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Photochemical and phytomass aspects of pomegranate seedlings grown under water salinity and foliar nitrogen fertilization

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ABSTRACT

Pomegranate is a fruit with potential for exploitation in the Brazilian Northeast as medicine and food. However, the qualitative and quantitative scarcity of water resources is common in this region, requiring the use of water with high salt contents in irrigated agriculture. The adoption of strategies to reduce the harmful effects of salt stress in plants is therefore necessary, and foliar nitrogen (N) fertilization is a promising alternative. The objective of the work, therefore, was to evaluate the photochemical and phytomass aspects of pomegranate seedlings grown under irrigation water salinity and foliar nitrogen fertilization. The experiment was performed in a randomized block design, in an incomplete factorial scheme, with five levels of electrical conductivity of irrigation water - ECw (0.50, 1.15, 2.75, 4.35 and 5.00 dS m^{-1}) and five nitrogen doses - N (0.0, 0.33, 1.15, 1.97 and 2.30 g L^{-1}), totaling nine combinations, generated by the Central Composite Design, with four repetitions and two plants per repetition. Foliar N application at a dose of 1.37 g L^{-1} increased the production of leaf and root dry phytomass at 90 days after emergence. Salt stress reduced the photochemical activity and phytomass production of pomegranate seedlings, while there was a stimulus by increasing N application up to 2.3 g L^{-1}.

Keywords: nitrogen, photochemical efficiency, *Punica granatum* L., salt stress.

Aspectos fotoquímicos e fitomassas de mudas romãzeira sob salinidade da água e adubação foliar nitrogenada

RESUMO

A romã é uma fruteira com potencial de exploração no Nordeste brasileiro com potencial de uso como composto medicinal e na alimentação. Porém, a escassez qualitativa e quantitativa dos recursos hídricos é comum nesta região, sendo necessário o uso de águas com elevados teores de sais na agricultura irrigada. Assim, é necessário a adoção de estratégias para reduzir os efeitos deletérios do estresse salino sobre as plantas, sendo a adubação foliar nitrogenada uma alternativa promissora. Assim, o objetivo do trabalho foi avaliar os aspectos fotoquímicos e as fitomassas da romãzeira em função da salinidade da água de irrigação e adubação foliar de nitrogênio (N). O experimento foi realizado em delineamento experimental de blocos casualizados, em arranjo fatorial incompleto, com cinco níveis de condutividade elétrica da água – CEa (0,50; 1,15; 2,75; 4,35 e 5,00 dS m^{-1}) e cinco doses de nitrogênio - N (0,0; 0,33; 1,15; 1,97 e 2,30 g L^{-1}), totalizando nove combinações, gerados pela matrix Composto Central de Box, com quatro repetições e duas plantas por repetição. A aplicação foliar de N na dose de 1,37 g L^{-1}, aumentou a produção de fitomassa seca de folhas e da raiz, aos 90 dias após a emergência. O estresse salino reduziu a atividade fotoquímica e a produção de fitomassa das plantas de romã, enquanto houve um estimulo pela aplicação crescente de N até 2,3 g L^{-1}.

Palavras-chave: eficiência fotoquímica, estresse salino, nitrogênio, *Punica granatum* L.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is a fruit with great potential for exploitation in the Northeast of Brazil. There is a growing demand for it in the market due to its nutraceutical properties and the strongly antioxidative capacities of substances contained in its fruit and seeds, such as phenolic compounds and acids, flavonoids, and tannins (Singh *et al.*, 2018).

In addition to its high nutritional value and medicinal properties, pomegranate is a species that is moderately tolerant to semi-arid conditions (Rashedy *et al.*, 2022), showing adaptability to different environmental conditions, withstanding high temperatures and dry periods (Farsi *et al.*, 2023).

Despite its adaptability to the conditions of the Brazilian semi-arid region, the qualitative and quantitative water scarcity requires the use of water with high salinity. Salinity compromises many biochemical, physiological, molecular and morphological processes in plants (Jahan *et al.*, 2020).

Water restriction imposed by the reduction in osmotic potential, ionic toxicity, especially of Na^+ and Cl^- ions, and nutritional imbalance is one of the main harmful effects caused by salt stress (Hu *et al.*, 2021). Toxic ions accumulation also induces the production of reactive oxygen species (ROS), promoting oxidative stress (Hasanuzzaman *et al.*, 2021).

