PHYSIOLOGICAL RESPONSES OF DWARF COCONUT PLANTS UNDER WATER DEFICIT IN SALT-AFFECTED SOILS[1]

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ABSTRACT - The objective of this study was to characterize the physiological acclimation responses of young plants of the dwarf coconut cultivar 'Jiqui Green' associated with tolerance to conditions of multiple abiotic stresses (drought and soil salinity), acting either independently or in combination. The study was conducted under controlled conditions and evaluated the following parameters: leaf gas exchange, quantum yield of chlorophyll *a* fluorescence, and relative contents of total chlorophyll (SPAD index). The experiment was conducted under a randomized block experimental design, in a split plot arrangement. In the plots, plants were exposed to different levels of water stress, by imposing potential crop evapotranspiration replacement levels equivalent to 100%, 80%, 60%, 40%, and 20%, whereas in subplots, plants were exposed to different levels of soil salinity (1.72, 6.25, 25.80, and 40.70 dS m^{-1}). Physiological mechanisms were effectively limited when water deficit and salinity acted separately and/or together. Compared with soil salinity, water stress was more effective in reducing the measured physiological parameters. The magnitudes of the responses of plants to water supply and salinity depended on the intensity of stress and evaluation period. The physiological acclimation responses of plants were mainly related to stomatal regulation. The coconut tree has a number of physiological adjustment mechanisms that give the species partial tolerance to drought stress and/or salt, thereby enabling it to revegetate salinated areas, provided that its water requirements are at least partially met.

Keywords: *Cocos nucifera* L.. Water and salt stress. Physiology.

RESPOSTAS FISIOLÓGICAS DE PLANTAS DE COQUEIRO ANÃO SOB DEFICIÊNCIA HÍDRICA, EM SOLOS AFETADOS POR SAIS

Resumo - Objetivou-se, com este trabalho, caracterizar as repostas fisiológicas aclimatativas de plantas jovens de coqueiro Anão, cultivar "Verde do Jiqui", associadas com a sua tolerância às condições de múltiplos estresses abióticos (deficiência hídrica e salinidade do solo), atuando isolados e/ou combinados. O estudo foi realizado sob condições controladas e avaliaram-se: as trocas gasosas foliares, o rendimento quântico da fluorescência da clorofila a e os teores relativos de clorofila total (índice Spad). O experimento foi conduzido sob delineamento estatístico de blocos casualizados, no arranjo de parcelas subdivididas, sendo as parcelas constituídas por diferentes níveis de deficiência hídrica, mediante a imposição de cinco distintos percentuais de reposição das perdas de água por evapotranspiração potencial da cultura (100; 80; 60; 40 e 20%) e as subparcelas constituídas pelos crescentes níveis de salinidade do solo (1,72; 6,25; 25,80 e 40,70 dS m^{-1}). Os mecanismos fisiológicos são efetivamente limitados quando a deficiência hídrica e a salinidade atuam isoladamente e/ou em conjunto. Os efeitos do estresse hídrico se mostram mais efetivos nos comprometimentos dos parâmetros fisiológicos, em detrimento à salinidade do solo. As magnitudes das respostas das plantas ao suprimento hídrico e à salinidade dependem das intensidades dos estresses e das épocas de avaliação. As respostas fisiológicas aclimatativas das plantas estão relacionadas, principalmente, à regulação estomática. O coqueiro apresenta uma série de mecanismos de ajustes fisiológicos que conferem à espécie uma parcial tolerância ao estresse hídrico e/ou salino, tornando-a capaz de revegetar áreas salinizadas, desde que as necessidades hídricas sejam ao menos parcialmente atendidas.

Palavras-chave: *Cocos nucifera* L.. Estresses hídrico e salino. Fisiologia.

INTRODUCTION

Despite an ever-increasing need for plant-based food, fiber, and energy, there remain vast areas of the world that have unfavorable environmental conditions for plant growth. This includes areas in which the soil and atmosphere are too dry and areas with high soil salinity, which are thus unsuitable for agriculture.

Plant growth results from regulated and integrated physiological processes (BEZERRA et al., 2005). Drought conditions and/or salt stress are known to induce various physiological disorders in crops, leading to reduced growth and yields (SILVA et al., 2010).

The use of plants that can tolerate high salt concentrations in the soil can be adopted as a strategy to transform barren areas (which are either naturally saline, or where salinity has been artificially increased by inadequate management of soil and water resources) into areas suitable for agricultural cultivation (SABRA; DAAYF; RENAULT, 2012).

Cultivation of coconut palm (*Cocos nucifera* L.) occurs mainly in coastal areas, dominated by "Typic Quartzipsamment" soils with sandy textures, generally characterized by their low fertility and/or high salinity. In the Morada Nova and Curu–Pentecoste Irrigation Perimeters in the state of Ceará, coconut palm is commonly cultivated in "Fluvisols," which present the most varied and diversified textural classes and degrees of salinity and/or sodicity; this is mainly attributable to inadequately managed irrigation and drainage systems. Even so, coconut cultivation is an important activity in Brazil, and particularly in this region, and in recent years it has seen significant growth worldwide (not just in Latin America) (SOUSA et al., 2011).

