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## Growth and gas exchange of corn under salt stress and nitrogen doses<sup>1</sup>

### Crescimento e trocas gasosas do milho sob estresse salino e doses de nitrogênio

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#### HIGHLIGHTS:

*The salinity of irrigation water affects the growth and gas exchange of corn at 30 and 45 DAS.*

*Nitrogen fertilization provides greater growth performance and gas exchange in corn plants.*

*The dose corresponding to 100% of the N recommendation (160 kg ha<sup>-1</sup>) mitigated salt stress in corn plants.*

**ABSTRACT:** The excess of salts can affect several processes in the crops, and nitrogen (N) can attenuate the depressive effect of salinity. The objective was to evaluate the influence of nitrogen doses on the growth and gas exchange of corn crop irrigated with saline water. The experiment was conducted from June to September 2019 at the University of International Integration of Afro-Brazilian Lusophony, Redenção, CE, Brazil. The experimental design was completely randomized, in a 2 x 3 factorial scheme (supply water of 0.3 dS m<sup>-1</sup> and saline solution of 3.0 dS m<sup>-1</sup>) and three nitrogen doses, 0, 80, and 160 kg ha<sup>-1</sup>, corresponding to 0, 50, and 100% of the recommended dose respectively, with six repetitions. At 30 and 45 days after sowing (DAS), plant height, leaf area, number of leaves, photosynthesis, transpiration, and stomatal conductance were evaluated. Saline stress affects plant height, leaf area, photosynthesis, transpiration, and conductance at 30 DAS. The doses of 80 and 160 kg ha<sup>-1</sup> provide greater performance in plant height, leaf area, photosynthesis, transpiration, and conductance at 30 DAS. The use of low salinity water and doses of 80 and 160 kg ha<sup>-1</sup> were more efficient in terms of plant height, leaf area, photosynthesis, transpiration, and conductance at 45 DAS. The dose of 160 kg ha<sup>-1</sup> of N attenuates the harmful effects of salts in AG 1051 hybrid maize plants, providing higher values of photosynthesis, transpiration, and stomatal conductance at 45 DAS when irrigated with water of 3.0 dS m<sup>-1</sup>.

**Key words:** *Zea mays*, saline stress

**RESUMO:** O excesso de sais pode afetar diversos processos nas culturas, e o nitrogênio (N) pode atenuar o efeito depressivo da salinidade. Objetivou-se avaliar a influência de doses de nitrogênio no crescimento e nas trocas gasosas da cultura do milho irrigado com água salina. O experimento foi conduzido no período de junho a setembro de 2019, na Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Redenção, CE. O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2 x 3 água de abastecimento de 0,3 dS m<sup>-1</sup> e solução salina de 3,0 dS m<sup>-1</sup>, e três doses de nitrogênio, 0, 80 e 160 kg ha<sup>-1</sup>, correspondendo a 0, 50 e 100% da dose recomendada, respectivamente, com seis repetições. Aos 30 e 45 dias após a semeadura (DAS) foram avaliadas altura de planta, área foliar, número de folhas, fotossíntese, transpiração e a condutância estomática. O estresse salino afeta a altura de plantas, área foliar, fotossíntese, transpiração e a condutância aos 30 DAS. As doses de 80 e 160 kg ha<sup>-1</sup> proporcionam maior desempenho em altura de plantas, área foliar, fotossíntese, transpiração e a condutância aos 30 DAS. O uso de água de baixa salinidade e as doses de 80 e 160 kg ha<sup>-1</sup> foram mais eficientes quanto à altura de plantas, área foliar, fotossíntese, transpiração e a condutância aos 45 DAS. A dose de 160 kg ha<sup>-1</sup> de N atenua os efeitos deletérios dos sais nas plantas de milho híbrido AG 1051 proporcionando maiores valores de fotossíntese, transpiração e condutância estomática aos 45 DAS, quando irrigadas com água de 3,0 dS m<sup>-1</sup>.

**Palavras-chave:** *Zea mays*, estresse salino

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## INTRODUCTION

Corn (*Zea mays* L.) is one of the main cereals produced in Brazil, widely used in human and animal nutrition. Even with great importance, part of the producers often cultivates corn inappropriately, requiring practical studies focused on irrigated agriculture combined with the correct fertilization management (Dantas Junior et al., 2016).

Irrigation with brackish water emerges as a necessary point in these practical studies since the soil and water salinity is one of the main obstacles to the crop production system, especially in regions where evapotranspiration exceeds rainfall, as in the Brazilian semiarid region (Silva et al., 2013).

The use of saline water negatively affects plant growth and metabolism, reducing gas exchange, nutrient absorption, and crop yield. Confirming this information, Sousa et al. (2016) observed that saline stress decreases photosynthesis, stomatal conductance, and transpiration and Costa et al. (2018) and Rodrigues et al. (2020) found negative effects on the absorption of mineral elements and yield of the corn crop, respectively.

