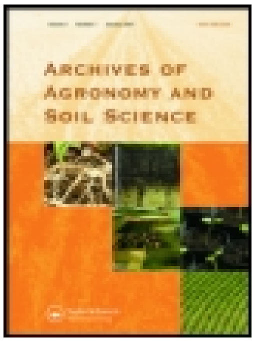

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Uses and losses of nitrogen by maize and cotton plants under salt stress

Aureliano De Albuquerque Ribeiro[a], Claudivan Feitosa Lacerda[a], Antônia Leila Rocha Neves[a], Carlos Henrique Carvalho De Sousa[a], Régis Dos Santos Braz[b], Adriana Cruz De Oliveira[a], Jordânia Maria Gabriel Pereira[a] and Jorge Freire Da Silva Ferreira[c]

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ABSTRACT

There is no consensus on how much N can be applied to plants under salt stress. In our research we tested the hypothesis that such response depends on salt tolerance of the plant species. So, this study aimed to evaluate the uses and losses of N by cotton (salt-tolerant) and maize (moderately salt-sensitive) irrigated with waters of different electrical conductivity (0.5, 2.0, 4.0, and 6.0 dS m^{-1}) and fertilized with nitrogen (60, 100, and 140% of the recommended dose for each crop). We found that nitrogen doses beyond the recommended values exacerbated the negative effects of salinity on growth and photosynthetic rates, especially in maize plants growing under moderate to high salinity. N rates over the recommended dose did not increase leaf nitrogenous compounds, believed to attenuate the negative impacts of salt stress. Our results indicate that the responses to additional nitrogen fertilization depend on crop salt tolerance and on the level of saline stress imposed. Increasing N rates beyond crop needs under salinity only increased leaching losses and reduced the nitrogen-use efficiency, indicating that such practice would result in economic losses and environmental N overload, especially when a supplemental dose is applied to a salt-sensitive crop.

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KEYWORDS
Salt tolerance; nitrogenous compounds; N-use efficiency; Nleaching

Introduction

Nitrogen is a chemical element of great importance in agriculture, significantly contributing to the increase in crop productivity. However, optimal rate of nitrogen can be affected by biotic and abiotic factors and varies depending on the crop system. For example, under rainfed agriculture, the recommended dose of N fertilizers can be related to the amount of rainfall along the crop cycle (Quemada and Gabriel 2016), while under irrigated systems the dose of N could depend only on crop demand. However, problems related to soil or irrigation water, such as salinity, can also affect productivity and nitrogen requirement by plants (Semiz et al. 2014; Lacerda et al. 2016).

Water and soil salinity problems are present in irrigated areas around the world, being more evident in arid and semi-arid regions (Singh 2017, 2018). High salinity may affect the physiological and biochemical functions of plant cells, limiting the growth and productive capacity of plants (Munns and Tester 2008; Schiattone et al. 2017; Chrysargyris et al. 2018; Carillo et al. 2019). These effects are independently associated with low osmotic potential in the soil solution, nutritional

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imbalance, ionic toxicity, hormonal imbalance, and induction of oxidative stress, or with a combination of these factors (Nadeem et al. 2014; Mansour and Ali 2017).

Excessive N fertilization is not only counterproductive, but is also a threat to the environment. So, fertilizer management practices are required to reduce N losses, such as nitrate leaching and ammonia volatilization, while maintaining farm productivity (Singh et al. 2012; Quemada and Gabriel 2016; Sigurdarson et al. 2018). Opposite to these ideas, high doses of nitrogen have been used as a management strategy to alleviate the effects of salt stress on plants (Zeng et al. 2014; Ma et al. 2016; Bezerra et al. 2019). This supposed beneficial effect may be related to the accumulation of nitrogenous organic compounds, such as proline, free amino acids, glycine betaine, and polyamines, which contribute directly to osmotic adjustment and protection of cellular structures and functions (Munns and Tester 2008; Ding et al. 2010; Zrig et al. 2011, 2018). In addition, some authors have reported that extra N application in plants cultivated under salt stress can significantly reduce Cl^- content and increase NO_3^- content in leaves, mitigating the effects of salt stress on plants (Abdolzadeh et al. 2008), but these findings have not been substantiated by others.

Despite the purported benefits of extra N application under salt stress, there is no consensus on the positive effects of such practice according to many reports in the literature (Chen et al. 2010; Azizian and Sepaskhah 2014; Min et al. 2014; Semiz et al. 2014; Zakery-Asl et al. 2014; Min et al. 2016). For many glycophytes a reduction in plant response to N fertilization is observed as salinity increases, resulting in economic losses and environmental contamination (Feitosa et al. 2016; Lacerda et al. 2016; Yasuor et al. 2017).

Several published reports indicate that the responses to supplemental N application under salinity conditions depend on edaphic conditions (soil fertility) and nutritional demand of the species (Grattan and Grieve 1998; Lacerda et al. 2016; Braz et al. 2019). Could this response also depend on crop salt tolerance? Could responses to N application in salt-tolerant plants be more expressive than in salt-sensitive ones? Comparison of results between halophytes and glycophytes seems to provide affirmative answers to these questions (Ding et al. 2010; Yuan et al. 2010; Lacerda et al. 2016). For example, the halophyte plant *Suaeda salsa* (Amaranthaceae) had significant biomass gains with high N levels at NaCl concentrations up to 300 mM, an EC_{iw} (electrical conductivity of irrigation water) of approximately 30 dS m^{-1} (Jiang et al. 2012), while no positive response was observed for maize at EC_{iw} higher than 2.5 dS m^{-1} (Lacerda et al. 2016). Despite these findings, there are no studies comparing the responses to N application in plants with different degrees of salt tolerance.

