Physiological responses of inoculated and uninoculated peanuts under saline stress

ABSTRACT

The objective of this work was to evaluate the effects of water salinity on the physiological indices in inoculated and non-inoculated peanut plants. The study was carried out in a protected environment, at the seedling production unit (UPMA), at Campus das Auroras, at the University for International Integration of the Afro-Brazilian Lusophony (UNILAB), Redenção, Ceará. The experimental design used was in a completely randomized (CRD), with treatments in a factorial arrangement, 5x2, referring to the five salinity levels of the irrigation water - CEa: 0.5, 1.5, 3, 4.5, and 6.0 dSm^{-1}, and inoculated and non-inoculated plants with a mix of rhizobia SEMIA 630, lot 0810, and SEMIA 6144, lot 0312, from *Bradyrhizobium* sp., isolated, with four replications. Moreover, recommended fertilization was done for phosphorus (62.5 kg ha^{-1} of P) and potassium (50 kg ha^{-1} of K) to supply the nutritional needs of the plants. The nutritional effect caused by symbiosis with *Bradyrhizobium* sp. favored inoculated plants to present greater tolerance to salt stress. The availability of nitrogen collaborated to increase the efficiency of plant physiological mechanisms. Uninoculated plants, even with a higher amount of chlorophyll and CO_2, were not efficient in the photosynthetic rate. Saline stress affected photosynthesis, transpiration, stomatal conductance, internal CO_2 concentration, water use efficiency, and chlorophyll; however, with less intensity when inoculated with *Bradyrhizobium* sp. The increase in salinity on irrigation water increased the leaf temperature.

Keywords: *Arachis hypogaea* L., *Bradyrhizobium*, plant-microbial relationship.

Respostas fisiológicas do amendoim inoculado e não inoculado sob estresse salino

RESUMO

Objetivou-se neste trabalho avaliar os efeitos da salinidade da água sobre os índices fisiológicos em plantas de amendoim inoculadas e não inoculadas. O trabalho foi desenvolvido em ambiente protegido, na unidade de produção de mudas (UPMA), no Campus das Auroras, na Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção, Ceará. O delineamento experimental utilizado foi o inteiramente casualizado (DIC), com tratamentos arranjados em esquema fatorial, 5 x 2, referentes aos cinco níveis de salinidade da água de irrigação - CEa: 0,5, 1,5, 3, 4,5 e 6 dSm^{-1}, e plantas inoculadas e não inoculadas com mix de rizóbios SEMIA 630, lote 0810 e SEMIA 6144, lote 0312, de *Bradyrhizobium* sp., isolado, com 4 repetições. Realizou-se também adubação recomendada para o fósforo (62,5 kg ha^{-1} de P) e o potássio (50 kg ha^{-1} de K) para suprir as necessidades nutricionais das plantas. O efeito nutricional ocasionado pela simbiose com *Bradyrhizobium* sp. favoreceram as plantas inoculadas a apresentarem maior tolerância ao estresse salino. A disponibilidade de nitrogênio ajudou na maior eficiência dos mecanismos fisiológicos da planta. As plantas não inoculadas, mesmo tendo uma maior quantidade de clorofila e CO_2, não foram eficientes na taxa fotossintética. O estresse salino afetou a fotossíntese, transpiração, condutância estomática concentração interna de CO_2, o uso e eficiência da água e a clorofila porém com menor intensidade quando inoculadas com *Bradyrhizobium* sp. O aumento da salinidade da água de irrigação aumentou a temperatura foliar.

Palavras-chave: *Arachis hypogaea* L., *Bradyrhizobium*, relação planta-micróbio.

1. INTRODUCTION

Peanut culture is found in several tropical countries, although, it is native from South America. The genus *Arachis* has more than 80 species, including cultivated peanuts (Kaprovickas and Gregory, 1994). The peanut crop currently demonstrates elevated economic importance, as it is the fourth most produced oilseed in terms of grain volume in the world (FAO, 2019).

