

# Ion accumulation in young plants of the ‘green dwarf’ coconut under water and salt stress<sup>1</sup>

## Acúmulo de íons em plantas jovens de coqueiro “anão verde” sob estresses hídrico e salino

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**ABSTRACT** - Water scarcity and salinity are two major limitations on agricultural production. The aim of this study was to investigate the biochemical mechanisms associated with the separate and/or concurrent effects of water deficit and soil salinity on young plants of the ‘green dwarf’ coconut from the responses of ion accumulation (potassium, sodium and chloride) in the leaves and roots. The experiment was carried out in a screened greenhouse in Fortaleza, in the State of Ceará, Brazil. A randomised complete block statistical design was used in a scheme of treatments in subdivided plots, to evaluate the effects of different levels of water deficiency (plots) using different percentages for the replacement of water lost through potential crop evapotranspiration (20, 40, 60, 80 and 100%), associated with sub-plots comprising increasing levels of soil salinity (1.72, 6.25, 25.80 and 40.70 dS m<sup>-1</sup>) found in soils collected in the Morada Nova Irrigated Perimeter (Morada Nova/Limoeiro do Norte, Ceará). Under conditions of water scarcity, K<sup>+</sup> is maintained and/or accumulated in the leaves; while in the roots, levels are jointly affected by water deficiency and soil salinity, where these stress agents act in an antagonistic way on the K<sup>+</sup> content. Conditions of salt stress lead to increases in the levels of Na<sup>+</sup> both in the leaves and especially in the roots. The Cl<sup>-</sup> ion content of the leaves and roots responds to the interaction between water deficiency and salinity, demonstrating synergy in the relationship between stress agents. The accumulation of inorganic solutes in the leaves and roots of the coconut follows the order: Cl<sup>-</sup> > K<sup>+</sup> > Na<sup>+</sup>.

**Key words:** *Cocos nucifera* L.. Multiple stress. Inorganic solutes.

**RESUMO** - A escassez de água e a salinidade são duas limitações importantes para a produção agrícola. Objetivou-se, com este trabalho, investigar os mecanismos bioquímicos associados às ações isoladas e/ou simultâneas do déficit hídrico e da salinidade do solo, em plantas jovens de coqueiro “anão verde”, através das respostas dos acúmulos de íons (potássio, sódio e cloreto), em folhas e em raízes. No experimento, conduzido em casa de vegetação, do tipo telado, em Fortaleza, Ceará, avaliaram-se, sob delineamento estatístico de blocos completos casualizados, no delineamento de tratamentos de parcelas subdivididas, os efeitos de diferentes níveis de deficiência hídrica (parcelas), mediante a imposição de distintos percentuais de reposições das perdas de água por evapotranspiração potencial da cultura (20; 40; 60; 80 e 100%), associados às subparcelas, constituídas pelos crescentes níveis de salinidade do solo (1,72; 6,25; 25,80 e 40,70 dS m<sup>-1</sup>), proporcionados pelos solos coletados no Perímetro Irrigado Morada Nova (Morada Nova/ Limoeiro do Norte, Ceará). Sob condições de insuficiência de água, ocorre manutenção e/ou acúmulo de K<sup>+</sup> nas folhas, ao passo que, nas raízes, os teores são mutuamente afetados pela deficiência hídrica e pela salinidade do solo, onde esses agentes estressores atuam de forma antagonica, sob os teores de K<sup>+</sup>. As condições de estresse salino provocam aumentos nos teores de Na<sup>+</sup>, tanto nas folhas como nas raízes, especialmente, nas raízes. Os teores foliares e radiculares do íon Cl<sup>-</sup> respondem à interação entre a deficiência hídrica e a salinidade, denotando-se a existência de sinergismo na associação entre os agentes estressores. O acúmulo de solutos inorgânicos foliares e radiculares, nas plantas de coqueiro, obedece à ordem: Cl<sup>-</sup> > K<sup>+</sup> > Na<sup>+</sup>.

**Palavras-chave:** *Cocos nucifera* L.. Estresses múltiplos. Solutos inorgânicos.

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## INTRODUCTION

Environmental stress, both biotic and abiotic, is a serious threat to the sustainability of global agriculture. In arid and semi-arid regions, drought and salinity can be considered as stress agents capable of causing the most severe damage to plants, as they result in large reductions in the cultivated area, in addition to compromising both crop productivity and quality (NAKABAYASHI; SAITO, 2015).

According to Ramegowda and Senthil-Kumarb (2015), the majority of species have developed complex adaptive mechanisms to dilute or counterbalance the effects of water and salt stress, through adjustments that may contribute to better plant adaptation to the environments in which such conditions predominate, the most common being osmotic adjustment, which is usually accompanied by the absorption of inorganic ions, where the plants adapt to the prevailing conditions, to maintain the water potential gradient and cell turgescence close to suitable levels (PEREIRA *et al.*, 2012).

