Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Gas exchange and osmotic adjustment in cotton cultivars subjected to severe salt stress

Trocas gasosas e ajuste osmótico em cultivares de algodão submetidas a estresse salino severo

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Abstract

Salinity is harmful to crops when the concentration of soluble salts overcomes the salinity threshold of the crop, causing osmotic stress and limitations in plant growth. In this scenario, adopting tolerant cultivars is the most adequate strategy to minimize agricultural losses. However, the inheritance of tolerance depends on the genotype. From this perspective, this study assessed the tolerance to severe salt stress in 11 cotton cultivars based on gas exchange parameters and the free proline content. The cultivars were grown in a greenhouse and subjected to 34 days of saline irrigation (10 dS m^{-1}), starting 45 days after seedling emergence (B1 phase). Plant growth was monitored weekly until the end of the salt stress period. The treatments consisted of a combination of two factors: eleven cultivars associated with two electrical conductivity levels of irrigation water (ECw: 0.3 and 10.0 dS m^{-1}). The experimental design was in randomized blocks in a 11 × 2 factorial arrangement with three replications (66 plots), with the experimental unit consisting of one plant per plot. Salinity impacted plant growth, being reflected on the gas exchange and free proline data of most cultivars. However, BRS 286, FMT 705, BRS 416, and BRS Acácia, and CNPA 7MH withstood the effects of stress and osmotically adjusted to the salt stress conditions, thus minimizing the damage to growth. Those cultivars are the most indicated for improvement programs aiming at tolerance to salt stress based on the results found in this research.

Keywords: *Gossypium hirsutum* L., abiotic stress, physiology.

Resumo

A salinidade é danosa as lavouras quando a concentração de sais solúveis ultrapassa a salinidade limiar da cultura, gerando estresse osmótico e limitações no crescimento das plantas. A adoção de cultivares tolerantes é a estratégia mais adequada para minimizar prejuízos agrícolas, porém a herança de tolerância é genótipo-dependente. Neste trabalho, a tolerância ao estresse salino severo em onze cultivares de algodão foi avaliada, baseando-se nas trocas gasosas e no teor de prolina livre. As cultivares foram crescidas em casa de vegetação e submetidas a 34 dias de irrigação salina (10 dS m^{-1}), iniciada aos 45 dias de emergência das plântulas (fase B1). O crescimento das plantas foi monitorado semanalmente até o final do período de estresse salino. Os tratamentos consistiram na combinação de dois fatores: 11 cultivares, associadas a dois níveis de condutividade elétrica da água de irrigação (0,3 e 10,0 dS m^{-1}). O delineamento foi em blocos ao acaso, em esquema fatorial 11 × 2, com 3 repetições, sendo a unidade experimental constituída por uma planta por parcela. A salinidade afetou o crescimento das plantas, refletido nos dados de trocas gasosas e prolina livre na maioria das cultivares, no entanto, BRS 286, FMT 705, BRS 416 e BRS Acácia e CNPA 7MH demonstraram habilidade para superar o efeito do estresse e se ajustar osmoticamente, minimizando os danos no crescimento. Essas cultivares são as mais indicadas para trabalhos de aprimoramento visando a tolerância ao estresse salino, de acordo com os resultados encontrados neste trabalho.

Palavras-chave: *Gossypium hirsutum* L., estresse abiótico, fisiologia.

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Received: May 4, 2023 – Accepted: October 4, 2023

1. Introduction

Salinity is a global soil issue that severely compromises the productivity of several crops. The worldwide extent of saline and sodic soils is 831 million ha, of which 46 million ha are in irrigated areas (FAO, 2022). However, despite those numbers, saline regions increase by nearly 10% every year due to climate change and inefficient management of irrigated areas, especially in arid and semi-arid regions where edaphoclimatic conditions such as low rainfall, high temperatures, and high evapotranspiration rates favor salt accumulation (Yadav et al., 2011; Hatam et al., 2020).

Saline soils are those with high levels of dissolved salts and high concentrations of sodium ions adsorbed in the soil matrix (Jesus et al., 2015). Na^+ accumulation due to irrigation with saline water damages the soil's colloidal aggregates, altering the soil structure and reducing permeability, thus impacting the clay-humic complex and interfering with the absorption of elements such as Ca^{++}, Mg^{++}, and K^+ (Navarro-Torre et al., 2023).

According to Machekposhti et al. (2017), even the use of quality water transports salts to the soil, thus increasing the initial salinity. When the concentration of salts in the external environment overcomes the tolerance threshold of crops, osmotic stress begins, increasing the difficulty in water absorption since the osmotic potential of the soil solution becomes more negative, which requires greater energy expenditure by plants. Such an imbalance can remove water from the cells, leading to dehydration and reducing the cytosolic and vacuolar volumes, so that the loss of intracellular water may set off a state of physiological drought. When the accumulation of unfavorable ions such as Na^+ is prolonged, plants suffer damage from ionic stress, manifesting cellular toxicity symptoms due to the imbalance in ionic homeostasis (Abdelraheem et al., 2019).

At the physiological level, the problems caused by salt stress include osmotic imbalance, stomatal closure, reduction in photosynthesis, deformation of chloroplasts, and ionic alterations. Such events may lead to toxicity, nutritional imbalance, and inhibition of cell division (Parida and Dias, 2005; Aslam and Bostan, 2011; Zhang et al., 2014).