In general, the northeast region of Brazil offers favorable climatic conditions for the development of peanut culture; however, the factors that hinder the crop expansion are the high evapotranspiration, low precipitation, and sometimes, the low irrigation water quality; these factors may limit the development and production of cultures worldwide (Freire *et al*., 2014).

Saline stress causes several physiological and biochemical disorders, as it reduces the osmotic potential of the soil, reducing water absorption; furthermore, these excess salts in the plant tissue become toxic to the plant leading to a nutritional disorder (Pereira Filho *et al*., 2019; Lima *et al*., 2020).

In legumes such as peanuts, it is known that nitrogen supply is facilitated by the presence of diazotrophic bacteria. Nevertheless, under ideal nutritional conditions, peanuts increase the nodulation rate, as the nitrogen provided by biological fixation will help in the production of organic molecules capable of resist to various stresses, that is, its antioxidant system is more efficient (Fukami *et al*., 2018; Freitas *et al*., 2020).

One of the strategies for growing peanuts under saline stress is the use of *Rhizobium spp.* and *Bradyrhizobium spp.* aiming to reduce the use of nitrogen fertilizers. These bacteria are able to fix the N necessary for better development of the culture and present positive effects for its yield (Rocha, Castro and Freitas 2019). According to Mondal, Mousumi et al. (2020b) this N does not only help in plant nutrition, but it can provide a more sustainable system for culture.

The objective of this work was to evaluate the effects of water salinity on the physiological indices in inoculated and non-inoculated peanut plants.

2. MATERIAL AND METHODS

The study was carried out in July 2019 in a protected environment, at the seedling production unit (UPMA), Campus Auroras, of University for International Integration of the Afro-Brazilian Lusophony (Portuguese: Universidade da Integração Internacional da Lusofonia Afro-Brasileira, UNILAB), located in Redenção, Ceará. According to Köppen, the climate of the region is classified as Aw’ type, that is, rainy tropical, very warm, with a predominance of rains in the summer and autumn seasons.

The experiment was conducted in 8L pots filled with soil from the Campus das Auroras and was removed from the 0-20 cm layer, classified as Red-Yellow Argisol (EMBRAPA, 2018). A sampling at a depth of 0-20 cm was sent for the analysis of chemical attributes to the Soil Laboratory, belong to the Federal University of Ceará, the results are shown in Table 1.

Table 1. Chemical attributes of the substrate used in the research.

pH H_20	P	$H+Al^{3+}$	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	SB	CEC	V	OM	CEes
	$Mg.kg^{-1}$				$cmol_c.kg^{-1}$				%	$g.kg^{-1}$	$dS.m^{-1}$
7.6	2	0.33	2.50	0.30	0.57	0.06	3.43	3.76	91	4.03	0.37

SB = sum of bases; CEC = cation exchange capacity; v= base saturation; OM = organic matter.

Sowing of cultivar BR-1 was carried out in the pot. After ten days of sowing, thinning was made, leaving only two plants per pot.

The experimental design used was in a completely randomized (CRD), with treatments in a factorial arrangement, 5x2, referring to the five salinity levels of the irrigation water - CEa: 0.5, 1.5, 3, 4.5, and 6.0 dSm^{-1}, and inoculated and non-inoculated plants with a mix of rhizobia

SEMIA 630 and SEMIA 6144, from *Bradyrhizobium* sp., with four replications measurements were made in all four by treatment.

The strains were activated according to the method described by Embrapa (1994), and posteriorly, its multiplication was conducted in a 125 ml flask, with 50 ml of liquid YM medium, incubated on a rotary shaker at 150 rpm and temperature of 28 °C and propagated in peat.

Two inoculations were performed, the first was made on peanut seeds using the mix of rhizobia spread in a peat medium and adhesive solution of 20% gum arabic as an adhesive solution. While the second, called reinforcement inoculation, occurred ten days after sowing (DAS), in which 2.0 mL of bacterial broth was added to the lap of each plant.