There are few studies evaluating simultaneously N utilization and losses in crops growing under salinity conditions. Thus, comparison between crops with differences in N requirement and degree of tolerance to salinity, such as maize and cotton, may be a way of helping clarify this scientific issue. Therefore, the hypothesis tested in this study was that plant response to N application under salinity depends on the degree of salt tolerance of the genotype, leading to a lesser N-use efficiency and higher N losses in the most salt-sensitive species. In this context, this study aimed to evaluate the growth, production, leaf gas exchange, accumulation of nitrogenous compounds, N-use efficiency, and soil nitrate loss by leaching, when comparing cotton (salt tolerant) and maize (moderately salt sensitive) plants irrigated with saline waters and provided N doses ranging from sub-optimal to supra-optimal.

Material and Methods

Experimental area location and characterization

The experiment was conducted in a greenhouse located at the Federal University of Ceará, in Fortaleza (3° 45′ S; 38° 33′ W and altitude of 19 m), Ceará, Brazil, under tropical climate (Aw), according to Köppen classification. In order to characterize the meteorological conditions along the experimental period, data of temperature, relative humidity, and light intensity were monitored using a datalogger installed in the center of the greenhouse. The daily average values of air temperature inside the greenhouse ranged from 26.4°C to 32.7°C, whereas the relative humidity fluctuated from 60.5 to 80.0%. The average daily value (8 am to 4 pm) and the maximum natural light intensity at noon were 597 and 985 μmol m^{-2} s^{-1}, respectively, with photoperiod of approximately 12 hours.

Experimental design and treatments

The study was carried out in a randomized block design, in a split-plot scheme, with two crops in the main plots (maize, considered moderately salt sensitive and cotton, considered salt tolerant), four levels of irrigation water salinity in the subplots (0.5, 2.0, 4.0, and 6.0 dS m^{-1}), and three N doses in the sub-subplots (60, 100, and 140% of the recommended dose for each crop). Four replicates were used, in a total of 96 experimental units, which consisted of PVC columns measuring 20 cm in diameter and 100 cm in length.

Characterization of the soil used

The soil used to fill the columns was an Ultisol, collected at depths of 0–15 cm (A horizon), 15–62 cm (E horizon) and 62–100 cm (B horizon). Table 1 presents the physical and chemical characterization of the soil horizons.

Assembling of soil columns

The soil columns were made of rigid PVC pipes, with internal diameter of 20 cm and length of 100 cm, closed and sealed at the base with PVC caps. A hole at the bottom of each column was connected to a hose, which was attached to a container to collect the leachate. The internal wall of the pipes received a layer of glue with sand to prevent the preferential water flow between the soil and the PVC column wall. A 10-cm-thick layer of sand was placed at the base of each pipe to facilitate the drainage of the leachate. The columns were completed with soil, leaving 5.0 cm free at the top to facilitate irrigation and fertilization.

The columns were marked on the inside every 10 cm that, once filled with the collected soil, was compacted to a density of 1.53 g cm^{-3}. The columns were assembled by following the same sequence of soil horizons found in the field. For this, the column was divided into three layers: the first one, 20 cm deep, filled with soil collected from the A horizon; the second one, 25 cm deep, filled with soil collected from the transition E horizon; and the third one, 40 cm deep, filled with material collected from the B horizon, making up for a soil core layer of 85 cm.

Table 1. Characterization of the soil used in the experiment.

Hor.	Depth (cm)	Texture	ρ_s	θ_{CAD}	Ca^{2+}	Mg^{2+}	Na^+	K^+	H^++Al^{3+}
			g cm^{-3}	g 100 g^{-1}	– – – – cmol$_c$ kg^{-1} – – – – – –				
A	0–15	Sandy Loam	1.47	2.21	0.80	0.70	0.09	0.05	0.83
E	15–62	Sandy Loam	1.46	2.67	0.60	0.60	0.09	0.11	1.65
B	62–100	Sandy Clay Loamy	1.35	4.98	0.80	0.70	0.09	0.15	1.98

Hor.	Depth(cm)	Al^{3+}	S	CEC	V	M	P	N	pH	ECe
		– – – cmol$_c$ kg^{-1} – – – – – –			– – – -% – – –		mg kg^{-1}	g kg^{-1}	H_2O	dS m^{-1}
A	0–15	0.05	1.6	2.5	64	3	6	0.23	6.7	0.06
E	15–62	0.15	1.4	3.0	47	10	4	0.18	5.4	0.04
B	62–100	0.50	1.7	3.7	46	23	2	0.34	5.0	0.05

Hor. = horizon; ρ_s = soil density; θ_{CAD} available water capacity; pH – hydrogen ion concentration in water (-log[H^+]); ECe – electrical conductivity of the soil saturated extract; S – sum of exchangeable bases; CEC – cation exchange capacity; V – base saturation; m – saturation by aluminum.

Experimental set-up and conduction

Sowing was performed by placing four seeds per column, at a 2 cm depth. Saline treatments started eight days after sowing (DAS). At 10 DAS, thinning was carried out, leaving one plant per column. The hybrid 'BRS 2020' was used for maize (*Zea mays* L.), and 'Fibermax 910' was used for cotton (*Gossypium hirsutum* L.).

The irrigation water with the lowest electrical conductivity (EC_{iw}) level (0.5 dS m^{-1}) was obtained by diluting well water (pH 6.9, EC = 1.0 dS m^{-1} and SAR 4.5) with rainwater (EC = 0.01 dS m^{-1}). The other concentrations (2.0; 4.0 and 6.0 dS m^{-1}) were obtained by adding sodium chloride (NaCl) and calcium chloride ($CaCl_2 2H_2O$) to the well water, in a 7:3 proportion, following the relationship between the irrigation water electrical conductivity (EC_{iw}) and their respective concentrations ($mmol_c$ L^{-1} = EC_{iw} x 10). The proportion of salts used in irrigation waters is a representative approximation of most sources of water available for irrigation in Northeastern Brazil.

