AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.17, n.2, e473, 2022
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v17i2a473 - Protocol 473
Subm. 21/04/2021 • App. 28/03/2022 • Pub. 23/05/2022

Physiological responses in sugar apple seedlings under irrigation with saline water and foliar nitrogen

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ABSTRACT: Irrigation water salinity is one of the most limiting factors to agriculture in the Northeast region of Brazil, compromising photosynthesis in most crops, especially in the early growth phase, thus requiring the development of research to mitigate its harmful effects. In this context, this study aimed to evaluate the effects of nitrogen fertilization for the mitigation of the salinity on the physiology of sugar apple seedlings. The experimental design was in randomized blocks, using five levels of electrical conductivity of irrigation water (0.5; 1.15; 2.75; 4.35 and 5.0 dS m^{-1}) and five nitrogen levels via foliar application (0.0; 0.33; 1.15; 1.97 and 2.3 g L^{-1}) in four replications and two plants per plot, totaling nine combinations generated by the Box Central Composite Design. Despite stimulating chlorophyll formation 70 DAS, saline water inhibits the fluorescence of chlorophyll a in photosystem II. Foliar nitrogen fertilization at the level of 1.62 g L^{-1} mitigates the effects of salinity on the gas exchange properties of sugar apple seedlings. The fluorescence activity and chlorophyll indices show positive responses to foliar nitrogen fertilization in sugar apple seedlings 70 DAS.

Key words: *Annona squamosa* L.; nitrogen fertilization; photosynthesis; salinity

Respostas fisiológicas em mudas de pinheira sob irrigação com águas salinas e aplicação foliar de nitrogênio

RESUMO: A salinidade da água de irrigação constitui um dos fatores mais limitantes à agricultura da região Nordeste por comprometer a fisiologia das culturas, principalmente na fase inicial de crescimento, o que tem exigido o desenvolvimento de pesquisas para amenizar esses efeitos danosos. Nesse contexto, objetivou-se avaliar os efeitos da adubação nitrogenada foliar visando atenuar efeitos da salinidade nas trocas gasosas, clorofila e fluorescência da clorofila de mudas de pinheira. O delineamento experimental foi em blocos casualizados, usando cinco valores de condutividade elétrica da água de irrigação (0,5; 1,15; 2,75; 4,35 e 5,0 dS m^{-1}) associados às doses de nitrogênio aplicado via foliar (0,0; 0,33; 1,15; 1,97 e 2,3 g L^{-1}) em quatro repetições e duas plantas por parcela, totalizando nove combinações geradas pela matriz Composto Central de Box. O aumento da salinidade da água de irrigação estimula a formação de clorofila e inibe as trocas gasosas e a fluorescência da clorofila a de mudas de pinheira aos 70DAS. A adubação nitrogenada foliar, na dose de 1,62 g L^{-1}, atenua os efeitos deletérios da salinidade nas trocas gasosas das mudas de pinheira. A atividade da fluorescência e os índices de clorofila apresenta respostas positivas a adubação foliar de N em pinheira aos 70 DAS.

Palavras-chave: *Annona squamosa* L.; adubação nitrogenada; fotossíntese; salinidade

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Associate Editor: Edivan Rodrigues de Souza

Introduction

Sugar apple (*Annona squamosa* L.), a member of the family Annonaceae, is a tropical fruit species with high adaptability to diverse climatic conditions, except for rainfall in the semi-arid areas of Northeastern Brazil. These advantages justify the position of that region as the leading sugar apple producer in Brazil, contributing 94% of the entire national production, which accounted for 21,087 t in 2012 (IBGE, 2021). The importance of this species is associated with the medicinal properties of its fruits, contributing to the socio-economic development of small and medium producers in Northeastern Brazil (Figueiredo et al., 2019).

Despite the expressiveness of the crop, the orchards in the Northeast region generally show poor production performance due to scarce information about crop management practices, irrigation technologies, and fertilization schedules (Lemos, 2014). In this scenario, besides adopting farming technologies to increase the fruit yield, it is indispensable to use high-quality biological materials for seedling formation in order to achieve economically viable results (Riikonen & Luoranen, 2018).

One of the limitations to production systems in the Brazilian semi-arid region is irrigation water restriction with regard to salinity (Ferreira et al., 2021). This situation is caused by prolonged drought periods, as seen in the last seven years, which, associated with high evapotranspiration rates in reservoirs, limit the availability of good-quality water (Melo et al., 2018). Such problems are common in the seedling formation phase of many species, including sugar apple, leading producers to use high-salinity water for seedling production (Oliveira et al., 2018).

According to Dell'Aversana et al. (2021), one of the effects of salinity is the reduction in the osmotic potential of the medium to values that restrict water uptake by plant roots. In such a situation, the low solution values absorbed by plants are concentrated in salts and/or specific ions, e.g., sodium (Na^+) and chloride (Cl^-), which cause nutritional and physiological imbalances in plants (Najar et al., 2019), including sugar apple seedlings (Andrade et al., 2018). From this perspective, Zörb et al. (2019) stated that toxic effects are involved in metabolic changes that contribute to forming reactive oxygen species (EROs), which interfere with the functioning and synthesis of plant photoassimilates, especially photosynthetic pigments and the activity of Rubisco.

The degradation of the photosynthetic apparatus in Annonaceae seedlings caused by salinity was already observed by Figueiredo et al. (2019) in sugar apple (*Annona squamosa* L.), by Veloso et al. (2021) in soursop (*Annona muricata* L.), and by Dalanhol et al. (2018) in araticum (*Annona emarginata*), thus requiring research development aiming to mitigate the deleterious effects of salinity.

Nitrogen fertilization could be an alternative to mitigate the degenerative effects of salinity, as observed by Melo et al. (2018), Oliveira et al. (2018), and Bezerra et al. (2019) in West Indian Cherry (*Malpighia emarginata*), jackfruit (*Artocarpus heterophyllus* L.), and yellow passion fruit, respectively (*Passiflora edulis* Sims). This nutrient participates in the synthesis of vital plant compounds, e.g., proteins and enzymes involved in photosynthesis (Lima et al., 2019). Additionally, nitrogen also contributes to the antioxidant and osmotic defense of the plant, processes that participate in the compartmentalization of salts, turgor maintenance, and free radical inhibition (Wanderley et al., 2020).