The accumulation of inorganic solutes (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>) has been demonstrated under saline conditions (SILVA *et al.*, 2009a). The following are important as ionic adjustments: extrusion and/or retention of ions by the roots, which avoids toxic levels being reached in the photosynthetic apparatus concentrated in the shoots; compartmentalisation of ions in the vacuoles; and regulation of ion concentrations by increases in the succulence of the plant tissue (MIRANDA *et al.*, 2013).

The use of salt-tolerant species has been a recommended strategy in agricultural crops to promote reintegration of soils degraded by excess salts and sodium. It is believed that the coconut, because of its social and economic relevance, and above all because of its known tolerance to salinity, considering that its natural dispersion is mainly along the coast where there is naturally a high salt concentration in the soil, may be a promising crop for the revegetation of salt-affected areas in the north-east of Brazil (FERREIRA NETO *et al.*, 2007; LIMA, 2014; MARINHO *et al.*, 2005).

For the appropriate selection of plant species capable of thriving in salt-affected soils in the north-east of Brazil, it is also necessary to evaluate the capacity of the crop for resistance to other environmental conditions, especially those of climate, which are particular to such regions, since in addition, there is a regular water deficit due to the prevailing rainfall regime, characterised by the temporal and spatial insufficiency and irregularity of the natural precipitation (PEREIRA *et al.*, 2012).

There are few reports in the literature that seek to demonstrate the joint effect of salinity and water stress

due to water deficit on the accumulation of inorganic solutes, which are compounds that exert a relevant function on plant growth under adverse conditions. This is particularly true for the coconut, where no studies have been carried out in this area. In this respect, the aim was to investigate the biochemical mechanisms associated with the separate and/or concurrent effects of water deficit and soil salinity on young plants of the 'green dwarf' coconut, evaluating ion accumulation (potassium, sodium and chloride) in the leaves and roots.

## MATERIAL AND METHODS

The experiment was carried out in a screened greenhouse, between October 2013 and February 2014, in the experimental area of the Federal University of Ceará, in the city of Fortaleza, located at 03°45' S and 38°33' W, at an altitude of approximately 19 m.

The experiment was conducted in a randomised complete block statistical design in a scheme of treatments in subdivided plots, comprising different levels of water deficiency, using five different percentages for the replacement of water lost through potential crop evapotranspiration - ET<sub>pc</sub> (20, 40, 60, 80 and 100% ET<sub>pc</sub>), and sub-plots comprising increasing levels of soil salinity (1.72, 6.25, 25.80 and 40.70 dS m<sup>-1</sup>). Four replications were used, with one plant per pot, giving a total of 80 experimental units.

The treatments allocated to the sub-plots were due to the soils, classified as Fluvic Neosols (EMBRAPA, 2013), collected at different places in plots representative of the Morada Nova Irrigated Perimeter, in the State of Ceará, Brazil. Soil samples from the 0-20 m layer were used, which were air dried and sieved through a 4 mm screen. When filling the pots, compound soil samples were removed for characterisation of the chemical attributes of the soil (Table 1).

Forty days after germination, seedlings of the 'Jiqui do Brazil green dwarf' coconut palm were transplanted to pots of flexible plastic material with a capacity of 25 L.

A drip irrigation system was adopted, using self-compensating emitters with a nominal flow rate of 4 L h<sup>-1</sup>. Irrigation management was based on the potential crop evapotranspiration - ET<sub>pc</sub>, as per the different treatments for water deficit.

Leaves and roots were collected 121 days after transplanting, lyophilised, macerated in a porcelain mortar in the presence of liquid nitrogen, and stored for later potassium, sodium and chloride analysis (MALAVOLTA, 1997).

**Table 1** - Chemical attributes of the soils used in the experiment as substrates for growing young plants of the 'green dwarf' coconut<sup>1</sup>

Salinity	pH (Water) (-)	EC (dS m <sup>-1</sup> )	Sorptions complex					Al <sup>3+</sup>
			Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Na <sup>+</sup>	H <sup>+</sup> + Al <sup>3+</sup>	
(----- cmol <sub>c</sub> kg <sup>-1</sup> -----)								
S1	6.8	1.72	6.58	4.34	0.06	4.32	2.56	0.00
S2	7.5	6.25	7.80	5.24	0.08	7.23	0.00	0.00
S3	7.4	25.80	7.51	5.69	0.05	15.78	0.00	0.00
S4	7.0	40.70	14.91	4.58	0.07	22.46	0.00	0.00

Salinity	S	CEC	V	PES	C	OM	PAssimilable	Classification
	(----- cmol <sub>c</sub> kg <sup>-1</sup> -----)	(----- % -----)	(----- g kg <sup>-1</sup> -----)	(--- mg kg <sup>-1</sup> ---)				
S1	15.30	17.86	86	24	12.26	21.13	30	Sodic
S2	20.35	20.35	100	36	16.64	28.66	30	Saline - sodic
S3	29.03	29.03	100	54	9.22	15.89	69	Saline - sodic
S4	42.02	42.02	100	53	20.23	34.88	82	Saline - sodic