Several studies state that tolerance to salinity depends on the genotype. Cultivars of the same species may show a variation in tolerance levels as a function of physiological and biochemical adjustments that are triggered when the stress signal is established (Uma and Patil, 1996; Zhang et al., 2014). Glycophytic species have greater sensitivity to salinity compared to halophytic ones, which have defense mechanisms that give them the ability for osmotic adjustment as well as compartmentalization and ionic exclusion, thus preventing the accumulation of toxic salt levels in the cytoplasm (Parida and Dias, 2005).

The cotton plant (*Gossypium hirsutum* L.) is a glycophytic species moderately tolerant to salinity, with a threshold of 5.1 dS m^{-1} in irrigation water and 7.7 dS m^{-1} in the soil saturation extract (Ayers and Westcot, 1999). Tolerance varies with the germplasm, growth phase, and stress duration. Zhang et al. (2014) tested cultivars sensitive and tolerant to salinity using increasing salt levels (80 to 240 mM NaCl) in the nutrient solution for seven days. The authors reported an effective protection mechanism in the tolerant cultivar, including mitigation of oxidative stress, lipid peroxidation, and an adequate performance in stomatal conductance, chlorophyll, and CO_2 assimilation. Superoxide Dismutase (SOD) activity also increased in leaves and roots, indicating a greater ability to reduce the oxidative damage produced by reactive oxygen species (ROS).

In another study, Wang et al. (2017) tested different biological strategies to assess the tolerance of cultivars subjected to NaCl (0.3%) for 72 h, obtaining expressive responses via molecular tools. According to those authors, the tolerant cultivar showed some effective physiological mechanisms, e.g., the ability to regulate K^+ and Na^+ transport, higher photochemical efficiency, and better antioxidant defense capacity. However, lower ROS values were found in mid-tolerant materials, which was attributed to a higher selective absorption of K^+ over Na^+ in roots across the membranes through SOS1 (plasma membrane Na^+/H^+ antiporter), AKT1 (inward-rectifier potassium channel), and HAK5 (high-affinity K^+ transporter) transcripts during salinity stress.

In Brazil, cotton is grown in regions of semi-arid climate and in the Cerrado ecosystem, with brackish water being commonly used for irrigation in order to preserve good water sources for other purposes. Adopting tolerant cultivars to saline environments is the most viable strategy to minimize possible losses in boll production. Also, knowing the sources of resistance to unfavorable conditions is essential for genetic improvement programs aiming at the introgression of favorable genes, with biological tools contributing significantly to selection procedures. From this perspective, aiming at contributing information about tolerance to salinity, this study assessed the behavior of a group of commercial cultivars under severe salt stress based on gas exchange and free proline data.

2. Material and Methods

2.1. Germplasm and assays

Seeds of 11 cotton cultivars (Table 1) were sown in pots (1 L) containing commercial substrate (Basaplant, Base) and maintained in a greenhouse under a 12/12 h light/dark photoperiod at 21.5 °C/43 °C (min/max) and 40%/86% relative air humidity. The seedlings were watered daily (0.3 dS m^{-1}) by maintaining 100% water retention capacity in the substrate (Oliveira et al., 2012).

The treatments consisted of a combination of two factors: eleven cultivars associated with two electrical conductivity levels of irrigation water (ECw: 0.3 and 10.0 dS m^{-1}). The experimental design was in randomized blocks, in 11 × 2 factorial arrangement with three replications (66 plots). The experimental unit consisted of one plant per plot. At the B1 stage (45 days after emergence), one seedling was randomly selected in each plot, and the salinity treatment was initiated, lasting for 34 days. An NaCl solution was added to the soil in each pot of the stressed treatments, maintaining the water electric conductivity (ECw) at 10 dS m^{-1}.

Table 1. Description of cotton cultivars used in this study.

Cultivar	Fiber	Owner	Cycle	Recommendation
1- BRS Seridó	White	Embrapa	Mid	SA/dry season
2- BRS 286	White	Embrapa	Mid	SA and SAV/dry season
3- FM 966	White	Bayer Crop seeds	Late	SAV/water season
4- CNPA 7MH	White	Embrapa	Earliness	SA/dry season
5- FMT 701	White	FMT	Late	SAV/dry season
6- CNPA ITA 90	White	Embrapa	Late	SAV/dry season
7- FMT 705	White	FMT	Late	SAV/water season
8- BRS RUBI	Brown	Embrapa	Earliness	SA/dry season
9- BRS 416	White	Embrapa	Mid	SA and SAV/dry season
10- MT 152	White	Embrapa	Mid	SA and SAV/dry season
11- BRS Acácia	White	Embrapa	Mid	SA, irrigated/SAV, dry season

Legend: SA: semiarid, SAV: Cerrado.

2.2. Growth and gas exchange

The plant height values were recorded during four weeks in each treatment, starting after 13 days of salt stress.

