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# Isolated and combined effects of soil salinity and waterlogging in seedlings of ‘Green Dwarf’ coconut

## Efeitos isolados e combinados da salinidade do solo e encharcamento em mudas de coqueiro ‘Anão Verde’

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### Abstract

Soil salinization is a problem commonly found in semi-arid regions. In addition, the problem of salinity is aggravated in clayey soils when accompanied by cycles of waterlogging in the rainy season or when excess irrigation is applied. In this work we evaluated the isolated and combined effects of soil salinity and waterlogging on the responses of young plants of ‘Green Dwarf’ coconut. The experiment was conducted under controlled environment in a complete randomized block design, arranged in split plots 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<sup>-1</sup>). Response of coconut seedlings to waterlogging was dependent on the level of soil salinity, with waterlogging significantly impairing biomass accumulation and leaf expansion at low soil salinity levels, but causing no additional harm at elevated salinity. Leaf gas exchange was reduced mainly due to soil salinity, and this response was related to stomatal and non-stomatal effects. Seedlings of ‘Green Dwarf’ coconut used in this study were classified as moderately-tolerant to salinity when grown in soils with an electrical conductivity up to 11.07 dS m<sup>-1</sup>, having the potential to be used in revegetation programs of salt-affected areas, provided that these areas are not exposed to frequent waterlogging cycles.

**Key words:** Salt stress. *Cocos nucifera*. Water excess.

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## Resumo

A salinização dos solos é um problema comumente encontrado em regiões semiáridas. Além disso, nos solos mais argilosos, o problema da salinidade vem acompanhado de ciclos de encharcamento do solo, no período de chuvas ou no caso de irrigação excessiva. Neste trabalho, avaliamos os efeitos da salinidade do solo e do encharcamento, de maneira isolada e combinada, nas respostas adaptativas de plantas jovens de coqueiro-anão-verde. O experimento foi conduzido em ambiente protegido, sob delineamento estatístico de blocos casualizados, arranjos em parcelas subdivididas com cinco repetições. As parcelas foram constituídas por cinco ciclos de encharcamento (0, 1, 2, 3 e 4), com duração de quatro dias cada, aos 30, 60, 90 e 120 dias do período experimental e as subparcelas foram constituídas por cinco níveis de salinidade do solo (1,70; 11,07; 16,44; 22,14 e 25,20 dS m<sup>-1</sup>). As respostas das mudas de coqueiro ao encharcamento dependeram do nível de salinidade do solo, os quais reduziram significativamente o acúmulo de biomassa e a expansão foliar em baixos níveis de salinidade do solo. Contudo, os níveis de encharcamento não causaram danos adicionais sob elevados níveis de salinidade. As trocas gasosas foliares foram reduzidas principalmente devido à salinidade do solo, e esta resposta pode estar relacionada aos efeitos estomáticos e não estomáticos. As mudas de coqueiro 'Anão Verde' utilizadas neste experimento foram classificadas como moderadamente tolerantes à salinidade, quando cultivadas em solos com condutividade elétrica de até 11,07 dS m<sup>-1</sup>, podendo ser utilizadas em programas de 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 several 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). The crop has been introduced in many countries such as Indonesia, Philippines, India, but Brazil stands out as the main producer (FAOSTAT, 2011). 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 (FERREIRA-SILVA et al., 2010).

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 excess salts. The moderate salt tolerance of 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 (YU et al., 2012), and information on the combined effects of salinity and waterlogging on this crop are unknown.

Thus, in this research we evaluate the morphometric and 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 the simultaneous stresses of salinity and excess water, while 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.: 3°45'S, Long.: 38°33'W, Alt.: 19 m). Air temperature, relative humidity, and luminosity during the experimental period were stored in 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 plots with five replications. The plots comprised of 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 transplanting (DAT). Each cycle lasted for four days, simulating the waterlogging caused by a typical rainfall in the region. After four days of waterlogging, the pots were drained and the excess water was 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, or ECe (S1 = 1.70, S2 = 11.07, S3 = 16.44, S4 = 22.14 and S5 =

25.20 dS m<sup>-1</sup>). The soil, classified as a Fluvic Neosol (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. The salinity levels used were representative of the different stages of soil salinization found within this irrigated area of Ceará.