It is assumed that coconut palm plants can grow in saline environments by developing a series of adaptive physiological mechanisms, and this, in concert with its economic potential, makes the coconut palm a promising plant for cultivation in salt-affected soils (LIMA, 2014; MARINHO et al., 2005).

The physiological effects of drought (GOMES et al., 2008; GOMES et al., 2010) and salinity of irrigation water (LIMA, 2014; MARINHO et al., 2005) on coconut palm trees have been reported previously. However, there have been no reports on the mutual influence of these stressors on this plant, nor is there information available regarding the physiological responses of these trees to the mutual influence of these multiple abiotic stresses.

Chaves, Flexas, and Pinheiro (2009) suggest that the physiological responses observed in species induced by a combination of stressors (e.g., drought and salinity) are unique, and cannot be extrapolated from the responses to single stress factors.

Thus, the objective of the present study was to characterize the physiological responses of Dwarf coconut palm saplings (cultivar 'Jiqui Green') associated with their tolerance to multiple abiotic stressors (drought and soil salinity), acting either in isolation or in combination, to elucidate the possible acclimatization strategies adopted by plants during the early stages of their growth.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse-like protected environment between October 2013 and February 2014, at the Experimental Area of the Agrometeorological Station of the Department of Agricultural Engineering, Federal University of Ceará (Pici *Campus*), in Fortaleza–CE, Brazil, with geographical coordinates: Latitude 03°45′South; Longitude 38°33′ West; elevation approximately 19 m.

An Onset Hobo® data-logger was installed in the center of the greenhouse to characterize and monitor environmental conditions (temperature, relative humidity, and luminosity) during the course of the experiment, with measurements being taken every 10 min. A Class "A"-type tank evaporimeter was also installed, with readings being performed daily. The mean values of temperature, relative humidity, atmospheric water vapor pressure deficit, luminosity, and water evaporation during the experimental period were $29.14 \pm 2.97°C$, $68.48\% \pm 12.60\%$, 3.34 ± 1.12 kPa, 0.076 ± 0.22 W m^{-2} (wavelength = 555 nm), and 5.70 ± 0.70 mm day^{-1}, respectively.

The experiment was conducted using a randomized block split-plot design, with the plots consisting of different water drought levels (D), by imposing five percentage values of the replacement of water loss by crop potential evapotranspiration (ETpc: D1 = 100% ETpc, D2 = 80% ETpc, D3 = 60% ETpc, D4 = 40% ETpc, and D5 = 20% ETpc), and subplots consisting of increasing levels of soil salinity (S: S1 = 1.72 dS m^{-1}, S2 = 6.25 dS m^{-1}, S3 = 25.80 dS m^{-1}, and S4 = 40.70 dS m^{-1}). Four replicates consisting of one plant per pot were used, for a total of 80 experimental units.

Dwarf coconut seedlings (cultivar 'Jiqui Green') obtained from a certified farmer were transplanted 40 days after germination into pots with a volumetric capacity of 25 L, filled on average with 32.57 kg of sodic Fluvisol (S1) or sodic saline Fluvisol (S2, S3, and S4), obtained from the topsoil at four different sites in the Morada Nova Irrigation Perimeter, located in the municipalities of Morada Nova and Limoeiro do Norte, in the State of Ceará (Latitude 5°10′South; longitude 38°22′West; elevation: approximately 80 m), which represented

substrates with increasing salinity levels. Composite soil samples were collected from the pots to characterize their physico-hydric and chemical attributes, the results of which are shown in tables 1 and 2, respectively. On the basis of these results, a chemical correction was performed with mineral fertilizers, the amounts of which were determined with reference to Fontes, Cintra and Carvalho-Filho (1998), who suggest the use of 200 g of a 15:10:15 formulation of nitrogen, phosphorus, and potassium, respectively. The equivalent to 30 g of the commercial formulation FTE BR 12 was also applied to meet micronutrient requirements.

Table 1. Physico-hydric attributes of soils used as substrates for the cultivation of Dwarf coconut palm saplings (cultivar 'Jiqui Green').

Salinity	Granulometric Composition			Textural class	Density		Humidity	
	Sand	Silt	Clay		Soil	Particles	33 kPa	1,500 kPa
	(---------- $g\,kg^{-1}$ ----------)			(-)	(--- $kg\,dm^{-3}$ ---)		(---- $m^3\,m^{-3}$ ----)	
S1	608	231	239	Sandy loam	1.23	2.52	19.59	12.10
S2	532	285	332	Sandy loam	1.33	2.56	19.63	11.55
S3	605	272	225	Sandy loam	1.38	2.60	17.42	9.11
S4	459	368	219	Loam	1.27	2.59	23.43	12.78

Table 2. Chemical attributes of soils used as substrates for the cultivation of Dwarf coconut palm saplings (cultivar 'Jiqui Green')[1].

