

COTTON GROWTH AND PRODUCTION UNDER SALINE STRESS AND INOCULATION WITH *AZOSPIRILLUM BRASILENSE*

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1 RESUMO

Objetivou-se com este estudo, avaliar o efeito da inoculação com *Azospirillum brasilense* na mitigação dos efeitos deletérios causados pela irrigação com águas salobras no crescimento e nos componentes de produção do algodoeiro de fibra branca BRS 286. A pesquisa foi conduzida em ambiente protegido (casa de vegetação) em lisímetros de drenagem. Os tratamentos resultaram da combinação entre dois fatores: cinco níveis de condutividade elétrica da água de irrigação - CEa (0,4; 3,1; 4,1; 6,1 e 7,1 dS m⁻¹) associados a inoculação ou não das sementes de algodão com *Azospirillum brasilense* - AZ (Com inoculação e sem inoculação), distribuídos no delineamento inteiramente casualizado, em arranjo fatorial 5 × 2, com seis repetições e uma planta por parcela, perfazendo o total de sessenta unidades experimentais. A inoculação das sementes com *Azospirillum brasilense* mitigou os efeitos deletérios do estresse salino sobre a área foliar e os componentes de produção quando submetido a CEa de até 3,1 dS m⁻¹. A irrigação com CEa de 0,4 dS m⁻¹ associado a inoculação das sementes com *Azospirillum brasilense* resultou nos maiores valores de área foliar, fitomassa seca da parte aérea, número de capulhos, número de sementes, massa de sementes e massa de algodão em pluma.

Palavras-chave: *Gossypium hirsutum* L., estresse abiótico, fixação biológica de nitrogênio.

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2 ABSTRACT

The objective of this study was to evaluate the effect of inoculation with *Azospirillum brasilense* in mitigating the deleterious effects caused by irrigation with brackish water on the growth and production components of BRS 286 cotton. The research was conducted in a protected environment (vegetation house) in drainage lysimeters. The treatments resulted from the combination of two factors: five levels of electrical conductivity of the irrigation water - EC_w (0.4, 3.1, 4.1, 6.1 and 7.1 dS m⁻¹) associated with inoculation or not of cotton seeds with *Azospirillum brasilense* - AZ (with inoculation and without inoculation), distributed in a completely randomized design, in a 5 × 2 factorial arrangement, with six repetitions and one plant per installment, making a total of sixty experimental units. Seed inoculation with *Azospirillum brasilense* mitigated the deleterious effects of saline stress on leaf area and yield components when subjected to an EC_w of up to 3.1 dS m⁻¹. Irrigation with an EC_w of 0.4 dS m⁻¹ associated with seed inoculation with *Azospirillum brasilense* resulted in the highest values of leaf area, shoot dry biomass, boll number, seed number, seed weight and cotton weight in feathery.

Keywords: *Gossypium hirsutum* L., abiotic stress, biological nitrogen fixation.

3 INTRODUCTION

The cotton plant (*Gossypium hirsutum* L.), belonging to the Malvaceae family, is an important crop used for the production of fibers and oil and is responsible for providing 35% of the world's total textile fiber (JIA *et al.*, 2022; NAZISH *et al.*, 2022). Its production must increase by 20% to meet the global demands of the increasing population (TARIQ *et al.*, 2017).

Brazil is one of the main producers and exporters of cotton fiber in the world (SILVA *et al.*, 2020a). In this sense, the Northeast region has stood out, with an estimated production of 1,668.2 thousand tons distributed among the states of Alagoas, Bahia, Paraíba, Ceará, Rio Grande do Norte, Piauí and Maranhão (ALGODÃO, 2023).

However, the semiarid region of the Northeast has water restrictions in terms of quality and quantity, making cotton production dependent on irrigation, which is often carried out with water that has a high salt content (SILVA *et al.*, 2021). The excess salts present in irrigation water, especially Na⁺ and Cl⁻, restrict the ability of roots to absorb water and nutrients from the soil

(SONI *et al.*, 2021). Furthermore, it causes osmotic stress and ionic imbalance, which directly affects plant growth and development (FAROUK; ELHINDI; ALOTAIBI, 2020).