The form of nitrogen application under salt stress conditions has shown diverging results in the literature (Bezerra et al., 2018; Figueiredo et al., 2019; Wanderley et al., 2020) depending on several factors, especially the competition between salts in root uptake sites, which tends to reduce nutrient availability to plants (Li et al., 2019).

As an alternative, foliar fertilization could contribute to improving nitrogen availability under salt stress conditions by inhibiting the action of severe physiological disturbs caused by salinity (Otálora et al., 2018). However, studies on this subject are still scarce and should be encouraged.

From this perspective, this study aimed to evaluate the effects of foliar nitrogen fertilization applied to mitigate salinity on the gas exchange, chlorophyll, and chlorophyll fluorescence in sugar apple seedlings.

Materials and Methods

The experiment was conducted from April to August 2019 in a protected environment at the Center of Agricultural Sciences of the Federal University of Paraíba, Areia, Paraíba, Brazil. The municipality is located at the geographic coordinates 6° 58' 00'' S and 35° 41' 00'' W, at an elevation of 575 m above sea level. According to the Köppen classification, the regional climate is classified as As', i.e., dry and hot summers and winter rainfall (Alvares et al., 2013). The mean temperature observed during the experimental period was 27.5 °C, ranging between a maximum temperature of 36.2 and a minimum of 18.8°C.

The experimental design was in randomized blocks with four replications and two plants per plot. The treatments were obtained using the Box Central Composite Design (Mateus et al., 2001) with five levels of electrical conductivity of irrigation water and five nitrogen levels supplied via foliar application (Table 1).

Table 1. Electrical conductivity of irrigation water (ECiw) and foliar nitrogen levels (L_{FN}) used to determine the treatments.

Treatments	Levels		Values	
	ECiw	L_{FN}	ECiw (dS m^{-1})	L_{FN} (g L^{-1})
1	-1	-1	1.15	0.33
2	-1	1	1.15	1.97
3	1	-1	4.35	0.33
4	1	1	4.35	1.97
5	-1.41(α)	0	0.50	1.15
6	1.41(α)	0	5.00	1.15
7	0	-1.41(α)	2.75	2.30
8	0	1.41(α)	2.75	0.00
9	0	0	2.75	1.15

The electrical conductivity of each irrigation water (ECiw) above 0.5 dS m^{-1} was obtained by diluting sodium chloride (NaCl) in tap water (0.5 dS m^{-1}) until obtaining the previously established value using an Instrutherm® portable microprocessor conductivity meter (model CD-860). The electrical conductivity values were selected according to Andrade et al. (2018), who recorded inhibitory salinity effects on sugar apple seedlings irrigated with ECiw levels from 0.5 to 4.5 dS m^{-1}.

The nitrogen levels were obtained based on the need for 300 mg per plant proposed by Novais et al. (1991) for an in-pot experiment with 1dm^3 pots in which the highest level evaluated was 400 mg per plant or the equivalent to 2.30 g L^{-1} of nitrogen. The nitrogen requirement was met using a commercial product (Nitrotecnia-20, Carbotecnia®) that contains 99 g L^{-1} of N and is based on urea.

The seeds used in the experiment came from ungrafted plants located at the Irrigation Perimeter Várzeas de Sousa, municipality of Aparecida, PB. Three seeds were sown per polyethylene bag with a capacity of 1150 mL. The bags were then kept at the moisture level equivalent to field capacity from sowing to germination, which occurred about 25 days after sowing when the plants were thinned to one seedling per plant. Prior to planting, the seeds were subjected to physical scarification using wood sandpaper to facilitate germination.

The polyethylene bags were filled with a substrate formed by 85% Latosol, 10% washed fine sand, and 5% cattle manure. The substrate was analyzed for its physical and chemical characteristics based on fertility and salinity according to the methodologies proposed by Embrapa (2017) and Richards (1954), as seen in Table 2.

Seedling irrigation with saline water began 35 days after sowing (DAS) with daily applications that provided the volume evapotranspirated on the previous day in order to restore soil moisture to field capacity (Bernardo et al., 2006). This practice consists of daily applying the evapotranspirated water volume, for which ten containers were selected and received collectors to determine, based on the difference between the water volume applied and the water volume drained in the previous irrigation, the necessary water volume to begin drainage in the container. A 10% leaching fraction was applied every 15 days during this period to leach the salts accumulated in the substrate by the irrigation events.

Foliar spraying with N began 40 DAS and was repeated every ten days through four applications and a total applied volume of 100 mL per plant. The product was diluted in distilled water on the day of application. Spraying was performed in the late afternoon using a manual sprayer.

The evaluations were performed 70 DAS from 9:00 to 10:00 a.m., referring to gas exchange properties, chlorophyll a fluorescence of photosystem II, and chlorophyll indices. The gas exchange properties were determined using a portable infrared gas analyzer - IRGA (model LI-6400XT, LI-COR®, Nebrasca, USA) with an airflow of 300 mL min^{-1} and a coupled light source of 1,200 μmol m^{-2} s^{-1}. The variables analyzed were: net CO_2 assimilation (A) (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s) (mol m^{-2} s^{-1}), internal CO_2 concentration (C_i) (μmol CO_2 m^{-2} s^{-1}), and transpiration (E) (mmol H_2O m^{-2} s^{-1}). These data were used to calculate the water-use efficiency (WUE = A/E), intrinsic water-use efficiency (iWUE= A/gs), and instantaneous carboxylation efficiency (iCE = A/Ci).

