ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.27, n.6, p.472-479, 2023
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v27n6p472-479

Foliar nitrogen fertilization attenuating harmful effects of salt stress on purple basil[1]

A adubação foliar com nitrogênio alivia os efeitos nocivos do estresse salino no manjericão roxo

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HIGHLIGHTS:
Irrigation water salinity limits growth and photochemical efficiency of purple basil.
The foliar fertilization at N improves photochemical efficiency of basil.
The Photochemical efficiency of basil is stimulated by nitrogen foliar application.

ABSTRACT: Salinity can have detrimental effects on plant development. To minimize this damage, strategies such as balanced maintenance of plants' nutritional status have been proposed previously. The objective of this study is to investigate whether the optimization of foliar nitrogen fertilization can promote the growth and photochemical efficiency of purple basil subjected to salt stress. The experiment was performed using a randomized block design with an incomplete 5 × 5 factorial arrangement generated through experimental matrix Central Composite Design with two parameters: salinity of irrigation water at 0.5, 0.81, 2.75, 4.69, and 5.50 dS m^{-1}; and nitrogen doses at 0, 58.58, 200, 341.42, and 400 mg L^{-1}. It was found that an increase in the salinity of irrigation water reduced the growth and photochemical efficiency of purple basil. However, the foliar application of nitrogen at the concentration of 400 mg L^{-1} attenuated the harmful effects of salinity on both the absolute and relative growth rates of stem diameter, the relative growth of plant height, and chlorophyll A fluorescence. These results showed that foliar nitrogen fertilization is a suitable strategy to help mitigate salt stress in basil plants.

Key words: *Ocimum basilicum*, abiotic stress, photochemical efficiency, foliar nitrogen fertilization, salt stress

RESUMO: A salinidade pode promover efeitos danosos ao desenvolvimento vegetal, sendo o necessário o uso de estratégias para minimizar os danos promovidos, como a manutenção equilibrada do estado nutricional da planta. Assim, o objetivo deste estudo foi avaliar o efeito da adubação nitrogenada foliar e da salinidade no crescimento e eficiência fotoquímica de manjericão roxo. O experimento foi conduzido com o delineamento de blocos casualizados, em arranjo fatorial incompleto 5 × 5 (salinidades da água de irrigação: 0,5; 0,81; 2,75; 4,69 e 5,50 dS m^{-1} × doses de nitrogênio: 0; 58,58; 200; 341,42 e 400 mg L^{-1}), gerados através da matriz experimental Composto Central de Box. O aumento da salinidade da água de irrigação reduz o crescimento e a eficiência fotoquímica das plantas de manjericão roxo. Todavia, a aplicação foliar de 400 mg L^{-1} de N atenuou os efeitos deletérios da salinidade sobre as taxas de crescimento absoluto e relativo para o diâmetro do caule e relativo para a altura de plantas, assim como na fluorescência da clorofila a. Esses resultados mostram que a adubação foliar nitrogenada é uma estratégia para mitigar o estresse salino em plantas de manjericão.

Palavras-chave: *Ocimum basilicum* L., estresse abiótico, eficiência fotoquímica, adubação foliar nitrogenada

• Ref. 265587 – Received 02 Jul, 2022
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• Accepted 04 Jan, 2023 • Published 11 Feb, 2023
Editors: Geovani Soares de Lima & Hans Raj Gheyi

Introduction

Basil (*Ocimum basilicum* L.) belongs to the Lamiaceae family and has a high potential for exploitation. The species is consumed and produced worldwide, and its aromatic properties are appreciated both in folk medicine and as a condiment (Tamfu et al., 2021). However, its exploitation in semi-arid regions, such as the Brazilian Northeast, may be limited by water scarcity and high salt content in the available irrigation water (Santos et al., 2019). The large number of saline ions, inadequate use of irrigation water, and lack of efficient drainage are limiting factors for agricultural production and contribute to soil salinization (Pessoa et al., 2022). Salt-induced stress influences several processes in plants, including a reduction in photochemical efficiency by damaging the photosynthetic apparatus (Figueiredo et al., 2021). A decrease in soil osmotic potential is the main damage caused by salt stress in plants because it limits their ability to absorb water, causing nutritional imbalance, oxidative stress due to reactive oxygen species (ROS) produced in the stress response, and toxicity due to high concentrations of Na^+ and Cl^- ions (Arif et al., 2020; Ondrasek et al., 2022).