The search for strategies that minimize the effects of saline stress on cotton is essential for the development of cotton farming in semiarid regions. In this sense, the use of growth-promoting bacteria (BPC) can act to induce tolerance to salt stress, bringing positive effects to plants (NASCIMENTO *et al.*, 2023). Within the group (BPC), the genus *Azospirillum* is one of the most studied and used for formulating inoculants in the world due to its ability to fix atmospheric nitrogen and stimulate plant growth (HUNGRIA *et al.*, 2010).

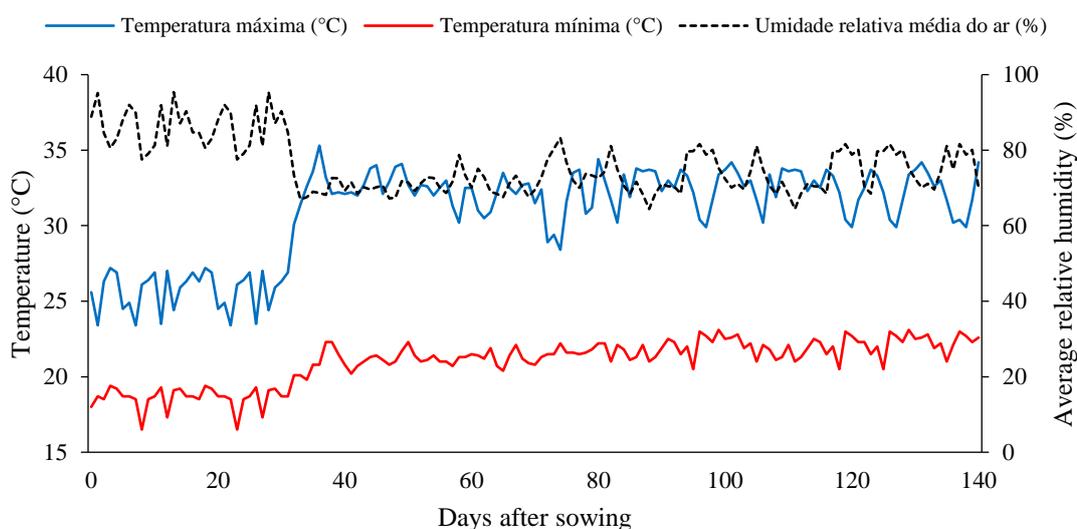
In view of the above, the objective of this study was to evaluate the effect of inoculation with *Azospirillum brasilense* in mitigating the harmful effects caused by irrigation with brackish waters on the growth and production components of the white fiber cotton plant BRS 226.

4 MATERIALS AND METHODS

The experiment was conducted from August 2019 to January 2020 in a protected environment (greenhouse) with translucent glass cover and sides located at EMBRAPA - Brazilian Agricultural Research Corporation, National Cotton Research

Center, located on Rua Osvaldo Cruz, Campina Grande-PB, whose geographic coordinates are 7° 13' 34.8" South latitude, 35° 54' 22.3" West longitude and average altitude of 550 m. The temperature data (maximum and minimum) and average relative humidity at the experiment site are shown in Figure 1.

Figure 1. Maximum and minimum temperature and average relative humidity observed during the experiment.



The treatments resulted from the combination of two factors: five levels of electrical conductivity of irrigation water - CEa (0.4; 3.1; 4.1; 6.1 and 7.1 dS m⁻¹) associated with inoculation or not of cotton seeds with *Azospirillum brasilense* - AZ

(with inoculation and without inoculation), distributed in a completely randomized design, in a 5×2 factorial arrangement, with six replications and one plant per plot (Table 1), making a total of sixty experimental units.

