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Physiological changes of pomegranate seedlings under salt stress and nitrogen fertilization[1]

Alterações fisiológicas de mudas de romãzeira sob estresse salino e adubação nitrogenada

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HIGHLIGHTS:
Irrigation water salinity alters gas exchange and biosynthesis of photosynthetic pigments in pomegranate.
The reduction in CO_2 assimilation in pomegranate plants under salt stress is related to non-stomatal factors.
Increasing nitrogen doses increase electrolyte leakage in pomegranate seedlings.

ABSTRACT: The semi-arid region of northeastern Brazil is characterized by scarce and irregular rainfall; in addition, the waters available for irrigation commonly contain high concentrations of salts, causing osmotic and/or ionic effects on plants. Thus, it is important to identify species adapted to the conditions of scarcity and lower quality of irrigation waters. In this context, the objective of this study was to evaluate the physiological changes of pomegranate when subjected to irrigation with saline waters and different nitrogen doses under greenhouse conditions. The study was carried out using a randomized block design in a 5 × 5 factorial arrangement, whose treatments consisted of five values of electrical conductivity of irrigation water - ECw (0.3; 1.8; 3.3; 4.8 and 6.3 dS m^{-1}) and five nitrogen doses (50, 75; 100; 125 and 150% of the recommended dose for pot experiments), with four replicates. Water salinity from 0.3 dS m^{-1} reduced the CO_2 assimilation rate, chlorophyll b concentration and total dry mass of pomegranate seedlings. However, stomatal conductance and leaf transpiration were not influenced by electrical conductivity of up to 4.0 and 3.6 dS m^{-1}, respectively. The increase in intercellular CO_2 concentration in pomegranate is related to factors of non-stomatal origin. Nitrogen doses did not attenuate the deleterious effects of salt stress on gas exchange, chlorophyll a concentrations and electrolyte leakage of pomegranate seedlings.

Key words: *Punica granatum* L., photosynthesis, mineral fertilization

RESUMO: O semiárido do Nordeste do Brasil se caracteriza por apresentar precipitações escassas e irregulares; além disso, as águas disponíveis para irrigação, comumente, possuem elevadas concentrações de sais, promovendo efeitos de natureza osmótica e/ou iônica sobre as plantas. Deste modo, é importante a identificação de espécies adaptadas às condições de escassez e qualidade inferior das águas de irrigação. Neste contexto, objetivou-se com este estudo avaliar as alterações fisiológicas da romãzeira quando submetida à irrigação com águas salinas e diferentes doses de nitrogênio sob condições de casa de vegetação. O estudo foi realizado utilizando-se o delineamento de blocos casualizados em arranjo fatorial 5 x 5, cujos tratamentos foram constituídos de cinco condutividade elétricas da água - CEa (0,3; 1,8; 3,3; 4,8 e 6,3 dS m^{-1}) e cinco doses de nitrogênio (50, 75; 100; 125 e 150% da dose recomendada para ensaios em vasos), com quatro repetições. A salinidade da água a partir de 0,3 dS m^{-1} diminuiu a taxa de assimilação de CO_2, clorofila b e fitomassa seca total das mudas de romãzeira. Contudo, a condutância estomática e a transpiração foliar não foram influenciadas pela condutividade elétrica de até 4,0 e 3,6 dS m^{-1}, respectivamente. O incremento na concentração intercelular de CO_2 na romãzeira está relacionado a fatores de origem não estomática. As doses de nitrogênio não atenuaram os efeitos deletérios do estresse salino sobre as trocas gasosas, teores de clorofila a e extravasamento de eletrólitos das mudas de romãzeira.

Palavras-chave: *Punica granatum* L., fotossíntese, adubação mineral

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Introduction

The semi-arid region of northeastern Brazil has climatic characteristics such as scarce and irregular rainfall, besides high evapotranspiration (Alves et al., 2011). In this region, it is common to find water sources with a high concentration of salts, mainly sodium, resulting in restrictions for use in agriculture (Walter et al., 2018).

One of the alternatives aimed at minimizing the deleterious effects of salt stress is the use of nitrogen fertilization, which in addition to promoting plant growth, can also reduce damage caused by salinity (Hessini et al., 2019). The explanation may be related to the fact that supplemental application of N increases the absorption of NO_3^-, to the detriment of Cl^-, reducing the Cl^-/N ratio in leaves, which can restore ionic homeostasis, reducing the effects of salt stress on plants (Ibrahim et al., 2018).

Among the species of economic importance for cultivation under salinity conditions is pomegranate (*Punica granatum* L.). This fruit crop shows adequate production suitability in arid and semi-arid regions, and its cultivation can be expanded to regions where the available water has high salt concentrations, because it is relatively tolerant to salt stress, with variations among cultivars (Okhovatian-Ardakani et al., 2010; El-Khawaga et al., 2013). Almeida et al. (2019), evaluating the effect of levels of irrigation water salinity on the growth and tolerance of pomegranate seedlings, found that pomegranate was tolerant to the effects of water salinity up to 6.0 dS m^{-1}.

However, little information is available about the interactive effects of water salinity and nitrogen fertilization on the physiological responses of pomegranate, and such information is important to improve the production practices of this crop in saline soils, or even under conditions where only waters with higher salt concentrations are available (Song et al., 2019). In this context, the objective of this study was to evaluate the physiological indices and the quality of pomegranate seedlings as a function of irrigation with waters of different salinity levels and nitrogen doses.