Proper fertilization helps to increase crop growth and yield. Among the fertilizer sources, nitrogenate sources provide nitrogen (N), one of the macronutrients most absorbed by the corn crop, playing an important role in its growth. It has been used in saline environments as an attenuator of the depressive effect of salinity, helping in the accumulation of nitrogenous organic compounds, such as proline and free amino acids, which directly contribute to the osmotic adjustment and protection of cellular structures and functions, resulting in better plant growth (Perveen & Nazir, 2018; Braz et al., 2019).

It is worth noting that in recent years, studies describe the benefits of nitrogen fertilization in plants grown in a saline environment (Silva et al., 2017). However, even with these benefits, there is no unanimity about the positive effects of such a practice; more studies are needed on the responses related to the interaction between nitrogen fertilization and saline stress.

Given this scenario, the objective was to evaluate the influence of nitrogen fertilization on growth and gas exchange of the corn crop irrigated with saline water.

## MATERIAL AND METHODS

The experiment was carried out in full sunlight in an area belonging to the Auroras Seedling Production Unit (UPMA), at the University of International Integration of Afro-Brazilian Lusophony (UNILAB), located at Campus das Auroras, in Redenção, CE, Brazil (4° 13' 33" S, 38° 43' 39" W, and altitude of 88 m) from June to September 2019. According to Köppen (1923), the region's climate is Aw-type, tropical climate with the dry winter season, the average air temperature of the warmest month above 38 °C and the coldest month below 20 °C.

The experimental design adopted was completely randomized (CRD) in a 2 x 3 factorial scheme, with six repetitions, corresponding to two values of electrical conductivity of the irrigation water - ECw (supply water 0.3 dS m<sup>-1</sup> and saline solution 3.0 dS m<sup>-1</sup>) associated with three nitrogen doses (0, 80, and 160 kg ha<sup>-1</sup>), estimated according to recommendation by Coelho (2006), corresponding to 0, 50

and 100% of the recommended dose, respectively. The nitrogen doses were applied in split form during the evaluation period, using urea as a source (45% N). Three applications (sowing and topdressing) of triple superphosphate and potassium chloride were also carried out, totaling 70 kg ha<sup>-1</sup> and 120 kg ha<sup>-1</sup>, respectively. So, for a 10,000-plant stand, the dosage per pot per plant was 16 g of N, 7 g of P<sub>2</sub>O<sub>5</sub>, and 12 g of K<sub>2</sub>O.

The hybrid corn seeds, AG 1051, were sown in pots of flexible plastic material with a volumetric capacity of 25 L adapted for drainage lysimeter, placing five seeds per pot, leaving, at 12 days after sowing (DAS), only one plant per pot, which were filled with substrate containing arisco, sand, and bovine manure in the proportion 3:1:1 (volume basis), respectively, whose chemical analysis revealed the following composition N = 0.26 g kg<sup>-1</sup>; P = 65 mg kg<sup>-1</sup>; K<sup>+</sup> = 0.65 cmol<sub>c</sub> kg<sup>-1</sup>; Ca<sup>2+</sup> = 1.20 cmol<sub>c</sub> kg<sup>-1</sup>; Mg<sup>2+</sup> = 1.20 cmol<sub>c</sub> kg<sup>-1</sup>; Na<sup>+</sup> = 0.33 cmol<sub>c</sub> kg<sup>-1</sup>; H<sup>+</sup>+Al<sup>3+</sup> = 1.32 cmol<sub>c</sub> kg<sup>-1</sup>; Al<sup>3+</sup> = 0.15 cmol<sub>c</sub> kg<sup>-1</sup>; S = 3.4 cmol<sub>c</sub> kg<sup>-1</sup>; CEC = 4.7 cmol<sub>c</sub> kg<sup>-1</sup>; V% = 72; ESP = 7%; OM = 4.34 g kg<sup>-1</sup>; pH = 6.2; EC = 1.19 dS m<sup>-1</sup>.

The irrigation water was prepared by dissolving salts (NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, and MgCl<sub>2</sub>·6H<sub>2</sub>O), in the equivalent proportion of 7:2:1 between Na, Ca, and Mg, obeying the relationship between ECw and its concentration (mmolc L<sup>-1</sup> = EC × 10), according to the methodology contained in Rhoades et al. (2000), with irrigation applied manually daily, calculated according to the drainage lysimeter principle (Bernardo et al., 2019), keeping the soil at field capacity.

Irrigation with water with higher saline concentration started at 13 DAS. The nitrogen doses were applied at 19 DAS, when the crops were fully established.