Salinity	pH	EC	Exchangeable cations					
	(Water)		Ca^{2+}	Mg^{2+}	K^+	Na^+	$H^+ + Al^{3+}$	Al^{3+}
	(-)	($dS\,m^{-1}$)	(-------------------------- $cmol_c\,kg^{-1}$ --------------------------)					
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	T	V	ESP	C	O.M.	$P_{assimilable}$	Classification
	(--- $cmol_c\,kg^{-1}$ --)		(----- % -----)		(--- $g\,kg^{-1}$ ---)		($mg\,kg^{-1}$)	
S1	15.30	17.86	86	24	12.26	21.13	30	Sodic
52	20.35	20.35	100	36	16.64	28.66	30	Sodic-saline
S3	29.03	29.03	100	54	9.22	15.89	69	Sodic-saline
S4	42.02	42.02	100	53	20.23	34.88	82	Sodic-saline

[1]pH, hydrogen potential; EC, electrical conductivity of the soil saturation extract; S, sum of bases; T, cation exchange capacity; V, base saturation; ESP, exchangeable sodium percentage; C, organic carbon; O.M., organic matter; $P_{assimilable}$, assimilable phosphorus; Classification, soil classification per salinity.

Irrigation was provided using a drip irrigation system equipped with autocompensating emitters, with a nominal flow rate of $4\,L\,h^{-1}$. The irrigation management method was selected based on weather conditions. The methodology proposed by Bernardo, Soares, and Mantovani (2006) was adopted to determine the potential crop evapotranspiration (ETpc). The duration of irrigation, controlled via independent records, was employed to differentiate water stress treatments.

The variables were analyzed at 30, 60, 90, and 120 days after seedling transplantation (DAT). Stomatal conductance (g_s), transpiration (E), and net photosynthesis (A) measurements were performed using a portable infrared gas analyzer (IRGA: LCpro; ADC, Hoddesdon, UK). Chlorophyll fluorescence emission was measured using a portable modulated light fluorometer (PEA Hansatech, Kings Lynn, UK). The relative chlorophyll contents (SPAD index) were determined using a portable meter (SPAD 502; Minolta Co., Ltd, Osaka, Japan).

The data were submitted to analysis of variance, according to a randomized block experimental design in a split-plot scheme, where the different drought levels were analyzed in the plots, the different soil salinity levels in the subplots, and the evaluation periods of these variables in sub-subplots.

Significant effects were further analyzed by regression. Upon observation of a significant effect of the interactions between the factors, multiple linear regression analysis was performed and the respective response surfaces were plotted.

Mathematical models were selected based on the significances of the regression coefficients using Student's *t*-test, of the coefficient of determination, and of the biological phenomenon being studied. Statistical analyses were performed using Microsoft Excel software (v. 2007), ASSISTAT (v. 7.6 beta), and STATISTICA (v. 7.0).

RESULTS AND DISCUSSION

The variables under analysis were significantly influenced by the following factors: drought, soil salinity, and time of evaluation (the exception being the quantum yield of chlorophyll a fluorescence: Fv Fm^{-1}), as indicated by the ANOVA results shown in Table 3. Additionally, significant effects were observed for all double and triple interactions.

Table 3. Summary of the analysis of variance for stomatal conductance (g_s), transpiration (E), photosynthesis (A), quantum yield of chlorophyll a fluorescence (Fv Fm^{-1} ratio), and relative total chlorophyll content (SPAD index) in Dwarf coconut saplings (cultivar 'Jiqui Green'), grown under different levels of water stress in salt-affected soils, assessed at 30, 60, 90, and 120 days after transplanting[1].

SV	D.F.	Mean squares				
		g_s	E	A	Fv Fm^{-1}	SPAD index
Blocks	3	0.00144*	0.26936**	2.47630**	0.00031^{ns}	9.39^{ns}
Drought (D)	4	0.22764**	46.08957**	518.24074**	0.00760**	62,710.32**
Residue – D	12	0.00037	0.03036	0.18508	0.00132	6.43
Plots	19					
Soil salinity (S)	3	0.02094**	3.49716**	36.81467**	0.00436*	4,325.10**
Interaction S × D	12	0.00396**	0.25696**	1.12011**	0.00372**	777.55**
Residue – S	45	0.00012	0.01072	0.10637	0.00131	1.75
Subplots	79					
Time of Evaluation (E)	3	0.22495**	62.41610**	907.68968**	0.00024^{ns}	251.71**
D × E interaction	12	0.02007**	2.29278**	40.74256**	0.00439**	65.63**
S × E interaction	9	0.00119**	0.04052**	2.39176**	0.00368*	4.67**
D × S × E interaction	36	0.00024**	0.07776**	0.64452**	0.00470**	9.64**
Residue – E	180	0.00010	0.00579**	0.03389	0.00152	1.87
Total	319					
C.V. - D (%)		24.24	21.40	18.72	4.65	14.47
C.V. - S (%)		13.88	16.78	16.61	4.63	12.33
C.V. - E (%)		12.36	14.98	13.73	4.99	12.41

[1]S.V.: sources of variation, D.F.: degrees of freedom, C.V.: coefficients of variation, *: significant at 5% probability, **: significant at 1% probability, ^{ns}: not significant by the F test.

In consideration of the specific purpose of the study, regardless of the significance of the drought × soil salinity × time of evaluation interaction, the drought × soil salinity double interaction was split.

Figure 1 shows that a linear polynomial is the best fit for the data, modeling the behavior of the stomatal conductance variable as a function of the level variations of the factors in the study, with a coefficient of determination (R^2) of 0.9651.

