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ISOLATED AND COMBINED EFFECTS OF SOIL SALINITY AND WATERLOGGING IN THE LEAF GAS EXCHANGE OF GREEN DWARF COCONUT SEEDLINGS

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ABSTRACT: Degraded soils with excess salinity are unproductive unless utilized to grow plant species that are tolerant to salinity. In addition, in clayey soils, the problem of salinity is aggravated when accompanied by cycles of waterlogging in the rainy season or when excess irrigation is applied. The aim of this work was to evaluate the effects of soil salinity and waterlogging, isolated and combined, on the physiological responses of young plants of green dwarf coconut. The experiment was conducted under controlled environment, in a complete randomized block design, arranged in split plot with five replications. The plots comprised five waterlogging cycles (0, 1, 2, 3 and 4), each with a duration of four days, and applied at 30, 60, 90 and 120 days into the experimental period, with the sub-plots consisting of five levels of soil salinity (1.70, 11.07, 16.44, 22.14 and 25.20 dS m^{-1}). Evaluation of the photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), and internal CO_2 concentration (Ci, μmol mol^{-1}) were performed before and after each waterlogging cycle at 30, 60, 90 and 120 days after transplanting (DAT). Leaf gas exchange was reduced which was associated more with soil salinity than with the waterlogging cycles so that the effects of excess water were seen only at the lower levels of salinity. This response was related to stomatal and non-stomatal effects.

KEYWORDS: salt stress, *Cocos nucifera*, water excess.

Considering the rate of photosynthesis the coconut seedlings were classified as moderately-tolerant to salinity when grown in soils with an electrical conductivity up to 11.07 dS m^{-1}, having the potential to be used in revegetation programs of salt-affected areas, provided that these areas are not exposed to frequent waterlogging cycles.

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EFEITOS ISOLADOS E COMBINADOS DA SALINIDADE DO SOLO E ENCHARCAMENTO NAS TROCAS GASOSAS DE MUDAS DE COQUEIRO-ANÃO-VERDE

RESUMO: Solos degradados pelo excesso de sais geralmente são improdutivos, a menos que sejam utilizados para cultivar espécies que sejam tolerantes à salinidade. Além disso, em solos argilosos, o problema da salinidade é agravado quando acompanhado por ciclos de encharcamento, na estação chuvosa ou quando se aplica irrigação em excesso. O objetivo deste trabalho foi avaliar os efeitos da salinidade e do encharcamento do solo, isolados e combinados, sobre as respostas fisiológicas de plantas jovens de coqueiro-anão-verde. O experimento foi conduzido em casa de vegetação, sob delineamento de blocos casualizados, em parcelas subdivididas, com cinco repetições. As parcelas foram constituídas por cinco ciclos de encharcamento (0, 1, 2, 3 e 4), cada ciclo com duração de quatro dias, aos 30, 60, 90 e 120 dias do período experimental, sendo as subparcelas constituídas por cinco níveis de salinidade do solo (1,70, 11,07, 16,44, 22,14 e 25,20 dS m^{-1}). As avaliações das taxas fotossintéticas (A, µmol CO_2 m^{-2} s^{-1}), condutância estomática (gs, mol m^{-2} s^{-1}), transpiração (E, mmol m^{-2} s^{-1}) e concentração interna de CO_2 (Ci, µmol mol^{-1}) foram realizadas antes e após cada ciclo de encharcamento, aos 30, 60, 90 e 120 dias após o transplantio (DAT). As trocas de gasosas foliares foram reduzidas, estando mais associada à salinidade do solo do que aos ciclos de encharcamento, de modo que os efeitos do excesso de água foram observados apenas em baixos níveis de salinidade. Essas respostas podem está relacionadas aos efeitos estomáticos e não-estomáticos. Considerando-se as reduções nas taxas fotossintéticas, as mudas de coqueiro foram classificadas como moderadamente tolerantes à salinidade, quando cultivadas em solos com condutividade elétrica de até 11,07 dS m^{-1}, podendo ser utilizadas para revegetação de áreas salinizadas, desde que essas áreas não estejam expostas a frequentes ciclos de encharcamento.

PALAVRAS-CHAVE: estresse salino, *Cocos nucifera*, excesso de água.

