

Initial Growth and Gas Exchanges of Plants of Colored Cotton Submitted to Saline Stress

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Abstract

The objective of the research was to evaluate the effects of saline stress on the initial growth and physiological parameters of three varieties of cotton plants, being two cultivars of colored fiber (BRS Topázio and BRS Verde) and one of white fiber (BRS 286). The experiment was conducted under greenhouse conditions. After chemical depletion with sulfuric acid, five seeds were seeded in polyethylene pots of 20 kg, leaving only one plant per pot after thinning. The experimental design was completely randomized, with a factorial scheme of 3×5 , with five replications, totaling 75 plots, being three cotton varieties and five saline stress treatments—1.1; 3.1; 5.1; 7.1; 9.1 dS m⁻¹. The evaluations occurred at 15 and 30 days after the beginning of the treatments (DAT) for growth variables (plant height, stem diameter, number of leaves and leaf area) and gas exchange (stomatal conductance, net photosynthesis rate and transpiration). The variety that was most resistant to the salt stress levels studied was BRS 286, followed by BRS Topázio and BRS Verde.

Keywords

Photosynthesis, Stomatal Conductance, Dry Mass, Resistance, Cultivars

1. Introduction

Salinity caused by the excessive concentration of salts in the soil solution, or even in the waters used for irrigation, is also considered an abiotic stress limiting the growth and production of plants [1]. High levels of salts in the soil solution reduce the capacity of water absorption by the roots, transport and use of the ions necessary for the growth and development of the plants [2].

Saline stress causes several physiological changes in the plant, such as reduction of leaf water potential, stomatal closure, reduction of photosynthetic rate,

decrease of aerial part, acceleration of senescence, leaf abscission, among others [3]. Therefore, the stomatal closure also becomes a defense mechanism of the plants to avoid water loss and eventual death by desiccation [4]. However, the process of photosynthesis is impaired, because it depends directly on the stomatal opening, for the assimilation of the carbonic gas in its biochemical stage.

The reduction of water absorption by plants, due to excess salts in the soil solution, is one of the main factors that undermine the photosynthetic process. According to [5], when it happens, the stomatal conductance is reduced, in the influx of CO₂ to the chloroplasts, and thus, the photosynthetic rate falls, also decreasing the transpiration.

The cotton crop is characterized as raw material of the agrotêxtil chain, being incorporated in about 90% of the total natural fibers consumed in Brazil. It is domesticated and cultivated in places where water deficiency is not necessarily an aggravating factor [6]. In addition, it is a culture considered to be tolerant to salinity [7].

Due to resistance to low rainfall and moderate levels of salinity, colored cotton has great importance in the semi-arid region. In addition, it is a crop used in the production of textiles due to the non-use of dyes or other chemical derivatives, thus adding more value to the finished product [8].

Therefore, this research had as objective to evaluate the initial growth, chlorophyll and the variables of gas exchange of different cotton cultivars, when submitted to saline stress.

2. Material e Methods

The research was carried out in a greenhouse, located at the Federal University of Campina Grande-PB, campus I, under the following geographic coordinates: 7° 13' 11" S and 35° 52' 31" W; altitude of 550 m. The duration of the survey was two consecutive months (September and October 2016). Cotton cultivars were evaluated, being one of white fiber (BRS 286) and two of colored fiber (BRS topaz and BRS Verde).

Before sowing, the seeds were chemically demarcated using sulfuric acid in the proportion of 7 kg of seeds per liter of acid. After delignification, these seeds were washed with 5% sodium hydroxide (NaOH) and water for the removal of linter and sulfuric acid residues. This process is necessary to prevent proliferation of fungi or other agents that may cause seed damage and subsequent germination.

For the distribution of the vessels in the experimental area, a completely randomized design was used, with a factorial scheme 3 × 5, with 5 replications, totaling 75 experimental plots. This factorial scheme is defined by three cotton cultivars (BRS Topázio, BRS Verde and BRS 286) and five different levels of electrical conductivity of water—1.1; 3.1; 5.1; 7.1; 9.1 dS m⁻¹.

Vessels with capacity of two liters were used, containing three holes in the base to allow drainage, and filled with 300 g of gravel to cover, subsequently,

filled with 3.5 kg of soil, classified as Argiloso Franco Latosol.

Only foundation fertilization was carried out, incorporating in each vessel amounts of 0.77 g of urea, 5.83 g of single superphosphate and 0.90 g of potassium chloride, according to [9]. Then the soil was placed in field capacity, using water supply.