Application of 20% ETpc to soils with a salinity equivalent to 40.70 dS m^{-1} resulted in the lowest values of stomatal conductance, transpiration, and photosynthesis, whereas the largest average values were obtained with the combined use of 100% ETpc and the lowest salinity level (1.72 dS m^{-1}) (Figures 1, 2, and 3).

Stomatal closure is one of the fastest and most intense responses that occur in plants subjected to stressors that compromise their water status, such as drought and salinity, and is considered to efficiently control water losses (GOMES; PRADO, 2007). This stomatal response is regulated by signaling pathways that occur in roots, and is modulated by the synthesis and distribution of abscisic acid (ORSINI et al., 2012).

$$g_s = -0.0341^* + 0.0036^* \, ETpc - 0.0002^* \, CE - 1.88 \cdot 10^{-5*} \, ETpc \cdot CE$$
$$R^2 = 0.9651$$
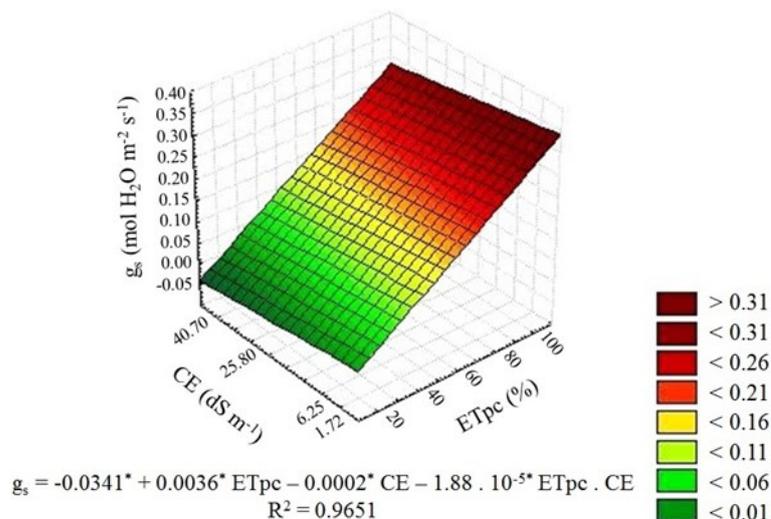
Figure 1. Response surface for stomatal conductance (g_s) data for Dwarf coconut saplings (cultivar 'Jiqui Green'), in response to combinations of different levels of water stress (% ETpc) and soil salinity levels (CE)[1].
[1](*) Significant at 5% probability using Student's *t*-test.

Figure 1 also shows that drought levels exert a significantly higher effect on stomatal conductance, indicating thereby that drought was the most limiting factor to stomatal opening compared to soil salinity. This inference can be made by comparing the line slope gradients that make up the response surface; those describing the drought factor are more pronounced than those of soil salinity levels. Thus, although the stomatal opening process is subjected to the combined influence of both stressors, it appears to be more sensitive to changes in water stress levels than to changes in soil salinity levels—the combination of 100% ETpc and CE = 1.72 dS m⁻¹ resulted in a mean g_s value of approximately 0.32 mol H_2O m⁻² s⁻¹, whereas the combination of 100% ETpc and CE = 40.70 dS m⁻¹ yielded an average g_s value of approximately 0.17 mol H_2O m⁻² s⁻¹ (Figure 1). Similar behaviors were also observed for the transpiration (Figure 2) and photosynthetic (Figure 3) variables.

Moreover, this behavior leads to the hypothesis that the effect of soil salinity may have accentuated further the degree of drought stress to which the plants were subjected, since salts interfere with the water absorption capacity of the root system (osmotic potential interference, and the reduction of the potential gradient between soil and root cells) (KUSVURAN, 2012). The stomatal conductance responses to different combinations of the stressors drought and soil salinity support this hypothesis. These inferences are supported by Ramegowda and Kumar (2015), who state that the increased concentration of salts in soil solution promotes an increase in retention forces due to their effect on the osmotic potential, thus amplifying the magnitude of drought in plants. According to these authors, an increase in osmotic potential, stemming from the

excess of soluble salts, may lead to a situation wherein plants will lack a satisfactory suction forces to overcome this osmotic potential, resulting in an inability to absorb water, even in moist soils.

Silva Júnior, Passos, and Gheyi (2002) also found that drought and the accumulation of salts in soil imposed severe restrictions on stomatal behavior in coconut palm plants. The authors also observed that exposure to high salt levels reduces stomatal opening in some salt-tolerant species and even in halophyte plant species, and that this in turn leads to decreased transpiration, and consequently to reduced primary production of photosynthates. Kusvuran (2012) states that high salinity has detrimental effects on the stomatal opening process in plants by increasing resistance to the diffusion of CO_2. Stomatal closure can act as an activation signal for multiple responses to stress, including inhibition of plant growth, which can be detrimental for yields. In turn, stomatal closure prevents damage to metabolic systems, by adjusting them to the water deficit in the plant (due to shortage of water and/or excess salts).

Our results corroborate those of Passos, Passos, and Prado (2005), who reported that under drought conditions during the dry season, "Dwarf" coconut ecotypes respond to drought effects by significantly reducing stomatal conductance. The effects of soil salinity levels in the present study corroborate the findings of Lima (2014), who also described a significant effect of irrigation water salinity on stomatal conductance. The findings of the present study are also consistent with those of Silva Júnior, Passos, and Gheyi (2002), who hypothesized that the coconut palm, when irrigated with saline water, has mechanisms that operate to prevent water stress by closing stomata, thereby substantially compromising gaseous exchange. Additionally,

Marinho et al. (2005) found that stomatal conductance in coconut palm plants decreased as the irrigation water salinity increased.