The gas exchange parameters were obtained through measurements performed in the third fully expanded leaf counting from the apex, collected after five and 34 days of salt stress, corresponding to phases B2 (second bud on the first reproductive branch) and F1 (occurrence of first flower), respectively. The intercellular CO_2 concentration (*Ci* - μmol CO_2 mol^{-1}), transpiration (*E* - mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs* - mol m^{-2} s^{-1}), and CO_2 assimilation rate (*A* - μmol CO_2 m^{-2} s^{-1}) were estimated using an Infra-Red Gas Analyzer (IRGA, ADC BioScientific Ltd, mod. LC-Pro) under natural conditions of air temperature, CO_2 concentration, and artificial radiation of 1,200 μmol m^{-2} s^{-1}. The instantaneous carboxylation efficiency (*iCE* - A/Ci) [(μmol m^{-2} s^{-1}) (μmol CO_2 mol^{-1})$^{-1}$] and instantaneous water use efficiency (*iWUE* - A/E) [((μmol m^{-2} s^{-1}) (mmol of H_2O m^{-2} s^{-1})$^{-1}$] were determined according to Dutra et al. (2018).

2.3. Free proline

Proline was estimated in the leaves of cotton plants in the B2 phase using the methodology described in Bates et al. (1973). Briefly, 3% (w/v) sulfosalicylic acid was used to homogenize the leaves (100 mg). The supernatant was supplemented with ninhydrin (47 mM), phosphoric acid (0.8 M), and glacial acetic acid (0.25 M) and further heated (98 °C) for 1 h. After cooling, the mixture was extracted with 0.3 vol of toluene, and the absorbance was read at 520 nm. The accumulated proline content was estimated through a standard curve and calculated based on the fresh weight. The analyses were performed in biological and experimental triplicates.

2.4. Statistical analyses

The data were tested for normality of distribution (Shapiro and Wilk, 1965) with subsequent analysis of variance (ANOVA) by the F-test using the software Sisvar (Ferreira, 2019). The multivariate (Principal Component Analysis - PCA) analysis was performed using the software Statistica v. 7.0 (Statsoft, 2004).

3. Results and Discussion

3.1. Growth of the cultivars under salt stress

Plant height was influenced by salt stress throughout plant growth, with different effects observed in the cultivars as a function of the C x S interaction (Table 2). The growth reduction was phenotypically noticeable early during the stress, especially in the cultivars BRS Seridó, BRS 286, FMT 705, and MT 152 (Figure 1). Only the cv. BRS Acácia had a virtually unaltered height throughout the assay, with a small reduction of just 4% at the end of the evaluation, after 34 days of salt stress (Figure 2). Cultivars BRS Rubi, FMT 701, and FMT 705 also remained adequate, with reductions of 10%, 11%, and 13%, respectively, which are reasonable values given they were all in the reproductive phase and the high ECw to which they were subjected. In the literature, studies involving salinity in cotton usually report the negative effects of salt on plant growth, even at electrical conductivity values lower than those used in the present study.

Silva et al. (2017) subjected the cultivar BRS Topázio to salinity at the ECw of 6 dS m^{-1} for 53 to 108 days and found moderate tolerance based on the variables used. The height reduction in plants ranged from 32% to 46% after 53 and 108 days of stress, respectively. Such values are close to those obtained with the cultivars of this study, although they were tested under more severe ECw conditions (10 dS m^{-1}). According to Souza et al. (2017), who studied the cv. BRS Topázio from emergence to early reproduction, this cultivar is tolerant to salinity and can be cultivated in soils with exchangeable sodium percentages up to 38.8%. The cv. BRS Topázio, as well as the cv. BRS Rubi used in this study, are colored-fiber cotton cultivars developed by EMBRAPA and are recommended for the semi-arid regions of Brazil (Furtado et al., 2013).

Table 2. Summary of the ANOVA for the plant height (cm) of cotton cultivars subjected to salt stress. Assessment performed at 13, 20, 27, and 34 days after the beginning of treatments.

SV	DF	Mean square			
		13	20	27	34
Cultivars (C)	10	98.77 **	96.88 **	102.66 **	108.01**
Salinity (S)	1	627.45 **	967.9 **	1258.91 **	1787.76 **
Interaction C x S	10	54.60 **	59.04 **	58.34 **	71.18 **
Blocks	2	5.39 ns	17.52 ns	9.91 ns	8.23 ns
Residual	42	6.11	6.40	6.80	7.25
CV (%)	-	6.1	5.9	6.0	6.0

SV- Source of variation, DF- degree of freedom, ns - not significant; **- significant ($p \leq 0.01$, F-test); CV- Coefficient of variation.