The water in use was stored in reservoirs of 100 L. The different levels of salinity in water (CEa) were obtained from the dissolution of sodium chloride in rainwater and water supply. These concentrations of NaCl, to be diluted to determine the desired CEa, were found based on the equation of [10], described below.

$$\text{Conc.NaCl} = \frac{585 \times (CEd - CEu) \times Vt}{1000} \quad (1)$$

where: Conc.NaCl—Concentration of sodium chloride (g);

CEd—desired electrical conductivity (dS m^{-1});

CEu—electrical conductivity of used water (dS m^{-1});

Vt—Total reservoir volume (L).

Five cotton seeds were sown per experimental plot, filled with soil at a depth of 5 cm. The emergence started 4 days after sowing (DAS), thinning at 10 DAS, leaving only one plant per pot. Soil scarification of the soil occurred before each irrigation and mentoring of some plants, in order to avoid tipping. In addition, there were applications of insecticides and fungicides when necessary.

Up to 10 DAS, irrigation was performed with moisture close to the field capacity, using only water supply. From then on, the irrigation occurred from two days in two days, always at 7 o'clock, applying in each vessel the appropriate treatment.

The evaluations occurred at 15 and 30 days after application of the treatments (DAT). The growth of the cotton cultivars was evaluated through the determination of plant height (AP), stem diameter (DC), number of leaves (NF) and leaf area (FA). The leaf area was determined by the equation proposed by [11], in leaves with length of main vein equal to or greater than 3 cm:

$$Y = 0.4322X^{2.3002} \quad (2)$$

where: Y = Leaf area, cm^2 ; X = Length of main vein of the leaf, cm.

The gas exchange variables of the plants, involving the stomatal conductance (gs) ($\text{mol m}^{-2}\cdot\text{s}^{-1}$), net photosynthesis rate (A) ($\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$) and transpiration (E) ($\text{mmol m}^{-2}\cdot\text{s}^{-1}$) were measured using the IRGA equipment, brand ADC, model LCpro-SD. These evaluations were carried out between 8 and 10 am, at 15 and 30 DAT. A source of artificial light was coupled to the apparatus in order to obtain a flux density of photosynthetic photons of $1200 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$.

At the end of the experiment, 45 DAS, the plants were removed from the vases, separating leaves, stem and root. After weighing the fresh mass of these materials, they were placed in a forced circulation oven at 65°C for 48 hours. After this period, there was a new weighing to obtain the dry mass of the different

parts.

The results obtained from the evaluated variables were submitted to analysis of variance through the statistical software SISVAR. The means were compared by the Tukey test at 5% probability. In the cases of significance, regression analysis was performed [12].

3. Results and Discussion

As observed in **Table 1**, the increasing salinity levels affected significantly ($p < 5\%$) all the growth variables evaluated, at 15 and 30 days after application of the treatments (DAT). In relation to the factor genotype (G) and interaction (SxG), the only variable that did not have a significant effect ($p < 5\%$) was the stem diameter (DC) for the two periods analyzed (15 and 30 DAT).

The average results for AP, DC, NF and AF, in the two evaluated periods, were adjusted to the linear regression model (**Figure 1**), indicating a reduction of these variables as a function of the increase of the salt level of the water applied to the soil. Probably, this was due to the reduction of water absorption by the roots of the plant, due to the accumulation of salts in the soil solution, which reduces its osmotic potential and, consequently, its water potential, making water absorption difficult.

At 15 DAT, the mean data of AP, DC, NF and AF decreased between the lowest (1.1 dS m^{-1}) and the highest (9.1 dS m^{-1}) saline level of 28.0%, 17.1%, 30.9% and 68.7%, respectively, resulting in a reduction of 3.5%, 2.1%, 3.9% and 8.6%, respectively. At 30 DAT, the linear trend of the mean results continued to decrease, but more sharply, being 39.3% for AP; 27.3% for DC; 57.5% for NF and 73.0% for AF, with a decrease, by unit increase of CEa, sequentially, of 4.9%, 3.4%, 7.2% and 9.1%.

According to [13], the reduction of growth of the plants when submitted to salinity conditions may be related to lower water absorption due to the decrease of the osmotic potential of the soil solution. The decrease of the leaf area and the

Table 1. Summary of ANOVA with the p-values regarding plant height (AP), stem diameter (DC), number of leaves (NF) and leaf area (FA), in the periods of 15 and 30 DAT, of cotton plants submitted to different levels of salinity.