Thus, the search for strategies that can reduce the damaging effect of salt stress in plants is necessary, and foliar N fertilization is a promising alternative. N is an element that is involved in a number of functions that increase plant tolerance to salinity, acting in the synthesis of enzymes, as a structural component of amino acids and proteins involved in the antioxidant defense system, which are able to promote osmotic adjustment (Lima *et al.*, 2019; Coulombier *et al.,* 2020). Positive effects of N fertilization have already been found in several studies, such as Sá *et al.* (2018) in acerola (*Malpighia emarginata* DC), Figueiredo *et al.* (2020) in passion fruit (*Passiflora edulis* Sims. f. flavicarpa), and Fatima *et al.* (2023) in custard apple (*Annona squamosa* L.).

N is a nutrient that can be made available by adding organic matter to the soil, by mineral fertilization via the soil and foliage, in the biological fixation of N from fixing bacteria or by the presence of the element in the soil. However, since the foliar application of N is a promising alternative to improve the performance of crop resistance to salinity, and in view of the lack of information related to its use in pomegranate trees, the objective of this study was to evaluate the photochemical and phytomass aspects of pomegranate seedlings grown under irrigation water salinity and foliar fertilization with nitrogen.

2. MATERIAL AND METHODS

The experiment was performed in a greenhouse of the Centro de Ciências Agrárias, of the

Universidade Federal da Paraíba (CCA-UFPB), municipality of Areia, Paraíba, Brazil. The municipality is located at the geographical coordinates 6°58’00’’ S and 35°41’00’’ W, at an altitude of 575 m. The climate of the region, according to the Köppen classification, is type As', dry, hot summer and rains in winter (Alvares *et al.*, 2013). During the experiment, the average air temperature and humidity were 28.4°C and 56.5%, respectively.

The experiment was performed in a randomized block design, in an incomplete factorial scheme, with five levels of electrical conductivity of irrigation water - ECw (0.50, 1.15, 2.75, 4.35 and 5.00 dS m^{-1}) and five nitrogen doses - N (0.0, 0.33, 1.15, 1.97 and 2.30 g L^{-1}), totaling nine combinations, generated by the Central Composite Design, with four repetitions and two plants per repetition. N doses were based on Fatima *et al.* (2022), using 5 doses because the Central Composite Design requires that the number of factors be equal to generate the combination of treatments.

To obtain seedlings, seeds were extracted from fully ripe fruits of the cv. Mollar from the orchard of the Universidade Federal Rural do Semi-Árido (UFERSA). After removal of the seeds, they were processed using a ¼ mesh sieve to remove the sarcotesta. Subsequently, sowing was performed, placing three seeds per polyethylene bag distributed equidistantly, keeping soil moisture near field capacity from sowing to germination. Twenty-five days after sowing, the excess plants were thinned, leaving only the most vigorous per bag.

Polyethylene bags with a capacity of 1.15 dm^3 were used for seedling formation, filled with substrate composed of 85% soil, 10% fine sand, and 5% tanned bovine manure. A physical, chemical (fertility) and salinity analysis, before the application of treatments, was performed to characterize the substrate (Table 1), according to the methodologies of Embrapa (2017) and Richards (1954).

Table 1. Physical, chemical and salinity analysis of the substrate used in the experiment.

Physical	Value	Chemical	Value	Salinity	Value
Sand (g kg^{-1})	639	pH in water (1:2.5)	7.00	pH	7.30
Silt (g kg^{-1})	227	P (mg dm^{-3})	146.32	ECe (dS m^{-1})	2.73
Clay (g kg^{-1})	134	K^+ (mg dm^{-3})	633.29	SO_4^{-2} ($mmol_c$ L^{-1})	1.02
Textural class	Sandy loam	Na^+ ($cmol_c$ dm^{-3})	0.27	Ca^{+2} ($mmol_c$ L^{-1})	16.00
		Al^{+3} ($cmol_c$ dm^{-3})	0.00	Mg^{+2} ($mmol_c$ L^{-1})	16.75
		H^++Al^{+3} ($cmol_c$ dm^{-3})	2.84	K^+ ($mmol_c$ L^{-1})	6.90
		Ca^{+2} ($cmol_c$ dm^{-3})	5.53	CO_3^{-2} ($mmol_c$ L^{-1})	0.00
		Mg^{+2} ($cmol_c$ dm^{-3})	1.70	HCO_3^{-2} ($mmol_c$ L^{-1})	40.00
		SB ($cmol_c$ dm^{-3})	9.12	Cl^- ($mmol_c$ L^{-1})	30.00
		CEC ($cmol_c$ dm^{-3})	11.96	SAR ($mmol_c$ L^{-1})	0.94
		OM ($cmol_c$ dm^{-3})	26.69	ESP (%)	0.13