INTRODUCTION

The green coconut crop is prominent in various countries because of its economic and social importance, which is due to the growing commercialization of a wide variety of products that can be obtained from the crop (YIN NG et al., 2015). In Brazil, the main producing states are Bahia and Ceará, both located in the northeast, with a large part of the cultivated area located at the semi-arid region, establishing the agronomic importance of the coconut in these areas.

Although the semi-arid has tropical conditions that are favourable to coconut farming, the water deficit, especially during the dry season, requires irrigation to attain high crop yield. However, inadequate irrigation management, water quality, and drainage problems can cause soil salinization, affecting the surrounding economy, society, and the environment.

Another factor associated with saline and saline-sodic soils in semi-arid regions is an excess of water (SINGH, 2015), especially during rainy season. Waterlogging is mainly associated with the limited drainage conditions found in part of the irrigated areas. These soils generally have physical attributes that favour this type of stress, i.e. a high clay content, reduced hydraulic conductivity, and unfavourable topographic conditions. Thus, extensive areas become predisposed to waterlogging in the rainy season because the soil lacks subsurface drainage systems (VELMURUGAN et al., 2016).

The use of salt-tolerant species has been a valid strategy recommended to promote the rehabilitation of soils degraded by an excess of salts. The moderate salt tolerance of the coconut (FERREIRA NETO et al., 2007; MARINHO et al., 2006) gives this crop the potential to be used in revegetation programs of salt-affected areas. However, the mechanisms of adaptation and/or tolerance that plants display when faced with simultaneous stresses are complex, and information on the combined effects of salinity and waterlogging on this crop are unknown.

Thus, in this research we evaluate the physiological responses of young plants of the green dwarf coconut cultivated in soils affected by salts and waterlogging cycles. The goal of this work was to test the establishment of coconut seedlings under simultaneous stress of salinity and excess of water, trying to determine the potential of this crop to be used in revegetation programs of salt-affected areas.

MATERIAL AND METHODS

Experimental conditions

The experiment was carried out in a greenhouse from June to October 2015, in the city of Fortaleza, Ceará, Brazil (Lat.: 3º45’S, Long.: 38°33’W, Alt.: 19 m). Air temperature, relative humidity, and luminosity during the experimental period was obtained with a datalogger (model HOBO® U12-012) and average values were 28.7 ° C, 68.6% and 5886.8 Lux, respectively.

Experimental design and treatments

The experiment was conducted in a complete randomized block design, arranged in split plot with five replications. The plots comprised five waterlogging cycles and the sub-plots were formed by five levels of soil salinity, totaling 125 experimental units.

The waterlogging cycles (0, 1, 2, 3 and 4) were imposed to the plants at 30, 60, 90 and 120 days after transplating (DAT). Each cycle had a duration of four days, simulating the occurrence of a tipical rainfall in the region. After four days of waterlogging, the pots were drained and the excess water collected in a container. This water was later returned to the pots to avoid the loss of salts by leaching.

The treatments in the sub-plots were composed of five increasing levels of soil salinity (S1 = 1.70, S2 = 11.07, S3 = 16.44, S4 = 22.14 and S5 = 25.20 dS m^{-1}). The soil, classified as Fluvic Neosols (EMBRAPA, 2013), was collected at different points of the Morada Nova Irrigated Perimeter in the state of Ceará, and located at 5°10' S and 38°22' W, representing the different stages of soil salinization found within this irrigated area.

Sixty-day-old seedlings of cultivar “Dwarf-Green” of Brazil de Jiqui to were transplanted into plastic containers with a capacity of 20 L, containing a drain in the lower part to remove any excess water after each waterlogging cycles. Plants were irrigated every other day with water from a well located in the experimental area, and having electrical conductivity (ECw) of 0.9 dS m^{-1}. The soil was maintened at field capacity, except during waterlogging cycles. A drip irrigation system was used to irrigate pots, employing self-compensating emitters with a flow rate of 4.0 L h^{-1}.

Leaf gas exchange

Evaluation of the gas exchange were performed before and after each waterlogging cycle, using an infrared gas analyser (LI-6400XT, Li-Cor, USA). The measurements were taken from mature leaves between 08:00AM and 10:00AM, under natural conditions of air temperature and CO_2 concentration, and employing an artificial source of radiation with an intensity of 1600 μmol m^{-2} s^{-1}.

