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Nitrogen improves biomass production and chlorophyll synthesis in basil plants grown under salt stress

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ABSTRACT

Basil (*Ocimum basilicum* L.), a medicinal and aromatic plant extensively cultivated in the Northeast region of Brazil, encounters growth challenges attributed to the salinity of irrigation water and soil. Nitrogen (N) is a crucial macronutrient employed to mitigate salt stress in plants. Therefore, this study aimed to evaluate the production of phytomass and chlorophyll synthesis in purple basil plants grown under salinity stress and nitrogen fertilization. The experiment was conducted in 2021 under protected environmental conditions at the Center for Agricultural Sciences, Universidade Federal Paraíba, Areia-PB, Brazil. Five levels of salt stress (0.0, 0.80, 2.75, 4.70, and 5.50 dS m^{-1}) and five doses of N (0.00, 58.58, 200.00, 341.42, and 400.00 mg L^{-1}) applied via foliar were studied. The results revealed that foliar fertilization with N increases plant tolerance to salt stress, promoting root fresh and dry mass accumulation at 294.96 and 205.36 mg L^{-1} and under ECw of 1.14 and 0.5 dS m^{-1}, respectively. Applying 217.39 and 231.30 mg L^{-1} of N improves the production of stem dry biomass and the shoot/root ratio of basil plants subjected to salinity of 0.5 dS m^{-1}. The electrical conductivity of irrigation water above 0.8 dS m^{-1} adversely affects biomass production. The salinity at 3.8 to 4.0 dS m^{-1} stimulated chlorophyll synthesis in purple basil plants. However, the foliar application of N proves to be a strategic approach to counteract these effects, resulting in increased total dry mass production and chlorophyll contents.

Keywords: *Ocimum basilicum* L., Abiotic stress, Fertilization, Plant nutrition.

Nitrogênio melhora a produção de biomassa e síntese de clorofila em manjericão cultivado sob estresse salino

RESUMO

O manjericão (*Ocimum basilicum* L.), planta medicinal e aromática amplamente cultivada na região Nordeste do Brasil, encontra desafios de crescimento atribuídos à salinidade da água de irrigação e do solo. O nitrogênio (N) é um macronutriente crucial empregado para mitigar o estresse salino nas plantas. Portanto, este trabalho teve como objetivo avaliar a produção de fitomassa e síntese de clorofila em plantas de manjericão roxo cultivadas sob estresse salino e adubação nitrogenada. O experimento foi realizado no ano de 2021 em condições de ambiente protegido no Centro de Ciências Agrárias, Universidade Federal da Paraíba, Areia-PB, Brasil. Foram estudados cinco níveis de estresse salino (0,0; 0,80; 2,75; 4,70 e 5,50 dS m^{-1}) e cinco doses foliares de N (0,00; 58,58; 200,00; 341,42 e 400,00 mg L^{-1}). Os resultados revelaram que a fertilização foliar com N aumenta a tolerância da planta ao estresse salino, promovendo o acúmulo de massa fresca e seca da raiz, nas doses de 294.96 e 205,36 mg L^{-1} e sob CEa de 1,14 e 0,5 dS m^{-1}, respectivamente. A aplicação de 217.39 e 231.30 mg L^{-1} de N melhora a produção de biomassa seca do caule e na relação entre a biomassa da parte aérea/raiz das plantas de manjericão submetidas a salinidade de 0,5 dS m^{-1}. A condutividade elétrica da água de irrigação acima de 0,8 dS m^{-1} afeta negativamente a produção de biomassa. A salinidade de 3,8 até 4,0 dS m^{-1} estimulou a síntese de clorofila das plantas de manjericão roxo. Contudo, a aplicação foliar de N revela-se uma abordagem estratégica para contrariar estes efeitos, resultando no aumento da produção de massa seca total e dos teores de clorofila.

Palavras-chave: *Ocimum basilicum* L., Estresse abiótico, Fertilização, Nutrição vegetal.