The irrigation waters were prepared using the NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ in the proportion 7:2:1, following the relationship between CEa and its concentration ($mmol_c\ L^{-1}$ = CE x 10) (Rhoades *et al*., 2000). Irrigation with saline water was started ten days after sowing with a daily irrigation frequency according to the drainage lysimeter principle (Puértolas *et al*., 2017), providing the volume of water losses by evapotranspiration in every 24 h, to maintain the soil with humidity corresponding to 90% of the field capacity.

Moreover, recommended fertilization was held for phosphorus (62.5 kg ha^{-1} of P) and potassium (50 kg ha^{-1} of K) to meet the nutritional needs of plants, following the recommendations of Fernandes (1993).

At 55 DAS, the following variables were analyzed: photosynthesis (A), transpiration (E), and stomatal conductance (gs), leaf temperature (LT), internal CO2 concentration (IC) in fully expanded sheets, to carry out these analyzes the portable infrared carbon gas analyzer was used (IRGA model LC-Pro-SD, Biosciences Inc., Lincon, Nebraska, USA), in an open system, with an airflow of 300 mL min-1 between 08:00 and 10:00 h, using light intensity active (PAR) constant (1300 μmol photons $m^{-2}\ s^{-1}$), concentration constant CO_2 (350ppm) with ambient air temperature and relative humidity, on average 30°C and 85% respectively. The instant water use efficiency (WUE), was determined from the ratio between A/E. Chlorophyll was measured with Minolta SPAD-502 portable meter.

The data of the evaluated variables were submitted to analysis of variance and, when significant, by the F test, and the means were compared by the Tukey test with $p<0.05$ using ASSISTAT, version 7.7 Beta (Silva & Azevedo, 2016). For the regression analysis, as a criterion for choosing the equations, the significance of the regression coefficients at the significance level of 0.01 and 0.05 probability was used by the F test and in the largest R^2.

3. RESULTS AND DISCUSSION

There was a significant effect on the interaction among salinity and inoculation for photosynthesis (A), conductance (gs), transpiration (E), internal CO_2 concentration (IC), water use efficiency (WUE), and chlorophyll. While, for leaf temperature (LT), there was an isolated effect for salinity and inoculation (Table 2).

Table 2. Summary of analysis of variance (ANOVA) for photosynthesis (A), conductance (gs), transpiration (E), leaf temperature (LT), internal CO_2 concentration (IC), water use efficiency (WUE) and chlorophyll.

SV	Medium square							
	DF	A	gs	E	LT	IC	WUE	Chlorophyll
Salinity (S)	4	2.38*	0.013**	0.645*	0.880*	3646.21**	3.58**	8.554^{ns}
Inoculation (I)	1	29.08**	0.011**	0.008^{ns}	1.067*	2736.16**	7.77**	5.675^{ns}
S x I	4	5.29**	0.019**	0.693**	0.393^{ns}	1074.64**	4.15**	65.54**
Residue	30	0.68	0.00074	0.170	0.223	110.53	0.71	4.663
CV (%)	-	16.02	20.27	21.71	1.53	3.54	29.7	6.22

SV: Source of variation, DF: Degree of freedom, I: Inoculation, S: Salinity, CV (%): Coefficient of variation, **: Significant at the level of 1% probability (p <0.01), *: Significant at 5% probability level (.01 = <p <.05), ns: Not significant (p> = 0.05).

About the result obtained for photosynthesis, it might be noticed (Figure 1A) that the increasing linear model was the one that best fit for the inoculated peanut, and decreasing for the non-inoculated with an increase in the electrical conductivity of the water (CEa).

This result may be associated with the efficiency of biological nitrogen fixation (BNF) due to the peanuts crop and the participation of bacteria, that is, the greater availability of nitrogen improved the physiological conditions of the plants. Tahjib-Ul-Arif et al. (2019) describe that salts can lead to a lower concentration of chlorophyll (pigment responsible for photosynthesis), however the N made available by bacteria may have attenuated the effects of stress caused by salts, favoring the photosynthetic process.