<sup>1</sup>pH - potential of hydrogen; EC - soil electrical conductivity of the saturation extract; S - sum of bases; CEC - cation exchange capacity; V - base saturation; PES - percentage exchangeable sodium; C - organic carbon; OM - organic matter; P<sub>Assimilable</sub> - Assimilable phosphorus

The Kolmogorov-Smirnov test at 5% probability was applied to verify data normality, and the F-test applied for the analysis of variance. When a significant effect was indicated, the data were analysed by simple and/or multiple linear regression, according to the statistically significant interactions between the factors under study. The mathematical models were chosen based on the significance of the regression coefficients (Fisher-Snedecor F-test at 5% probability), on the coefficient of determination, and on the biological phenomenon under study. Statistical analyses were carried out using the Microsoft Excel 2007, ASSISTAT 7.6 beta, and STATISTICA 7.0 software.

## RESULTS AND DISCUSSION

From the Kolmogorov-Smirnov test (not shown), the analysed variables presented a normal distribution, which according to Koch and Link (1971) can be verified by the values for the coefficient of variation (CV) being close to 30% (Table 2), and which, according to the, Pimentel-Gomes and Garcia (2002) classification, further shows that in general there was high level of test precision.

The results of ANOVA presented in Table 2 show that the different levels of water deficiency under evaluation (D) significantly affected the potassium ion - K<sup>+</sup><sub>L</sub> and chloride ion - Cl<sup>-</sup><sub>L</sub> content of the leaves (p≤0.01), and the chloride ion - Cl<sup>-</sup><sub>R</sub> content of the roots (p≤0.05). The factor of soil salinity (S) promoted a statistical significance in the

sodium ion - Na<sup>+</sup><sub>L</sub> content of the leaves (p≤0.05), and the potassium ion - K<sup>+</sup><sub>R</sub> (p≤0.05), sodium ion - Na<sup>+</sup><sub>R</sub> (p≤0.01) and Cl<sup>-</sup><sub>R</sub> chloride ion (p≤0.05) content of the roots. On the other hand, the factors of water deficiency (D) and soil salinity (S) acted together, causing changes in the chloride ion - Cl<sup>-</sup><sub>L</sub> content of the leaves (p≤0.05) and potassium ion - K<sup>+</sup><sub>R</sub> (p≤0.05) and chloride ion - Cl<sup>-</sup><sub>R</sub> content of the roots (p≤0.01).

The water deficit increased the concentration of K<sup>+</sup> in the leaves of the coconut, irrespective of the level of soil salinity (Figure 1a), so that for each unit increase in % ETpc, the linear model that best fit the data (R<sup>2</sup> = 0.8967, F-test: p≤0.05), gave an estimated decrease of approximately 0.06 g K<sup>+</sup> kg<sup>-1</sup> DM (0.32%).

From the results, it was estimated that the maximum value, corresponding to 16.48 g K<sup>+</sup> kg<sup>-1</sup> DM, resulted from the water regime of 20% ETpc, while the lowest value, 12.00 g K<sup>+</sup> kg<sup>-1</sup> DM, lower by approximately 27% compared to the maximum, was obtained under the control conditions of water supply (100% ETpc) (Figure 1a). It can however be seen that irrespective of the water supply, the potassium content of the leaves exceeded the limit of 8 g K<sup>+</sup> kg<sup>-1</sup> DM recommended by Silva *et al.* (2009b) as the critical level of K<sup>+</sup> for the species.

These results differ from those obtained by Valicheskii *et al.* (2011), which indicated no statistically significant effect on the K<sup>+</sup> content of the leaves for water availability.

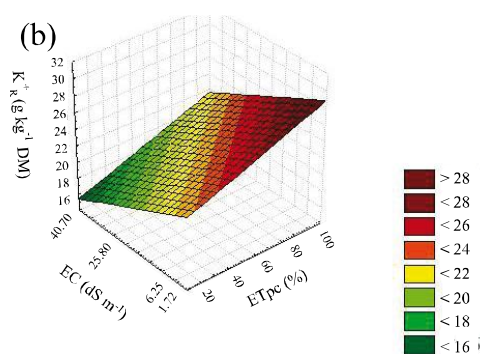
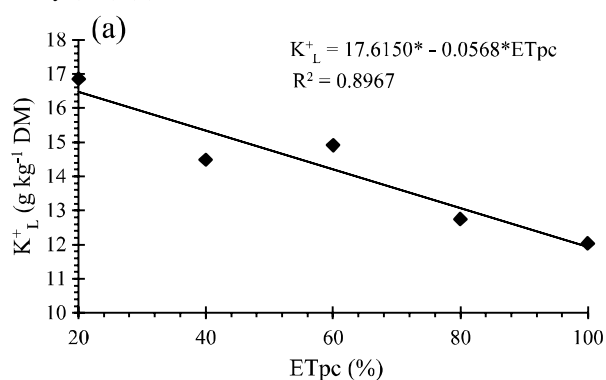
The greater K<sup>+</sup> content of the leaves at the lowest levels of water deficiency can be attributed to

