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Growth of lettuce (*Lactuca sativa* var. iceberg) irrigated with brackish water under competition

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Abstract

The objective of this work was to evaluate the effects of salt stress and competition on the development and growth of lettuce plants (*Lactuca sativa* var. iceberg). The plants were grown under greenhouse conditions with 50% interception of solar radiation. Iceberg lettuce seedlings were transplanted to 8-liter pots with surface area of 4.9 dm^2 filled with 5 kg of a substrate composed of soil, sand, and manure at the ratio of 3:1:1 v v^{-1}. The experiment was set up in a completely randomized design with a 6 × 2 factorial arrangement (six salinity levels and one or two plants per pot), and three replications. An electrical conductivity meter was used to determine the salinity level; NaCl was added to the water until reaching 0, 2, 4, 6, 8, and 10 dS m^{-1}. The lettuce plants were sensitive to salt stress; however, the plants can be irrigated with brackish water with electrical conductivity equal to or lower than 2 dS m^{-1} without significant decreases in shoot fresh weight, therefore meeting the social demand for use low quality water in agriculture. The morphophysiological plasticity of lettuce plants increases the competitive potential of plants in high density crops, with one plant per 2.45 dm^2.

Keywords: Development; growth analysis; lettuce; olericulture; salinity.

Abbreviations: AS1P or AS2P_no salt with one or two plants; BIOM_biomass; CAR_carotenoid contents; Chl_chlorophyll contents; Ca_leaf calcium content; *dS*_decisiemens; *E*_transpiration rate; Fv'/Fm' PSII maximum efficiency; HS1P or HS2P_high salt with one or two plants per pot; LS1P or LS2P_low salt with one or two plants; NaCl_sodium chloride; MS1P or MS2P_median salt with one or two plants; Na_leaf sodium contents; NSL_number of senescent leaves; Pe_absorbed light that was not photochemically used or thermally dissipated; RWC_relative water content; RWR_root weight ratio; SFM_shoot fresh weight.

Introduction

In Brazilian agriculture, stands out the demand for labor in vegetable cultivation. According to IBGE (2017) data, the sector was responsible for more than 2 million direct jobs, providing financial activities of approximately R$ 23.2 billion reais. Among most consumed vegetable in Brazil, lettuce (*Lactuca sativa* L.), which belongs to the Asteraceae family, is widespread approved in tthe market; in 2017, it was cropped over approximately 15 thousand acres, most of them in the Brazilian Southeast region (IBGE, 2017; Mendonça et al., 2019).

The main lettuce types are curly, 70% of the demand in Brazil, iceberg, and romaine varieties, with 15% and 5% of the Brazilian preference (Leite et al., 2019). Lettuce shows great importance in human diet; it is rich in vitamin A and C, and nutrients like calcium, phosphorus, and potassium. The main consumption of lettuce is as fresh salad, however, it can be consumed in other ways, like juices and cigarettes without nicotine, made with dry leaves (Echer et al., 2016).

According to Potrich et al. (2012), the seek for healthy eating habits has increasing vegetable consumption in Brazil in the last years, and lettuce outstands in distinct diet groups (vegetarian, vegan, and omnivorous).

Most vegetables crops are irrigated and demand considerable amount of water for their development, because these species are not tolerant to water deficit. Abiotic stresses like water deficit and salt stress limits yield (Zörb et al., 2019). The salinization process in soils occurs mainly in irrigated areas. Due to increased shortage of good quality water, more low-quality water has been used; considering that the population growth, rainfall seasonality, and hazards to aquifers has increased, populations claim to rational use of water in agriculture (Leite et al., 2019).

It is considered that there is approximately two billion acres of saline soils in the world, in Brazil, approximately 25% of areas used for irrigate crops are affected with this problem (Pedrotti et al., 2015). Besides natural factors that result in saline soils like the parent material, excessive chemical fertilization together with poor irrigation management contribute to increase soil salinity levels. These effects are more pronounced under protected cultivation conditions, which are used for most vegetables produced in Brazil (Gomse et al., 2015).