Table 1. Description of the treatments analyzed

	Electrical conductivity of irrigation water (ECa)				
	0.4 dS m ⁻¹	3.1 dS m ⁻¹	4.1 dS m ⁻¹	6.1 dS m ⁻¹	7.1 dS m ⁻¹
No inoculation	S1AZ0	S2AZ0	S3AZ0	S4AZ0	S5AZ0
With inoculation	S1AZ1	S2AZ1	S3AZ1	S4AZ1	S5AZ1

The seeds used in the experiment were those of the BRS 286 cultivar provided by the National Cotton Research Center (CNPA), selected at random. Before sowing, it was necessary to delint the seeds. The BRS 286 cultivar originated from a biparental

crossing between the varieties CNPA ITA 90 and CNPA 7H, which occurred in 2000.

The plants were grown in plastic pots adapted to drainage lysimeters with a capacity of 20 L (35 cm high, 31 cm upper diameter, 20 cm lower diameter), with a fine mesh screen at the base and connected to a

water collector. Water drained through a hose with a diameter of 15 mm. Above the screen, the pots received a 3 cm thick layer of gravel and 24 kg of Neosol Regolith with a sandy-clayey texture collected at a depth of 0-20 cm from the rural area of the municipality of Campina Grande, PB, being duly crushed and sieved, whose chemical characteristics were determined according to the methodology proposed by Teixeira *et al.* (2011): Ca²⁺, Mg²⁺, Na⁺, K⁺, Al³⁺ + H⁺ = 22.7, 9.2, 6.9, 2.27 and 0.0 mmolc dm⁻³, respectively; pH (water 1:2.5) = 7.1; organic matter (g kg⁻¹) = 5.5.

Base fertilization with N, P₂O₅ and K₂O was carried out in accordance with the recommendation of the soil laboratory of the Brazilian Agricultural Research Corporation - EMBRAPA, using ammonium sulfate (nitrogen), monoammonium phosphate (phosphorus) and potassium chloride (potassium) as sources.

Azospirillum brasilense strain (AbV5/AbV6), supplied by the company Total Biotecnologia, based in Curitiba - PR, Brazil. For inoculation, the methodology indicated by the company was used, which consists of mixing the seeds with the substance until they are all surrounded by a uniform layer of inoculant in a proportion of 16 mL of the substance in 10,000 seeds. The entire procedure was carried out in the shade, and then sowing was carried out.

The saline waters were prepared to have an equivalent ratio of 7:2:1 between Na:Ca:Mg from the dissolution of the salts NaCl, CaCl₂·2H₂O and MgCl₂·6H₂O in local supply water (0.4 dS m⁻¹), considering the relationship between ECa and salt concentration according to Richards (1954), according to Eq. 1. After preparing the water, the electrical conductivity was checked and adjusted before use.

$$Q \approx 10 \times Ce_a \quad (1)$$

On what:

Q - sum of cations (mmol c L⁻¹);

CEa - electrical conductivity of water (dS m⁻¹).

Fifteen days after seed emergence, irrigation with saline water began, adopting a two-day irrigation shift, applying water to each lysimeter, according to treatment, to maintain soil moisture close to field capacity, with the applied volume determined according to the water needs of the plants, estimated by the water balance in the soil, being determined by Eq. 2:

$$VI = \frac{(Va - Vd)}{(1 - FL)} \quad (2)$$

On what:

VI - volume of water to be used in the irrigation event (mL);

Va - volume of water applied in the previous irrigation event (mL);

Vd - volume drained after the previous irrigation event (mL);

FL - leaching fraction of 0.10, applied every 30 days to avoid excessive accumulation of salts.

The effects of the treatments were evaluated by analyzing the growth variables: plant height - AP (cm), stem diameter - DC (mm), number of leaves, leaf area - (cm²) and dry phytomass of the aerial part - FSPA (g per plant) and of the production components: number of bolls - NC, number of seeds - NS, seed mass - MS (g per plant) and cotton lint mass - MAP (g per plant). The production components were quantified at the end of the production cycle (140 DAS), according to the Embrapa Algodão methodology.

The growth of the BRS 286 cotton plant was measured 110 days after sowing. Plant height (AP) was measured taking as a reference the distance from the plant neck to the insertion of the apical meristem, the stem diameter (DC) was measured 2 cm from the plant neck, the number of leaves (NF) was obtained by counting fully expanded leaves with a minimum length of 3 cm in each plant and the leaf area was determined, according

to the equation of Grimes and Carter (1969), measuring the length of the main vein of all leaves of the plant, considering it if Eq. 3.

$$AF = \sum(0,4322 \times P)^{2,3002} \quad (3)$$

On what:

AF = leaf area (cm²); It is

P = length of the central rib.