The splitting of the association effects between treatments for the variable transpiration by polynomial regression analysis showed a linear effect for the drought and soil salinity levels, as well as for the interaction between these factors, with a coefficient of determination (R^2) of 0.9925 (Figure 2).

$$E = 0.7356^* + 0.0386^* \, ETpc - 0.0182^* \, CE + 8.10 \cdot 10^{-5*} \, ETpc \cdot CE$$
$$R^2 = 0.9925$$
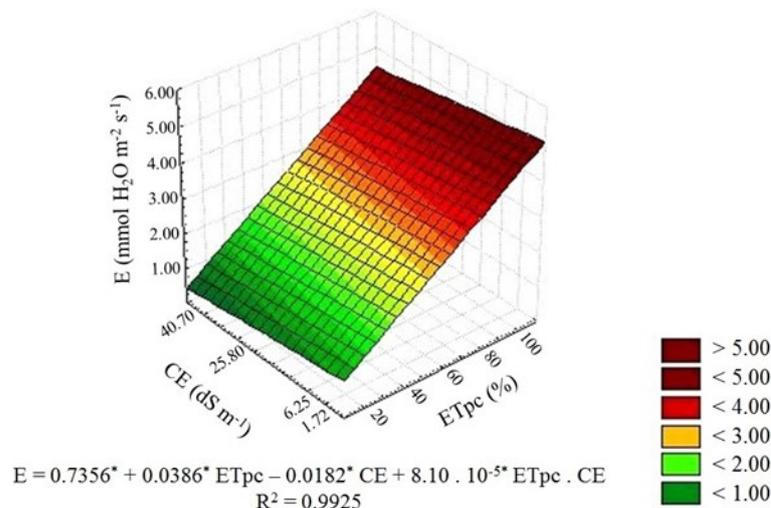
Figure 2. Response surface for transpiration (E) data for Dwarf coconut saplings (cultivar 'Jiqui Green'), in response to combinations of different levels of water stress (% ETpc) and soil salinity levels (CE)[1].
[1](*) Significant at 5% probability using Student's *t*-test.

As the water supply becomes limiting, transpiration decreases (GONÇALVES et al., 2010). The lower transpiration values in plants under drought and salt stresses precisely reflect the effects that such stressors exert on stomatal closure (Figure 2). This closure is the result of a reduction in the soil's osmotic potential stemming from the increasing salt levels. This phenomenon, along with the ionic effect, may induce stomatal closure, ionic imbalance, nutritional deficiency, and consequently a decrease in biomass production (KUSVURAN, 2012). An adequate water supply appears to mitigate the effect of salinity on the transpiration rates, i.e., results in smaller decreases (Figure 2).

It should be noted that the lowest transpiration values were observed for the combination of treatments that also resulted in the lowest values of stomatal conductance. This direct correspondence between stomatal conductance and transpiration is expected, taking into consideration the fact that stomatal closure reduces water vapor outflow into the atmosphere (and consequently also reduces transpiration rates) (GONÇALVES et al., 2010).

The present results indicate that the reduction in stomatal conductance and transpiration can partially compromise photosynthetic activity. When plants were exposed to different levels of salinity, transpiration rate decreases were estimated to be 1.08%, 0.72%, 0.38%, 0.30%, and 0.20% per unit increase of CE, and in ETpc replacement levels equivalent to 20%, 40%, 60%, 80%, and 100%, respectively (Figure 2). However, Orsini et al. (2012) inferred that such responses can also be viewed as a means of protecting plants from rapid dehydration, assuring survival under stress conditions. Moreover, through stomatal regulation, plants can prevent the incidence of several other signals that would further affect their respective agronomical performance.

According to Sabra, Daayf, and Renault (2012), plants tend to close their stomata under saline conditions, thus reducing the amount of transpired water, which can contribute to a reduction in the absorption and loading of toxic ions such as Na^+ and Cl^- into their interior, thus constituting an additional adaptive strategy for survival under the predominance of these stressful conditions. Hence, the inferences described above can explain plant survivability based upon an association between ETpc- and CE-stressing agents, assuming that the regulation of stomatal opening, and consequently control of water loss, is a mechanism adopted by plants to adjust to adverse conditions (CHAVES; FLEXAS; PINHEIRO, 2009). These assertions are corroborated by Sucre and Suárez (2011), who hypothesized that plants respond to drought and salinity by closing stomata, thus reducing leaf transpiration and/or preventing the development of an excessive water deficit situation in tissues. This response enables the leaf to retain a water potential capable of maintaining its adjustment to and/or recovery from stressor-induced damage, whether these stressors act in isolation or in combination.