**Table 2** - Summary of the analysis of variance for the levels of the inorganic solutes potassium ( $K^+$ ), sodium ( $Na^+$ ) and chloride ( $Cl^-$ ) in the leaves ( $K^+_L, Na^+_L$  and  $Cl^-_L$ ) and roots ( $K^+_R, Na^+_R$  and  $Cl^-_R$ ) of young plants of the 'green dwarf' coconut grown under different levels of water deficiency in salt-affected soils<sup>1</sup>

SV	DF	Square root					
		$K^+_L$	$K^+_R$	$Na^+_L$	$Na^+_R$	$Cl^-_L$	$Cl^-_R$
Blocks	3	2.74 <sup>ns</sup>	7.41 <sup>ns</sup>	55.84*	8.54 <sup>ns</sup>	121.28*	8.29 <sup>ns</sup>
Water deficit (D)	4	57.56**	19.42 <sup>ns</sup>	5.25 <sup>ns</sup>	8.90 <sup>ns</sup>	240.41**	262.12*
Residual - D	12	5.45	37.98	11.58	11.50	28.02	64.55
Plots	19						
Soil salinity (S)	3	0.89 <sup>ns</sup>	200.53*	26.47*	75.11**	87.09 <sup>ns</sup>	92.70*
Interaction S x D	12	8.51 <sup>ns</sup>	16.10*	11.72 <sup>ns</sup>	10.64 <sup>ns</sup>	90.53*	148.33**
Residual - S	45	6.56	60.13	8.20	5.73	43.12	30.71
Total	79						
CV - D (%)		16.43	27.06	24.15	23.52	25.13	28.65
CV - S (%)		18.04	24.05	27.16	16.61	31.18	19.76

<sup>1</sup>SV: sources of variation, DF: degrees of freedom, CV: coefficients of variation, \*: significant at 5% probability, \*\*: significant at 1% probability, <sup>ns</sup>: not significant by F-test

**Figure 1** -  $K^+$  ion content in leaves of young coconut plants for different levels of water deficit (% ETpc) (a). Response surface for the  $K^+$  ion content in roots of young coconut plants, for combinations of different levels of water deficit (% ETpc) and soil salinity (EC) (b)<sup>1</sup>



$$K^+_R = 21.5603* + 0.0706*ETpc - 0.1326*EC - 0.0005*ETpc*EC$$

$$R^2 = 0.8711$$

<sup>1</sup>(\*), significant at 5% probability by the Fisher-Snedecor F-test

the effects of concentration, an hypothesis based on the fact that water shortage resulted in less growth and higher concentrations of this ion (BALIZA *et al.*, 2010).

This greater accumulation of  $K^+$  in the leaves under conditions of water stress can also be explained considering that under such conditions the demand of the plant for  $K^+$  is increased to maintain photosynthesis and protect the chloroplasts (CATUCHI *et al.*, 2012).

Furthermore, the accumulation of  $K^+$  in the leaves can be generally explained by the relevant performance of

this ion in osmotic regulation; especially under conditions of water restriction, where it ensures the turgescence of guard cells by a reduction in osmotic potential (MENDES *et al.*, 2013).

According to Silva *et al.* (2010), an increase in the concentration of  $K^+$  ions in response to water deficit is not usually found in plant tissue. However, for those authors, the importance of  $K^+$  in the osmotic adjustment of plants under water stress is widely known, due to the great solubility of  $K^+$  together with its potential for playing a key role in osmoregulation.

The fact that the  $K^+$  content of the leaves was not statistically influenced by the isolated and/or joint effects of soil salinity can be explained by information given by Silva *et al.* (2009a), who stated that reductions in  $K^+$  content caused by salinity are far more intense in the roots than the leaves. For those authors, such reductions may be related to the direct exposure of the roots to salts.

The  $K^+$  content of the roots varied from 18.00 to 29.80  $g\ kg^{-1}\ DM$ , the lowest values being obtained at 20% ETpc together with an EC of 40.70  $dS\ m^{-1}$ , while the highest values resulted from the use of 100% ETpc with an EC of 1.72  $dS\ m^{-1}$  (Figure 1b).

The use of higher levels of ETpc in soils characterised with a lower salt content resulted in increases in the levels of  $K^+$  in the roots, which possibly indicates an antagonistic effect between the factors under study, given that further water increased, and salt stress reduced, the concentration of  $K^+$  in the roots (Figure 1b). This can be explained by taking into account the osmotic effect caused by the high concentration of soluble salts in the soil, which compromises water relations (LIMA *et al.*, 2014).