According to Matos et al. (2019), saline soils reduce plant length, number of leaves, stem diameter, relative water content, transpiration, and biomass. The increase in quantity of salt absorbed by plants cause cell dehydration, inhibiting many enzymes activities. Vegetables are more vulnerable to saline stress, considering their short-life cycle, and more

prone to physiological disturbs, due to their fast vegetative growth (Almeida et al., 2019).
Increases in the use of waters without defined criteria is increasing in small production areas. This practice often cause competition between plants for water, light, and nutrients, and reductions in crop yields (Mali et al., 2019). High density cultivation is beneficial, mainly for horticulture production, since high plant densities maintain soil humidity, reduce weeds, and increase the efficiency of agricultural supplies applications (Silva and Junqueria, 2018).
Recent studies focused on understand the effects of plant population on yield of vegetables has shown that are few differences between planting spacings (Benetti et al., 2019), and denser spacings cause no significant yield reductions (Nomura et al., 2015); however, these experiments evaluated yields without considering salinity aspects.
Therefore, considering the significance of lettuce production in the Brazilian agriculture and the advances in areas with saline soils, especially under protected environments, and the increasing need for the use of low-quality water, the objective of this work was to evaluate the effects of salt stress and competition on the development and growth of lettuce plants.

Results

Physiological and growth variables

According to the analysis of variance (Table 1), the competition of plants in the pots promoted significant difference in number of senescent leaves and leaf sodium contents. The number of senescent leaves of plants grown alone was 48% lower than that of plants in pots with two plants (considering the senescent value as the sum of all senescent leaves of the two plants in the same pot). The leaf sodium contents in plants grown alone was 17% lower than that of pants grown in pots with two plants. The other variables showed no significant differences for the different number of plants per pot. Only leaf calcium content (Ca) showed no significant fit to linear or quadratic regression models (Table 1).
The analysis of variance of the absorbed light that was not used in photochemically or thermally dissipated (Pe), shoot fresh weight, biomass, root weight ratio, and carotenoid and chlorophyll contents are shown in Table 2. Shoot fresh weight and biomass were, respectively, 61% and 62% lower in plants grown in pots with two plants (considering the sum of the shoot fresh weight and biomass of the two plants) when compared to plants grown alone. The other variables showed no significant difference between treatments with one and two plants per pot. Only biomass and carotenoid content showed no significant fit to linear or quadratic regression models.

Regression analysis

Leaf sodium contents, relative water content, number of senescent leaves, transpiration, shoot fresh weight, and root weight ratio fitted to linear regression model (Figure 1). Na leaf contents in both treatments (one and two plants per pot) increased linearly as the electrical conductivity was increased. Na contents were 543% (one plant per pot) and 628% (two plants per pot) lower in control plants when compared to plants under 10 dS m^{-1}. Relative water content was 15% lower in plants under 10 dS m^{-1} when compared to control plants, for plants grown with two plants per pot.
The number of senescent leaves of plants in the treatments with 10 dS m^{-1} were 97% (one plants per pot) and 201% (two plants per pot) higher than that of plants in the treatment with 0 dS m^{-1}. The absorbed light that was not photochemically or thermally dissipated (Pe) increased 77% in plants in the treatments with 10 dS m^{-1}, when compared to control plants, in pots with one plant.
Irrigation with brackish water changed the biomass partitioning. Plants under the treatment with 10 dS m^{-1} allocated less biomass to roots than the control plants, reaching a difference of 36%. However, control plants allocated, on average, 48% and 42% more biomass to leaves in tthe treatments with one and two plants per pot, respectively, when compared to plants in the treatment with 10 dS m^{-1}.
The PSII maximum efficiency (Fv'/Fm') and chlorophyll contents fitted to a linear regression model (Figure 2). PSII maximum efficiency (Fv'/Fm') increased 11% and chlorophyll contents increased sharply as the salinity level was increased. Mean chlorophyll contents of control plants were 37% higher than that of plants under 10 dS m^{-1}.
The principal component analysis (Figure 3) ordered the variables in two axes, with 73% of data variation. According to the importance of the variables, it enabled to name axis 1 as “leaf hydration” and axis 2 as “growth restriction”; therefore, despite the non-formation of a clear group, it is was possible to identify the treatments with two plants or under salt stress at the first and second quarters, denoting growth restrictions in both situations.
The multiple regression analysis (Table 3) showed that increases in shoot fresh weight, commercial interest part of lettuce, are directly related to increases in biomass, and inversely related to root weight ratio and leaf sodium contents.

