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Photosynthetic pigments and biomass in noni irrigated with saline waters with and without leaching

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Key words:
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ABSTRACT

The response of plants to salt stress is an extremely complex phenomenon that involves morphological, physiological and biochemical changes, modifying the leaf contents of chlorophyll and carotenoids, among others and affecting plant growth, development and production. An experiment was carried out from July 2010 to June 2011, in order to evaluate the contents of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, the chlorophyll a/b ratio and biomass accumulation of shoots and roots of noni plants, when subjected to irrigation and leaching with water of increasing salinity. The experiment was carried out in a greenhouse, at the Center of Agricultural Sciences of the Federal University of Paraíba, in the municipality of Areia-PB, Brazil. Treatments were distributed in randomized blocks, in a 5 × 2 factorial scheme with four replicates and two plants per plot, and corresponded to levels of electrical conductivity of irrigation water (0.5; 1.5; 3.0; 4.5 and 6.0 dS m^{-1}) in pots with and without leaching. The increase in irrigation water salinity impairs the leaf contents of chlorophyll a, b, total chlorophyll, carotenoids and biomass production of noni plants, but to a lesser extent in all the treatments in which same irrigation water was used for leaching.

Palavras-chave:
Morinda citrifolia L.
drenagem
clorofila
estresse salino

Pigmentos fotossintéticos e biomassa em noni irrigado com águas salinas com e sem lixiviação

RESUMO

A reposta das plantas ao estresse salino é um fenômeno extremamente complexo, que envolve alterações morfológicas, fisiológicas e bioquímicas alterando, dentre outros, os teores foliares de clorofila e carotenoides, comprometendo o crescimento, o desenvolvimento e a produção das plantas. O experimento foi desenvolvido em ambiente protegido do Centro de Ciências Agrárias da Universidade Federal da Paraíba, Areia, PB, no período de julho de 2011 a junho de 2012. Este trabalho foi realizado com o objetivo de avaliar os teores de clorofila a, clorofila b, clorofila total, relação clorofila a/b e produção de biomassa da parte aérea e radicular em plantas de noni submetidas à irrigação e lavagem do solo com águas de salinidade crescente. Os tratamentos foram distribuídos em blocos ao acaso num fatorial 5 × 2 com quatro repetições e duas plantas por parcela, correspondentes aos níveis de condutividade elétrica de 0,5; 1,5; 3,0; 4,5 e 6,0 dS m^{-1} da água de irrigação, em vasos sem e com dreno na parte final inferior para a lixiviação dos sais do solo. O aumento da salinidade da água de irrigação compromete os teores foliares de clorofila a, b, total, carotenoides e a produção de biomassa de plantas de noni mas em menor intensidade nas plantas de todos os tratamentos com a lixiviação dos sais com as próprias águas utilizadas na irrigação.

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Introduction

The global scarcity of water is a problem found especially in countries with large semiarid regions, such as the Brazilian northeast, where more than 60% of its territory is characterized by low rainfall and high evapotranspiration rates, which limits the growth and development of commercially important crops (Sá et al., 2013).

Besides the limitation caused by water scarcity, the qualitative aspect of the water stored in surface water sources, like weirs, dams and lakes, as well as groundwaters, is another factor that hampers the productive capacity of crops, due to the high concentrations of soluble salts and specific ions in the water, such as sodium, chlorides and sulfates (Barroso et al., 2011).

The response of plants under conditions of high salinity involves morphological and growth changes with negative consequences for physiological and biochemical processes (Graciano et al., 2011). The results of Pak et al. (2009) indicate that the saline stress impairs the photosynthetic process because of the degradation of pigments like chlorophyll through the chlorophyllase; the degradation is related to the photoinhibition or the formation of reactive oxygen species, due to the production of peroxidases (Aghaleh et al., 2009).

Given the scarcity of good-quality water and in the volume demanded by agriculture, including the effects of salts in plants, research projects and the generation of technologies that allow the use of saline water in food production become important (Paulus et al., 2010).

