

NK combinations do not alleviate the effects of salt stress on gas exchange, photosynthetic pigments and growth of cotton (*Gossypium hirsutum* L.)

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Abstract

The water resources in the semi-arid region of Northeast Brazil commonly have high concentrations of salts, compromising the quality of the water used in irrigation. Thus, it is necessary to study the feasibility of using these resources in agriculture. The present study aimed to evaluate gas exchanges, photosynthetic pigments and growth of white cotton cv. BRS 368 RF using irrigation water of different salinity levels and fertilization with combinations of nitrogen and potassium, under greenhouse conditions in the municipality of Campina Grande-PB, Brazil. Treatments were arranged in randomized blocks in a 5 x 4 factorial scheme, with five levels of electrical conductivity of water-ECw (0.7, 2.2, 3.7, 5.2 and 6.7 dS m⁻¹) and four combinations of fertilization with nitrogen and potassium (70/50, 100/75, 130/100, 160/125% of the recommended dose), with three replicates. There was degradation in chlorophyll *a* and *b* levels and in cotton growth, but the cultivar BRS 368 RF markedly increased carotenoid biosynthesis at 75 days after sowing. Nitrogen and potassium application in the combination of 100/75% of their respective recommendations led to increase in plant height. The supply of nitrogen in combination with potassium did not alleviate the deleterious effects of salt stress on gas exchange, photosynthetic pigments and growth of cotton.

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

Abbreviations: DAS_ days after sowing; ECw_electrical conductivity of water; PH_plant height; SD_stem diameter, NL_number of leaves LA_leaf area; *gs*_stomatal conductance; *A*_assimilation rate, *E*_transpiration; *Ci*_internal CO₂ concentration; *Chl a*_chlorophyll *a*; *Chl b*_chlorophyll *b*; Car_carotenoids.

Introduction

Herbaceous cotton (*Gossypium hirsutum* L.) is one of the main crops exploited in Brazil, especially for the production of fiber, raw material of the Brazilian and global textile industries. Cotton cultivation generates products with various industrial applications, and all plant parts are used. Cotton fruit is composed of seeds (52%), fibers (40%) and other botanic structures (8%) (Brasil, 2007). The ripe capsule, or cotton boll, is harvested and both its seeds and its fibers can be used after ginning. The fiber has greater economic importance, and its main product is the lint, which is used in a series of textile varieties. From the seed, part is used as animal feed without any processing and the remaining portion is crushed in machines to obtain oil, cake and meal (Abrapa, 2013).

Brazil is currently the fifth largest cotton producer, with 6.5% of the total world production, only behind India, China,

USA and Pakistan. In addition, the country is the third largest exporter, exporting about 50% of the national production. In the 2016/2017 season, mean seed cotton yield was estimated at 2.38 t ha⁻¹, in a planted area of approximately 938,000 hectares (Conab, 2017).

The area cultivated with cotton in the Northeastern semi-arid region is very small, due to the low yield and competitiveness caused by several factors, particularly including disorganization in the regional production chain, occurrence of water deficit and low levels of technology, such as mechanization, increasing the costs with labor, which has currently become scarce and expensive (Embrapa, 2017). In addition, the availability of water for irrigation has been gradually reduced in both quality and quantity, making it necessary to use waters with high concentrations of salts in order to meet the water requirements of crops in this

region (Silva et al., 2014). For this region, the use of saline waters has stood out as an alternative capable of minimizing water scarcity. High concentrations of salts in water and/or soil are among the main environmental factors limiting the growth and yield of crops, besides reducing the osmotic potential of the soil solution, compromising water absorption, possibly leading to toxic effects on plants, and causing functional disorders and damage in their metabolism (Silva et al., 2009). Although cotton is considered as a salt-tolerant crop, its salinity threshold is 5.1 dS m^{-1} in the irrigation water and 7.7 dS m^{-1} in the soil saturation extract (Ayers and Westcot, 1999), salinity can cause deleterious effects on cultivars of the same species, depending on factors such as type and concentration of salts, intensity and duration of stress, fertilization management and nutritional status of the crop (Munns and Tester, 2008). Some techniques have been developed in an attempt to minimize the deleterious effects of excess salts in water and/or soil on plants. Fertilization management stands out as an important strategy, based on the use of fertilizers, capable of favoring nutrient acquisition by plants under saline conditions (Silva et al., 2011). According to Wei et al. (2018), fertilization is one of the main technologies used to increase crop yield and profitability, and nitrogen is one of the main responsible for such increment because this macronutrient is a component of several molecules which are important for plant metabolism, such as amino acids, proteins, enzymes, RNA, DNA, ATP and chlorophyll. Potassium participates in several stages of the metabolism, such as the reaction of phosphorylation, synthesis of carbohydrates, proteins, respiration and regulation of stomatal opening and closure. It is important in root development and essential in fruit production and maturation, because it is responsible for converting starch into sugars (Taiz et al., 2017). According to Venkajesan et al. (2004), there is a positive correlation between the activity of the nitrate reductase enzyme and potassium fertilization. According to Matsumoto et al. (2013), combined nitrogen and potassium fertilizations contribute to the increase of leaf nutrient ratios, reducing $\text{Cl}^- / \text{NO}_3^-$ and Na^+ / K^+ ratios, restoring nutritional equilibrium and mitigating the toxic effects of Na^+ and Cl^- ions on the plant, consequently promoting an increase in crop tolerance. Gurgel et al. (2010) showed that potassium fertilization reduced the deleterious effects of high-salinity water on the melon cv. 'Goldex'. In this context, this study aimed to evaluate gas exchanges, photosynthetic pigments and growth of white cotton cv. BRS 368 RF as a function of irrigation with saline water and different combinations of fertilization with nitrogen and potassium, at 45 and 75 days after sowing.

