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Organic solutes in coconut palm seedlings under water and salt stresses¹

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multiple stresses
biochemical mechanisms

ABSTRACT

The objective of this study was to investigate the biochemical mechanisms associated with isolated and/or concurrent actions of drought and soil salinity in seedlings of coconut tree, through the accumulation of organic solutes (soluble carbohydrates, soluble amino N and free proline) in leaves and roots. The experiment, conducted in a protected environment, in Fortaleza, Brazil, in a randomized block design, in a split-plot arrangement, evaluated the effects of different levels of water stress (plots) by imposing distinct percentages of replacement of water losses through crop potential evapotranspiration - ET_{pc} (20, 40, 60, 80 and 100%), associated with subplots consisting of increasing levels of soil salinity in saturation extract (1.72, 6.25, 25.80 and 40.70 dS m⁻¹) provided by the soils collected in the Irrigated Perimeter of Morada Nova. Salinity did not change the concentration of organic solutes; however, there were increases in leaf and root levels of free proline in response to water stress, which contributes to the osmoregulation and/or osmoprotection of the species under adverse conditions of water supply.

Palavras-chave:

Cocos nucifera L.
estresses múltiplos
mecanismos bioquímicos

Solutos orgânicos em plantas jovens de coqueiro sob estresses hídrico e salino

RESUMO

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 através da mensuração do acúmulo de solutos orgânicos (carboidratos solúveis, N - aminosolúveis e prolina livre) em folhas e em raízes. No experimento conduzido em casa de vegetação, em Fortaleza, CE, avaliaram-se, sob delineamento estatístico de blocos casualizados, no arranjo de parcelas subdivididas, os efeitos de diferentes níveis de deficiência hídrica (parcelas) mediante a imposição de distintos percentuais de reposição das perdas de água por evapotranspiração potencial da cultura - ET_{pc} (20; 40; 60; 80 e 100%), associados às subparcelas, constituídas pelos níveis de salinidade do solo no extrato de saturação (1,72; 6,25; 25,80 e 40,70 dS m⁻¹), proporcionados pelos solos coletados no Perímetro Irrigado Morada Nova. A salinidade não alterou os teores dos solutos orgânicos; todavia, verificaram-se incrementos nos teores foliares e radiculares de prolina livre em resposta à deficiência hídrica. Este fato corrobora com uma possível contribuição à osmorregulação e/ou osmoproteção da espécie em condições adversas de suprimento hídrico.



INTRODUCTION

Under natural conditions, plants may be subjected to a simultaneous combination of various types of abiotic stresses, such as the associations between the effects of water deficit and salinity. Recent studies have shown that the biochemical responses exhibited by the plants to the interaction between multiple stress agents are singular and can not be directly fixed based on those that are commonly observed in the individual actions of the stress agents (Ahmed et al., 2015).

Among the important plant species in Northeast Brazil, the coconut palm (*Cocos nucifera* L.) occupies a prominent position in the regional scene, especially for its socioeconomic importance. In addition, it should be pointed out that this species occurs preferentially in coastal regions, characterized by high contents of salts in the soil (Machado et al., 2014).

Because of its occurrence in areas with problems of salinity and water deficit, the coconut palm is expected to show biochemical characteristics adjusted to these conditions. Such aspects raise the hypothesis that the coconut palm may fit among the relevant species for revegetation of areas with salinized soils.

Studies related to the independent effects of water and salt stresses have already been conducted (Marinho et al., 2005; Gomes et al., 2010). However, for coconut palm, there are no studies involving the simultaneous influence of these stress agents, especially those that encompass biochemical responses.

Therefore, this study aimed to investigate the biochemical mechanisms associated with the isolated and/or simultaneous actions of water deficit and soil salinity on seedlings of coconut palm, evaluating the accumulation of organic solutes in leaves and roots.

MATERIAL AND METHODS

The experiment was conducted in a screened greenhouse from October 2013 to February 2014, at the Experimental Area of the Federal University of Ceará, in the municipality of Fortaleza, CE, Brazil (03° 45' S; 38° 33' W; 19 m), which has Aw' climate, rainy tropical, according to Köppen's classification.

The experiment was conducted in a randomized block design arranged in a split-plot scheme, in which the plots consisted of different water deficit levels, through the imposition of five percentages of replacement of water losses through crop potential evapotranspiration - ET_{pc} (20; 40; 60; 80 and 100% ET_{pc}), while subplots consisted of levels of soil salinity in saturation extract (1.72; 6.25; 25.80 and 40.70 dS m⁻¹), with six replicates and one plant per pot, totaling 120 experimental units.

