

## Emergence, morpho-physiology and flowering of colored-fiber cotton (*Gossypium hirsutum* L.) submitted to different nitrogen levels and saline water stress irrigation

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### Abstract

The decrease in the availability of water resources, especially in semi-arid regions, increases every day the need for solutions to supply this deficit water demand. Under low supply of good-quality water, the generation of technologies and research that allow the use of saline waters is currently an essential activity. Hence, this study aimed to evaluate the effect of different saline levels of the irrigation water and nitrogen (N) fertilization doses on the emergence, morpho-physiology and flowering of colored-fiber cotton, cv. 'BRS Rubi', in an experiment conducted in lysimeters under greenhouse conditions in a Eutrophic Neolithic soil of a sandy-loam texture, in the municipality of Campina Grande-PB, Brazil. The experiment was conducted in a randomized block design and the treatments consisted of five levels of irrigation water salinity with the following electrical conductivities - EC<sub>w</sub> (5.1, 6.1, 7.1, 8.1 and 9.1 dS m<sup>-1</sup>), combined with five N doses - ND (65; 100; 135; 170; 205 mg of N kg<sup>-1</sup> of soil), distributed in a 5 x 5 factorial scheme with three replicates. The effects of the treatments were evaluated, through variables: emergence percentage, emergence speed index, gas exchanges, stomatal conductance, transpiration, CO<sub>2</sub> assimilation rate, internal CO<sub>2</sub> concentration, instantaneous carboxylation efficiency, instantaneous water use efficiency, stem diameter, plant height, leaf area, number of days for flower bud opening and flower abortion rate. Germination, gas exchanges and growth of the cotton cv. 'BRS Rubi' are negatively affected by irrigation water salinity from 5.1 dS m<sup>-1</sup> on wards. Irrigation using water with EC<sub>w</sub> higher than 5.1 dS m<sup>-1</sup> prolonged the period of opening of flower buds and promoted increase in flower abortion rate and internal CO<sub>2</sub> concentration. Increasing doses of N led to increment in cotton stomatal conductance and the highest value was obtained at the dose of 205 mg of N kg<sup>-1</sup> of soil; irrigation water salinity levels and N fertilization doses acted as independent factors for the studied variables of the cotton crop.

**Key words:** *Gossypium hirsutum* L., BRS Rubi, saline water, nitrogen fertilization.

**Abbreviations:** EP\_ emergence percentage; ESI\_ emergence speed index; *g<sub>s</sub>*\_stomatal conductance; *E*\_transpiration; *A*\_CO<sub>2</sub> assimilation rate, *C<sub>i</sub>*\_internal CO<sub>2</sub> concentration; EICI\_instantaneous carboxylation efficiency; WUE\_instantaneous water use efficiency; SD\_stem diameter; PH\_ plant height; LA\_leaf area; NFO\_number of days for flower bud opening; FAR\_flower abortion rate; EC<sub>w</sub>\_electrical conductivity of the irrigation water.

### Introduction

Belonging to the Malvaceae family, cotton (*Gossypium hirsutum* L.) is an oilseed crop cultivated in various regions of Brazil and worldwide, due to the versatility of its production, being the main raw material for the textile industry, because of the characteristics and utilities of its fiber, being also used for the production of oil and other byproducts (Viana, 2014), besides constituting an important option for economy, by both keeping labor at the field and generating jobs.

In the semi-arid region, especially as occurs in Northeast Brazil, due to the scarcity of rainfall and high evaporation demand, there is a deficit of moisture in the soil during most

of the year. Thus, the adoption of technology such as irrigation is fundamental to guarantee the agricultural production with guarantee (Oliveira et al., 2010). In this region, water quality has variability in both geographical and seasonal terms, and it is common to find water sources with high concentration of salts (Bezerra et al., 2010).

In the case of irrigation with saline waters, the salt stress occupies a prominent position, because the concentration of salts, at levels higher than those tolerated by plants, affects water availability, leads to decrease in the osmotic potential, toxicity by specific ions, nutritional imbalance and other damages related to indirect effects through physical and

chemical alterations in the soil (Neves et al., 2009); thus, the osmotic adjustment is essential for plant growth in a saline environment.

The presence of salts in excess can inhibit germination and cause non-uniformity in the growth of crops, trigger various events that interact, including the inhibition of enzymatic activity in metabolic pathways, besides disturbing the physiological functions, due to the mechanism of stomatal closure, reducing water losses through transpiration and decreasing the supply of CO<sub>2</sub> to the leaves (Amorim et al., 2010). However, these effects on plants depend on other factors, such as species, cultivar, types of salt, intensity and duration of the stress, crop and irrigation management, edaphoclimatic conditions and fertilization (Deuner et al., 2011).

Hence, the utilization of water with high concentrations of salts is conditioned to the tolerance of the crops to salinity and to the management practices of irrigation and fertilization, which must avoid environmental impacts and consequent damages to the crops. In this context, the mineral nutrition through fertilizer management stands out among the main strategies employed to increase crop yield and profitability, and nitrogen (N) is one of the main macronutrients responsible for this increase (Chaves et al., 2011). This dependence of crops on N can be related to the functions of this element in the plants, since it plays structural function, participating in various essential organic compounds, especially amino acids, proteins, co-enzymes, nucleic acids, vitamins, chlorophyll, among others.

Given its importance as a constituent of these compounds, N is involved in the processes that occur in the protoplasm, in enzymatic reactions and in photosynthesis, resulting in the stimulus to growth (Epstein & Bloom, 2006). Various studies have found that the accumulation of these organic solutes increases the capacity of osmotic adjustment of the plants to salinity (Barhoum et al., 2010).

In this way, osmotic homeostasis is essential for the growth and development of plants grown under saline conditions and any failure in this balance causes injuries similar to drought, such as loss of turgidity and reduction in growth and production (Ashraf & Harris, 2004). Thus, the most important mechanism to regulate osmotic stress in crops is the selective absorption of ions, since tolerant plants have the capacity to extract essential nutrients from the saline solution, whose concentration of non-essential ions (toxic) is higher (Fageria, 1989).