Material and Methods

The experiment was carried out in a 24-m-long, 8-m-wide arched greenhouse, with 150-micron low-density polyethylene cover, at the Center for Sciences and Agri-Food Technology (CCTA/UFCG) located in the municipality of Pombal, PB, Brazil, at the geographic coordinates 6° 47' 20" South latitude and 37° 48' 01" West longitude, at an altitude of 194 m. Figure 1 shows the meteorological data observed along the experiment between November 12, 2018 and August 8, 2019.

The experimental design was in randomized blocks, using a 5 x 5 factorial arrangement, whose treatments consisted of five values of electrical conductivity of irrigation water - ECw (0.3; 1.8; 3.3; 4.8 and 6.3 dS m^{-1}) and five nitrogen doses (50, 75, 100, 125 and 150% of the recommendation of Novais et al., 1991). Four replicates were used, with one plant in each experimental unit, totaling one hundred experimental units. The dose referring to 100% corresponded to 100 mg of N kg^{-1} of soil.

The water used in the irrigation of the treatment of lowest salinity (0.3 dS m^{-1}) came from the public supply system of Pombal, PB, Brazil; with this same water, the other salinity levels were prepared in such a way to have an equivalent proportion of 7:2:1, of Na:Ca:Mg, respectively, from the salts NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$, a proportion that prevails in sources of water used for irrigation in small properties of northeastern Brazil (Audry & Suassuna, 1995).

Containers with capacity of 1.5 dm^3 of substrate were filled with a mixture in the proportion of 2:1:1 of a Entisol with sandy loam texture, sand and organic matter (aged bovine manure was used as source), from the rural area of the municipality of São Domingos, PB, Brazil, whose chemical and physical-hydraulic characteristics were obtained according to the methodology proposed by Donagema et al. (2011): Ca^{2+} = 2.42 $cmol_c$ kg^{-1}; Mg^{2+} = 5.84 $cmol_c$ kg^{-1}; Na^+ = 0.09 $cmol_c$ kg^{-1}; K^+ = 0.21 $cmol_c$ kg^{-1}; $H^+ + Al^{3+}$ = 0.00 $cmol_c$ kg^{-1}; CEC = 8.56 $cmol_c$ kg^{-1}; organic matter =3.80 dag kg^{-1}; P = 11.99 mg kg^{-1}; pH in water (1:2.5) = 7.00; electrical conductivity of saturation

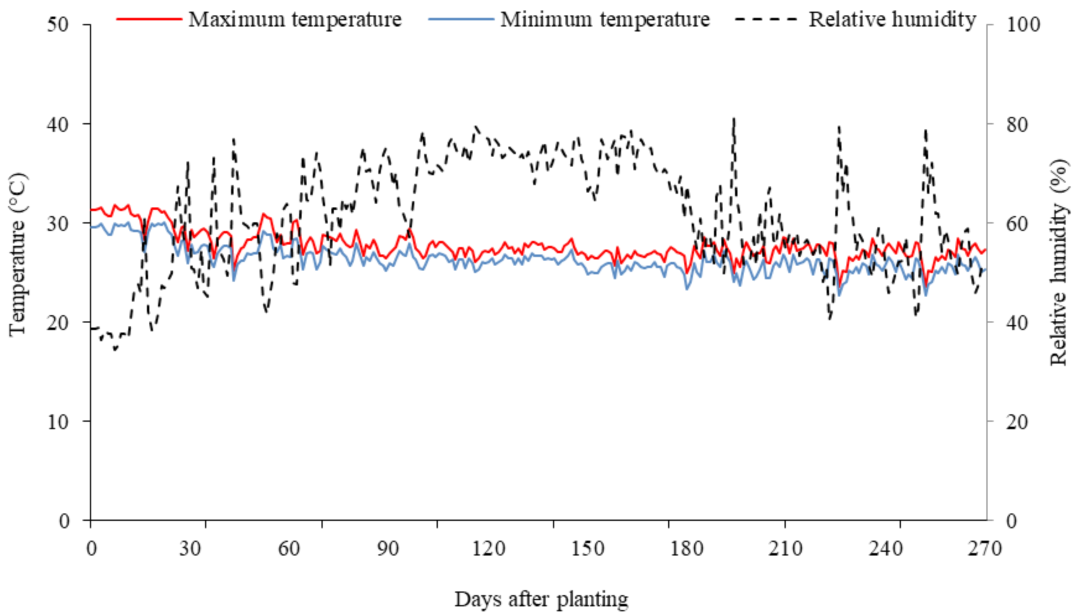


Figure 1. Climatic data of maximum and minimum temperature (°C) and relative air humidity (%) during the experimental period

extract = 0.20 dS m^{-1}; sand = 846.3 g kg^{-1}; silt =137.4 g kg^{-1}; clay = 16.3 g kg^{-1}; moisture at 33.42 kPa = 11.16 dag kg^{-1}; moisture at 1519.5 kPa = 4.23 dag kg^{-1}.

Prior to sowing, the soil moisture content was increased until reaching field capacity, using the respective water of each treatment. After transplanting, irrigation was performed daily, applying in each container a volume of water sufficient to maintain soil moisture close to field capacity. The applied volume was determined according to the water requirement of the seedlings, estimated by the water balance: volume applied minus volume drained in the previous irrigation, plus a leaching fraction of 0.10 every 15 days.