The use of leaching to reclaim the soil is necessary when high salt contents in the root zone hamper crop growth and yield; thus, it is necessary to leach the soluble salts from the root zone of the plants (Ayers & Westcot, 1999; Veras et al., 2011).

Another expressive factor to be considered is the selection of crops tolerant or moderately tolerant to salinity, which include noni (*Morinda citrifolia* L.). This crop proved to adjust osmotically when subjected to the saline stress of the irrigation water and, based on this information, can be an alternative for areas affected by salinity in semiarid regions (Nivas et al., 2011; Souto et al., 2013). This crop originated in Asia and has been used in alternative medicine in many countries worldwide (Wang et al., 2002; Singh, 2012; Rentala et al., 2013).

Given the above, this study aimed to evaluate the contents of photosynthetic pigments and the biomass accumulation of noni plants subjected to irrigation with water of increasing salinity, in the soil with and without salt leaching.

Material and Methods

The experiment was carried out from July 2011 to June 2012 in a greenhouse of the Soil and Rural Engineering Department, at Center of Agricultural Sciences (CCA) of the Federal University of Paraíba (UFPB), in Areia-PB, Brazil. The soil used as a substrate was collected in the Chã do Jardim Farm, which belongs to the CCA/UFPB, from the layer of 0-20 cm, and is classified as distrophic Red Yellow Latosol, based on the criteria of the Brazilian Soil Classification System - SiBCS (EMBRAPA, 2013).

The characterization of the soil for fertility and physical attributes, according to the methodology recommended by EMBRAPA (1997), and for salinity, according to Richards (1954), is shown in Table 1.

The experiment was set in a randomized block design, with four replicates and two plants in each plot, using a 5 × 2 factorial scheme. The treatments consisted of five levels of electrical conductivity in the irrigation water (0.5; 1.5; 3.0; 4.5 and 6.0 dS m^{-1}) in substrate with and without soil leaching. Irrigation waters were prepared by diluting moderately saline water (7.35 dS m^{-1}) from a surface dam in non-saline water (0.50 dS m^{-1}).

Each treatment was represented by two plastic pots, with capacity for 20 dm^3, with one plant each. The bottom of the pots was covered with 2 cm of sand and 2 cm of crushed stone. In the treatments with soil leaching, a PVC hose (5/16" x 0.8 mm) was installed connecting the bottom of the pots to a 2-L collector, in order to store the leachate.

In the first 90 days after emergence (DAE), plants under the treatments with and without leaching were irrigated using the weighing method, which consists in supplying the volume of respective water evapotranspired in the previous day, in order to raise the water content to field capacity. From 90 DAE on, in the treatments with leaching, the soil was weekly washed using the respective irrigation water.

During the first days of each week until the end of the experiment, plants were irrigated with a certain volume of water and the daily values were recorded. On the seventh day, water was gradually applied until the drainage began. Then, a

Table 1. Chemical attributes regarding fertility and salinity and physical attributes, before installing the experiment

Fertility		Salinity		Physical attributes	
pH in water (1:2.5)	5.07	pH	5.29	Soil bulk density (kg dm^{-3})	1.35
P (mg dm^{-3})	1.78	Ca^{2+} ($mmol_c$ L^{-1})	0.30	Soil particle density (kg dm^{-3})	2.69
K (mg dm^{-3})	46.80	Mg^{2+} ($mmol_c$ L^{-1})	0.40	Total porosity (%)	49.7
Ca^{2+} ($cmol_c$ dm^{-3})	0.35	K^+ ($mmol_c$ L^{-1})	0.11	Sand (g kg^{-1})	557
Mg^{2+} ($cmol_c$ dm^{-3})	0.30	Na^+ ($mmol_c$ L^{-1})	0.25	Silt (g kg^{-1})	63
Na^+ ($cmol_c$ dm^{-3})	0.11	Cl^- ($mmol_c$ L^{-1})	1.50	Clay (g kg^{-1})	380
SB ($cmol_c$ dm^{-3})	0.88	CO_3^{2-} ($mmol_c$ L^{-1})	-	Clay disp. in water (g kg^{-1})	26
Al^{3+} ($cmol_c$ dm^{-3})	0.90	HCO_3^- ($mmol_c$ L^{-1})	1.13	Degree of flocculation (%)	93.0
$H^+ + Al^{3+}$ ($cmol_c$ dm^{-3})	5.70	SO_4^{2-} ($mmol_c$ L^{-1})	0.25	Index of dispersion (%)	7.0
CEC ($cmol_c$ dm^{-3})	6.58	ECse (dS m^{-1})	0.17	Water content $_{FC}$ (g kg^{-1})	128
V (%)	13.37	SAR (mmol L^{-1})^{0,5}	3.47	Water content $_{PWP}$ (g kg^{-1})	45
OM (g dm^{-3})	18.25	ESP (%)	1.67	Available water (g kg^{-1})	83