Several strategies have been proposed to increase plants' tolerance to the harmful effects of salinity, including nitrogen fertilization, which is known to promote plant growth and development (Sousa et al., 2021). Nitrogen acts in many important cellular pathways as a constituent of chlorophyll, amino acids, nucleic acid, enzymes, proteins, and osmo-protective compounds. Nitrogen is also known to increase plants' tolerance to salt stress (Singh et al., 2019).

The accumulation of toxic ions and production of reactive oxygen species promote the denaturation of chlorophyll by destructing the proteins involved in its synthesis, compromising the activity of the photosystem II (PSII) and the photosynthetic apparatus. Analyzing chlorophyll a fluorescence is important to determine the photochemical efficiency of plants submitted to salt-induced stress conditions (Saddiq et al., 2021). Given this context and the need for information on how foliar nitrogen application can improve basil tolerance to salt stress, the objective of this study was to evaluate the growth and photochemical efficiency of purple basil (*Ocimum basilicum*) subjected to both salt stress and foliar nitrogen fertilization.

Material and Methods

This study was carried out in a greenhouse at the Agricultural Sciences Center (CCA) of the Universidade Federal da Paraíba(UFPB), Areia, Paraíba, Brazil (6º 57' 42", 35º 41' 43", altitude 573 m), with climate type As', having dry and hot summers and rainy winters according to the Köppen classification (Alvares et al., 2013). The average daily temperature observed during the experiment was 28.4 °C, and the relative humidity of air was 54.8%.

The experiment was performed using a randomized block design with an incomplete 5 × 5 factorial scheme composed of two variable parameters: salinity of irrigation water (0.5, 0.81, 2.75, 4.69, and 5.50 dS m^{-1}) and nitrogen dose (0, 58.58, 200, 341.42, and 400 mg L^{-1}). A design of experiments approach was used to generate the experimental matrix shown in Table 1. The matrix is composed of nine treatments and was generated through a central composite design (Hang et al., 2011).

Table 1. Treatments generated by the experimental matrix Central Composite Design

Treatments	ECw (dS m^{-1})	ND (mg L^{-1})
T1	0.81	58.58
T2	4.69	58.58
T3	0.81	341.42
T4	4.69	341.42
T5	2.75	0
T6	2.75	400.00
T7	5.50	200.00
T8	0.5	200.00
T9	2.75	200.00

ECw - Electrical conductivity of irrigation water; ND - Nitrogen doses

Basil seeds cv. Italiano Roxo (*Ocimum basilicum* var. *purpurascens* Benth.) were used. The plants were grown in polyethylene bags with a capacity of 1.2 dm^3 and filled with a substrate composed of soil mixture (Oxisol – EMBRAPA, 2018), decomposed bovine manure, and washed sand (3:1:1, v:v). The substrate had the following chemical characteristics: pH = 7.8; P = 85.5 mg kg^{-1}; K^+ = 693.6 mg kg^{-1}; Na^+ = 0.23 $cmol_c$ dm^{-3}; $H^+ + Al^{+3}$ = 0 $cmol_c$ dm^{-3}; Al^{+3} = 0 $cmol_c$ dm^{-3}; Ca^{2+} = 2.9 $cmol_c$ dm^{-3}; Mg^{2+} = 1.59 $cmol_c$ dm^{-3}; SB = 6.5; CEC = 6.5 g kg^{-1}; OM = 22.2 g kg^{-1}.

Irrigation with saline water was started 10 days after seedlings emerged. Water batches with different salinities were prepared by adding sodium chloride to municipal supply water (0.5 dS m^{-1}) until the required levels of conductivity were reached. The values were calibrated with a portable Instrutherm® microprocessor-based conductivity meter (model CD-860). The volume of the water used for irrigation was determined by a drainage lysimeter (Eq. 1), with the volume of water lost through evapotranspiration the previous day being replenished daily, thus maintaining the soil at field capacity.

$$VI = \frac{(Va - Vd)}{(1 - LF)} \tag{1}$$

where:

VI - volume of water to be used in the irrigation (mL);
Va - volume of water applied in the previous irrigation event (mL);
Vd - drained volume (mL); and,
LF - leaching fraction (0.10).