Herbaceous cuttings of the 'Molar' variety from several parent plants were used. The cuttings were collected early in the morning and then placed in a polystyrene box, being arranged in layers and covered by moistened paper towels, to avoid dehydration. Then, they were washed in running water and disinfested with 2% sodium hypochlorite solution for 5 min and standardized for size, establishing a length of 15 cm and mean diameter of 4.5 mm.

Fertilizations were performed as topdressing. Fertilizations with phosphorus and potassium were based on the recommendation of Novais et al. (1991), applying the equivalent to 300 mg P_2O_5 and 150 mg K_2O kg^{-1} of soil, using single superphosphate and potassium chloride, respectively; all phosphorus recommendation was applied at planting, while potassium was applied via irrigation water at 30 and 50 days after planting (DAP). Nitrogen fertilization was performed via fertigation, at intervals of ten days, and the doses were applied per container according to the recommendation of N (100 mg N kg^{-1} of soil), using urea.

The effects of the different treatments on pomegranate crop were measured at 270 DAP, through the determination of gas exchange: stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci) and CO_2 assimilation rate (A). These measurements were performed with a gas exchange meter in plants, containing an infrared gas analyzer (IRGA, LCpro - SD model, ADC Bioscientific, UK), under photosynthetic photon flux density of 1,200 μmol m^{-2} s^{-1} and air flow of 200 mL min^{-1}.

In the same leaves, photosynthetic pigments were determined: chlorophyll a (Chl a) and chlorophyll b (Chl b) and carotenoid (Car) concentrations. Chlorophyll a and b contents were determined according to the laboratory method developed by Arnon (1949), by means of samples of 5 discs of the lamina of the third mature leaf from the apex; chlorophyll and carotenoid concentrations in the solutions were determined by measuring absorbance (ABS) at wavelengths of 470, 646, and 663 nm, using a spectrophotometer (Spectrum SP-88 1105). Pigment concentrations were estimated by the following equations: Chlorophyll a (Chl a) = 12.21 ABS663 −2.81 ABS646; Chlorophyll b (Chl b) = 20.13 A646−5.03 ABS663; Total carotenoids (Car) = (1000ABS470 −1.82 Chl a− 85.02 Chl b)/198. The values obtained for chlorophyll a, chlorophyll b and carotenoid contents in the leaves were expressed in mg g^{-1} of fresh matter (mg g^{-1} FM).

Total dry mass (TDM) was also measured at 270 DAP. TDM was obtained by summing the dry mass of leaves, stems and roots (data not shown), after drying in an oven at a constant temperature of 65 °C for 48 hours. In the same period, electrolyte leakage was also determined, as recommended by Campos & Thi (1997).

The data obtained were subjected to the verification of homogeneity of variances and subsequently to analysis of variance and, in cases of significance, linear and quadratic polynomial regression analysis was performed, using the statistical program SISVAR (Ferreira, 2011). When there was significant interaction between the factors salinity levels (SL) and nitrogen doses (ND), TableCurve 3D software was used to obtain the response surface curves.

Results and Discussion

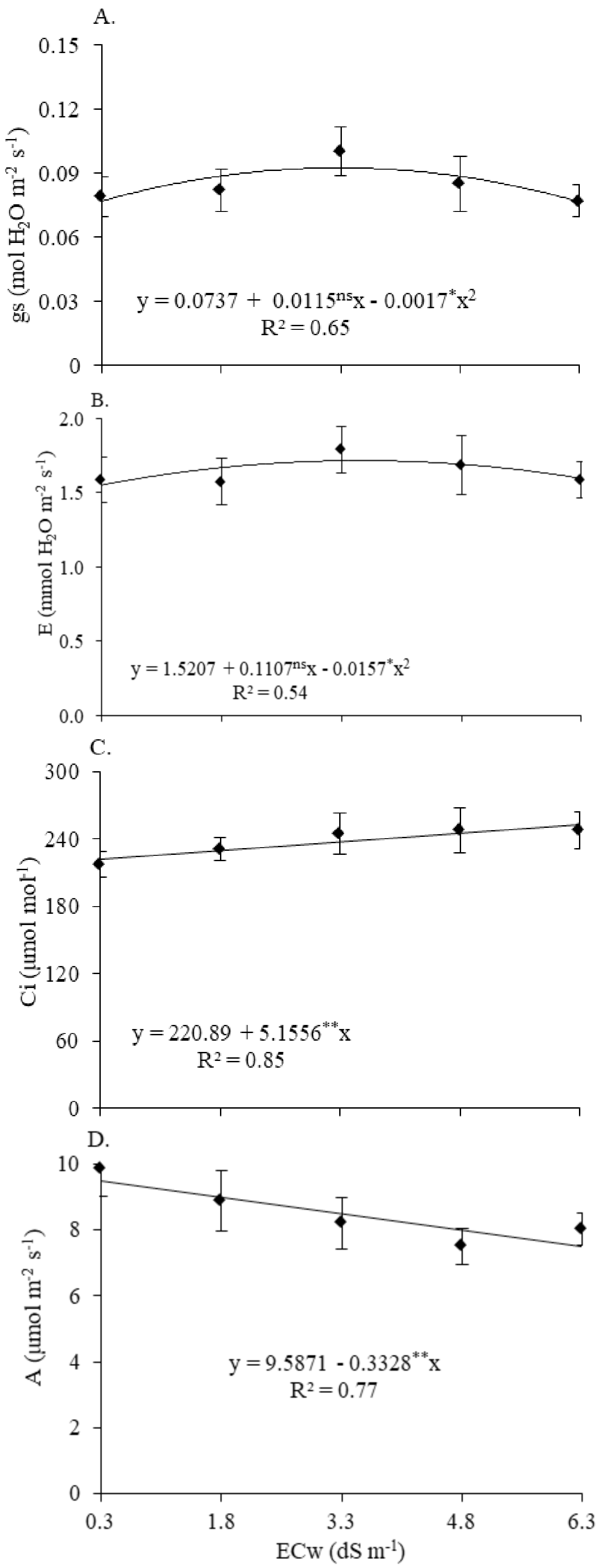
According to the summary of the analysis of variance, the salinity levels (SL) had a significant influence on stomatal conductance (gs), transpiration (E), intercellular CO_2 concentration (Ci) and CO_2 assimilation rate (A). Nitrogen doses (ND) and the interaction between factors (SL x ND) did not significantly influence any of the physiological variables analyzed (Table 1).