Variation Factor	GL	ANOVA-SALINE STRESS							
		AP-15 cm	AP-30 cm	DC-15 mm	DC-30 mm	NF-15	NF-30	AF-15 cm ²	AF-30 cm ²
Sal. (S)	4	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
Gen. (G)	2	0.0000*	0.0053*	0.0661 ^{ns}	0.1308 ^{ns}	0.0000*	0.0000*	0.0000*	0.0000*
S x G	8	0.0327*	0.8229 ^{ns}	0.6981 ^{ns}	0.1478 ^{ns}	0.0017*	0.0281*	0.0081*	0.0049*
CV (%)		9.80	8.06	7.40	5.10	11.87	12.70	20.85	18.39

ns: not significant; * $p < 5\%$ by the Tukey test.

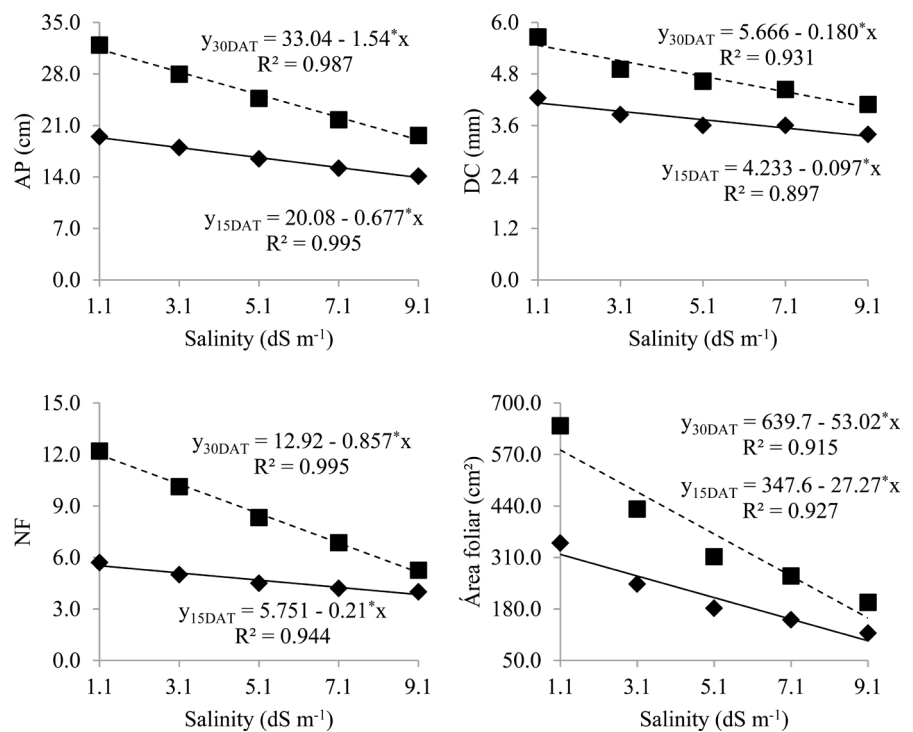


Figure 1. Plant height (AP), stem diameter (DC), number of leaves (NF) and leaf area (PA), as a function of increasing water salinity levels (dS m⁻¹), at 15 and 30 days DAT.

number of leaves can show a physiological mechanism of adaptation of the plants to the saline stress, to avoid the loss of water by transpiration [14]. In addition, the decrease in leaf area may have occurred, possibly due to the reduction of the number of leaves, being this, a response of the plants to saline stress, aggravating during the time of this exposure.

Similar results were obtained by [15], which studied the morphology and production of BRS Topazio colored cotton, irrigated with saline waters, observed reductions in plant height, stem diameter and leaf area as a function of salinity increase.

According to **Figure 2**, the cultivar BRS Topázio differed significantly from BRS 286 in AP, suffering less damage to the growth, at the different levels of salts applied in the soil, at 15 DAT (17.6 cm) and 30 DAT (26.3 cm). In NF, these two cultivars were only statistically different from the 30 DAT, whereas in AF there was not significant distinction between them. This difference in growth between varieties of the same crop may be related to the better capacity of osmotic adaptation of genotypes, allowing a greater or lesser absorption of water in salinity conditions [16].

