Tolerance to irrigation water salinity in *Physalis peruviana* L. plants[1]

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ABSTRACT

Abiotic factors, such as saline stress, can happen in agricultural areas and can harm the metabolism of the plants. Studies that seek to evaluate the acting of *P. peruviana* L. under stress conditions are still incipient. Therefore, the objective was to evaluate the tolerance to salinity in plants of *P. peruviana* L. under different saline levels of irrigation water. The work was developed in the experimental farm of the Universidade Federal de Campina Grande, Campus Pombal, PB, from January to March 2017. Four saline concentrations of irrigation water were used (0.3; 1.2; 2.1; and 3.0 dS m^{-1}) with five repetitions. At 60 days after the transplant, were evaluated the leaf area, intercellular CO_2 concentration, stomatal conductance, transpiration, photosynthetic rate, intrinsic water use efficiency, instantaneous carboxylation efficiency, electrolyte extravasation, chlorophyll a, b and carotenoids. The results show that saline levels of irrigation water up to 3.0 dS m^{-1} did not provide physiological damages in plants of *P. peruviana* L. until 60 DAT, except for extravasation of electrolytes and chlorophyll a and b, characterizing the plant as tolerant to salinity until the studied level.

Keywords: physalis; salinity; gas exchange; water use efficiency.

INTRODUCTION

The *Physalis peruviana* L. is a vegetable fruit that has as the center of origin in countries like Bolivia, Colombia, Peru, and Ecuador (Muniz *et al*., 2011), while other authors report its production in certain areas of South Africa (Puente *et al*., 2011). In Brazil, concerning studies to *P. peruviana* L. they are still incipient, having some works accomplished with this species, mainly in the coldest regions of the country (Lima, 2009; Muniz *et al*., 2015). Production data for the species in Brazil, despite being scarce, esteem values from two to three annual tons (Melo *et al*., 2017).

Referring to the productivity index observed in Brazil, many of the times, it can be related to the cultural handling used in field cultivation; that due to the physiologic alterations in the plant, provoked by abiotic factors, as observed by Ianckievicz *et al*. (2013), Zeist *et al*. (2014) and Lima *et al*. (2015). This way, when in stress conditions, the hormonal and metabolic alterations in the plant can result in unfavorable aspects to the production.

The amount of water supplied to the plants during the stages of, in agreement with Silva *et al*. (2015), on the ability of plants to perform gas exchange and photosynthesis, so that excess can affect root aeration and nutrient leaching, while lack of water can reduce growth and affect numerous physiological processes and, therefore, production.

Regarding the use of saline water in irrigation, Ianckievicz *et al*. (2013) report that plants of *P. peruviana* L., submitted to saline concentrations of nutrients corresponding to 3.0 dS m^{-1} presented a larger fresh mass of the fruits, while Campos e Cavalcante (2009) observed that the bell pepper (*Capsicum annuum* L.) tolerates up to 1.88 dS m^{-1} of salts in irrigation water if bovine biofertilizer is used as a salinity attenuator.

According to Dias and Blanco (2010), when under saline conditions, growth and fruit production are significantly reduced, which can be attributed to the effect of toxic ions present in the water and osmotic stress According to some authors, changes in morphology, anatomy, water relations, photosynthesis, hormonal profile, distribution of toxic ions and biochemical adaptation (such as the response of the antioxidative metabolism) are strategies adopted by plants, including vegetables, to tolerate the salt stress of irrigation water (Acosta-Motos, *et al.*, 2017).