Discussion

Competition can negatively affect the development of most plants. The results found for lettuce plants were conclusive regarding competition aspects, denoting the existence of competition for soil resources between lettuce plants.
Shoot fresh weight decreased and root weight ratio increased as the salinity was increased for plants grown alone. The salinity negatively affects plant growth and hinders absorption in the soil solution, regardless of the number of plants per pot. The multiple regression analysis showed that increases in leaf sodium accumulation and root weight ratio contribute negatively for shoot fresh weight. The results corroborate those found by Santos et al. (2019), who evaluated production variables of lettuce irrigated with brackish water and observed negative effects on all variables related to lettuce production.
Chlorophyll content increased as the salinity was increased, denoting that the growth of two plants per pot resulted in competition for light. According to Taiz et al. (2017), increases in chlorophyll are important physiological adjustments to intercept solar radiation. The result can be related to the toxic effect of salt, acceleration of chlorophyll degradation and, consequently, need for synthesis. Nevertheless, PSII maximum efficiency (Fv'/Fm') and light dissipation (Pe) increased in plants grown alone as the salinity was increased. This response may be related to reductions in stomatal conductance, low CO_2 influx, and

Table 1. Analysis of variance and mean test for number of senescent leaves (NSL), relative water content (RWC), transpiration rate (*E*), leaf sodium contents (Na), leaf calcium content (Ca), and PSII maximum efficiency (Fv'/Fm') in lettuce (*Lactuca sativa*) plants irrigated with brackish water and under competition.

Source of Variation	Mean Squares					
	NSL	RWC (%)	*E* ($g.H_2O.day^{-1} pot^{-1}$)	Na (ppm)	Ca (ppm)	Fv'/Fm'
N. of Plants (NP)	148.03^{**}	1.1571^{ns}	693.44^{ns}	5980.4^{**}	5377.8^{ns}	0.004^{ns}
Salinity (S)	23.961^{**}	169.25^{**}	1310.2^{**}	35539^{**}	2824.4^{ns}	0.004^{ns}
NP x S	7.5611^{ns}	29.812^{ns}	341.71^{ns}	1078.9^{ns}	2784.4^{ns}	0.003^{ns}
CV (%)	32.79	11.13	27.64	20.91	25.23	5.51
Treatments	Means					
One plant	3.83 b	62.07 a	60.67 a	123.94 b	224.44 a	0.796 a
Two plants	7.89 a	62.43 a	51.89 a	149.72 a	200.00 a	0.817 a
Regression	Significance					
Linear	**	*	*	**	ns	**
Quadratic	ns	ns	ns	ns	ns	ns

*significant at 5% of probability; ** significant at 1% of probability; ns = not significant by the F test. Means followed by the same lowercase letter did not differs from each other at 5% of probability by the Tukey test.