The BRS Verde obtained mean data significantly different from the others, in all growth variables, obtaining the lowest values. [17] observed in their studies with BRS Verde cotton that the effects of salinity were quite negative on initial growth, especially for plant height, number of leaves and leaf area.

Regarding the variables of fresh and dry mass (**Table 2**), it can be seen that

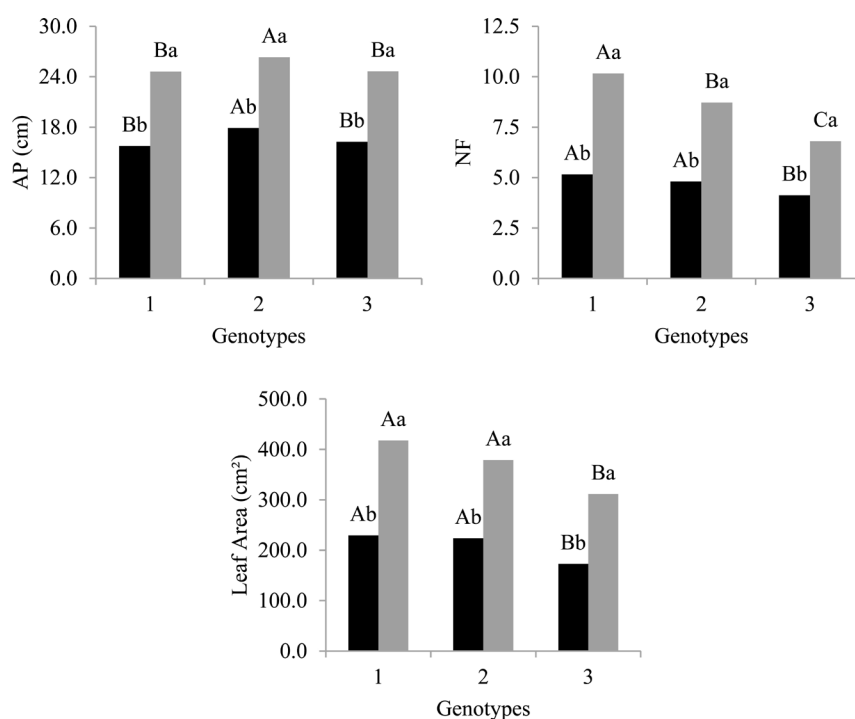


Figure 2. Plant height (AP), number of leaves (NF) and leaf area (AF) for the genotype factor. Equal capital letters do not differ between genotypes. Equal lowercase letters do not differ between times. 1—BRS 286; 2—BRS Topázio; 3—BRS Verde. ■—Average values at 15 DAT; ▒—Average values at 30 DAT.

Table 2. Summary of ANOVA with the p-values of fresh masses of leaves, stem and roots (MFF, MFC, MFR) and dry masses of leaves, stem and roots (MSF, MSC, MSR) of cotton plants submitted to different salinity levels.

VariationFactor	GL	ANOVA-SALINE STRESS					
		MFF	MSF	MFC	MSC	MFR	MSR
		g					
Sal. (S)	4	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
Gen. (G)	2	0.0000*	0.0000*	0.5499 ^{ns}	0.0000*	0.0001*	0.0009*
S x G	8	0.0000*	0.0062*	0.0734 ^{ns}	0.0025*	0.0090*	0.0043*
CV (%)		13.13	11.58	10.20	12.83	4.64	15.33

ns: not significant; *p < 5% by the Tukey test.

there was a significant effect of the isolated factor S for all these variables. The G factor and interaction (S x G) did not exert significance only in the fresh stem mass variable (MFC).

According to **Figure 3**, the average results obtained by the variables of fresh and dry masses of leaves, stem and roots were adjusted to the linear regression equation, occurring reduction of these variables, according to the increase of salts in the substrate. The highest increase of fresh and dry weight in leaves, stem and roots occurred at the salinity level of 1.1 dS m⁻¹, and the lowest in the CEA of

