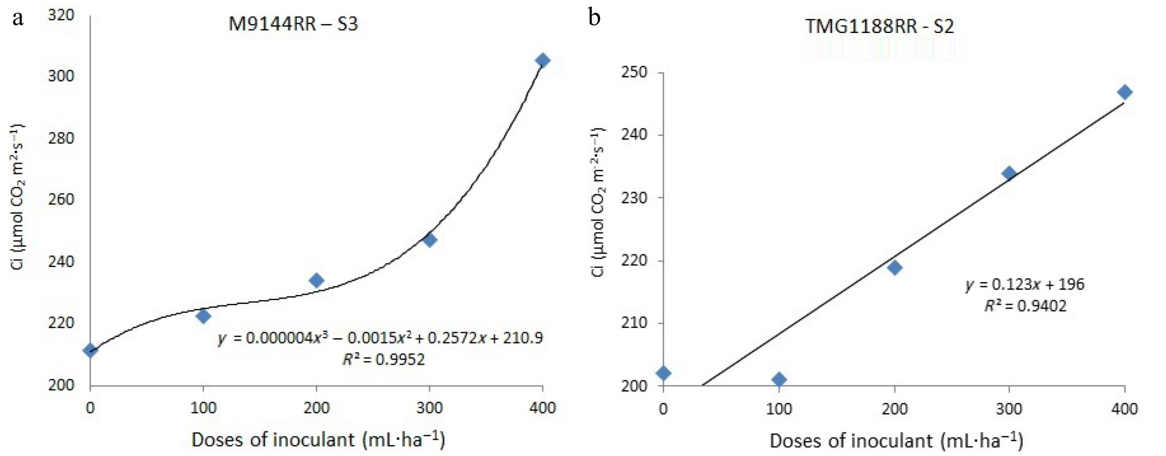

Fig. 3 Intercellular CO_2 concentration as a function of foliar application of different doses of *A. brasilense* in soybean phenology stage V5, on three sowing dates.

Table 5. Means of S × C × D interaction for net photosynthesis rate (*A*) of two soybean cultivars, on three sowing dates and different doses of *A. brasilense*.

Sowing dates	Cultivars	*A* (µmol CO_2 $m^{-2}{\cdot}s^{-1}$)				
		Dose mL ha^{-1}				
		0	100	200	300	400
S1	M9144RR	20.35Aa	21.05Aa	20.10Aa	21.70Aa	23.10Aa
	TMG132RR	18.90Ab	19.00Aa	17.75Ab	20.85Aa	23.70Aa
S2	M9144RR	16.0Ba	15.60Ca	16.25Ca	17.05Ba	17.20Cb
	TMG132RR	16.55Ba	16.95Ba	15.65Ba	15.80Bb	21.60Ba
S3	M9144RR	17.15Ba	18.75Ba	18.55Ba	17.40Ba	20.00Ba
	TMG132RR	14.30Cb	14.65Cb	13.50Cb	14.00Cb	12.75Cb

Means between seasons, within the same cultivar, followed by the same capital letter in the column do not differ by the Scott Knott test at 5% probability. Means between cultivars, within the same season, followed by the same lowercase letter in the column do not differ by the Scott Knott test at 5% probability. * S1: sowing date 1; S2: sowing date 2; S3: sowing date 3.

The net photosynthetic rate can be considered as a product of gas exchange, which begins with stomatal conductance and is dependent on factors such as water availability, light and temperature. However, *A* can be compromised, not only by reducing *gs*, but also by reducing the rate of electron transport and photophosphorylation, both processes in the photochemical step of photosynthesis[23].

Costa & Marenco[24] found a low correlation between *A* and *gs*, explaining that both photosynthesis and stomatal conductance are plant parameters that respond to a set of factors that interact in a coordinated but highly complex manner.

Therefore, for cultivar TMG1188RR, what would possibly explain the increase in *A*, even with a reduction in *gs*, could be an additional external factor. Thus, the inoculation seems to have protected the photosynthetic apparatus, guaranteeing chain reactions, even with the plant under unfavorable environmental conditions.