According to the proposed mathematical model, unit increases in ETpc resulted in increases of 0.0706  $K^+\ kg^{-1}\ DM$  (0.33%) in the  $K^+$  content of the roots, while increases in EC resulted in reductions of 0.1326  $g\ K^+\ kg^{-1}\ DM$  (0.61%); whereas the association between the above factors also reduced root concentrations of this ion, with the parameter corresponding to this interaction participating with small reductions of 0.0005  $g\ K^+\ kg^{-1}\ DM$ . It can therefore be seen that soil salinity proved more limiting compared to the water deficit, in relation to the potassium content of the root tissue. This is consistent with Silveira *et al.* (2009), who reported that salt stress often causes a reduction in the  $K^+$  ion content of plant tissue, especially of the roots, as a direct consequence

of the competition of  $K^+$  with  $Na^+$  at the level of the root plasmalemma.

According to Silva *et al.* (2009a), a reduction in the accumulation of  $K^+$  in the roots in response to salinity, may be related to the roots having direct contact with the salts, which causes changes in the integrity and selective permeability of the plasma membrane.

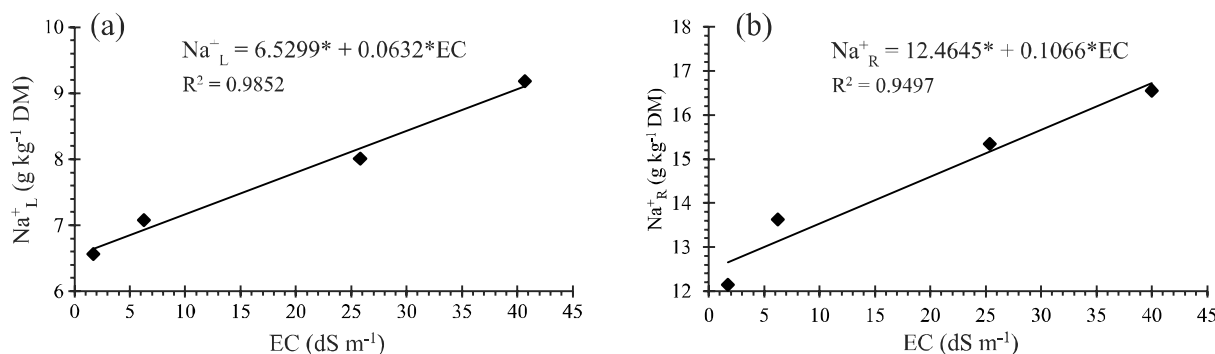
These results are similar to those of Lima (2014), who showed that concentrations of  $K^+$  in the roots of this species reduced linearly with salinity. Comparing the different parts of the plants, the highest potassium content was generally found in the roots (22.78  $g\ kg^{-1}\ DM$ ), around 1.6 times the content seen in the leaves (14.20  $g\ kg^{-1}\ DM$ ) irrespective of the level of water deficiency and/or salinity. This suggests that the species does not have a mechanism for the distribution of  $K^+$  to the leaves to the detriment of the root cells, which is considered an important plant attribute for tolerating salinity (ARAÚJO *et al.*, 2014). It is possible that the accumulation of  $K^+$  in the root system compared to the leaves is a defence strategy adopted by the plant against the excess of  $Na^+$  in the soil solution (INOCENCIO; CARVALHO; FURTINI NETO, 2014).

According to Feijão *et al.* (2013), maintaining high levels of  $K^+$  in plant tissue is considered a common protective mechanism, adopted against the toxic effects of salts, with a view to suitable cellular ion homeostasis in the cytosol.

The salinity of the soil resulted in an increase in the  $Na^+$  content of the leaves, with values varying from 6.64 to 9.10  $g\ kg^{-1}\ DM$  for soils with an electrical conductivity of 1.72 to 40.70  $dS\ m^{-1}$  respectively (Figure 2a).

It was found from the regression analysis that the significant effect from the increase in the levels of soil salinity resulted in a linear increase of 0.0632  $g\ Na^+\ kg^{-1}$

**Figure 2** -  $Na^+$  ion content in the leaves (a) and roots (b) of young coconut plants for different levels of soil salinity (EC) at 121 days after transplanting<sup>1</sup>



<sup>1</sup>(\*), significant at 5% probability by the Fisher-Snedecor F-test

DM (0.97%) in the leaf tissue of the coconut for each unit increase in soil EC (Figure 2a).

Increases in the leaf content of the Na<sup>+</sup> ion under saline conditions were also found by Silva *et al.* (2009a), and can be explained by the increasing availability of this element in the soil solution along the salinity gradient being evaluated (Table 1).

In the present work, although the salinity gradient of the evaluated soil resulted in increments in the Na<sup>+</sup> content of the leaves, it should be noted that it did not cause visual symptoms characteristic of Na<sup>+</sup> phytotoxicity, such as wilting and/or yellowing of the leaves (INOCENCIO; CARVALHO; FURTINI NETO, 2014). It is therefore suggested that in the coconut the effect of salinity may be more damaging due to the physical effect (e.g. a reduction in the amount of water available to the plants), to the detriment of the direct toxic effect of Na<sup>+</sup>, which gives the species a wide tolerance to excess salts, especially to sodium (CRUZ *et al.*, 2006). This is consistent with Ferreira Neto *et al.* (2007), who report that the coconut can ensure an appropriate cationic balance, even when subjected to high salinity.