Regarding photosynthesis, the proposed model indicates that drought and soil salinity effects increase and reduce photosynthetic rates, respectively, and can be described using a linear polynomial with a coefficient of determination (R^2) of 0.9866 (Figure 3).

$$A = 2.7489^* + 0.1307^* \, ETpc - 0.0724^* \, CE - 0.0003^* \, ETpc \cdot CE$$
$$R^2 = 0.9866$$
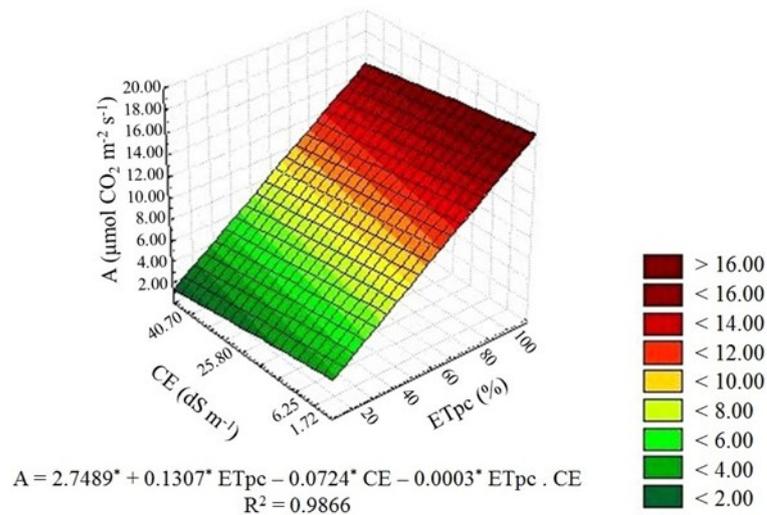
Figure 3. Response surface for photosynthesis (A) data for Dwarf coconut saplings (cultivar 'Jiqui Green'), in response to combinations of different levels of water stress (% ETpc) and soil salinity levels (CE)[1].
[1](*) Significant at 5% probability using Student's t-test.

In the present study, the highest photosynthetic rates were obtained with a drought control level of 100% ETpc and the lowest salinity level (1.72 dS m^{-1}). Conversely, the lowest photosynthetic rates were measured when a drought level corresponding to 20% of ETpc in association with the highest level of soil salinity (40.70 dS m^{-1}) were imposed; the extent of reduction was extremely dependent on the intensity of drought and salt levels (Figure 3). This behavior contrasts with the observations reported by Sucre and Suárez (2011), wherein some studies showed that plant survivability capacity increases when affected by a combination of salinity and drought, i.e., compared to situations in which the plants are only affected by drought or salinity alone, plant physiology is less compromised. Thus, the general trends shown in the present study support the hypothesis that stressor combinations may potentiate the effects of their action when acting alone (CHAVES; FLEXAS; PINHEIRO, 2009).

Similar to the observation on cashew plants reported by Bezerra et al. (2005), it is assumed that much of the reduction in photosynthesis in coconut plants stems from stomatal limitation. Silva et al. (2010) reported similar results in *Jatropha* plants, which also exhibited a strong restriction of photosynthesis via stomatal closure, as a prevention strategy for both stress conditions (drought and salinity). Silva et al. (2011a) also reported a decrease in photosynthesis, associated with low leaf stomatal conductance in plants simultaneously subjected to salinity and drought.

Although a decrease in photosynthesis is mostly due to a decrease in stomatal conductance, effects such as increased resistance to internal transport of CO_2, damage to the photosynthetic machinery caused by stressors, biochemical alterations that can directly compromise the photosynthetic efficiency, and even salt effects on chloroplasts (particularly on the transport of electrons and on the secondary processes), may also have occurred. Furthermore, a decrease in photosynthesis under stress conditions can also stem from leaf size reduction (SANTOS et al., 2012).

According to Gomes and Prado (2007), drought-induced reductions in photosynthetic rates in coconut palm plants are initially ascribed to a limitation in CO_2 diffusion from the atmosphere into the intercellular spaces as a result of stomata closure. The authors also observed that factors unrelated to stomata contribute to a reduction in photosynthesis, both during a period of severe drought and during the recovery phase after water supply is resumed. Indeed, during the latter, factors unrelated to stomata are more relevant to photosynthetic modulation.

According to Sabra, Daayf, and Renault (2012), the non-stomatal factors potentially capable of restricting photosynthetic activity are biochemical in nature, and include the inhibition of Rubisco activity and ATP synthesis; these responses are complementary to the stomatal responses to stress in plants.

The water favoritism, conditioned by increasing levels of ETpc, promoted an increase in the Fv Fm^{-1} ratio. However, the magnitude of the response was restricted by the influence of increasing soil salinity (Figure 4).