The treatments allocated in the subplots were provided by soils classified as Fluvic Neosols, collected in different areas of

representative lots of the Irrigated Perimeter of Morada Nova, in the state of Ceará, Brazil. Soil samples from the layer of 0-0.20 m were air-dried and passed through a 4-mm-mesh sieve. During the filling of the pots, composite soil samples were collected for the characterization of the chemical attributes and the results are presented in Table 1.

The seedlings of coconut palm, cultivar "Anão Verde do Brasil de Jiqui", with 40 days of germination, were transplanted to flexible plastic pots with capacity for 25 L.

A drip irrigation system was used, with pressure compensating emitters, with nominal flow rate of 4 L h⁻¹. The adopted method of irrigation management was based on the climatic conditions. Crop potential evapotranspiration - ET_{pc} was determined using the methodology proposed by Bernardo et al. (2006). The water deficit treatments were differentiated through the variation of the irrigation time, controlled using independent valves. The total water depths applied, at the end of the treatment, were equal to 62.40, 124.80, 187.20, 249.60 and 312.00 mm for the replacement levels of 20, 40, 60, 80 and 100%, respectively.

At 121 days after transplanting, leaves and roots were collected and lyophilized, macerated in porcelain mortar, in the presence of liquid nitrogen, and stored for subsequent analysis of soluble carbohydrates - SC (Dubois et al., 1956), soluble amino N - AA (Yemm & Cocking, 1955) and free proline - Pro (Bates et al., 1973).

The data were subjected to analysis of variance and, in case of significant effect were analyzed through regression. The mathematical models were selected based on the significance of the regression coefficients using the t-test, on coefficient of determination and on the studied biological phenomenon.

RESULTS AND DISCUSSION

The results of the ANOVA (Table 2) indicated that the contents of soluble carbohydrates (leaves) and free proline (leaves and roots) were significantly influenced by the factor water deficit ($p \leq 0.01$ or $p \leq 0.05$); however, there was no significant effect of soil salinity ($p \geq 0.05$) and of the interaction between salinity and water deficit ($p \geq 0.05$) on the contents of the studied organic solutes.

It can be assumed that the salt stress imposed on the plants during the initial development stages (until 121 DAT) was not sufficient to cause significant changes in the contents of organic solutes in the studied plants.

The absence of effects of salinity and interaction are contrary to various reports in the literature, which confirm that the accumulation of compatible solutes is associated with the tolerance to abiotic stresses, during the various phenological

Table 1. Chemical attributes of the soils used as substrates for the cultivation of seedlings of coconut palm, cultivar "Anão Verde", in the experiment¹

Salinity	pH (Water)	EC _{SE} (dS m ⁻¹)	Exchange complex						S	T	V	ESP	OC	OM	P _{Assimilable} (mg kg ⁻¹)	Classification
			Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	H ⁺ + Al ³⁺	Al ³⁺								
S1	6.8	1.72	6.58	4.34	0.06	4.32	2.56	0.00	15.30	17.86	86	24	12.26	21.13	30	Sodic
S2	7.5	6.25	7.80	5.24	0.08	7.23	0.00	0.00	20.35	20.35	100	36	16.64	28.66	30	Saline – sodic
S3	7.4	25.80	7.51	5.69	0.05	15.78	0.00	0.00	29.03	29.03	100	54	9.22	15.89	69	Saline – sodic
S4	7.0	40.70	14.91	4.58	0.07	22.46	0.00	0.00	42.02	42.02	100	53	20.23	34.88	82	Saline – sodic

¹pH - potential of hydrogen, EC_{SE} - electrical conductivity of the soil saturation extract; S - sum of bases; T - cation exchange capacity; V - base saturation; ESP - exchangeable sodium percentage; OC - organic carbon; OM - organic matter; P_{Assimilable} - Assimilable phosphorus

Table 2. Summary of the analysis of variance for the contents of the organic solutes - soluble carbohydrates (SC), soluble amino N (AA) and free proline (Pro) in leaves (SC_{leaf} , AA_{leaf} and Pro_{leaf}) and roots (SC_{root} , AA_{root} and Pro_{root}) of seedlings of coconut palm, cv. "Anão Verde", cultivated under different water deficit levels, in salt-affected soils, evaluated at the end of the experimental period (121 DAT)¹