The Northeast area of Brazil presents a low rainfall index and high temperatures in certain periods (Silva *et al.*, 2011), besides, a great part of underground water possesses a high content of salts in its composition, which can be an added difficulty in the production of *P. peruviana* L., for instance. However, under conditions of saline stress, some plants can tolerate the salinity, such as the *Atriplex nummularia* (Melo *et al.*, 2017), that gets to accumulate salts inside its cells, while others manage to exclude Cl⁻ from the root system, or to accumulate these ions in the cell vacuole (Flowers & Colmer, 2015), as well as to reduce the photosynthetic rate, water absorption or increase the osmotic potential, according to Lima *et al.* (2015) and Taiz *et al.* (2017). Besides, it is possible to observe mechanisms of exclusion of the elements sodium and chlorine in some morphophysiological structures in the plant (Deinlein *et al.*, 2014).

In this context, this work aimed to evaluate the tolerance to salinity in plants of *P. peruviana* L. under different saline levels of irrigation water.

MATERIAL AND METHODS

Conduction and design of the experiment

The study was developed in a protected atmosphere in the experimental farm of the Center for Agri-Food Science and Technology of the Federal University of Campina Grande, located in São Domingos' city, PB (6°48'41.7 "S of latitude and 37°56'13.8 "W of longitude with an altitude of 190 meters) from January to March 2017, lasting 60 days. For the extraction of seeds, the fruits were cut in the middle with bistoury and the seeds were washed in running water with aid of a mesh sieve die for the complete removal of the pulp. Afterward, the seeds were put to dry in atmospheric conditions in the laboratory for three days on two sheets of Germitest paper.

The seedlings' production was accomplished according to the methodology described by Silva *et al.* (2018), adopting, therefore, polypropylene containers with a capacity of 50 ml, filled out with commercial substratum Basaplant®, sowed to 0,5 cm of depth and irrigated three times a day with water of urban provisioning (0.3 dS m⁻¹)

until the 30 days after the sowing (DAS) (Muniz *et al.*, 2015), being transplanted for plastic vases with capacity for 12 dm⁻³, however, containing 10 soil dm⁻³, sand and manure in the proportions 2:1:1, the chemical and physical characteristics of the substratum used in the production of seedlings (SPM) and in the cultivation (SCV) of the plants they can be observed in Table 1.

The water used in the irrigation was originating from an artesian well and the urban net, being mixed to obtain the electric conductivities (0.3; 1.2; 2.1 and 3.0 dS m⁻¹), its chemical characteristics are presented in Table 1. The maximum level of 3.0 dS was adopted due to the natural salinity in the artesian well, being close to this value. During the first seven days after the transplant, it was applied only water of urban provisioning (0.3 dS m⁻¹) for the good initial establishment of the transplanted plants. From transplanting to the end of the experiment, irrigations were performed manually, using a 1000 mL beaker.

The weekly ECw check was performed with the aid of a portable conductivity meter. The volume drained and the electrical conductivity of the drain (ECd) was estimated by collecting some samples 24 hours after irrigation. To determine the daily volume of water required, the following equation was used:

$$Vi = \frac{Va - Vd}{1 - LF} \tag{1}$$

Where: Vi = Volume to be irrigated; Va = Volume applied; Vd = Volume drained after 24 hours of application; LF = Leaching factor (10%).

We used a randomized block design with five repetitions of three plants, the treatments were characterized through the irrigation water with different electric (CEa) conductivity (0.3; 1.2; 2.1 and 3.0 dS m⁻¹), constituting an experimental area of 60 plants. Plants were arranged in the crop row using East-West direction., in agreement with Rodrigues *et al.* (2013), spaced 1.5 m between lines and 1.0 m between plants.

Daily, with the aid of a thermohygrometer, the temperature (°C) and air humidity (%) values were quantified at 60 DAT. Both data are expressed in Figure 1.