$$Fv\, Fm^{-1} = 0.7539^{*} + 0.0005^{*}\, ETpc - 0.0003^{*}\, CE^{*} + 1.24 \cdot 10^{-7*}\, ETpc \cdot CE$$
$$R^2 = 0.9718$$
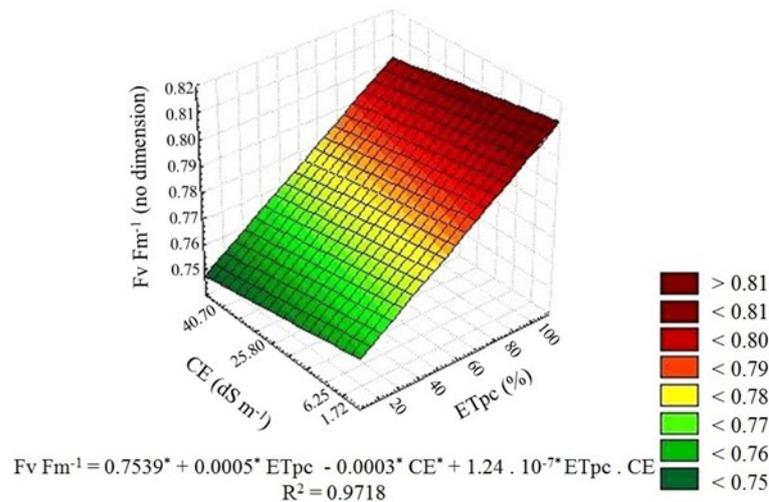
Figure 4. Response surface for chlorophyll *a* fluorescence quantum yield (Fv Fm⁻¹ ratio) data for Dwarf coconut saplings (cultivar 'Jiqui Green'), in response to combinations of different levels of water stress (% ETpc) and soil salinity levels (CE)[1].
[1](*) Significant at 5% probability using Student's *t*-test.

Generally, and consistent with the findings of the study by Silva et al. (2014) on citrus hybrids, the quantum yield of chlorophyll fluorescence upon imposition of saline stress responds in a manner similar to the variables related to gas exchange in leaves. When we analyzed the effect of salinity in isolation, we found that our results differ from those obtained by Marinho et al. (2005), who found that the salinity of the irrigation water did not affect variables pertaining to the efficiency of the photosynthetic machinery in coconut palms, at any given evaluation point of that assay. However, our results are similar to those obtained by Lima (2014), who also observed decreases in the Fv Fm⁻¹ ratio when coconut palms were subjected to irrigation with saline water.

According to Azevedo Neto et al. (2011), the Fv Fm⁻¹ ratio is an estimate of the maximum quantum efficiency of the photochemical activity of photosystem II (PS2) when all its reaction centers are open. Disturbances in this ratio are ascribed to salinity stress effects on the photosynthetic system, considering that its reduction suggests a decline in the photochemical efficiency of PS2, in addition to indicating the occurrence of disturbances and/or damage to the photosynthetic machinery in response to the inherently adverse salinity effects. Furthermore, considering that the values obtained for the Fv Fm⁻¹ ratio when plants were subjected to the combination of the most severe drought level (20% ETpc) and the lowest salinity level (1.72 dS m⁻¹) were lower than those obtained for plants subjected to the combination of full water supply (100% ETpc) and the highest salinity (40.70 dS m⁻¹), it can be reasoned that drought conditions exerted a more pronounced effect, in such a way that adequate water supply appears to mitigate the adverse effects of salinity in the photosynthetic apparatus (Figure 4).

According to Araújo et al. (2010), a decrease in the Fv Fm⁻¹ ratio when plants are subjected to a given stressor is related to photoinhibitory effects, and can represent either a reversible photoprotective regulation or an irreversible inactivation of PS2. For these authors, the implications arising from the effects of drought on this variable show that plants often exhibit a remarkable inhibitory effect on photosynthesis, which is characterized by a significant decrease in PS2 quantum yield. Gomes et al. (2008) also observed a decrease in Fv Fm⁻¹ ratios of two coconut palm ecotypes grown under conditions of water scarcity, which led to the assumption that water stress causes photoinhibition in coconut genotypes. According to the authors, in addition to high irradiation, any environmental restriction that can directly or indirectly limit the photosynthetic capacity is potentially capable of inducing photoinhibition. Moreover, corroborating Araújo et al. (2010), the authors also hypothesize that decreases in Fv Fm⁻¹ ratios can actually reflect certain photochemical adjustments that plants may adopt under adverse weather and soil conditions, and is not a consequence of damage to PS2, noting that the decrease in the Fv Fm⁻¹ ratio can result from an increase in the initial fluorescence (Fo). This finding is consistent with that of Cruz et al. (2009), who reported that an increase in Fo causes increased loss of excitation energy during its transfer among antenna pigments and reaction centers, and thereby constitutes a factor that contributes significantly to a decrease in the Fv Fm⁻¹ ratio.

According to Gonçalves et al. (2010), there is no consensus in the literature regarding what effects a decrease in soil water availability may produce on the Fv Fm⁻¹ ratio. Some authors state that, in general, plants show a rapid decrease in the ratio when the water content in soil is reduced, whereas others argue that the electron transport capacity is not altered by water stress, which reflects the invariance

of the $Fv\,Fm^{-1}$ ratio under drought predominance. Although statistically significant, the differences in $Fv\,Fm^{-1}$ ratios between the different levels of drought and soil salinity observed in the present study were relatively small and independent of the time of evaluation. In general, their values fell within the range 0.49–1.00, with an overall mean value of 0.78 ± 0.04, which indicates that some of the combinations between the studied factor levels at different evaluation times resulted in damage to the PS2 reaction centers, as shown in Figure 4. Such inferences are based on the findings of Silva et al. (2013), who stated that the $Fv\,Fm^{-1}$ ratio should always be approximately 0.8 for satisfactory efficiency, since values below 0.75 indicate a reduction of the photosynthetic potential of plants due to the incidence of certain forms of stress, whereas the capability of maintaining a high $Fv\,Fm^{-1}$ ratio (>0.80) indicates a highly efficient use of radiation by carbon assimilation reactions, despite the prevalence of conditions that are adverse to plants.

