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Physiological Indicators in Two Lettuce Cultivars Under Saline Stress

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Abstract

Lettuce (*Lactuca sativa* L.) is considered as the main leafy vegetable in Brazil. In the last decades, there had been many changes in the predominant varietal types in the country, however, issues regarding the use of saline water inhibit the growth by the osmotic effect. The aim of this study is avaliate the effect of water salinity on physiological in lettuce cultivars. The experiment was carried out at the Alagoas Federal University, Arapiraca *Campus*, in a completely randomized design and with a 5 × 2 factorial scheme, with six replications. Five treatments of water salinity levels were analyzed (ECw: 0.14, 1.54, 2.94, 4.34, and 5.74 dS m^{-1} at 25 °C) in two types of lettuce crops (Saia Véia and Vitoria Verdinha). Stomatal conductance, net photosynthesis, transpiration rate, water use efficiency, leaf temperature, and green index were assessed at 10, 20, and 30 days after the application of the treatments. The saline stress caused by the increase in saline concentrations decreased the photosynthesis and transpiration rates, which were associated with the reduction of stomatal conductance in both cultivars. Nevertheless, Saia Véia cultivar was higher tolerance in all tested saline levels compared to Vitória Verdinha. The green index for Vitoria Verdinha was seven times higher when compared to Saia Véia from the lowest to the highest saline levels. The cultivars differ in salt sensitivity, which could be useful for producers to choose the cultivar that is most adapted to the region and breeders regarding improvement prospects for adaptation of the lettuce under saline stress. In addition to osmotic stress, which is the first to happen, there are others.

Keywords: *Lactuca sativa* L., plant ecophysiology, salinity

1. Introduction

Soil salinity and irrigation water have been arousing interest since they are regarded as worldwide issues in agricultural production. It is estimated that soils affected by salt occupy about 10% of land surface and 50% of irrigated land in the world (Ruan et al., 2010). The effects of salinisation can be observed in numerous vital ecological and non-ecological soil functions. Drivers of salinisation can be detected both in the natural and man-made environment, with climate and the foreseen climate change also playing an important role. Global annual losses in agricultural production due to salt-affected soils can be as high as US$ 12 billion (Qadir et al., 2008; Flowers et al., 2010). The salinization process is due to environmental characteristics and anthropic actions (Daliakopoulos et al., 2016). Natural characteristics include the transport of salt sediments from salinized areas to unsalted sites; actions of ascent by capillarity of the soils to surface; high rates of evapotranspiration, among other factors (Pedrotti, 2015; Walter et al., 2018).

Soil salinity, whether natural or human induced, is a major geo-hazard in arid and semi-arid landscapes. In agricultural lands, it negatively affects plant growth, crop yields, whereas in semi-arid and arid non-agricultural areas it affects urban structures due to subsidence, corrosion and ground water quality, leading to further soil erosion and land degradation (Abuelgasim & Ammad, 2019).

The Brazilian Semi-arid (BSA) region known as the Sertão is located in the Northeast part of the country and is one of the two areas most affected by climate change in Brazil (Seddon et al., 2016). The Sertão is characterized by the unique Caatinga biome - mostly consisting in deciduous forests, with uneven rainfall patterns and land distribution, climatic variation and social disparities. Several public policies have been adopted since 1877 to address the issues of the region, predominantly related to water scarcity; yet, the Sertão remains marginalized. Family farmers are the area's most vulnerable social group, in particular diffuse farmers (Machado & Revere, 2018). In these regions, agricultural activity has been a great challenge, especially for family farmers who do not have technical assistance and, plant empirically, leafy vegetables that are highly sensitive to soil salination (Qin et al., 2010; Medeiros et al., 2016).

Among the leafy vegetables sensitive to soil salinity, lettuce stands out (*Lactuca sativa* L.), consumed worldwide and is an important crop of agrosystems in semi-arid regions (Ribeiro et al., 2003). Saline environments are mainly located in coastal zones, but they can also be found inland. Natural causes for inland salinization are the presence of salt-rich rainwater or saline groundwater, high evaporation and low precipitation. In such regions, upward-directed soil water movement by capillary rise is a dominant process and rates of precipitation are too low to leach out salts. This leads to an accumulation of salts within the topsoil or at the soil surface. Typically, such conditions are found in arid regions (Colombani, Mastrocicco, & Giambastiani, 2015; Walter et al., 2018).

Accumulation of salts in the soil can alter the water uptake by plants, reducing assimilation and nutrient utilization (Parida & Das, 2005; Tavakkoli et al., 2012). The accumulation of Na^+ and/or Cl^- in chloroplasts, affects important biochemical and photochemical processes involved in photosynthesis and gas exchange (Xu & Mou, 2015; Amorim et al., 2010). Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity and eventually die). Majority of major crop species belong to this second category. Thus salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide (Flowers, 2004; Munns & Tester, 2008). In addition, the tolerance a crop has to salinity can vary between genotypes of the same species as well as the plant's development stage (Gheyi et al., 1997).