The total quantities of N applied per column, at the different doses, and for each crop, are shown in Table 2. The reference dose (100%) corresponded to 210 kg ha^{-1} of N for maize, with 80,000 plants per hectare, and to 120 kg ha^{-1} of N for cotton, with 75,000 plants per hectare.

For maize, application of nitrogen (126; 210 and 294 kg ha^{-1} of N, equivalent to 60, 100 and 140% of N recommendation for the crop) and potassium (80 kg ha^{-1} of K_2O) were split into four applications: 15% at thinning, 25% at 15 and 30 days after thinning, and 35% at 45 days after thinning. The other nutrients were applied before planting, including 6.25 and 1.0 g per column of single superphosphate and a mixture of micronutrients and macronutrients (1.0% Zn, 2.5% Mn, 17% Fe, 0.1% B, 1.0% Cu, 0.05% Mo, 6.0% Ca, 3.0% Mg and 12% S), respectively.

For cotton, nitrogen (72, 120 and 168 kg ha^{-1} of N, equivalent to 60, 100 and 140% of N recommendation for the crop) and potassium (50 kg ha^{-1} of K_2O) fertilizations were split into three applications: 25% at thinning, and 75% into two equal portions at 15 and 30 days after thinning. At sowing, each column received 7.7 and 1.0 g of single superphosphate and a mixture of micronutrients and macronutrients (the same used for maize), respectively. In both crops, the sources of N and K were urea (45% N) and potassium chloride (60% K_2O and 48% Cl), respectively.

The irrigation depth necessary to meet the water requirement of the crops was obtained based on the soil water balance, by the difference between the volume applied and the volume drained in the previous irrigation, plus a leaching fraction of 0.15. Water was applied every other day and directly onto the soil surface, in order to avoid its direct contact with the leaves.

Photosynthetic parameters

At 25, 40, 58 and 65 days after treatments (DAT), net photosynthetic rate (*A*), stomatal conductance (g_s) and internal CO_2 concentration (*Ci*) were measured with a portable infrared gas analyzer (Li – 6400XT model, LiCor, USA). Fully expanded leaves were subjected to saturating irradiance (1,800 µmol of photons m^{-2} s^{-1}) and maintained under ambient conditions of CO_2 and air temperature. At 65 DAT, readings of relative chlorophyll index (RCI) were taken using a portable meter (SPAD 502, Minolta Co, Ltd, Osaka, Japan), and the results were expressed in the reading units generated by the device. Readings were taken in the same leaves used for gas exchange readings.

Table 2. Total quantities of nitrogen (N) applied (g $column^{-1}$), based on the recommended N dose (100%) for each crop.

Crop	N dose (g $column^{-1}$)		
	60%	100%	140%
Cotton	0.95	1.60	2.22
Maize	1.56	2.62	3.66

Data collection during vegetative and reproductive growth

At 72 DAT, plants were collected and leaf area (LA) was obtained using an area integrator (Area meter, LI-3100, Li-Cor, Inc., Lincoln, NE, USA). At harvest, roots, vegetative and reproductive shoots were placed in paper bags and dried in a forced-air circulation oven (65 to 70°C). After drying, each sample was weighed on an analytical scale to obtain the total dry mass (TDM). Dry biomass data were used to estimate the partition index (PI) based on the relationship between boll biomass for cotton or ear biomass for maize and the total dry biomass of the plants.

Nitrogen-use efficiency

Nitrogen-use efficiency was obtained using the following ratios: net photosynthetic rate/total N applied (A/Napp) and total dry mass/total N applied (TDM/Napp).

ΔN-proline and ΔN-amino in leaves

At 71 DAT, five leaves were collected per cotton plant (from the 5th leaf down in each main stem) and three leaves were collected per maize plant (opposite and located below the first upper ear). These leaves were freeze-dried and then ground in a Wiley mill. After that, 0.5 g of the ground material was weighed and transferred to test tubes, which received 10 mL of deionized water. The samples were kept for 1 hour in a water bath at 45°C ± 2°C and shaken every 15 minutes. Subsequently, the supernatant (aqueous extract) was collected, filtered in qualitative filter paper (pore diameter of 14 µm) and the filtrate was stored in glass vials. Proline and amino acids contents were determined in this extract using the methodologies described by Bates et al. (1973) and Yemm and Cocking (1955), respectively. The variations in proline and N-amino contents in the leaves were obtained by the following expression:

ΔN-proline or ΔN-amino = content at supra-optimal dose – content at recommended dose

ΔN-nitrate in soil

At the end of the experiment (70 DAT), soil samples were collected in the 45–85 cm layer and nitrate concentrations were determined in the soil saturation extract, using the salicylic acid method according to Cataldo et al. (1975). The variations in nitrate concentration in the soil were obtained by the following expression:

ΔN-nitrate = concentration at supra-optimal dose – concentration at recommended dose

Statistical analysis

The results were subjected to analysis of variance by F test and, in case of significance, regression analysis was performed for the salinity factor and comparison test for means was carried out for crop and N doses. Regression models were selected according to the best fit based on the coefficient of determination (R^2). Statistical analyses were performed using the statistical program SISVAR®, version 5.3 (Ferreira 2011).