Water salinity levels affected the stomatal conductance of pomegranate seedlings in a quadratic way (Figure 2A) and, according to the regression equation, the maximum estimated value of 0.093 mol H_2O m^{-2} s^{-1} was obtained in plants subjected to water salinity of 4.0 dS m^{-1}, decreasing from this level, reaching the value of 0.078 mol H_2O m^{-2} s^{-1} when using the highest water salinity level (6.3 dS m^{-1}). This reduction in the gs of pomegranate seedlings occurs due to stomatal closure caused by osmotic effects, which reduces the water or ionic potential due to the effect of specific ions or nutritional stress, caused by salinity, as a defense mechanism of the plant to minimize water losses through the leaves and, consequently, the absorption of water and salts from the soil solution (Hussain et al., 2012). Dias et al. (2018) evaluated the effects of water salinity (ECw of 0.8 and 3.8 dS m^{-1}) on the gas exchange of West Indian cherry cv. BRS 366 Jaburu and also found that the increase in ECw caused reduction in the stomatal conductance, at 180 days after transplanting.

Table 1. Summary of the analysis of variance for stomatal conductance (gs), transpiration (E), intercellular CO_2 concentration (Ci) and CO_2 assimilation rate (A) of pomegranate var. Molar at 270 days after planting (DAP), irrigated with saline waters and fertilized with nitrogen

Source of variation	DF	Mean squares			
		gs	E	Ci	A
Water salinity levels (SL)	4	0.0017^{**}	0.3647^{**}	3539.59^{**}	15.19^{**}
Linear regression	1	0.0006^{ns}	0.0890^{ns}	11960.94^{**}	12.53^{**}
Quadratic regression	1	0.00006^{*}	0.00007^{*}	2091.89^{*}	0.25^{ns}
Nitrogen doses (ND)	4	0.0001^{ns}	0.1036^{ns}	626.59^{ns}	1.09^{ns}
Linear regression	1	0.0004^{ns}	0.3419^{*}	747.49^{ns}	4.33^{ns}
Quadratic regression	1	0.00004^{ns}	0.0009^{ns}	296.29^{ns}	0.04^{ns}
Interaction (SL x ND)	16	0.0006^{ns}	0.1231^{ns}	2124.69^{ns}	5.03^{ns}
Blocks	3	0.0010^{*}	0.7065^{**}	7224.00^{**}	9.35^{**}
Residual	72	0.0003	0.0704	564.77	2.14
CV (%)		20.70	16.54	9.99	17.90
Overall mean		0.08	1.56	218.66	8.78

CV - Coefficient of variation; DF - Degrees of freedom; ns, *, ** - Not significant, significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively



ns, *, ** - Not significant, significant at $p \le 0.05$ and at $p \le 0.01$ by F test, respectively

Figure 2. Stomatal conductance - gs (A), transpiration - E (B), intercellular CO_2 concentration - Ci (C) and CO_2 assimilation rate - A (D) of pomegranate seedlings var. Molar, as a function of electrical conductivity of irrigation water - ECw at 270 days after transplantation (DAT)

As observed for gs (Figure 2A), the transpiration of pomegranate seedlings was quadratically influenced by water salinity. According to the regression equation (Figure 2B), irrigation using water with electrical conductivity of 3.6 dS m^{-1} caused a higher estimated value of E (1.716 μmol mol^{-1}). Mastrogiannidou et al. (2016) stated that the reduction of transpiration in plants under salt stress is related to stomatal closure in response to osmotic stress caused by the increase in salinity. Studying the gas exchange of grafted West Indian cherry as a function of salt stress (ECw of 0.8 and 3.8 dS m^{-1}), Dias et al. (2019) observed, as in this study, that the increase in water salinity from 0.8 to 3.8 dS m^{-1} sharply reduced leaf transpiration of plants at 400 days after transplanting.