Physiological analysis

At 60 days after the transplant (DAT), was determined the leaf area through the measurement of the length (C) and width (L) of all leaves of the plants, in agreement with Reis *et al.* (2013), being applied the following equation: AF = C*L*f, where: AF - area to foliate (m²); C - length of the leaf (m); L - width of the leaf (m); and f - correction factor (0.59);

At 60 DAT, were evaluated the gaseous changes in intermediate leaves of plants of *P. peruviana* L. with the aid of IRGA (InfraRed Gas Analyzer, LCi-SD), measuring

the intercellular concentration expressed as CO_2 (Ci) μmol (CO_2) m^{-2} s^{-1}, stomatal conductance (gs) in mol (H_2O) m^{-2} s^{-1}, transpiration (E) in mmol (H_2O) m^{-2} s^{-1} and the photosynthetic tax (A) μmol (CO_2) m^{-2} s^{-1}, using these data was calculated the intrinsic efficiency in the use of water ($EIUA$) for the reason between A and E, ($EIUA=A/E$) expressed in [ìmol (CO_2) m^{-2} s^{-1}] [mmol (H_2O) m^{-2} s^{-1}]$^{-1}$ and the instantaneous efficiency of the carboxylation (EICi) becoming separated to A for Ci ($EICi=A/Ci$) and expressed in [mmol (CO_2) m^{-2} s^{-1}] [ìmol (CO_2) mol^{-1}]$^{-1}$ (Zeist *et al*., 2014; Barbosa *et al*., 2015).

For evaluation of cellular damages, the electrolytes extravasation technique was used (Bajji *et al*., 2001), being collected eight foliate disks with a diameter of 1.2 cm^2 each, later transported to the Laboratory of Analyses of Seeds and Seedlings of the Federal University of Campina Grande, where it was washed immediately with distilled water seeking the removal of broken cells and other electrolytes adhered to the disks foliate.

After the washing, the disks were dried in a paper towel and put in beakers containing 25 ml of distilled water at 25 ºC, for 4h00min; after this period the electric (C1) conductivity was measured with a bench conductivity meter (mCA 150) and the beakers with the disks were put in a kiln at 90 ºC, for 2h00min; afterward, after the temperature equalization, the maximum electric conducti-

Table 1: Chemical and physical composition of substrates used in the production of seedlings (SPM) and in the cultivation (SCV) of *Physalis peruviana* L. under different levels of irrigation water salinity and the chemical composition of irrigation water used in the cultivation of *Physalis peruviana* L. under different levels of irrigation water salinity

	Chemical and physical composition of substrates							
	pH	**P**	**S-SO$_4$$^{-2}$**	**K$^+$**	**Na$^+$**	**H$^+$+Al^{+3}**	**Al^{+3}**	**Ca^{+2}**
	Water (1:2.5)	**mg dm^{-3}**		**cmol$_c$ dm^{-3}**				
SPM	5.5	257.30	-	2.00	0.43	8.57	0.05	2.69
SCV	7.4	733.39	-	2.73	1.20	0.00	0.00	1.23
	Mg^{+2}	**SB**	**CTC**	**M.O.**	**Sand**	**Silt**	**Clay**	**Textural class**
	cmol$_c$ dm^{-3}			**kg^{-1}**				
SPM	1.46	3.89	12.46	233.51	878	113	9	-
SCV	1.09	6.25	6.25	10.34	789	155	56	Frank sand

		Chemical composition of irrigation water									
CEa	**pH**	**Ca^{+2}**	**Mg^{+2}**	**K$^+$**	**Na$^+$**	**CO$_3$$^{2-}$**	**HCO$_{3-}$**	**Cl$^-$**	**SO$_4$$^{2-}$**	**CSR**	**RAS**
		mmol$_c$ L^{-1}									
0.3	7.20	0.30	0.65	0.12	1.30	0.00	2.15	1.60	0.02	1.20	2.67
1.2	6.33	3.05	2.50	0.67	3.90	0.00	6.20	6.20	0.00	0.65	3.31
2.1	7.38	2.03	1.47	0.28	3.07	0.49	6.23	10.73	0.15	3.22	3.33
3.0	8.60	2.75	1.25	0.05	4.01	1.48	10.34	24.40	0.44	7.82	4.01