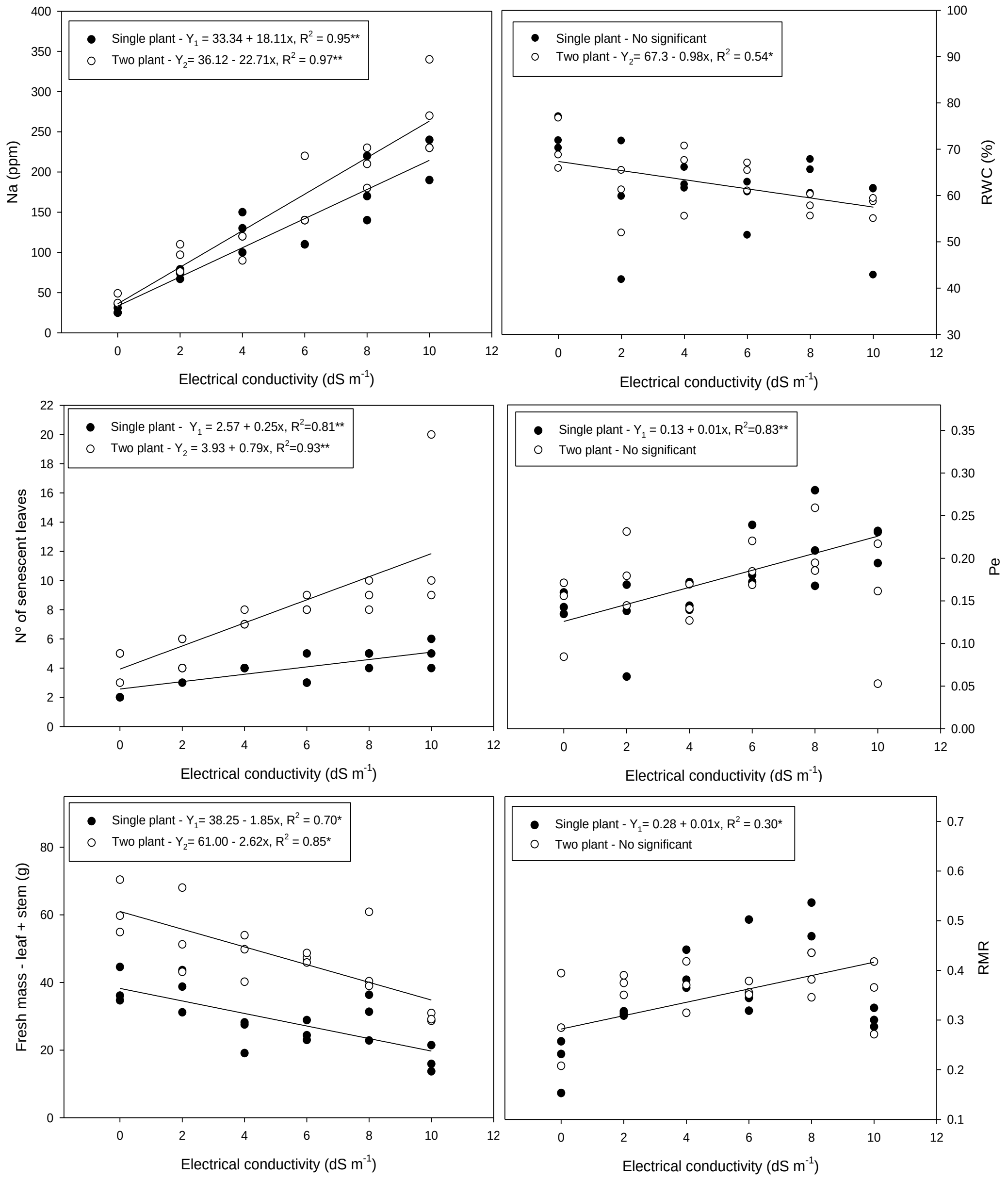

Fig 1. Regression equations for leaf sodium contents (Na), relative water content (RWC), number of senescent leaves (NSL), absorbed light that was not photochemically used or thermally dissipated (Pe), shoot fresh weight, and root weight ratio (RWR) in lettuce (*Lactuca sativa*) plants irrigated with brackish water and under competition. *significant at 5% of probability; ** significant at 1% of probability; ns = not significant by the F test.

Table 2. Analysis of variance for absorbed light that was not photochemically used or thermally dissipated (Pe), shoot fresh weight (SFM), biomass (BIOM), root weight ratio (RWR), carotenoid (CAR) and chlorophyll *a* + *b* contents in lettuce (*Lactuca sativa*) plants irrigated with brackish water and under competition.