Corroborating this information, Lucio *et al*. (2013), when inoculating and irrigating the melon culture with saline water, found an increase in the nitrogen concentration in the leaves, providing a higher photosynthetic rate in relation to the treatment without inoculation.

Concerning the stomatal conductance (Figure 1B), for the inoculated plants, the model that best fitted was a polynomial, with a maximum conductance of 0.17 mol m^{-2} s^{-1} to a CEa of 3.59 dS m^{-1}. While for non-inoculated plants, the decreasing linear model was used.

Mbarki et al. (2017) exposes that microorganisms when present in association with plants, can improve the positive health of the soil, in addition to providing important compounds to mitigate the negative effects of salts, and thus improve the mechanisms of areas of plants in a state of stress . The inoculated plants obtained better results, that is, the nitrogen that was fixed by the inoculation bacteria, produces molecules such as (proline) capable of attenuating the effect of salinity (Taiz et al., 2017).

Alike, Kaschuk *et al*. (2009) concluded in their study that the nutritional effect brought by symbiosis can improve the gas exchange in plants. Lucio *et al*. (2013), working with the culture of inoculated and uninoculated melon under saline stress, also found a result similar to the present study.

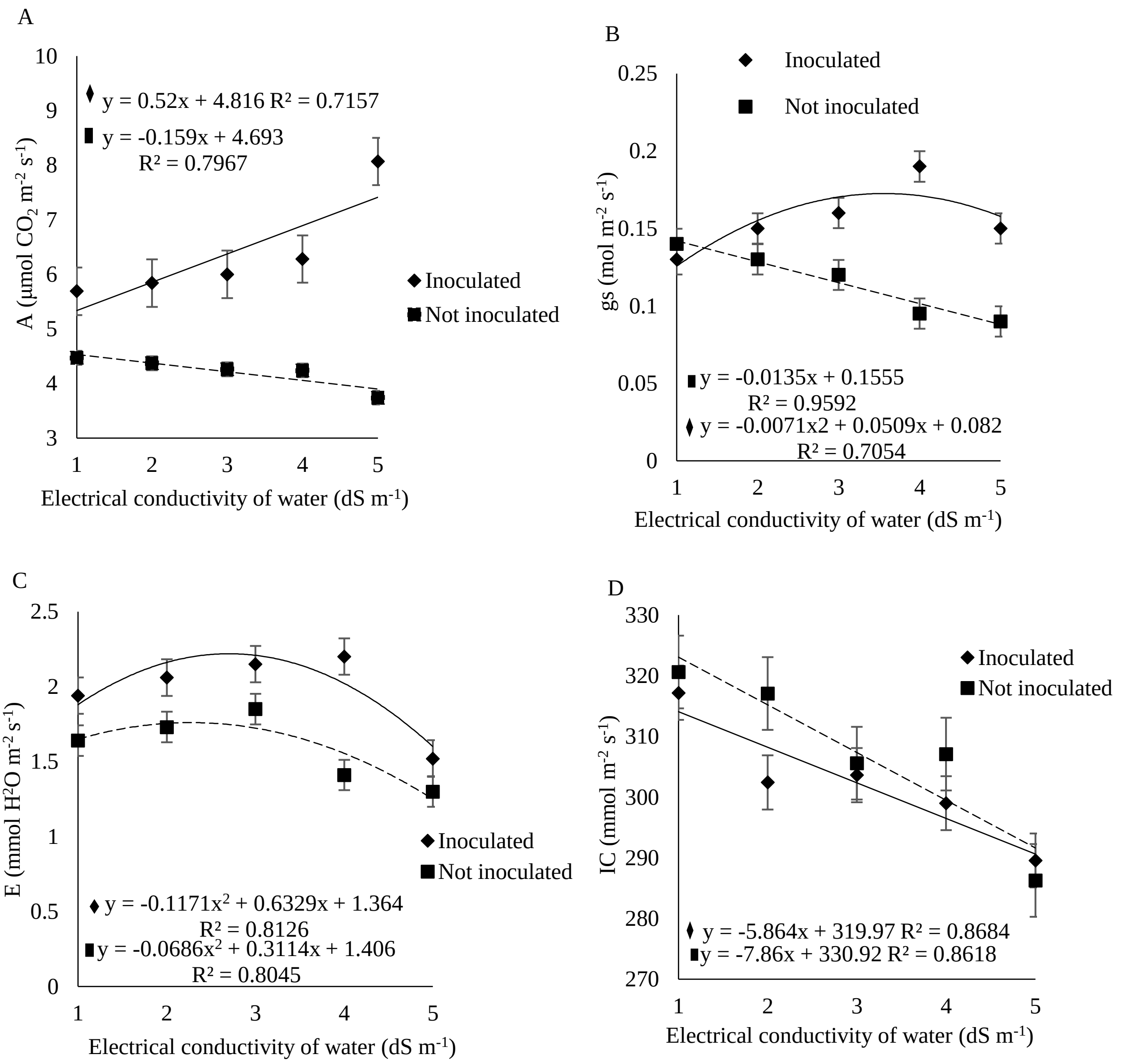

Figure 1. A: Photosynthesis (A), gs: stomatal conductance (B), E: Transpiration (C) and IC: Internal concentration of CO_2 (D), of inoculated and non-inoculated peanuts, submitted to different levels of salinity

According to Figure 1C, both trend lines fit into a quadratic polynomial. The inoculated plants had their maximum point 2.22 mmol H_2O m^{-2} s^{-1} for a CEa of 2.7 dS m^{-1}, the non-inoculated plants, the maximum transpiration was 1.76 mmol H_2O m^{-2} s^{-1} with water of 2.27 dS m^{-1}.

It should be noted that saline stress causes a reduction in water absorption and, consequently, a lower transpiratory rate. Pereira *et al*. (2019) state that Na^+ and Cl^- when present in the soil alter the osmotic potential, causing the plant to absorb less water, showing a partial closure of the stomata and, therefore, less transpiration.