Results

Growth and partition index

The interaction between salinity and N doses significantly affected leaf area ($p < 0.01$), total dry mass ($p < 0.01$), and partition index (PI) ($p < 0.01$). When comparing cotton plants irrigated with the highest salinity water ($EC_{iw} = 6.0$ dS m^{-1}) with plants irrigated with control salinity ($EC_{iw} = 0.5$ dS m^{-1}), there were leaf area reductions of 23.96%, 19.36% and 41.98% for the N doses of 60, 100 and 140% of crop recommendation, respectively (Figure 1A). For maize (Figure 1B), these reductions were 24.4%, 30.3% and 51.5%, respectively.

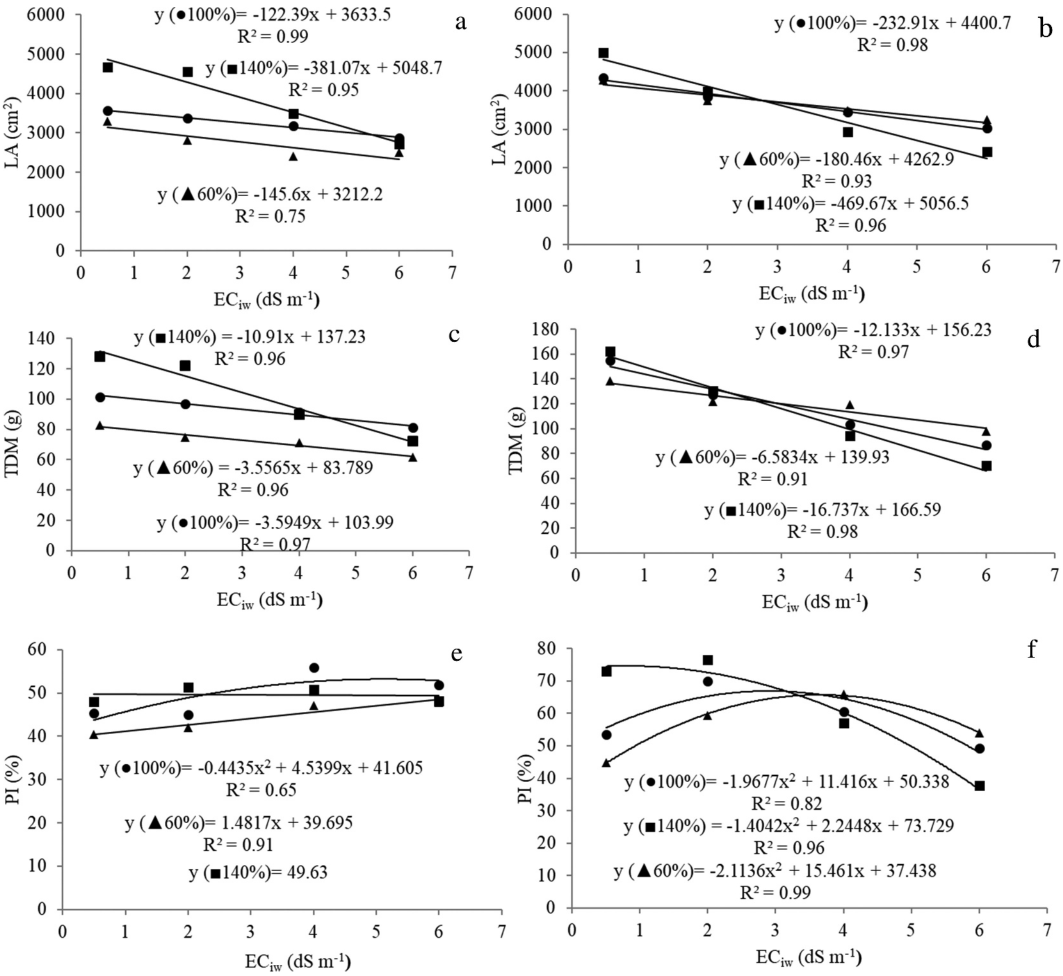

Figure 1. Leaf area (LA), total dry mass (TDM), and partition index (PI) of cotton (a, c and e) and maize (b, d and f) plants as a function of irrigation water salinity and N doses (60, 100 and 140% of recommended dose for each crop).

When we compared cotton plants subjected to EC_{iw} = 6.0 dS m^{-1} with the ones irrigated with control salinity (EC_{iw} = 0.5 dS m^{-1}), total dry mass (TDM) was reduced in 25.2, 19.9, and 43.6% at the doses of 60, 100 and 140% of the crop N recommendation, respectively (Figure 1C). In maize, these reductions were equal to 29.0, 43.7 and 56.6%, respectively (Figure 1D).

As water salinity increased from 0.5 to 6.0 dS m^{-1}, there was a relative increase of 18.30% in cotton PI at the lowest N dose. In plants cultivated at the dose recommended for the crop, a maximum estimated PI of 53.2% was obtained for the salinity of 5.11 dS m^{-1}. At the highest N dose, the mean value of PI was 49.6%, with no salinity effect (Figure 1E). For maize plants fertilized with N doses of 60, 100 and 140%, the maximum estimated PI values were 67.7, 66.9 and 74.6% under irrigation with waters of EC_{iw} of 3.6, 2.9, and 0.8 dS m^{-1}, respectively, decreasing after these EC_{iw} levels (Figure 1F).

Gas exchanges and relative chlorophyll index

The interaction between salinity and N doses was significant for leaf gas exchanges and relative chlorophyll index ($p < 0.05$). The salinity increment in irrigation water led to reductions of approximately 16 and 37% in stomatal conductance (g_s) of cotton at the N doses of 100 and 140% of crop recommendation (Figure 2A). In maize, values of g_s were 16, 54.5 and 67.6% lower at the highest salinity level for the N doses of 60, 100 and 140% of crop recommendation, respectively (Figure 2B). In both crops, the highest g_s values were obtained in plants under the control treatment (EC_{iw} = 0.5 dS m^{-1}) and at the highest N dose, while the lowest values were observed at the highest level of salinity (EC_{iw} = 6.0 dS m^{-1}) combined also with the highest N dose (Figure 2A and 2B).