Through the response surface study for the variable relative total chlorophyll content (SPAD index), the highest values were obtained under full irrigation conditions (100%), whereas the lowest values were observed under the most severe level of drought stress (20% ETpc), as shown in Figure 5.

SPAD index = $7.8615^{*} + 0.9474^{*}$ ETpc $+ 0.1576^{*}$ CE $- 0.0083^{*}$ ETpc . CE
$R^2 = 0.8243$
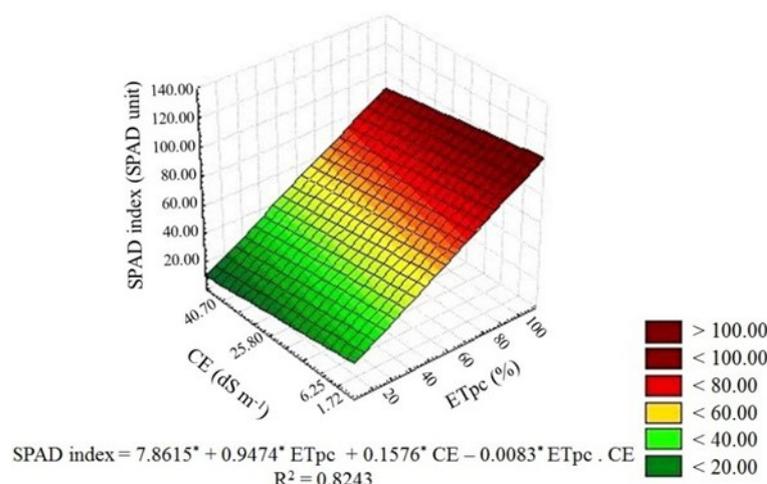
Figure 5. Response surface for relative total chlorophyll content (SPAD index) data for Dwarf coconut saplings (cultivar 'Jiqui Green'), in response to combinations of different levels of water stress (% ETpc) and soil salinity levels (CE)[1].
[1](*) Significant at 5% probability using Student's *t*-test.

Therefore, it can be inferred that an increase in the SPAD index with increasing water supply is a clear reflection of the sensitivity of relative chlorophyll contents in coconut plant saplings to water stress. This is consistent with the findings of Silva et al. (2011b), who showed that drought stress is typically characterized by a loss of chlorophyll, associated with a progressive decline in the photosynthetic capacity of plants. This behavior can be explained by the more prominent effect caused by drought on this variable, coupled with the likely ability that an adequate water supply has in mitigating the potential adverse effects that salinity alone could exert on total chlorophyll content. This is based on the observation that an increase in water availability yielded higher chlorophyll values, and that the intensity of the response variable was compromised as a result of the adverse effects of salinity.

According to Chaves, Flexas, and Pinheiro (2009), the interactions between stressors in plants may retard, disguise, or even transform the plants' responses to an isolated stress factor. Lima (2014) found significant linear reductions in relative total chlorophyll contents with increasing salinity in irrigation water. The decreases in SPAD index readings reflect decreases in chlorophyll content, as a response to salt stress (SILVA et al., 2011a). These authors speculate that this behavior may be associated with the degradation of chlorophyll pigments through increased chlorophyllase activity and instability of the protein complex under salt stress. However, the authors also argue that instead of chlorophyll degradation, this decrease could be attributed to the influence of ions on the synthesis of new proteins that are structural components of chlorophylls.

Generally, SPAD index values for the different combinations between treatments ranged between 13.4 and 134.1 SPAD units, with an overall average value of 56.7 ± 24.0 SPAD units (Figure 5). Given that the lowest values were obtained with combination treatments that represented the most severe stress levels, it can be deduced that, among other causes, the prevalence of these conditions affected the photosynthetic process due to the onset of a chlorophyll deficit. According to Torres Netto et al. (2005), this aspect becomes clear among different plant species showing SPAD index readings below 40 SPAD units. These results corroborate the information reported by Cardoso et al. (2011) on the existence of factors that may influence SPAD

readings, such as environmental conditions (temperature, light, water stress, soil salinity, etc.), nutritional deficiency, and growth stage.

According to Jesus and Marenco (2008), SPAD index readings can relate to the chlorophyll content in leaves—chlorophylls are related to the photosynthetic efficiency of plants, and consequently their growth and adaptability to different environments. In this sense, the SPAD index can be indicative of reduced photosynthetic capacity due to drought and salt stress, since the content of photosynthetic pigments is reduced by the action of adverse climatic and soil effects, compounded by the prolonged duration of exposure to stressors (TORRES NETTO et al., 2005).

CONCLUSIONS

Leaf gas exchange, the quantum yield of chlorophyll fluorescence, and the relative chlorophyll index are effectively limited both when drought and salinity act in isolation and when they occur in conjunction.

The coconut palm saplings are physiologically more susceptible to drought than to increases in soil salinity.

The acclimative and physiological responses of coconut palm saplings to drought and salinity factors, and to a combination of both, mainly comprise stomatal regulation.

ACKNOWLEDGMENTS

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