According to the researchers Gupta & Huang (2014) a comprehensive understanding on how plants respond to salinity stress at different levels and an integrated approach of combining physiological tools with and biochemical techniques are imperative for the development of salt-tolerant varieties of plants in salt-affected areas. Recent research has identified various adaptive responses to salinity stress at physiological levels, although mechanisms underlying salinity tolerance are far from being completely understood. Lettuce is considered 'moderately sensitive' to salinity and its potential yield is reached when the electrical conductivity of the saturated extract reaches the threshold value of 1.3 dS m^{-1}, with a 13% reduction in yield per unit increase of salinity above the value (Ayers & Westcot 1991). In this context, this paper aims evaluate the effect of water salinity on physiological in lettuce cultivars.

2. Methods

2.1 Environment and Meteorological Parameters

The experiment was carried out in a greenhouse with 50% shading and anti-UV treatment, located at the Alagoas Federal University (UFAL), Arapiraca *Campus* (latitude 09°41′53.6″S, longitude 36°41′26.3″W and 244 m altitude) Northeast of Brazil. The outside meteorological parameters in the greenhouse obtained from the university's automatic weather station (maximum, minimum temperature, precipitation and global radiation). The inside meteorological parameters in the greenhouse: photosynthetically active radiation obtained from spectroradiometer (Apogee, model SS-110) which measures received by pots were performed in two moments: 7:00 a.m. to 11:00 a.m. and 12:00 p.m. to 4:00 p.m. The temperature and humidity were measured with the aid of a thermo-hygrometer (model HT-208) and it was installed at the center of the protected environment, 1.0 m above from the ground level.

The climate of the region is of the AS tropical type (rainy with a dry summer), according to the classification of Köppen (1948) and Dubreuil et al. (2018). It features two well established climatic seasons: a warm and dry summer, with occasional rainfall (September to March), and wet and rainy winter (April to August). The mean annual precipitation ranges from 750 to 1000 mm (Xavier & Dornellas, 2005).

2.2 Lettuce Varieties

Sáia véia: The cycle of 35 to 40 days after transplantation. Optimum germination range: 10-27 °C. Plant with moderately wrinkled, large, and elliptical smooth leaves, with light green color. It does not form a head and has white seeds.

Vitória verdinha: The cycle of 35 to 40 days after transplantation. Optimum germination range: 10-27 °C. It has smooth leaves, slightly wrinkled and soft texture, dark green in color. It does not form a head and has white seeds.

These two lettuce cultivars used in this experiment are the most planted in the APL region (Aranjo Produtivo Local) in the municipality of Arapiraca in Northeast of the Brazil. The APL is reference in the production of fruits and vegetables and it distributes to consumer markets in Brazil.

2.3 Soil Analyses

A soil sample of 1.0 kg was collected at the UFAL area in a 0-0.20 m layer that represents the effective depth of the lettuce root system. Samples were sent for chemical and physical analyses, respectively, to the Soil Fertility Laboratory and Soil Physics Laboratory (Department of Environmental Resources, School of Agronomic Sciences, UNESP, Botucatu, São Paulo).

Soil analysis classified it as a sandy loam soil and showed the following features: pH in water of 6.3; 18 mg/dm^3 of P; 3.6 mmol$_c$/dm^3 of K; 44 mmol$_c$/dm^3 of Ca; 16 mmol$_c$/dm^3 of Mg; 0 mmol$_c$/dm^3 of Al; 11 mmol$_c$/dm^3 of H$^+$ Al; 17 mg/dm^3 of Fe; 4.7 mg/dm^3 of Mn; 0.5 mg/dm^3 of Cu; 1.4 mg/dm^3 of Zn; 25 mmol$_c$/dm^3 of S; 63 mmol$_c$/dm^3 of base sum (BS); 85% base saturation (V), 74 mmol$_c$/dm^3 cation exchange capacity (CEC) and 18 g/dm^3 organic matter (OM). The physical analyzes quantified the levels of sand (484 g/kg), silt (62 g/kg), and clay (162 g/kg), soil with a medium texture.

2.4 Water Analyses

The water (control) used in the experiment had the following elements: 3 mg/L of N; 0.3 mg/L of P; 12 mg/L of K; 5 mg/L of Ca; 3 mg/L of Mg; 4 mg/L of S; 4.6 mg/L of Na; 0.12 mg/L of B; 0 mg/L of Cu; 0 mg/L of Fe; 0 mg/L Mn and 0 mg/L of Zn.

The electrical conductivity curves were obtained as a function of sodium chloride (NaCl) concentration, in which the desired electrical conductivity (dS m^{-1}) was multiplied by 640 mg L^{-1}, according to Richards (1954). The different saline levels were obtained by dissolving sodium chloride (NaCl) in water, that was obtained from the supply system of UFAL, in buckets of 14 L. Saline water that was used for treatments irrigation was verified before each irrigation with the aid of a Portable Digital Conductivity Meter (Mod. Cd-4301).