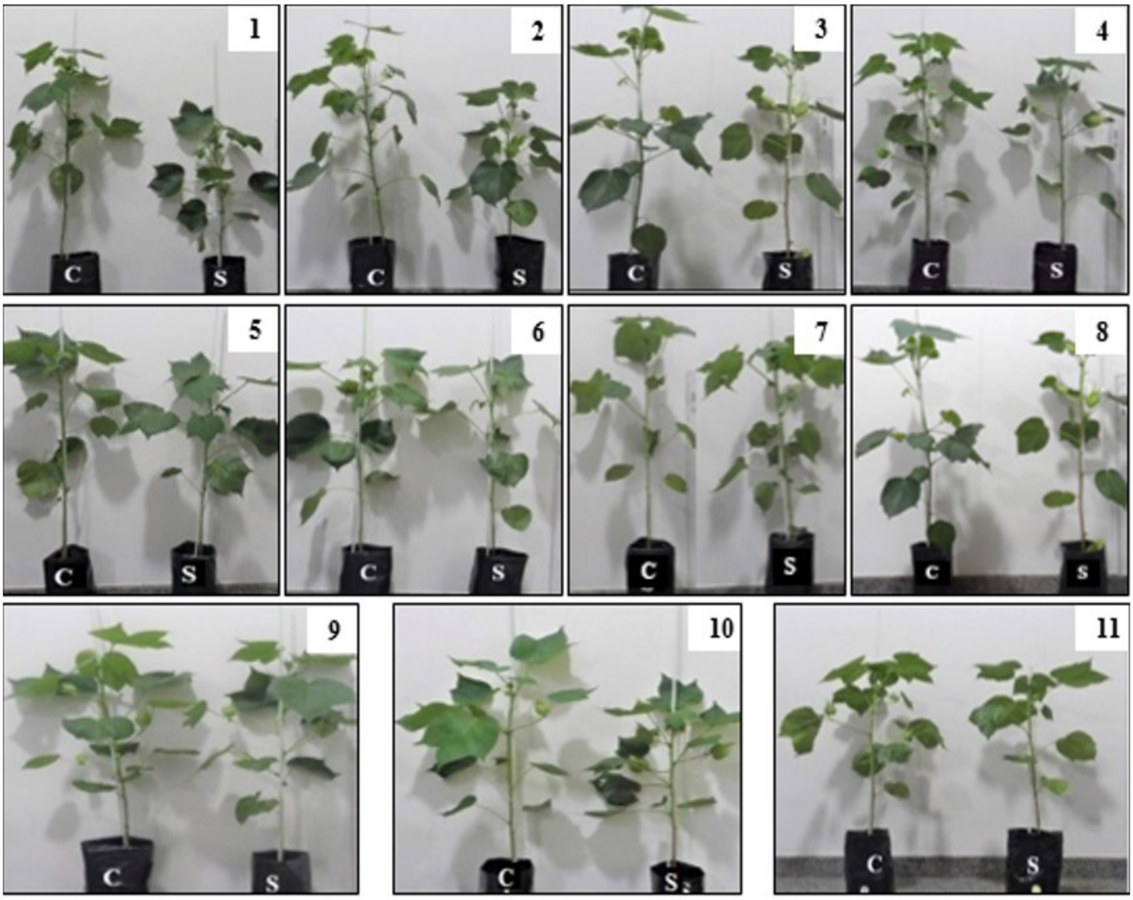

Figure 1. Profile of the cotton cultivars subjected to 13 days of severe salt stress. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7- FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11- BRS Acácia. C - control, S- stress.

With genetically modified cotton, Dias et al. (2020) subjected the cultivar BRS 368 RF to salinity at different ECw values (0.7 to 6.7 dS m^{-1}) for 107 days, observing growth reductions even at the minimum concentration. According to those authors, plant height was linearly inhibited by the increase in salinity, reaching 65.13% at 6.7 dS m^{-1} at the end of the assay.

The height reduction in plants grown in saline environments is one of the first phenotypically visible symptoms due to osmotic damage or ion toxicity as a function of salt accumulation in leaves, affecting cell turgor and expansion (Acosta-Motos et al., 2017; Dias et al., 2020). The tolerant genotypes also exhibited salt stress symptoms. However, they responded by using other strategies to escape the damage caused by stress, e.g., redirecting energy to maintaining biochemical and physiological activities, in addition to the compartmentalization and exclusion of Na^+ ions (Zhang et al., 2014; Sharif et al., 2019). This response could explain the behavior of the cv. BRS Acácia in the plants that received the experimental treatments, which remained virtually similar to the control plants (Figures 1 and 2). Such a result corroborates Braz et al. (2019), who subjected the cv. BRS Acácia to 95 mM NaCl for 72 h, starting in phase V3 (21 DAE). The authors found that the cultivar reduced evapotranspiration and water consumption as a strategy to decrease the uptake of ions from the soil. This mechanism explained the good performance of this material when grown in a saline environment.

3.2. Free proline and gas exchange in cotton cultivars subjected to salt stress

Proline accumulation in the plants was assessed five days after the beginning of stress, whereas the gas exchange parameters were estimated five and 34 days after the onset of stress. Statistically significant differences were found between cultivars, salinity levels, and for the effect of the C x S interaction, indicating the cultivars responded differently to the ECw imposed and used peculiar adjustments to withstand stress (Table 3). Most cultivars showed an increase in proline accumulation (Figure 3), with peaks of 249% and 147% for BRS Seridó and BRS 286, respectively, indicating that those materials invested differently in their cell reserves to adjust osmotically since the increase in proline levels in plants under stress is a response to maintain cell integrity and support the antioxidative defense (Macêdo et al., 2019; Per et al., 2017).

The greater the input of this osmolyte in the cell, the higher the demand of energy required to prevent cell damage. The accumulation seen in this research was close to the mean values found by Guo et al. (2020), who subjected cotton plants to different soil salinity levels using NaCl, Na_2SO_4, and Na_2CO_3 + $NaHCO_3$. Those authors reported a significant increase in proline accumulation, amounting to 230% in the treatment with 1.39 dS m^{-1} (NaCl) and 264% in alkaline soil (Na_2CO_3 + $NaHCO_3$ 0.63 dS m^{-1}). In a study with two contrasting cotton cultivars, Zhang et al. (2014) reported that salt concentrations up to 240 mM NaCl increased the level of free proline in both cultivars, but more so in the sensitive cultivar, which was more affected by the increase in salinity.