Results and Discussion

Effect of salt stress and nitrogen and potassium fertilization on cotton fiber growth

There was no significant ($p>0.05$) effect (Table 1) of the interaction between salinity levels and combinations of N and K (SL x NK), on the variables analyzed. Considering the factors separately, water salinity levels had significant ($p<0.01$) effect on plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA) of cotton in both periods of evaluation. On the other hand, the combinations

of fertilization with N and K (NK) had significant influence on plant height ($p<0.01$) at 75 DAS. According to the regression equations obtained for plant height (Fig 1A), this variable decreased linearly as the levels of irrigation water salinity increased, with reductions of 8.12 and 10.09% per unit increase in ECw at 45 and 75 DAS, respectively. Lower deleterious effect of irrigation water salinity on PH was observed at 45 DAS, whereas at 75 DAS the stress on the crop was intensified and there was greater reduction compared with the first evaluation. Thus, it is possible to note the sensitivity of this crop as a sign of the negative effect caused by the salt stress on the plants. Salinity affects water absorption and growth of plants due to the decrease in the water potential of the soil solution through the osmotic effect of Na^+ and Cl^- salts (Jouyban, 2012). Thus, the reduction in the PH of the cotton cv. BRS 368 RF, due to the increase in ECw, is probably related to the lower absorption of water caused by the decrease in the osmotic potential of the soil solution (Oliveira et al., 2012). The height of cotton plants cv. BRS 368 RF (Fig 1B) at 75 DAS was significantly different according to the different combinations of N and K. Based on the means comparison test (Fig 1B), it can be observed that plants receiving 100/75% of N and K showed the greatest PH (59.73 cm), and the lowest value (51.14 cm) was observed in plants subjected to the treatment with 160/125% of the recommendation for N and K. On the other hand, plants subjected to fertilization with 75/50, 100/75 and 130/100% of N and K did not differ. The reduction in cotton height is possibly related to the competitive inhibition of the ions, because this nutrient is frequently absorbed by many species in quantities greater than those required, and under these conditions the excess of K may interfere with the absorption of other cations by crops, especially when they compete for the same absorption sites in root tissues, inhibiting the absorption of Ca^{2+} and Mg^{2+} (Meurer, 2006). Stem diameter was also negatively and linearly affected by the increment in the levels of irrigation water salinity and, based on the estimates of the regression equations (Fig 2A), there were reductions of 0.348 and 0.547 mm per unit increase in irrigation water electrical conductivity, at 45 and 75 DAS. Plants grown under the highest water salinity level (6.7 dS m^{-1}) showed reductions of 35.72 and 44.67%, respectively, compared with those irrigated with 0.7 dS m^{-1} water. According to Taiz et al. (2017), physiological processes are affected by water stress because, under conditions of lower water availability, cell turgor is decreased, leading to a reduced rate of cell expansion and division, which compromises plant growth in height and stem diameter. Lima et al. (2017), studying the impacts of different levels of irrigation water salinity (5.1 to 9.1 dS m^{-1}) on the colored cotton 'BRS Rubi', also found significant effects of ECw on stem diameter, with reductions of 1.83 mm (22.34%) per unit increase in ECw at 83 days after sowing. As observed for SD (Fig 2A), the number of leaves (Fig 2B) also decreased due to the increase in irrigation water salinity, with reductions of 10.18 and 13.49% per unit increase in ECw, which corresponded to decreases of 13.33 and 37.46 leaves in cotton plants irrigated with 6.7 dS m^{-1} water in comparison to those subjected to the lowest salinity level (0.7 dS m^{-1}), at 45 and 75 DAS respectively. The reduction in the number of leaves under high-salinity conditions (6.7 dS m^{-1}) is probably related to the action of the osmotic component, besides the

possibility of occurrence of ionic toxicity, which makes it difficult for water to enter plant cells (Ambede et al., 2012), resulting in alterations in the photosynthetic capacity, consequently inhibiting plant growth. In addition, the reduction in the number of leaves can be considered as an adaptive mechanism of the plant because, under salt stress conditions, morphological and anatomical alterations are common, which lead to reduction of transpiration as an alternative to maintain low absorption of toxic ions, especially Na^+ and Cl^- (Bezerra et al., 2018).

The leaf area of cotton cv. BRS 368 RF was also negatively influenced by irrigation water salinity, and a decreasing linear model fitted best to the data for both periods of evaluation (45 and 75 DAS). The reductions in leaf area were equal to 12.81 and 14.28% per unit increase in ECw and corresponded to 268.75 and 603.36 cm^2 per unit increase in ECw at 45 and 75 DAS, respectively (Fig 2C). Leaf area is one of the growth variables most affected by salinity because, under salt stress, the photosynthetic apparatus contributes to the decline in the capacity of production of organic assimilates and results in the inhibition of plant growth, leading to reductions in transpiration and net CO_2 assimilation rate, caused by stomatal closure in response to the low water potential in the soil, due to the high concentration of salts. The reduction in leaf area represents a mechanism of defense of plants under water and salt stress conditions, reducing water losses through transpiration (Bezerra et al., 2018). According to Gratão et al. (2005), leaves are more vulnerable to Na^+ and Cl^- than roots and stem simply because they accumulate higher concentrations of these ions, which results in reduction in cell expansion, causing a decrease in plant growth, since both are transported through the transpiration stream in the xylem and accumulate in the leaves when water is transpired.