2.5 Experimental Design

The experiment was assessed in three periods at interval of 10 days. Saline treatments were applied three days after the seedlings were established, thus starting the first evaluation period. The experimental design was completely randomized, arranged in a 5 × 2 factorial scheme (five salt levels and two lettuce cultivars), with six replicates. The experimental unit was represented by a pot with a plant. The treatments resulted from the combination of five irrigation water salinity levels, as follows: 0.14 (control); 1.54; 2.94; 4.34; and 5.74 dS m^{-1}. The effect of irrigation water salinity levels on the analyzed variables was measured through the analysis of variance, whose results of the treatments were studied through polynomial regression analysis. The effect of the cultivars was compared by the Tukey's test at 0.05 probability. Statistical tests were performed using the SISVAR software, version 5.3Build 77 (Ferreira, 2014).

2.6 Cultivations Conditions

Initially, seedlings of lettuce (*Lactuca sativa* L.) of the Saia Véia and Vitória Verdinha varieties were produced in Styrofoam trays with 200 cells, filled with the Bioplant substrate. Seedlings were irrigated daily with water (electrical of conductivity of 0.14 dS m^{-1}) and every 7 days were fertigated (0.5% of urea in the trays). The transplanting was performed manually 30 days after sowing (DAS), where seedlings that showed more vigor were selected and transplanted into plastic containers and one plant was conditioned per pot, with seedlings with four to six leaves. As a form of irrigation control, plastic vats with a capacity of five liters were drilled and connected to collecting hoses to conduct the fraction of drained water. Each pot had in the bottom filled with 500 g of gravel (3 cm high) and the remaining volume was completed with soil, totaling 5.5 kg. Pots were placed on a bench at 1.0 m of distance from the ground level.

The beginning of the experiment was characterized by raising the soil to the field capacity; for this, recipients were saturated with water and wrapped individually in plastic, to force the loss of water only by drainage. When the drainage ceased, the plastic covers were removed and the recipients were weighed on a digital scale (0.1 g precision), thus obtaining the control weight, corresponding to the field capacity, which was approximately 6.8 kg for each recipient; finally, seedlings were transplanted.

After the beginning of treatments, irrigation was performed twice a day (at 8:00 am and 4:00 p.m.), based on the water consumption of the plants of the previous day, keeping the soil moisture close to the field capacity.

The estimated water volume for daily irrigation was divided by the factor 0.9, to restore soil moisture to field capacity, also, to obtain a leaching fraction (FL) of 10%, using the following formula: (VI-VD)/(1-FL), where VI: is the volume of water to be applied on the irrigation; VA: is the volume of water applied in the previous irrigation, and VD: is the volume of drained water in the previous irrigation (VI, VA, and VD were described in mL).

2.7 Physiological Analysis

The physiological analysis was performed with an infrared gas analyzer IRGA (LI 6400, LICOR, Lincoln, USA), with a photosynthetic photon flux density (PPFD) according to the environment of 800 μmol photons m^{-2} s^{-1}. Analyses were performed between 8:00 a.m. and 10:00 a.m., at 10, 20, and 30 days after the application of treatments (DAAT). In fully expanded and non-senescent leaves, the following measures were performed: net photosynthesis (*A*-μmol CO_2 m^{-2} s^{-1}) and transpiration (*E*-mmol H_2O m^{-2} s^{-1}) rates, stomatal conductance (*gs*-H_2O m^{-2} s^{-1}), leaf temperature (T °C), and water use efficiency (WUE-μmol CO_2 $mmol^{-1}$ H_2O) through the *A*/*E* ratio. The green index (GI) was measured in 10 fully expanded and non-senescent leaves. For each leaf, 10 readings were collected, and a mean of the green index was obtained. The readings were obtained with a Portable Chlorophyll Meter (Model SPAD-502®, Soil Plant Analyzer Development, Minolta, Japan).

3. Results and Discussion

3.1 Data From the Automatic Meteorological Station

The recorded, for the greenhouse proximal area, where the pots were allocated with the seeds, from germination to the end of the plants' biocycle, temperatures that ranged from 24 to 36 °C (Figure 1A) and global radiations that ranged from 5 to 28 MJ m^{-2} day^{-1} (Figure 1B). Inside the greenhouse, the photosynthetically active radiation was measured, ranging from 558 μmol m^{-2} s^{-1} from 7:00 a.m. to 11:00 a.m. and 678 μmol m^{-2} s^{-1} from 12:00 p.m. to 4:00 p.m. The temperature inside the greenhouse ranged from 40 °C (May) to 28 (June) and relative humidity ranged from 30 to 80%.

The precipitation average for the year of this experiment is in line with the averages recorded for the region, as can be seen in the author's paper Xavier and Dornella (2005), in which municipality of Arapiraca has a rainfall regime concentrated in the autumn-winter period, confirming the regional dynamics. Most of your rainfall precipitates in just 3 months (usually, May, June and July).

In the month in which the experiment started (May), the highest temperature of the year was recorded, a fact that repeated itself at the end of November (Figure 1A), which increased the internal temperature of the greenhouse, as well as the relative humidity. Temperature and relative humidity influence the metabolic functions, transpiration, and mechanisms of thermal control of plants (Teruel, 2010).