The intercellular CO_2 concentration of pomegranate increased linearly as a function of the increase in water salinity levels. According to the regression equation (Figure 2C), there were increments in Ci of 2.33% per unit increase in ECw, that is, when using water with electrical conductivity of 6.3 dS m^{-1} there was an increase of 13.90% (30.93 μmol mol^{-1}) compared to plants cultivated under ECw of 0.3 dS m^{-1}. The increase in the intercellular CO_2 concentration is indicative of deterioration of the photosynthetic structure, because the damage caused to the structures responsible for CO_2 fixation is not only due to stomatal factors, but also to the accumulation of salts in the leaves (Hussain et al., 2012). The increase in the internal CO_2 concentration as a function of the increase in salinity has also been observed in other crops such as West Indian cherry (Dias et al., 2018; Dias et al., 2019) and cotton (Lima et al., 2017).

As for stomatal conductance and transpiration, the increase in electrical conductivity negatively affected the CO_2 assimilation rate (A), which decrease by 21.04% as the salinity of irrigation water increased from 0.3 to 6.3 dS m^{-1} (Figure 2D). This decrease in the CO_2 assimilation rate of pomegranate seedlings with the increase in salinity levels of irrigation water is directly related to the reduction of stomatal conductance, in addition to the increase in intercellular CO_2 concentration, clearly indicating that salinity may have increased mesophilic resistance to the entry of atmospheric CO_2 into carboxylation sites and/or reduced enzymatic activities associated with the photosynthetic carbon metabolism, and this effect is common in plants grown under saline conditions (Cruz et al., 2017; Olmo et al., 2019).

Based on the results of the analysis of variance (Table 2), there was a significant effect of the irrigation water salinity levels (SL) on chlorophyll a (Chl a) and chlorophyll b (Chl b). In relation to the nitrogen doses factor, there was a significant effect on total dry mass (TDM) and electrolyte leakage (EL). Regarding the interaction between the factors (SL x ND), a significant effect was observed only on chlorophyll a. For the carotenoid (Car) concentrations, there was no effect of the studied factors, which strengthens the idea that the treatments corresponding to irrigation with salinized water during the production of pomegranate seedlings led to positive results, because carotenoids are accessory pigments in the absorption and transfer of radiant energy and protectors of chlorophyll against photooxidation.

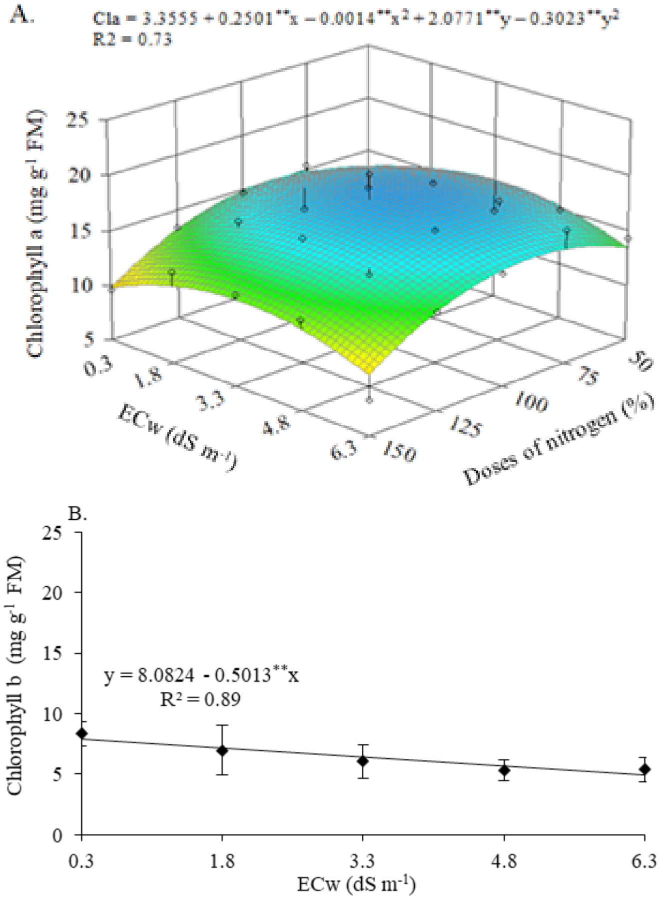
Table 2. Summary of the analysis of variance for chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car), total dry mass (TDM) and electrolyte leakage (EL) of pomegranate var. Molar, irrigated with saline water and fertilized with nitrogen, at 270 days after planting (DAP)

Source of variation	DF	Mean squares				
		Chl a	Chl b	Car	TDM	EL
Water salinity levels (SL)	4	46.69**	23.88**	0.11^{ns}	53.68^{ns}	4.73^{ns}
Linear regression	1	148.90**	56.74**	0.27^{ns}	31.27^{ns}	14.67*
Quadratic regression	1	0.03^{ns}	1.11^{ns}	0.13^{ns}	50.29^{ns}	2.69^{ns}
Nitrogen doses (ND)	4	22.57*	12.50^{ns}	0.25^{ns}	90.04**	7.62*
Linear regression	1	0.11^{ns}	1.98^{ns}	0.31^{ns}	69.03**	5.39*
Quadratic regression	1	0.96^{ns}	6.67^{ns}	0.16^{ns}	173.32**	1.12^{ns}
Interaction (SL x ND)	16	29.13**	7.00^{ns}	0.54^{ns}	24.26^{ns}	4.17^{ns}
Blocks	3	82.98^{ns}	22.43^{ns}	0.33^{ns}	322.49**	2.37^{ns}
Residual	72	8.82	5.97	0.28	26.90	2.60
CV (%)		20.58	38.02	13.23	19.82	16.51
Overall mean		14.43	6.43	4.03	26.04	9.78