Irrigation water salinity did not affect cotton photosynthetic rate at any of the N doses tested, and the mean values were equal to 28.2, 29.2 and 28.5 µmol m^{-2} s^{-1} for the N doses of 60, 100 and 140% of crop recommendation, respectively (Figure 2C). On the other hand, maize plants had reductions of 19.0, 36.3 and 46% in net photosynthesis between plants under the highest salinity level and those grown under control salinity, at the N doses of 60, 100 and 140% of crop recommendation, respectively (Figure 2D).

Compared to the control (0.5 dS m^{-1}), the internal CO_2 concentration (*Ci*) of cotton decreased linearly by 4.2, 2.7 and 7.8 µmol mol^{-1} per unit increase in ECiw, corresponding to reductions of 8.6, 6.4 and 14.2% in th *Ci* of plants irrigated with water of ECiw = 6.0 dS m^{-1} at N doses of 60, 100 and 140% of crop recommendation, respectively (Figure 2E). In maize, irrigation water salinity caused reductions of 4.3, 10.9, and 18.5 µmol mol^{-1} per unit increase in EC_{iw} at N doses of 60, 100 and 140% of crop recommendation (Figure 2F). When comparing the data obtained in maize plants irrigated

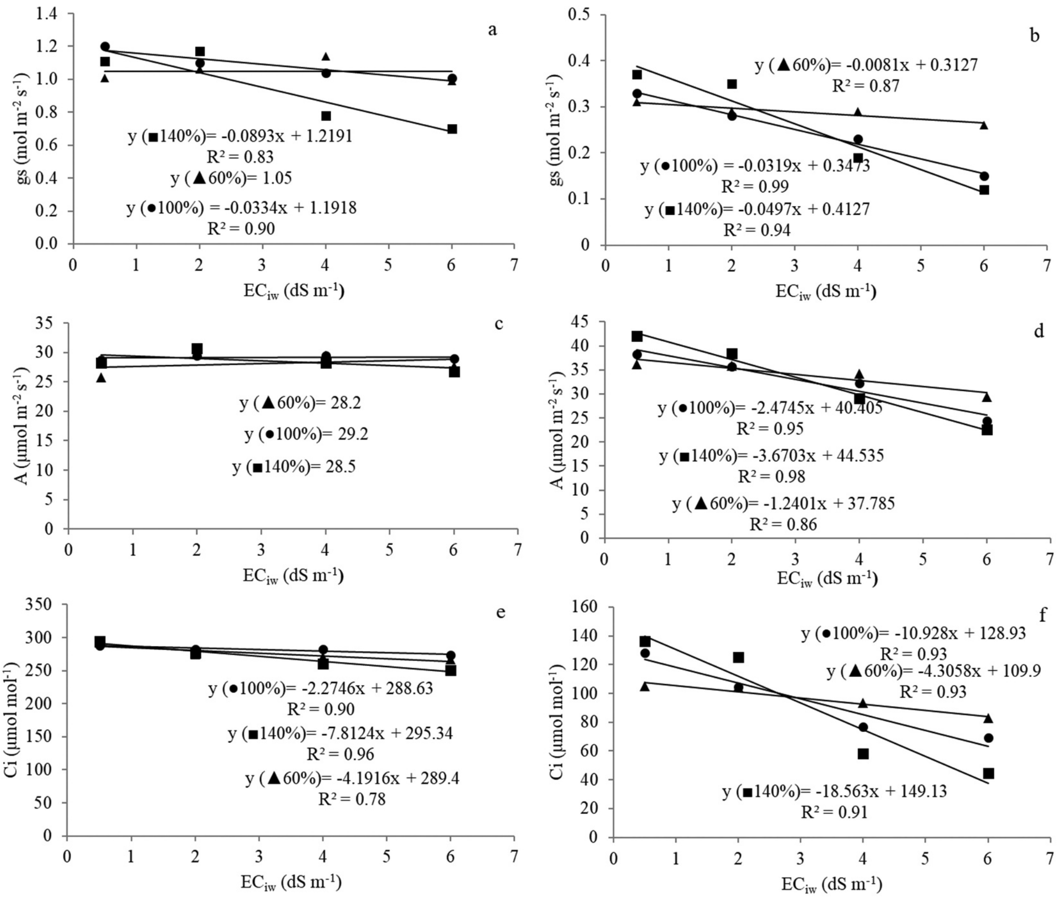

Figure 2. Stomatal conductance (g_s), net photosynthesis (*A*), and internal CO_2 concentration (*Ci*) in cotton (a, c and e) and maize (b, d and f) plants as a function of irrigation water salinities and N doses (60, 100 and 140% of recommended dose for each crop).

with water of the highest salinity ($EC_{iw} = 6.0$ dS m^{-1}) to those plants cultivated under control salinity ($EC_{iw} = 0.5$ dS m^{-1}), there were reductions of 21, 46 and 67% in *Ci* at the N doses of 60, 100 and 140% of crop recommendation, respectively (Figure 2F).

When salinity increased from control to the highest level, there was a 21.0% increase in the relative chlorophyll index (RCI) of cotton for the N dose of 60% of crop recommendation (Figure 3A). In plants cultivated at N doses of 100 and 140%, the maximum estimated RCI values of 44.5 and 47 were obtained at salinity levels of 6.15 and 4.25 dS m^{-1}, respectively. In maize, there was a linear reduction in RCI, as a function of salinity, at the lowest N dose tested with a total reduction of 12%. At N doses of 100 and 140%, the estimated maximum RCI values were 46 and 53, recorded at the salinity levels of 1.71 and 2.08 dS m^{-1}, respectively, decreasing after these values of ECiw (Figure 3B).