Sixty-day-old seedlings of the coconut cultivar Dwarf Green Brasil de Jiqui were transplanted into 20-L plastic containers, equipped with a drain in the lower part to remove excess water after each waterlogging cycles. During the cultivation, 200 g of a NPK formulation (15-10-15) was added, following technical fertilizer recommendations for the coconut crop (FONTES et al., 1998). In addition, 30 g of the commercial formulation FTE BR 12 was applied to prevent micronutrient deficiencies.

Plants were irrigated every other day with water from a well located in the experimental area, which water had an electrical conductivity (ECw) of 0.9 dS m<sup>-1</sup>. The soil was maintained at maximum water holding capacity (field capacity), except during waterlogging cycles. The pots were irrigated with a drip irrigation system employing self-compensating emitters with a flow rate of 4.0 L h<sup>-1</sup>.

### *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<sub>2</sub> concentration, and employing an artificial source of radiation with an intensity of 1600 μmol m<sup>-2</sup> s<sup>-1</sup>.

### *Plant growth*

Plants were harvested 124 DAT to determine total leaf area (LA) using a LI-3100 area integrator (Li-Cor, Inc., Lincoln, NE, USA) and shoot dry

biomass. These parameters were evaluated for the different soil salinity levels and water logging cycles tested, and compared to the control treatment.

#### *Tolerance of plants*

The relative salt and/or waterlogging tolerance were/was obtained by considering the reductions in shoot dry biomass production quantified for different levels of salinity and waterlogging, and comparing them to the control (plants grown in non-saline soil and without waterlogging), according to Fageria et al. (2010). Thus, the plants, according to their growth reduction, were 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*

Data were submitted to analysis of variance at the probabilities of 5% and 1%. If a significant effect was found for any isolated parameter (salinity and waterlogging) or for their interaction, data were then submitted to regression analysis using the SISVAR® 5.5 software (FERREIRA, 2010).

## **Results**

#### *Plant growth*

Leaf area and shoot dry weight of the ‘Green Dwarf’ coconut plants were affected by the interaction waterlogging x soil-salinity ( $p < 0.05$ ). Plants grown in a soil of lower salinity (1.7 dS  $m^{-1}$ ) markedly reduced their leaf area (Figure 1A) when exposed to more than one waterlogging cycle, with reductions of 5, 45, 48 and 51% when subjected to one, two, three and four waterlogging

events, respectively. Their related reduction in shoot biomass (Figure 1B) were 3, 41, 42 and 45%, respectively. On the other hand, the effect of waterlogging decreased when the level of soil salinity increased, and no influence of waterlogging was seen at salinity higher than 11.07 dS  $m^{-1}$ .