Effect of salt stress and nitrogen and potassium fertilization on the photosynthetic pigments of white cotton

Based on the summary of analysis of variance (Table 2), the interaction between factors (SL x NK) had no significant ($p>0.05$) influence on any of the variables evaluated. Separately, only water salinity levels caused significant ($p<0.01$) effect on the contents of chlorophyll *a* (*Chl a*), *b* (*Chl b*), and carotenoids (*Car*).

The increment in water salinity levels led to reduction in chlorophyll *a* content (Fig 3A), 11.73% per unit increase in ECw, which corresponded to a decrease of 76.69% in *Chl a* content in the leaves of plants irrigated with water of highest salinity (6.7 dS m^{-1}) compared with those under ECw of 0.7 dS m^{-1} . Reduction in chlorophyll *a* synthesis, due to salt stress, cannot be interpreted only as a consequence of the deleterious effects of salts on plant metabolism, because the decrease in chlorophyll *a* contents can be a response of acclimation to the action of stressing agents, adopted by some plants in the attempt to save energy and, therefore, capture less light energy, thus avoiding possible photo-oxidative stress (Tabot et al., 2013).

For the content of chlorophyll *b*, the regression equation (Fig 3B) revealed a negative linear effect as a function of the increase in ECw, and the reduction was approximately 11.75% per unit increase in ECw, i.e., plants subjected to irrigation with 6.7 dS m^{-1} water showed reduction of 76.84%

in *Chl b* content, compared with those irrigated with 0.7 dS m^{-1} . Reduction in *Chl b* content in cotton plants subjected to salt stress may have occurred because of the increase in the synthesis of the enzyme chlorophyllase, which acts on the degradation of the molecules of this photosynthesizing pigment (Campos et al., 2012).

The content of carotenoids (*Car*) in the cotton cv. BRS 368 RF increased linearly as a function of irrigation with increasing levels of salinity and, according to the regression equation (Fig 3C), it increased by 27.80% per unit increase in electrical conductivity. The contents of carotenoids decreased by 58.26% in plants irrigated with 6.7 dS m^{-1} water, compared with those subjected to the lowest level of water salinity (0.7 dS m^{-1}). Such situation is extremely important for crops under saline conditions, because carotenoids act in the control of free radicals produced in greater quantity when the plant is under stress. Sayyad-Amin et al. (2016) mention that under such conditions there is an increase in carotenoid contents and reduction in chlorophyll biosynthesis, which causes lower absorption of energy by leaves and, therefore, an increase in visible reflectance, characterized by the yellow or chlorotic color in the plants.

Effect of salt stress and nitrogen and potassium fertilization on the gas exchanges of white cotton

There was no significant ($p>0.05$) effect of the interaction between salinity levels and combinations of N and K (SL x NK) on the gas exchange variables analyzed (Table 3). Considering the factors separately, fertilization with NK caused no significant ($p>0.05$) effect, but water salinity levels significantly ($p<0.01$) affected stomatal conductance (*gs*), transpiration (*E*), CO_2 assimilation rate (*A*), internal CO_2 concentration (*Ci*), instantaneous water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*EiCi*) in cotton plants cv. BRS 368 RF, at 75 days after sowing.

Increase in the electrical conductivity of irrigation water caused linear decreases in stomatal conductance (*gs*) (Fig 4A), of the order of 10.44% per unit increase in ECw. There was a decrease of 67.63% in the *gs* of plants subjected to the highest salinity level (6.7 dS m^{-1}), compared with those under the lowest concentration of salts (0.7 dS m^{-1}). According to Sousa et al. (2018), the reduction in stomatal conductance occurs because plants under salt stress close their stomata to reduce water loss by transpiration, resulting in a lower photosynthetic rate, due to reduced partial pressure of CO_2 in the intercellular spaces or in the substomatal chamber, which is one of the causes for the reduced growth of species cultivated under high-salinity conditions.

The transpiration of the cotton cv. BRS 368 RF was negatively affected by irrigation water salinity and, according to the regression equation (Fig 4B), there was a reduction of 0.2173 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ per unit increase in ECw. In addition, when plants were irrigated using water of highest salinity level (6.7 dS m^{-1}), transpiration decreased by 56.24% in comparison to plants under ECw of 0.7 dS m^{-1} . Furthermore, reduction in *E* can be attributed to the partial closure of the stomata, as a strategy of the plants to avoid excessive dehydration of guard cells, or to water imbalance, a mechanism which leads to reduction in CO_2 diffusion and, consequently, reduction in CO_2 assimilation rate (Time et al., 2018). According to Gonçalves et al. (2010), a close relation

Table 1. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA) of white cotton cv. BRS 368 RF irrigated with saline waters and fertilized with different combinations of nitrogen and potassium, at 45 and 75 days after sowing (DAS).