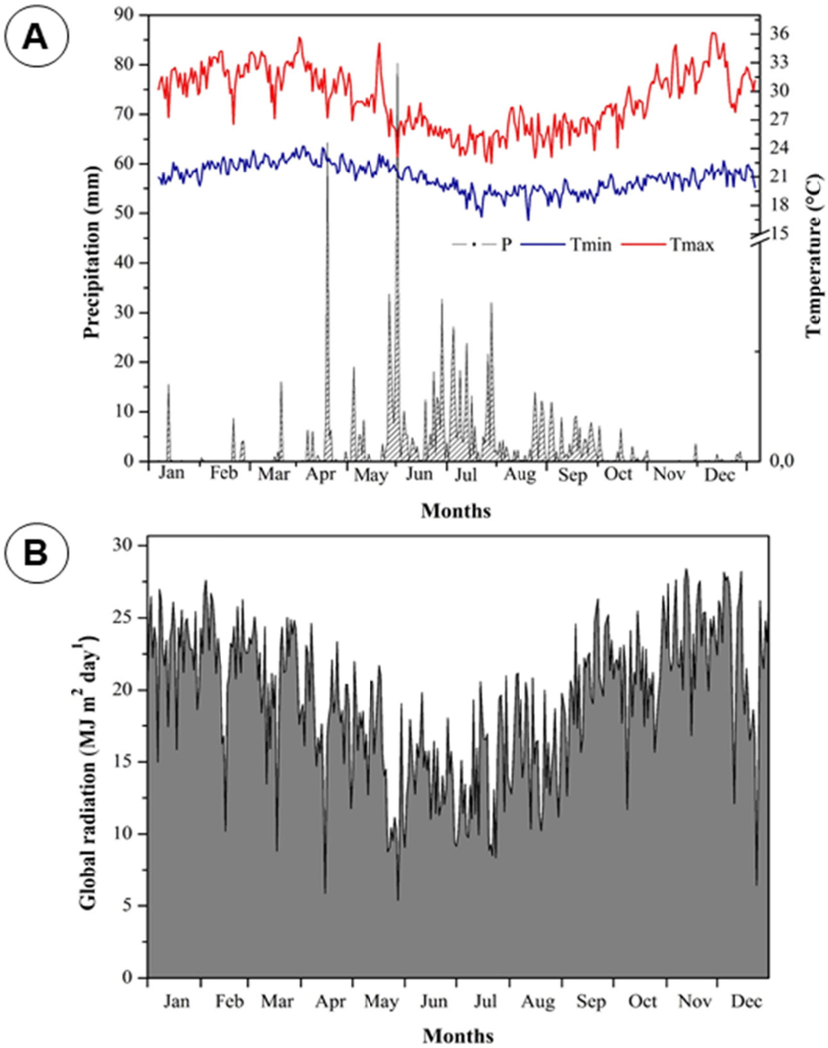

Figure 1. Climatic data obtained from the automatic weather station. A- Maximum and minimum temperatures and precipitation levels recorded in the city of Arapiraca. B- Global radiation registered in the outside area of the greenhouse. Year 2016

3.2 Physiological Parameters

There was a significant effect of irrigation water salinity during the gas exchanges in the two lettuce cultivars. However, there was no interaction between salinity and cultivars. Regarding transpiration rates (*E*) in both cultivars, the interaction effect was observed only at 20 DAAT (Table 1).

Table 1. Summary of variance analysis for photosynthesis (*A*), transpiration (*E*) and stomatal conductance (*gs*) for Saia Véia and Vitória verdinha cultivars, as a function of the electrical of conductivity water) of irrigation, at 10, 20 and 30 days after application of treatments (DAAT)

Sources of Variation	GL	Mean Squares (DAAT)								
		A			*E*			*Gs*		
		10	20	30	10	20	30	10	20	30
Salinity (S)	4	1.25 **	2.30 **	3.62 **	1.38 **	2.49 **	3.42 **	0.0001 **	0.0070 **	0.0005 **
Linear Regression	1	4.90 **	9.16 **	14.20 **	5.48 **	3.85 **	13.46 **	0.0009 **	0.0249 **	0.0020 **
Quadratic Regression	1	0.01 NS	0.01 NS	0.22 **	0.02 NS	0.06 **	0.18 **	0.0000 NS	0.0020 NS	0.0000 NS
Deviation regression	2	0.04 NS	0.02 NS	0.03 NS	0.01 NS	0.04 *	0.03 NS	0.0000 NS	0.0005 NS	0.0000 NS
Cultivar (C)	1	0.01 NS	1.31 **	1.11 NS	0.15 NS	1.15 **	0.12 **	0.0015 **	0.0366 **	0.0000 NS
S × C	4	0.01 NS	0.05 NS	0.01 NS	0.34 NS	0.02 *	0.01 NS	0.0000 NS	0.0034 NS	0.0000 NS
Residual	10	0.02	0.03	0.02	0.01	0.01	0.01	0.0000	0.0009	0.0000
CV (%)		0.81	0.89	0.71	1.94	1.06	1.16	0.70	8.39	1.46
Salinity (S)		------- μmol CO_2 m^{-2} s^{-1} -------			------ mmol H_2O m^{-2} s^{-1} ------			------------ mol H_2O m^{-2} s^{-1} ------------		
S1 (0.14 dS m^{-1})		16.96	19.02	20.76	6.60	7.74	8.56	0.3074	0.4201	0.5217
S2 (1.54 dS m^{-1})		16.66	18.45	20.45	6.29	7.00	8.07	0.3029	0.3601	0.5144
S3 (2.94 dS m^{-1})		16.41	18.10	19.86	5.98	6.64	7.58	0.2957	0.3511	0.5120
S4 (4.34 dS m^{-1})		15.85	17.61	19.10	5.49	6.14	7.04	0.2922	0.3230	0.5000
S5 (5.74 dS m^{-1})		15.61	17.05	18.45	5.15	5.69	6.18	0.2894	0.3141	0.4937
Cultivar (C)										
C1 (Saia Véia)		16.32a	18.30 a	19.72 a	5.93 a	6.88 a	7.56 a	0.3061 a	0.3965 a	0.5094 a
C2 (Vitória Verdinha)		16.27 a	17.79 b	19.72 a	5.87 a	6.40 b	7.41 b	0.2889 b	0.3109 b	0.5073 a
MSD		0.13	0.16	0.14	0.11	0.07	0.09	0.0021	0.0295	0.0074