Source of Variation	Mean Squares					
	Pe	SFM	BIOM	RWR	CAR	Chl (*a* + *b*)
		(g)	(g vaso^{-1})	(%)	(g kg^{-1})	(g kg^{-1})
Numb. of Leaves (NP)	0.00^{ns}	3221.7**	47.22**	0.00^{ns}	0.00^{ns}	0.01^{ns}
Salinity (S)	0.00**	515.46**	5.19**	0.02**	0.00^{ns}	0.21**
NP x S	0.00^{ns}	29.09^{ns}	0.43^{ns}	0.01^{ns}	0.00^{ns}	0.05^{ns}
CV (%)	25	18.29	20.11	15.77	87.76	21.53
Treatments	Means					
One plant	0.17 a	28.98 b	3.81 b	0.34 a	0.0018 a	1.25 a
Two plants	0.16 a	47.90 a	6.10 a	0.35 a	0.0014 a	1.28 a
Regression	Significance					
Linear	**	*	ns	*	ns	*
Quadratic	ns	ns	ns	ns	ns	ns

*significant at 5% of probability; ** significant at 1% of probability; ns = not significant by the F test. Means followed by the same lowercase letter did not differs from each other at 5% of probability by the Tukey test.

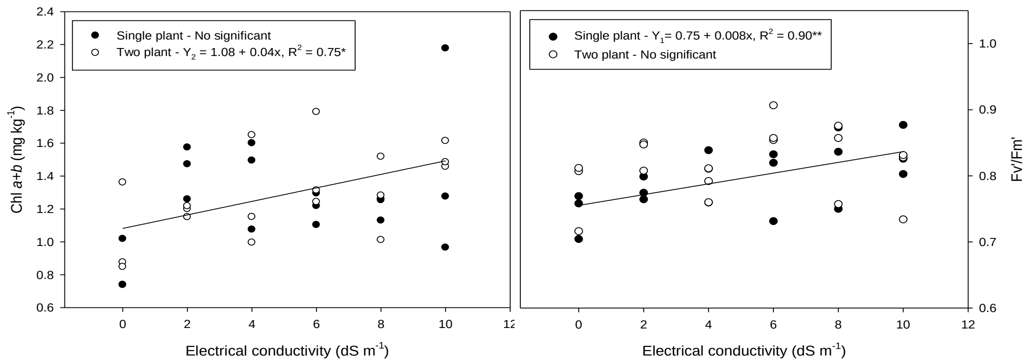

Fig 2. Regression equations for chlorophyll content (Chl) and PSII maximum efficiency (Fv'/Fm') in lettuce (*Lactuca sativa*) plants irrigated with brackish water and under competition. *significant at 5% of probability; ** significant at 1% of probability; ns = not significant by the F test.

Table 3. Multiple regression model evaluating the importance of the effect of variables on fresh leaf weight of lettuce (*Lactuca sativa*) plants irrigated with waters with different electrical conductivities.

Fresh leaf weight	R^2= 0.887		F (11.24) =17.22		p<0.0000	
	Beta	Std.Err. of Beta	B	Std.Err. of B	t (24)	p-level
Intercept			13.784	25.318	0.544	0.591
Biomass	0.996	0.11	8.447	0.93	9.081	0.000
Root weight ratio	-0.412	0.09	-74.661	16.273	-4.588	0.000
Sodium contents	-0.354	0.143	-0.065	0.027	-2.467	0.021