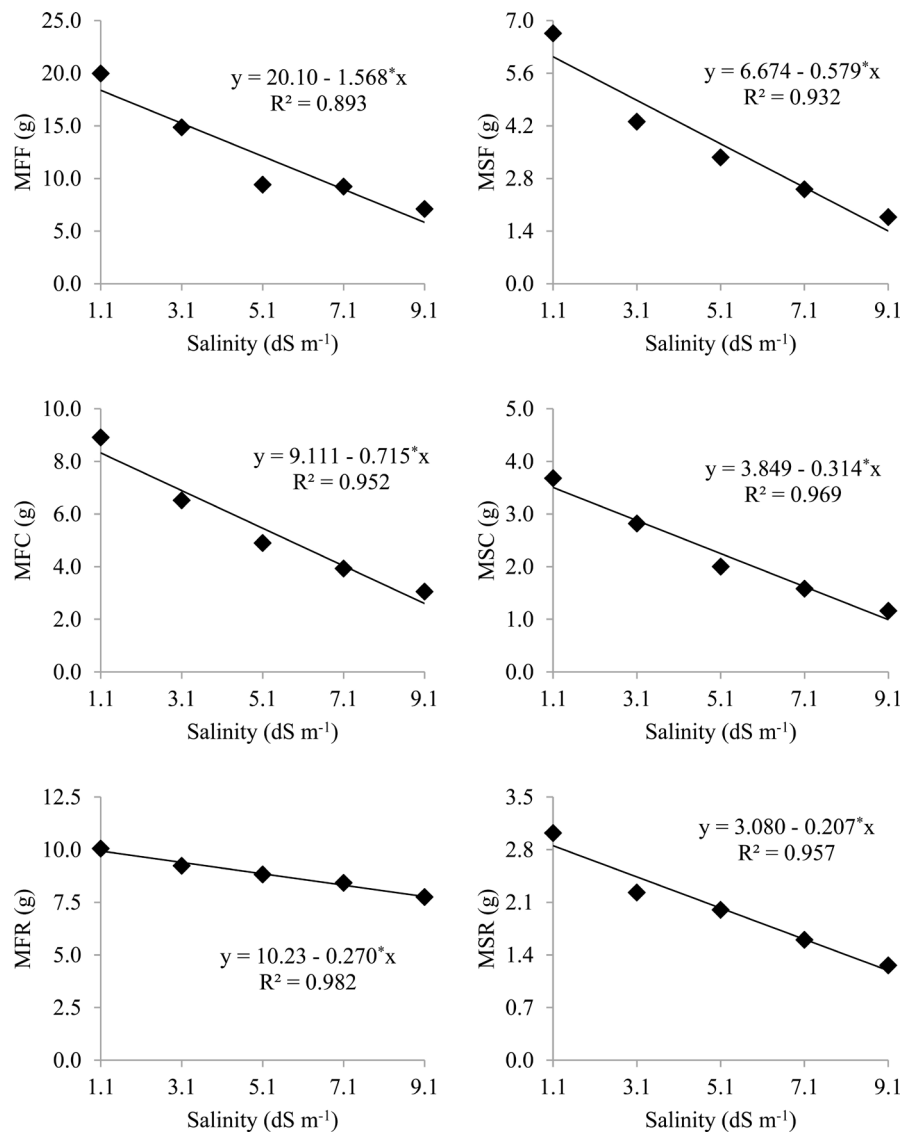


Figure 3. Fresh masses of leaves, stem and roots (MFF, MFC, MFR) and dry masses of leaves, stem and roots (MSF, MSC, MSR), as a function of increasing levels of CEa (dS m⁻¹).

9.1 dS m⁻¹. This is probably due to the difficulty that plants have to absorb water in environments with low osmotic potential, which leads to less accumulation of mass.

This decrease of fresh and dry biomass of the different parts of the plant may also be related to the reduction of the leaf area, because for [18], the part of the plant most sensitive to high levels of salts is the leaf. According to [19], with the reduction of leaf area, several simultaneous processes of plant development are impaired, such as reduction of the photosynthetic process, water absorption and nutrients, affecting growth and, consequently, biomass production. [20], evaluating the initial growth of three cotton cultivars under saline stress, also observed a decrease in fresh and dry masses of leaves, stem and roots when the salt

levels increased in irrigation water.

It is observed in **Figure 4** that the white cotton cultivar, BRS 286, differed statistically from the other genotypes studied in all variables, obtaining better average results for MFF, MSF, MFR and MSR.

In relation to the gas exchange variables evaluated (**Table 3**), it can be observed at 15 DAT that the S factor had a significant effect on all the gas exchange variables, except on the stomatal conductance (gs). At 30 DAT, the increasing salinity levels affected negatively all variables analyzed.