OM = Organic Matter; SB = Sum of Bases (Na^+ + K^+ + Ca^{2+} + Mg^{2+}); CEC = Cation Exchange Capacity = SB + (H^+ + Al^{3+}); ECe = Electrical conductivity of the saturation extract; SAR = Sodium Adsorption Ratio = Na^+ × [(Ca^{2+} + Mg^{2+})/2]-1/2; ESP = Exchangeable Sodium Percentage (100 × Na^+/CTC).

Sodium chloride (NaCl) was used for the preparation of saline water, diluted in water from the UFPB supply system (0.5 dS m^{-1}) until reaching the electrical conductivities of irrigation water (ECw), with values measured with an Instrutherm® microprocessed portable conductivity meter (Model CD-860).

Irrigation with saline water began 10 days after emergence (DAE), and was performed daily manually. The volume of water applied was according to the needs of the crop, established by the drainage lysimetry method (Bernardo *et al.*, 2019). A depth of leaching (10%) based on the applied volume in this period was applied to reduce the accumulation of salts in the substrate.

The N doses were established according to the methodology proposed by Novais *et al.* (1991) for cultivation in 1 dm^3 pots, based on a requirement of 300 mg per plant, with the highest dose tested being 400 mg per plant, equivalent to 2.30 g L^{-1} of N. The commercial product Nitro Tecnia-20® containing in its composition 99 g L^{-1} of N (urea base) was used as N source.

Foliar N applications began at 15 DAE, diluting the doses (0, 58.16, 200, 341.84 and 400 g L^{-1}) of the fertilizer in distilled water and spraying the plants using an atomizer. Seven applications were made with a volume of 175 mL per plant, at 10-day intervals.

The measurement of phytomass accumulation of pomegranate seedlings was performed 90 days after treatment application (DTA). The stem (SM), leaves (LM) and roots (RM) were separated and placed in Kraft paper bags and dried in a forced air circulation oven at 65ºC until reaching constant weight, and the samples were weighed on a precision analytical balance (0.001 g). The shoot dry mass (SDM) was obtained by the sum of the SM and LM, while the total dry mass (TDM) was quantified by the sum of the SM, LM and RM.

The photochemical efficiency of the pomegranate plants was evaluated by fluorescence indices in the light phase, obtaining the relative electron transport rate (ETR), the chlorophyll fluorescence after the saturation pulse (Fs), photochemical extinction (qP) and non-photochemical extinction (qN), using a portable infrared gas analyzer - IRGA (model LI-6400XT, LI-COR®, Nebraska, USA) with airflow of 300 mL min^{-1} and coupled light source of 1200 μmol m^{-2} s^{-1}, with measurements taken from 9:00 to 11:00 am.

The data obtained were subjected to the normality test (Shapiro-Wilk) and homogeneity of variances (Bartlett). Then, it was applied to analysis of variance ($P \leq 0.05$), and in cases of significant effect to the regression analysis, using the statistical program R (R Core Team, 2022).

3. RESULTS AND DISCUSSION

There was a significant effect for the interaction between salinity of irrigation water (ECw) and foliar N fertilization for leaves dry matter (LDM) and root dry mass (RDM) of pomegranate seedlings (Figures 1A and 1B) at 90 days after emergence. For leaves' dry mass (Figure 1A), it appears that the maximum increment in production was 2.14 g per plant, obtained in plants subjected to ECw of 1.84 dS m^{-1} and 1.36 g L^{-1} of N. There were 90% decreases in salinity from 5.0 dS m^{-1} with increasing ECw. This effect demonstrates that the foliar application of N increases the pomegranate tree's tolerance to saline stress, possibly by virtue of increasing the capacity to produce photoassimilates, resulting in increased production of leaf biomass (Figueiredo *et al.*, 2020).

For root dry mass (RDM), foliar N application reduced the harmful effect of salt stress, with the highest accumulation (1.96 g per plant) observed in plants subjected to ECw of 2.02 dS m^{-1} and 1.37 g L^{-1} of N, reducing as the salinity increased, reaching a reduction of 84% in the accumulation of RDM in the ECw of 5.0 dS m^{-1} (Figure 1B). This effect may be associated with the fact that N stimulates plant growth, as well as the production of osmolytes that act on the plant's antioxidant defense system (Braz *et al.*, 2019), providing the greatest accumulation of dry phytomass in the root of seedlings of pomegranate, under the salinity of 2.02 dS m^{-1}.