Note. (*) Significant at 0.05 and (**) at 0.01 probability; (NS) Not Significant; (MSD) Minimum Significant Difference. Means followed by the same letter do not differ from each other, in columns, by the Tukey test at $p < 0.05$ level.

On the other hand, when the isolated effect of salinity levels was analyzed, there was an effect ($p < 0.01$) for the three variables (*A*, *E*, and *gs*) in the different periods assessed, which highlights the direct influence of saline water on growth and development of the two lettuce cultivars (Table 1).

The presence of NaCl in the irrigation water influenced the photosynthesis of the assessed cultivars only at 20 DAAT, where the cultivar C1 showed an increase of approximately 3% in this variable compared to C2. Compared to C2, cultivar C1 showed higher values for *E* at 20 (7%) and 30 (2%) DAAT and *gs* at 10 (5.6%) and 20 (21.6%) DAAT (Table 1).

Saline stress caused linear reductions in *gs* in all assessed periods; however, at 20 DAAT, the decrease was more expressive with approximately 5% per unit increase of ECw. The decrease of the lowest (0.14 dS m^{-1}) to the highest saline level (5.74 dS m^{-1}) was 24.7% (Figure 2A).

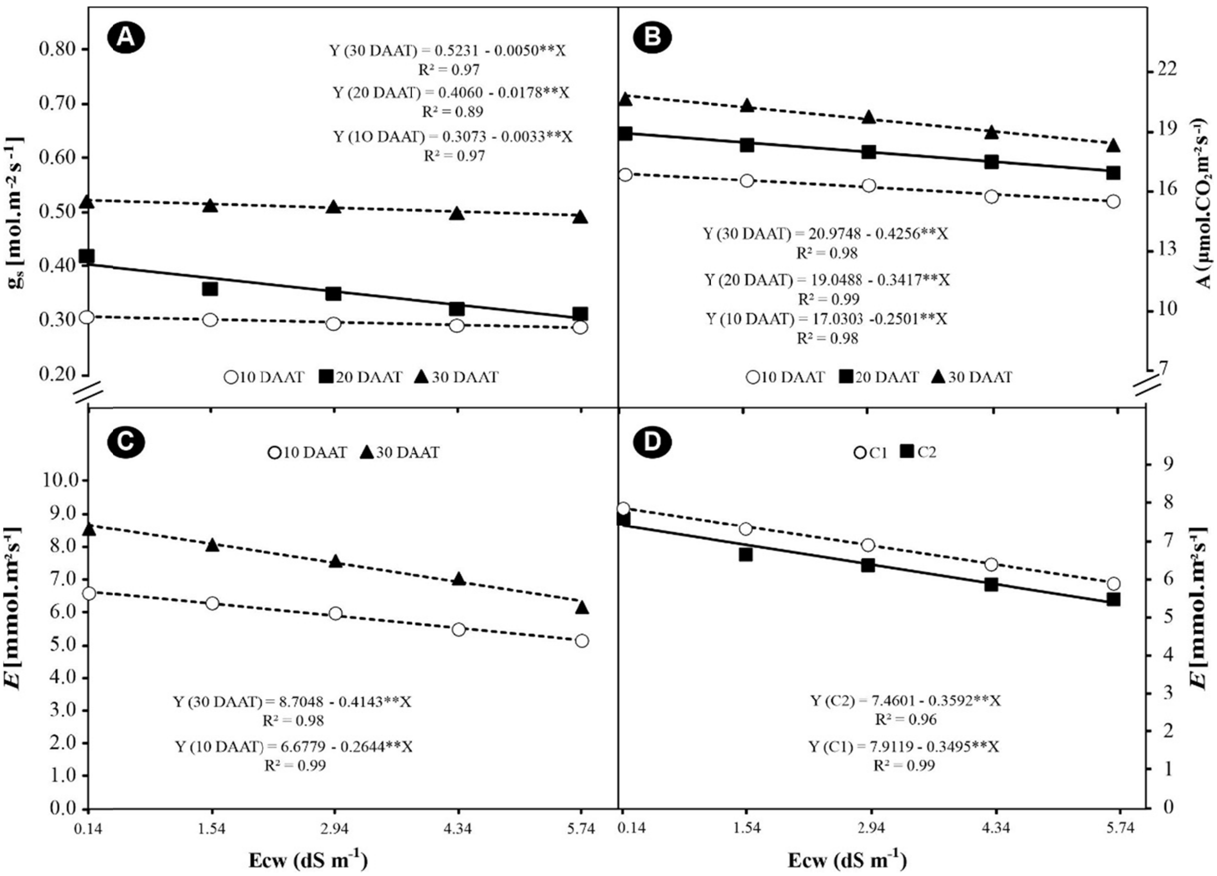

Figure 2. Physiological parameters of two lettuce cultivars submitted to increasing salinity levels. (A) Stomatal conductance, (B) photosynthesis, (C) Transpiration only for salinity levels and (D) Transpiration only cultivars effect

In the photosynthesis analysis, there were linear reductions of 1.5, 1.8, and 4.8%, at 10, 20, and 30 DAAT, respectively (Figure 2B), for each increased unit of ECw. The decrease in the lowest level concerning higher saline levels was 8.2, 10, and 26.8% at 10, 20, and 30 DAAT, respectively.

The transpiration rate at 10 and 30 DAAT was only effective for the salinity levels, with linear reductions of 4.1 and 4.8%, respectively, for each increased unit of ECw (Figure 2C). The decrease in the lowest level concerning the highest saline level was 22.3 and 26.8%, respectively, at 10 and 30 DAAT.

At 20 DAAT, cultivars presented linear adjustments, with reductions of 4.5 and 4.9% with a unit increment of salinity, respectively. The decrease between the lowest and highest saline levels was 24.9 and 27.2% for C1 and C2, respectively (Figure 2D).