SV	DF	Mean square					
		SC_{leaf}	SC_{root}	AA_{leaf}	AA_{root}	Pro_{leaf}	Pro_{root}
Blocks	3	3,752.23*	188.91 ^{ns}	15.34 ^{ns}	27.47 ^{ns}	0.42 ^{ns}	15.02 ^{ns}
Water deficit (D)	4	5,160.56*	733.97 ^{ns}	18.39 ^{ns}	55.05 ^{ns}	4.11**	74.39**
Residual - D	12	963.28	2,191.91	4.69	20.26	0.60	4.79
Plots	19						
Soil salinity (S)	3	567.33 ^{ns}	959.26 ^{ns}	9.64 ^{ns}	4.30 ^{ns}	0.23 ^{ns}	7.24 ^{ns}
Interaction S x D	12	1,272.13 ^{ns}	2,449.56 ^{ns}	14.77 ^{ns}	23.71 ^{ns}	1.24 ^{ns}	5.57 ^{ns}
Residual - S	45	843.00	1,506.39	5.79	21.67	0.79	4.52
Total	79						
CV - D (%)		24.17	21.29	23.27	23.63	23.35	26.10
CV - S (%)		22.61	24.23	28.07	24.44	72.64	24.50

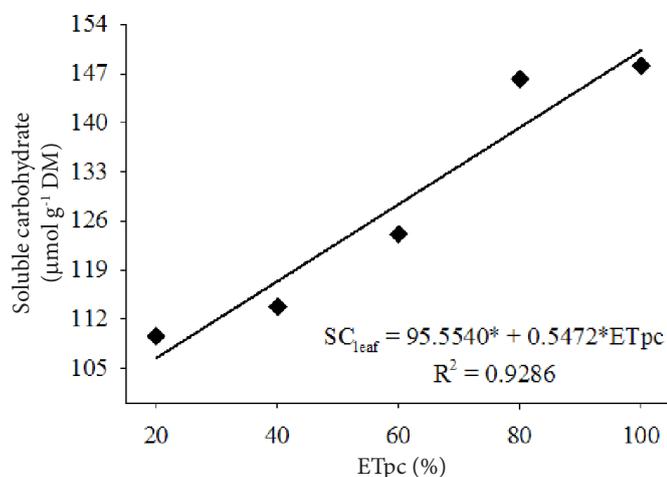
¹SV: Sources of variation, DF: Degrees of freedom, CV: coefficients of variation, * significant at 0.05 probability level, **: significant at 0.01 probability level, ^{ns}: not significant by F test

stages, especially regarding salinity, indicating that these solutes play a fundamental role in the osmoprotection (Simpson et al., 2014).

As to the effects of different water deficit levels on the leaf contents of soluble carbohydrates, the values remained between 106.50 and 150.27 $\mu\text{mol g}^{-1}$ DM, so that the extreme contents of this variable were obtained at levels of replacement of crop potential evapotranspiration equivalent to 20 and 100% ET_pc, respectively (Figure 1).

According to the regression analysis, the leaf contents of soluble carbohydrates followed a linear model of prediction as a function of the evaluated water deficit levels ($R^2 = 0.92$), in which the treatment with 100% ET_pc was superior in approximately 41%, compared with 20% ET_pc. So that it is possible to estimate, through the mathematical model to which the data best fitted, that each unit increase in ET_pc resulted in increments of approximately 0.55 $\mu\text{mol g}^{-1}$ DM (0.57%), as shown in Figure 1.

According to Vitorino et al. (2012), plants usually react osmotically to the reduction in soil water availability through increments in the contents of soluble carbohydrates in their



(*), significant at 0.05 probability level by t-test

Figure 1. Contents of soluble carbohydrates in leaves (SC_{leaf}) of seedlings of coconut palm as a function of water deficit levels (% ET_pc)

tissues in order to contribute to the adaptation or simply for the adjustment to the imposed water stress. However, the results observed in the present study demonstrate the contrary, i.e., coconut palm plants actually exhibited an apparent reduction in the content of this solute, under the condition of water scarcity, which may also be related to the lower mobilization of reserves.

According to Akinci & Losel (2009), reductions in the contents of soluble carbohydrates under water deficit conditions indicate the limited osmoregulatory potential of the plants, and this probable reduction in the leaf contents of soluble carbohydrates is related to the consumption of these compounds to guarantee the survival of the species under adverse environmental conditions. In addition, specifically under water deficit, these organic solutes can be altered both quantitatively and qualitatively, while these evident modifications can be interpreted as a relevant metabolic signal, triggered in response to the stress due to water restriction.

According to Pedrosa et al. (2014), under environmental conditions adverse to the plants, the soluble carbohydrates synthesized in the leaves are used to maintain the metabolic activities that ensure plant survival, so that the reductions in the leaf concentration of these compounds are frequently reported under severe water deficit conditions.