The growth and gas exchange analyzes were performed at 30 and 45 (DAS), where the variables analyzed were: plant height (PH) using a measuring tape, measuring the distance between the soil surface and the plant apex (cm); leaf area (LA), calculated through linear measures of maximum leaf length (L) and width (W) (cm), multiplying the values by the correction factor (0.75), according to the equation LF = 0.75 (LxW), as the methodology by Oliveira et al. (2016), and the number of leaves (NF) through direct counting of fully expanded leaves. To determine photosynthesis (A), stomatal conductance (gs), and transpiration (E), fully expanded leaves from each plant were chosen, and an IRGA infrared gas analyzer (LI 6400 XT from LICOR) was used in an open system with a flow of air of 300 mL min<sup>-1</sup>; measurements were made between 10:00 a.m. and 12:00 a.m.

The data after collected were submitted to analysis of variance (ANOVA). In cases of the significance of the interaction or isolated factors, the Tukey test was performed at p ≤ 0.01 and p ≤ 0.05 using the software Assistant. 7.7 Beta (Silva & Azevedo, 2016).

## RESULTS AND DISCUSSION

It is observed in Table 1, at 30 DAS, that the variables plant height, leaf area, photosynthesis, transpiration, and stomatal conductance were influenced in isolation by the factors, salinity and nitrogen doses, and the variable number of leaves by the interaction between factors. At 45 DAS, the

**Table 1.** Summary of analysis of variance for plant height (PH), leaf area (LA), number of leaves (NL), photosynthesis (A), transpiration (E), and stomatal conductance (gs) at 30 and 45 DAS, in corn plants under different water electrical conductivities and nitrogen fertilization doses

SV	DF	Mean square					
		PH	LA	NL	A	E	gs
30 DAS							
Salinity (S)	1	66.15*	11417.56**	10.02**	100.36**	2.25**	0.22**
Doses (D)	2	91.12**	7479.43*	13.08**	69.32**	0.63**	0.20**
S x D	2	1.80 <sup>ns</sup>	515.27 <sup>ns</sup>	1.69*	4.90 <sup>ns</sup>	0.01 <sup>ns</sup>	0.009 <sup>ns</sup>
Treatments	5	50.40**	5481.39*	7.91**	49.76**	0.71**	0.13**
Residue	30	9.09	1505.52	0.43	6.17	0.02	0.02
CV (%)	-	8.12	17.42	6.79	8.05	4.47	33.96
45 DAS							
Salinity (S)	1	1116.22**	50948.95**	11.11**	31.21**	0.30**	0.004**
Doses (D)	2	1325.69**	108764.49**	30.52**	56.52**	1.05**	0.01**
S x D	2	62.49 <sup>ns</sup>	1022.53 <sup>ns</sup>	4.19**	12.00*	0.01*	0.001**
Treatments	5	778.52**	54104.60**	16.11**	33.65**	0.48**	0.008**
Residue	30	34.72	3548.40	0.32	2.19	0.002	0.00007
CV (%)	-	11.08	12.97	5.81	9.06	2.97	4.45

SV - Source of variation; DF - Degrees of freedom; CV - Coefficient of variation; \*, \*\*, ns - Significant at  $p \leq 0.05$ ,  $p \leq 0.01$  and not significant, respectively, by F test

factors in isolation influenced plant height and leaf area. In contrast, the number of leaves, photosynthesis, transpiration, and stomatal conductance variables suffered a significant effect of the interaction between factors.

Plants irrigated with water of  $0.3 \text{ dS m}^{-1}$  grew more in height in both evaluations, at 30 and 45 days (Figures 1A, C). Although exceeding the absence of nutrient addition, nitrogen doses did not differ between 30 and 45 DAS. (Figures 1B, D).