The same period was used to determine the chlorophyll fluorescence using a modulated fluorometer (Sciences Inc.- Model OS-30p, Hudson, USA). For that purpose, tweezers were fixed 30 minutes before the readings to adapt the leaves to the dark and thus obtain the parameters of initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence ($F_v = F_m - F_0$), the ratio of variable fluorescence to initial fluorescence F_v/F_0, and the quantum yield of photosystem II (F_v/F_m). Chlorophyll a, chlorophyll b, and total chlorophyll were obtained by a non-destructive method using a portable chlorophyll meter (ClorofiLOG®, model CFL 1030, Porto Alegre, RS), with values measured as Falker chlorophyll index (ICF).

The data were subjected to analysis of variance and regression using the statistical software R (R Core Team, 2020).

Results and Discussion

According to the summary of the analysis of variance (Table 3), the N levels and the electrical conductivity of

Table 2. Physical and chemical attributes of the substrate used in the experiment.

Physical	Value	Chemical	Value	Salinity	Value
Sand (g kg^{-1})	639	pH in water (1: 2.5)	7.00	pH	7.30
Silt (g kg^{-1})	227	P (mg dm^{-3})	146.32	CEes (dS m^{-1})	2.73
Clay (g kg^{-1})	134	K^+ (mg dm^{-3})	633.29	$SO4^{-2}$ ($mmol_c$ L^{-1})	1.02
Textural class	Sandy-loam	Na^+ ($cmol_c$ dm^{-3})	0.27	Ca^{+2} ($mmol_c$ L^{-1})	16.00
		Al^{+3} ($cmol_c$ dm^{-3})	0.00	Mg^{+2} ($mmol_c$ L^{-1})	16.75
		H^++Al^{+3} ($cmol_c$ dm^{-3})	2.84	K^+ ($mmol_c$ L^{-1})	6.90
		Ca^{+2} ($cmol_c$ dm^{-3})	5.53	CO_3^{-2} ($mmol_c$ L^{-1})	0.00
		Mg^{+2} ($cmol_c$ dm^{-3})	1.70	HCO_3^{-2} ($mmol_c$ L^{-1})	40.00
		SB ($cmol_c$ dm^{-3})	9.12	Cl^- ($mmol_c$ L^{-1})	30.00
		CEC ($cmol_c$ dm^{-3})	11.96	Na^+ ($mmol_c$ L^{-1})	0.89
		OM ($cmol_c$ dm^{-3})	26.69	RAS ($mmol_c$ L^{-1})	0.94
				PST (%)	2.25
				Classification	Non-saline

OM = organic matter; SB = sum of bases (Na^+ + K^+ + Ca^{2+} + Mg^{2+}); CEC = cation exchange capacity = SB + (H^+ + Al^{3+}); ECse = electrical conductivity of the saturation extract; RAS = sodium adsorption ratio = Na+ × [(Ca^{2+} + Mg^{2+})/2] -1/2; PST = percentage of exchangeable sodium (100 × Na^+/ CEC).

Table 3. Summary of the analysis of variance for the physiological variables of stomatal conductance (gs), CO_2 concentration in intercellular spaces (Ci), CO_2 assimilation rate (A), leaf transpiration rate (E), water-use efficiency (WUE), intrinsic water-use efficiency (iWUE), and instantaneous carboxylation efficiency (iCE) in sugar apple seedlings irrigated with saline water (ECiw) and foliar nitrogen levels (L_{FN}) analyzed 70 days after sowing (DAS).

Source of variation	DF	Mean square						
		gs	Ci	E	A	WUE	iWUE	iCE
Blocks	3	$3.3e^{-4**}$	962.0^{**}	0.23^{ns}	0.12^{ns}	0.61^{**}	465.2^{**}	$4.71e\text{-}6^{**}$
Treatments	(8)	$9.5e^{-4**}$	598.3^{**}	0.65^{**}	3.55^{**}	0.42^{**}	327.9^{**}	$4.36e\text{-}5^{**}$
L_{FN} (L)	1	0.043^{**}	17.1^{**}	1.09^{**}	2.40^{**}	$5.8e\text{-}4^{ns}$	6.46^{ns}	0.008^{**}
L_{FN} (Q)	1	0.003^{ns}	20.6^{**}	0.13^{ns}	0.63^{**}	0.62^{**}	15.89^{**}	0.003^{**}
ECiw (L)	1	0.004^{ns}	25.58^{**}	0.21^{ns}	0.75^{**}	0.76^{**}	20.06^{**}	0.003^{**}
ECiw (Q)	1	0.004^{ns}	13.31^{*}	0.22^{ns}	0.29^{ns}	0.38^{*}	11.34^{**}	0.001^{**}
L_{FN} (L) x ECiw (L)	1	0.011^{**}	5.21^{*}	0.33^{ns}	0.78^{**}	0.06^{ns}	2.49^{ns}	0.003^{**}
CV (%)		12.9	3.6	14.3	9.4	11.3	10.5	5.5

ns, **, * respectively not significant, significant at $p \leq 0.01$ and significant at $p \leq 0.05$ by the F-test.

irrigation water significantly influenced the water-use efficiency (WUE) and the intrinsic water-use efficiency (iWUE) 70 DAS, whereas the N levels influenced transpiration (E) in the same period. Furthermore, the interaction between water salinity and nitrogen levels significantly influenced the stomatal conductance (gs), internal CO_2 concentration (Ci), CO_2 assimilation (A), and instantaneous carboxylation efficiency (ICE) to 70 DAS.