SB - Sum of bases; CEC - Cation exchange capacity; V - Exchangeable base saturation; OM - Organic matter in the soil; ECse - Electrical conductivity in the soil saturation extract; SAR - Sodium adsorption ratio; ESP - Exchangeable sodium percentage; Water content $_{FC}$ - Water content at field capacity, tension of -0.010 MPa; Water content $_{PWP}$ - Water content at the permanent wilting point, tension of -1.5 MPa leaching fraction equivalent to 10% was applied and the volume was recorded. After that, all the values were added in order to obtain the total volume applied each week (Ayers & Westcot, 1999; Diniz et al., 2013). For the treatments without leaching, irrigation continued to be based on the weighing method.

At the end of the experiment (330 DAE), two leaves were collected from the middle third section of the plants for the characterization of photosynthetic pigments. The leaves were immediately placed in aluminum envelopes, stored in thermally isolated recipients containing dry ice and taken to the laboratory. Then, using a circular cutter, circles of plant tissue with masses ranging from 0.1 and 0.2 g were removed from the central part. After that, the material was macerated and placed in a 50-mL recipient coated with aluminum foil, in which 25 mL of 80% acetone were added.

The recipients were kept in an environment under controlled refrigeration (8 °C) for 24 h. Later, the solutions containing acetone + plant tissue were filtered in paper for 5 min (Arnon, 1945). The readings of absorbance were obtained through spectrophotometry at the wavelengths of 470 (A470), 647 (A647) and 663 nm (A663), and 80% acetone was used for the blank reading. The readings were converted into contents of chlorophyll a, b, total chlorophyll and carotenoids (CHLa, CHLb, CHLt and CAR, respectively), using the equations described by Lichtenthaler (1987):

$$\text{Chlorophyll a} = (12.25 * \text{A663}) - (2.79 * \text{A647})$$

$$\text{Chlorophyll b} = (21.50 * \text{A647}) - (5.10 * \text{A663})$$

$$\text{Total chlorophyll} = (7.15 * \text{A663}) + (18.71 * \text{A647})$$

$$\text{Total carotenoids} = \left[(1000 * \text{A470}) - (1.82\text{Cla}) - (85.02\text{Clb})\right]/198$$

The obtained values were transformed into contents of chlorophyll a, b and carotenoids in the leaves and expressed in units of mass per mass of fresh matter (mg g^{-1} fresh matter).

In the same period, the total dry biomass was collected, divided into dry biomass of roots and shoots (stem, branches, leaves and fruits) and were dried in a forced-air oven at 65 °C, for 72 h. Then, the values of dry biomass of roots and each part of shoot were obtained.

At the end of the experiment, soil samples of each treatment were collected in all blocks for the evaluation of electrical conductivity of the saturation extract, according to the methodology proposed by Richards (1954).

The results were subjected to the analysis of variance by F test to verify the effects of water salinity levels, salt leaching and the interaction between both; means of the soil with and without leaching were compared by F test, which is conclusive for the factors with and without, and for means related to water salinity levels regression analysis were performed, using the statistical software SISVAR (Ferreira, 2011).

Results and Discussion

According to the summary of the analysis of variance (Table 2), the interaction water salinity x salt leaching, except for root dry matter, caused significant effects on the electrical conductivity of the soil saturation extract and all the other plant variables.