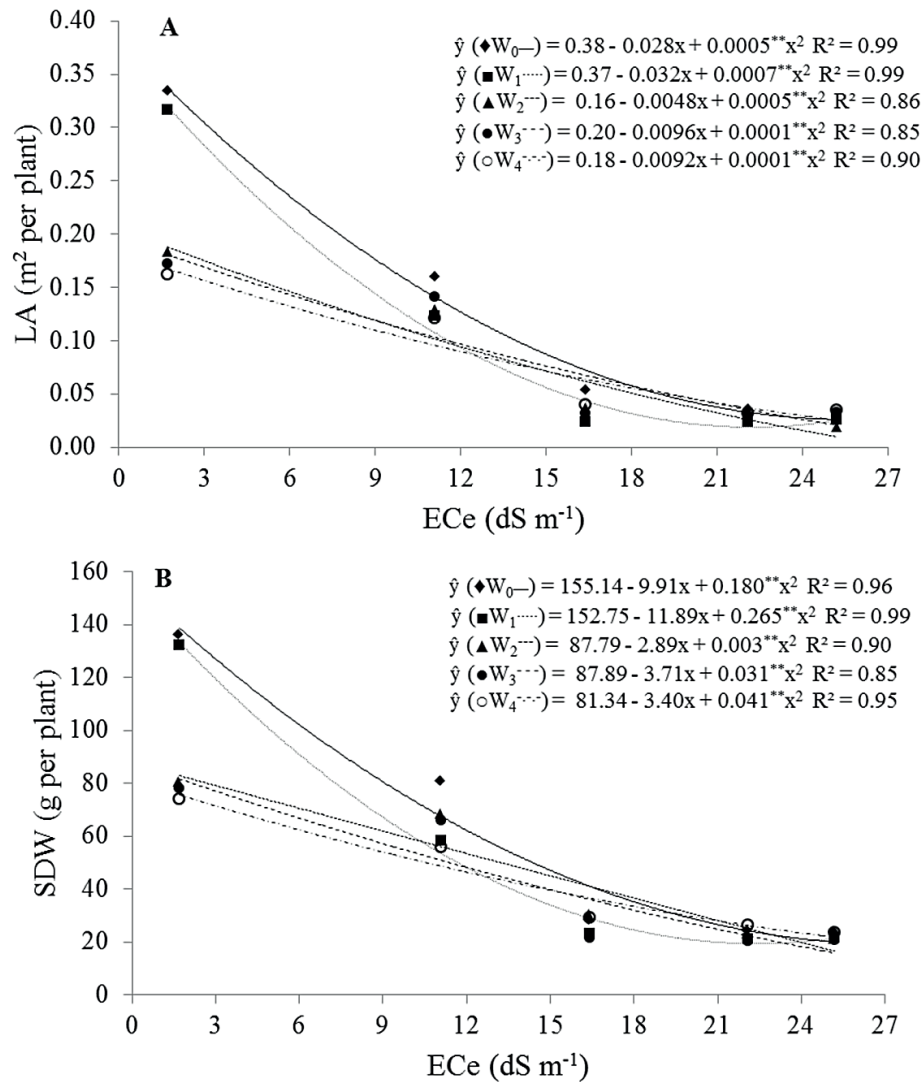
The increase in soil salinity caused severe inhibition on leaf area expansion (Figure 1A), resulting in reductions of 42, 84, 87 and 88% for salinity levels of 11.07, 16.44, 22.14 and 25.20 dS  $m^{-1}$ , respectively, compared to the mean values for plants grown in soil of low salinity (EC = 1.70 dS  $m^{-1}$ ). These decreases in shoot biomass (Figure 1B) were 34, 74, 77 and 78% for soil salinity levels of 11.07, 16.44, 22.14 and 25.20 dS  $m^{-1}$ , respectively.

#### *Tolerance of plants*

Young plants of ‘Green Dwarf’ coconut were classified as tolerant when cultivated in non-saline soil (ECe = 1.70 dS  $m^{-1}$ ) and subjected to only one waterlogging cycle (Table 1). When the plants were exposed to more than one waterlogging cycle, even if under control salinity, they proved to be moderately tolerant or moderately sensitive to waterlogging with shoot biomass reductions of 40-45%, respectively (Table 1).

Salinity, expressed by the electrical conductivity of the saturated soil extract (ECe), allowed the plants to be classified as moderately tolerant to ECe of 11.07 dS  $m^{-1}$ , without waterlogging, and as moderately sensitive when exposed to one or more waterlogging cycles. On the other hand, at the more severe levels of soil ECe (16.44, 22.14 and 25.20 dS  $m^{-1}$ ), waterlogging had no significant influence on dry shoot biomass, and plants were classified as sensitive, regardless of the number of waterlogging cycles (Table 1).

**Figure 1. A,** Leaf area (LA) and **B,** shoot dry weight (SDW) of seedlings of 'Green Dwarf' coconut according to soil salinity and waterlogging cycles.



**Table 1.** Reduction in shoot dry weight (SDW) production (%) and classification of salt/waterlogging tolerance in young plants of 'Green Dwarf' coconut.