In this sense, Del Amor et al. (2000) report that there is evidence of competition in nitrate and chloride uptake, so that an increase in nitrate concentration in the root zone may inhibit chloride uptake by the plant. In this context, this study aimed to evaluate the emergence, morpho-physiology and flowering of colored-fiber cotton as a function of irrigation with water of different saline levels and N doses.

## Results and discussion

### *Effect of saline stress and nitrogen doses on colored-fiber cotton emergence*

According to the summary of the analysis of variance (Table 1), there was significant influence of the levels of irrigation water salinity on the index and percentage of emergence ( $p \leq 0.05$ ). However, there was no significant effect of the N doses ( $p \leq 0.05$ ) and the interaction between the factors (saline levels x nitrogen doses - SL x ND) on any of the analyzed variables. Different results were observed by Sousa Junior et al. (2005), who evaluated the germination and growth of the colored cotton 'BRS Verde' irrigated with saline water (ECw

varying from 2.0 to 9.5 dS m<sup>-1</sup>) and found no significant difference for the variables ESI and EP, with increasing ECw levels were used.

Hence, this difference of responses can be related to the genetic material used in this study, because Sousa Junior et al. (2005) studied the cultivar 'BRS Verde' and in the present study the cultivar used was 'BRS Rubi'. Thus, this result is an indication that the effects of salinity on cotton depend on the cultivar, types of salts present in the water, intensity and duration of the stress and managements of crop, irrigation and fertilization (Deuner et al., 2011).

The increase in ECw negatively affected the emergence speed index of 'BRS Rubi' cotton and, the linear model adjusted best to the ESI data, with decrease of 6.75% per unit increase in ECw (Fig 1A), i.e., the ESI of plants under irrigation with salinity level of 9.1 dS m<sup>-1</sup> decreased by 0.35 seedlings per day or 27.01%, in relation to plants irrigated with ECw of 5.1 dS m<sup>-1</sup>. During the emergence of the seedlings, water is the factor with highest influence on the emergence process, mainly because of the rehydration of the tissues, followed by the intensification of respiration and other metabolic activities (Carvalho and Nakagawa, 1998).

Therefore, the reduction of ESI in 'BRS Rubi' cotton plants (Fig 1A) due to the increment in irrigation water salinity can be attributed to the lesser absorption of water caused by the concentration of soluble salts in the soil, as well as by the entry of ions in amount sufficient to cause toxicity to the embryo and/or cells of the endosperm membrane. In addition, the toxic concentrations of ions (Na<sup>+</sup> and Cl<sup>-</sup>) affect other processes, including cell division and differentiation, activity of enzymes and capture and distribution of nutrients, which may cause delay in seedling emergence and mobilization of reserves or even reduce seed viability (Voigt et al., 2009).

The emergence percentage of 'BRS Rubi' cotton linearly decreases as a function of the irrigation water salinity and, according to the regression equation (Fig 1B), there were decreases of 5.62% per unit increase in ECw, or reduction of 22.50% under ECw of 9.1 dS m<sup>-1</sup>, compared with plants irrigated with water of 5.1 dS m<sup>-1</sup>. In this context, Abbasdokht et al. (2012) cite that salinity usually damages germination, germination speed and the stand of plants tends to be irregular. Lopes & Silva (2010), studying the effect of different saline concentrations (0, 2, 4, 6 and 8 g.L<sup>-1</sup>) on the germination of 'BRS Rubi' cotton, observed a linear reduction in EP, which reached values lower than 30% at concentrations higher than 4 g of NaCl.L<sup>-1</sup> (7.5 mS.cm<sup>-1</sup>) in the solution of irrigation.

### *Effect of saline stress and nitrogen doses on colored-fiber cotton gas exchanges*

Based on the summary of the analysis of variance (Table 1), there was significant difference of the distinct saline levels on transpiration, CO<sub>2</sub> assimilation rate, internal CO<sub>2</sub> concentration and instantaneous carboxylation efficiency ( $p < 0.01$ ), and on stomatal conductance and instantaneous water use efficiency ( $p \leq 0.05$ ). There was significant effect of the N doses on the stomatal conductance, transpiration and internal CO<sub>2</sub> concentration of the 'BRS Rubi' cotton. The interaction between factors (SL x ND) did not influence significantly ( $p \leq 0.05$ ) any of the analyzed variables.

The stomatal conductance of 'BRS Rubi' cotton was linearly inhibited by the increase in irrigation water salinity. Based on the regression equation referring to  $g_s$  (Fig 2A), there was a reduction of 8.30% per unit increase in ECw, i.e., a decrease of 33.20% in the  $g_s$  of plants irrigated with water of 9.1 dS m<sup>-1</sup> in relation to those under ECw of 5.1 dS m<sup>-1</sup>. The reduction in stomatal conductance in 'BRS Rubi' cotton

may be related to the decrease in the osmotic potential and, consequently, to the reduction in the availability of water in its tissues. Thus, possibly there was stomatal closure and the consequent reduction in the partial pressure of CO<sub>2</sub> in the intracellular spaces or in the substomatal chamber, which resulted in alterations between the appropriate balance and the transport of electrons, carbon metabolism and the consumption of ATP and NADPH, which, combined, rendered the photosystem II inefficient, significantly compromising the production of photosynthates (Tezara et al., 2005). Ribeiro et al. (2009) also add that the partial closure of the stomata can be considered as a strategy to avoid excessive dehydration or a consequence of the water imbalance in the epidermis of the leaves. However, Silva et al. (2010) cite that one of the consequences of the stomatal closure is the restriction to the entry of CO<sub>2</sub> into the cells, which could increase the susceptibility to photochemical damages, because low CO<sub>2</sub> assimilation rates cause excessive luminous energy in the photosystem II. Reductions in stomatal conductance due to the salinity of the environment have been observed in different crops, such as jatropha (Sousa et al., 2012), castor bean (Lima et al., 2014) and sunflower (Gomes et al., 2015).