The G factor, on the other hand, only presented significance at 30 DAT, only in the mean data of stomatal conductance (gs) and transpiration (E). The

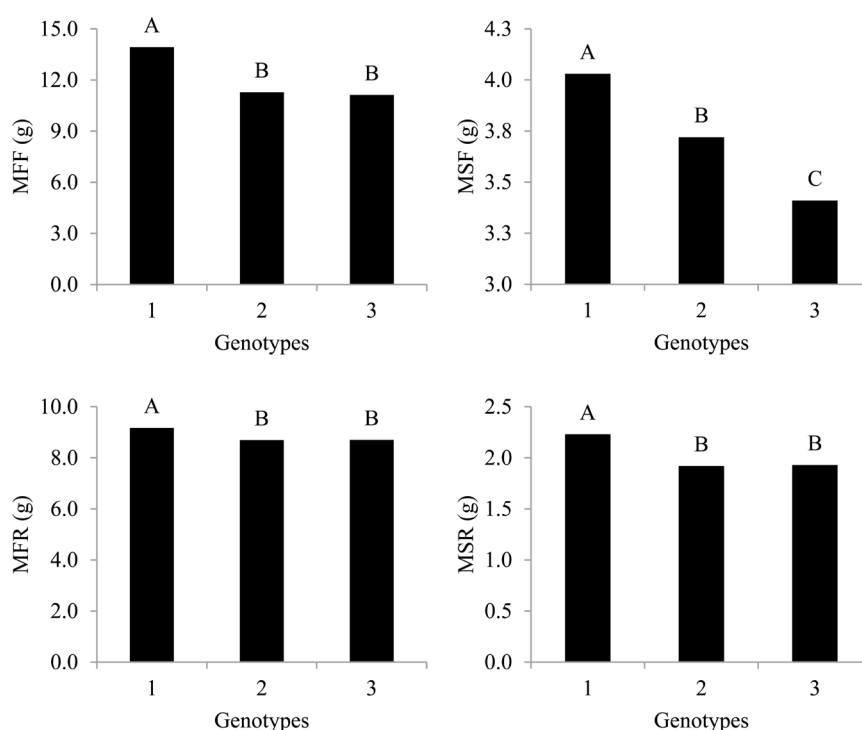


Figure 4. Fresh masses of leaves and roots (MFF, MFR) and dry masses of leaves and roots (MSF, MSR) for the genotype factor. Averages with the same letter do not differ significantly from each other. 1—BRS 286; 2—BRS Topázio; 3—BRS Verde.

Table 3. Summary of ANOVA with the p-values regarding stomatal conductance (gs), liquid photosynthesis rate (A) and transpiration (E) of cotton plants submitted to different levels of salinity.

Variation Factor	GL	ANOVA-SALINE STRESS					
		gs-15	gs-30	A-15	A-30	E-15	E-30
Sal. (S)	4	0.8962 ^{ns}	0.0104*	0.0000*	0.0000*	0.0000*	0.0076*
Gen. (G)	2	0.7196 ^{ns}	0.0039*	0.3906 ^{ns}	0.3843 ^{ns}	0.1070 ^{ns}	0.0045*
S x G	8	0.1547 ^{ns}	0.3354 ^{ns}	0.1217 ^{ns}	0.5951 ^{ns}	0.7011 ^{ns}	0.7157 ^{ns}
CV (%)		30.23	32.39	7.41	11.29	10.66	23.36

ns: not significant; *p < 5% by the Tukey test.

interaction ($S \times G$) did not present a significant effect on the gas exchange variables studied.

At 30 DAT, the stomatal conductance (g_s) of the cotton cultivars presented a linear decrease, with the increase of saline levels (**Figure 5(a)**), obtaining a reduction, between CEa of 1.1 and 9.1 dS m^{-1} , of 33.3%, which is equivalent to a decrease of 4.1% per unit increase of CEa. [16] and [21] also observed reduction of stomatal conductance in cotton genotypes under salt stress. This behavior was also found for other crops such as castor bean, cowpea, and eggplant [22] [23] [24].