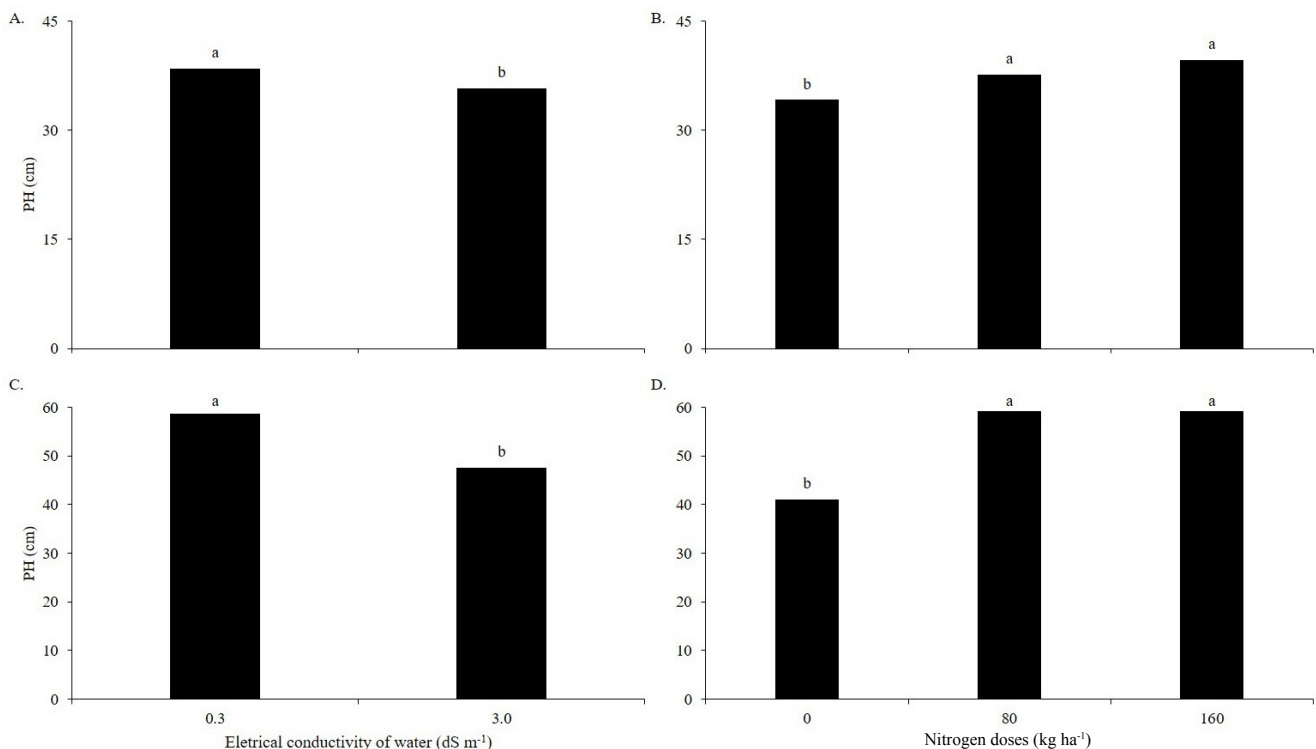
The reduction in plant height with an increase in the electrical conductivity of irrigation water can be explained by the fact that water with a high concentration of salts decreases cell turgor potential, affecting their growth (Braz et al., 2019). This plant height response regarding to salinity was

also observed by Sousa et al. (2016) when working with corn, obtaining lower values from irrigation with water of  $1.5 \text{ dS m}^{-1}$ .

The positive effect of nitrogen for this variable is related to its structural function since this nutrient is part of enzymes and hormones that favor plant growth (Prado, 2008), which was also observed by Khan et al. (2014), who obtained higher plant height in the corn crop when increased with nitrogen fertilization similar to this study ( $150 \text{ kg ha}^{-1}$ ).

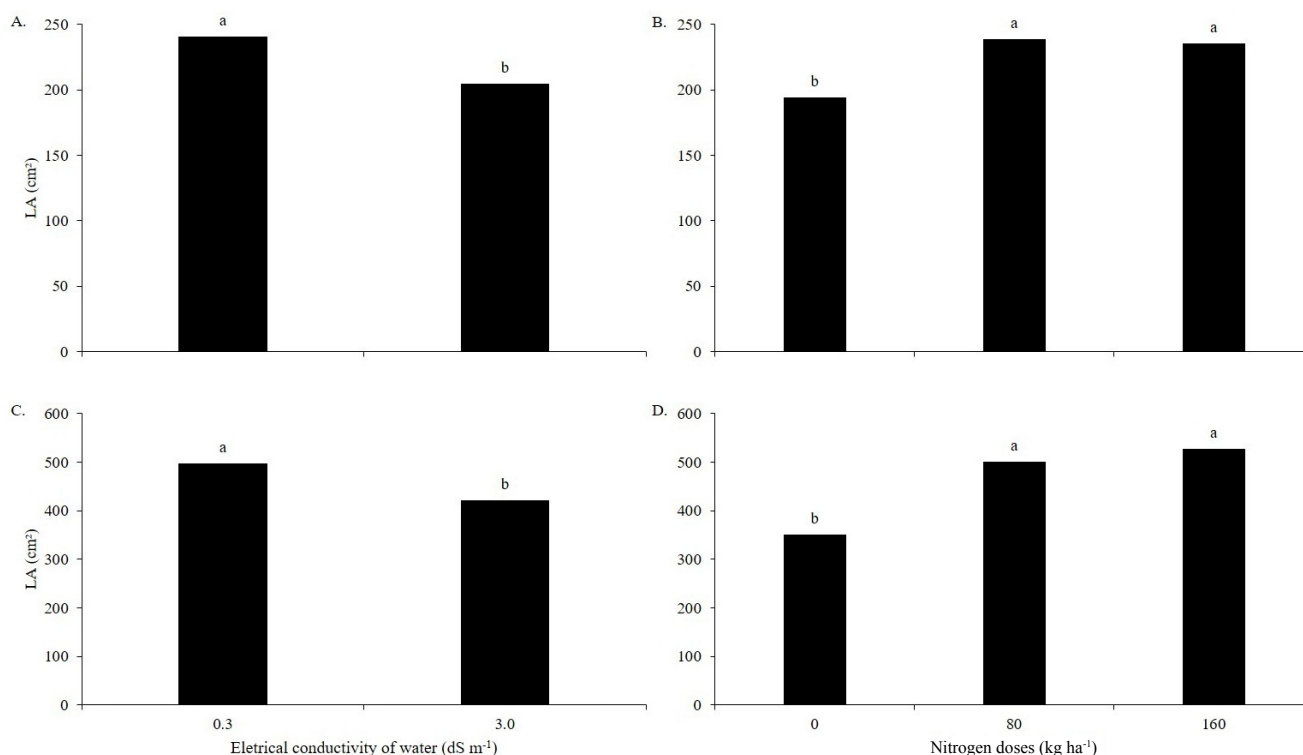
The water of  $0.3 \text{ dS m}^{-1}$  provided the best leaf area results (Figures 2A, C). The treatments that received 160 and  $80 \text{ kg ha}^{-1}$  provided the best leaf area results (Figures 2B, D).