Source of variation	Mean squares							
	PH		SD		NL		LA	
	Days after sowing							
	45	75	45	75	45	75	45	75
Saline levels (SL)	1386.43**	5585.96**	9.55**	22.57**	399.51**	2929.91**	5904739.15**	27226269.55**
Nitrogen/Potassium (NK)	57.91 ^{ns}	190.19**	0.35 ^{ns}	0.23 ^{ns}	16.17 ^{ns}	80.02 ^{ns}	75074.29 ^{ns}	441401.01 ^{ns}
Interaction (SL x NK)	14.63 ^{ns}	27.53 ^{ns}	0.30 ^{ns}	0.38 ^{ns}	4.42 ^{ns}	26.16 ^{ns}	95532.46 ^{ns}	871718.59 ^{ns}
Blocks	156.21 ^{ns}	173.37 ^{ns}	0.05 ^{ns}	0.60 ^{ns}	36.15 ^{ns}	6.81 ^{ns}	732308.43 ^{ns}	961657.01 ^{ns}
Residual	33.69	34.75	0.40	0.42	11.06	39.71	109684.07	1117223.90
CV (%)	15.38	10.73	13.17	11.45	24.46	27.20	30.03	53.0

ns, **, * respectively, not significant, significant at $p < 0.01$ and $p < 0.05$.

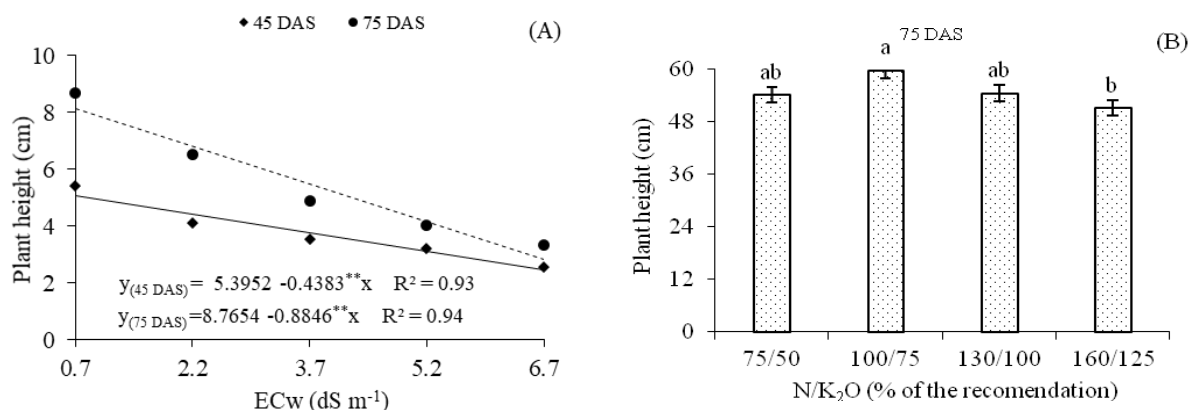


Fig 1. Plant height of white cotton cv. BRS 368 RF as a function of irrigation water salinity – ECw (A), at 45 and 75 days after sowing (DAS), and of the combination of fertilization with nitrogen and potassium (B), at 75 DAS. Means followed by the same letter do not differ by Tukey test ($p < 0.05$); Bars represent standard error of the mean ($n = 3$).

Table 2. Summary of analysis of variance for the contents of chlorophyll *a* (*Chl a*), *b* (*Chl b*), and carotenoids (*Car*) in white fiber cotton cv. BRS 368 RF irrigated with waters of different salinity levels and combinations of fertilization with nitrogen and potassium, at 75 days after sowing.

Source of variation	Mean squares		
	<i>Chl a</i>	<i>Chl b</i>	<i>Car</i>
Saline levels (SL)	48.1175**	9.7026**	3.0340**
Nitrogen/Potassium (NK)	1.0904 ^{ns}	0.1895 ^{ns}	2.2819 ^{ns}
Interaction (SL x NK)	2.7228 ^{ns}	1.7051 ^{ns}	0.5054 ^{ns}
Blocks	0.6465 ^{ns}	2.7251 ^{ns}	0.2374 ^{ns}
Residual	2.2315	1.0538 ^{ns}	0.9428 ^{ns}
CV (%)	18.07	16.14	12.63

ns, **, * respectively, not significant, significant at $p < 0.01$ and $p < 0.05$.

Table 3. Summary of analysis variance for stomatal conductance (*gs*), transpiration (*E*), CO₂ assimilation rate (*A*), internal CO₂ concentration (*Ci*), instantaneous water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*EiCi*) of white cotton plants cv. BRS 368 RF irrigated with waters of different salinity levels and combinations of fertilization with nitrogen and potassium, at 75 days after sowing.