Tolerance of plants

The relative salt and/or waterlogging tolerance was obtained by considering the reductions in photosynthetic rates quantified for different levels of salinity and waterlogging, comparing them to the control (plants grown in non-saline soil and wihtout waterlogging), according to Fageria et al. (2010). The plants were thus classified as tolerant (0 to 20% reduction), moderately tolerant (20.1 to 40% reduction), moderately sensitive (40.1 to 60% reduction) and sensitive (reduction greater than 60%).

Statistical analysis

The data were submitted to analysis of variance at the probabilities of 5% and 1%; where a significant effect was found, they were submitted to regression analysis using the SISVAR® 5.5 software (FERREIRA, 2010).

RESULTS

Leaf gas exchange

Leaf gas exchange were affected by waterlogging x soil-salinity interaction (Figure 1). Higher mean values for stomatal conductance (0.12 mol m^{-2} s^{-1}) were seen in the control plants; however, imposition of waterlogging caused decreases in stomatal conductance in plants of this treatment. With the increases in soil salinity, significant decreases in stomatal conductance were also noted, and these effects were independent of the number of waterlogging cycles, especially at the soil salinity levels higher than 11.07 dS m^{-1} (16.44, 22.14 and 25.20 dS m^{-1}).

For any period of evaluation, an increase in soil salinity (11.07 to 25.20 dS m^{-1}) resulted in limited stomatal conductance, leading to average restrictions of 38, 70, 77, 76% and of 40, 76, 83 and 81%, before and after waterlogging, respectively (Figures 1A, B), when compared to the average values presented by control plants grown in non-saline soil (ECe = 1.70 dS m^{-1}). Similar results were seen for the rate of transpiration (Figures 1C, D).

The lowest values for A were seen in plants grown in soils with EC of 16.44, 22.14, and 25.20 dS m^{-1}, irrespective of the number of waterlogging cycles (Figures 1E, F); whereas the greatest mean values for A were found when the plants were grown in soil of a lower salinity (1.70 dS m^{-1}) and not subjected to any waterlogging cycle. Comparing the measurements for A before and after the waterlogging cycles, reductions of 71, 83, and 78%, and of 77, 86, and 87%, were seen for photosynthetic rates of plants grown in soils with the greatest salinities (16.44, 22.14 and 25.20 dS m^{-1}), respectively. Minimum values of 2.02 μmol m^{-2} s^{-1} were estimated for plants grown in soil of the highest salinity before imposition of the fourth waterlogging cycle, and of 0.99 μmol m^{-2} s^{-1} after the fourth cycle (Figure 1F).

Mean values for internal CO_2 concentration (Ci) were 233 and 202 μmol mol^{-1} for plants subjected to 1.7 and 11.07 dS m^{-1} respectively, with significant increases recorded at the lowest salinity level when plants were subjected to three and four waterlogging cycles (Figure 1G, H). However, the greatest values for Ci were found in plants grown in soils at higher salinity levels combined with increases in the number of waterlogging cycles. Before being exposed to waterlogging, at the highest levels of soil salinity (16.44, 22.14 and 25.20 dS m^{-1}), plants displayed Ci mean values of 297.5, 290.5 and 324.9 µmol mol^{-1} when exposed to two, three and four waterlogging cycles respectively (Figure 1G). After exposure to waterlogging, Ci values were 344.9, 316.6 and 326.1 µmol mol^{-1} respectively (Figure 1H).

Tolerance of plants

Young plants of green dwarf coconut were classifield as tolerant when cultivated in non-saline soil (EC = 1.70 dS m^{-1}) same subjected to the waterlogging cycles (Table 1). When the plants were exposed to three waterlogging cycles, still under control salinity, they proved to be moderately tolerant to waterlogging with in photosynthetic rates reductions of 23% (Table 1).

Regarding salinity, expressed by electrical conductivity (EC), the plants were classifield as moderately tolerant to EC of 11.07 dS m^{-1}, and as moderately sensitive when exposed to three waterlogging cycles. On the other hand, at the more severe levels of soil EC (16.44, 22.14 and 25.20 dS m^{-1}), the waterlogging had no significant influence on photosynthetic rates, and plants were classified as sensitive, regardless of the number of waterlogging cycles (Table 1).