Grain yield (GY)

When comparing the averages (Table 6), it was possible to observe that, between the sowing dates, the cultivar M9144RR obtained the highest average productivity value in S2, at the highest dose applied, differing from the control.

Considering that S2 presented unfavorable environmental conditions (Fig. 2), this difference may be related to inoculation responses in plants under water stress, as observed by Cohen et al.[25] in corn plants grown under similar conditions. In addition, in S2, the same cultivar showed better response to *gs* (Fig. 2a) and *A* (Fig. 4a), at the highest applied dose.

In cultivar TMG1188RR, the highest yield occurred at the lowest dose applied, also in S2 (Table 6), in addition to an increase in *gs* (Fig. 2b), indicating an antagonistic response to that observed for cultivar M9144RR. In this case, it can be considered that the cultivar TMG1188RR was more sensitive to the effects of inoculation within the same sowing date. However, at the highest applied dose, productivity decreased.

It is important to note that, in the control treatment, there was no mean difference between the cultivars in each season (Table 6), that is, without the presence of *A. brasilense,* the behavior of the materials in the face of environmental variations was similar for productivity. However, after the control, this behavior changed, indicating that the response to inoculation really is multifactorial and dependent on factors such as the type of cultivar and the environmental conditions to which they are subjected.

For the M9144RR cultivar, dose adjustment occurred only in S3, using a linear equation (Fig. 5a), with the best response at the maximum dose applied (1,725.45 kg·ha^{-1}). However, the effects of the bacteria were also observed in S2. The low productivity observed in S3 may have occurred due to multiple environmental factors. It is important to note that the averages observed in *gs* and *A* were also low in S3, especially for the cultivar TMG1188RR, which showed the lowest productivity. Furthermore, the low rainfall at the beginning of sowing, before inoculation with *A. brasilense*, may have been an additional factor that contributed to the low yield in this condition.

For the cultivar TMG1188RR, the adjustment occurred in S1 and S2, by means of cubic equation (Fig. 5b & c). In the first sowing season, with the highest dose applied, the average yield reached was 3,339.71 kg·ha^{-1}. This result shows how environmental conditions can interfere differently in the plant/bacteria relationship, even in the same cultivar.

Considering the second sowing date to be the one in which the environmental conditions were less favorable, the cultivar TMG1188RR showed greater sensitivity to the effects of the bacteria, responding at the lowest applied doses. This result was also observed for the physiological parameters. However,