Nitrogen doses were established according to requirements (300 mg per plant) for one dm^3 pot, as proposed by Novais et al. (1991). To meet the need for nitrogen, the commercial product Nitrotecnia-20 (Carbotecnia®), containing 99.0 g L^{-1} of nitrogen based on urea, was used. The total foliar nitrogen fertilization per plant was divided into five parts and then sprayed every seven days using a manual sprayer in the late afternoon, applying a total volume of 175 mL per plant.

Absolute and relative growth rates of plant height and stem diameter (AGRph and AGRsd, and RGRph and RGRsd) of basil plants were determined 45 days after the start of irrigation with saline water, according to the methodology described by Benincasa (2003), and are represented in Eqs. 2 and 3.

$$\mathrm{AGRph} = \frac{(\mathrm{Ap2} - \mathrm{Ap1})}{(\mathrm{t2} - \mathrm{t1})}$$
$$\mathrm{AGRsd} = \frac{(\mathrm{sd2} - \mathrm{sd1})}{(\mathrm{t2} - \mathrm{t1})} \quad (\text{cm per day}) \tag{2}$$

where:

AGRph and AGRsd: absolute growth rates of plant height and stem diameter, respectively;

Ap1 and sd1- evaluated values at the 15th day after sowing;

Ap2 and sd2 - evaluated values at the 45th day after sowing;

t1 - number of days of the first evaluation (15 days); and

t2 - number of days of the second evaluation (45 days),

$$\mathrm{RGRph} = \frac{(\ln \mathrm{Ap2} - \ln \mathrm{Ap1})}{(\mathrm{t2} - \mathrm{t1})}$$
$$\mathrm{RGRsd} = \frac{(\ln \mathrm{sd2} - \ln \mathrm{sd1})}{(\mathrm{t2} - \mathrm{t1})} \quad (\text{cm cm}^{-1} \text{ per day}) \tag{3}$$

where:

RGRph and RGRsd - absolute growth rate for plant height and stem diameter;

lnAp1 and lnsd1- evaluated value at the 15th day after sowing;

lnAp2 and lnsd2 - evaluated value at the 45th day after sowing;

t1 - number of days of the first evaluation (15 days); and

t2 - number of days of the second assessment (45 days).

Dickson's Quality Index (Dickson et al., 1960) was evaluated according to Eq. 4;

$$\mathrm{DQI} = \frac{\mathrm{TDP}}{\left(\frac{\mathrm{PH}}{\mathrm{SD}}\right) + \left(\frac{\mathrm{SDP}}{\mathrm{RDP}}\right)} \tag{4}$$

where:

DQI - Dickson Quality Index;

PH - plant height (cm);

SD - stem diameter (mm);

TDP - total dry phytomass (g);

SDP - shoot dry phytomass (g); and

DPR - root dry mass (g).

Fluorescence indices were measured to determine the photochemical efficiency of the basil plants, including electron transport rate (ETR), steady-state fluorescence yield (F_s), photochemical quenching (qP), and nonphotochemical quenching (qN). To evaluate dark-adapted fluorescence indices, clips were placed on leaves for 30 min. The initial (F_0), maximum (F_m), and variable ($F_v = F_m - F_0$) fluorescence indices were measured. The F_v/F_0 ratio and quantum yield of the PSII (F_v/F_m) were also evaluated. A portable infrared gas analyzer (IRGA, model LI-6400XT, LI-COR®, Nebraska, USA) with an airflow of 300 mL min^{-1} and an attached light source of 1200 μmol m^{-2} s^{-1} was used for analyses. Measurements were taken from 09:00 a.m. to 11:00 a.m.

Data were subjected to normality (Shapiro-Wilk) and homogeneity of variances (Bartlett) tests. Subsequently, an analysis of variance ($p \leq 0.05$) was performed, and a regression analysis was carried out in cases of significance. The statistical program R was used (R Core Team, 2021).

Results and Discussion

Foliar nitrogen application attenuated the harmful effects of salt stress on the relative growth rate of the purple basil plant height and the absolute and relative growth rates of stem diameter (Figure 1).