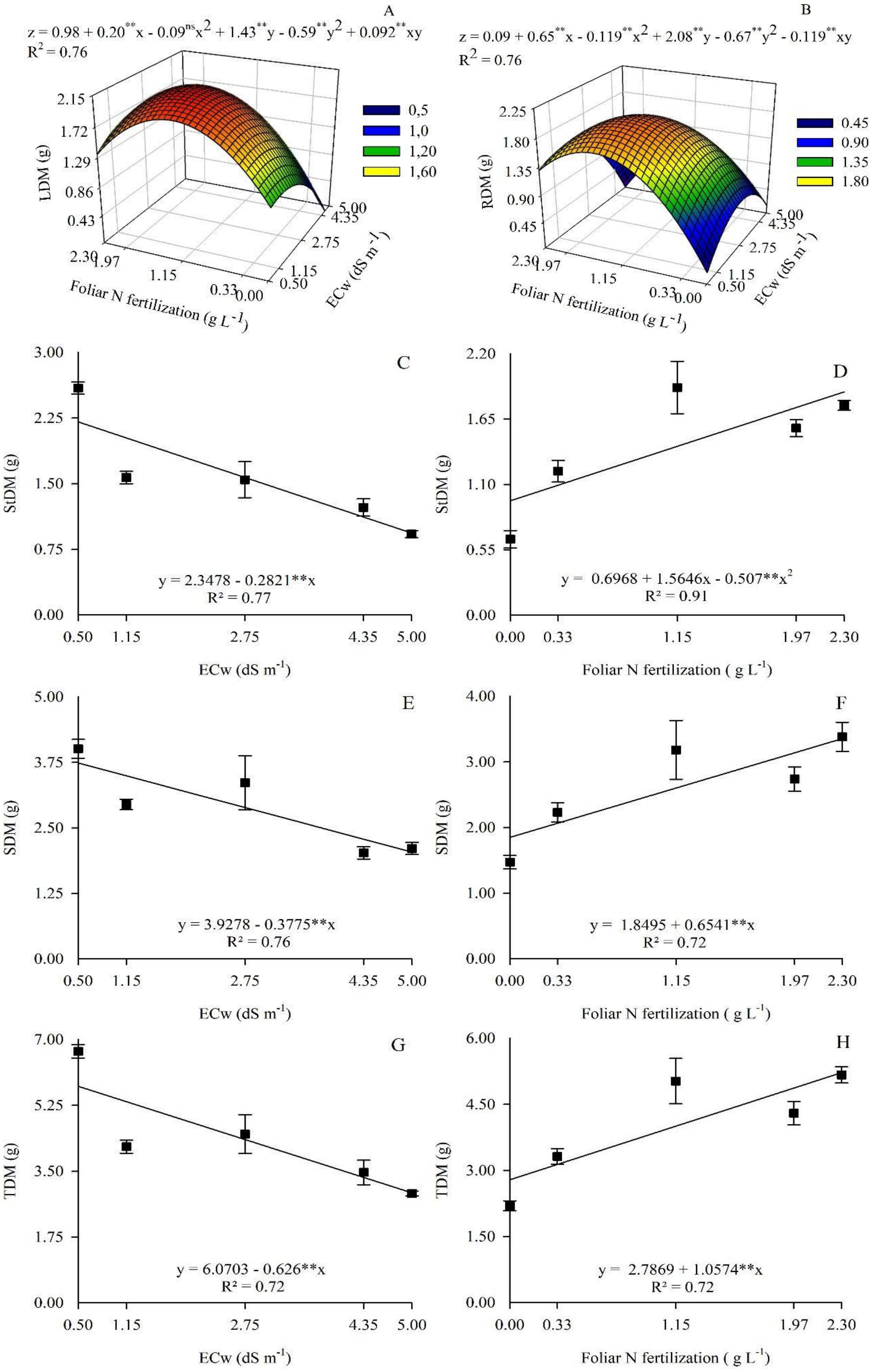

Figure 1. Leaf dry mass – LDM (A), root dry mass – (B), stem dry mass – StDM (C e D), shoot dry mass – SDM (E and F) and total dry mass - TDM (G and H) of pomegranate seedlings subjected to salinity of irrigation water (ECw) and foliar N fertilization at 90 days after emergence.