Regardless of the treatments, with and without leaching (Figure 1), the increment in water salinity drastically increases soil salinity, to a point where it impairs the activity of photosynthetic pigments and the biomass production of shoots and roots of plants in general, including noni (Nivas et al., 2011; Souto et al., 2013).

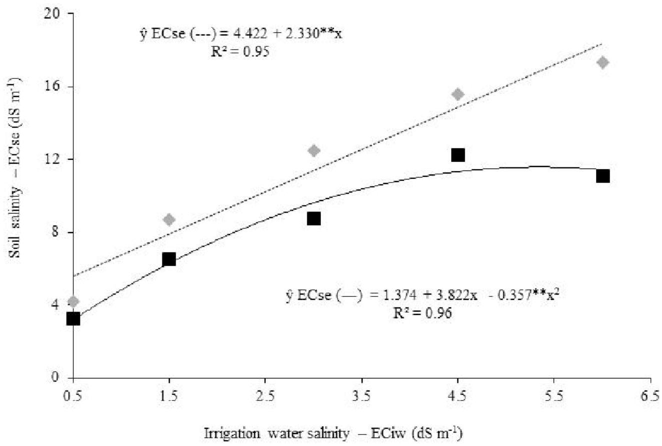

Figure 1. Electrical conductivity of the saturation extract (ECse) of the soil irrigated with water of increasing salinity (ECiw), with (—) and without (- - -) leaching

Table 2. Summary of the analysis of variance for the effects of irrigation water salinity (W) and leaching (L) on the electrical conductivity of the soil saturation extract (ECse), chlorophyll contents (CHL*a*, CHL*b* and total CHL_T), chlorophyll a/b ratio (CHLa/CHLb), carotenoids (CAR), shoot dry matter (SDM) and root dry matter (RDM) of noni (*Morinda citrifolia* L.)

SV	DF	Mean Square							
		ECse	CHLa	CHLb	CHL_T	CHLa/CHLb	CAR	SDM	RDM
Blocks	3	0.10^{ns}	0.54^{ns}	0.29^{ns}	1.47^{ns}	0.11^{ns}	11879.77^{ns}	166.65^{ns}	98.08^{ns}
Water (W)	4	162.28**	186.6**	33.47**	378.18**	4.25**	691025.27**	41602.82**	5193.8**
Leaching (L)	1	108.90**	26.50**	4.32**	52.21**	1.12**	101651.76**	5970. 93**	2899.8**
W X L	4	7.83**	20.07**	3.20**	38.95**	0.47**	47385.41**	1810.22**	342.19^{ns}
Linear (W)	1	610.51**	637.88**	110.61**	1280.32**	10.38**	2456651.00**	132356.45**	20352.20**
Quadr. (W)	1	31.08**	33.31**	9.43**	78.28**	4.23**	54513.34*	505.75^{ns}	51.57^{ns}
Linear (WxL_1)	1	442.22**	464.37**	84.41**	944.97**	9.40**	1841675.70**	71318.02**	-
Quadr. (WxL_1)	1	13.01^{ns}	1.34^{ns}	2.00^{ns}	6.61^{ns}	3.25**	16493.84^{ns}	780.01^{ns}	-
Linear (WxL_2)	1	193.60**	200.74**	32.32**	394.50**	2.22**	738763.27**	61230.62**	-
Quadr. (WxL_2)	1	18.28**	49.05**	8.58**	98.84**	1.22**	40408. 52**	3568.01**	-
Residue	27	0.32	2.52	0.49	51.17	0.18	8486.16	301.16	238.25
CV (%)		6.76	18.73	18.34	18.46	23.61	16.84	22.74	42.92

Although the soil without leaching has higher saline level compared to the soil with leaching when irrigated with non-saline water (0.5 dS m^{-1}), the increment of salts in the irrigation water significantly increased the saline condition of the soil in the treatments with leaching. This situation, as also observed by Diniz et al. (2013), evidences that, despite the positive effect of soil leaching, this practice does not avoid the increment of salts in the soils through the irrigation with water of increasing salinity.