CV - Coefficient of variation; DF - Degrees of freedom; ns, *, ** - Not significant, significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively

According to the regression equations for chlorophyll a concentrations as a function of water salinity levels and nitrogen fertilization doses (Figure 3A), plants that received doses of 75 and 100 mg N kg^{-1} of soil and were irrigated using water with ECw of 4.8 dS m^{-1} showed higher chlorophyll a concentrations (17.24 and 17.34 mg g^{-1} FM), respectively. For plants grown under the doses of 150 mg N kg^{-1} of soil irrigated with ECw of 0.3 and 6.3 dS m^{-1}, the lowest average chlorophyll a concentrations were 9.96 and 10.45 mg g^{-1} FM, respectively (Figure 3A). Several authors have demonstrated a reduction in chlorophyll content, which can be explained in part by the antagonism in nitrogen absorption due to the NO_3^-/Cl^- interaction at the ion transport sites, because chloride results in severe membrane depolarization associated with non-competitive inhibition in nitrate absorption (Chen et al., 2010; Farooq et al., 2015). Studies involving pomegranate genotypes showed a decrease in chlorophyll content in response to salt stress (Melgar et al., 2008; Mastrogiannidou et al., 2016). In the present study, however, depending on the N dose, there was increase, reduction or no effect of salinity on chlorophyll a concentrations, indicating that this response may vary with genotype and level of stress.

It is verified that only the water salinity factor the affected the chlorophyll b concentration (Table 2), with a linear decrease of 6.20% per unit increase in ECw (Figure 3B), that is, a reduction of 37.91% (3.0 mg g^{-1} FM) in the Chl b of plants irrigated with water of 6.3 dS m^{-1} compared to those subjected to 0.3 dS m^{-1}. In general, chlorophyll concentration in plants has a strong negative correlation with salinity, due to the absorption of some ions, such as Mg^{+2} and Fe^{+2}, which are involved in chlorophyll formation. In addition, chlorophyll-degrading enzyme, chlorophyllase, is more active under salt stress (Florina et al., 2013). Similarly, Mastrogiannidou et al. (2016) evaluated the effects of NaCl, KCl and Na_2SO_4 on the growth, tissue ions, chlorophyll content and antioxidant response of *P. granatum* and found that excess salts resulted in a significant decrease in chlorophyll concentrations in the leaves of pomegranate seedlings, under treatment with KCl at a concentration of 120 mM, leading to the lowest values compared to the control.

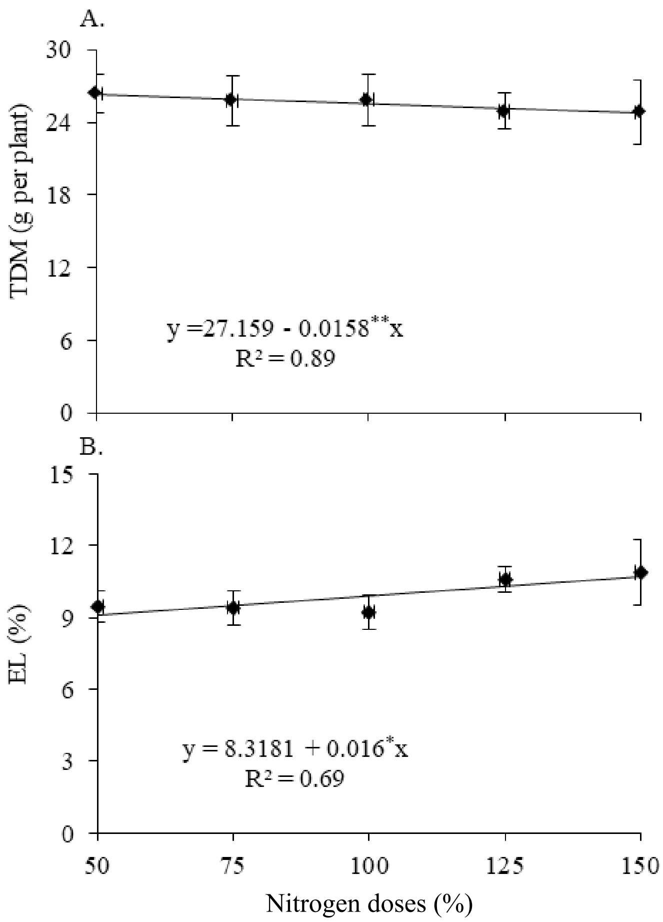


ns, *, ** - Not significant, significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively; Figure 3A: X and Y correspond to the nitrogen dose and ECw, respectively

Figure 3. Chlorophyll a concentrations (A) of pomegranate as a function of electrical conductivity of irrigation water (ECw) and doses of nitrogen fertilization, and chlorophyll b concentrations (B) as a function of electrical conductivity of irrigation water – ECw, at 270 days after transplantation (DAT)

The total dry mass (TDM) of pomegranate was negatively influenced by the increase in nitrogen doses and, according to the regression equation (Figure 4A), there were linear reductions of 1.45% for each 25% increase in N dose. When the TDM of plants that received N doses of 150% was compared with the TDM of those that were fertilized with the lowest dose (50%), there was a decrease of 1.58 g per plant. According to Alcarde et al. (2007), the nutritional requirements of plants vary according to development stage. Therefore, when a quantity greater than those required by the species is applied, there may be antagonistic effects, causing soil chemical degradation, contributing to increased salinization and acidification, and compromising plant growth (Oliveira et al., 2017).