Nitrogen fertilization linearly increased the stomatal conductance of 'BRS Rubi' cotton (Fig 2B) and, based on the regression equation, there was an increment in *g<sub>s</sub>* of 19.44% per 35% increase in N dose. Comparing plants subjected to N dose of 205 mg kg<sup>-1</sup> of soil with those that received 65 mg of N, there was an increment in *g<sub>s</sub>* of 77.76%. According to Correia et al. (2005), the increment in N dose usually leads to positive effects on stomatal conductance, since this nutrient is part of the main components of the photosynthetic system, such as chlorophylls, ribulose 1,5-biphosphate carboxylase/oxygenase (RuBisCO) and phosphoenolpyruvate carboxylase (PEPcase). Such superiority in the values of *g<sub>s</sub>* reflects the greater stomatal opening as a mechanism to meet the absorption of CO<sub>2</sub> from the external medium and regulate leaf temperature through transpiration (Pompeu et al., 2010). As to leaf transpiration of 'BRS Rubi' cotton (*E*), there was a linear and negative effect (Fig 3A), with reduction of 5.56% per unit increase in EC<sub>w</sub>. According to the regression equation (Fig 3A), plants irrigated with water of highest saline level (9.1 dS m<sup>-1</sup>) showed a reduction in *E* of 22.26% in relation to those irrigated with water of 5.1 dS m<sup>-1</sup>. Stomatal opening is directly related to the stomatal conductance and depends on the degree of water saturation of the stomatal cells. There might be restriction of transpiration if the water deficit in the plant is high, and the intensity varies with the action of internal factors, superficial area, distribution and number of stomata and external factors, such as light, relative air humidity, temperature and water availability in the soil (Cerqueira et al., 2004). Decrease in leaf transpiration, due to the increment in EC<sub>w</sub>, was also observed by Gomes et al. (2015), who evaluated the gas exchanges of sunflower irrigated with water of different salinity levels.

Unlike the result for *g<sub>s</sub>* (Fig 2A) and *E* (Fig 3A), the internal CO<sub>2</sub> concentration of 'BRS Rubi' cotton linearly increased as a function of water salinity and, according to the regression equation (Fig 3B), there was a linear increment in *C<sub>i</sub>* of the order of 13.62% per unit increase of EC<sub>w</sub>. The highest values of *C<sub>i</sub>* observed in cotton plants under the highest saline levels (Figure 3B) indicate that the carbon entering the leaf mesophyll cell was not being fixed in the carboxylation phase, possibly due to metabolic restrictions in the Calvin cycle because of the condition of salt stress to which these plants were exposed. Additionally, such

increment in internal CO<sub>2</sub> concentration is an indication that there was no restriction in the acquisition of CO<sub>2</sub> by the crop; however, when the CO<sub>2</sub> reached the mesophyll cells, the process of fixation during the carboxylation phase was compromised (Habermann et al., 2003), a fact that can be related to the degradation of the photosynthetic apparatus in response to the process of leaf senescence of the tissues, resulting from the stress caused by the excess of salts (Silva et al., 2013).

The CO<sub>2</sub> assimilation rate (Fig 3C), instantaneous carboxylation efficiency - EICI (Fig 3D) and instantaneous WUE (Fig 3E) were linearly and negatively affected by the increasing levels of EC<sub>w</sub>. According to the regression equation (Fig 3C), there was a reduction of 6.93% in the CO<sub>2</sub> assimilation rate per unit increase in EC<sub>w</sub>, indicating that plants subjected to EC<sub>w</sub> of 9.1 dS m<sup>-1</sup> showed a decrease of 27.72% in CO<sub>2</sub> assimilation rate, in relation to those irrigated under the threshold water salinity for the crop (5.1 dS m<sup>-1</sup>). Hence, the reduction observed in the CO<sub>2</sub> assimilation rate (Fig 4A), especially in plants irrigated with water of high salinity, may have been due to the reduction in stomatal conductance (Fig 2A), transpiration (Fig 3A) and to the increase in the internal CO<sub>2</sub> concentration (Fig 3B), demonstrating the low efficiency of use of the CO<sub>2</sub> that enters the cell. In addition, the decreases in the CO<sub>2</sub> assimilation rate of the 'BRS Rubi' cotton observed in the present study may be related to the stomatal closure associated with the osmotic and ionic effects caused by irrigation water salinity (Larcher, 2006). Similar results have also been observed in cotton (Meloni et al., 2003) and in jatropha (Sousa et al., 2012).

As to the variable instantaneous carboxylation efficiency (Fig 3D), cotton plants subjected to EC<sub>w</sub> of 9.1 dS m<sup>-1</sup> showed EICI values of 0.0076 [(μmol m<sup>-2</sup> s<sup>-1</sup>) (μmol mol<sup>-1</sup>)<sup>-1</sup>], which corresponded to a reduction of 24.24%, in relation to those irrigated with the lowest saline level (5.1 dS m<sup>-1</sup>) (Fig 3D). The expressive reduction of EICI in plants subjected to the highest EC<sub>w</sub> levels are reflexes of the low CO<sub>2</sub> assimilation rates (Fig 3C), in relation to the CO<sub>2</sub> found in the substomatal chamber (Fig 2A), because if the internal CO<sub>2</sub> concentration increases and there is a decrease in CO<sub>2</sub> consumption in the chloroplasts due to the reduction in photosynthetic activity. In this context, Machado et al. (2005) comment that the instantaneous carboxylation efficiency (EICI) is closely related to the CO<sub>2</sub> assimilation rate and to the internal CO<sub>2</sub> concentration.

In agreement with the results obtained for stomatal conductance (Fig 2A), leaf transpiration (Fig 3A) and CO<sub>2</sub> assimilation rate (Fig 3C), according to the regression equation (Fig 3E), there was lower instantaneous water use efficiency (WUE) in plants irrigated with EC<sub>w</sub> of 9.1 dS m<sup>-1</sup>, on average 4.92 [(μmol m<sup>-2</sup> s<sup>-1</sup>) (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], while plants under EC<sub>w</sub> of 5.1 dS m<sup>-1</sup> reached 7.49 [(μmol m<sup>-2</sup> s<sup>-1</sup>) (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], resulting in decreases of 65.68% in WUE, respectively. The gas exchanges, regulated by stomatal opening and closure, promote water loss due to the entry of CO<sub>2</sub>, and the decrease of such loss also restricts CO<sub>2</sub> entry (Damayanthi et al., 2010; Anjum et al., 2011).