In the treatments without salt leaching, the electrical conductivity of the soil increased linearly by 2.33 dS m^{-1} per unit increase of water salinity, from 5.58 to up to 18.40 dS m^{-1}. In the treatments with salt leaching, soil electrical conductivity increased as a function of the water salinity up to the maximum value of 11.57 dS m^{-1}, corresponding to the maximum estimated value of ECiw, 5.33 dS m^{-1}.

Although high, the values of soil electrical conductivity in the treatments with leaching are significantly lower for any irrigation water used and express the positive effects of the drainage on the leaching of salts from the root zone. The results, in both situations, agree with those obtained by Blanco & Folegatti (2001) and Nunes et al. (2012), who observed that leaching reduces salt content in the soil, while the increment in water salinity increases the saline character of the soils.

The increase in electrical conductivity of the irrigation water inhibited the production of chlorophyll a, chlorophyll b and total chlorophyll, with higher intensity in the soil with salt leaching, in plants irrigated with water of salinity higher than 4.0 dS m^{-1} (Figure 2A, B, C). Similar behavior was reported by Graciano et al. (2011) and Freire et al. (2014), who observed that the increase in water salinity promoted a reduction in the activity of the photosynthetic process of peanut (*Arachis hypogaea*) and yellow passion fruit (*Passiflora edulis*).

In the treatments without leaching, the increase in water salinity linearly inhibited the contents of chlorophyll a, b and total chlorophyll of noni plants by 2.431, 1.042 and 3.474 mg g^{-1} of fresh matter with per unit increase of electrical conductivity. These reductions resulted in decreases from 13.98 to 0.61 mg g^{-1} (Figure 2A), 6.21 to 0.48 mg g^{-1} (Figure 2B) and 20.21 to 1.09 mg g^{-1} (Figure 2C), with losses of 95.6, 92.3 and 94.6% in the contents of chlorophyll a, b and total chlorophyll between plants irrigated with water of 0.5 and 6.0 dS m^{-1}, respectively.

In plants of the treatments with salt leaching, the increase in water salinity from 0.5 to 1.72, 1.78 and 1.74 dS m^{-1} increased the contents of photosynthetic pigments until the maximum estimated values of 12.38 mg g^{-1} for chlorophyll a (Figure 2A), 5.42 mg g^{-1} for chlorophyll b (Figure 2B) and 17.80 mg g^{-1} for total chlorophyll (Figure 2C). The irrigation using water of salinity levels above these values caused the degradation of leaf pigments in noni plants.

Considering the importance of chlorophyll in the first step of photosynthesis, characterized by the photochemical stage, the high content of salts absorbed by the plants contributed to the increase in the activity of chlorophyllase, an enzyme that degrades chlorophyll (Lima et al., 2004), hampering the formation of these pigments and inhibiting the photosynthetic activity of the plants in general (Munns & Tester, 2008, Taiz & Zeiger, 2013). Comparatively, the effects are similar to those reported by Graciano et al. (2011) in peanut plants and by Cavalcante et al. (2011) in yellow passion fruit, who observed that irrigation with saline water causes a reduction in the chlorophyll activity of these variables in the respective crops.