With regard to the gas exchange parameters, the cultivars showed significant statistical differences in all variables analyzed at five and 34 days of salt stress in both treatments, with a strong effect of the C x S interaction (Figures 4 and 5). Overall, the cultivars BRS 286, FMT 705, BRS 416, and BRS Acácia were the most effective in fighting salt stress based on the results of stomatal conductance (*gs*), CO_2 assimilation rate (*A*), instantaneous carboxylation efficiency (*iCE*), and instantaneous water use efficiency (*iWUE)*, which did not differ statistically from the control in both phases analyzed. These results suggest a tolerance condition since the plants maintained the capacity of adjusting to adverse conditions with little or no reduction in the CO_2 assimilation rate (Figure 4), leading to an increase in the maintenance of the *iWUE* (Figure 5).

Table 3. Summary of the ANOVA for proline and gas exchange parameters in the cotton cultivars subjected to salinity.

SV	DF	5 days of salt stress						
		Mean square						
		Proline	***Ci***	***E***	***gs***	***A***	***iCE***	***iWUE***
Cultivars (C)	10	0.030 **	2945.0 **	0.87 **	0.0030 **	18.2 **	0.0005 **	2.34 *
Salinity (S)	1	0.110 **	6842.0 **	1.32 **	0.0003 **	157.3 **	0.0041 **	10.10 **
C x S	10	0.006 **	4822.0 **	1.18 **	0.0010 **	14.3 **	0.0002 *	2.04 *
Blocks	2	0.001	394.6	0.43	0.0008	7.3	0.0002	0.85
Residual	42	0.001	202.4	0.10	0.0003	3.1	0.0000	0.85
CV (%)	-	9.4	6.1	13.0	13.2	16.9	20.9	21.3
		34 days of salt stress						
			Ci	*E*	*gs*	*A*	*iCE*	
Cultivars (C)	10		1943.07 **	0.215 **	0.001 **	13.44 **	0.001 *	
Salinity (S)	1		6206.06 **	4.682 **	0.011 **	193.0 **	0.007 **	
C x S	10		2277.01 **	0.480 **	0.003 **	21.60 **	0.000 **	
Blocks	2		1611.40	0.281	0.001	6.92	0.001	
Residual	42		510.43	0.063	0.000	3.34	0.000	
CV (%)	-		11.6	22.9	32.3	7.3	32.7	

SV- Source of variation, DF- degree of freedom; *; **- significant ($p \leq 0.05$ and $p \leq 0.01$, respectively by the F-test); CV- Coefficient of variation. C- Cultivar, S - salinity, *Ci* - Intercellular CO_2 concentration, *E* - Transpiration, *gs* -Stomatal conductance, *A* - CO_2 assimilation rate, *iWUE* - Instantaneous water use efficiency, and *iCE* - Instantaneous carboxylation efficiency.