With the increase in water salinity, the osmotic potential of the soil decreases, making the plant unable to adjust osmotically, thus reducing water absorption by the roots and



Means followed by the same letters do not differ by the Tukey test at  $p \leq 0.05$

**Figure 1.** Plant height of corn plants (PH) at 30 (A-B) and 45 (C-D) days after sowing (DAS) under different electrical conductivities of irrigation water (A, C) and nitrogen fertilization doses (B, D)



Means followed by the same letters do not differ by the Tukey test at  $p \leq 0.05$

**Figure 2.** Leaf area of corn plants (LA) at 30 days (A-B) and 45 days (C-D) after sowing (DAS) under different electrical conductivities of irrigation water (A, C) and nitrogen doses (B, D)

the amount of water in the plant, causing the plant to decrease its leaf area and thus its water loss due to transpiration (Taiz et al., 2017). Similar results to this study were found by Sousa et al. (2016) when irrigating the corn crop with water of increasing salinity (0.8 to 6.0 dS m<sup>-1</sup>). Braz et al. (2019) also observed a reduction in corn plants' leaf area with salts' accumulation in irrigation water.

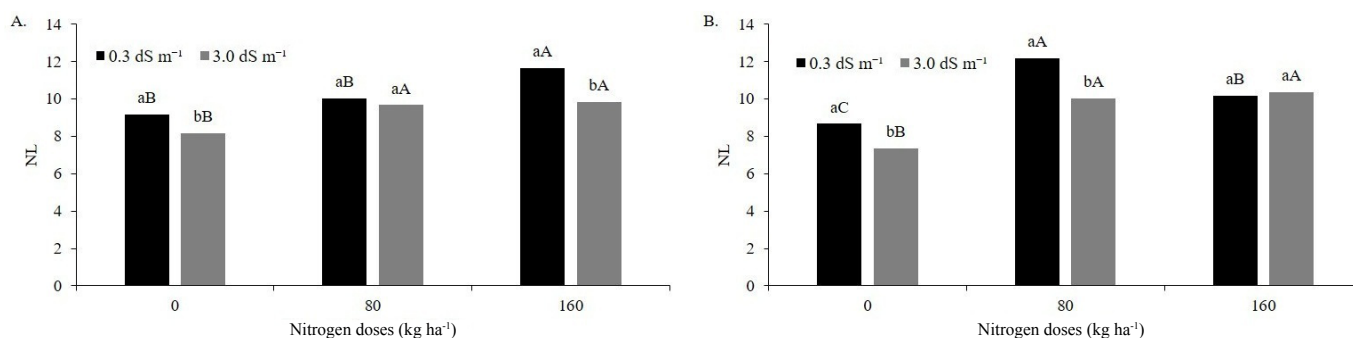
For the effect of nitrogen doses, the dose of 100% (160 kg ha<sup>-1</sup>) provided greater growth of plants causing greater expansion and cell division and, consequently, a larger leaf area. Troyjack et al. (2018) worked with the corn crop and obtained a larger leaf area with the increase of nitrogen fertilization. Braz et al. (2019), working with corn, obtained similar results; that is, the higher nitrogen dose provided greater the leaf area.

The highest number of leaves at 30 DAS was found when the plants were irrigated with low salinity water under the dose of 160 kg ha<sup>-1</sup> of nitrogen (Figure 3A). At 45 DAS (Figure 3B), treatments with irrigation with water of 0.3 dS m<sup>-1</sup>, associated

with fertilization of 80 kg ha<sup>-1</sup> of nitrogen, provided a greater number of leaves.

Saline stress was more aggressive in treatments without the application of nitrogen doses, which can be attributed to osmotic effect intensified by leaf fall due to the ionic effect of the salts, leading to the burning and subsequent senescence of the leaves, both intending to reduce water loss (Lima et al., 2014). The treatments that received N had the less harmful effects of the salts, possibly linked to nitrogen's function in increasing the number of leaves of most plant species (Prado, 2008). The result agrees with that obtained by Martínez-Eixarch et al. (2013) when they obtained interaction between salinity and nitrogen fertilization for the number of leaves in two rice cultivars. Both showed a higher number of leaves with an increase in fertilization.