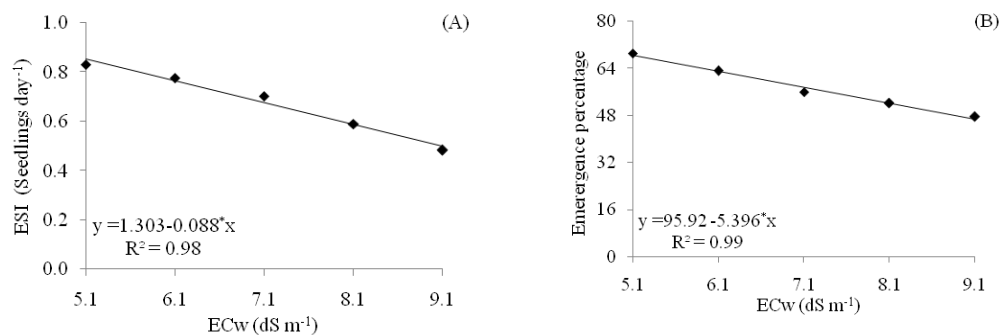
#### ***Effect of saline stress and nitrogen doses on colored-fiber cotton growth and flowering***

According to the summary of the analysis of variance (Table 2), there was significant effect of the different saline levels on stem diameter, plant height, leaf area, number of days for flower bud opening and flower abortion rate of the cotton cv. 'BRS Rubi'. For the N doses and the interaction between

**Table 1.** Summary of the analysis of variance referring to the emergence percentage (EP), emergence speed index (ESI), stomatal conductance (*gs*), transpiration (*E*), CO<sub>2</sub> assimilation rate (*A*), internal CO<sub>2</sub> concentration (*Ci*), instantaneous carboxylation efficiency (EICI) and intrinsic water use efficiency (WUE) of cotton plants, cv. ‘BRS Rubi’, irrigated with waters of different saline levels and nitrogen doses.

Source of variation	DF	Mean squares							
		ESI	EP	<i>gs</i>	<i>E</i>	<i>A</i>	<i>Ci</i>	EICI	WUE
Saline levels (SL)	4	0.30*	1125.99*	0.003*	0.10**	11.02**	17895.68**	0.0003**	14.68*
Linear regression	1	1.15*	4295.95*	0.01*	0.38**	40.00**	67213.30**	0.001**	56.14**
Quadratic regression	1	0.02 <sup>ns</sup>	41.10 <sup>ns</sup>	0.0008 <sup>ns</sup>	0.01 <sup>ns</sup>	0.74 <sup>ns</sup>	642.14 <sup>ns</sup>	0.00002 <sup>ns</sup>	0.07 <sup>ns</sup>
N doses (ND)	4	0.07 <sup>ns</sup>	330.29 <sup>ns</sup>	0.002*	0.03 <sup>ns</sup>	0.43 <sup>ns</sup>	3163.21 <sup>ns</sup>	0.00001 <sup>ns</sup>	2.11 <sup>ns</sup>
Linear regression	1	0.17 <sup>ns</sup>	48.98 <sup>ns</sup>	0.004*	0.02 <sup>ns</sup>	0.004 <sup>ns</sup>	2642.64 <sup>ns</sup>	0.00004 <sup>ns</sup>	2.91 <sup>ns</sup>
Quadratic regression	1	0.06 <sup>ns</sup>	168.82 <sup>ns</sup>	0.002 <sup>ns</sup>	0.003 <sup>ns</sup>	0.08 <sup>ns</sup>	7662.46 <sup>ns</sup>	0.00004 <sup>ns</sup>	0.38 <sup>ns</sup>
Interaction (SLxND)	15	0.16 <sup>ns</sup>	165.77 <sup>ns</sup>	0.0009 <sup>ns</sup>	0.01 <sup>ns</sup>	0.91 <sup>ns</sup>	1514.84 <sup>ns</sup>	0.00001 <sup>ns</sup>	4.70 <sup>ns</sup>
Blocks	2	0.04 <sup>ns</sup>	412.67	0.001 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	3699.01 <sup>ns</sup>	0.00001 <sup>ns</sup>	2.05 <sup>ns</sup>
Residual		16.69	15.35	13.89	15.35	10.77	10.28	14.51	17.09

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

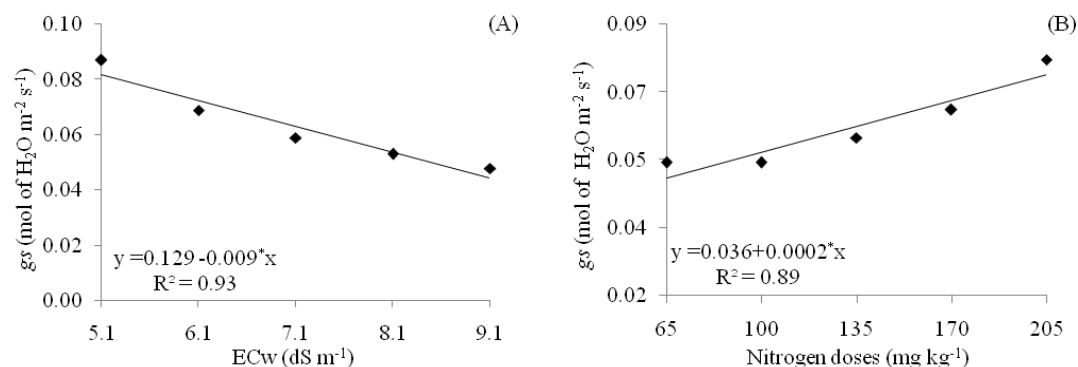


**Fig 1.** Emergence speed index - ESI (A) and emergence percentage – EP of cotton, cv. ‘BRS Rubi’, as a function of the electrical conductivity of the irrigation water – ECw.