Waterlogging Cycles	Reduction in SDW (%)				
	Soil salinity (dS m <sup>-1</sup> )				
	1.70	11.07	16.44	22.14	25.20
0	Control	26 (MT)	79 (S)	82 (S)	83 (S)
1	3 (T)	57 (MS)	83 (S)	84 (S)	84 (S)
2	40 (MT)	50 (MS)	83 (S)	83 (S)	83 (S)
3	40 (MT)	51 (MS)	84 (S)	85 (S)	85 (S)
4	45 (MS)	59 (MS)	78 (S)	81 (S)	83 (S)

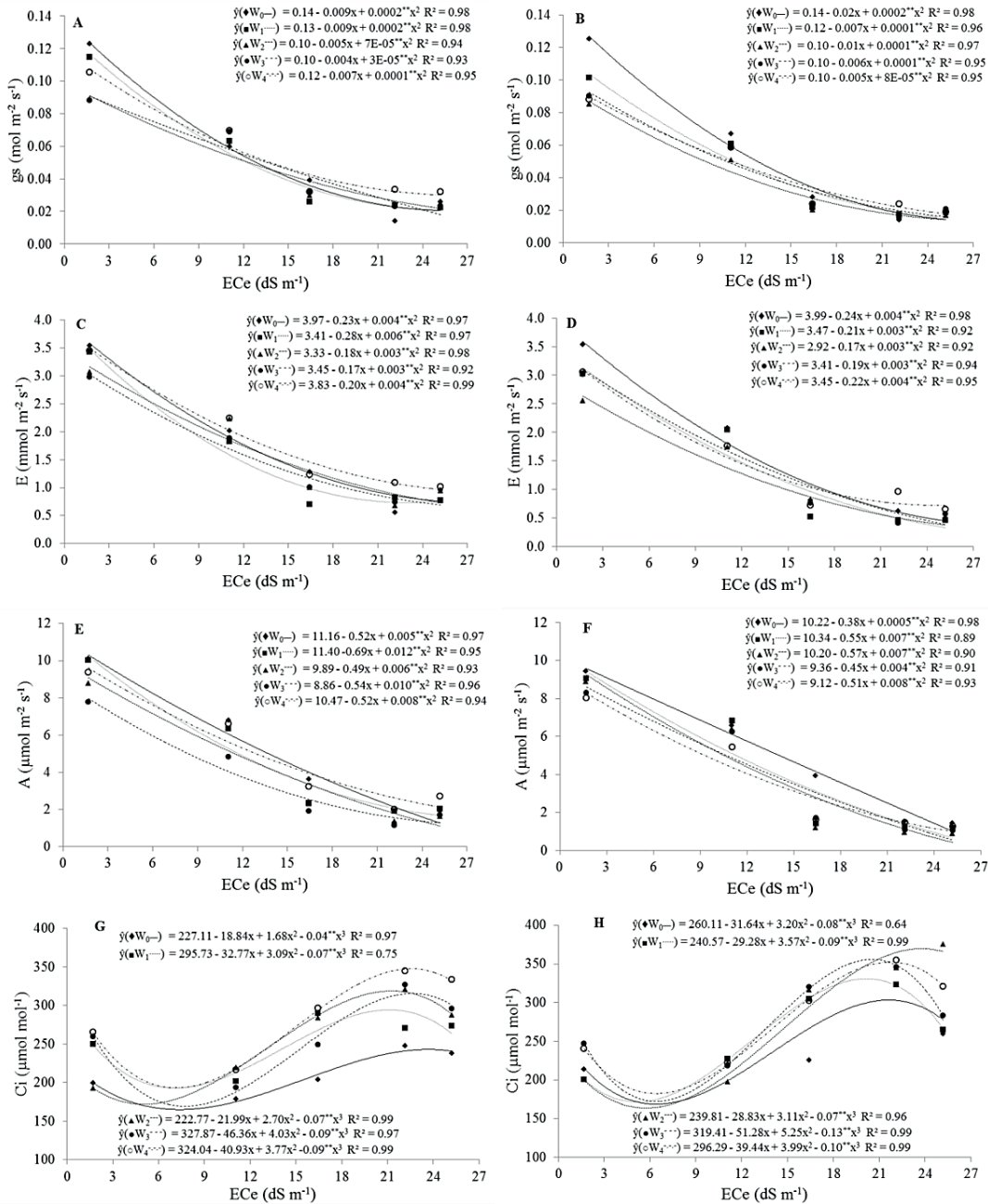
T= tolerant, MT= moderately tolerant, MS= moderately sensitive, S= sensitive.