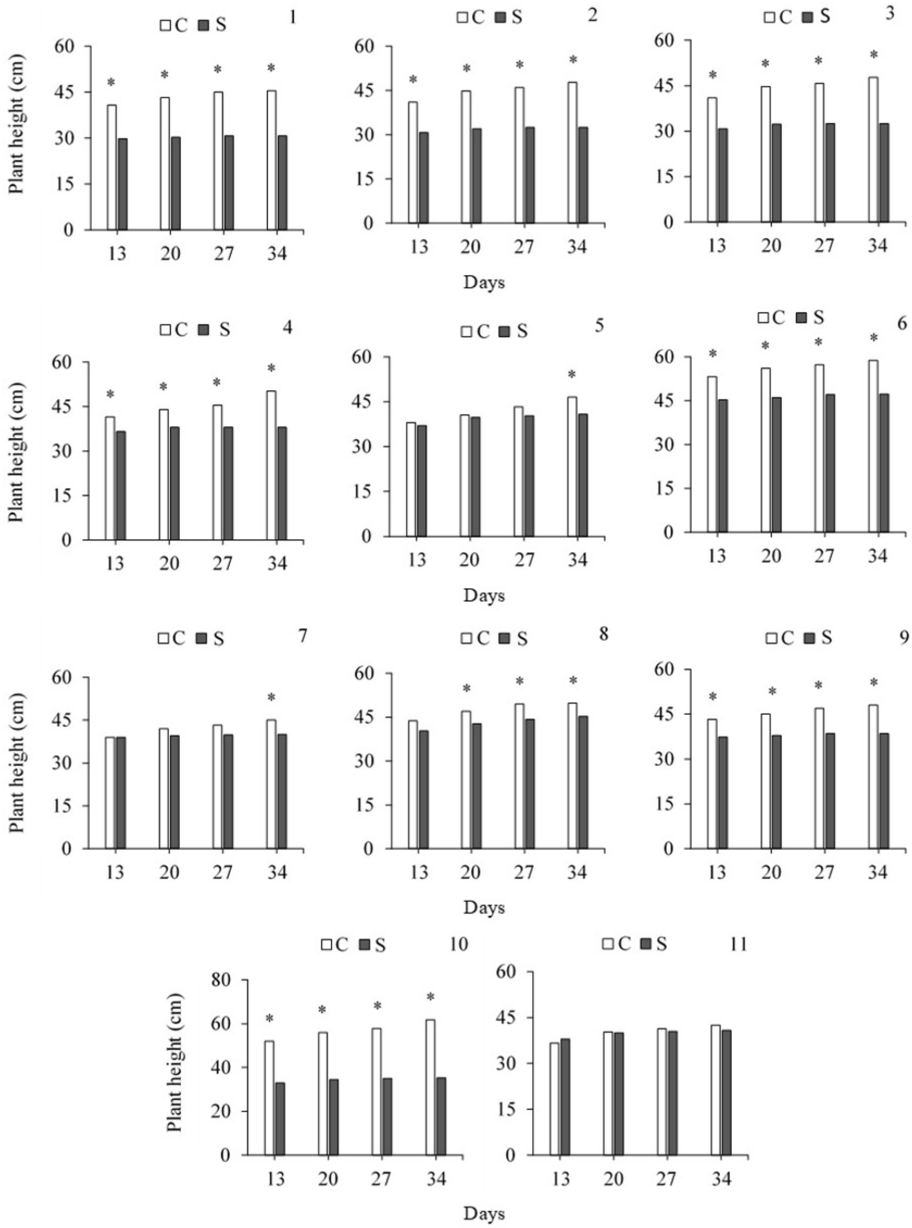

Figure 2. Height of cotton cultivars subjected to salt stress and evaluated at 13, 20, 27, and 34 days after the beginning of treatments. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7- FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11- BRS Acácia. C - control, S- stress. * between treatments indicates a significant statistical difference by the F-test (p ≤ 0.05).

Another interesting result was seen in the cv. CNPA 7MH, which showed reductions of 33% and 44% in the CO_2 assimilation rate (*A*) and instantaneous carboxylation efficiency (*iCE*) in the beginning of stress, respectively. However, by the end of stress, the cultivar adjusted so as to have greater control of stomatal conductance (*gs*) and *A*, with consequent maintenance of *iCE* (Figure 5 at five days). That strategy matches the genetic base of the cultivar, which responds satisfactorily when it faces water stress situations in a semi-arid environment (Farias et al., 1997).

Cultivars BRS Seridó, FM 966, CNPA ITA 90, and BRS Rubi were the most sensitive to the EC_w adopted in this study, showing worsened physiological effects by the end of stress based mainly on the CO_2 assimilation rate, with reductions of 37%, 36%, 39%, and 42%, respectively (Figure 4). In the cultivars BRS Seridó and FM 966, the drop in *A* occurred as a result of high transpiration rates and reduced carboxylation efficiency over the period assessed. In a study by Marcelino et al. (2022), who subjected those same cultivars to moderate salinity

(6 dS m^{-1}) for 35 days, the authors reported that the cultivars BRS Seridó, CNPA ITA 90, and BRS Rubi behaved as tolerant cultivars as a function of the adjustment seen in the *gs*, *E*, and *A* of plants under stress. Under the severe stress condition used in the present study, tolerance changed due to the greater accumulation of salts in the cells, affecting the *A* and *iWUE*. According to the literature, the reduction in *A* is a response to the reduction in the osmotic potential of the soil due to the increase in the content of salts in irrigation water and the accumulation of Cl^- and Na^+ ions, which hinders compartmentalization by the cell (Zhang et al., 2014; Peng et al., 2016; Dias et al., 2020).

Cultivars FMT 701 and MT 152 were the most sensitive, exhibiting drastic reductions in the CO_2 assimilation rate of 81% and 70%, respectively, which compromised the adjustment of the other physiological descriptors (Figure 4). The result of the cv. FMT 701 was also observed by Braz et al. (2019), who subjected the same cultivar to severe salt stress (approximately 95 dS m^{-1}) for 72 h, verifying that the genotype did not adapt to irrigation water with excess salts.

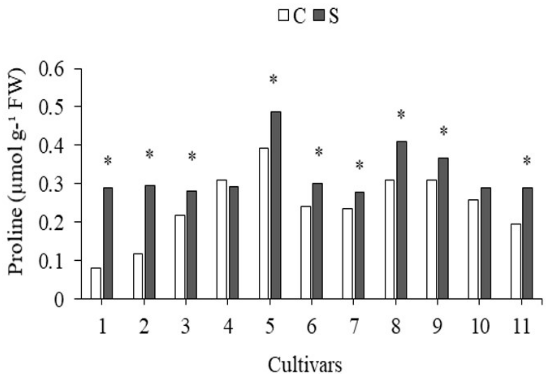

Figure 3. Accumulation of free proline in the cotton cultivars subjected to five days of severe salt stress (10 dS m^{-1}). Cultivars 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11 - BRS Acácia. C - control, S- stress. *between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

3.3. Clustering of cultivars via Principal Component Analysis (PCA)

The Principal Component Analysis was performed based on the descriptors of stressed plants, whose values and eigenvalues were employed to obtain variance estimates (eigenvalues and cumulative variation) (Table 4) in order to place the cultivars in a scatter plot. Since about 89% of the total variation was explained by the first two eigenvalues, the data could be represented in a two-dimensional scatter plot (Figure 6).