About the variables of photosynthesis, transpiration, and stomatal conductance at 30 DAS (Figures 4A, C, and E, respectively), it was observed that the best results were obtained



Means followed by the same lowercase letters at the same nitrogen dose or uppercase letters at the same water electrical conductivity do not differ significantly by the Tukey test ( $p \leq 0.05$ )

**Figure 3.** Number of leaves of corn plants (NL) at 30 days (A) and 45 days (B) after sowing (DAS) under different electrical conductivities of irrigation water and nitrogen doses

when the corn plants were irrigated with water of 0.3 dS m<sup>-1</sup>, being negatively affected by the increase in ECw. As for the nitrogen doses (Figures 4B, D, and F), the application of 160 kg ha<sup>-1</sup> of N favored a better result of the studied variables than the doses of 0 and 80 kg ha<sup>-1</sup>.

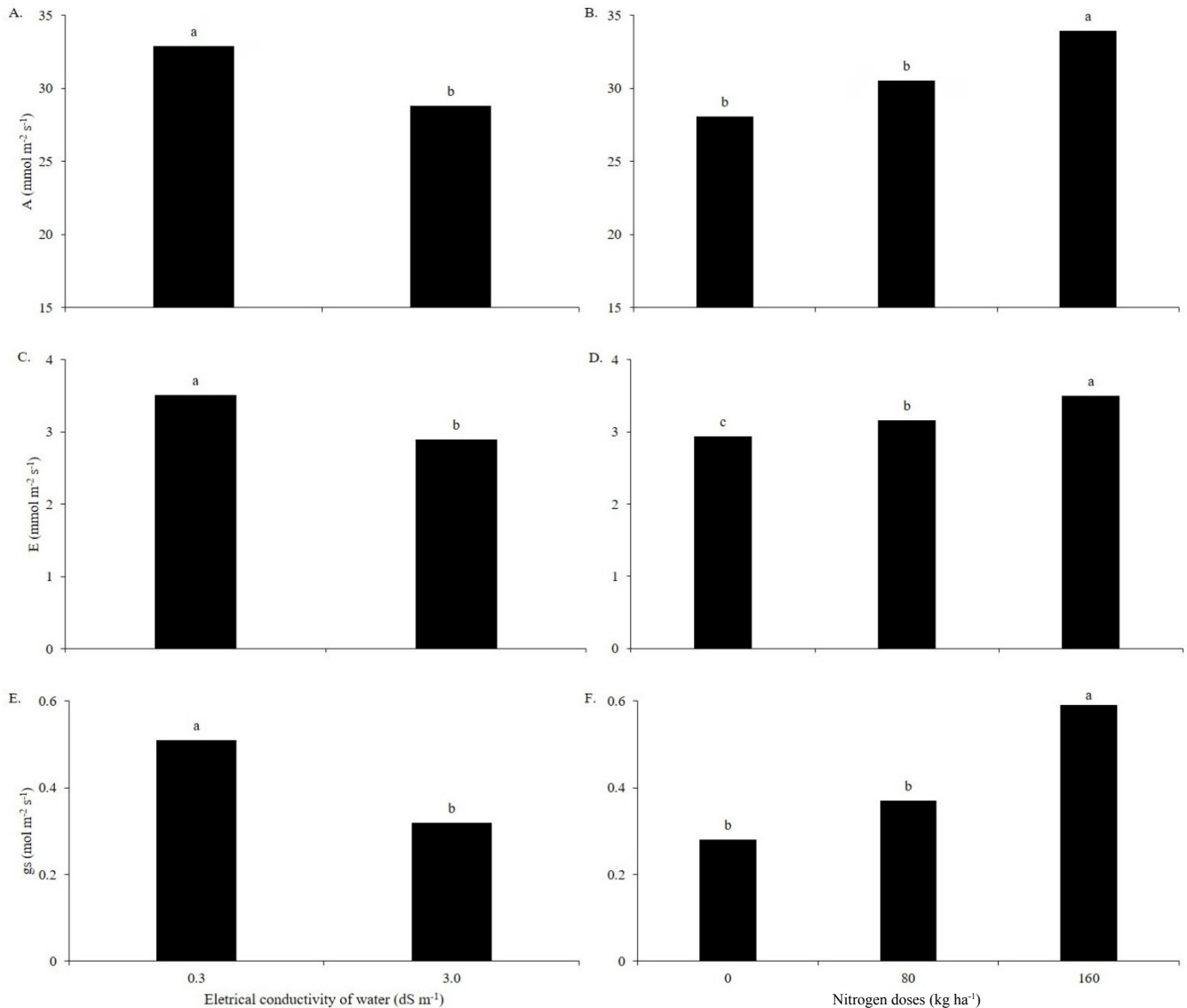
The reduction in the photosynthetic rate (Figure 4A) is due to the osmotic adjustment that the plant exerts to continue its metabolic processes; at the beginning of saline stress, the plant responds only with stomatal control, but with the increase in the intensity of stress, biochemical mechanisms are also triggered (Braz et al., 2019). Souza et al. (2019), working with the fava bean crop, obtained results similar to the present study; that is, with the increase in the electrical conductivity of the irrigation water, the rate of photosynthesis was reduced.