Source of variation	Mean squares					
	<i>gs</i>	<i>E</i>	<i>A</i>	<i>Ci</i>	<i>WUEi</i>	<i>EiCi</i>
Saline levels (SL)	0.0120**	3.18**	164.35**	14545.49**	12.82**	0.0076**
Nitrogen/Potassium (NK)	0.0009 ^{ns}	0.23 ^{ns}	8.58 ^{ns}	2412.26 ^{ns}	2.89 ^{ns}	0.0003 ^{ns}
Interaction (SL x NK)	0.0004 ^{ns}	0.09 ^{ns}	2.84 ^{ns}	2390.03 ^{ns}	2.14 ^{ns}	0.0004 ^{ns}
Blocks	0.0009 ^{ns}	1.68 ^{ns}	11.04 ^{ns}	385.66 ^{ns}	7.09 ^{ns}	0.0005 ^{ns}
Residual	0.0004 ^{ns}	0.13 ^{ns}	4.37 ^{ns}	1925.60 ^{ns}	2.60 ^{ns}	0.0002 ^{ns}
CV (%)	26.44	21.71	26.83	20.78	34.95	38.77

ns, **, * respectively, not significant, significant at $p < 0.01$ and $p < 0.05$.

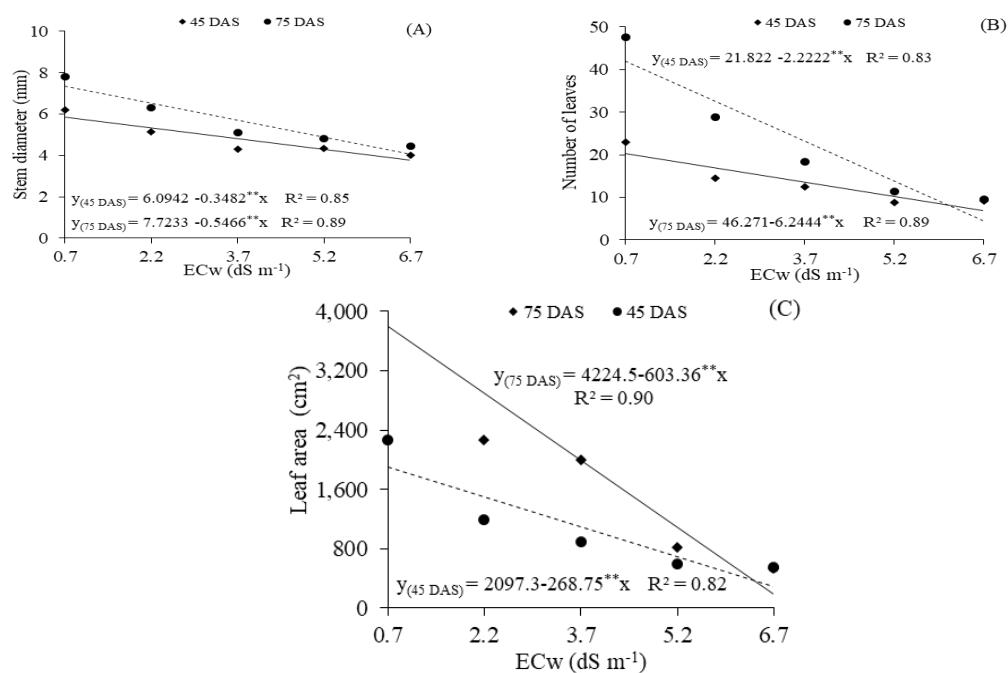


Fig 2. Stem diameter (A), number of leaves (B) and leaf area (C) of white cotton cv. BRS 368 RF, as a function of irrigation water salinity – ECw, at 45 and 75 days after sowing (DAS).