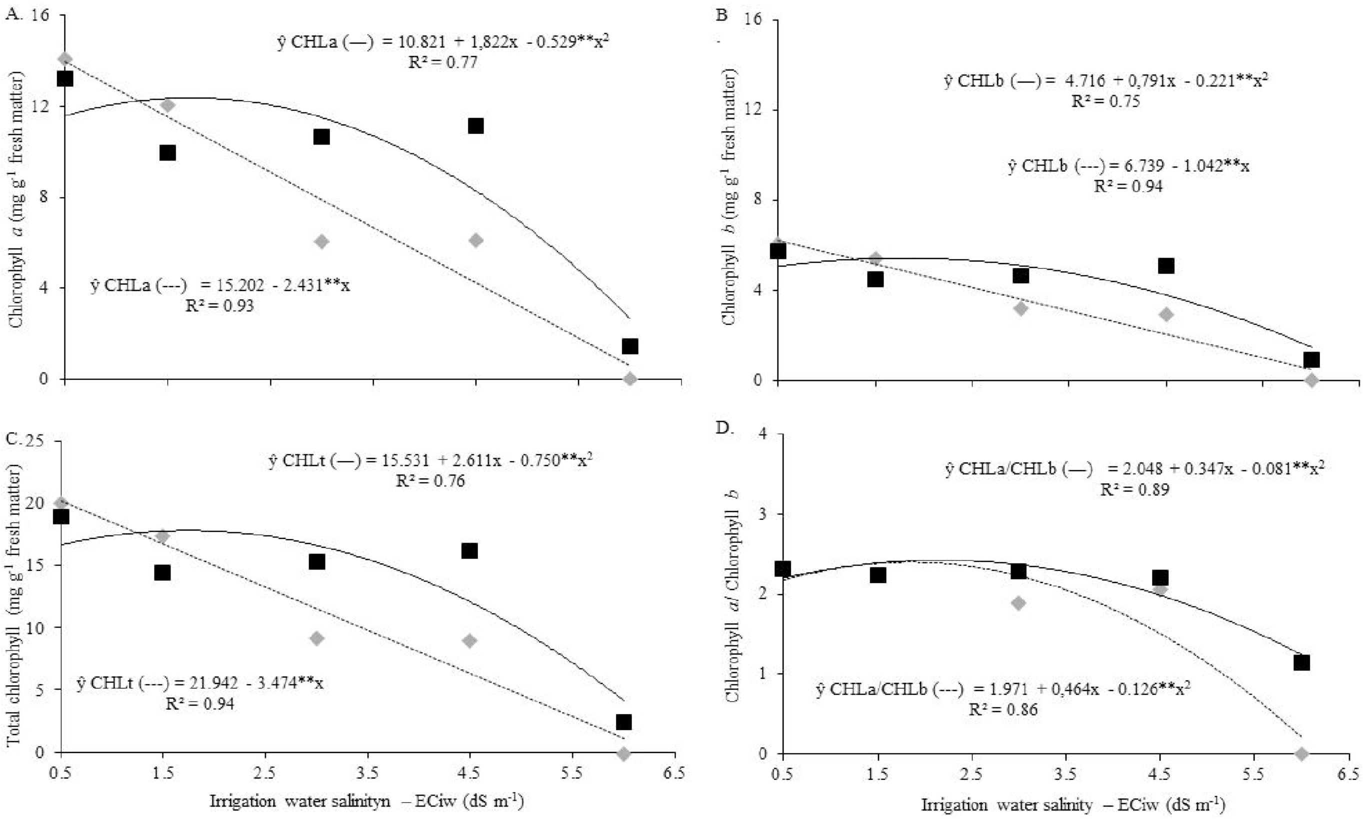

Figure 2. Leaf contents of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and the chlorophyll a/b ratio (D) of noni (*Morinda citrifolia* L.) irrigated with water of increasing salinity levels in soil with (—) and without (- - -) leaching

The values of the chlorophyll a/b ratio (Figure 2D) increased in the treatments without leaching until the ECiw of 1.84 dS m^{-1}, showing a value of 2.39. From this point on, a reduction in the chlorophyll a/b ratio was observed with the increase in the salt concentration of the irrigation water, with the lowest value of 0.22 for the ECiw of 6.0 dS m^{-1}. The treatments with and without leaching showed similar tendency; however, the former showed higher values of the ratio in waters of higher salt contents. Under salt leaching conditions, the chlorophyll a/b ratio was high, reaching a maximum value of 2.41 for water of estimated electrical conductivity of 2.14 dS m^{-1}. From this point on, the values of the chlorophyll a/b ratio decreased with the increase in the electrical conductivity of the irrigation water, but it reached a minimum value of 1.21 in the water of the highest salt content (6.0 dS m^{-1}).

In plants irrigated with less saline water, the photosynthetic activity is less impaired, compared with those irrigated with water of high salt contents. This leads to higher contents of chlorophyll a (Figure 2A) in relation to chlorophyll b (Figure 2B) and, consequently, higher values of the chlorophyll a/b ratio (Figure 2D). The superiority of the coefficients, according to Lima et al. (2004), is due to the higher contents of chlorophyll a in the leaves and to the increase of the efficiency of photosynthetic reactions. This pigment actively participates in the first photochemical process of the photosynthesis, while chlorophyll b is an accessory pigment that has as its main function the absorption of light in the wavelength spectrum not absorbable by chlorophyll a (Taiz & Zeiger, 2013).