The FSPA was determined at the end of the production cycle (140 DAS). To obtain dry phytomass, the stem of each plant was cut close to the ground, and then the different parts (stem and leaf) were separated and placed in a paper bag; subsequently, they were placed to dry in an oven with forced air ventilation at a temperature of 65 °C until constant weight was obtained. Subsequently, the material was weighed, obtaining the dry phytomass of the leaves (FSF) and the stem (FSC), with the FSPA being the sum of the dry phytomass of the leaves and the stem.

The multivariate structure of the results was evaluated through principal component analysis (PCA), synthesizing the amount of relevant information contained in the original data set in a smaller number of dimensions, resulting from linear combinations of the original variables generated from the eigenvalues ($\lambda \geq 1.0$) in the correlation matrix, explaining a percentage greater than 10% of the total variance (GOVAERTS *et al.*, 2007).

After reducing the dimensions, the original data of the variables of each component were subjected to multivariate

analysis of variance (MANOVA) using the Hotelling test *et al.* (1947) at 0.05 probability for the electrical conductivity levels of irrigation water and the inoculation or not of cotton seeds with *Azospirillum brasilense*, as well as for interaction between them. Only variables with a correlation coefficient greater than or equal to 0.6 were kept in each principal component (PC) (HAIR *et al.*, 2009). For statistical analyses, we used the software Statistica v. 7.0 (STATSOFT, 2004).

5 RESULTS AND DISCUSSION

According to the summary of multivariate analysis of variance (Table 2), the original variables were presented in two main components (CP1 and CP2) with eigenvalues greater than $\lambda \geq 1.0$, according to Kaiser (1960). The eigenvalues and percentage of variation explained for each component (Table 2) together represented 91.63% of the total variation. CP1 explained 77.87% of the total variance, formed by most of the variables analyzed, except the number of leaves (NF). CP2 represented 13.76% of the remaining variance, being formed by the NF variable.

ECa), and the inoculation or noninoculation of cotton seeds with *Azospirillum* was observed. *Brazilian* (AZ) for the two main components (CP1 and CP2). A significant effect ($p \leq 0.01$) of the factors was also found when analyzed in isolation.

Table 2. Eigenvalues, percentage of total variance explained, in multivariate analysis of variance (MANOVA) and correlation coefficients (r) between original variables and the main components.