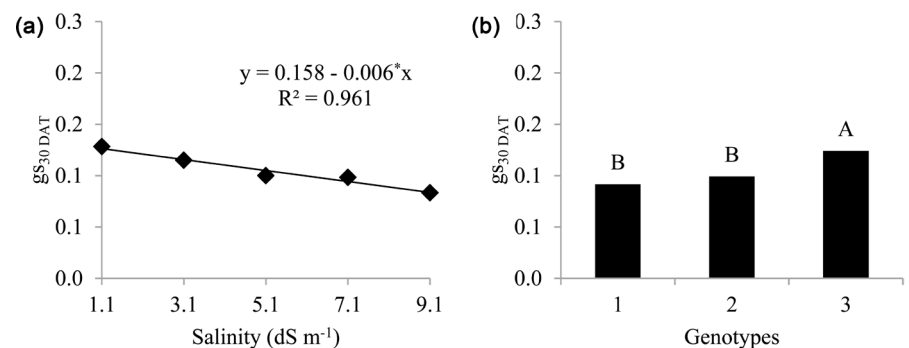
This stomatal closure, due to the application of more salinized water, may be related to the osmotic effect associated with the accumulation of salts in the soil solution, besides the reduction of the hydraulic conductivity of the root system, in accordance with the increase of the suberization and lignification of the tissues of the roots of plants submitted to salinity [25].

In IRGA readings at 30 DAT, the mean values obtained by the BRS Verde variety differed statistically from those found for BRS 286 and BRS Topázio, showing higher average g_s results. BRS 286 and Topázio did not differ statistically among genotypes, however, BRS Topázio obtained average results of g_s slightly higher than those of BRS 286 (**Figure 5(b)**).

The variables A and E had decreasing average results, with the increase of salinity in the substrate, at 15 and 30 DAT, according to the linear regression equation (**Figure 6**). It may be evidenced by the stomatal closure, which reduces the absorption of atmospheric CO_2 and, consequently, the activity of Rubisco and other enzymes, causing negative effects on the carboxylation reactions, important for the accomplishment of the biochemical stage of photosynthesis.

The liquid photosynthesis rate (A) and transpiration (E) decreased by a unit increase of CEa at 15 and 30 DAT, respectively, of 4.2% and 3.9%, and 7.1% and 5.9%. Behaviors also found by [24] and [22] in their research.

With the reduction of stomatal conductance, other subsequent processes may be impaired, among them, the decline of liquid photosynthesis, due to the decrease of CO_2 partial pressure, in the intercellular spaces or in the substomatic



Figures 5. Stomatal conductance (g_s) at 30 days DAT, as a function of increasing levels of CEa (dS m^{-1}) and genotypes. Averages with the same letter do not differ significantly from each other. 1—BRS 286; 2—BRS Topázio; 3—BRS Verde.

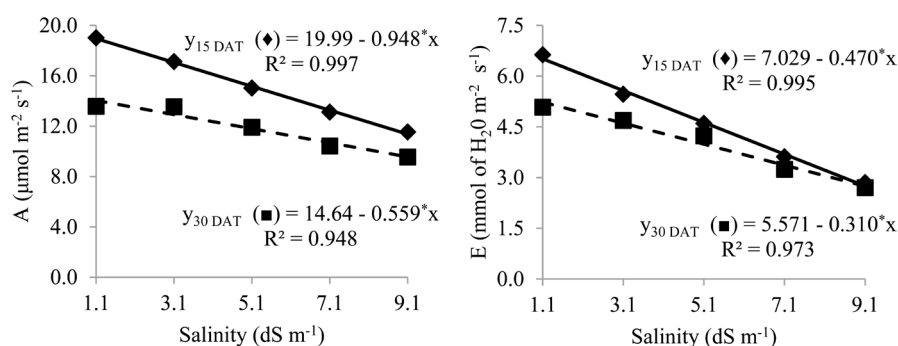


Figure 6. Liquid photosynthesis rate (A) and transpiration (E), at 15 and 30 days DAT, as a function of increasing water salinity levels (dS m^{-1}).

chamber [26]. In addition, stomatal closure restricts the process of transfer of water vapor from the plant to the atmosphere, that is, it reduces the transpiration process [27].

According to [28], the reduction of the growth and development of the crops, in relation to the salt stress, is linked to three factors: stomatal closure, reduction of leaf expansion and acceleration of leaf senescence.

4. Conclusions

Increasing CEa levels affected significantly the initial growth and gas exchange of cotton cultivars, both at 15 and 30 DAT.

In general, the white cotton cultivar, BRS 286, showed to be more resistant to the levels of salts analyzed, among the cultivars studied. Among the varieties of colored cotton, BRS Topázio proved to be more resistant to saline stresses, obtaining, in some cases, results similar to cultivar white fiber. BRS Verde was the most sensitive among all evaluated varieties.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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