1. Introduction

Basil (*Ocimum basilicum* L. - Lamiaceae) is a medicinal, aromatic, and ornamental plant cultivated globally, owing to its significant importance in various market sectors. It is classified as a sub-shrub plant and can be cultivated perennially or annually (Bharti et al., 2016; Silva et al., 2019). The leaves and inflorescences of basil feature essential oil-secreting glands, with linalool being its primary component. This compound is employed in numerous syntheses of cosmetics, pharmaceuticals, and perfumery industries. Furthermore, it is utilized for flavoring food, beverages, and environments (Souza et al., 2013).

Basil is extensively cultivated across the Brazilian territory, predominantly by small-scale producers engaged in family farming. These producers market basil leaves as an aromatic condiment for culinary purposes. While basil exhibits adaptability to diverse climatic conditions, its optimal development occurs in hot and humid climates, prevalent in northeastern Brazil (Palaretti et al., 2015). This cultivation pattern contributes significantly to local economies and evidences the basil's adaptability to varying environmental conditions. The preference for hot and humid climates aligns with the regional characteristics of northeastern Brazil, highlighting basil's resilience and suitability for cultivation in specific climatic niches.

In semi-arid regions, particularly within the extensive Northeast Brazil region, irrigation stands out as a pivotal technology significantly shaping the growth of cultivated plants. This is attributed to its ability to mitigate the adverse effects of erratic precipitation patterns (Oliveira et al., 2014). Nevertheless, it is noteworthy that irrigation water in these regions frequently exhibits elevated salt levels, often exceeding 3.0 dS m^{-1}. This high salinity is commonly sourced from deep wells, presenting the advantageous characteristic of lower costs for perforation (Silva et al., 2018).

Salinity poses a significant challenge, particularly in arid and semi-arid regions, as it constrains plant growth, development, and yield (Jiang et al., 2017; Konuşkan et al., 2017). This limitation arises initially from the osmotic effect, which hampers water absorption. As ions, particularly Na^+ and Cl^-, accumulate in the plant, there is an elevation in reactive oxygen species, a decline in gas exchange, degradation of photosynthetic pigments, and various other detrimental effects (Nóbrega et al., 2024). These consequences lead to reduced photosynthesis, impaired absorption of essential elements, and morphological damage. In extreme cases of prolonged exposure, salinity can even result in the death of the plant (Munns and Gilliham, 2015; Silva et al., 2019).

The use of saline water for irrigation, along with the exploration of plant genotypes exhibiting tolerance potential and the implementation of soil and water management strategies, including mineral fertilization, present a significant challenge for producers and researchers. These individuals actively develop studies to mitigate salts' adverse impacts on plants and enhance overall yield (Naveed et al., 2020). Successful irrigation with saline water relies on a combination of crop tolerance to salinity and effective management practices in terms of irrigation and fertilization (Lima et al., 2015; Sá et al., 2018).

Nitrogen (N) stands out as one of the indispensable nutrients crucial for plant development, representing a key component in the growth process (Costa et al., 2015). Beyond its role in fostering plant growth, nitrogen plays a pivotal role in mitigating the detrimental effects of salinity stress. This is attributed to its involvement in the composition of vital cellular components such as proteins, nucleic acids, photosynthetic pigments, proline, and quaternary ammonium compounds, including betaine, glycine, and enzymes. These elements are essential for cell development and plant growth, influencing leaves and roots (Souza et al., 2019).

Information on foliar fertilization with N to mitigate salinity stress in basil is scarce in the literature, and studies are needed to elucidate the information gaps. Therefore, this study aimed to evaluate the production of phytomass and chlorophyll synthesis in purple basil plants grown under salinity stress and nitrogen fertilization.

2. Material and Methods

The experiment was conducted in a protected environment at the Center for Agricultural Sciences, the Department of Plant and Environmental Sciences of the Federal University of Paraíba, Areia, Paraíba – Brazil, located at 6º58'00" S and 35º41'00" W with an altitude of 575 m. The average temperature recorded during the experiment was 28.4 °C and a relative humidity of 54.8%. The experiment was conducted in a protected environment, with a transparent plastic cover, the sides were made of screen with 50% shading, and the floor and benches were made of masonry.