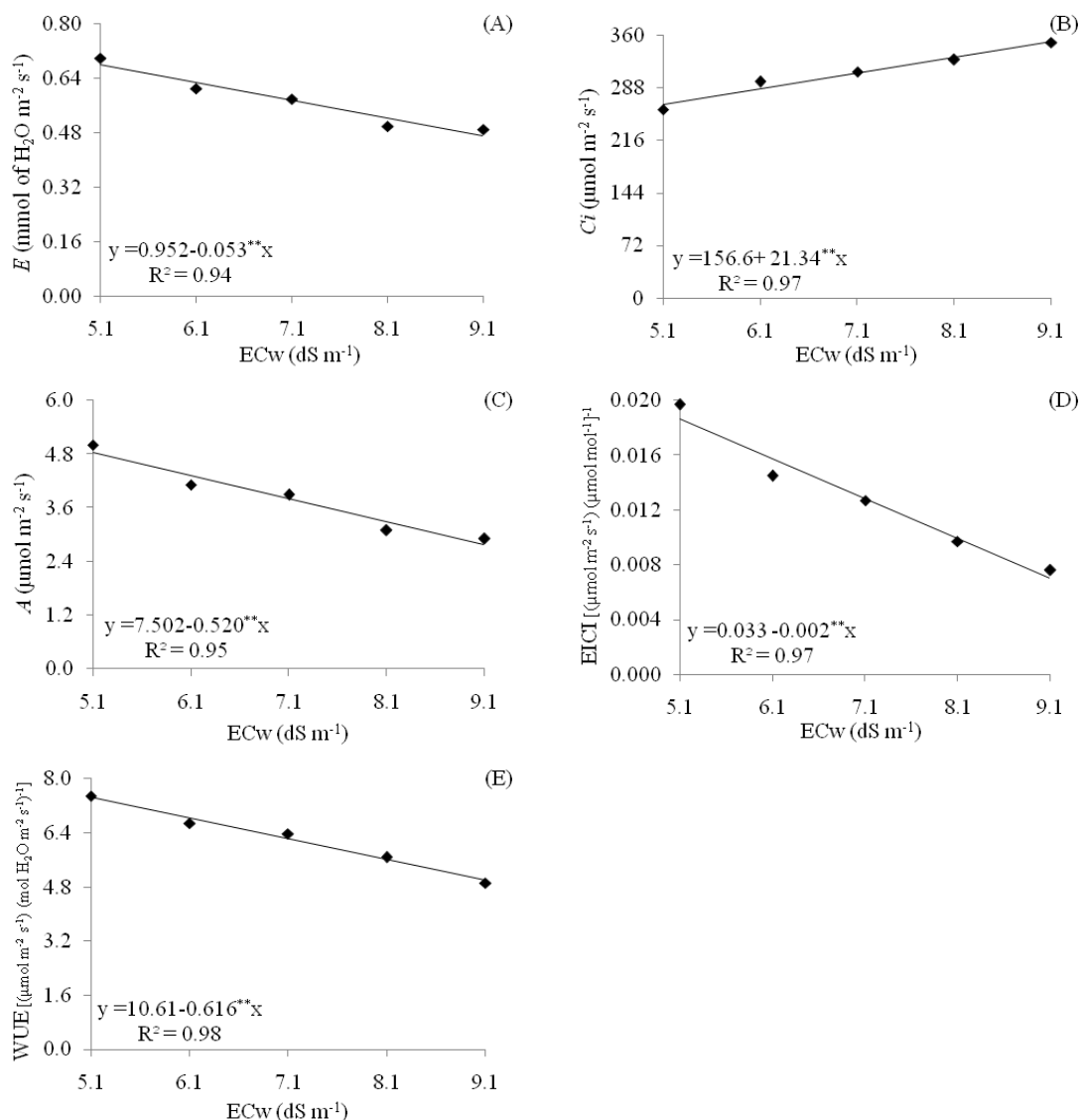
**Table 2.** Summary of the analysis of variance referring to stem diameter (SD), plant height (PH) leaf area (LA), number of days for flower bud opening (NFO) and flower abortion rate (FAR) of ‘BRS Rubi’ cotton cultivated under irrigation with saline waters and doses of nitrogen.

Source of variation	DF	Mean squares				
		SD	PH	LA	NFO	FAR
Saline levels (SL)	4	8.31**	826.56**	3882863.74**	324.72**	5502.81**
Linear regression	1	31.16**	3123.47**	14017969.94**	1191.86**	20019.39**
Quadratic regression	1	0.84 <sup>ns</sup>	65.01 <sup>ns</sup>	867757.67*	1.90 <sup>ns</sup>	837.74 <sup>ns</sup>
N doses (ND)	4	0.13 <sup>ns</sup>	3.03 <sup>ns</sup>	37331.57 <sup>ns</sup>	60.10 <sup>ns</sup>	415.95 <sup>ns</sup>
Linear regression	1	0.14 <sup>ns</sup>	3.34 <sup>ns</sup>	4126.81 <sup>ns</sup>	179.30 <sup>ns</sup>	234.20 <sup>ns</sup>
Quadratic regression	1	0.31 <sup>ns</sup>	0.43 <sup>ns</sup>	114599.48 <sup>ns</sup>	0.00 <sup>ns</sup>	918.84 <sup>ns</sup>
Interaction (SLxND)	15	0.15 <sup>ns</sup>	17.09 <sup>ns</sup>	70921.23 <sup>ns</sup>	27.23 <sup>ns</sup>	406.21 <sup>ns</sup>
Blocks	2	0.10 <sup>ns</sup>	17.08 <sup>ns</sup>	140981.16 <sup>ns</sup>	101.37 <sup>ns</sup>	667.85 <sup>ns</sup>
Residual		11.47	15.68	26.54	6.97	19.93

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



**Fig 2.** Stomatal conductance–*gs* of cotton, cv. ‘BRS Rubi’, as a function of the electrical conductivity of the irrigation water - ECw (A) and nitrogen doses (B).