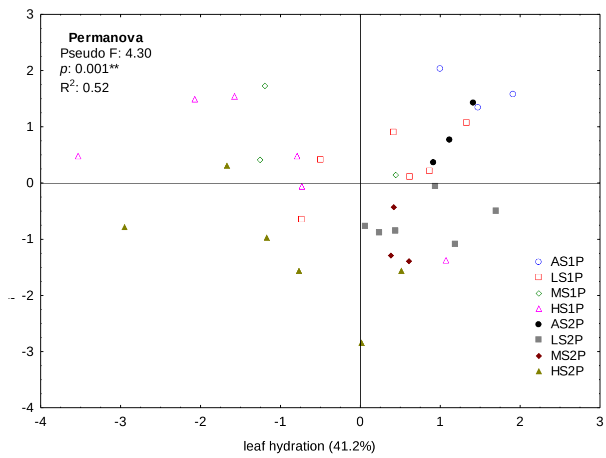

Fig 3. Principal component analysis for lettuce (*Lactuca sativa*) plants irrigated with waters with different electrical conductivities distributed as follows: no salt with one or two plants (AS1P or AS2P), low salt with one or two plants (LS1P or LS2P), median salt with one or two plants (MS1P or MS2P), and high salt with one or two plants per pot (HS1P or HS2P).

excess of photochemical energy that need to be dissipated for processes other than photosynthesis.

The higher number of senescent leaves and lower transpiration of lettuce plants grown in pots with two plants indicate negative effects of competition in the soil solution for water and nutrients. The results corroborate those found by Silva et al. (2018) and Santos et al. (2019), who found decreases in number of leaves as the water salinity was increased. In addition, plants grown under competition, with two plants per pot had higher leaf sodium contents and lower shoot fresh weight than plants grown alone, indicating that water salinity significantly affect lettuce plant growth.

According to Matos et al. (2019), the low osmotic potential of brackish water hinders plant absorption in the soil solution due to the low water potential; thus, the competition in pots with two plants made water absorption difficult. Plants under competition showed decreases in water relative content as the water salinity was increased. The multiple regression and principal component analysis denoted the negative effects of salt and sodium accumulation in tissues and lettuce growth.

Despite shoot fresh weight and biomass of plants grown with two plants per pot were not two-fold that of plants grown alone due to competition, the commercial part of lettuce plants (shoot) grown with two plants por pot exhibited higher shoot fresh weight than that of plants grown alone, with the same aspects and acceptable appearance for commercialization (Table 2). Thus, despite the negative effects of competition and lower fresh shoot weight of plants grown alone, the growth of one plant per 2.45 dm^2 is recommended due the greater commercial value of two plants in 4.9 dm^2.

The negative effects of irrigation with brackish water on the growth of lettuce plants indicated that the specie is sensitive to salt stress, according to Souza et al. (2015) classification. Plants irrigated with brackish water (electrical conductivity above 4 dS m^{-1}) showed decreases of more than 30% in shoot fresh weight when using one plant per pot, and more than 20% when using two plants per pot; however, the use of brackish water with 2 dS m^{-1} electrical conductivity cause less than 10% reductions in plant growth in plants grown with two plants per pot.

Materials and methods

Experimental design

The study was conducted at the State University of Goiás, Ipameri campus (17°42'59.12''S, 48°08'40.49''W, and altitude of 773 m), in Ipameri, GO, Brazil. The experiment was carried out in a greenhouse covered with transparent plastic, with a shade cloth on the sides resulting in a 50% light interception. The region has a tropical climate with dry winter and wet summer (Aw), according to the Köppen classification, and mean temperature of 20 °C (Alvares et al., 2013). Two lettuce seedlings were transplanted to 8-liter pots with surface area of 4.9 dm^2 filled with 5 kg of a substrate composed of soil, sand, and manure at the ratio of 3:1:1 v v^{-1}. The chemical analysis of the substrate showed the following values: pH (CaCl2) 5.4; 16 g dm^{-3} organic matter; 68 mg dm^{-3} of P; 6.81 mmolc dm^{-3} of K (Mehlich-1); 22 mmolc dm^{-3} (SMP) of H + Al; 31 mmolc dm^{-3} of Ca; 15 mmolc dm^{-3} of Mg; 53 mmolc dm^{-3} of sum of bases; 75 mmolc dm^{-3} of cation exchange capacity, and 71% base saturation.

The experiment was set up in a completely randomized design, in a 6 × 2 factorial arrangement (six salinity levels and one or two plants per pot), with three replications. An electrical conductivity meter was used to determine the salinity level; NaCl was added to the water until reaching 0, 2, 4, 6, 8, and 10 dS m^{-1}.