Five classification groups were identified, containing the following cultivars: I- FM 966, II- CNPA 7MH and BRS Rubi, III- MT 152, IV- BRS Seridó and FMT 701, and V- BRS 286, CNPA ITA 90, FMT 705, BRS 416, and BRS Acácia.

The composition of the groups generated shows coherence with the previously discussed results, so that the genotypes that showed a markedly sensitive behavior, e.g., FM 966 and MT 152, were assigned to unitary groups, whereas BRS 286, BRS 416, FMT 705, CNPA ITA 90, and BRS Acácia occupied the same group, showing biological proximity based on the variables adopted in this research. In the physiological context, however, cultivars FMT 705, BRS 416, BRS Acácia, and CNPA 7MH were considered the most tolerant, although they use different strategies to withstand salt stress. That is justifiable because tolerance to salinity is governed by multigenic factors that lead to plants showing varied responses when facing stress situations as a function of gene interaction (Morton et al., 2019).

In a transcriptome analysis study with salinity-tolerant and -sensitive cotton plants, Guo et al. (2015) reported that distinct metabolic pathways operate in salt-tolerant and salt-sensitive upland cotton cultivars subjected to salinity, showing different levels of expression. According to those authors, genes induced in tolerant genotypes are, at the same time, repressed in sensitive genotypes, depending on the biosynthetic network that involves the trait, including transcription factors and hormone metabolism, among others. Peng et al. (2014) found 129 unigenes that were differentially expressed in salinity-tolerant cotton genotypes, including transcription factors, in plants subjected to 24 h of exposure to 200 mM NaCl. Those results are interesting as they show the ability of plants to reprogram in face of stress, activating new genes that

Table 4. Eigenvalues (variance), percentages of isolated and cumulative variance of the principal components obtained from the matrix formed by the physiological parameters, and growth of cotton plant genotypes subjected to salt stress.

Component	Eigenvalue	Variance (%)	Cumulative variance (%)
PC 1	5.05	72.19	72.19
PC 2	1.15	16.47	88.66
PC 3	0.59	8.46	97.12
PC 4	0.18	2.61	99.74
PC 5	0.01	0.23	99.97
PC 6	0.00	0.02	99.99
PC 7	0.00	0.00	100.0