Given the obtained results, it is supposed that the increasing linear accumulations of soluble carbohydrates recorded with the increments in soil water availability evidence the probable contribution that these solutes had on the supply of part of the metabolic demand of the plants under water deficit conditions, which probably prevented its respective accumulation under water shortage conditions, which leads to the supposition that they do not participate in the probable phenomenon of osmoregulation in the species.

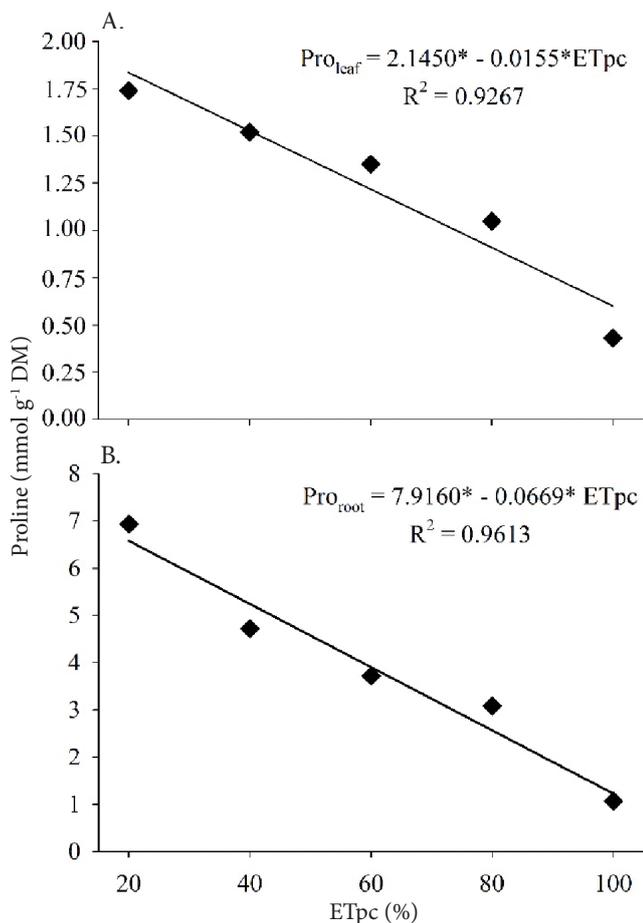
The leaf contents of free proline varied from 0.60 $\mu\text{mol g}^{-1}$ DM in fully irrigated plants (100% ET_pc) to 1.84 $\mu\text{mol g}^{-1}$ DM in those cultivated under the most severe level of water stress (20% ET_pc), totaling a percent increment of approximately 67%, comparing the extreme values obtained for this solute. These results shows that, in coconut palm plants, the leaf contents of proline increased with the reduction in soil water availability (Figure 2A).

The regression analysis for the leaf contents of free proline as a function of the different levels of water deficit (Figure 2A) indicated that the data best fitted to a decreasing linear model ($R^2 = 0.92$), which was able to predict that each unit increase in the percent replacement of water losses led to a reduction of approximately 0.72% in the leaf contents of free proline (0.0155 $\mu\text{mol g}^{-1}$ DM).

These results are in agreement with the data of Filippou et al. (2014), who reported that, frequently, increments in the leaf contents of free proline have been observed as one of the inherent effects of water stress as way of mitigating the deleterious implications of water shortage on plant species.

According to Alvarenga et al. (2011), proline acts in osmotic adjustment, stabilization of subcellular structures and elimination of free radicals or, also, it can constitute a potential stock of nitrogen and carbon, which can be metabolically used after the period of exposure to the stress.

On this matter, Slabbert & Krüger (2014) claimed that plants can accumulate compatible solutes, such as proline, in response



* significant at 0.05 probability level by t-test

Figure 2. Contents of free proline in leaves - Pro_{leaf} (A) and roots - Pro_{root} (B) of seedlings of coconut palm as a function of different water deficit levels (% ETpc)

to stresses, to facilitate water absorption and protect the cells against the excessive accumulation of reactive oxygen species. According to these authors, the accumulation of proline was correlated with a variety of edaphoclimatic conditions adverse to plants and this osmolyte is apparently an important antioxidant agent of non-enzymatic nature.

According to Silva et al. (2012), in plants subjected to water deficit, the increments in proline contents may be associated with the increase in the activity of proteolytic enzymes, which, under the predominance of these conditions adverse to plants, start to promote a greater availability of this free amino acid.