About the positive effect of inoculation, Doni *et al*. (2014) affirm in their study that the symbiosis between plants and bacteria/fungi may enhance the entire physiological system of plants and thus, improve their performance in perspiration. Additionally, Lucio *et al*. (2013) got superior results of sweating in treatments that were inoculated compared to those not inoculated.

Regarding the internal CO_2 concentration parameter (Figure 1D), the results showed that both trend lines fit into a decreasing linear model; however, the inoculated plants obtained lower results. Plants under salt stress end up closing their stomata as a strategy to prevent water loss, however, they also reduce CO_2 assimilation and the net rate of their concentration in cells (Taiz & Zeiger 2017 & Tahjib-Ul-Arif et al, 2019)

Another aspect may be related to the N provided by the symbiosis, and this mineral element provides greater enzymatic activity and better osmotic adjustment of the plants (Mondal, Mousumi et al. 2020b) and K + by fertilization, that is, both improved the structure of RuBP (ribulose-bisphosphate carboxylase / oxygenase), making it more efficient (Alvarenga et al. 2019), showing that the inoculated plants were more suitable in the use of available CO_2.

Another relevant factor for the behavior of CO_2 is related to RuBP (ribulose-bisphosphate carboxylase / oxygenase), which, when working under favorable conditions, increases carboxylation and suppresses the oxygenation activity of the photosynthetic system, improving the use of CO_2, ie , the nitrogen supplied in biological fixation improved the conditions of RuBP and favored its functioning (Hsiao et al., 1999).

Besides, Oliveira *et al*. (2017), working with cowpea under saline stress, also registered a decrease in the internal concentration of CO_2. These same authors also point out that this result may have been caused by the lower diffusion of CO_2 evidenced by stomatal closure.