Leaf gas exchange

Leaf gas exchange were also affected by waterlogging x soil-salinity interaction (Figure 2). Higher mean values for stomatal conductance ( $0.12 \text{ mol m}^{-2} \text{ s}^{-1}$ ) were seen in the control plants; however, imposition of waterlogging caused decreases in stomatal conductance in plants under

this treatment. With the increases in soil salinity, significant decreases in stomatal conductance were also observed, and these effects were independent of the number of waterlogging cycles, especially at the soil salinity levels higher than  $11.07 \text{ dS m}^{-1}$  ( $16.44, 22.14$  and  $25.20 \text{ dS m}^{-1}$ ).

**Figure 2.** Stomatal conductance - gs (A and B), transpiration - E (C and D), photosynthetic rate - A (E and F), and internal  $\text{CO}_2$  concentration (G and H) in young plants of ‘Green Dwarf’ coconut, according to soil salinity and waterlogging cycles. Measurements were performed before (A, C, E and G) and after (B, D, F and H) exposure to waterlogging.



For any period of evaluation, an increase in soil salinity from 11.07 to 25.20 dS m<sup>-1</sup> 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 2A, B), when compared to the average values presented by control plants grown in non-saline soil (ECe = 1.70 dS m<sup>-1</sup>). Similar results were seen for the rate of transpiration (Figures 2C, D).

The lowest values for photosynthetic rate (A) were seen in plants grown in soils with ECe of 16.44, 22.14, and 25.20 dS m<sup>-1</sup>, irrespective of the number of waterlogging cycles (Figures 2E, F); whereas the greatest mean values for A were found when the plants were grown in soil of control salinity (1.70 dS m<sup>-1</sup>) 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 recorded for plants grown in soils with the greatest salinities (16.44, 22.14 and 25.20 dS m<sup>-1</sup>), respectively. Minimum values of 2.02 μmol m<sup>-2</sup> s<sup>-1</sup> were estimated for plants grown in soil of the highest salinity before imposition of the fourth waterlogging cycle, and of 0.99 μmol m<sup>-2</sup> s<sup>-1</sup> after the fourth cycle (Figure 2F).

Mean values for internal CO<sub>2</sub> concentration (Ci) were 233 and 202 μmol mol<sup>-1</sup> for plants subjected to ECe of 1.7 and 11.07 dS m<sup>-1</sup> respectively, with significant increases recorded at the lowest salinity level when plants were subjected to three and four waterlogging cycles (Figure 2G, H). However, the greatest values for Ci were found in plants grown in soils of 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<sup>-1</sup>), plants displayed Ci mean values of 297.5, 290.5 and 324.9 μmol mol<sup>-1</sup> when exposed to two, three, and four waterlogging cycles respectively (Figure 2G). After exposure to waterlogging, Ci values were 344.9, 316.6 and 326.1 μmol mol<sup>-1</sup> respectively (Figure 2H).

## Discussion

Plant species are often limited by multiple stress factors operating simultaneously (LACERDA et al., 2016; LENSSEN et al., 2003; SILVA et al., 2016) such as soil salinity and waterlogging (GARCÍA; 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 effect of a stress factor depends on the level of the other factor to which the plant is subjected. Analysing the degree of inhibition of leaf area and biomass accumulation (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<sup>-1</sup>). Increased salinity, ranging from 16 to 25 dS m<sup>-1</sup>, produced a salt stress condition so severe that increases in waterlogging cycles had little additional negative effect on morphometric and physiological response patterns provoked by high soil salinity.