The foliar nitrogen levels did not increase the stomatal conductance (Figure 1 A) and CO_2 concentration in intercellular spaces (Figure 1 B), with the highest respective values of 0.109 mol m^{-2} s^{-1} and 301.78 µmol CO_2 m^{-2} s^{-1} being achieved in the treatments without nitrogen associated with the ECiw of 0.50 dS m^{-1} for gs and 1.79 dS m^{-1} for Ci. On the other hand, the lowest gs values (0.028 mol m^{-2} s^{-1}) were observed without nitrogen fertilization but associated with the ECiw of 5.00 dS m^{-1}. These results differ from the Ci variable, whose lowest values corresponded to the level of 2.30 gL of N and the ECiw of 0.5 dS m^{-1}, achieving 245.29 µmol CO_2 m^{-2} s^{-1}. According to Dell'Aversana et al. (2021), the reduction in the stomatal

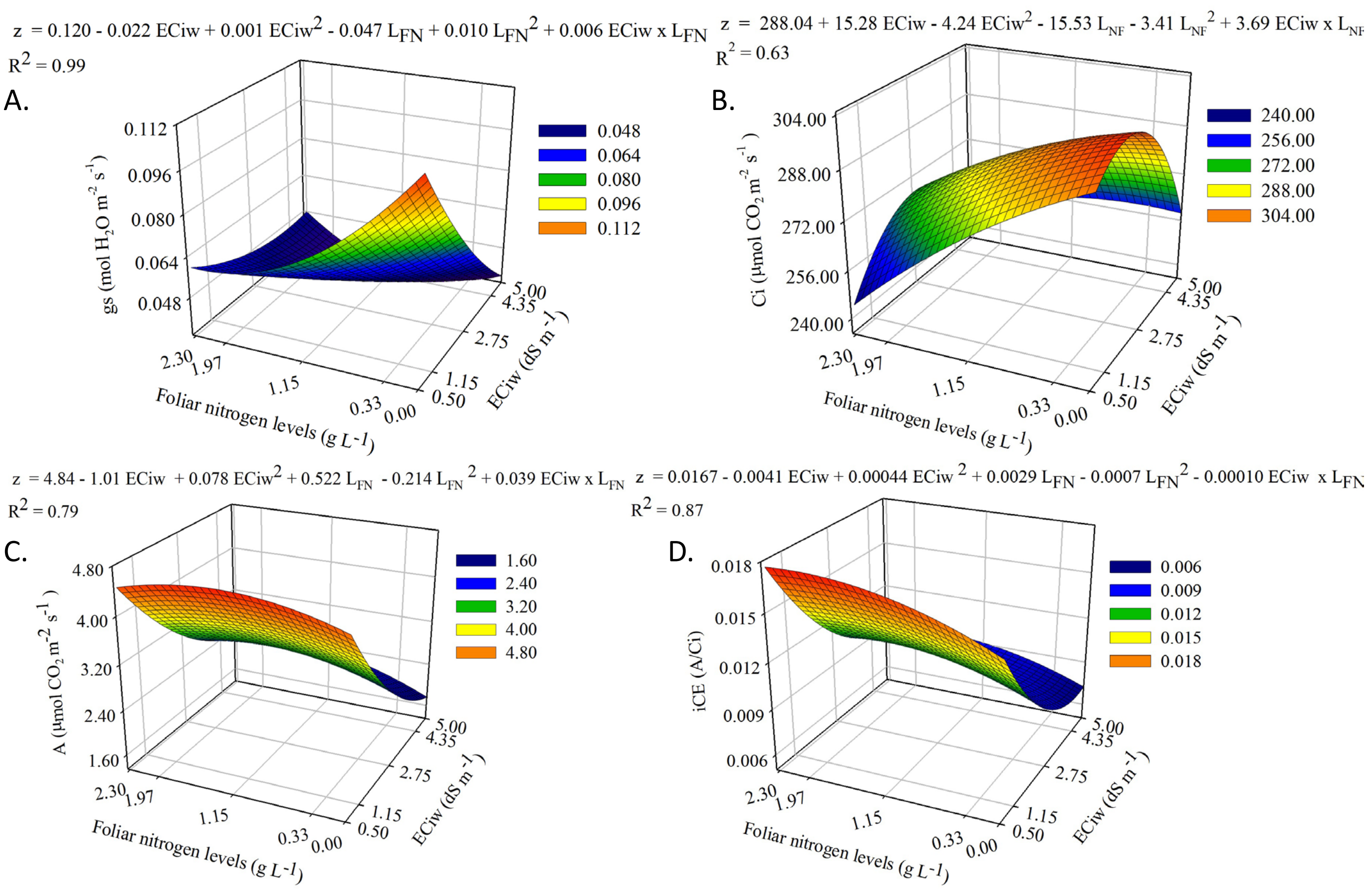


Figure 1. Stomatal conductance (A), internal CO_2 concentration (B), net photosynthesis (C), and instantaneous carboxylation efficiency (D) of sugar apple seedlings as a function of the electrical conductivity of irrigation water and foliar nitrogen levels 70 DAS.

opening is a plant response mechanism to salt stress as a strategy to decrease water losses and salt uptake even with a reduced CO_2 entry into the substomatal chambers.