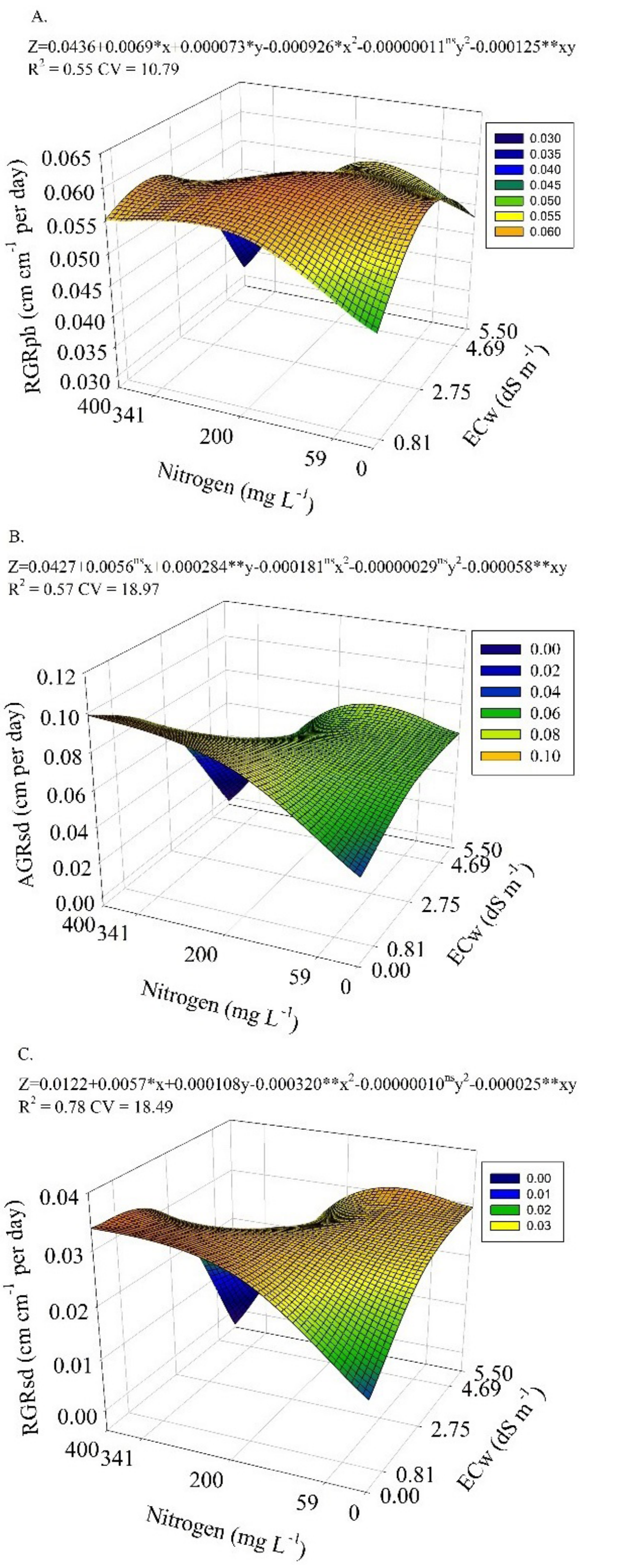
A dose of 0.08 mg L^{-1} of nitrogen reduced the harmful effects of salinity on the relative growth rate of plant height, up to an electrical conductivity of the irrigation water (ECw) of 3.7 dS m^{-1}, with a maximum value of 0.056 cm cm^{-1} per day (Figure 1A). The attenuating effect of foliar nitrogen fertilization was also observed in the absolute and relative growth rates of stem diameter, with the largest increments of 0.036 cm per day and 0.099 cm cm^{-1} per day, respectively, observed in plants subjected to doses of 392 and 400 mg L^{-1} of nitrogen and in an ECw of 0.51 and 0.54 dS m^{-1}, respectively (Figures 1B and C).

Improvements in the relative growth rate of plant height and the absolute and relative growth rates of stem diameter indicate that nitrogen mitigates the effects of salt stress. Nitrogen plays a key role in a series of mechanisms that increase plant tolerance and accumulation of protective substances and regulate stomatal adjustment and physiological processes, such as water usage efficiency and photosynthesis (Zhu et al., 2022). These aspects act as defense mechanisms against stress conditions and enhance plant growth rates (Saud et al., 2017).

Foliar nitrogen fertilization mitigated the harmful effects of salt stress on the growth of purple basil plants, thereby improving the plants' response under saline conditions. Nitrogen is a component in several organic compounds that can improve plant performance under saline conditions by providing osmotic adjustment (Sousa et al., 2021). Among these compounds are proline, betaines, and free amino acids, which can play a key role in protecting cellular structures and their functions (Wanderley et al., 2018; Braz et al., 2019).