Nitrogen uses and losses

The interaction between salinity and crop affected the N-use efficiency obtained by the A/Napp ($p < 0.01$) and TDM/Napp ($p < 0.01$) ratios. The values of A/Napp in maize decreased by 0.92 µmol CO_2 m^2 s^{-1}/g N per unit increase in EC_{iw}, resulting in a 30.5% reduction in plants irrigated with water of $EC_{iw} = 6.0$ dS m^{-1}, compared to plants irrigated with water of $EC_{iw} = 0.5$ dS m^{-1} (Figure 4A). The same trend was observed for TDM/Napp values in the maize crop, with a total reduction of 39.2% (Figure 4B). In cotton, irrigation water salinity affected only the TDM/Napp ratio, reaching a 28.3% lower value at the highest salinity level.

Application of the supra-optimal N dose did not result in nitrogenous compound accumulation, especially at high salinity levels (Figure 5). For cotton plants treated with 6.0 dS m^{-1}, application of supra-optimal N dose, compared to the recommended dose, resulted in reductions of 5.5 and 11% of the N-proline content (Figure 5A) and total dry biomass (Figure 5C), respectively, and an increment of 15.3% in N-amino (Figure 6B). For maize, we observed reductions in proline (Figure 5A), N-amino (Figure 5B), and total dry biomass (Figure 5C) of 14.0, 27.3 and 19.3%, respectively.

Excessive N fertilization caused accumulation of N-nitrate in subsoil as salinity increased, and this response was evident only for maize (Figure 5D). For example, when the highest salinity level ($EC_{iw} = 6.0$ dS m^{-1}) was associated with the highest N dose it was found that N-nitrate soil accumulation was 26.36% higher compared to the dose recommended for this crop. In addition, under the same treatment, it was observed that approximately 70.5% of N-nitrate accumulation was located at the greatest soil depth (data not shown). For the sub-optimal dose, it was found that the N-nitrate contents in subsoil were much lower than those related to the recommended dose. However, the difference was smaller at the highest levels of salinity (data not shown).

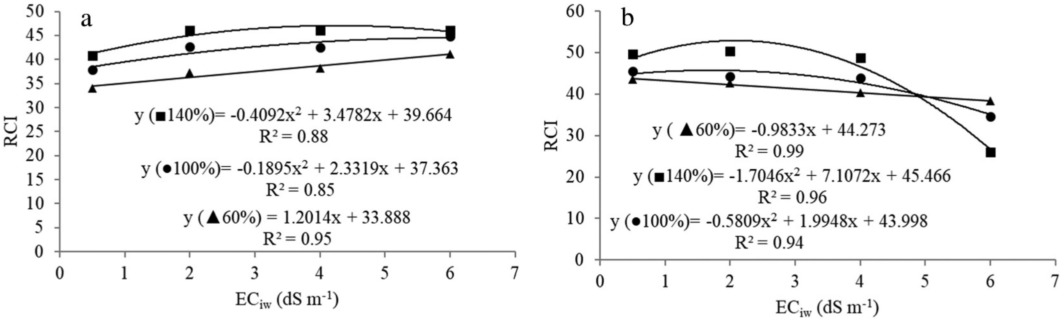

Figure 3. Relative chlorophyll index (RCI) of cotton (a) and maize (b) plants in response to irrigation water salinities and N doses (60, 100 and 140% of recommended dose for each crop).

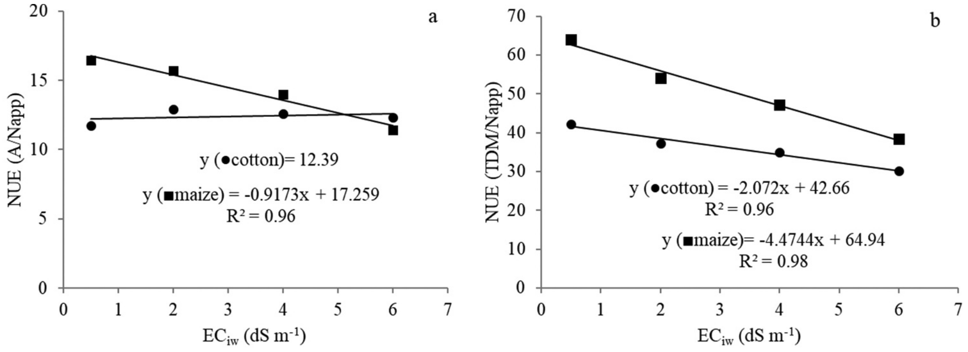

Figure 4. Nitrogen-use efficiency, obtained by the ratios *A*/Napp – μmol CO_2 m^{-2} s^{-1}/g N (a), and TDM/Napp – g of total dry mass/g N (b), in cotton and maize as a function of irrigation water salinity.