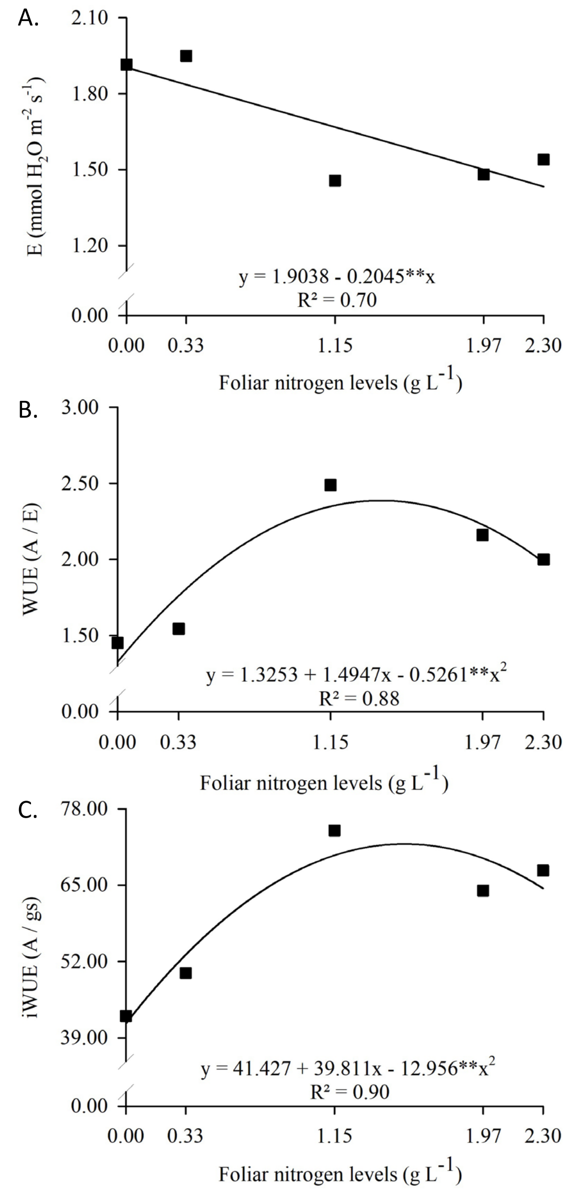
However, the foliar nitrogen applications increased the net photosynthesis and carboxylation efficiency of sugar apple seedlings irrigated with saline water 70 DAS (Figure 1C and D), favoring the maximum values of A (4.70 μmol CO_2 m^{-2} s^{-1}) and iCE (0.0177) at the N levels of 1.79 and 1.27g L^{-1}, both associated with the ECiw of 0.5 dS m^{-1}. These results show that, even after a short period of foliar nitrogen application, the rapid absorption of the element by the sugar apple plant increases its photosynthetic efficiency. This improvement could be a response to the nitrogen roles in composing enzymes and proteins involved in photosynthesis, including ATP synthase and Rubisco, essential for the efficient fixation of carbon in the plant (Wanderley et al., 2020).

Under the conditions of the present experiment, the salt stress resulting from salt accumulation in the soil due to irrigation caused expressive losses in the functioning of the photosystem due to the degradation of proteins involved in the photosynthetic activity (Zörb et al., 2019). These results explain the depreciation in photosynthesis and the intrinsic carboxylation efficiency of plants without any N level and irrigated with the highest ECiw, with losses amounting to 61.43 and 59.32% compared to the values obtained at the points of maximum gain.

As seen in Figure 2, although the increase in the foliar N levels linearly inhibited transpiration (E), the increase from 0.00 to 1.42 and 1.53 g L^{-1} increased the water-use efficiency (WUE) and intrinsic water-use efficiency (iWUE). The increase in the N levels via foliar application reduced transpiration by 24.74% among sugar apple seedlings treated with the lowest and highest N levels (Figure 2A). Conversely to transpiration, the increase in N from 0.00 to 1.42 g L^{-1} increased the water-use efficiency from 1.32 to 2.39 (Figure 2B) and the intrinsic water-use efficiency when passing from the level of 0.00 to 1.53 g L^{-1} of N, changing from 41.43 to 72.02 (Figure 2C), respectively increasing by 81.06 and 73.84%.

Therefore, the reduction in transpiration with the increase in N contributed to increasing the water-use efficiency among plants that received nitrogen fertilization, agreeing with Oliveira et al. (2019) when those authors concluded that the decrease in transpiration improved the WUE due to the balance in the photosynthetic activity between water translocation and nutrient dynamics in the xylem, contributing to maintaining cell turgor due to the high loss of water as vapor by the stomata. The iWUE is related to the high photosynthetic performance, which, when associated with reduced stomatal opening, increases the use of the carbon available in the substomatal chambers (Evans & Clarke, 2019).

With regard to the effects of the electrical conductivity of irrigation water on the water-use efficiency and intrinsic water-use efficiency (Figure 3A and B), the best results were achieved at the ECiw of 5.00 dS m^{-1}, corresponding to 2.49 for WUE and 76.90 for iWUE, values 3.01 and 10.42% higher than those observed in the plants irrigated with the ECiw of 0.5 dSm^{-1}. Since the salt stress condition was initial, the highest salt concentrations in the water partially closed the stomata through an osmotic effect that improved the use efficiency of the compounds used in the biochemical phase of photosynthesis (Najar et al., 2019).

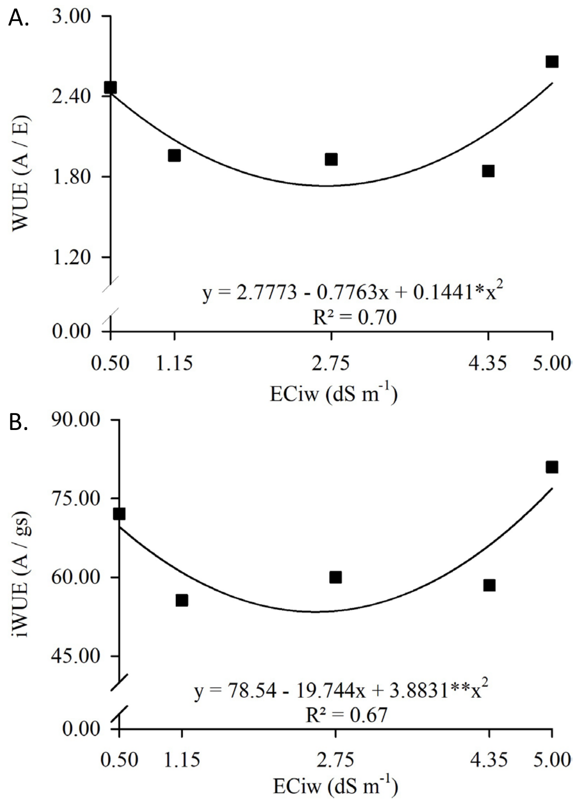


**: Significant at $p \leq 0.01$ by the F-test.

Figure 2. Transpiration (A), water-use efficiency (B), and intrinsic water-use efficiency (C) of sugar apple seedlings as a function of foliar nitrogen levels 70 DAS.

For the chlorophyll and fluorescence indices (Table 4), there was an isolated effect of the foliar N levels and water electrical conductivity on chlorophyll a, b, and total chlorophyll, maximum fluorescence, and variable fluorescence. On the other hand, the interaction of the factors was only noted on the initial fluorescence, and the factors did not significantly influence the other variables during the evaluated period.