Basil seeds of the purple cultivar were used. The seedlings were produced in polyethylene bags of 1.3 dm^3 capacity filled with substrate formulated from a mixture of soil classified as Latossolo (Santos et al., 2018), washed sand, and cattle manure (3:1:1 v/v), with the following chemical properties: pH = 7.8, organic matter (g kg^{-1}) = 22.2, ECse = 2.0 dS m^{-1}, phosphorus (mg kg^{-3}) = 85.5, K^+ (mg kg^{-3}) = 693.6, Ca^{+2} ($cmol_c$ dm^{-3}) = 2.9, Mg^{+2} ($cmol_c$ dm^{-3}) = 1.59, Na^+ ($cmol_c$ dm^{-3}) = 0.23, sum of bases ($cmol_c$ dm^{-3}) = 6.5, H^++Al^{+3} ($cmol_c$ dm^{-3}) = 0.00, Al^{+3} ($cmol_c$ dm^{-3}) = 0.0, and CEC ($cmol_c$ dm^{-3}) = 6.5.

A randomized block experimental design arranged in an incomplete factorial scheme 5 x 5 with four replications was used. Five electrical conductivity of irrigation water (ECw = 0 (distilled water), 0.8, 2.75, 4.7, and 5.5 dS m^{-1}) and five nitrogen doses (0, 58.58, 200, 341.42, and 400 mg L^{-1}) were assessed. The incomplete factorial was generated through the Central Composite Design experimental matrix, resulting in nine combinations (Table 1).

The salinity levels of irrigation water were prepared by introducing sodium chloride (NaCl) into the water supply (0.5 dS m^{-1}) until they reached the specified electrical conductivities. The values were calibrated using a portable conductivity meter, specifically the microprocessor model Instrutherm® (CD-860 model). Saline water irrigation commenced ten days after the emergence of the plants, with water application facilitated through the drainage lysimeter method (Bernardo et al., 2019).

Nitrogen (N) doses were determined considering a requirement of 300 mg $plant^{-1}$ for a 1 dm^3 pot (Novais et al., 1991). A commercially available product containing 99 g L^{-1} of N, derived from urea, served as the nitrogen source. Foliar applications of nitrogen were conducted at seven-day intervals, totaling five applications. A hand sprayer was employed for the application, with a spray volume of 175 mL per plant administered in the late afternoon on each scheduled day.

The assessment of fresh mass of root, stem, leaves, shoot, and total was conducted 45 days after the initiation of saline water irrigation. Plant material was weighed using a precision analytical scale. Subsequently, the plant material was individually placed in Kraft paper bags and dried in a laboratory oven with forced air circulation set at 65 ºC until a constant mass was achieved. The recorded data were expressed in grams per plant. A non-destructive method was employed to determine chlorophyll *a*, *b*, and total indexes utilizing a portable chlorophyll meter (ClorofiLOG®, model CFL 1030, Porto Alegre, RS). The values obtained were calibrated in the Falker chlorophyll index (FCI).

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

Levels		Doses	
ECw	N	ECw	N
-1	-1	0.80	58.16
-1	1	0.80	341.84
1	-1	4.70	58.16
1	1	4.70	341.84
-α	0	0.00	200.00
α	0	5.50	200.00
0	A	2.75	400.00
0	-α	2.75	0.00
0	0	2.75	200.00

ECw: Electrical conductivity of irrigation water

The data were subjected to analysis of variance ($p \leq 0.05$). In cases of significance, a regression analysis was conducted. Response surface plots were generated. For isolated effects, either linear or quadratic regression analyses were applied. The statistical software R (R Core Team, 2022) was used for all analyses.

3. Results and Discussion

Influence from the interaction between the factors (nitrogen foliar fertilization and electrical conductivity of irrigation water - ECw) was noted on the root fresh and dry mass, stem dry mass, and shoot/root ratio (Figure 1). The highest root fresh mass (3.42 g) was observed at ECw of 1.14 dS m^{-1} and with a nitrogen dose of 294.96 mg L^{-1}, indicating that the application of nitrogen mitigated the harmful impact of salt stress (Figure 1A).