P, K, Na: Extractor Mehlich 1; SB: Sum of Exchangeable Bases; H + Al: Extractor Calcium Acetate 0.5 M, pH 7,0; CTC: Cation Exchange Capacity; Al, Ca, Mg: Extractor KCl 1 M; M.O.: Organic Matter – Walkley-Black.
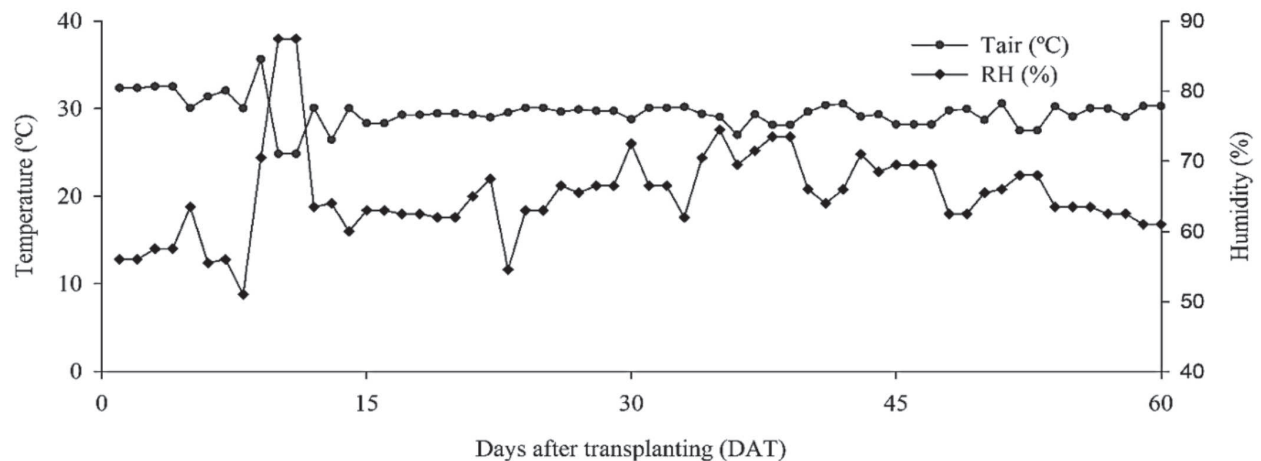

Figure 1: Precipitation, average temperature (Tair), and average relative air humidity (Humidity - RH) during the cultivation of *Physalis peruviana* L.

vity was measured (C2) and the extravasation of electrolytes (EE) was calculated through the following equation: EE = (C1/C2) x 100.

For the determination of chlorophyll content a, b and carotenoids also eight foliate disks were collected, 1.2 cm^2 each, coming from the medium third of each plant. The extraction of the pigments was accomplished by maceration with acetone 80% and $CaCO_3$ in the darkness, being later filtered and completed up to 25 mL with acetone 80% and the readings were made with aid of a spectrophotometer in the wavelengths of 663; 645 and 470 nm. The results were calculated according to Lichtenthaler (1987) and expressed in grams by square meter of leaf blade (g m^{-2}).

Statistical analysis

The data obtained was analyzed for normality (Shapiro Wilk test) and homoscedasticity (Hartley test) and, subsequently, subjected to analysis of variance (p d" 0.05) and, when significant, polynomial regression was applied at the level of 5% of probability, using the Sisvar 5.3 (Ferreira, 2011).

RESULTS AND DISCUSSION

In agreement with the summary of the variance analysis presented in Table 2, the unitary increase of the salinity of irrigation water did not significantly influenced (p < 0.05) in the physiologic characteristics in plants of *P. peruviana* until 60 DAT, except for the variables extravasation of electrolytes and chlorophyll a and b.