The contents of carotenoids in noni leaves (Figure 3) showed a behavior similar to that of chlorophyll, with superiority in plants of the treatments with leaching. In the soil without salt leaching, the contents of carotenoids decreased linearly from 976.90 to 59.49 mg g^{-1}, indicating a loss of 98.9% between plants irrigated with waters of 0.5 and 6.0 dS m^{-1} and corresponding to a reduction of 152.9 mg g^{-1} with per unit increase of the salinity in the irrigation water.

For noni plants in the soil with leaching, the increase in water salinity from 0.5 to up to 6.0 dS m^{-1} (Figure 3) also inhibited the production of carotenoids in the leaves, but always in lower proportions for plants in the soil without drainage. Increases of 30.3, 75.50 and 348.9% can be observed between plants in soil with and without leaching by comparing the estimated values of 675.22, 506.88 and 267.08 mg g^{-1} with 518.20, 288.85 and 59.50 mg g^{-1}, for the ECiw values of 3.0, 4.5, and 6.0 dS m^{-1}, respectively.

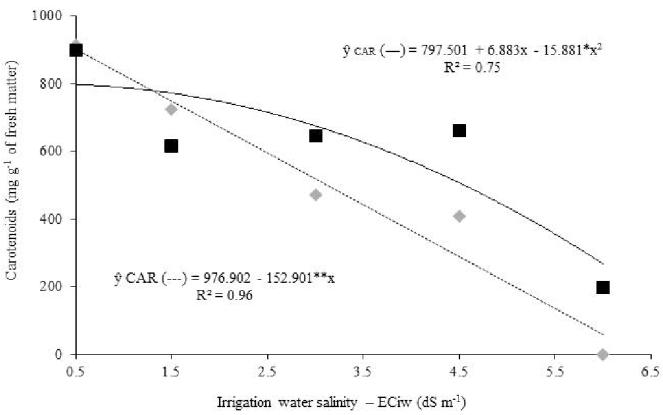

Figure 3. Contents of carotenoids in noni (*Morinda citrifolia* L.) irrigated with water of increasing salinity, in soil with (—) and without (---) leaching

Comparatively, the results do not agree with those reported by Cavalcante et al. (2011), who concluded that irrigation water salinity did not have significant effects on the contents of carotenoids. For Munns & Tester (2008), the reduction in the contents of carotenoids in noni leaves occurs because high salinity causes degradation of ß-carotene, besides promoting the reduction in the synthesis of zeaxanthin, which are structural pigments of carotenoids involved in the protection of chlorophyll.

The increase in water salinity inhibited the production of shoot dry biomass (Figure 4A), but with lower intensity in plants under treatments with salt leaching. Plants in the treatments without leaching did not survive to irrigation with water 5.15 dS m^{-1} or higher, as also observed for the contents of chlorophyll a, b, total chlorophyll and carotenoids, as shown in Figure 2 and 3, respectively.

In the treatments without leaching, the value of shoot dry matter decreased by 30.401 g with per unit increase in the electrical conductivity of the irrigation water. In the non-saline water (0.5 dS m^{-1}), the estimated value was 158.302 g, which was reduced to nil in the water of 5.20 dS m^{-1} on (Figure 4A).