Salinity causes difficulties for the plant to absorb water from the soil, which reduces water loss, with a decrease in stomatal conductance (Souza et al., 2019); consequently, when closing stomata, plants reduce their transpiration (Figure 4C). Souza et al. (2016) evaluated the use of saline water in the corn crop and concluded that transpiration is reduced under salt stress conditions.

The lower stomatal conductance (Figure 4E) is due to the presence of salts in the root zone, which causes less water and nutrients absorption by the plant in detriment to the closure of stomata, causing a reduction in the normal flow of CO<sub>2</sub> towards the carboxylation site (Bosco et al., 2009). Similar results were noticed by Sousa et al. (2014), where they describe that saline stress decreases stomatal conductance in cowpea plants.

The nitrogen dose of 160 kg ha<sup>-1</sup> provided greater liquid photosynthesis (Figure 4B). This higher value can be explained by the greater synthesis of photosynthetic components, of which N is a constituent (Pompeu et al., 2010), such as rubisco and chlorophyll molecules. Feijão et al. (2013), studying the effect of nitrogen source on the growth and accumulation of inorganic and organic solutes in corn plants subjected to salinity, observed that the increase in N provides a greater accumulation of organic compounds acting in the cellular osmotic adjustment, therefore contributing to photosynthetic efficiency. Braz et al. (2019) also showed higher photosynthesis rates with increased nitrogen doses in corn plants.

About the effect of nitrogen fertilization, Taiz et al. (2017) highlight that this essential nutrient, as well as K, both being



Means followed by the same letters do not differ by the Tukey test at p ≤ 0.05

**Figure 4.** Photosynthesis - A (A-B), transpiration - E (C-D), stomatal conductance - gs (E-F) at 30 days after sowing (DAS), of corn plants under different electrical conductivities of water and nitrogen doses

quite required during the formation of chloroplasts, protein synthesis, and thylakoid membranes, having a primary role in transpiration (Figure 4D). The result obtained in the present study is consistent with Braz et al. (2019) when analyzing transpiration in corn plants. These same authors obtained higher rates in treatments with a higher dose of nitrogen.

The result of the reduction of  $g_s$  in plants with less nitrogen (Figure 4F) reports the explanation of Kumagai et al. (2009), where the nitrogen deficiency induces a sharp decline in the  $CO_2$  assimilation, a decrease in the capacity of light absorption and photosynthetic activity in photosystem II, reducing the stomatal conductance of corn plants.

Similar results were found by Silva et al. (2017) with reduced gas exchange in corn with salt stress and mineral fertilization in the nutritional composition of the crop, and by Sousa et al. (2016), who observed that irrigation with saline water causes a reduction in stomatal conductance in corn plants.

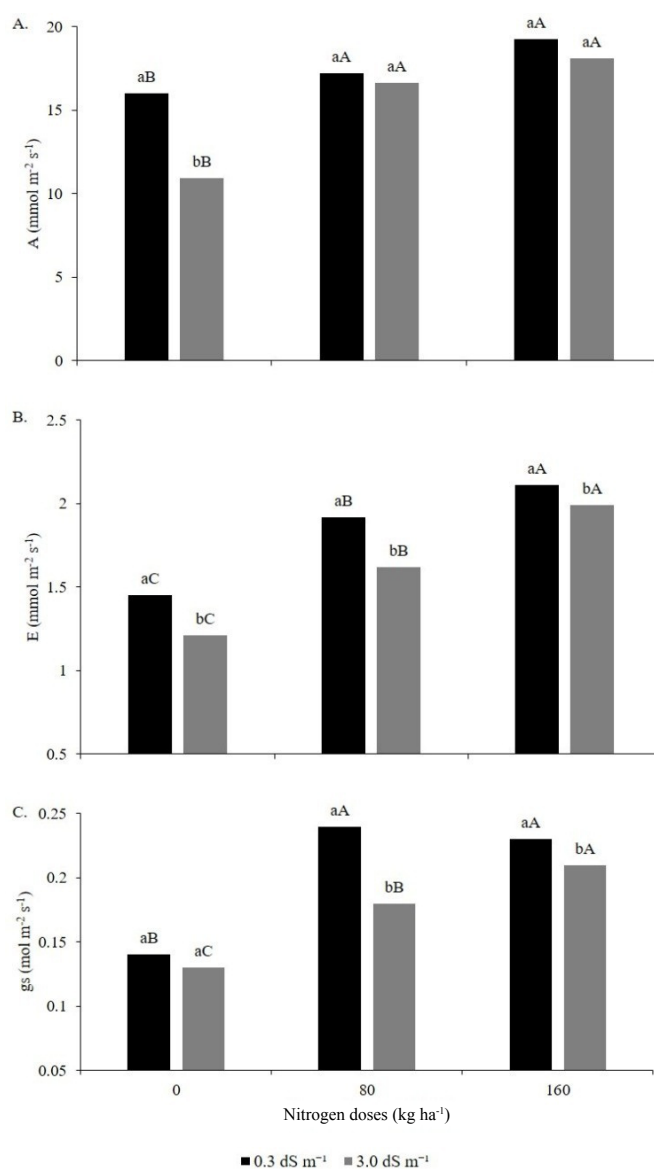
The water with low salinity associated with nitrogen fertilization doses (80 and 160 kg ha<sup>-1</sup>) provided the best photosynthesis results with 17.23 and 19.26 mmol m<sup>-2</sup> s<sup>-1</sup>, respectively, at 45 DAS (Figure 5A).