When evaluating the intracellular concentration of CO_2 in plants of *P. peruviana* L., the average of 234.61 μmol (CO_2) m^{-2} s^{-1} presented in Table 2 were close to those observed by Silva *et al.* (2015), who obtained 244.87 μmol

m^{-2} s^{-1} when evaluating *Ci* in eggplant (*Solanum melongena* L.) plants at 100 DAT, using 66% more of the irrigation depth recommended for the culture; while Zeist *et al.* (2014) verified variation between 378.29 and 382.94 μmol (CO_2) m^{-2} s^{-1} in *P. peruviana* L. cultivated in two atmospheres (greenhouse and field) at 86 DAT in the municipal district of Guarapuava, PR.

In a similar way, the unitary increase in CEa in levels up to 3.0 dS m^{-1} did not promote alterations in the stomatal conductance (*Gs*) in plants of *P. peruviana* L. to 60 DAT, probably in the function of the osmotic adjustment during the growth of the plants, however, it could commit productive aspects. Climatic factors such as the high temperature at the time of the assessment (10:00 a.m.) and a certain water limitation may have contributed to the achievement of these results. However, the natural process would be for the plants to show differences in stomatal conditions due to the increase in saline levels.

According to Kerbauy (2012), the capacity of some plants in regulating the stomatal opening and the transpiration, associated with assimilation of CO_2, is influenced by factors as hydric availability in the soil, air humidity, and temperature.

The verification of stomatal conductance in plants of *Physalis peruviana* becomes valid because, through this variable, it is possible to evaluate the CO_2 influx in the substomatal cavity and the O_2 efflux as a byproduct of the photosynthesis, so that, as larger is the stomatal opening great will be the CO_2 diffusion (Silva *et al.*, 2015).

In agreement with stomatal conductance and transpiration data (Table 2), it is verified that there was no reduction in the loss of water for the atmosphere due

Table 2: Summary of the variance analysis for the variables foliate area (AF), intercellular concentration of CO_2 (*Ci*), stomatal conductance (*Gs*) transpiration (*E*), photosynthetic rate (*A*), intrinsic efficiency in the use of water (*EIUA*), instantaneous efficiency of the carboxylation (*EICi*), extravasation of electrolytes (EE), chlorophyll a (*Ca*), chlorophyll b (*Cb*) and carotenoids (*Carot*) in plants of *P. peruviana* L. at 60 DAT, under different levels of salinity of the irrigation water

| | Middle square | | | | |
	Salinity	Block	Error	Average	CV%
AF	0.002 [ns]	0.001	0.003	0.27	20.23
Ci	124.355 [ns]	76.089	57.704	234.61	3.24
E	0.043 [ns]	0.723	0.064	1.69	14.97
Gs	0.000 [ns]	0.000	0.000	0.09	16.11
A	0.270 [ns]	0.809	0.235	6.60	7.35
EIUA	0.629 [ns]	1.341	0.126	3.72	9.56
EICi	0.000 [ns]	0.000	0.000	0.03	12.73
EE	116.611 [**]	3.239	4.609	11.34	18.92
Ca	0.065 [**]	0.045 [*]	0.009	1.38	6.96
Cb	0.006 [**]	0.004 [*]	0.000	0.42	6.75
Carot	0.003 [ns]	0.004 [ns]	0.002	0.32	14.32
GL	3	4	12	-	-

** = significant at 1% (p < 0.01); * = significant at 5% (p < 0.05); ns = not significant (p > 0.05). GL = Degree of freedom.

to saline effects, it could be resulting from the temperature oscillation (Figure 1) and hydric availability in the soil. Therefore, as reported by Silva *et al*. (2015), the reduction in the hydric availability can cause stomatal closing, limiting the stomatal conductance and the transpiration, which reduces, consequently, the photosynthetic rate.

Considering the evapotranspiration of the culture (ETc), plants of *Solanum melongena* L. that received 59.48% more water, in relation to ETc, resulted in values of stomatal conductance equal to 0.24 mol of H_2O m^{-2} s^{-1} at 100 DAT (Silva *et al*., 2015), therefore, greater than these observed in this work. These results are evidence that is necessary to reduce the irrigation intervals, which means apply water more frequently and in the same volume when plants of *P. peruviana* are in the flowering period, which starts at 45 DAT.