On the other hand, it is assumed that the coconut used the abundant Na<sup>+</sup> ion as a relatively “cheap” osmotic from the point of view of energy, for guaranteeing turgescence. It is also possible that the excess Na<sup>+</sup> was stored in the vacuoles of mesophilic cells to ensure low concentrations in the cytosol, demonstrating the probable and relevant contribution of this solute to osmotic adjustment (MIRANDA *et al.*, 2013).

Values for the Na<sup>+</sup> ion content of the roots were between 12.65 and 16.80 g kg<sup>-1</sup> DM, such that the lowest value was obtained in the soil with the lowest salinity (1.72 dS m<sup>-1</sup>), while the maximum, approximately 33% higher in comparison, was found at a salinity corresponding to 40.70 dS m<sup>-1</sup> (Figure 2b).

Through regression analysis, it was found that in a similar way to that seen in the leaf tissue, increases in the levels of soil salinity resulted in linear increases in the Na<sup>+</sup> content of the roots, of such magnitude that the mathematical model which best fit the data estimated a mean increase of 0.1066 g Na<sup>+</sup> kg<sup>-1</sup> DM (0.85%) in the roots for each unit increase in EC (Figure 2b).

Similarly, Lima (2014) also verified linear increases Na<sup>+</sup> content of the leaves and roots in young plants of the coconut grown under increasing levels of irrigation-water salinity. On the other hand, the mean values found by that author were lower than measured in the present study, in both the leaves (1.96 ± 0.71 g Na<sup>+</sup> kg<sup>-1</sup> DM) and roots (0.68 ± 0.14 g Na<sup>+</sup> kg<sup>-1</sup> DM).

The increases in mean Na<sup>+</sup> content of the roots due to the different levels of salinity under evaluation

were expected, as increases in their respective levels in the roots are routinely accompanied by increases in the concentrations of Na<sup>+</sup> in the soil solution (BALIZA *et al.*, 2010). Such increases may also be related to the direct contact of the roots with Na<sup>+</sup>, which, among other damage, is potentially capable of causing changes in the integrity and selective permeability of the plasma membranes of the cells that make up the root tissue, contributing to its increased absorption and consequent accumulation in that organ. This demonstrates that the accumulation of Na<sup>+</sup> in the roots cannot be interpreted simply as a direct consequence of the high levels of this ion in the soil (BELKHEIRI; MULAS, 2013).

Comparing the different parts of the plants, it can be seen that the highest sodium content was found in the roots (14.42 g Na<sup>+</sup> kg<sup>-1</sup> DM), which in terms of mean value is about 1.9 times greater than that seen in the leaves (7.71 g Na<sup>+</sup> kg<sup>-1</sup> DM).

The results suggest that the coconut, when exposed to salinity, tends to retain higher concentrations of Na<sup>+</sup> in the roots, avoiding high levels of this ion reaching the leaves, irrespective of the water supply. According to Hasegawa (2013), the transport systems that limit Na<sup>+</sup> uptake to the xylem vessels in the root are responsible for reducing movement of this ion to the leaves.

According to Miranda *et al.* (2013), plants can retain ions in the roots, limiting the salinity of the xylem sap and reducing the amount of ions reaching the leaves. For those authors, this ability is important for reducing the probable harmful effects of excess Na<sup>+</sup> on the photosynthetic tissue, favouring gas exchange and efficient water use.

The Cl<sup>-</sup> ion content of the leaf varied from 5.19 to 32.54 g kg<sup>-1</sup> DM, the lowest value being obtained with the application of 20% ETpc for the soil with a salinity of 1.72 dS m<sup>-1</sup> while the greatest value, approximately 527% higher, resulted from the use of 100% ETpc at a salinity of 40.70 dS m<sup>-1</sup> (Figure 3a).

In view of the present results, it can be inferred that the plants were adequately supplied with Cl<sup>-</sup>, since respective leaf content was higher than the level reported by Teixeira *et al.* (2005) as critical to the species (2.5 g kg<sup>-1</sup> DM).

Studying the associations between the treatment levels under evaluation, a significant fit was seen to the response surface model that best described the behaviour of the data for leaf Cl<sup>-</sup> content (R<sup>2</sup> = 0.7711, F-test: p ≤ 0.05), showing a linear and increasing response for the ion with increases in water depth and soil salinity, while each unit increase of ETpc and EC resulted in an increase of 0.1751 and 0.1831 g Cl<sup>-</sup> kg<sup>-1</sup> DM respectively. In turn, the joint action between these factors resulted in a change in the

nature of the response, giving rise to reductions, albeit insignificant, of 0.0012 g Cl<sup>-</sup> kg<sup>-1</sup> DM (Figure 3a).