A decreasing linear effect of the absolute growth rate of plant height was observed for increasing ECw and nitrogen doses, resulting in variations of 19.8% and 14.7%, respectively, comparing the highest and lowest values observed (Figures 2A and B). This reduction in the growth rate occurred because of the harmful effects of salt-induced stress, such as loss of turgor, which hinder cell-expansion processes and compromise growth, as observed in *Mesosphaerum suaveolens* (L.) Kuntze (Nóbrega et al., 2020).

Looking at the evolution of the Dickson quality index as a function of the ECw, basil plants kept growing up until a maximum ECw of 3.78 dS m^{-1}, reaching a DQI of 7.28, after which the DQI started decreasing as the salinity increased further (Figure 2. C). Regarding the effect of foliar nitrogen fertilization, there was a reduction in the DQI for doses below



*, ** - Significant at p ≤ 0.05 and p ≤ 0.01, respectively; ns - Not significant, by F test

Figure 1. Relative growth rate of plant height (A), absolute (B), and relative (C) growth rates of stem diameter of purple basil subjected to salinity - ECw and foliar nitrogen fertilization

200 mg L^{-1}; for higher nitrogen doses, there was a proportional increase in the DQI as a function of the increase in the nitrogen dose, reaching the highest value of 7.40 at a dose of 400 mg L^{-1} (Figure 2D). This effect may be associated with the ability of the plant to adjust to its environment during the experimental period, with the quality of their seedlings increasing by 5.4% (Figure 2D).

Although foliar nitrogen fertilization attenuated the harmful effects of salt stress, the plants still experienced an absolute growth rate of plant height and a DQI that decreased with increasing salinity. This is due to a few factors: i) the reduction in water absorption imposed by the increase in soil osmotic potential; ii) increased toxicity caused by Na^+ and Cl^- ions; and iii) nutritional imbalance, which reduces plant development (Wang et al., 2019; Zulfiqar & Ashraf, 2021; Ondrasek et al., 2022). A reduction in plant development caused by an increase in the salinity of the irrigation water has also been observed in basil (*Ocimum basilicum* L.) (Santos et al., 2019; Silva et al., 2022).

The plant height/stem diameter ratio decreased with increasing nitrogen dose, reaching a decrease of 21% for a dose of 400 mg L^{-1} (Figure 2E). This effect occurred because the plants grew less in height as the nitrogen dose increased, indicating that the foliar application of nitrogen favored the growth of the stem diameter against the plant height. This is because nitrogen is directly involved in cell division and expansion, thereby regulating plant growth and development (Singh et al., 2019).