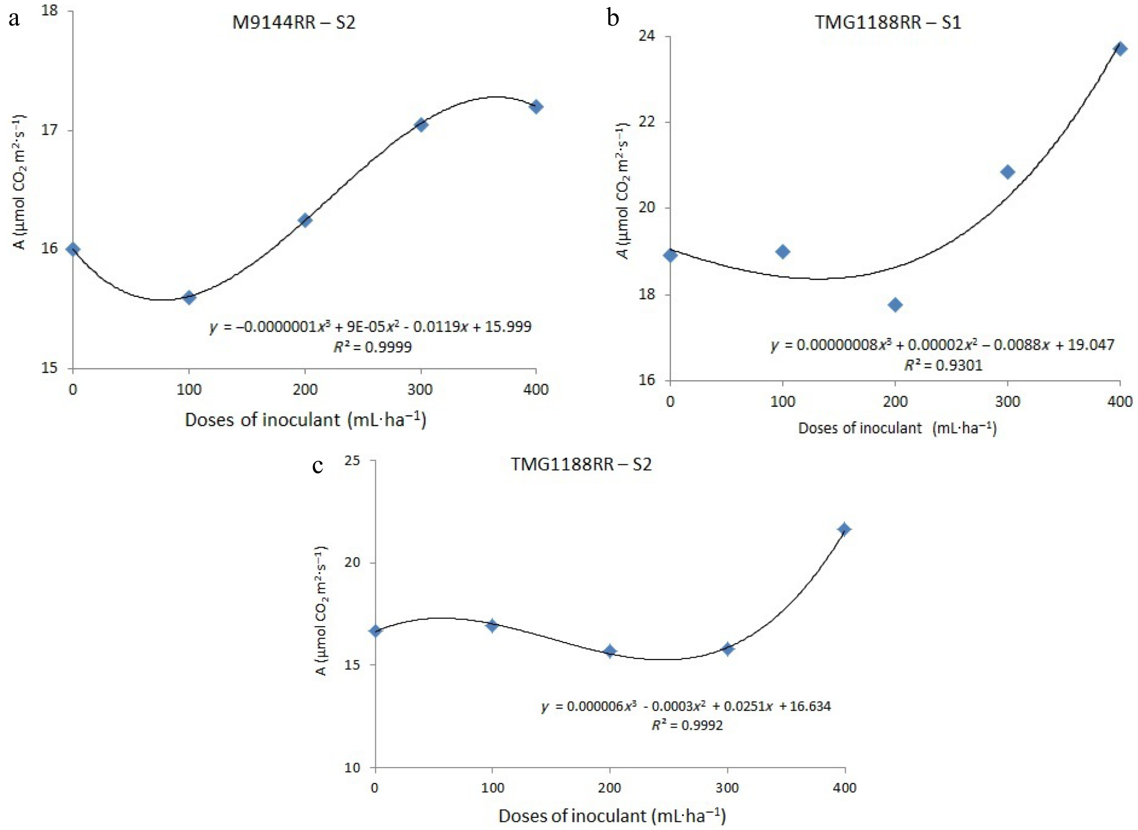

Fig. 4 Net photosynthesis rate as a function of foliar application of different doses of *A. brasilense* in soybean at phenological stage V5, on three sowing dates.