Results similar to the present study were obtained by Gomes et al. (2010), who also reported an expressive accumulation of proline in the leaves of coconut palm seedlings in response to water deficit. Based on the results, these authors inferred that, apparently, this accentuated accumulation of free proline was better correlated with the functions of protection, instead of osmoregulation.

While many studies indicate high correlation between proline accumulation and the increase in drought tolerance, others suggest that the accumulation is simply an effect of the stress (Ribeiro et al., 2014).

Thus, despite the reports in the literature that point out the role of proline followed by its participation in osmotic adjustment as an important mechanism capable of providing tolerance to water deficit in various coconut palm genotypes,

Gomes et al. (2010) still question the relevance of this strategy for drought tolerance in the species.

Based on the obtained responses, however, it is supposed that these leaf accumulations of free proline, associated with the beneficial implications that are inherent to this osmolyte, clearly contribute to the probable resistance to water stress exhibited by part of the coconut palm seedlings in the present study, through either osmoregulation or osmoprotection, or even through the synergism between these phenomena.

As to the root contents of free proline, coconut plants showed a maximum value of 6.58 $\mu\text{mol g}^{-1}$ DM, at the water deficit level equivalent to 20% ETpc, while the treatment in which water requirements were fully met (100% ETpc) led to the lowest value of this variable (1.23 $\mu\text{mol g}^{-1}$ DM). Hence, the increment observed in the root contents of proline between the highest and lowest levels of replacement of water losses (ETpc) was approximately 82%, and the amplitude between the extreme values was approximately 5.35 $\mu\text{mol g}^{-1}$ DM (Figure 2B).

The data best fitted to a decreasing linear equation ($R^2 = 0.96$), with a reduction of 0.0669 μmol of free proline g^{-1} DM, corresponding to 0.85%, per unit increase in the level of ETpc replacement (Figure 2B).

There is a similarity between leaf and root contents of free proline and these results can be partially explained by taking into consideration the data of Rhein et al. (2011). According to these authors, in plants under water stress, the free proline synthesized in leaf tissues can be easily translocated through the phloem until the roots, where it accumulates to preserve and maintain the turgor of root cells, which are the first organs to detect water scarcity in the soil.

Based on the results, there is the hypothesis that these prominent accumulations of free proline evidenced in the root system and in leaves of coconut palm seedlings possibly result from a series of disorders that occur in the protein metabolism due to water stress, since, under the predominance of these adverse conditions, there may be substantial changes in the proportions of amino acids that, in short, favor the biosynthesis of free proline (Bortolo et al., 2009).

Comparing the mean values of leaf and root contents of free proline, it is inferred that this solute was more accumulated in the roots (3.90 $\mu\text{mol g}^{-1}$ DM) than in the leaves (1.21 $\mu\text{mol g}^{-1}$ DM), and the contents of this osmolyte in the root system were about 3.22 times higher than those in the leaves, regardless of the considered level of water deficit.

Such observations suggest that the root system, besides communicating with the shoots, plays an important function in the osmoregulatory mechanisms shown by coconut palm seedlings under water stress conditions, based on the confirmed accumulation of substances of low molecular weight, especially the organic solute proline.

This accumulation of free proline in the roots, compared with that in the leaves, can be explained through the data reported by Signorelli et al. (2013), who suggested that proline is synthesized in the shoots and then translocated to the roots, where its metabolism is necessary to promote survival and/or plant growth under conditions of low water potentials in the soil.

Although the accumulation of free proline during water stress is well documented in the literature for the most plant species

(Campos et al., 2011), in the present study, it is inferred that the alterations in root and leaf contents of proline, through the increased syntheses and/or the decreased metabolic uses, were not sufficient to precisely predict whether the actual role of these compounds in the response of coconut palm plants to water deficit of either osmoregulation or osmoprotection.

In the specific case of free proline and based on the propositions of Lacerda et al. (2004), the fact that the leaf and root contents remained always very low can mainly indicate the small effective contribution that this solute may have had on the mechanisms that, in association, promote tolerance to water deficiency in coconut palm seedlings, despite the observed increments due to the reduction in water availability.

Thus, it can be inferred that, as observed by Gomes et al. (2010), leaf and root accumulations of free proline under water deficit possibly caused more relevant effect of osmoprotection, in detriment of osmoregulation itself.

CONCLUSIONS

1. Salinity, isolated or associated with water deficiency, does not alter leaf or root contents of soluble carbohydrates, soluble amino N and free proline of coconut palm seedlings.

2. The leaf and root contents of free proline increased in response to water deficit in coconut palm seedlings.

3. Under adverse conditions of water supply, the accumulation of free proline is associated with osmoregulation and/or osmoprotection in coconut palm seedlings.

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