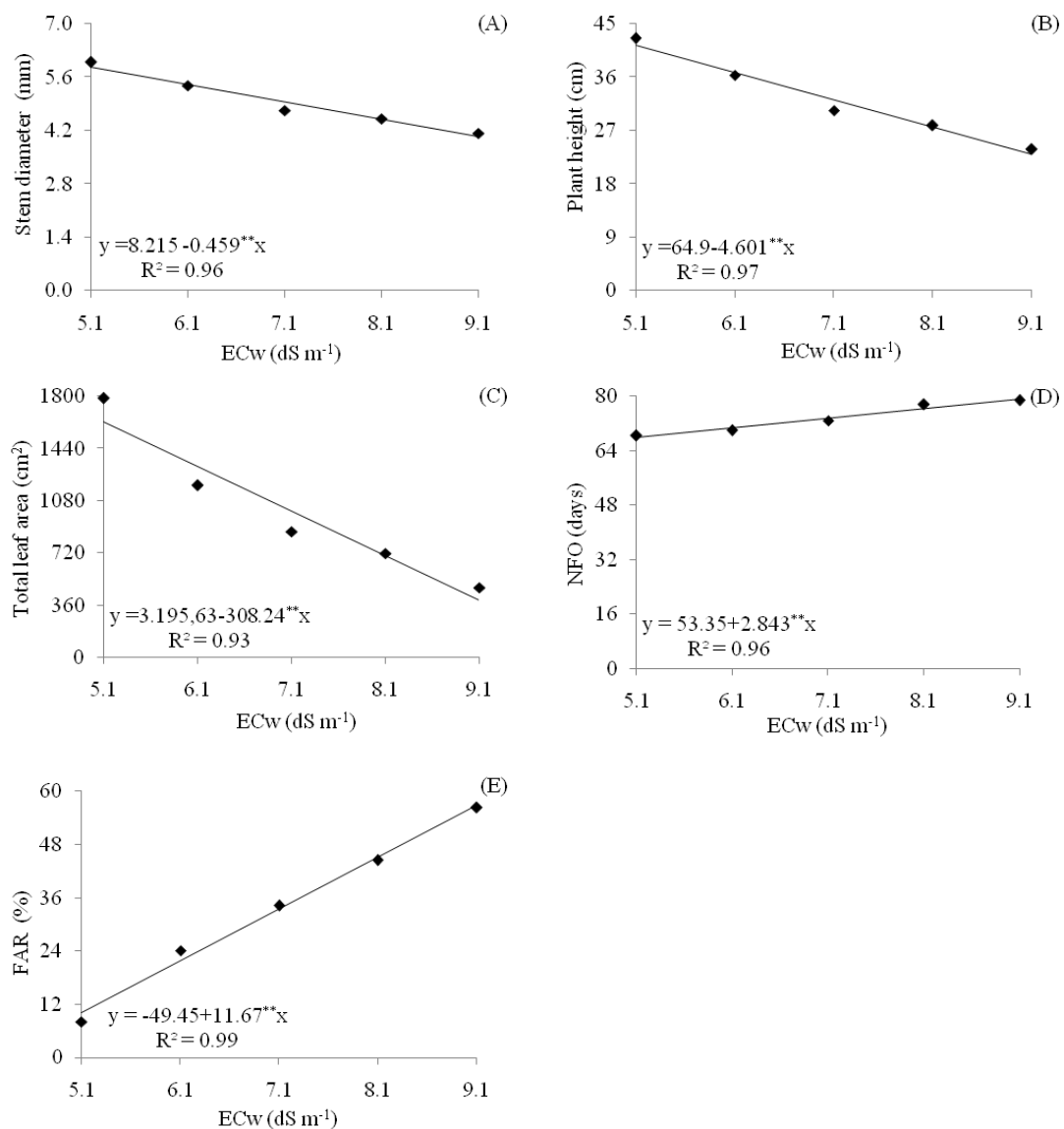
**Fig 3.** Transpiration  $-E$  (A), internal CO<sub>2</sub> concentration  $-Ci$  (B), CO<sub>2</sub> assimilation rate  $-A$  (C), instantaneous carboxylation efficiency  $-EiCI$  (D) and intrinsic water use efficiency  $-WUE$  (E) of cotton, cv. 'BRS Rubi', as a function of the electrical conductivity of the irrigation water  $-ECw$ .

factors (SL x ND), there was no significant difference for any of the studied variables, which indicates that the N doses showed similar behavior at different saline levels of the water used in irrigation.

The increase in the electrical conductivity of the irrigation water negatively affected the stem diameter and plant height of 'BRS Rubi' cotton (Fig 4A and 4B) showing, according to the regression equations, more accentuated effects on SD and PH in plants irrigated using water with ECw of 9.1 dS m<sup>-1</sup>. Comparing the values obtained in plants subjected to ECw of 9.1 dS m<sup>-1</sup> with those of plants irrigated with water of 5.1 dS m<sup>-1</sup>, there were reductions of 1.83 mm (22.34%) in SD and 1.84 cm (28.35%) in PH. Thus, it can be noted (Fig 4B) that the damages due to the salt stress were more pronounced on the growth in height of the cotton, cv. 'BRS Rubi'. The reduction of growth observed through the decrease in SD and PH were probably due to the reduction of the osmotic potential of the soil solution resulting from the increment in the salinity levels, directly affecting water absorption, cell division and elongation and inhibiting plant growth (Graciano et al., 2011). Santos et al. (2012) also add that this trend of decrease in the growth of 'BRS Rubi' cotton can be attributed

to the fact that the plant, in order to adjust osmotically, spends a certain amount of energy to accumulate sugars, organic acids and ions in the vacuole, energy that could have been used for growth.