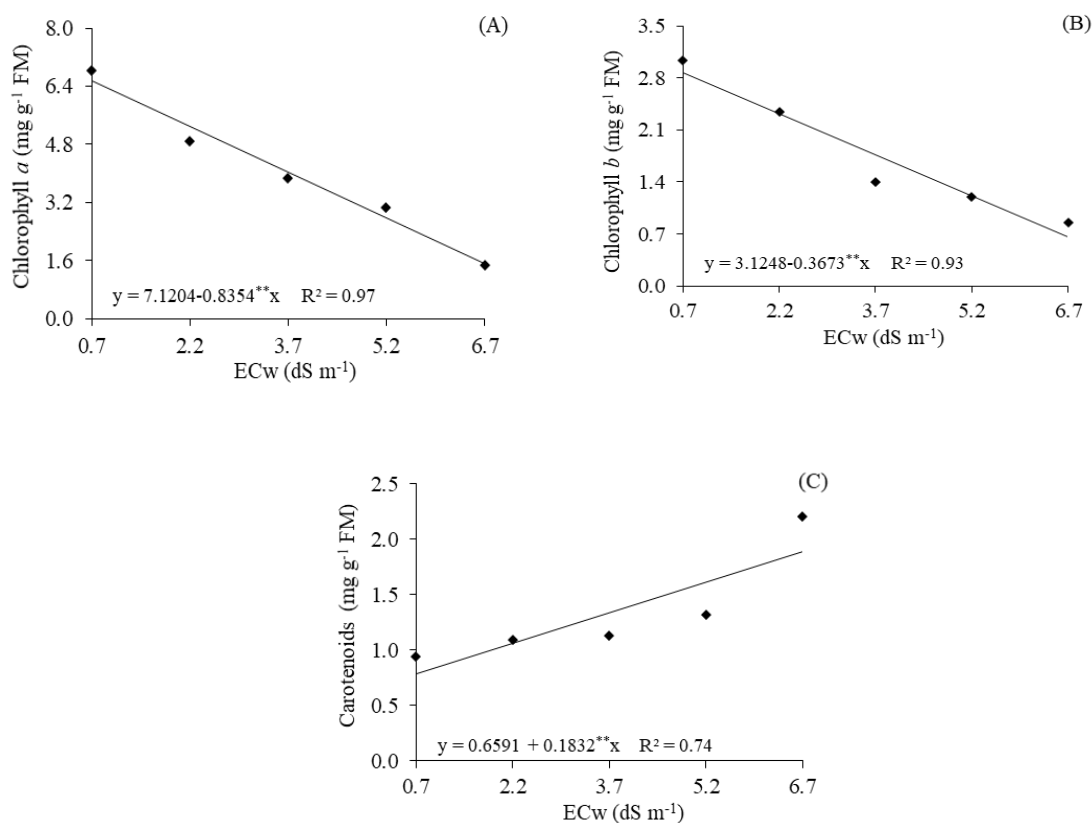


Fig 3. Contents of chlorophyll a (A), chlorophyll b (B) and carotenoids (C) in white cotton cv. BRS 368 RF, as a function of irrigation water salinity – ECw, at 75 days after sowing.

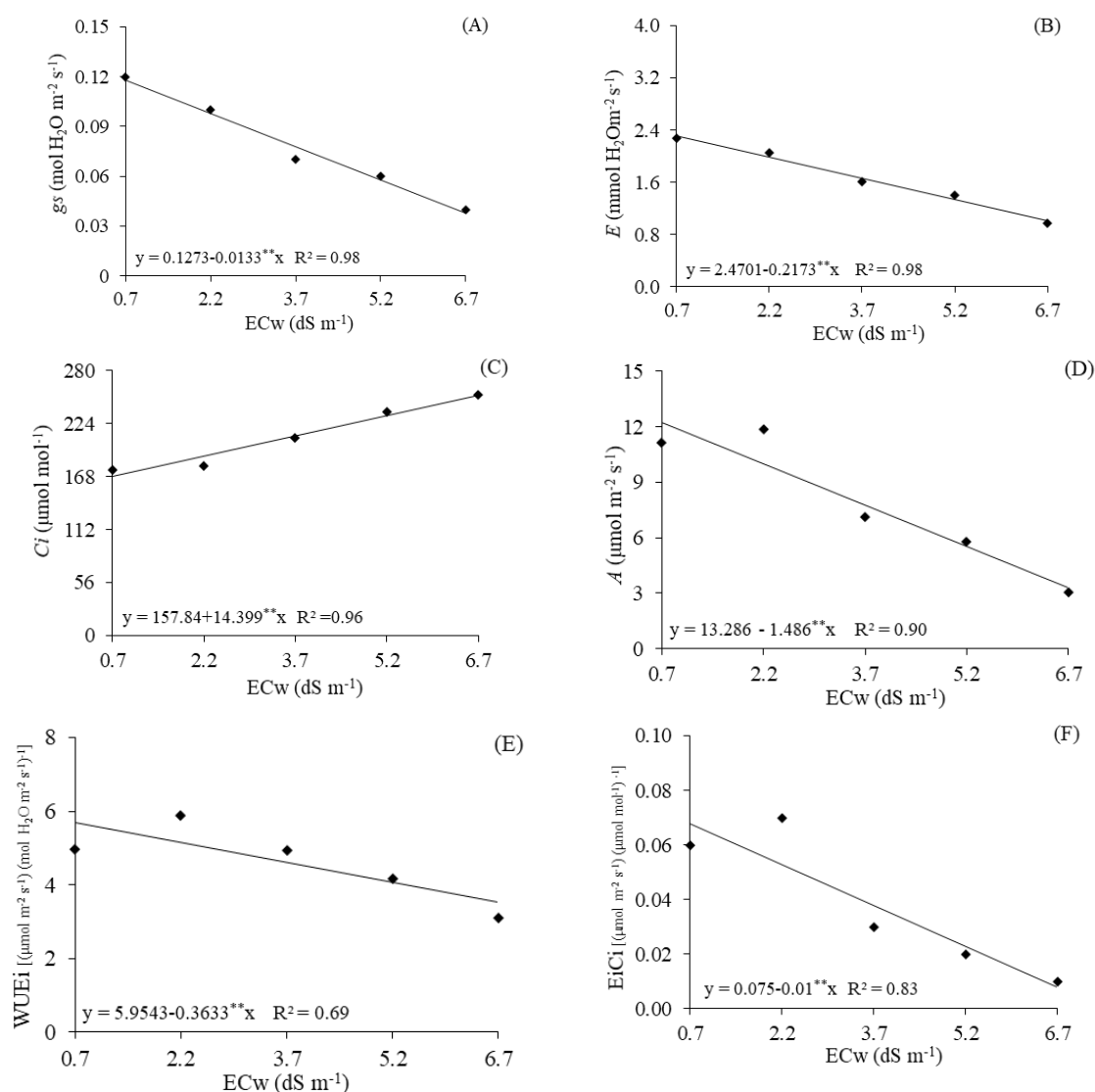


Fig 4. Stomatal conductance – *g_s* (A), transpiration – *E* (B), internal CO₂ concentration – *C_i* (C), CO₂ assimilation rate – *A* (D), instantaneous water use efficiency – WUE_i (E) and instantaneous carboxylation efficiency – EiCi (F) of white cotton cv. BRS 368 RF, as a function of irrigation water salinity – ECw, at 75 days after sowing.