It can be inferred that mean values for the Cl<sup>-</sup> anion in the root tissue were between 12.04 and 37.29 g Cl<sup>-</sup> kg DM<sup>-1</sup>. The associations between the treatment levels for water deficit and soil salinity that resulted in the highest and lowest Cl<sup>-</sup> content in the leaves were respectively 100% ETpc with 40.70 dS m<sup>-1</sup> and 20% ETpc with 1.72 dS m<sup>-1</sup>. These combinations also conditioned the extreme values for the Cl<sup>-</sup> content of the roots, leading to the assumption that in the present study the Cl<sup>-</sup> content of the leaves and roots were proportional, which may reflect, albeit in simple terms, the probable increasing concentrations of the Cl<sup>-</sup> ion in the soil solution throughout the increasing salinity levels under evaluation, despite it not being possible to determine the levels of Cl<sup>-</sup> in the soil (Figure 3a).

Similar to the Cl<sup>-</sup> ion content of the leaves, the mathematical model that best described the responses of this variable to the different levels of the factors under study (R<sup>2</sup> = 0.8613, F-test: p ≤ 0.05) also indicated positive linear increases for the levels of ETpc and EC, where each unit increase in the levels of the above factors resulted in an increase of 0.2412 and 0.2827 g Cl<sup>-</sup> kg DM<sup>-1</sup> respectively (Figure 3b).

On the other hand, the interaction between ETpc and EC altered the nature of the responses of the isolated factors for the variable in question, resulting in a decrease of 0.0028 g Cl<sup>-</sup> kg DM<sup>-1</sup> for each unit increase in the respective factors under study (Figure 3b).

It can be inferred that the high levels of ETpc, combined with high levels of soil salinity, caused increases

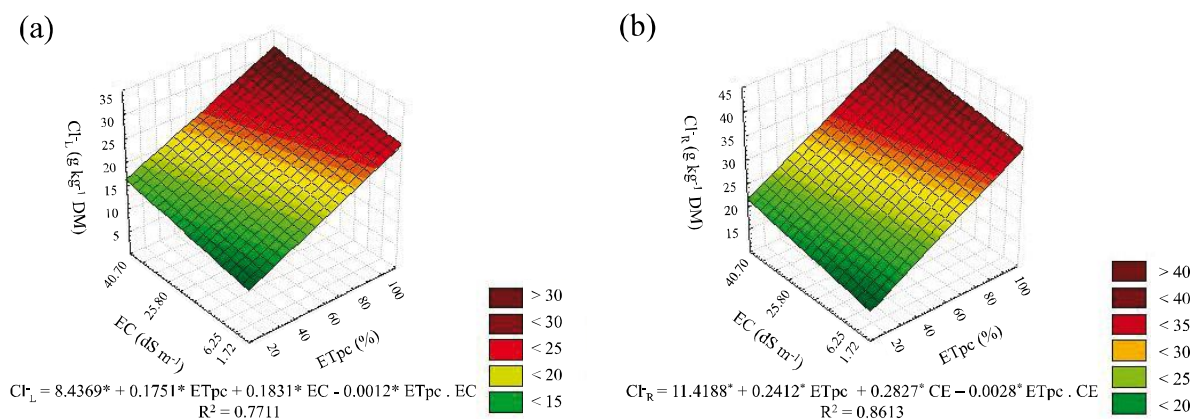
in the Cl<sup>-</sup> content of the leaf and root tissue, which demonstrates the probable additive effect of further water on the Cl<sup>-</sup> content of the leaves and/or roots. The results show that the absorption of Cl<sup>-</sup> ions from the soil solution, and their consequent accumulation in the plant tissue, is directly related to their availability in the soil as well as to water uptake by the plants (CUNHA *et al.*, 2012).

According to Valicheski *et al.* (2011), the mobility of Cl<sup>-</sup> in the soil preferably occurs by mass flow, therefore the lower levels of water deficiency probably resulted in less water absorption, and consequently of Cl<sup>-</sup>. It is assumed that water deficiency restricted the rate of transpiration, thereby reducing water consumption, and obviously reducing the absorption of the probable constituent elements of the soil solution (STEIDLE NETO; ZOLNIER; MAROUELLI, 2010).

For the positive linear increases in the Cl<sup>-</sup> ion content of the leaves and roots as a function of the increasing levels of salinity under evaluation, it can be inferred that this behaviour was a direct indication of the greater absorption of this ion as the soils became more saline due to probable increases in the respective Cl<sup>-</sup> concentrations in the soil solution. These results are consistent with those of Ferreira Neto *et al.* (2007) and Lima (2014), who also found Cl<sup>-</sup> accumulation in leaves of the coconut with increased salinity.

It is important to note that although Cl<sup>-</sup> is recognised as a micronutrient, when absorbed and accumulated in sufficiently high concentrations, it can trigger a series of physiological and/or metabolic problems, above all in the functions of enzymatic systems and protein synthesis, finally compromising photosynthetic efficiency (FREIRE *et al.*, 2013).