The rise in the salinity of the irrigation water increased the leaf temperature linearly (Figure 2A). This is possible because plants under salt stress have great difficulty in absorbing water from the soil, leading to stomatal closure, and consequently, reducing perspiration (Taiz *et al*., 2017), increasing leaf temperature. Sousa *et al*. (2012), when assessing physiological responses of physic nut under salt stress, also identified a linear increase in leaf temperature.

The non-inoculated plants showed higher average leaf temperature values than those not inoculated (Figure 2B). The symbiosis between plants and bacteria provides better osmotic adjustment, improving perspiration, and decreasing leaf temperature. It is worth stand out that BNF increases nitrate reductase activity, thus increasing the production of plant hormones and improving the antioxidant system of plants (Fukami *et al*., 2018).

Doni *et al*. (2014), when inoculating the rice culture, affirm that the presence of nitrogen for the inoculated plants can help in the osmotic adjustment of the plants, increasing, therefore, their transpiration rate, and in this way, corroborating the decrease in leaf temperature.

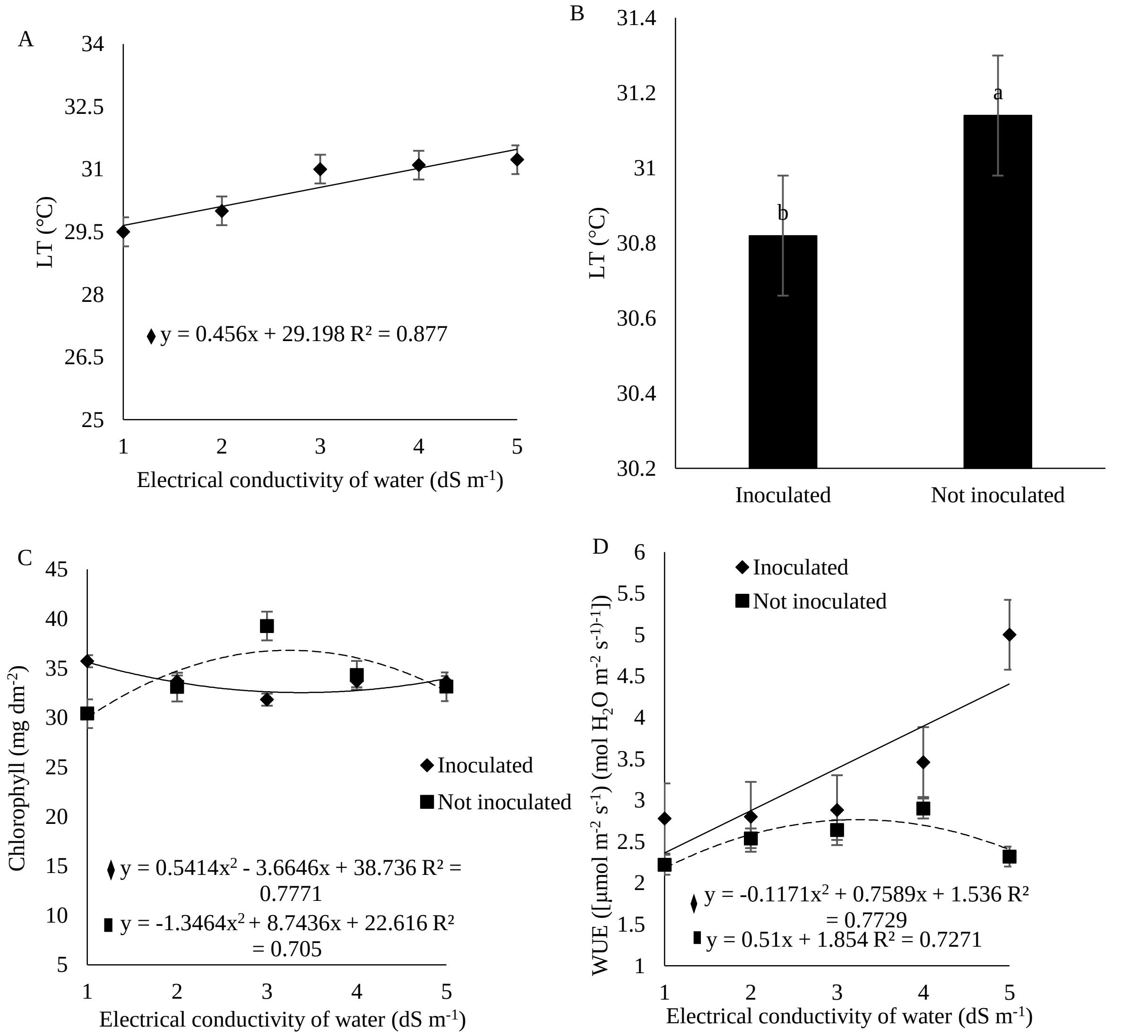

Figure 2. LT: Leaf temperature for the salinity factor (A), LT: leaf temperature for the inoculation factor (B), Chlorophyll (C) and WUE: water use efficiency (D), of the inoculated and non-inoculated peanuts, submitted to different levels of salinity

The results also permitted to affirm that for chlorophyll, the quadratic polynomial model was the one that best fitted the data, as shown in Figure 2C. The inoculated plants had a maximum amount of chlorophyll of 32.535 mg dm^{-2} in the water of 3.38 dS m^{-1}, while the non-inoculated plants had their maximum of 36.81 mg dm^{-2} for the water of 3.25 dS m^{-1}.

Moreover, the result is in accordance with those reported in the study conducted by Zhang *et al*. (2010) when that found the salt stress causes swelling and rupture of the thylakoids and the chloroplast layer, due to the excess of Na^+ and Cl^- ions that also inhibit the synthesis of new chlorophyll molecules.