DISCUSSION

Plant species are often limited by multiple stress factors operating simultaneously (LENSSEN et al., 2003; LACERDA et al., 2016; SILVA et al., 2016), such as soil salinity and waterlogging (GARCÍA and MENDOZA 2014; SINGH, 2015), and plant response in these cases can be a consequence of amplified or hierarchical effects (LENSSEN et al., 2003). The results of the present work suggest that the action of a stress factor depends on the level of the other factor to which the plant is subjected. Analysing the degree of inhibition of gas exchanges and photosynthetic rates (Figure 1 and Table 1), the influence of waterlogging was clearly expressed only at the first two levels of soil salinity (1.7 and 11.07 dS m^{-1}). Increased salinity ranging from 16 to 25 dS m^{-1} produced a salt stress condition so severe that increases in waterlogging cycles did little contributed to worsen the negative physiological response patterns provoked by high soil salinity.

Other results obtained from the interaction between salinity and waterlogging maybe explained, at least in part, by the degree of severity of the two stress factors involved. In this work, physiological responses were mainly affected by soil salinity (Figure 1), but with some similarities when compared to the growth data (MEDEIROS et al., 2017). Waterlogging significantly decreased leaf gas exchange at lower salinity levels, but salt stress effects prevailed especially at higher levels of soil salinity. Zheng et al. (2009), studying the individual and combined effects of salinity and waterlogging on wheat, obtained similar results where the main stressing factor was salinity, which severely reduced leaf gas exchange, while waterlogging had practically no influence when combined with salinity.

Decrease in stomatal conductance and rate of transpiration are probably the first defences of the plant in detriment to increases in salinity, alone or associated with another stress factor (SUÁREZ 2011), as observed in the present study. Stomatal closure can also result in the impairment of photosynthetic capacity, by reducing both the influx of CO_2 and the internal CO_2 concentration (ORSINI et al., 2012; SLAMA et al., 2015). However, under severe stress conditions, non-stomatal responses, such as alterations in carboxylating enzymes or pigment degradation, may decrease photosynthetic rates. This was demonstrated in the present study, because reducted photosynthesis was acompanied by significant increases in the internal CO_2 concentration, specially at the higher levels of soil salinity.

Our results illustrate three different and interesting situations, linking stomatal responses to, possibly, non-stomatal responses, according to the interaction and severity of salt and waterlogging stresses: 1) plants at the lowest salinity level (1.7 dS m^{-1}) and under waterlogged conditions (three and four cycles) had increased Ci, demonstrating a certain degree of severity of waterlogging to coconut seedlings; 2) at an intermediate level of salinity (11.07 dS m^{-1}), there was a tendency to maintain or decrease Ci in relation to the control level, demonstrating the occurrence of stomatal effects and the lesser severity of the two stress factors imposed simultaneously on the plants; 3) plants subjected to highest levels of salinity (16 to 25 dS m^{-1}) had the most significant increases in Ci, clearly demonstrating the severity of soil salinity on the photosynthetic process. These last results leave no doubt that stomatal closure was not the only cause of the reduction in photosynthetic rate in coconut seedlings under severe salt stress.

CONCLUSIONS

Our results showed that response of coconut seedlings to waterlogging depends on the level of soil salinity, with waterlogging significantly impairing seedling in the gas exchang at low soil salinity levels, but causing no additional harm at elevated salinity.

Leaf gas exchange was reduced mainly by soil salinity, being slightly more intense when the stresses were combined. These responses however, were linked to stomatal and non-stomatal causes, and depended on the interaction and on the severity of waterlogging and salinity.

Coconut seedlings were classified as moderately tolerant to salinity when grown in soils with an electrical conductivity of up to 11.07 dS m-1. So, coconut seedlings are of potential use in revegetation programs of salt-affected areas, provided that these areas are not exposed to frequent waterlogging cycles.