For electrolyte leakage (EL) as a function of nitrogen doses (Figure 4B), there was an increase in EL with the increase of nitrogen doses, where plants that received the highest dose (150 mg of N per kg^{-1}) had an EL of 10.71%. Thus, the increase in nitrogen doses led to the increase of electrolyte leakage, due to damage to the cell membrane, mainly caused by the influx of K^+ and the so-called counterions (Cl^-, HPO_4^{2-}, NO_3^-, citrate^{3-} and malate^{2-}), which move to balance the efflux of positively charged potassium ions (Bajji et al., 2002).



*, ** - Significant at p ≤ 0.05 and at p ≤ 0.01 by F test, respectively

Figure 4. Total dry mass - TDM (A) and electrolyte leakage - EL (B) of pomegranate seedlings as a function of the doses of nitrogen fertilization, at 270 days after transplant (DAT)

Conclusions

1. Irrigation water electrical conductivity from 0.3 dS m^{-1} reduced the CO_2 assimilation rate, chlorophyll b concentration and total dry mass of pomegranate seedlings. However, stomatal conductance and leaf transpiration were not influenced by electrical conductivity of up to 4.0 and 3.6 dS m^{-1}, respectively.

2. The increase in intercellular CO_2 concentration in pomegranate is related to factors of non-stomatal origin.

3. Nitrogen doses did not attenuate the deleterious effects of salt stress on the gas exchange, chlorophyll a concentrations and electrolyte leakage of pomegranate seedlings.

Literature Cited

Alcarde, C. A. Fertilizantes. In: Novaes, R. F.; Alvarez, V. V. H.; Barros, N. F.; Fontes, R. L. F.; Cantarutti, R. B.; Neves, J. C. L. Fertilidade do solo. Viçosa: Sociedade Brasileira de Ciência do Solo, 2007. p.737-768.

Almeida, J. P. N. de; Mendonça, V.; Alves, A. A.; Cardoso Neto, R.; Costa, L. P.; Silva, F. S. O. Morphometric responses and tolerance of pomegranate seedlings irrigated with saline water. Revista Brasileira de Engenharia Agrícola e Ambiental, v.23, p.341-346, 2019. https://doi.org/10.1590/1807-1929/agriambi.v23n5p341-346

Alves, M. S.; Soares, T. M.; Silva, L. T.; Fernandes, J. P.; Oliveira, M. L. A.; Paz, V. P. S. Estratégias de uso de água salobra na produção de alface em hidroponia NFT. Revista Brasileira de Engenharia Agrícola e Ambiental, v.15, p.491-498, 2011. https://doi.org/10.1590/S1415-43662011000500009

Arnon, D. I. Copper enzymes in isolated chloroplasts: Polyphenoloxidases in *Beta vulgaris*. Plant Physiology, v.24, p.1-15, 1949. https://doi.org/10.1104/pp.24.1.1

Audry, P.; Suassuna, J. A. A qualidade da água na irrigação do trópico semi-árido - um estudo de caso. In: Seminário Franco-Brasileiro de Pequena Irrigação. Recife, Anais... Recife: CNPq, SUDENE, 1995, p.147-153.

Bajji, M.; Kinet, J. M.; Lutts, S. Osmotic and ionic effects of NaCl on germination, early seedling growth and ion content of *Atriplex halimus* (Chenopodiaceae). Canadian Journal of Botany, v.80, p.297-304, 2002. https://doi.org/10.1139/b02-008

Campos, P. S.; Thi, A. T. P. Effects of an abscisic acid pretreatment on membrane leakage and lipid composition of *Vigna unguiculata* leaf discs subject to osmotic stress. Plant Science, v.130, p.11-18, 1997. https://doi.org/10.1016/S0168-9452(97)00199-4

Chen, W.; Hou, Z.; Wu, L.; Liang, Y.; Wei, C. Effects of salinity and nitrogen on cotton growth in arid environment. Plant Soil, v.326, p.61-73, 2010. https://doi.org/10.1007/s11104-008-9881-0

Cruz, J. L.; Coelho Filho, M. A.; Coelho, E. F.; Santos, A. A. dos. Salinity reduces carbon assimilation and the harvest index of cassava plants (*Manihot esculenta* Crantz). Acta Scientiarum. Agronomy, v.39, p.545-555, 2017. https://doi.org/10.4025/actasciagron.v39i4.32952

Dias, A. S.; Lima, G. S. de; Pinheiro, F. W. A.; Gheyi, H. R.; Soares, L. A. A. Gas exchanges, quantum yield and photosynthetic pigments of West Indian cherry under salt stress and potassium fertilization. Revista Caatinga, v.32, p.429-439, 2019. https://doi.org/10.1590/1983-21252019v32n216rc

Dias, A. S.; Lima, G. S. de.; Sá, F. V. S.; Gheyi, H. R.; Soares, L. A. A.; Fernandes, P. D. Gas exchanges and photochemical efficiency of West Indian cherry cultivated with saline water and potassium fertilization. Revista Brasileira de Engenharia Agrícola e Ambiental, v.22, p.628-633, 2018. https://doi.org/10.1590/1807-1929/agriambi.v22n9p628-633

Donagema, G. K.; Campos, D. V. B.; Calderano, S. B.; Teixeira, W. G.; Viana, J. H. M. (Org.). Manual de métodos de análise de solo. 2. ed. Rio de Janeiro, RJ: Embrapa Solos. 2011. 230 p.