The foliar nitrogen fertilization increased the chlorophyll indices of sugar apple plants 70 DAS, with the chlorophyll b contents increasing linearly along with foliar N (Figure 4B),

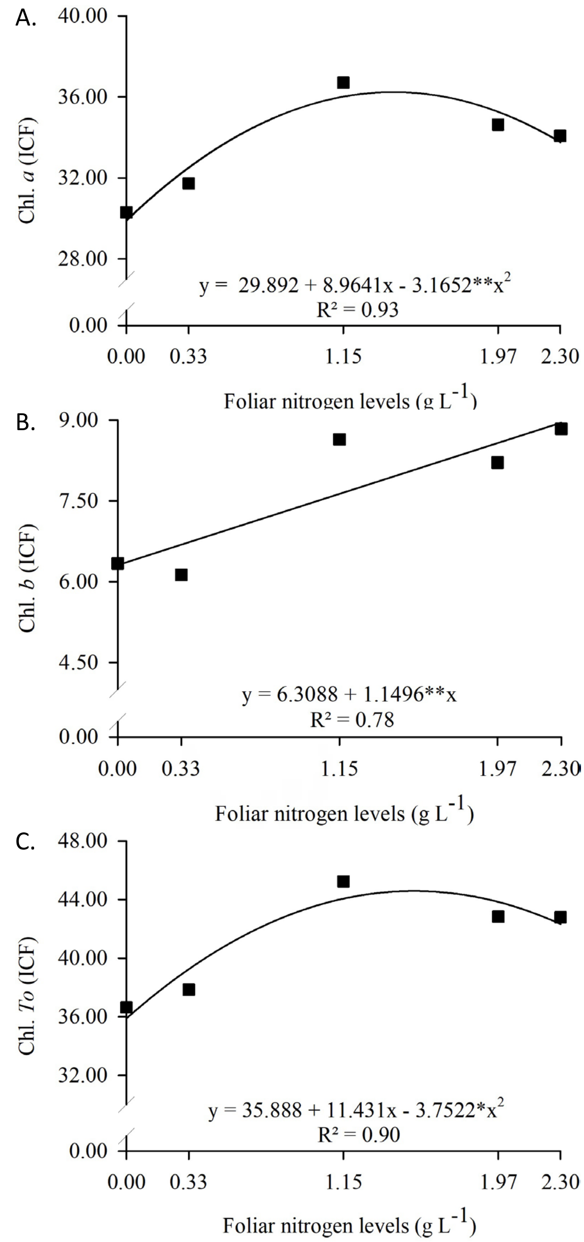


**: Significant at $p \leq 0.01$ by the F-test.

Figure 3. Water-use efficiency (A) and intrinsic water-use efficiency (B) of sugar apple seedlings as a function of the electrical conductivity of irrigation water 70 DAS.

resulting in a 41.84% increase at the N level of 2.30 g L^{-1} (8.95) compared to the results observed in the plants without nitrogen fertilization (6.31). On the other hand, the contents of chlorophyll a and total chlorophyll showed a quadratic behavior (Figure 4A and C), with maximum gains at the N levels of 1.41 and 1.52 g L via foliar application, corresponding to 36.24 and 44.59, which were higher by 21.24 % for Chl. a and by 24.25% for total chlorophyll compared to sugar apple plants without nitrogen fertilization, which showed 29.89 and 35.89, respectively.

The increases in chlorophyll due to nitrogen fertilization are directly related to the participation of this element in



**: Significant at $p \leq 0.01$ by the F-test.

Figure 4. Chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) of sugar apple seedlings as a function of foliar nitrogen levels 70 DAS.

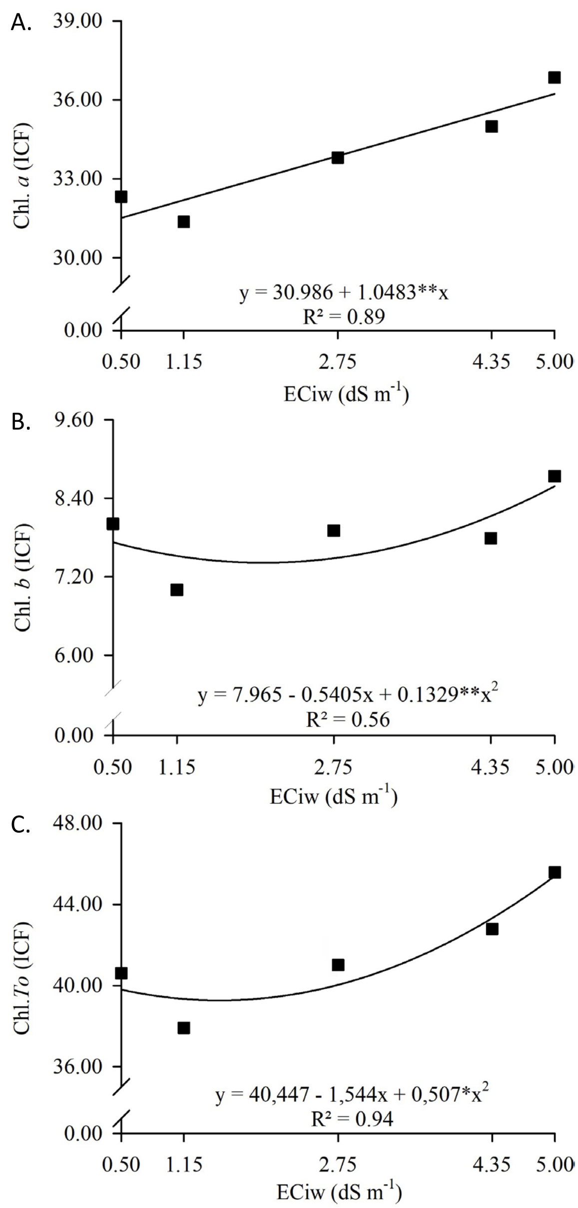
Table 4. Summary of the analysis of variance for chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll (Chl. To), and ratio of chlorophyll a to chlorophyll b (Chl. a/ Chl. b), initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), quantum yield of photosystem II (Fv/Fm), and ratio of variable fluorescence to initial fluorescence (Fv/Fo) in sugar apple seedlings irrigated with saline water (ECiw) and foliar nitrogen levels (L_{FN}) analyzed 70 DAS.