**Figure 3** - Response surface for the Cl<sup>-</sup> ion content in the leaves (a) and roots (b) of young coconut plants for combinations of different levels of water deficit (% ETpc) and soil salinity (EC) at 120 DAT<sup>1</sup>



<sup>1</sup>(\*), significant at 5% probability by the Fisher-Snedecor F-test

According to Teixeira *et al.* (2005), the coconut especially has a high demand for this anion, of such magnitude that its adequate supply becomes relevant. For those authors, when the coconut is grown far from the sea, this deficiency in  $\text{Cl}^-$  is easily noted; this probably explains, at least partially, increases in the leaf and root content of this anion ( $\text{Cl}^-$ ) due to salinity, that have been demonstrated in the literature, since with increases in the salt content of the soil, greater availability of this anion in the solution is almost universally expected.

Gomes and Prado (2007) report that in the coconut,  $\text{Cl}^-$  has important and distinct metabolic functions, especially in water relations, leading to the supposition that in the present study the above ion, once absorbed and accumulated, may still have contributed positively to the ability of the species to tolerate stress agents.

In short, for the above authors there is clear evidence in this species that this ion is important in regulating stomatal opening, to such an extent that it has been suggested that the role of  $\text{Cl}^-$  is physiologically dominant in stomatal regulation in the coconut to the detriment of the recognized performance of the  $\text{K}^+$  ion in the process.  $\text{Cl}^-$  also plays an essential role in the physiological and metabolic integration of a series of mechanisms that, isolated and/or combined, are able to confer greater tolerance to water scarcity on the coconut. In this way, any possible deficiency of this nutrient drastically reduces the osmoregulatory capacity of the species under adverse conditions of water supply, since there are strong indications that  $\text{Cl}^-$  acts as an effective osmolyte, at least at the level of the cells making up the stomatal complex.

Similar to that found for the  $\text{Na}^+$  ion, by evaluating the mean levels of  $\text{Cl}^-$  in the different parts of the plant, an accumulation of the ion, around 1.3 times greater, was seen in the roots (28.04 g  $\text{Cl}^-$   $\text{kg}^{-1}$  DM) to the detriment of the leaves (21.06 g of  $\text{Cl}^-$   $\text{kg}^{-1}$  DM), irrespective of water supply and/or soil salinity. This result provides strong evidence that salt tolerance in fruit species is associated with effective mechanisms for the retention of toxic ions in the roots, which limits their accumulation in quantities capable of causing damage to photosynthetic tissue (ARAÚJO *et al.*, 2014).

According to Hasegawa (2013), the mechanisms associated with the exclusion of toxic ions such as  $\text{Na}^+$  and  $\text{Cl}^-$  depend on both the absorption selectivity of the root system, and on the resistance of these ions to transfer from the roots to the leaves, so as to maintain adequate levels of ionic concentrations in the leaves.

On the other hand, due to the probable importance of an adequate supply of the chloride ion ( $\text{Cl}^-$ ) to metabolism in the coconut, especially faced with the adverse water

supply, it can be assumed that the high levels of this ion, which might in fact have reached the leaves, may indeed have had a positive contribution.

According to Sobral and Nogueira (2008), this is confirmed by the fact that in the coconut, chlorine is considered the third most-exported nutrient, to the point that some authors in fact consider  $\text{Cl}^-$  a macronutrient, in which it is quantitatively similar to the demand for N and K.

In relation to the comparative contribution of the evaluated inorganic solutes to the possible osmotic adjustment affected by the roots and leaves of the coconut, a greater relative contribution of the  $\text{Cl}^-$  ion can be seen, followed by  $\text{K}^+$  and, last but no less important, by  $\text{Na}^+$ . Such behaviour goes against the general tendency shown by plants that demonstrate a high affinity for saline ions, since in these species there is a higher relative contribution of  $\text{K}^+$ , followed by  $\text{Cl}^-$ , to the detriment of  $\text{Na}^+$  (SILVEIRA *et al.*, 2009). On the other hand, these differences can be explained by the essentiality of chlorine to the coconut, together with the relevance of potassium to water relations, even where there is evidence of  $\text{Na}^+$  acting as a probable substitute for  $\text{K}^+$  under high levels of salt, especially of sodium in the soil.

## CONCLUSIONS

1. Under conditions of water scarcity,  $\text{K}^+$  is maintained and/or accumulated in the leaves; while in the roots, levels are jointly affected by water deficiency and soil salinity, where these stress agents act in an antagonistic way on the  $\text{K}^+$  content through increases and reductions, due to greater water availability and salinity respectively;
2. Conditions of salt stress lead to increases in the levels of  $\text{Na}^+$  both in the leaves and especially in the roots;
3. The  $\text{Cl}^-$  ion content of the leaves and roots respond to the interaction between water deficiency and salinity, demonstrating synergy in the relationship between stressors such that increases in the replacement level of water and/or salinity increase the levels of  $\text{Cl}^-$  in the leaves and roots;
4. The accumulation of inorganic solutes in the coconut follows the order:  $\text{Cl}^- > \text{K}^+ > \text{Na}^+$ .

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