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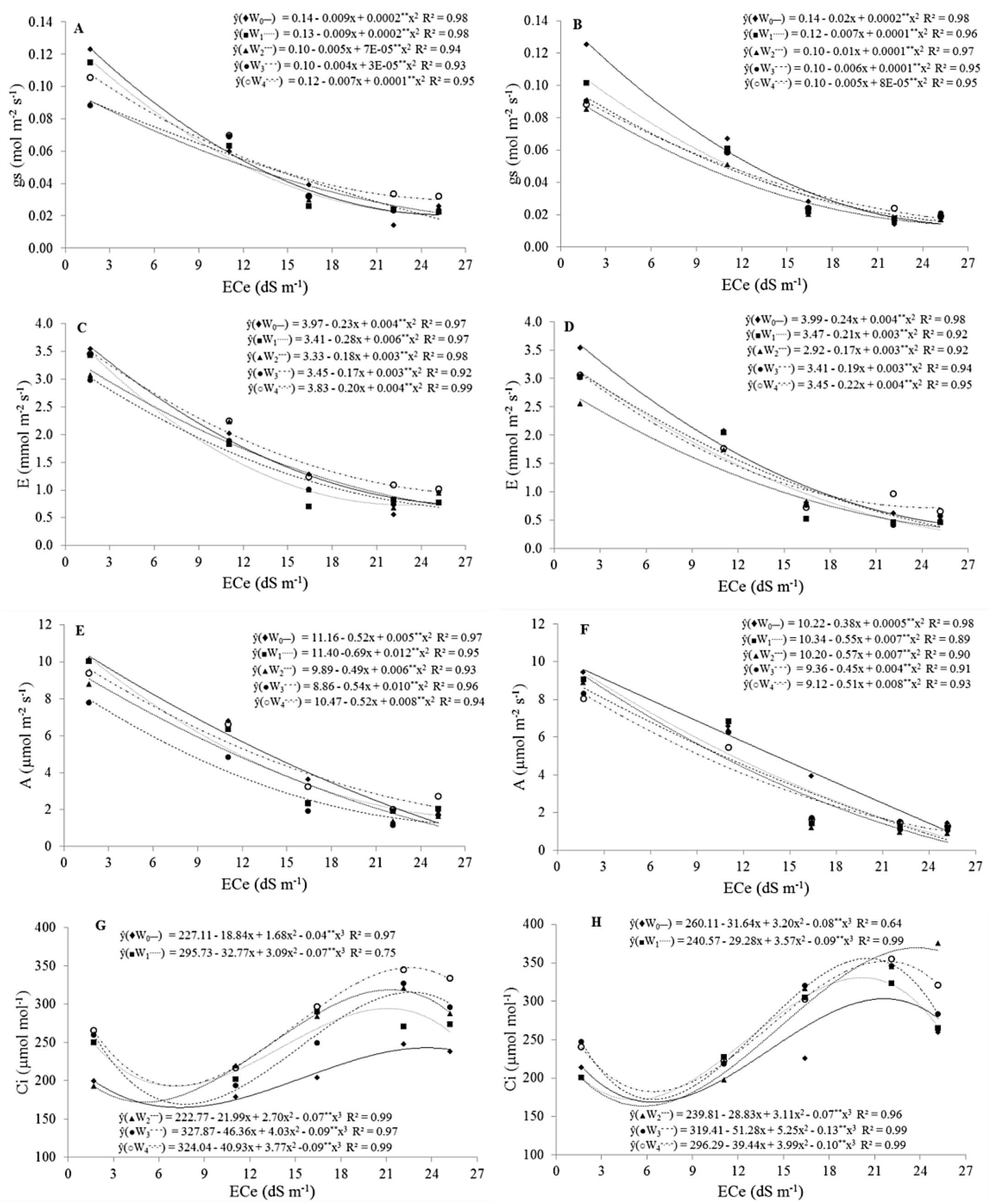

Figure 1. Stomatal conductance - gs (A and B), transpiration - E (C and D) and photosynthetic rate - A (E and F), Internal CO_2 concentration (G and H) in young plants of the green dwarf coconut, as a function of soil salinity and waterlogging cycles. Measurement were performed before (A, C, E and G) or after (B, D, F and H) exposure to waterlogging.

Table 1. Reduction in photosynthetic rates (%) and classification of salt/waterlogging tolerance in young plants of green dwarf coconut

Waterlogging Cycles	Reduction in photosynthetic rates (%)				
	Soil salinity (dS m^{-1})				
	1.70	11.07	16.44	22.14	25.20
0	Control	33 (MT)	64 (S)	87 (S)	81 (S)
1	1 (T)	37 (MT)	77 (S)	81 (S)	80 (S)
2	13 (T)	37 (MT)	77 (S)	86 (S)	84 (S)
3	23 (MT)	52 (MS)	81 (S)	89 (S)	83 (S)
4	7 (T)	35 (MT)	68 (S)	80 (S)	73 (S)
Médias	11 (T)	39 (MT)	73 (S)	85 (S)	80 (S)

T= tolerant, MT= moderately tolerant, MS= moderately sensitive, S= sensitive.