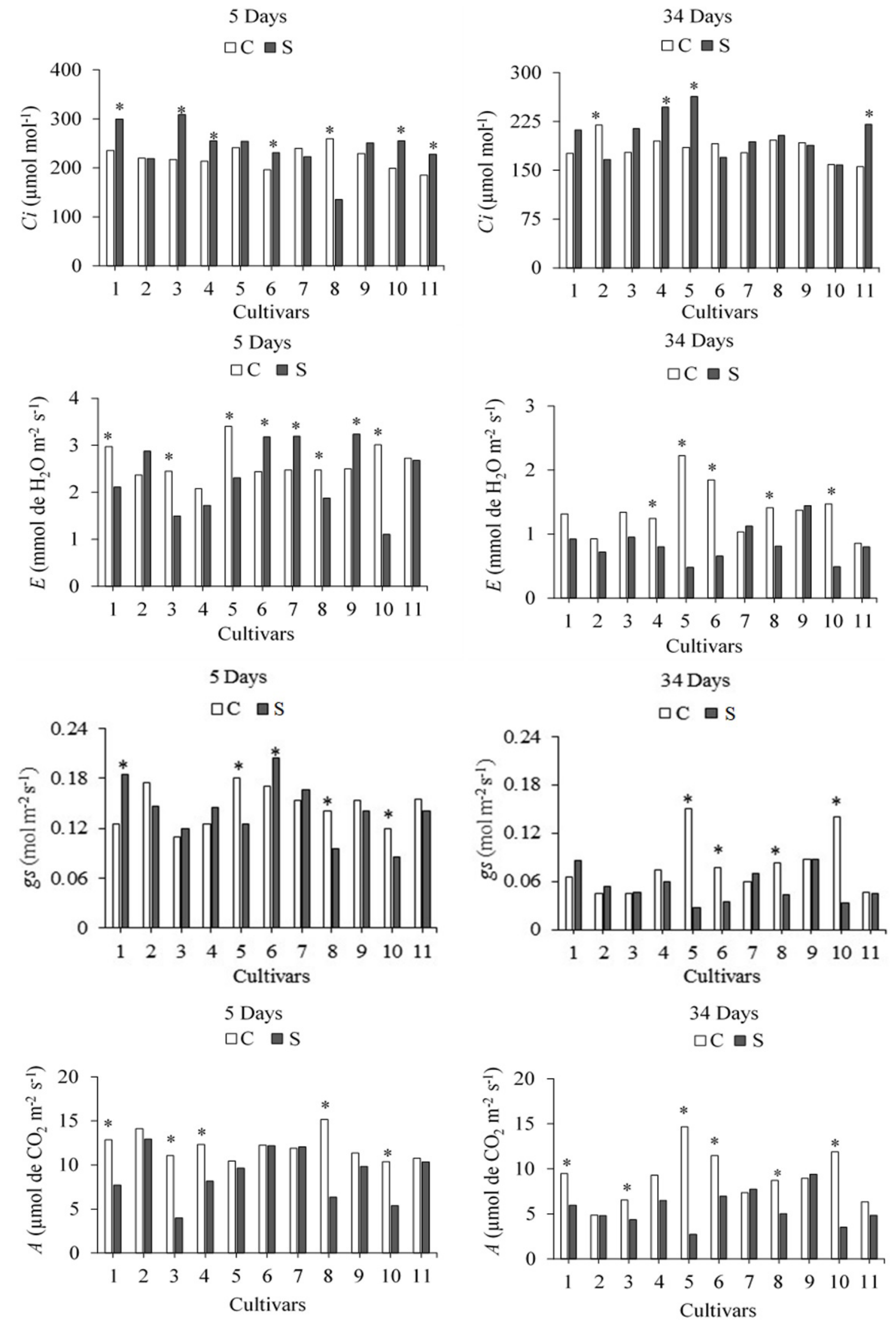

Figure 4. Gas exchange variables in the cotton cultivars subjected to five and 34 days of severe salt stress (10 dS m^{-1}). *Ci* - Internal carbon, *E* - Transpiration, *gs* - Stomatal conductance, *A* - CO_2 assimilation rate, *iCE* - Instantaneous carboxylation efficiency. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11 - BRS Acácia. C - control, S- stress. *between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

may be useful candidates in assisted selection processes aiming at identifying tolerant materials.

The results presented in this study show that, although the cultivars employed in this study have high agronomic value, they differ widely regarding the level of tolerance to salinity. Hence, robust germplasm databases are recommended to serve as genetic resources in improvement programs aiming at adding biological and agronomical value to the cotton agribusiness. Based on the physiological and osmoregulatory descriptors, the genotypes BRS Acácia, BRS 286, and BRS 416 were the most tolerant to the severe salt stress condition (10 dS m^{-1}).

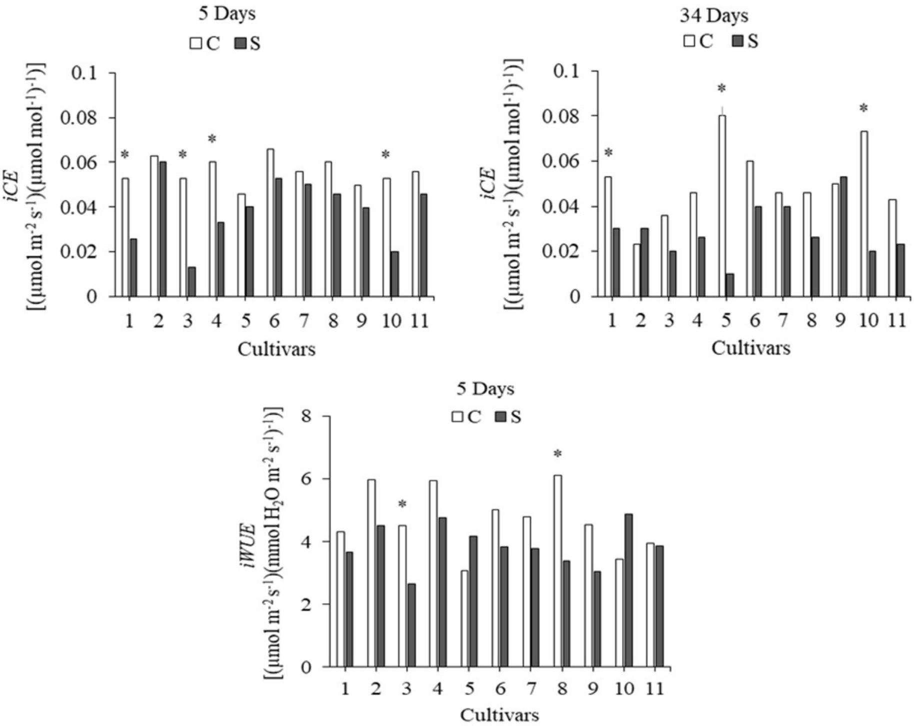

Figure 5. Instantaneous carboxylation efficiency (*iCE*) of cotton cultivars subjected to five and 34 days of severe salt stress and instantaneous water-use efficiency (*iWUE*) in the plants after five days of salt stress. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11 - BRS Acácia. C - control, S - stress. *between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

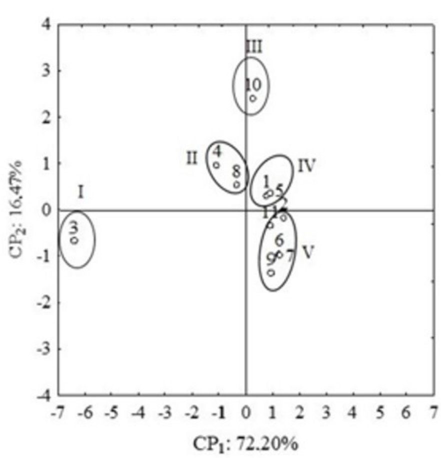

Figure 6. Scatter plot of eleven cotton cultivars subjected to salt stress. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, and 11 - BRS Acácia.

Acknowledgements

The authors gratefully acknowledge the Brazilian Agricultural Research Corporation - EMBRAPA Algodão, and the Coordination for the Improvement of Higher Education Personnel (CAPES).

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