Irrigation with waters of different salinity levels negatively affected the leaf area of 'BRS Rubi' cotton. Based on the regression equation (Fig 4C), a reduction of 9.64% per unit increase in ECw was observed. In addition, the regression equation (Fig 4C) also indicates that 'BRS Rubi' cotton plants, when irrigated with water of 9.1 dS m<sup>-1</sup>, exhibited a reduction in leaf area of 1232.96 cm<sup>2</sup> in relation to those subjected to ECw of 5.1 dS m<sup>-1</sup>. The reduction of leaf area in cotton plants irrigated with water of high salinity level reflects the decrease observed in stomatal conductance (Fig 2A), transpiration (Fig 3A), CO<sub>2</sub> assimilation rate  $-A$  (Fig 3C) and in the instantaneous water use efficiency (Fig 3E). Additionally, this decrease in leaf area is probably a result of the reduction in the volume of cells; with the reduction in the transpiring surface (Fig 4C), the water losses through stomatal flow are minimized (Fig 2A), thus contributing to the maintenance of a high water potential in the plant (Nascimento et al., 2011).



**Fig 4.** Stem diameter (A), plant height (B), total leaf area (C), number of days for flower bud opening - NFO (D) and flower abortion rate – FAR(E)of cotton, cv. ‘BRS Rubi’, as a function of the electrical conductivity of the irrigation water – ECw.

The number of days for flower bud opening of ‘BRS Rubi’ cotton significantly increased as a function of the irrigation water salinity levels. According to the regression equation (Fig 4D), as the levels of irrigation water salinity increased, there was an increment in the number of days for flower bud opening of 5.32% per unit increase in ECw. Comparing cotton plants under highest level of electrical conductivity (9.1 dS m<sup>-1</sup>) with those irrigated with the lowest ECw level (5.1 dS m<sup>-1</sup>) in Fig 4D, there was an increase of approximately 11 days (21.31%) in the number of days for flower bud opening. Thus, the highest values of NFO obtained in plants irrigated with waters of highest ECw probably result from the deviation of energy, due to the progressive salt stress caused by the different levels of water salinity, as explained by Silva et al. (2012). In this context, Cavalcante et al. (2010) add that plants cultivated under salt stress conditions are able to stimulate and assimilate micronutrients that influence the capacity to anticipate or retard the stage of development and production of the plants, for directly compromising the time for flower bud opening, of open flowers and fruits. In a study with the castor bean cultivar ‘BRS Energia’, Lima et al. (2015) also observed that the irrigation with saline waters

(ECw from 0.3 to 3.9 dS m<sup>-1</sup>) prolongs the time of production of the raceme, and the increment is equal to ten days in plants under ECw of 3.9 dS m<sup>-1</sup> in comparison to those under ECw of 0.3 dS m<sup>-1</sup>. As observed for NFO (Fig 4D), the flower abortion rate of ‘BRS Rubi’ cotton also increased linearly and, according to the regression equation (Fig 4E), there was an increment in FAR of 23.59% per unit increase in ECw, i.e., plants irrigated with water of 9.1 dS m<sup>-1</sup> showed an increment in flower abortion rate of 94.39%, compared with those subjected to ECw of 5.1 dS m<sup>-1</sup>. Thus, it can be inferred that the expressive increase in FAR can be related to the low CO<sub>2</sub> assimilation rates (Fig 3C) and stomatal conductance (Fig 2A) and to the increment in the internal CO<sub>2</sub> concentration (Fig 3B), a fact that reflected in decrease of growth (SD, PH and LA) of the ‘BRS Rubi’ cotton and, consequently, it may have contributed to prolong such parameter. It should be noted that the excess of salts in the root zone of the plants also has harmful effects on flowering, because of the greater osmotic effect outside the root and restriction to the water flow from the soil to the plants.

## Materials and methods

### Localization, experimental procedure, treatments and plant material

The experiment was conducted in pots adapted as drainage lysimeters under greenhouse conditions, during March and July 2016, 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 the geographic coordinates of 7°15'18" S, 35°52'28" W and altitude of 550 m.

The experimental design was in completely randomized blocks in a 5 x 5 factorial scheme, with three replicates, and the treatments were composed of five levels of irrigation water electrical conductivity - ECw (5.1; 6.1; 7.1; 8.1 and 9.1 dSm<sup>-1</sup>) and five N doses - ND (65; 100; 135; 170; 205 mg of N kg<sup>-1</sup> of soil), which according to the recommendation of N fertilization for pot experiments of Novais et al. (1991), corresponded to 65, 100, 135, 170 and 205%.

This study used the cotton cultivar 'BRS Rubi', it is a genetic material indicated for cultivation in the semi-arid region of Northeast Brazil. It is a cultivar with dark brown or reddish brown fiber, mean plant height of 1.10 m and cultivation cycle of 120 to 140 days (EMBRAPA, 2011).

### Establishment and management of the experiment

Plants were grown in drainage lysimeters with capacity for 20 L (height of 35 cm, bottom diameter of 20 cm and upper opening diameter of 31 cm), perforated at the bottom to allow drainage, connected to a drain with diameter of 4 mm at the base. The tip of the drain inside the lysimeter was involved in a nonwoven geotextile (Bidim OP 30) to avoid obstruction by soil material and, below each drain, a plastic bottom was placed to collect the drained water and estimate plant water consumption.

The lysimeters were filled with a 0.5-kg layer of crushed stone (size 0), followed by 26 kg of Eutrophic Regolithic Neosol, of a sandy loam texture (0-20 cm), whose chemical and physico-hydraulic characteristics were determined according to the methodologies proposed by Claessen (1997): Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup> and H<sup>+</sup> + Al<sup>3+</sup> = 3.49, 2.99, 0.17, 0.21 and 5.81 cmolckg<sup>-1</sup>, respectively; organic matter = 1.83 dag kg<sup>-1</sup>; P = 18.2 mg kg<sup>-1</sup>; pH in water (1:2.5) = 5.63; electrical conductivity of the saturation extract = 0.61 dS m<sup>-1</sup>; water content at 33.42 and 1519.5 kPa = 12.68 and 4.98 dag kg<sup>-1</sup>. The irrigation waters were prepared using NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O, at the proportion equivalent to 7:2:1, between Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>, respectively, adjusting the concentration of the public supply water from the municipality of Campina Grande-PB, considering the relationship between ECw and the concentration of salts (10\*mmol<sub>c</sub> L<sup>-1</sup> = ECw dS m<sup>-1</sup>), according to Richards (1954). Before sowing, soil water content was increased up to field capacity (33.42 kPa), using the respective water of each treatment. After sowing, irrigation was daily performed, by applying in each lysimeter a water volume to maintain soil moisture close to field capacity, and the volume to be applied was determined according to the water requirement of the plants, estimated through the water balance: applied volume minus the volume drained in the previous irrigation. Eight seeds of cotton, cv. 'BRS Rubi', were planted in each lysimeter at depth of 1.5 cm, equidistantly distributed. At 15 and 25 days after sowing (DAS), thinnings were performed to leave only one plant per lysimeter.