The accumulation of stem dry mass (StDM), shoot dry mass (SDM) and total dry mass (TDM) was severely reduced by salt stress, with the largest increases (2.21, 3.73 and 5.76 g per plant, respectively) observed in plants subjected to ECw of 0.5 dS m^{-1}, followed by decreases of 57.5, 45.4 and 48.9%, respectively, in plants subjected to ECw of 5.0 dS m^{-1} (Figures 1C, 1E and 1G). The reduction in the accumulation of dry phytomass in pomegranate seedlings occurs due to the effects of osmotic stress that limits water availability to the plant, compromising the processes of cell division and expansion (Hasanuzzaman *et al.*, 2021). In addition, toxicity of ions such as Na^+ and Cl^- can promote ion imbalance, causing changes in ion homeostasis (Figueiredo *et al.*, 2020; Roque *et al.*, 2022).

The reduction in biomass accumulation due to osmotic and ionic effects arising from saline stress is reported by other authors, such as Ferreira *et al.* (2023) who observed a 50% reduction in phytomass in guava seedlings irrigated with an ECw of 4.3 dS m^{-1}. Fatima *et al.* (2023) found a reduction of 94.6% in the phytomass of sugar apple seedlings irrigated with salinity water of 5.0 dS m^{-1}.

Foliar N fertilization had a beneficial effect on the accumulation of dry phytomass, with the dose of 2.30 g L^{-1} providing the highest values (1.61, 3.35 and 5.22 g per plant) in StDM, SDM and TDM, with equivalent increases of 57, 44.9 and 46.6%, respectively, when compared with the values of the control treatment (Figures 1D, 1F and 1H). N is an element directly involved in the physiological processes of cell division and expansion, enhancing growth, and consequently, stimulating the production and accumulation of phytomass in the different plant organs (Nóbrega *et al.*, 2022).

The salinity of irrigation water reduced the chlorophyll fluorescence after the saturation pulse (Fs). Plants subjected to the lowest ECw (0.5 dS m^{-1}) had the highest value (799.45), followed by decreases that reached 37% at an ECw of 5.0 dS m^{-1} (Figure 2A). This behavior indicates that salt stress promoted changes in the primary photosynthetic reactions, such as in energy storage and dissipation, electron transport, and excitation of energy between antenna complexes (Küpper *et al.*, 2019), directly influencing the plant photosynthetic performance.

Increasing salinity up to an ECw of 2.2 dS m^{-1} stimulated photochemical extinction (qP) showing the highest value (0.1984), followed by a decrease as ECw increased (Figure 2C). This effect is an indication that pomegranate seedlings were able to increase qP in order to protect the reaction centers, since it reflects the degree of PSII openness, as well as reflects the PSII quinone status (QA), and may indicate plant resilience to stress conditions (Li *et al.*, 2019). Bashir *et al.* (2021) in research with *Moringa oleifera* Lam. also observed that water salinity increased qP indices to 0.505 when subjected plants to 400 mM NaCl.

Salinity reduced the relative electron transport rate (ETR), with the highest value (102.95) observed at an ECw of 0.5 dS m^{-1}, reaching decreases of 12% with increasing ECw to 5.0 dS m^{-1} (Figure 2F). The reduction in ETR results from the partial closure of stomata as a mechanism to reduce water loss, which consequently reduces light uptake and reactions for NADPH and ATP synthesis (Hussain *et al.*, 2020).

On the other hand, foliar N application stimulated the chlorophyll fluorescence indices after the saturation pulse (Fs), photochemical extinction (qP) and non-photochemical extinction (qN), with gains being observed with increasing N doses up to 400 mg L^{-1}, reaching increments of 28, 25 and 17% at the dose of 2.3 g L^{-1}, respectively (Figures 2B, 2D and 2E). Foliar N application was beneficial to pomegranate seedlings, enabling improvements in photosynthetic activity from energy dissipation and excitation and the better utilization of energy by the PSII reaction centers (Wu *et al.*, 2019).