When evaluating the results obtained for photosynthesis in plants of *P. peruviana* (Table 2) the average of 6.60 μmol (CO_2) m^{-2} s^{-1} can be considered low in agreement with 17.2 and 24.7 ìmol (CO_2) m^{-2} s^{-1} found by Flores (2007) when evaluating different tomato accesses in greenhouse conditions, in Viçosa, MG. However, the shading proportionated by the number of leaves and, consequently, the leaf area (0.27 m^2) could have limited the photosynthetic rate in *P. peruviana* at 60 DAT, because, according to Zeist *et al*. (2014), protected atmospheres as greenhouse provide increments in the speed of leaves expansion, mainly, due to high temperature and relative humidity conditions

To evaluate the influence of the number of leaves and the foliate area in cultures as *Solanum lycopersicum* (Reis *et al*., 2013; Moura *et al*., 2017) and *Solanum melongena* L. (Lima *et al*., 2015) researches were carried out with the intention of predicting agronomic values and adaptations in cultivated plants, creating relations among the number of leaves, the leaf area, and photosynthetic efficiency. Reis *et al*. (2013) explain that the leaf area can provide a larger use of solar energy, capturing photons and using them in photosynthetic processes and in the translocation of photoassimilates. However, according to results observed by Zeist *et al*. (2014), the cultivation environment can be a limiting factor for this variable.

Regarding the intrinsic efficiency in the use of water (Table 2), which represents the reason between the amount of assimilated CO_2 and the water perspired by the plant, it was verified that the different studied saline levels did not provide significant differences for this characteristic in plants of *P. peruviana* L. at 60 DAT, suggesting certain tolerance to the saline stress as a consequence of the growing levels of CEa. Besides, the reason between the photosynthetic tax and the intracellular concentration of CO_2 suggests poor assimilation of CO_2 and accumulation

of reserves in the plant because there was low photosynthesis to the detriment of the high intracellular concentration of CO_2 (Table 2).

Lima *et al*. (2015) mention that some morphologic alterations in plants happen due to hydric, nutritional, and hormonal imbalance, then influencing in the stomatal leaf closing and reduction in the transpiration and absorption of water. However, in agreement with Souza *et al*. (2011), the ions accumulation in the vacuoles, as well as of organic solutes, allow the hydric potential gradient maintenance, resulting in the translocation of water by the plant's root to the aerial part.

Some plants can accumulate salts inside their cells, while others can exclude Cl$^-$ from the root system, or accumulate these ions in the cell vacuole, as well as reducing photosynthetic rate, water absorption, or increasing osmotic potential. In addition, there may have been mechanisms for the exclusion of the element's sodium and chlorine in some morphophysiological structures in *P. peruviana* L. When the extravasation of electrolytes was evaluated in plants of *P. peruviana* L. (Figure 2A) it was verified that the unitary increase in CEa resulted in the increment of 70.88 percentile points among to smallest and the largest saline concentration, which were observed the occurrence of cellular damages in relation to the plants that received saline water. Therefore, high levels of salinity applied in the irrigation of plants of *P. peruviana* L. can damage essential structures of the cells, mainly of the plasmatic membrane.

In agreement with Langaro *et al*. (2014) the extravasation of electrolytes is the last stage observed in function of the oxidative stress, suggesting that there were a breaking of the cellular membrane, so that, the greater is the accumulation of ions in the vacuoles greater will be the cellular extravasation, which implicates, indirectly, in leaves of larger thickness and dark coloration, affecting the chlorophyll contents a and b (Figure 2B). In this way, the capacity of accumulate salts absorbed by the roots and translocate to the leaves does the *P. peruviana* L. a plant considered tolerant at saline levels up to 3.0 dS m^{-1}.