Source of variation	DF	Mean square								
		Chl *a*	Chl *b*	Chl *To*	Chl *a*/Chl *b*	Fo	Fm	Fv	Fv/Fm	Fv/Fo
Blocks	3	26.50^{**}	9.75^{**}	67.96^{**}	1.53^{**}	537^{ns}	4204^{ns}	$7.8e^{3ns}$	$2.0e^{-3ns}$	0.22^{ns}
Treatment	8	16.13^{*}	2.88^{**}	30.82^{**}	0.47^{ns}	1337^{*}	$1.2e^{4*}$	$1.6e^{4*}$	$9.0e^{-4ns}$	0.61^{ns}
L_{FN} (L)	1	2.83^{*}	1.10^{**}	3.93^{*}	0.29^{ns}	11.3^{ns}	11.2^{ns}	73.2^{ns}	0.015^{ns}	0.33^{ns}
L_{FN} (Q)	1	2.64^{**}	0.31^{ns}	2.95^{*}	0.15^{ns}	11.8^{ns}	99.3^{**}	80.3^{*}	0.011^{ns}	0.48^{ns}
ECiw (L)	1	3.40^{**}	0.77^{ns}	4.17^{**}	0.05^{ns}	14.1^{ns}	108.8^{**}	86.9^{*}	0.014^{ns}	0.61^{ns}
ECiw (Q)	1	2.10^{ns}	1.47^{**}	3.57^{*}	0.66^{ns}	5.7^{ns}	16.4^{ns}	9.8^{ns}	0.009^{ns}	0.37^{ns}
L_{FN} (L) x ECiw (L)	1	0.008^{ns}	0.08^{ns}	0.09^{ns}	0.05^{ns}	9.1^{*}	3.6^{ns}	27.5^{ns}	0.004^{ns}	0.04^{ns}
CV (%)		5.40	9.40	5.60	8.10	7.30	2.50	3.20	2.30	7.70

ns, **, * respectively non-significant, significant at $p < 0.01$, and significant at $p < 0.05$ by the F-test.

the composition of the chlorophyll molecule. As reported by Evans & Clarke (2019), the photosynthetic reaction centers concentrate about 75% of all the leaf nitrogen. These results agree with Cavalcante et al. (2012), who studied the nitrogen/chlorophyll ratio in sugar apple and observed a positive correlation between nitrogen supplementation and the chlorophyll a and total chlorophyll contents.

The chlorophyll indices were also increased by irrigation with saline water 70 DAS (Figure 5), with the plants irrigated with the ECiw of 5.00 dS m^{-1} showing the highest values of chlorophyll a (36.23), b (8.58), and total chlorophyll (45.40), resulting in a 14.48% increase in Chl. a (Figure 5A), 11.00% in Chl. b (Figure 5B), and 14.07% in total chlorophyll (Figure 5C) in relation to the plants irrigated with tap water. These findings highlight the plant adaptation to the initial salt stress



**: Significant at $p \leq 0.01$ by the F-test.

Figure 5. Chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) of sugar apple seedlings as a function of the electrical conductivity of irrigation water 70 DAS.

condition and are related to the rapid synthesis of chlorophyll under this condition to avoid compromising the capture and dissipation of light energy, which is affected by the metabolic damage resulting from prolonged salt stress and aggravated by oxidative stress (Akrami & Arzani, 2018). Similar responses were reported by Figueiredo et al. (2019) in sugar apple seedlings (*Annona squamosa* L.) irrigated with water salinity levels up to 4.0 dS m^{-1}.

With regard to initial fluorescence (Figure 6), the association between the ECiw of 2.28 dS m^{-1} and the N level of 1.34 g L^{-1} resulted in the highest Fo value (257.05). However, the combination between the highest irrigation water salinity and the highest foliar N level resulted in the lowest Fo value (222.08), which, compared to the highest point, showed a 13.60% reduction in the fluorescence activation value. For Veloso et al. (2021), the Fo consists of the loss of photochemical energy released by chlorophyll a molecules in the light-harvesting antenna of the photosystem. Therefore, the foliar N levels and irrigation with electrical conductivity levels up to 2.28 dSm^{-1} increase such losses. Under these conditions, the energy requirements of the photosystem can still be met without adaptation, which was not observed at the other ECiw levels.

The maximum fluorescence (Figure 7A) and variable fluorescence (Figure 7B) showed a quadratic behavior for nitrogen fertilization 70DAS, with a point of maximum gain at the N level of 1.02 g L^{-1} for Fm (2179.55) and 0.91 g L^{-1} for Fv (1928), resulting in respective increases of 2.27 and 2.54% in relation to the fluorescence observed in the plants without nitrogen fertilization. Increases in the Fm and Fv variables have been associated with the excitation potential of the chlorophyll a molecules of photosystem II, contributing to the synthesis of ATP and NADPH for the carbon assimilation phase (Lima et al., 2019).