Results published by others showed that, under severe conditions of salinity, responses to water availability are greatly affected. Silva et al. (2016), studying the interactions between soil salinity and water deficit in seedlings of dwarf coconut, found that water deficit accentuated plant susceptibility to salinity. However, this interaction was evident only at low salinity levels. Those authors reported that seedlings under a soil salinity of 6.25 dS m<sup>-1</sup> were classified as tolerant (3% reduction in biomass production) under no water stress, but their classification changed to moderately sensitive (48% reduction) when the water supply was reduced to 20% of control. When grown in soil with ECe = 25.8 dS m<sup>-1</sup>, the plants were classified as moderately sensitive (with a reduction of about 50% in biomass), regardless of the level of water supply (100 or 20%).

It is clear from the present study, that the high salinity levels resulted in a significant degree of severity on growth responses, which were not substantially altered even after the addition of

waterlogging (Table 1, Figure 1). However, the effects of waterlogging were observed in plants grown at the two lowest levels of soil salinity (1.7 and 11.07 dS m<sup>-1</sup>), and this should not be ignored considering that salinity levels as high as 11.7 dS m<sup>-1</sup> are found in many areas degraded by salinization.

Other results obtained from the interaction between salinity and waterlogging may be explained, at least in part, by the degree of severity of the two stress factors involved. For example, biomass accumulation in seedlings of *Lotus tenuis* subjected to soil salinity did not decrease when waterlogging was imposed (GARCÍA; MENDOZA, 2014). On the other hand, the halophyte *Suaeda maritima*, under conditions of flooding and salinity, displayed reduced shoot biomass accumulation (32%) when submitted to a combination of salinity and waterlogging, compared to plants subjected only to salt stress (ALHDAD et al., 2013).

In this work, physiological responses were mainly affected by soil salinity (Figure 2), but with some similarities when compared to the growth data (Figure 1). 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 stress factor was salinity, which severely reduced leaf gas exchange, while waterlogging had practically no influence when combined with salinity.

According to Taiz et al. (2017), plants that are sensitive to waterlogging are severely impaired after only 24 hours of stress exposure, while tolerant plants can cope for a few days but do not withstand long periods of hypoxia or anoxia. Similarly, Medri et al. (2012) state that stomatal closure and decreased photosynthesis are common responses to the restriction or lack of oxygen in the soil caused by waterlogging. Our results obtained with coconut seedlings show that cycles of four days of hypoxia were enough to cause reductions in leaf gas

exchange and growth, especially at the lower levels of soil salinity.

Decrease in stomatal conductance and decrease in the rate of transpiration are probably the first defenses of the plant in response to increased salinity, alone or associated with another stress factor (LI et al., 2013; 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<sub>2</sub> and the internal CO<sub>2</sub> concentration (BHUIYAN et al., 2015; 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 (SANTOS et al., 2012). This was demonstrated in the present study because reduced photosynthesis was accompanied by significant increases in the internal CO<sub>2</sub> concentration, specially at the higher levels of soil salinity.

Our results illustrate three different, and interesting situations, possibly linking stomatal responses to non-stomatal responses, and according to the interaction and severity of salt and waterlogging stresses: 1) plants at the lowest soil salinity level (1.7 dS m<sup>-1</sup>) 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<sup>-1</sup>), there was a tendency to maintain or decrease Ci in relation to the control level, demonstrating the occurrence of stomatal effects and a lesser severity of the two stress factors imposed simultaneously on the plants; 3) plants subjected to highest levels of soil salinity (16 to 25 dS m<sup>-1</sup>) 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, and agree with results obtained by others (MEDRI et al., 2012).



## Conclusions

Our results indicated that the seedling response of the 'Dwarf Green' coconut to waterlogging depended on the level of soil salinity, with waterlogging significantly impairing seedling biomass accumulation at low soil salinity levels, but causing little to 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<sup>-1</sup>, and without exposure to waterlogging cycles. 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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