Regarding the chlorophyll content a and b (Figure 2B), it was observed growing linear effect significant for both variables, with an increase of 7.56 and 8.17 percentile points for the chlorophyll values a and b (*Ca* and *Cb*), respectively, when it was used growing levels until 3.0 dS m^{-1} in CEa until 60 DAT (Figure 2B). The results observed for chlorophyll values a and b do not corroborate with the statements by Freire *et al*. (2013), where plants exposed to the salinity of the irrigation water tend to reduce the chlorophyll contents in reason of the enzyme chlorophyllase increase, which degrades the molecules of this pigment.
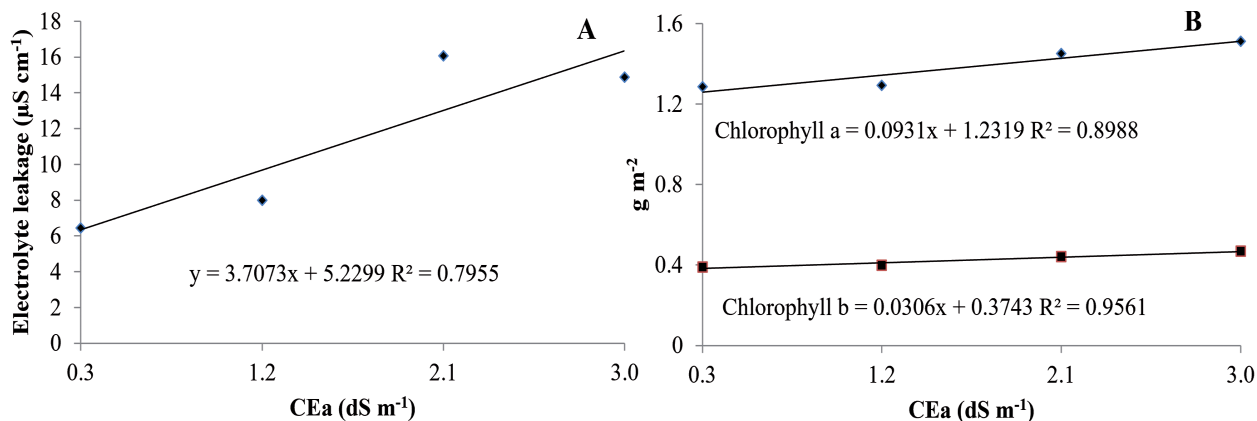
Figure 2: Extravasation of electrolytes (A) and chlorophyll a and b (B) in *P. peruviana* L. plants under different levels of irrigation water salinity at 60 DAT.

The contents of *Ca*, *Cb*, and carotenoids may have been affected due to the pH and to the content of K^+ in the water (Table 1) and in the soil (Table 1), causing the competitive inhibition of the nutrient Mg^{2+}, because it acts in the plant as a cofactor of enzymes phosphorylating, forming a bridge between the pyrophosphate of ATP or ADP and the enzyme's molecule, and, consequently, in the photosynthesis (Taiz *et al.* 2017).

According to Lima *et al.* (2012) the chlorophyll a and b are hydrophobic structures, therefore, insoluble in water, and their main function is to convert luminous energy into chemical energy, a process that happens in the chloroplasts, while the carotenoids are pigments liposoluble with yellow-orange coloration, which can be less visible due to presence of chlorophyll a and b, also considered as antioxidants, which explains the no significant results ($p < 0.05$) for carotenoids contents, expressed in Table 2.

CONCLUSIONS

With the results obtained in this work, it can be concluded that *Physalis peruviana* L. is a species tolerant to salinity in levels up to 3.0 dS m^{-1} and that it develops mechanisms such as reduction in stomatal activity, sweating, and photosynthesis without presenting significant effect in the evaluated physiological characteristics until 60 DAT, except for the variables extravasation of electrolytes and chlorophyll a and b.

ACKNOWLEDGMENTS

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