The root dry mass declined with the rise in ECw. A more pronounced increase in root dry mass (0.58 g) occurred at ECw of 0.5 dS m^{-1} and nitrogen dose of 205.36 mg L^{-1} (Figure 1B). Conversely, stem dry mass demonstrated a notable increase (0.57 g) at the dose of 217.39 mg L^{-1} of N and an ECw of 0.5 dS m^{-1} (Figure 1C), indicating that foliar fertilization with nitrogen mitigated the impact of salt stress. The most substantial increase in the shoot/root ratio (0.28) occurred at the dose of 231.30 mg L^{-1} of N and an ECw of 0.5 dS m^{-1} (Figure 1D).

Foliar fertilization with nitrogen alleviated the effects of salinity stress induced by irrigation water, increasing the root fresh and dry mass, stem dry mass, and the shoot/root ratio. As an essential macronutrient, nitrogen plays a vital role in various metabolic pathways, including forming ATP, NADH, NADPH, nucleic acids, proteins, enzymes, and chlorophyll (Souza et al., 2019). Consequently, this enhances plant tolerance to salinity stress (Bezerra et al., 2018).

The mitigation of salt stress through nitrogen application is attributed to the accumulation of nitrogen and/or the facilitation of ionic homeostasis (Miranda et al., 2015). Additionally, nitrogen promotes homeostasis and plays a role in producing osmoprotectants, enabling the plant to tolerate salt stress (De la Torre-González et al., 2019). The efficacy of nitrogen in alleviating salinity stress has been observed in various plant species, including *Gossypium hirsutum* L. (Lima et al., 2019), *Malpighia emarginata* D.C. (Alvarenga et al., 2019), *Psidium guajava* L. (Bezerra et al., 2018), and *Cucumis sativus* L. (Ma et al., 2020).

Salinity induced an independent effect on the leaf fresh mass, stem fresh mass, shoot fresh and dry mass, and total fresh and dry mass (Figure 2). The rise in ECw linearly reduced the leaf fresh mass, with the maximum value (14.8 g) observed in the control; there were losses of 50% in the highest salinity level compared to the control treatment (Figure 2A).