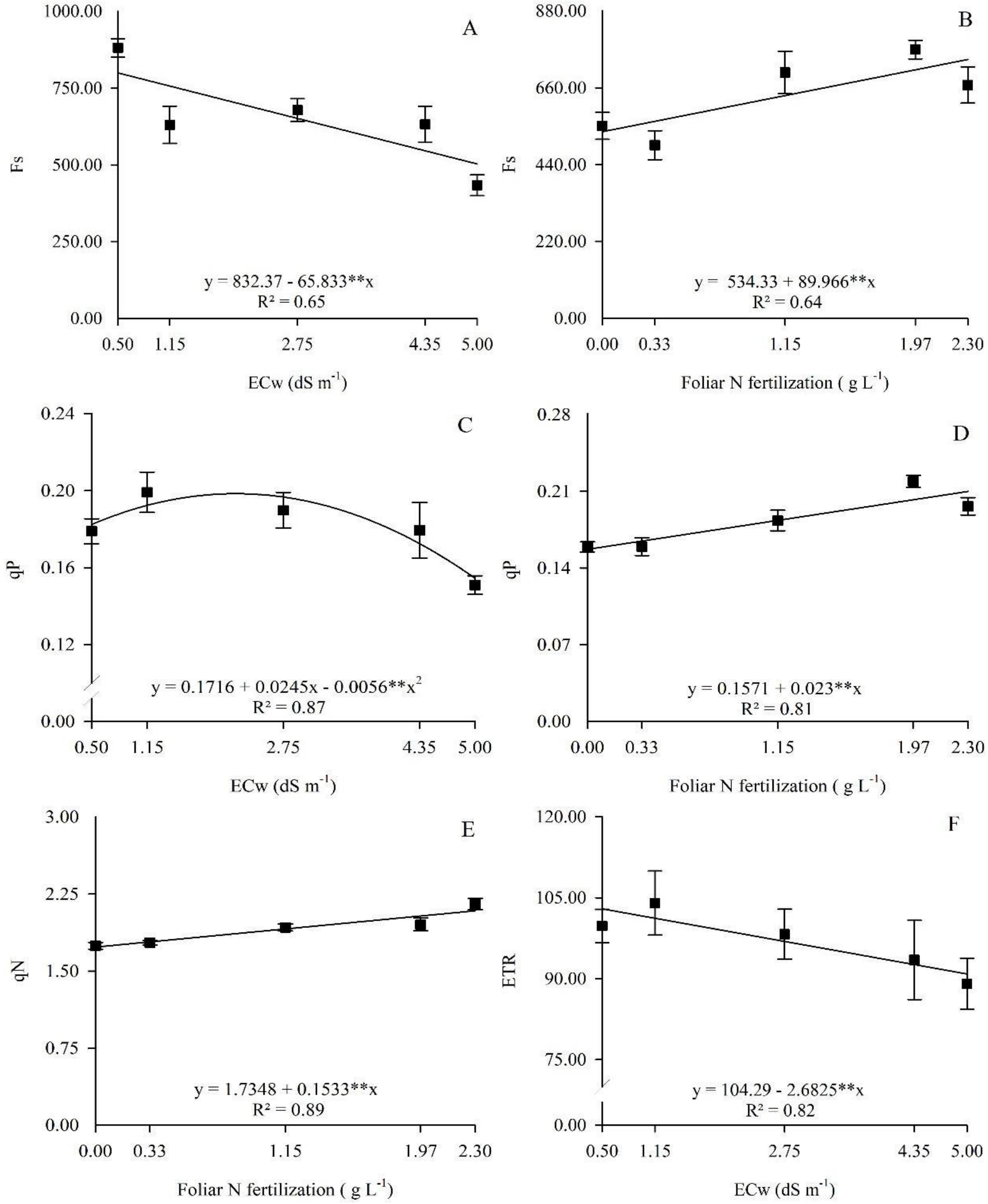

Figure 2. Chlorophyll fluorescence after the saturation pulse – Fs (A and B), photochemical extinction – qP (C e D), non-photochemical extinction – qN (E) relative electron transport rate - ETR (F) of pomegranate seedlings subjected to salinity of irrigation water (ECw) and foliar N fertilization at 90 days after emergence.

4. CONCLUSIONS

Foliar application of N up to a dose of 1.37 g L^{-1} reduces the harmful effect of salt stress on the accumulation of dry mass of leaves and roots up to ECw of 1.94 and 2.02 dS m^{-1}, respectively, in pomegranate seedlings at 90 days after emergence.

Water salinity reduces photochemical activity and inhibits dry phytomass formation, while foliar N application stimulates it in pomegranate seedlings.

Foliar N fertilization stimulates the accumulation of phytomass in the different organs of pomegranate seedlings.

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