In the two cultivars evaluated, the rates of *A* and *E* were directly influenced by *gs*. Under conditions of stress, especially water and salt, the stomatal closure can be seen as a positive response of the plant to the maintenance of water balance (Yousifet al., 2010). It is well known that salt stress reduces root hydraulic conductivity resulting in decreased water flow from roots to shoot, even in osmotically adjusted plants (O'Leary, 1969; Prisco & Gomes-Filho, 2010). This decrease in water flow due to salt stress may cause a lowering in leaf water content, that would result in stomatal closure in order to maintain their water status (Azevedo-Neto et al., 2004). Thus, *gs* may have been reduced due to lower water availability of the roots (Tatagiba et al., 2014).

Similar results were observed by Han and Lee (2005) in which lettuce plants submitted to salt stress showed severe reductions in *A*, *E*, and *gs*. Similar results were found by Moles et al. (2016), where severe restrictions on *A* and *E* rates were observed in two tomato cultivars submitted to different saline levels.

Water use efficiency (WUE) and leaf temperature (T) were not influenced by the interaction of salinity levels and cultivars (Table 2); however, Green Index (GI) was significant only at 30 DAAT.

Table 2. Summary of variance analysis for Green Index (GI), Water Use Efficiency (WUE), and Leaf Temperature (LT) of cultivars Saia Véia and Vitória verdinha, as a function of the electrical conductivity of water of irrigation at 10, 20, and 30 days after application of the treatments (DAAT)

Sources of Variation	GL	Mean Squares (DAAT)								
		GI			WUE			LT		
		10	20	30	10	20	30	10	20	30
Salinity (S)	4	1.67 **	11.23 **	11.64 **	0.14 **	0.17 **	0.18 **	1.84 **	2.27 **	4.78 **
Linear Regression	1	6.64 **	42.03 **	42.03 **	0.54 **	0.69 **	0.68 **	6.78 **	8.61 **	18.53 **
Quadratic Regression	1	0.02 NS	0.35 NS	3.81 **	0.01 NS	0.00 NS	0.03 **	0.00 NS	0.37 *	0.17 NS
Deviation regression	2	0.00 NS	1.28 **	0.36 *	0.00 NS	0.00 NS	0.01 *	0.28 NS	0.04 NS	0.20 *
Cultivars (C)	1	0.04 NS	73.35 **	46.82 **	0.00 NS	0.08 **	0.02 **	1.88 **	0.02 NS	0.03 NS
S × C	4	0.04 NS	0.20 NS	1.80 **	0.01 NS	0.00 NS	0.00 NS	0.14 NS	0.02 NS	0.02 NS
Residual	10	0.05	0.09	0.05	0.00	0.00	0.00	0.17	0.05	0.04
CV (%)		0.75	1.01	0.74	2.32	1.25	1,53	1.25	0.67	0.61
Salinity (S)		--- Dimensionless greatness ---			------ CO_2 mmol^{-1} H_2O ------			--------------- °C ---------------		
S1 (0.14 dS m^{-1})		29.95	27.58	29.03	2.57	2.46	2.42	32.39	32.53	32.71
S2 (1.54 dS m^{-1})		29.58	28.00	29.40	2.65	2.64	2.54	33.08	32.79	33.43
S3 (2.94 dS m^{-1})		29.23	30.18	30.30	2.74	2.73	2.62	33.38	33.04	33.67
S4 (4.34 dS m^{-1})		28.75	30.65	31.05	2.89	2.87	2.71	33.45	33.77	34.77
S5 (5.74 dS m^{-1})		28.33	31.38	33.33	3.03	3.00	2.99	34.26	34.36	35.45
Cultivars (C)										
C1 (Saia Véia)		29.21 a	27.64 b	29.09 b	2.77 a	2.68 b	2.63 b	33.00 b	33.33 a	33.97 a
C2 (Vitória Verdinha)		29.12 a	31.47 a	32.15 a	2.78 a	2.80 a	2.69 a	33.62 a	33.27 a	34.04 a
MSD		0.22	0.30	0.23	0.06	0.01	0.04	0.42	0.22	0.21