Fertilization with phosphorus and potassium was performed according to the recommendation of Novais et al. (1991), applying 300 mg of P<sub>2</sub>O<sub>5</sub> and 150 mg of K<sub>2</sub>O kg<sup>-1</sup> of soil, using single superphosphate and potassium chloride. Phosphorus was entirely applied as basal dose. The N source was urea. Fertilizations with K<sup>+</sup> and the different N doses were split equally; K<sup>+</sup> was applied at 12, 28 and 42 DAS, while N was applied at 15, 30, 45 and 60 DAS. In order to correct the deficiencies of micronutrients, 3 L of solution containing 2.5 g L<sup>-1</sup> of ubyfol [(N (15%); P<sub>2</sub>O<sub>5</sub> (15%); K<sub>2</sub>O (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%)] were applied through foliar spray at 25, 40 and 55 DAS. The phytosanitary management was performed preventively, by applying insecticides from the Neonicotinoid chemical group, fungicide from the Triazole chemical group and acaricide from the Abamectin chemical group, at the doses of 5.4, 7.0 and 3.5 g L<sup>-1</sup>, respectively. Manual weedings and superficial scarification of the soil were weekly performed before each irrigation event.

### Traits measured

The effects of the treatments on the cotton crop, cv. 'BRS Rubi', were evaluated at 83 DAS, through variables related to germination: emergence percentage (EP), emergence speed index (ESI); gas exchanges: stomatal conductance (g<sub>s</sub>), transpiration (E), CO<sub>2</sub> assimilation rate (A), internal CO<sub>2</sub> concentration (C<sub>i</sub>), instantaneous carboxylation efficiency (E<sub>IC</sub>), intrinsic water use efficiency (WUE); growth: stem diameter (SD), plant height (PH) and leaf area (LA); the variables related to flowering were measured at 100 DAS, through determination of number of days for flower bud opening (NFO) and flower abortion rate (FAR)

Seed germination was evaluated until stabilization of emergence, recording daily the number of emerged seedlings and adopting as criterion the appearance of the epicotyl on soil surface. Then, the emergence percentage - EP was measured (number of emerged seedlings in relation to the number of planted seeds). At 10 DAS, the emergence speed index (ESI) was determined according to methodology described by Vieira and Carvalho (1994), using Eq.1

$$ESI \text{ (seedlings day}^{-1}\text{)} = \frac{\sum_1}{N_1} + \frac{\sum_2}{N_2} + \dots + \frac{\sum_n}{N_n}$$

Where:  $\sum_1, \sum_2, \dots, \sum_n$  represent the number of emerged seedlings in the first, second and last counts,  $N_1, N_2, \dots, N_n$  are the number of days from sowing to the first, second and last counts, respectively.

Stomatal conductance (mol of H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration (mmol of H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> assimilation rate (μmol m<sup>-2</sup> s<sup>-1</sup>) and internal CO<sub>2</sub> concentration (μmol m<sup>-2</sup> s<sup>-1</sup>) were evaluated on the third leaf counted from the apex, using the portable device for photosynthesis measurement "LCPro+" from ADC BioScientific Ltda. These data were used to quantify intrinsic WUE (A/E) [(μmol m<sup>-2</sup> s<sup>-1</sup>) (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>] and the instantaneous carboxylation efficiency (A/C<sub>i</sub>) [(μmol m<sup>-2</sup> s<sup>-1</sup>) (μmol mol<sup>-1</sup>)<sup>-1</sup>] (Konrad et al., 2005; Jaimez et al., 2005).

Plant height was obtained based on the distance from the base of the plant until the insertion of the apical meristem. Stem diameter was measured 5 cm from the base of the plant. Leaf area was obtained by measuring the midrib length of all leaves of the plants, taking into consideration the methodology described by Grimes y Carter (1969), according to Eq 2:  $y = \sum (0,4322x^{2,3002})$ , where: y - total leaf area per plant (cm<sup>2</sup>), x - midrib length (cm) of the main leaf. The total leaf area per plant was determined by the sum of the leaf area of all leaves. The NFO was estimated through the daily

monitoring of flower bud opening, while flower abortion rate was obtained through the relationship between the number of aborted flower buds and the total number of flowers produced, in percentage

### Statistical analysis

The data were evaluated using analysis of variance by F test at 0.05 and 0.01 probability levels and, in cases of significance, linear and quadratic polynomial regression analyses were applied, using the statistical program SISVAR-ESAL (Ferreira, 2011).

### Conclusion

The germination, gas exchanges and growth of cotton, cv. 'BRS Rubi', are negatively affected by irrigation water salinity from 5.1 dS m<sup>-1</sup> on; Irrigation with water of electrical conductivity higher than 5.1 dS m<sup>-1</sup> prolongs the period for flower bud opening and promotes increase in flower abortion rate and internal CO<sub>2</sub> concentration of cotton, cv. 'BRS Rubi'; Nitrogen fertilization promotes increment in the stomatal conductance of the cotton crop, and the highest values were obtained at the dose of 205 mg of N kg<sup>-1</sup> of soil; The salt stress caused by the different levels of irrigation water salinity and nitrogen fertilization doses act as independent factors for the studied variables of the cotton crop.

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