It is important to highlight that even with the highest amount of chlorophyll, the non-inoculated plants failed to develop well in photosynthesis because the lower availability of nitrogen in their organism led to greater damage to the physiological system (Taiz *et al*., 2017). On the other hand, the presence of nitrogen for the inoculated plants made them more efficient in the use of CO_2 and chlorophyll. This macronutrient is part of the rubisco structure, thus making the enzyme more effective in its physiological activities (Alvarenga *et al*. 2019).

Cha-um *et al*. (2013), also found a decline in chlorophyll concentration due to the increase in soil salinity in cowpea and jack bean (*Canavalia ensiformis*) plants.

In Figure 2D, it is possible to observe for the parameter, water use efficiency (WUE), that the growing linear model was the one that best fits for the inoculated plants. Differently, for the non-inoculated plants, the quadratic polynomial model was the best adjusted, with a maximum WUE of 3.56 ([μmol m^{-2} s^{-1}) (mol H2O m^{-2} s^{-1}) $^{-1}$]) obtained with the use of water with 3.84 dS m^{-1}.

Tahjib-Ul-Arif et al. (2019) warn that if the transpiration rate is compromised, the WUE values decrease, however, the circumstances that provided better use and water efficiency to the inoculated plants can be explained by the adequate nutritional supply and, consequently, by the osmotic adjustment that improved the transpiration and its ability to absorb water and the production of photoassimilates.

(Campelo *et al*., 2019). Contrary to the results found in this study, Cha-um *et al*. (2013), when working with cowpea and jack bean cultures, there was a reduction in water use and efficiency.

4. CONCLUSIONS

Saline stress affected photosynthesis, transpiration, stomatal conductance, internal CO_2 concentration, water use efficiency, and chlorophyll; however, with less intensity when inoculated with *Bradyrhizobium* sp.

The increase in salinity on irrigation water increased the leaf temperature in peanut plants.

The inoculation with mix of rhizobia SEMIA 630 and SEMIA 6144, of Bradyrhizobium sp is an efficient alternative to attenuate the saline stress in peanut plants regarding the physiological responses.

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