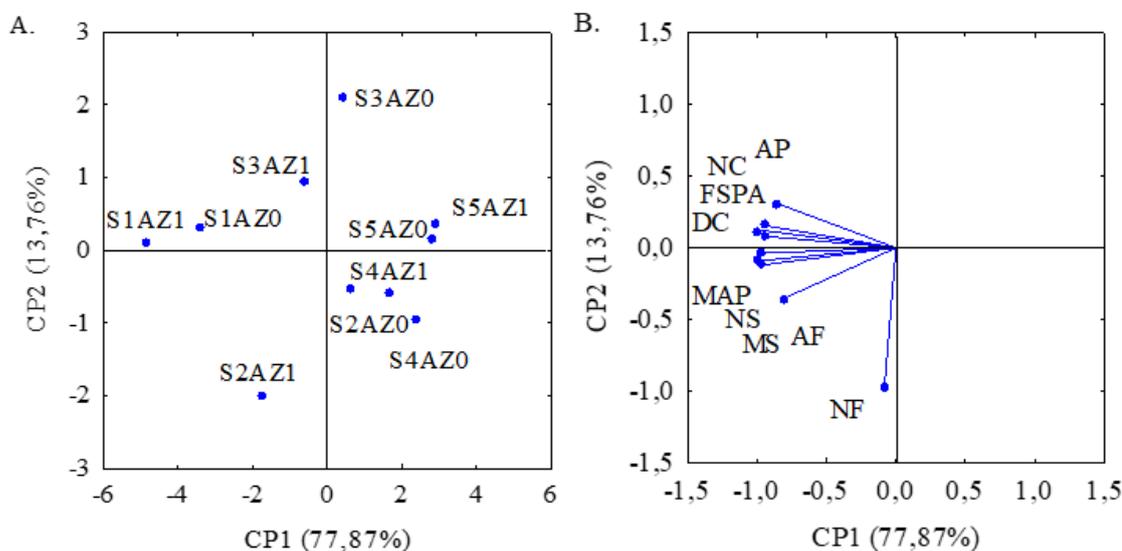
		Componentes principais							
		CP1	CP2						
Autovalores (λ)		7,01	1,24						
Porcentagem de variância total ($S^2\%$)		77,87	13,76						
Teste de Hotelling (T^2) para condutividade elétrica da água (CEa)		0,01	0,01						
Teste de Hotelling (T^2) para inoculação de <i>Azospirillum brasilense</i> (AZ)		0,01	0,01						
Teste de Hotelling (T^2) para interação (CEa \times AZ)		0,01	0,01						
CPs	Coeficiente de correlação								
	AP	DC	NF	AF	FSPA	NC	NS	MS	MAP
CP1	-0,85	0,95	-0,08	-0,81	-0,99	0,94	-0,97	-0,98	-0,99
CP2	0,31	0,08	-0,97	-0,35	0,13	0,15	-0,04	-0,12	-0,09
Tratamentos	Valores médios								
	AP	DC	NF	AF	FSPA	NC	NS	MS	MAP
S1AZ0	80,60	7,40	31,33	1816,2	14,00	7,67	190,17	19,13	13,98
S2AZ0	74,67	7,02	40,83	1136,6	10,24	6,33	152,67	14,15	10,36
S3AZ0	77,33	6,88	31,67	1088,9	9,45	7,00	168,50	14,56	9,80
S4AZ0	68,33	6,48	22,00	1047,1	6,65	5,50	143,17	12,32	8,59
S5AZ0	73,83	6,22	31,00	877,7	6,91	5,33	132,67	11,39	8,03
S1AZ1	83,85	7,82	30,17	2436,3	16,67	9,00	201,50	21,36	15,14
S2AZ1	73,17	7,40	57,00	1640,5	11,32	7,67	186,00	18,49	12,56
S3AZ1	74,71	7,38	20,17	1586,0	10,83	8,00	156,50	14,52	10,59
S4AZ1	68,33	6,80	33,67	1476,0	7,05	5,50	145,83	13,52	8,88
S5AZ1	65,83	6,77	25,17	893,8	6,60	5,67	128,33	11,29	7,75

S1 (0.4 dS m⁻¹); S2 (3.1 dS m⁻¹); S3 (4.1 dS m⁻¹); S4 (5.1 dS m⁻¹); S5 (7.1 dS m⁻¹); AZ0 (without *Azospirillum inoculation Brazilian*); AZ1 (inoculated with *Azospirillum Brazilian*); AP (plant height - cm); DC (stem diameter - mm); NF (number of sheets); AF (leaf area - cm²); FSPA (dry biomass of the aerial part - g per plant); NC (number of bolls per plant); NS (number of seeds per plant); DM (seed mass - g per plant); MPA (mass of cotton lint - g per plant).

The two-dimensional projections of the effects of treatments and variables on the first and second principal components (CP1 and CP2) are shown in Figure 2A and 2B. In the first main component (CP1), a process was identified, possibly characterized by the

effect of the interaction between the electrical conductivity levels of the irrigation water and the inoculation of *Azospirillum brasilense*. The correlation coefficients for AP, DC, NF, AF, FSPA, NC, NS, MS and MAP were greater than 0.80 (Table 2).

Figure 2. Two-dimensional projection of the main component scores for the electrical conductivity factors of irrigation water (S) and *Azospirillum brasilense* inoculation (AZ) (A) and the analyzed variables (B) in the two main components (CP1 and CP2).