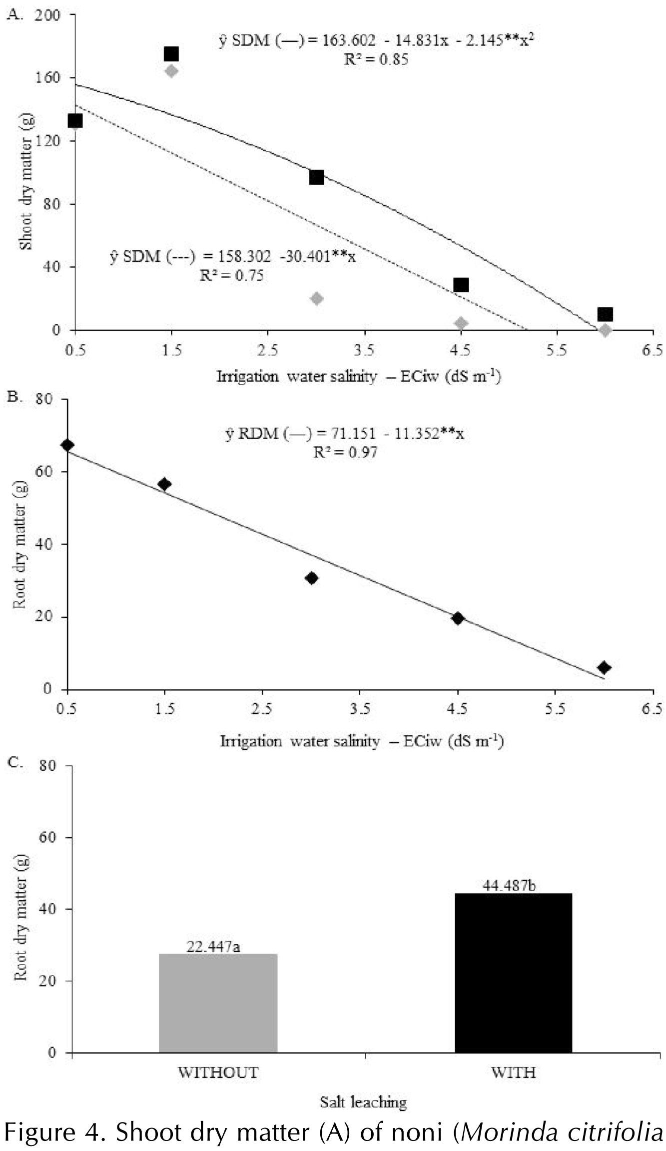

Figure 4. Shoot dry matter (A) of noni (*Morinda citrifolia* L.) plants irrigated with water of increasing salinity, in soil with (—) and without (- - -) leaching, root dry matter as a function of water salinity (B) and salt leaching (C)

On the other hand, soil leaching promoted higher biomass accumulation compared with soil without leaching. For the root biomass of noni plants, there was no significant effect of the interaction between water salinity and leaching, but it responded to the isolated effects of both (Figure 4B and 4C). Considering that this interaction had significant effects on all the other variables studied (Table 2), for which the coefficients of variation were substantially lower, its high value of 42.92% must have masked the effects of this interaction on the production of root biomass in noni plants.

The increase in water salinity caused a linear decrease of 11.352 g per unit increase of electrical conductivity in the irrigation water in root dry matter, resulting in a reduction from 71.151 to 3.04 g plant^{-1}, which corresponded to a loss of 95.7% between plants irrigated with waters of 0.5 and 6.0 dS m^{-1} (Figure 4B). However, the practice of leaching salts from the soil stimulated the accumulation of root dry matter from 22.447 to 44.487 g, with a percent gain of 98.18% (Figure 4C).

Considering that the saline stress reduced the production of pigments that are essential to the photosynthetic process of the plants (Taiz & Zeiger, 2013), the high contents of salts incorporated to the soil through the irrigation resulted in lower growth and development of noni plants, evaluated through plant dry matter (Figure 4). Freire et al. (2010), studying the effects of irrigation water salinity on neem plants, observed that plant growth was also hampered with the increase in the electrical conductivity of the irrigation water. Gabriel et al. (2012) observed that the leaching of salts from the root zone allowed a higher gain of biomass in maize plants, which was also observed in noni plants under saline conditions in the presence of salt leaching through the drainage.

Conclusions

1. The saline character increases as a function of the electrical conductivity of the irrigation water, but in lower proportion in the treatments with soil salt leaching.
2. The increase in irrigation water salinity impairs the leaf contents of chlorophyll a, b, total chlorophyll, carotenoids and the production of biomass of noni plants, but to a lesser extent in all the treatments in which the irrigation water was used for leaching.

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