between *g_s* and *E* is expected because of the decrease in water vapor flux to the atmosphere, and therefore in transpiration, as the stomata close. The internal CO₂ concentration of cotton cv. BRS 368 RF increased linearly as the levels of irrigation water salinity increased. According to the regression equation (Fig 4C), it was equal to 254.31 μmol mol⁻¹ in plants irrigated with 6.7 dS m⁻¹ and to 167.92 μmol mol⁻¹ in plants subjected to the lowest salinity level (0.7 dS m⁻¹). In other words, irrigation using water with high conductivity led to increment of 51.45% in the *C_i* of cotton plants cv. BRS 368 RF. The increase in *C_i* is related to the low values of leaf water potential, resulting in the reduction of *g_s* (Fig 4A), caused by the decline in the osmotic potential of the solution, in addition to the reduction in the activity of enzymes involved in CO₂ fixation. Under salt stress conditions, the increase of CO₂ in the substomatal chamber indicates that it is not being used for the synthesis of sugars during the photosynthetic process, which leads to

accumulation of this gas, suggesting the interference of a non-stomatal factor in this process, which may result in damage to the photosynthetic apparatus of the plants (Morais et al., 2018). Contrary to what was observed for *C_i* (Fig 4C), the CO₂ assimilation rate (Fig 4B) of cotton plants cv. BRS 368 RF decreased linearly as irrigation water salinity increased. According to the regression equation (Fig 4B), there was a reduction in CO₂ assimilation rate of 11.18% per unit increase in ECw, which is equivalent to a decline of 90.74% in the *A* of cotton plants irrigated with 6.7 dS m⁻¹ in comparison with those subjected to ECw of 0.7 dS m⁻¹. The reduction in CO₂ assimilation rate in response to the increase in irrigation water salinity may have occurred due to the osmotic effect associated with the accumulation of soluble salts in the soil and the decrease in root hydraulic conductivity caused by the increase in suberization and lignification of the vascular tissues of the roots of plants under salt stress conditions (Lima et al., 2014). The

instantaneous water use efficiency (WUEi) decreased linearly with the increase in irrigation water salinity, by 6.10% per unit increase in ECw, i.e., plants irrigated with of 6.7 dS m⁻¹ water had a 38.24% decrease in comparison to those subjected to ECw of 0.7 dS m⁻¹ (Fig 4E). As observed in the case of the instantaneous water use efficiency (WUEi), the instantaneous carboxylation efficiency (EiCi) also decreased linearly with the increase in irrigation water salinity, by 13.33% per unit increase in ECw, i.e., plants irrigated with 6.7 dS m⁻¹ showed reduction of 88.23% in comparison to those subjected to ECw of 0.7 dS m⁻¹ (Fig 4F). Such result is probably a consequence of greater CO₂ assimilation compared with the CO₂ found in the substomatal chamber because, if Ci increases due to the rate of stomatal opening, the entry of CO₂ in the leaf mesophyll may have decreased because of the reduction in gs, but CO₂ consumption in the chloroplast may have been reduced as well (Ouyang et al., 2017). According to Soares et al. (2018), osmotic stress, associated with accumulation of salts in the soil, reduces water availability to plants and, therefore, can affect their gas exchanges and growth.

Materials and methods

Plant material

The cotton cultivar used in the experiment was BRS 368 RF, which is a transgenic material, resistant to the herbicide glyphosate, with fiber yield of 40% and production potential between 4,200 and 4,500 kg ha⁻¹. It is a dwarf cultivar, with cycle from medium to early, first flower opening at 55 days and crop cycle between 165 and 170 days (Embrapa, 2017).

Localization, experimental procedure, treatments

The study was carried out under greenhouse conditions at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), located in the municipality of Campina Grande, PB, situated at local geographic coordinates 7° 15' 18" S, 35° 52' 28" W and altitude of 550 m. The experimental design was in randomized blocks, with three replicates, using a 5 x 4 factorial scheme, and treatments consisted of five levels of irrigation water electrical conductivity – ECw (0.7, 2.2, 3.7, 5.2 and 6.7 dS m⁻¹) and four combinations of fertilization with nitrogen and potassium – NK (70:50, 100:75, 130:100, 160:125% of the respective recommendation). The dose relative to 100%, as recommended by Novais et al. (1991) for pot experiments, corresponded to 100 mg kg⁻¹ of N and 150 mg kg⁻¹ of K₂O.

Saline waters were prepared by dissolving the salts NaCl, CaCl₂·2H₂O and MgCl₂·6H₂O, at equivalent proportion of 7:2:1, respectively, in water (ECw = 0.6 dS m⁻¹) from the public supply system of Campina Grande-PB, and their quantities were determined based on the relationship between ECw and the concentration of salts (10*mmol_c L⁻¹ = ECw dS m⁻¹) according to Richards (1954).

Establishment and management of the experiment

Drainage lysimeters with capacity for 20 L were used to grow cotton plants. At the bottom of each lysimeter, a drain was installed using a 4-mm-diameter tube to drain the leachate

to a container to evaluate the drained water and determine water consumption of plants. The tip of the drain inside the lysimeter was involved in a nonwoven geotextile (Bidim OP 30) to avoid clogging by soil material.