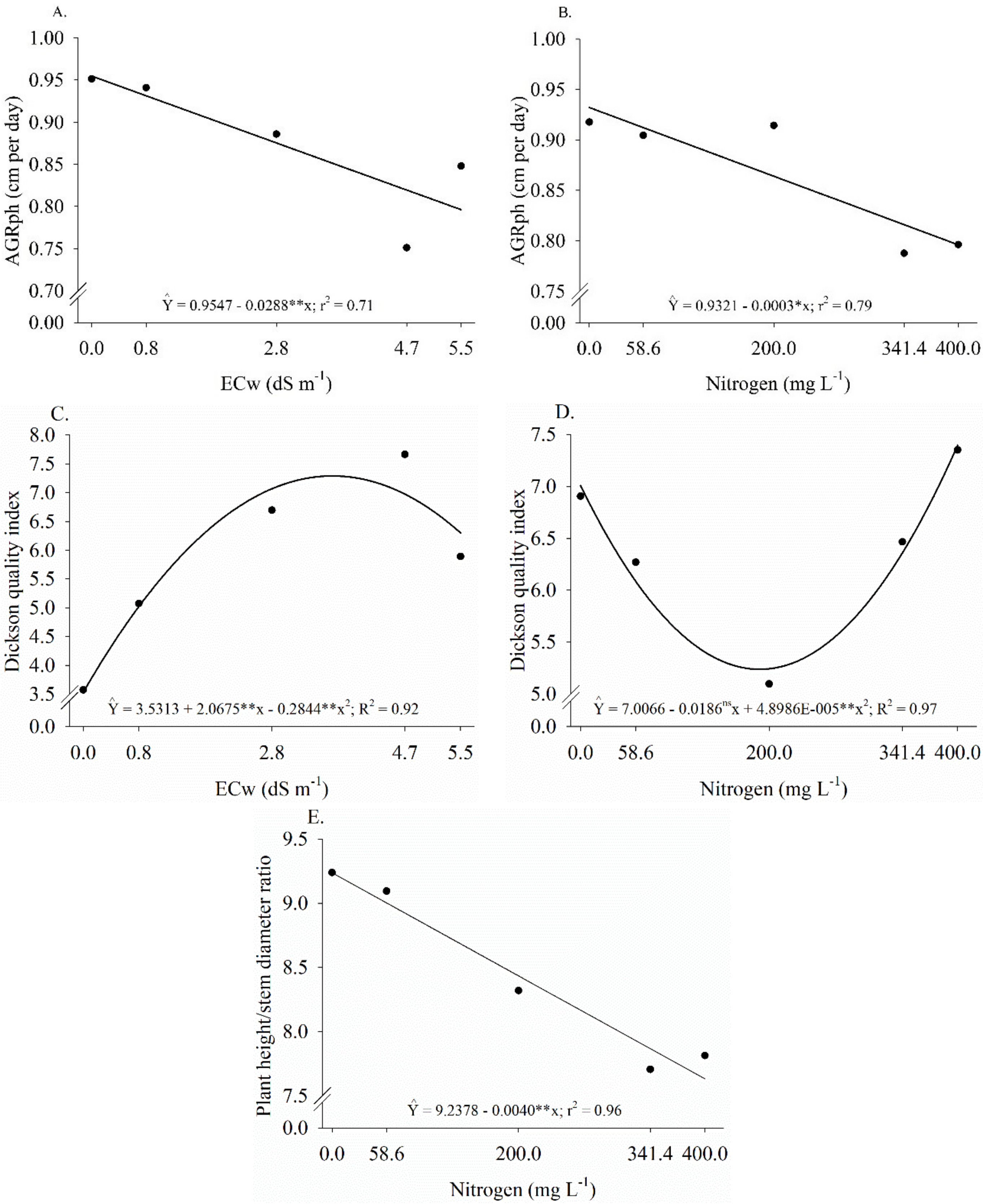
Foliar nitrogen fertilization promoted the photochemical efficiency of basil plants grown under salt stress (Figure 3). A dose of 399 mg L^{-1} of nitrogen provided the highest value of 320.96 quantum^{-1} electrons in the initial fluorescence indices (F_0) when plants were subjected to an ECw of 0.5 dS m^{-1} (Figure 3A).

The maximum fluorescence observed at 1993,537 electron quantum^{-1} was for an ECw of 0.5 dS m^{-1} and an application of 193 mg L^{-1} of nitrogen (Figure 3B). When saline water with an ECw of 5.49 dS m^{-1} was used, the F_v/F_0 ratio was increased by foliar nitrogen fertilization at a dose of 399 mg L^{-1}, indicating that foliar nitrogen application increases the photochemical efficiency of purple basil plants.

The beneficial effects of foliar nitrogen application were not only observed in the growth rates but also in the photochemical efficiency of purple basil plants. When the plants are subjected to salt stress, they tend to increase F_0 because of the damage induced by the reaction centers, limiting energy transfer between photosystems (Sá et al., 2018). Nitrogen reduces this damage, presenting lower F_0 indices with increasing salinity in basil plants. However, there is an increase in the F_m, F_v/F_0, and F_v/F_m ratios, which indicates that the reaction centers are potentially active, even under adverse conditions, inducing a greater photochemical activity, which is characterized by an increase in the fluorescence indices, probably because nitrogen is a constituent of the chlorophyll molecule (Taiz et al., 2017).

Non-photochemical quenching is affected by salinity, with the highest value of 1.74 observed in plants subjected to the lowest ECw (Figure 4A). However, the effect of the foliar nitrogen fertilization increased the qN ($y = 0.9327 + 0.0037^{ns}x - 9.4900E\text{-}006^{*}x^2$; $R^2 = 0.41$) until a dose of 195 mg L^{-1}, followed by a decrease in the qN on increasing the applied nitrogen dose.