For transpiration (Figure 5B), the highest values were presented from the irrigation with water of 0.3 dS m<sup>-1</sup> associated with a dose of 160 kg ha<sup>-1</sup> (100%) of nitrogen obtaining 2.11 mmol m<sup>-2</sup> s<sup>-1</sup>. In the stomatal conductance variable (Figure 5C), the treatments that received doses of 80 and 160 kg ha<sup>-1</sup> of N with irrigation water of low electrical conductivity were higher than those with values of 0.24 and 0.23 mol m<sup>-2</sup> s<sup>-1</sup>, respectively.

In the present study, nitrogen showed the ability to reduce the harmful effects of salts, assisting in realizing vital physiological processes through its translocation, as in the example of photosynthesis. Feijão et al. (2011) obtained similar results with sorghum-Sudan grass cultivation; finding better photosynthesis results in treatments with increased nitrogen fertilization under irrigation with saline water.

The transpiration can be explained by the stomatal effects, where plants in the presence of Cl<sup>-</sup> and Na<sup>+</sup> close their stomata, inhibiting physiological factors. Thus, all treatments, regardless of the recommendations used that received irrigation with saline water, suffered a decrease for the transpiration variable concerning low salinity water, with that it is understood that osmotic effects prevail over those related to nutritional status, as stated by Munns & Tester (2008), especially when they are subjected to salt stress. The result is similar to that of Lacerda et al. (2016) when they obtained lower transpiration values for treatments with increased saline water regardless of nitrogen fertilization.

The lowest values of stomatal conductance under irrigation with water of 3.0 dS m<sup>-1</sup> can be understood as plants' behavior in response to salt stress, and treatments with 0 and 80 kg ha<sup>-1</sup> of N, the plants possibly compromised stomatal opening to avoid water loss. In the treatment with 160 kg ha<sup>-1</sup>, it is observed that there is an adjustment in the physiological process that allowed similar values between the two electrical conductivity (0.23 mol m<sup>-2</sup> s<sup>-1</sup> for 0.3 dS m<sup>-1</sup> and 0, 21 mol m<sup>-2</sup> s<sup>-1</sup> to 3.0 dS m<sup>-1</sup>), indicating that this dose was efficient in nourishing the plant even with the presence of toxic ions such as Na<sup>+</sup> and Cl<sup>-</sup>, showing a positive relationship between gas exchange and N nutrition as reported by Cechin & Fumis (2004).



Means followed by the same lowercase letters at the same nitrogen dose or uppercase letters at the same water electrical conductivity do not differ significantly by the Tukey test ( $p \leq 0.05$ )

**Figure 5.** Photosynthesis - A (A), transpiration - E (B), stomatal conductance -  $g_s$  (C) at 45 days after sowing, of corn plants under different electrical conductivities of water and nitrogen fertilization doses

Similarly, Feijão et al. (2011) found higher values of stomatal conductance in sorghum-Sudan grass plants with nitrogen fertilization under saline water irrigation.

## CONCLUSIONS

1. Saline stress affects plant height, leaf area, photosynthesis, transpiration, and conductance at 30 days after sowing (DAS).
2. The doses of 80 and 160 kg ha<sup>-1</sup> provide higher performance in plant height, leaf area, photosynthesis, transpiration, and conductance at 30 DAS.
3. The use of low salinity water and doses of 80 and 160 kg ha<sup>-1</sup> were more efficient in terms of plant height, leaf area, photosynthesis, transpiration, and conductance at 45 DAS.
4. The dose of 160 kg ha<sup>-1</sup> of N attenuates the harmful effects of salts in AG 1051 corn hybrid plants, providing higher values

of photosynthesis, transpiration, and stomatal conductance at 45 DAS when irrigated with water of 3.0 dS m<sup>-1</sup>.

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