With regard to salinity, the maximum fluorescence decreased linearly with the increase in the electrical conductivity of irrigation water (Figure 8A), ranging from an Fm of 2188.76 at the CE of 0.5 dS m^{-1} to 2116.43 at the ECiw of

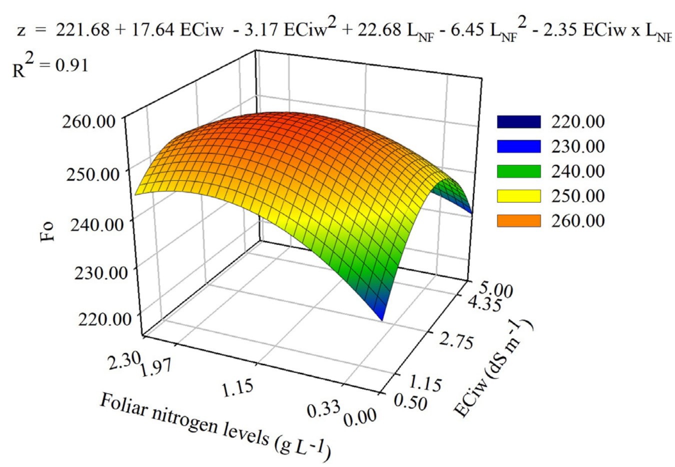
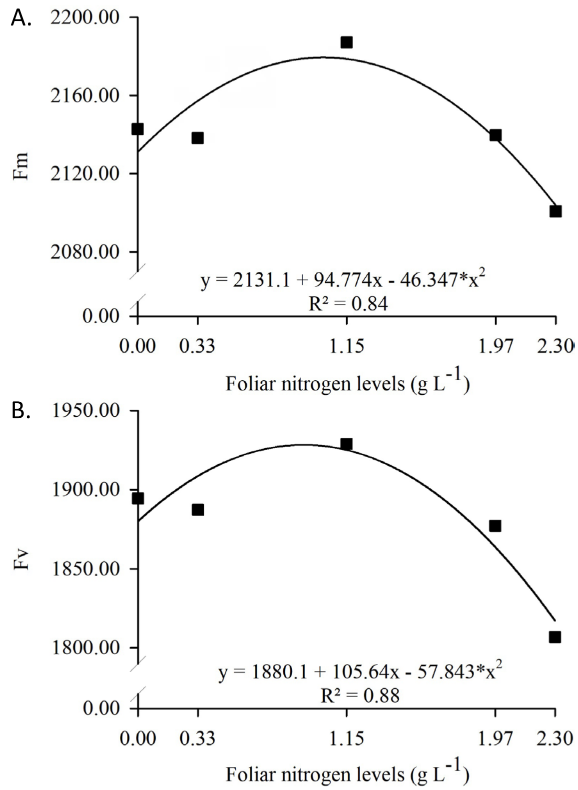
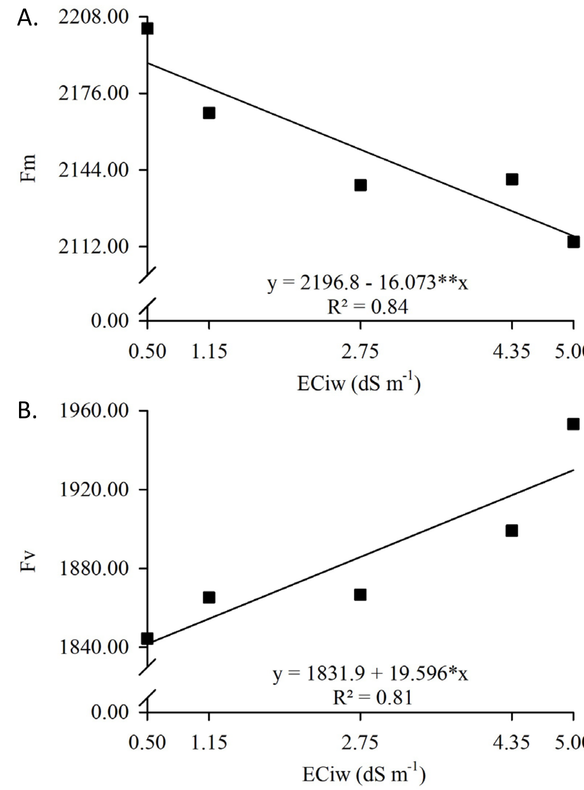


Figure 6. Initial fluorescence of sugar apple seedlings as a function of the electrical conductivity of irrigation water and foliar nitrogen levels 70 DAS.



**: Significant at p ≤ 0.01 by the F-test.

Figure 7. Maximum fluorescence (A) and variable fluorescence (B) of sugar apple seedlings as a function of foliar nitrogen levels 70 DAS.



**: Significant at p ≤ 0.01 by the F-test.

Figure 8. Maximum fluorescence (A) and variable fluorescence (B) of sugar apple seedlings as a function of the electrical conductivity of irrigation water 70 DAS.

5.0 dS m^{-1}, with a loss of 3.30%. On the other hand, the variable fluorescence increased linearly with the increase in irrigation water salinity (Figure 8B), with the highest ECiw (1929.88) resulting in a gain of 4.79% in relation to the plants irrigated with tap water at the ECiw of 0.5 dSm^{-1} (1841.70). For Ferreira et al. (2021), the Fm restriction due to salt stress indicates a slowdown in the photosynthetic activity to mitigate the toxic effects of salinity. Conversely, improvements in the Fv express the adaptation occurred in the use of photochemical energy (Najar et al., 2019).

Conclusions

The increase in irrigation water salinity stimulates the formation of chlorophyll and inhibits the gas exchange and chlorophyll a fluorescence in sugar apple seedlings 70 DAS.

Foliar nitrogen fertilization at the level of 1.62 g L^{-1} mitigates the deleterious effects of salinity on the gas exchange properties of sugar apple seedlings.

The activity of fluorescence and the chlorophyll indices showed positive responses to foliar nitrogen fertilization in sugar apple seedlings 70 DAS.

Acknowledgments

To the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) for the concession of the scholarships.

Compliance with Ethical Standards

Author contributions: Conceptualization: RTF; Data curation: RTF, JTAF, WFC, JESR; Formal analysis: RTF; Funding acquisition: WEP; Investigation: JSN, JTAF, WFC; Methodology: RTF, JSN; Project administration: WEP; Resources: FRAF; Supervision: MBP; Validation: MBP; Visualization: JESR, WEP; Writing – original draft: RTF, JSN; Writing – review & editing: FRAF, JESR, MBP, WEP.

Conflict of interest: The authors declare that there isn't conflict of interest.

Financing source: The Coordination for the Improvement of Higher Education Personnel (CAPES) – Finance Code 001 and the National Council for Scientific and Technological Development (CNPq).

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