Note. (*) Significant at 0.05 and (**) at 0.01 probability; (NS) Not Significant; (MSD) Minimum Significant Difference. Means followed by the same letter do not differ from each other, in columns, by the Tukey test at $p < 0.05$ level.

When analyzing the isolated effect of salinity levels ($p < 0.01$) for the three variables (GI, WUE, and T), the different evaluation periods were observed, and interaction was observed between salt levels and cultivars exclusively for GI at 30 DAAT. The cultivar factor was significant ($p < 0.01$) at 20 and 30 DAAT. Regarding cultivars C1 and C2, higher values were observed for GI 13.9 and 10.5% at 20 and 30 DAAT, respectively (Table 2).

The variable WUE was influenced by the presence of NaCl at 20 and 30 DAAT, where cultivar C2 increased by 4.5 and 2.3%, respectively, when compared to C1 (Table 2). At 10 DAAT, LT was higher for C2, which presented an increase of 1.9% about C1 (Table 2).

At 10 DAAT, each increased unit of ECw showed a linear decrease of 1% for the outcome GI; this decrease reached 5.3% when the lowest and highest saline levels were compared (Figure 3A). At 20 DAAT, plants presented a linear increase of 1.5% at each unit increase of ECw; this increase reached 14.7% when the lowest and highest saline levels were compared (Figure 3A).

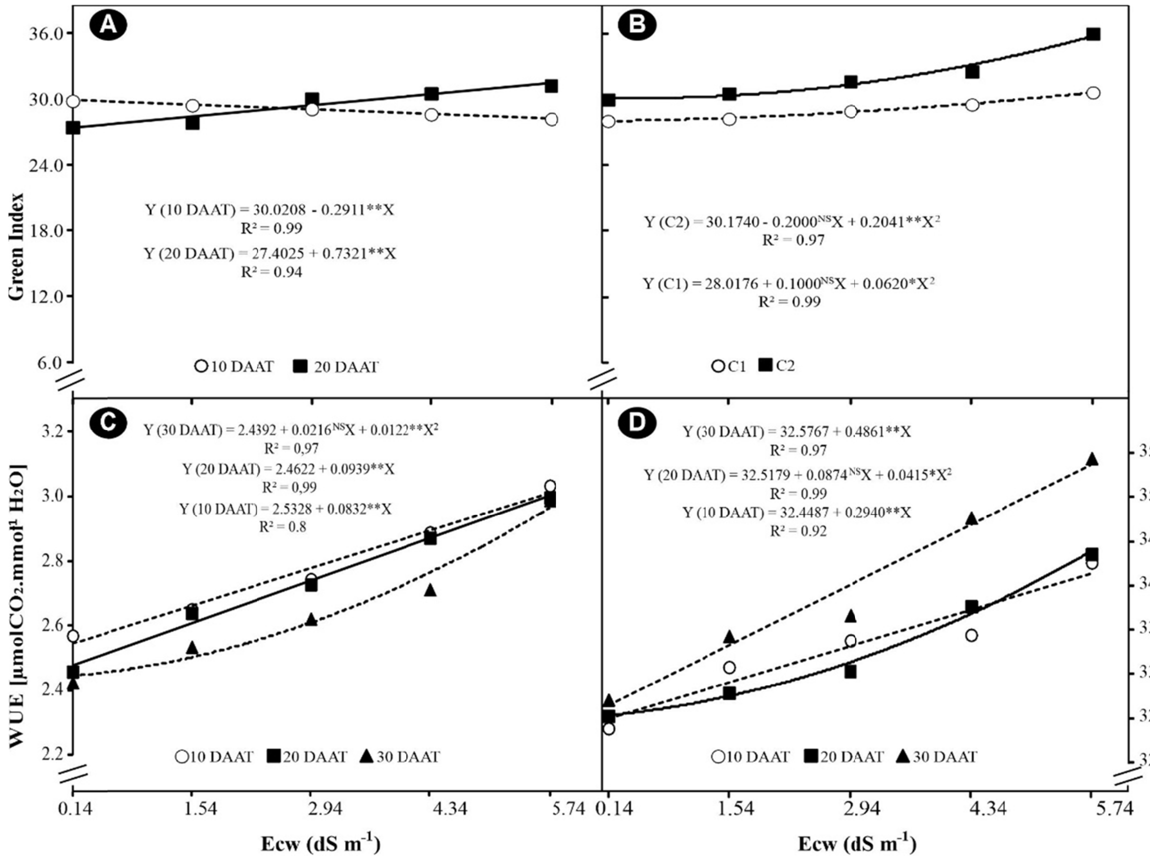

Figure 3. A and B: Green Index (GI), C: Water Use Efficiency (WUE), D: Temperature (T) as a function of the electrical conductivity of the irrigation water, at 10, 20, and 30 days after application of the treatments (DAAT)

At 30 DAAT, the outcome GI was effective ($p < 0.01$) for the interaction between the salinity levels and the cultivar, in which a linear increase occurred for each increased unit of ECw in C1 and C2 of 2.2 and 3.4%, respectively; also, an increase in GI in C1 and C2 of 9.4 and 19.1% occurred, respectively, from lowest to highest saline levels (Figure 3B).