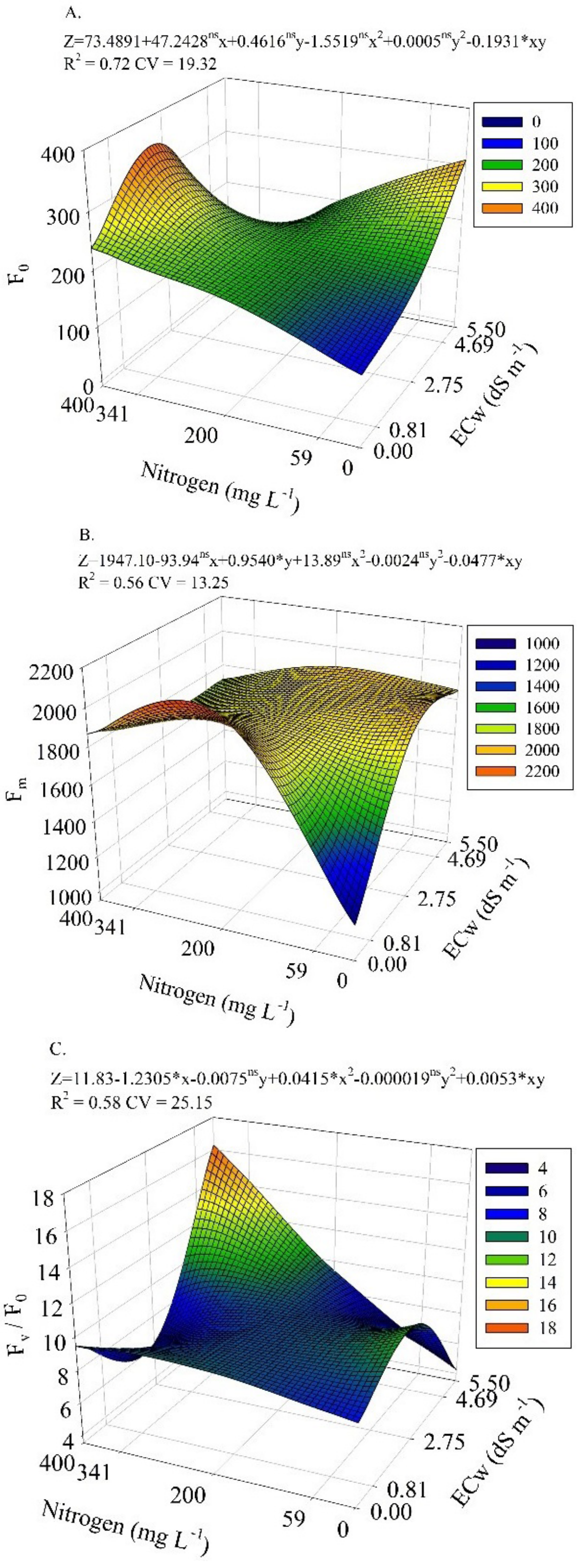
The electron transport rate increased with nitrogen fertilization, reaching its maximum level of 134,318 at the highest nitrogen dose of 400 mg L^{-1}, with gains of 12% compared to plants that were not fertilized with nitrogen (Figure 4B). Similarly, an increase in the steady-state fluorescence yield



*, ** - Significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, ^{ns} Not significant, by F test

Figure 2. Absolute growth rate based on plant height (A and B), Dickson quality index (C and D), and plant height/stem diameter ratio (E) of purple basil subjected to salinity and foliar nitrogen fertilization