El-Khawaga, A. S.; Zaeneldeen, E. M. A.; Yossef, M. A. Response of three pomegranate cultivars (*Punica granatum* L.) to salinity stress. Middle East Journal of Agriculture Research, v.1, p.64-75, 2013.

Farooq, M.; Hussain, M.; Wakeel, A.; Siddique, K. H. M. Salt stress in maize: effects, resistance mechanisms, and management. A review. Agronomy for Sustainable Development, v.35, p.461-481, 2015. https://doi.org/10.1007/s13593-015-0287-0

Ferreira, D. F. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia (UFLA), v.35, p.1039-1042, 2011. https://doi.org/10.1590/S1413-70542011000600001

Florina, F.; Giancarla, V.; Cerasela, P.; Sofia, P. The effect of salt stress on chlorophyll content in several Romanian tomato varieties. Journal of Horticulture, Forestry and Biotechnology, v.17, p.363-367, 2013.

Hessini, K.; Issaoui, K.; Ferchichi, S.; Abdelly, C.; Siddique, K. H. M.; Cruz, C. Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize. Plant Physiology and Biochemistry, v.139, p.171-178, 2019. https://doi.org/10.1016/j.plaphy.2019.03.005

Hussain, S.; Luro, F.; Costantino, G.; Ollitrault, P.; Morillon, R. Physiological analysis of salt stress behavior of citrus species and genera: Low chloride accumulation as an indicator of salt tolerance. South African Journal of Botany, v.81, p.103-112, 2012. https://doi.org/10.1016/j.sajb.2012.06.004

Ibrahim, M. E. H.; Zhu, X.; Zhou, G.; Ali, A. Y. A.; Ahmad, I.; Farahk, G. A. Nitrogen fertilizer alleviated negative impacts of NaCl on some physiological parameters of wheat. Pakistan Journal of Botany, v.50, p.2097-2104, 2018.

Lima, G. S. de; Dias, A. S.; Soares, L. A. A.; Gheyi, H. R.; Nobre, R. G.; Souza, L. P. Growth and yield of colored-fiber cotton grown under salt stress and nitrogen fertilization. Revista Brasileira de Engenharia Agrícola e Ambiental, v.21, p.415-420, 2017. https://doi.org/10.1590/1807-1929/agriambi.v21n6p415-420

Mastrogiannidou, E.; Chatzissavvidis, C.; Antonopoulou, C.; Tsabardoukas, V.; Giannakoula, A.; Therios, I. Response of pomegranate cv. wonderful plants to salinity. Journal of Soil Science and Plant Nutrition, v.16, p.621-636, 2016. https://doi.org/10.4067/S0718-95162016005000032

Melgar, J. C.; Syvertsen, J. P.; Martinez, V.; Garcia-Sanchez, F. Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. Biologia Plantarum, v.52, p.385-390, 2008. https://doi.org/10.1007/s10535-008-0081-9

Novais, R. F.; Neves, J. C. L.; Barros, N. F. Ensaio em ambiente controlado. In: Oliveira, A. J. (ed.) Métodos de pesquisa em fertilidade do solo. Brasília: Embrapa-SEA. p.189-253. 1991.

Okhovatian-Ardakani, A. R.; Mehrabanian, M.; Dehghani, E.; Akbarzadeh, A. Salt tolerance evaluation and relative comparison in cuttings of different pomegranate cultivars. Plant Soil and Environment, v.56, p.176-185, 2010. https://doi.org/10.17221/158/2009-PSE

Oliveira, F. Í. F. de; Souto, A. G. de L.; Cavalcante, L. F.; Medeiros, W. J. F. de; Bezerra, F. T. C.; Bezerra, M. A. F. Quality of jackfruit seedlings under saline water stress and nitrogen fertilisation. Semina: Ciências Agrárias, v.38, p.2337-2350, 2017. https://doi.org/10.5433/1679-0359.2017v38n4SUPLp2337

Olmo, A.; Garcia-Sanchez, F.; Simon, I.; Lidon, V.; Alfosea-Simon, M.; Camara-Zapata, J. M.; Martinez-Nicolas, J. J.; Simon-Grao, S. Characterization of the ecophysiological responses of three pomegranate cultivars to salinity. Photosynthetica, v.57, p.1015-1024, 2019. https://doi.org/10.32615/ps.2019.103

Song, X.; Zhou, G.; Ma, B.; Wu, W.; Ahmad, I.; Zhu, G.; Yan, W.; Jiao, X. Nitrogen application improved photosynthetic productivity, chlorophyll fluorescence, yield and yield components of two oat genotypes under saline conditions. Agronomy, v.9, p.115-122, 2019. https://doi.org/10.3390/agronomy9030115

Walter, J.; Lück, E.; Bauriegel, A.; Facklam, M.; Zeitz, J. Seasonal dynamics of soil salinity in peatlands: A geophysical approach. Geoderma, v.310, p.1-11, 2018. https://doi.org/10.1016/j.geoderma.2017.08.022