The plants were irrigated with 300 ml of water until for seven days after germination and then subjected to the treatments. The amount of water to be supplied was stablished using the method proposed by Padilha et al. (2016) based on the pot weight. The following analysis were carried out at 35 days after emergence: number of senescent leaves, biomass, shoot fresh weight, root weight ratio, relative water content, transpiration rate, carotenoid and chlorophyll contents, chlorophyll *a* fluorescence, and leaf Ca and Na contents.

Growth variables

The number of senescent leaves was evaluated at 35 days after the application of the treatments. The plants were weighed to obtain the shoot fresh weight, dried in an oven at 65 ºC for 48 hours until constant weight and then weighed again. The dry weight data was used to calculate the biomass by the sum of all parts. The results of senescent leaves, shoot dry weight, and biomass expressed the data of only one plant in the treatment with one plant, and the sum of two plants in the treatment with two plants.

Relative water content

The relative water content was obtained using five leaf discs of 1.2 cm diameter, taken from fully expanded leaves, weighed to record the fresh weight (FM), and saturated for 24 hours in petri dishes with distilled water; they were again weighed to determine the turgid weight (TM) and then dried at 65 ºC for 48 hours. The dry weight (DM) was obtained and then the relative water content was calculated by the following equation: $[(FM – DM)/(TM – DM) – 100]$.

Transpiration

The total daily transpiration of the plants was measured by the difference in pot weight. Each pot was placed into a plastic bag and fixed with rubber band at the plant stem, leaving only the aerial part (leaves and stem) external to the bag and weighed (weight 01), and after 24 hours they were weighed again (weight 02). Total transpiration was estimated by the difference between weight 011 and 02.

Photosynthetic pigments

Leaf discs of 0.6 mm diameter were taken from fully expanded leaves and placed in test tubes containing dimethyl sulfoxide (DMSO) to determine total chlorophyll and carotenoid contents. The extraction was carried out in a water bath at 65 °C for one hour. Aliquots were removed for spectrophotometric readings at 480, 649, and 665 nm. Chlorophyll *a* (Cl *a*), chlorophyll *b* (Cl *b*), and total carotenoid (Car) contents were determined according to the equation proposed by Wellburn (1994).

Fluorescence

Maximum quantum efficiency of photosystem II (Fv/Fm) was measured using a fluorometer (Junior-Pam, Walz, Germany). The readings were performed with a magnetic leaf clip attached to the fluorometer in a middle region of the adaxial side of the leaf, with 0.3 second of light saturation with pulse emissions under 0.6 KHz frequency. The fluorescence data were automatically computed, stored, and transferred to a computer using the WinControl-3 program.

CA and NA

Calcium and sodium concentrations were evaluated using three leaf discs of third pair of completely developed leaves. The leaves were crushed in 5 mL of distilled water. Fractions were removed for readings in a mobile sodium and calcium meter (LAQUAtwin) and the values were obtained in ppm.

Statistical procedures

The data were subjected to analysis of variance by the test F (1% and 5 % of probability) and thee means were compared by the Tukey test at 5% of probability. Regression analysis was carried out using the SigmaPlot 10.0 program (Systat, 2006). The coefficient of determination (R^2) was calculated by the ratio between the sum of squares of the regression and the total sum of squares. Multivariate analysis was carried out by multiple regression using the forward stepwise model (Sokal and Rolf, 1995) and principal component analysis, using a permutational multivariate analysis of variance (Permanova - Anderson, 2001). The Statsoft (Statistica, 2007) and SigmaPlot 10.0 (Systat Software, 2006) programs were used to carry out these analyses.

Conclusions

Lettuce plants (*Lactuca sativa* var. iceberg) are sensitive to salt stress; however, these plants can be irrigated with brackish water with electrical conductivity equal or lower than 2 dS m^{-1} without significant decreases in shoot fresh weight, meeting the consumer demands for low-quality water use in agriculture. The morphophysiological plasticity of lettuce plants increases the competitive potential of plants in high density crops, with one plant per 2.45 dm^2.

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