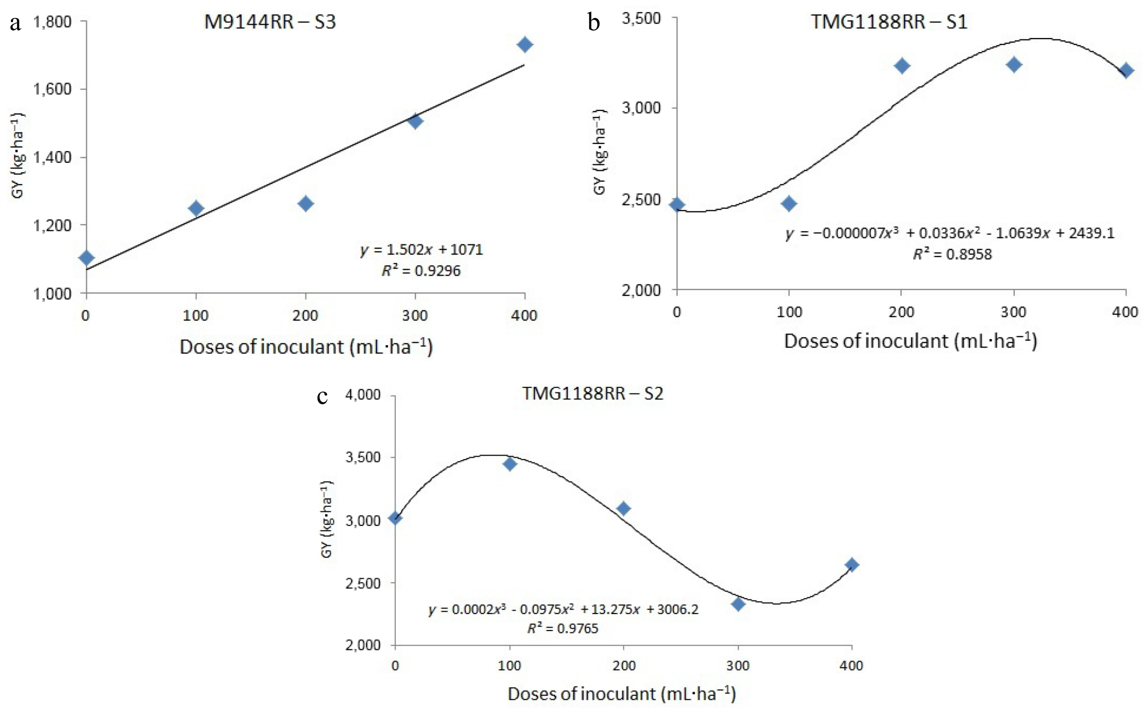

Fig. 5 Grain yield as a function of foliar application of different doses of *A. brasilense* in soybean at phenological stage V5, on three sowing dates.

under more adequate conditions of water availability (S1), the benefits attributed to the inoculation were achieved in the highest doses applied.

That way, the productivity response can be attributed to the physiological parameters, which were influenced by the bacteria. Neto et al.[26] observed significant changes in dry biomass

Table 6. Means of S × C × D interaction for grain yield (GY) of two soybean cultivars, on three sowing dates and different doses of *A. brasilense*.

Sowing dates	Cultivars	GY ($kg \cdot ha^{-1}$)				
		Doses ($mL \cdot ha^{-1}$)				
		0	100	200	300	400
S1	M9144RR	2,512 Aa	2,438 Aa	2,578 Bb	2,737 Ab	2,529 Bb
	TMG1188RR	2,471 Ba	2,472 Ba	3,235 Aa	3,239 Aa	3,210 Aa
S2	M9144RR	2,792 Aa	2,658 Ab	3,212Aa	2,844 Aa	3,441 Aa
	TMG1188RR	3,022 Aa	3,450 Aa	3,094 Aa	2,331 Bb	2,644 Bb
S3	M9144RR	1,106 Ba	1,508 Ba	1,265 Cb	1,251 Bb	1,730 Ca
	TMG1188RR	1,553 Ca	1,506 Ca	1,609 Ba	1,562 Ca	1,311 Cb

Means between seasons, within the same cultivar, followed by the same capital letter in the column do not differ by the Scott Knott test at 5% probability. Means between cultivars, within the same season, followed by the same lowercase letter in the column do not differ by the Scott Knott test at 5% probability. * S1: sowing date 1; S2: sowing date 2; S3: sowing date 3.

and root structure at different doses of *A. brasilense* in corn. Naoe et al.[11] also observed different responses in the productivity of soybean inoculated with *A. brasilense* under different environmental conditions. On the other hand, Zuffo et al.[27] did not observe beneficial effects on the productivity of soybean inoculated with the bacteria.

Thus, in unfavorable environmental conditions, *A. brasilense* seems to exert an additional benefit to the plants. However, in this study, the way in which the cultivars responded to this interaction was different, although both were favored directly and/or indirectly by inoculation.

Conclusions

The foliar application of *A. brasilense* on soybean plants influenced the physiological parameters and grain yield differently in the two evaluated cultivars.

At the dose of 100 $mL \cdot ha^{-1}$, the TMG1188RR cultivar showed greater yield (3,450 $kg \cdot ha^{-1}$) and *gs* (4.89 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$). While the cultivar M9144RR showed higher yield (3,441 $kg \cdot ha^{-1}$), *gs* (3.35 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$) and *A* (17.32 $\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$), at the dose of 400 $mL \cdot ha^{-1}$.

The foliar inoculation with *A. brasilense* can be recommended as a biotechnological technique that influences the physiological characteristics of soybeans and improves yield in unfavorable environmental conditions.

Author contributions

The authors confirm contribution to the paper as follows: Study conception and design: Reina E, Peluzio JM; data collection: Reina E, Monteiro FJF; analysis and interpretation of results: Reina E, Peluzio JM, Naoe AML; draft manuscript preparation: Naoe AML, Reina E ; review & editing: Naoe AML. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data in this paper are free from any conflict of interest. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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