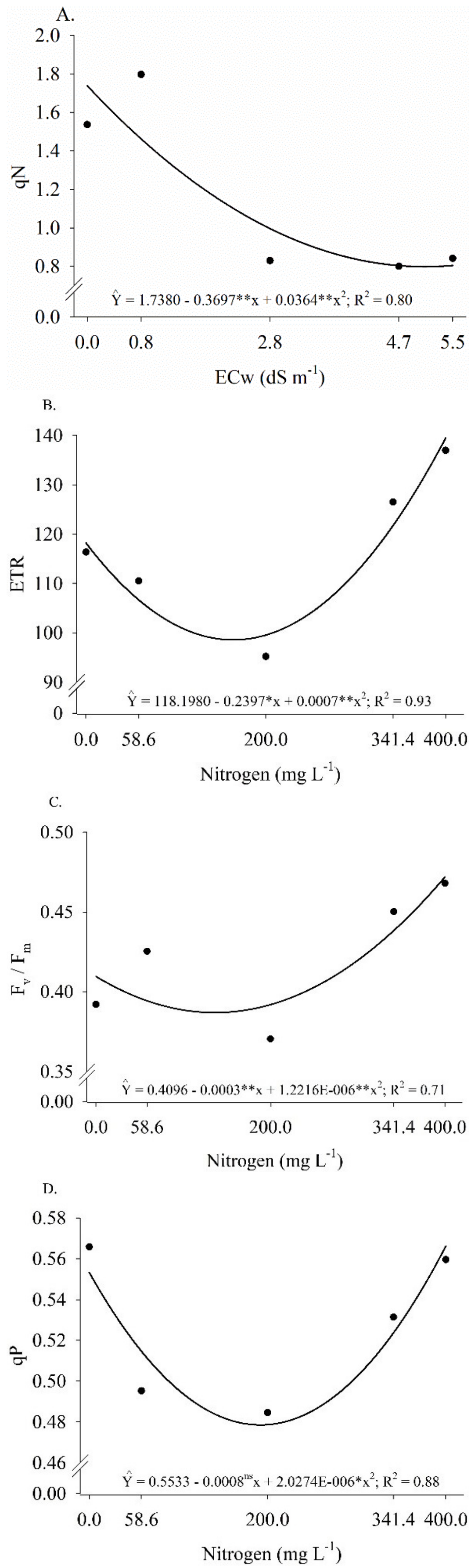
($F_s - y = 521.7944 - 0.2955^{*}x + 0.0011^{**}x^2$, $R^2 = 0.54$), and the quantum yield of PSII (F_v/F_m) was observed, reaching their maximum values of 579.59 and 0.4850, respectively, at the highest nitrogen dose of 400 mg L^{-1}, which corresponds to gains of 10 and 15%, respectively, compared to plants that were not fertilized with nitrogen (Figure 4C). Photochemical quenching was also stimulated by foliar nitrogen fertilization, with the highest value of 0.5576 observed at a nitrogen dose of 400 mg L^{-1}, indicating that the application was beneficial to the photosynthetic apparatus of basil plants (Figure 4D).



*, ** - Significant at p ≤ 0.01 and p ≤ 0.05, respectively; ns - Not significant, by F test

Figure 3. Initial fluorescence (A), maximum fluorescence (B), and F_v/F_0 ratio (C) of purple basil subjected to salinity and foliar nitrogen fertilization

Salinity reduced the non-photochemical quenching, while the foliar application of nitrogen increased the non-photochemical quenching until a dose of 195 mg L^{-1}. With this protection, basil plants maintained their photosynthetic activity because the qN plays a photoprotective role by dissipating excess energy and helping protect the PSII reaction centers (Lassouane et al., 2013).



*, ** - Significant at p ≤ 0.05 and p ≤ 0.01, respectively, ns - Not significant, by F test

Figure 4. Non-photochemical quenching (A), electron transport rate (B), quantum yield of the PSII (C), and photochemical quenching (D) of purple basil when subjected to varying salinity and foliar nitrogen fertilization conditions

An increase in photochemical efficiency due to nitrogen supply has been observed in *Oryza sativa* L. (Peng et al., 2021), *Anonna squamosa* L. (Figueiredo et al., 2019), *Zea mays* L. (Wu et al., 2019), and *Malpighia emarginata* L. (Sá et al., 2018). In the current study, it was found that in addition to the increase in the qN, nitrogen fertilization also increased the ETR, F_v/F_m, and qP, thereby improving the photochemical performance of the basil plants. Nitrogen is a fundamental element for plant development, influencing photosynthetic activity through energy dissipation by the qP and qN and enhancing energy utilization by the PSII, thus increasing the plant's ability to tolerate stress (Wu et al., 2019). Under adequate levels, nitrogen improves the quantum and photochemical efficiency of plants, resulting in a higher photosynthetic yield (Peng et al., 2021). Mitigating abiotic stresses can increase the productivity of crops. The search for alternatives, such as the foliar application of nitrogen, is a fundamental step in reducing the harmful effects on the environment and ensuring the success of crops.

Conclusions

Exposure to salinity conditions reduced the growth and photochemical efficiency of purple basil plants, but the foliar application of 400 mg L^{-1} nitrogen attenuated the harmful effects of salinity on the growth rates and photochemical efficiency.

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