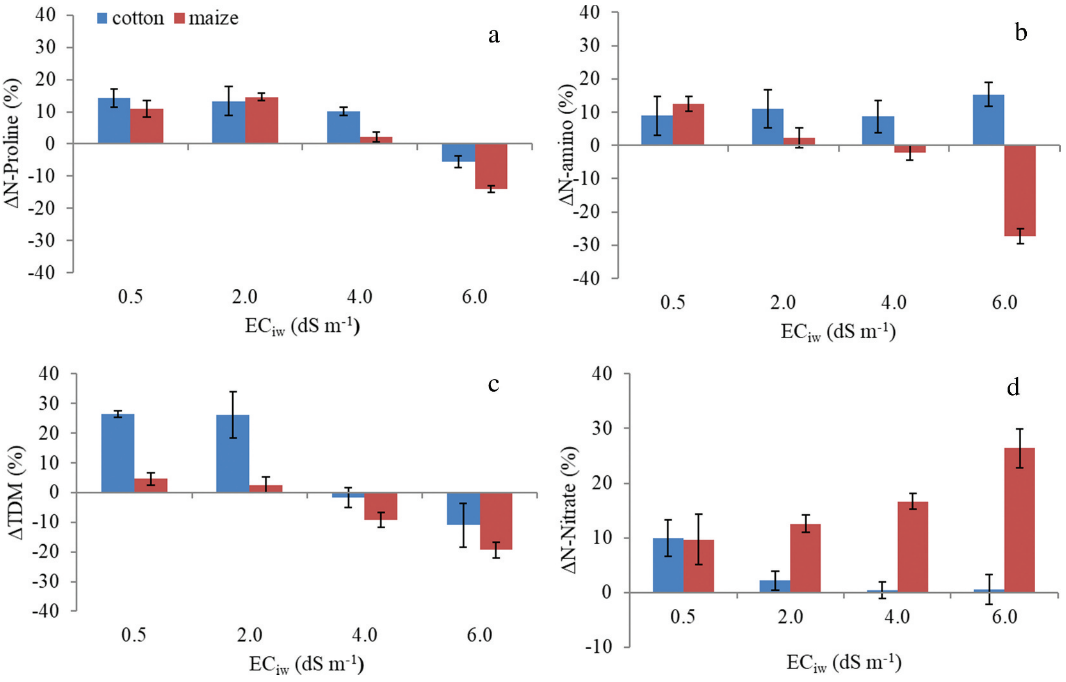

Figure 5. ΔN-proline (a), ΔN-amino (b) in leaves, Δtotal dry biomass (c) and ΔN-nitrate in subsoil (45 to 85 cm) (d), obtained by the difference between the supra-optimal and the recommended doses of N, for cotton and maize plants in response to irrigation water salinity. Vertical bars represent errors of the means (n = 4). The values were expressed as percentage of increase or decrease in relation to recommended N rate.

Discussion

Some authors suggest that the cellular demand for N increases under salt stress conditions (Zeng et al. 2014; Ma et al. 2016; Bezerra et al. 2019), considering the accumulation of nitrogenous compounds that contribute to osmotic adjustment and other N compounds and enzymes with roles in protecting the cell against oxidative damage (Munns and Tester 2008; Barhoumi et al. 2010; Ding et al. 2010). From these assumptions, many studies have been conducted aiming to mitigate the effects of salt stress on plants by applying more N than the recommended dose for different crops (Shenker et al. 2003; Chen et al. 2010; Min et al. 2014; Lacerda et al. 2016; Yasuor et al. 2017).

However, in many cases, no positive responses were observed and most studies did not consider N-use efficiency, possible N losses, or environmental contamination.

Nitrogen losses can be significant when the stress level exceeds the salinity threshold of a specific crop, as defined by Maas and Hoffman (1977), because the osmotic and toxic effects of salts result in clear reductions in biomass production (Munns 2002; Rahnama et al. 2010; Munns and Gilliham 2015), water consumption, and soil nutrient extraction (Neves et al. 2009; Lacerda et al. 2016). Based on these findings, one can suggest that the discrepancies in the responses of a certain species to the addition of N under salinity conditions can be explained, at least in part, by variations in the crop salinity threshold and in the rate of yield reduction as salinity stress intensifies. Our data on leaf area and total biomass production demonstrated this type of response as cotton, a salt tolerant crop, when compared to maize, a moderately salt sensitive crop, showed a positive effect for additional N fertilization up to the salinity of 2.0 dS m^{-1}. However, no benefit of additional N was observed for maize (Figure 1).

In cotton, Chen et al. (2010) also found that when soil salinity was low (EC_e = 2.4 dS m^{-1}), an increment in N application rate alleviated the adverse effects of salinity. However, soil salinity at higher levels limited plant growth and additional application of N promoted no benefit. Min et al. (2014) observed that under high salinity conditions, the application of high N rates had no effect on biomass production of cotton. In maize, Lacerda et al. (2016) verified that there was no positive effect of N on plant growth when irrigation water salinity was higher than 2.5 dS m^{-1}. In pepper, Yasuor et al. (2017) found that, under high salinity conditions (3.7–5.7 dS m^{-1}), crop growth was limited by the salts and not by the competition between Cl^- and NO_3^-, and the response to N fertilization disappeared or decreased with increasing salinity of irrigation water.

As observed for growth, the effects of salinity and N on the partition index differed greatly between crops (Figure 1), with more severe impacts on maize than on cotton. For maize, it was verified that the supra-optimal N dose favored the biomass partition for reproductive growth at low levels of salt stress, but this effect was completely reversed under irrigation water salinities of 4.0 and 6.0 dS m^{-1}. For cotton, the partition index tended to increase as salinity increased, with the sub-optimal dose leading to a lower percentage of partition to reproductive organs compared to the other doses. These results demonstrate that the discrepancy in the response to N in salt-sensitive and salt-tolerant species depends on the salt stress level, taking into account both shoot growth or biomass partitioning.

In maize, Azizian and Sepaskhah (2014) observed that biomass partition to reproductive organs was statistically similar at water salinities of EC_{iw} equal to 2 (43%) and 4 dS m^{-1} (42%), but significantly lower than that (46%) of plants irrigated with control salinity (0.6 dS m^{-1}). Moreover, there was no difference in the partition between the doses of 150 and 300 kg N ha^{-1}. In cotton, Zhang et al. (2012) verified that the largest biomass partition to reproductive organs (50.5%) was found in plants that were cultivated under high salinity, but did not receive N fertilization, and that high N rate stimulated vegetative growth during the stage of boll formation, probably being detrimental to the development of reproductive organs.