In main component 1, it is possible to notice that cotton plants irrigated with ECa of 0.4 dS m^{-1} that had their seeds inoculated with *Azospirillum brasilense* (S1AZ1) stood out in relation to the other treatments, presenting the highest values (Table 2) of AP (83.85 cm), DC (7.82 mm), AF (2436.6 cm^2), FSPA (16.67 g per plant), NC (9 bolls), NS (201.5 seeds), MS (21.36 g) and MAP (15.14 g). When comparing the results obtained in plants from the S1AZ1 treatment with those grown under S1AZ0, an increase of 4.03% (3.25 cm) was observed; 5.68% (0.42 mm); 34.14% (620.1 cm^2); 19.07% (2.67 g); 17.34% (1.33 bolls); 5.96% (11.33 seeds); 11.66% (2.23 g) and 8.30% (1.16 g) in AP, DC, AF, FSPA, NC, NS, MS and MAP, respectively.

Inoculation with *Azospirillum brasilense* also promoted an increase in AF, FSPA, NC, NS, MS and MAP of cotton irrigated with ECa of 3.1 dS m^{-1} (Table 2), demonstrating the effect of *Azospirillum brasilense* in inducing tolerance to salinity. For main component 2 (PC 2), inoculation with *Azospirillum brasilense* promoted an increase in the number of leaves of cotton

irrigated with a CEa of 3.1 dS m^{-1} (Table 2), obtaining the highest NF value (57).

Azospirillum brasilense promotes plant growth through different mechanisms, including the synthesis of phytohormones (for example, auxins, cytokinins and gibberellins) and other important compounds, which contribute to root development, favoring the absorption of water and nutrients (FUKAMI *et al.*, 2018). Another important advantage is its ability to fix nitrogen in plant roots (NASCIMENTO *et al.*, 2023). Nitrogen is an essential constituent of many biomolecules, such as enzymes, structural proteins, nucleic acids, porphyrins, alkaloids and N-glycosides, and plays a crucial role in several physiological processes in plants (LEGHARI *et al.*, 2016).

Due to the great importance of nitrogen, many studies have been carried out aiming to maximize the efficiency of N use by plants through biological nitrogen fixation (BNF) or nitrogen absorption by the soil (SILVEIRA *et al.*, 2016). In this sense, inoculation with *Azospirillum brasilense* can increase nitrogen (N) mobility, bringing positive effects to plants under saline stress. For Ashraf *et al.* (2018), an adequate

nitrogen concentration can contribute to the greater synthesis of low molecular weight compounds, such as glycine, betaine and proline, which act as membrane osmoprotectors and macromolecules, which help in the osmotic adjustment of plants to salinity.

It is worth mentioning that irrigation with ECa above 3.1 dS m⁻¹ negatively affected cotton growth and production components, regardless of inoculation with *Azospirillum brasilense*. Salt stress reduces the activity of ions in solution and alters the processes of absorption, transport, assimilation and distribution of nutrients in the plant, consequently causing a reduction in growth and production components (SILVA *et al.*, 2022).

Similar results were observed in other studies with cotton; for example, Silva *et al.* (2020b), evaluating the production of cotton genotypes (BRS Safira and BRS 368 RF) under saline stress (ECa ranging from 1.5 to 7.5 dS m⁻¹), found that irrigation with ECa from 1.5 dS m⁻¹ caused reductions in all production variables analyzed. In research developed by Capitulino *et al.* (2020), it was found that irrigation with water with electrical conductivity above 1.5 dS m⁻¹ negatively affected the growth and production of colored fiber cotton, with cotton lint mass being the most sensitive variable to saline stress.

6 CONCLUSION

The increase in the electrical conductivity of irrigation water reduced the growth and production components of cotton BRS 286. However, the inoculation of seeds with *Azospirillum brasilense* mitigated the deleterious effects of saline stress on the leaf area and production components when subjected to a CEa of up to 3.1 dS m⁻¹. Irrigation with an ECa of 0.4 dS m⁻¹ associated with inoculation of seeds with *Azospirillum brasilense* resulted in the

highest values of leaf area, dry phytomass of the aerial part, number of bolls, number of seeds, seed mass and cotton mass in feathers.

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