The intensity and duration of stress progression influence the plant's responses to water scarcity and salinity since these factors influence the processes associated with acclimatization (Chaves et al., 2009). The increase in GI of the plants at 20 DAAT compared to 10 DAAT, suggests acclimatization of the plants to the different levels of NaCl.

The increase in GI shows that the plant can use mechanisms such as a greater thickness of the mesophyll and an increase in the number of thylakoids and chloroplasts, which indicate activation in the process of protection to the photosynthetic apparatus (Lacerda et al., 2006).

Some studies showed that increases in GI were observed in response to salt stress in *Vigna unguiculta* L. (Lacerda et al., 2006) and *Arachis hypogaea* L. (Graciano et al., 2011); on the other hand, *Vigna unguiculta* L (Praxedes et al., 2009) showed a reduction in GI, which might be attributed to a weakening of the pigment-protein complex in most sensitive cultivars due to exposure to salinity (Taffouo et al., 2009).

WUE rates were inversely proportional to A and E at all assessments, confirming the effects of saline stress with linear increases at all periods (Figures 2B, C, D, and 3C). There was a unit increase of ECw of 3.3, 3.8, and 3.9% for 10, 20, and 30 DAAT, respectively. Regarding the lowest and highest saline levels, values of 18.3, 21.3, and 21.8% were obtained at 10, 20, and 30 DAAT, respectively (Figure 2C).

The increase in the influence of salt levels to WUE was reported for two tomato cultivars (Moles et al., 2016) and Citrus plants (Syvertsen et al., 2010) under different saline levels. This evidence sustains the hypothesis that an increase in WUE, under salt stress, can be interpreted as a mechanism of tolerance to NaCl in lettuce plants.

The increase in the electrical conductivity of water promoted linear increases in leaf temperature in all evaluated periods (Table 2). However, at 30 DAAT, there was an increase for each unit increase of ECw of 1.5%, with a rise in temperature from the lowest to the highest salt levels of 8.3%, equivalent to 2.7 °C (Figure 2D).

In Figure 4, it is possible to observe at 30 days after the application of saline levels the aspect of each cultivar and it is noticeable that the cv 'Vitória Vedinha', has superior green index (visual aspect), however, the cv 'Saia Véia' has with physiological performance higher.

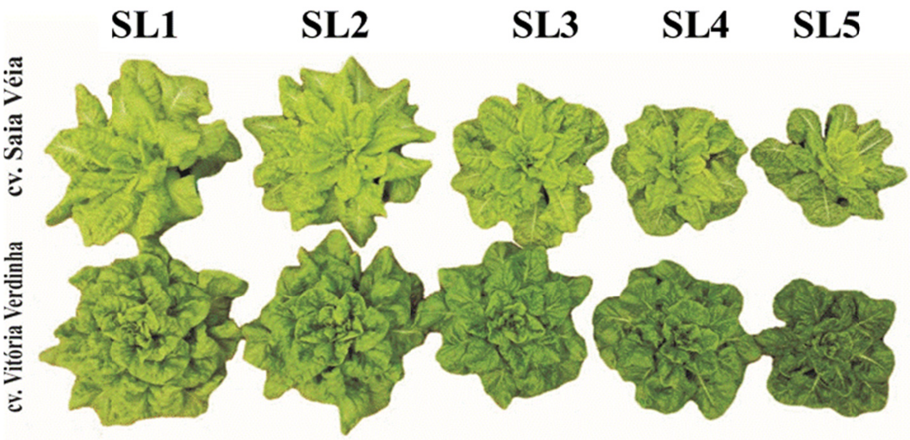

Figure 4. Morphological aspect of the two varieties of lettuce submitted to different saline levels 30 days after treatments. SL: Saline Level. 1 = 0.14; 2 = 1.54; 3 = 2.94; 4 = 4.34 and 5 = 5.74 dS m^{-1}

The results of the present study were similar to those described by Viana et al. (2004), who observed that an increase in salinity may promote leaf temperature elevation, since by osmotic effect, the restriction of the water flow towards the soil - plant - atmosphere and, consequently, transpiration, resulting in elevation of LT.

4. Conclusion

The cv. Saia Véia was higher values of gs, *A*, and *E*, whereas cv. Vitoria Verdinha showed higher tolerance to salt stress, indicated by the higher values of WUE and GI in different levels of NaCl. Results from the present study highlight the need for new studies regarding the genetic improvement of the species so breeders can find genes that can be cloned and later incorporated into new extremely productive varieties, that are not yet sensitive or not tolerant to different levels of saline soil or irrigation water.

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