The negative effects of salts on maize leaf gas exchanges were the highest for plants cultivated under 140% N dose (Figure 2), possibly because the additional application of N exacerbated the osmotic effects of saline treatments. As in the present study, Tabatabaei (2006) found that increased N concentration in the cultivation medium caused reductions in g_s, A, and E (transpiration) in olive plants under salt stress. On the other hand, Zeng et al. (2014) observed that sunflower photosynthetic rates increased with the application of N under low and moderate levels of salinity, whereas excess N reduced photosynthesis under severe salt stress.

Plant growth is controlled by many physiological, biochemical, and molecular processes, particularly photosynthesis (Ashraf and Harris 2004; Ashraf and Foolad 2013). The impact of salinity on photosynthesis is strongly dependent on N fertilization, tolerance to salinity of a species, and both duration and intensity of salt stress (Hessini et al. 2013). In our study, positive effects of N fertilization on leaf gas exchanges of cotton and maize were evident only at the lowest levels of salinity (Figure 2), and the use of supra-optimal doses intensified the effects of salt stress, mainly on maize plants.

The relative chlorophyll index was also significantly different in response to salinity and N doses, in both studied crops (Figure 3). While salinity and the increment in N doses increased the chlorophyll content of cotton, these values tended to decrease in maize beyond moderate levels of salinity, even when supra-optimal N doses were applied. Our results for maize were similar to those reported by Lacerda et al. (2016), who observed that the positive effect of high N dose on plants disappeared when salinity increased from low to moderate. According to Jamil et al. (2007), salinity reduced chlorophyll content in plants susceptible to salinity and increased it in salt-tolerant plants. As observed for cotton, the increase in N levels increased chlorophyll contents in soybean (Zhang et al. 2013) and wheat (Vafadar et al. 2014; Ibrahim et al. 2018). This response, however, may also be associated to leaf morphophysiological characteristics and to defense mechanisms of each species in response to salt stress (Lacerda et al. 2006).

The results of the present study indicate that salinity reduces N-use efficiency (Figure 4), and this response was more evident in maize plants, which are more sensitive to salinity than cotton plants. The reduction in N-use efficiency can be exacerbated when using N rates above the recommended value, especially under moderate to high salinity (Zhang et al. 2012; Lacerda et al. 2016). For these treatments, excessive N application did not result in accumulation of nitrogenous compounds (Figure 5), which have been reported to attenuate the effects of salt stress (Munns and Tester 2008; Ding et al. 2010). Our results also demonstrate that the application of supra-optimal N rates resulted in losses by leaching (Figure 5D), especially in soil columns with maize plants. In this regard, the additional application of N to plants under salt stress may become an inefficient agricultural practice from both economic and environmental standpoints (Neves et al. 2009; Segal et al. 2010; Ramos et al. 2012; Semiz et al. 2014; Feitosa et al. 2016; Lacerda et al. 2018). According to Zhang et al. (2017), a more sustainable management of N fertilization is needed in modern farming systems, aiming to mitigate environmental pollution resulting from N losses.

Although nitrate losses from leaching are significant in tropical agriculture (Huddell et al. 2020), it is necessary to consider other possible N losses in the soil, such as ammonia volatilization or denitrification reactions, which were not evaluated in the present study. According to Huddell et al. (2020), high temperatures observed during the day in tropical regions favor gaseous N losses and this problem can be enhanced due to global warming. Ammonia emissions from the agricultural enterprise, originating especially from application of manure slurry and urea-based mineral fertilizers, lead to numerous environmental and economic concerns (Sigurdarson et al. 2018). Laboratory experiments showed that high soil salinity also increases ammonia volatilization (Akhtar et al. 2012), especially when combined with high soil pH (Gandhi and Paliwal 1976). However, soil conditions during our study (pH ranging from 5.4 to 6.7 in the soil column and unsaturated soil with aerobic conditions) were not favorable to ammonia volatilization and denitrification (Singh et al. 2012; Ghaly and Ramakrishnan 2013; Sigurdarson et al. 2018). According to Sigurdarson et al. (2018), at an acidic soil pH (pH < 7), most of the NH_3 formed during urea hydrolysis by ureases is converted to cationic ammonium that cannot volatilize (app. 99% on NH_4^+ form at pH 5.5). In addition, fertilization of soil columns was performed in a way that minimized gaseous N losses. Urea was distributed along the cycle of both crops and incorporated into the soil at the end of the day due to lower temperatures. However, the controlled conditions used in our study are not always achieved under extensive commercial agricultural systems. Therefore, due to the high temperatures often observed in tropical regions, future studies related to nitrogen-use efficiency under salinity conditions should include evaluations of N losses due to volatilization.

Conclusions

Our results strongly indicate that the responses to additional nitrogen fertilization depend on crop salt-tolerance and on the level of saline stress imposed. Excess N application was only beneficial to cotton, and only up to an EC_{iw} = 2.0 dS m^{-1}. In fact, fertilization with 60% of recommended N dose was plenty for biomass production of maize under 6 dS m^{-1}, while 100% was better for cotton.

Nitrogen doses beyond the recommended values exacerbated the negative effects of salinity on growth and photosynthetic rates, especially on maize plants growing under moderate to high salinity conditions. The supra-optimal N rate had no effect on increasing leaf nitrogenous compounds, believed to attenuate the negative impacts of salt stress. On the contrary, increasing N rates only increased nitrate leaching losses and reduced the nitrogen-use efficiency, indicating that such practice would result in economic losses and environmental N overload, especially if a supplemental dose is applied to a salt-sensitive crop.

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Disclosure statement

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