The lysimeters were filled with a 0.5-kg layer of crushed stone (No. 0), followed by 25 kg of a eutrophic Regolithic Neosol with sandy loam texture collected in the 0-20 cm layer, in the rural area of the municipality of Lagoa Seca, PB. This material was properly pounded to break up clods and sieved, and its physical and chemical characteristics were determined according to the methodologies proposed by Donagema et al. (2011): exchangeable Ca²⁺, Mg²⁺, Na⁺, K⁺, Al³⁺ + H⁺ = 2.60, 3.66, 0.16, 0.22 and 1.93 cmol_c kg⁻¹, respectively; pH (soil:water 1:2.5) = 5.9; ECse (dS m⁻¹) = 1.0; organic matter (%) = 1.36; sand, silt and clay = 732.9, 142.1, and 125.0 g kg⁻¹, respectively; apparent density (kg dm⁻³) = 1.39; moisture content at 33.42 and 1519.5 kPa = 11.98 and 4.32 dag kg⁻¹, respectively.

Before sowing, the soil in all lysimeters was brought to field capacity using the respective waters of each treatment. After sowing, irrigation was performed every day, applying in each lysimeter a volume of water sufficient to maintain the soil close to field capacity. The applied volume was determined according to the water requirement of the plants, estimated by water balance: volume applied minus volume drained in the previous irrigation.

Fertilization with phosphorus was carried out as recommended by Novais et al. (1991), using 300 mg of P₂O₅ per kg of soil before planting. Nitrogen and potassium were applied according to the treatments, as top-dressing, in five applications by fertigation at 15-day intervals, with the first one at 10 DAS. Monoammonium phosphate, urea and potassium chloride were used as sources of P, N and K, respectively.

Micronutrients were applied at 20, 40 and 60 days, using 4.0 L of a solution (1.0 g L⁻¹) of Ubyfol (N-15%; P₂O₅-15%; K₂O-15%; Ca-1%; Mg-1.4%; S-2.7%; Zn-0.5%; B- 0.05%; Fe-(0.5%; Mn-0.05%; Cu-0.5%; Mo-0.02%) sprayed on cotton leaves until full wetting of the adaxial and abaxial faces.

Traits measured

The growth of 'BRS 368 RF' cotton was evaluated at 45 and 75 days after sowing (DAS) based on plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA). PH was considered as the distance between collar and apical meristem. SD was measured at 2 cm from plant collar, whereas NL was obtained by counting the leaves of each plant. LA was obtained by measuring the midrib length of all leaves, following the methodology described by Grimes et al. (1969), according to Eq 1:

$$y = 0.26622 x^{2.3002} \quad (1)$$

Where: y is leaf area per leaf, and x is the midrib length of the cotton leaf. Leaf area per plant was determined by the sum of the area of all leaves.

At 75 DAS the contents of chlorophyll *a* (Chl *a*), *b* (Chl *b*) and carotenoids (Car) were determined following the laboratory method developed by Arnon (1949), in samples of 5 leaf discs from the lamina of the third mature leaf from the apex of the quaternary branch. Their extracts in 80% acetone were used to determine the contents of chlorophyll and carotenoids in the solution with a spectrophotometer at the

absorbance wavelength (ABS) (470, 646, and 663 nm), through the equations: Chlorophyll *a* (*Chl a*) = 12.21 ABS663 – 2.81 ABS646; Chlorophyll *b* (*Chl b*) = 20.13 ABS646 – 5.03 ABS663; Total carotenoids (*Car*) = (1000 ABS470 – 1.82 *Chl a* – 85.02 *Chl b*)/198. The obtained contents of chlorophyll *a*, *b* and carotenoids were expressed in mg g⁻¹ of fresh matter (FM).

At this same evaluation period (75 DAS), the gas exchanges of cotton plants were measured by determining stomatal conductance (*gs* - mol H₂O m⁻² s⁻¹), CO₂ assimilation rate (*A* - μmol m⁻² s⁻¹), transpiration (*E* - mmol H₂O m⁻² s⁻¹) and internal CO₂ concentration (*Ci* - μmol mol⁻¹), using a portable infrared gas analyzer (IRGA), model LCPro+ Portable Photosynthesis System®. These data were used to quantify the instantaneous water use efficiency (WUEi) (*A/E*) [(μmol m⁻² s⁻¹) (mmol H₂O m⁻² s⁻¹)⁻¹] and instantaneous carboxylation efficiency (EiCi) (*A/Ci*) [(μmol m⁻² s⁻¹) (μmol mol⁻¹)⁻¹].

Statistical analysis

The collected data were subjected to analysis of variance by F test. When significant, means comparison test (Tukey test at 0.05 probability level) was carried out for the combinations of fertilization with N and K and polynomial regression analysis was performed for water salinity levels, using the statistical software SISVAR-ESAL.

Conclusion

There is degradation in the contents of chlorophyll *a* and *b* and in cotton growth, but the cultivar BRS 368 RF sharply increases the biosynthesis of carotenoids at 75 days after sowing. Nitrogen and potassium supply in the combination of 100/75% of respective recommendations leads to increase in the height of cotton cv. BRS 368 RF. The supply of nitrogen in combination with potassium does not alleviate the deleterious effects of salt stress on gas exchange, photosynthetic pigments and growth of cotton.

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