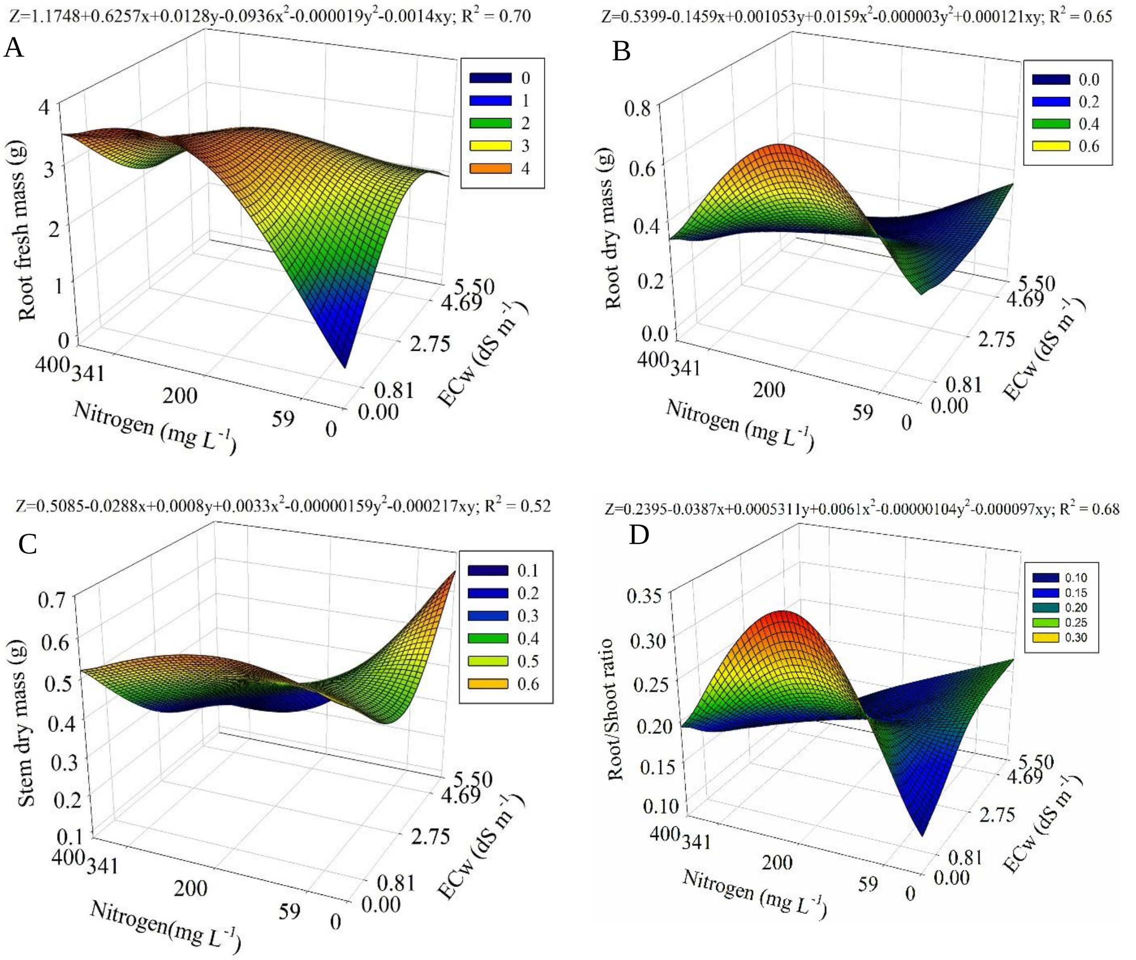

Figure 1. Root fresh mass (A), root dry mass (B), stem dry mass (C), and shoot/root ratio (D) of purple basil according to the salinity level of irrigation water and foliar fertilization with N.

The stem fresh mass decreased (1.4 g) with the increasing salinity, resulting in losses of 31.8% comparing the highest and lowest ECw values (Figure 2B). The shoot fresh and dry mass exhibited a linear decrease with the increase in ECw, with the highest values (19.0 g and 2.18 g, respectively) observed in the control treatment. These values decreased by 47.3% and 58.7%, respectively, comparing the highest and lowest ECw values, corresponding to shoot fresh and dry mass values of 9.0 g and 1.28 g, respectively (Figure 2C and D). The total fresh and dry mass of the basil plants decreased linearly with the increase in ECw. The highest values (22 and 1.2 g) occurred at the lowest salinity level, followed by losses of 45.4% and 60%, respectively, at the highest ECw (Figure 2E and 2F).

The decline in the leaf fresh mass, stem dry mass, shoot dry mass, and total fresh and dry mass with the increasing ECw can be attributed to the restriction in water absorption by the roots due to the elevated osmotic potential in the nutrient solution. Additionally, this decrease may be associated with the imbalance and ionic toxicity induced by the excessive accumulation of salts in the plant tissues (Huang, 2018). Such a response can be interpreted as an adaptive mechanism, facilitating water loss reduction through transpiration and thereby aiding in the retention of toxic ions within the roots (Acosta-Motos et al., 2017).

The presence of ions, particularly Na^+, and alterations in the Na^+/K^+, Na^+/Ca^{+2}, Na^+/Mg^{+2}, and Cl^-/NO_3^- ratios lead to ionic toxicity, significantly impacting various aspects of plant metabolism. This includes disruptions in protein metabolism, respiratory chain function, photophosphorylation, and nutrient assimilation, among other organic compounds, thereby inducing widespread changes in overall plant metabolism (Viudes and Santos, 2014). These changes are corroborated by reductions in biomass accumulation, as evidenced by the decrease in the fresh and dry mass of basil plants in this study, attributed to the increasing salinity of the irrigation water.

Conversely, the buildup of Na^+ and Cl^- ions in leaf tissue can lead to stomatal closure, potentially causing damage to the photosynthetic machinery. This, in turn, results in reduced CO_2 assimilation by plants and consequently leads to a decrease in the accumulation of fresh and dry mass (Abdelgawad et al., 2016). The salinity induced alterations in the chlorophyll content of basil plants, with quadratic behavior of chlorophyll *a*, *b*, and total (Figure 3).

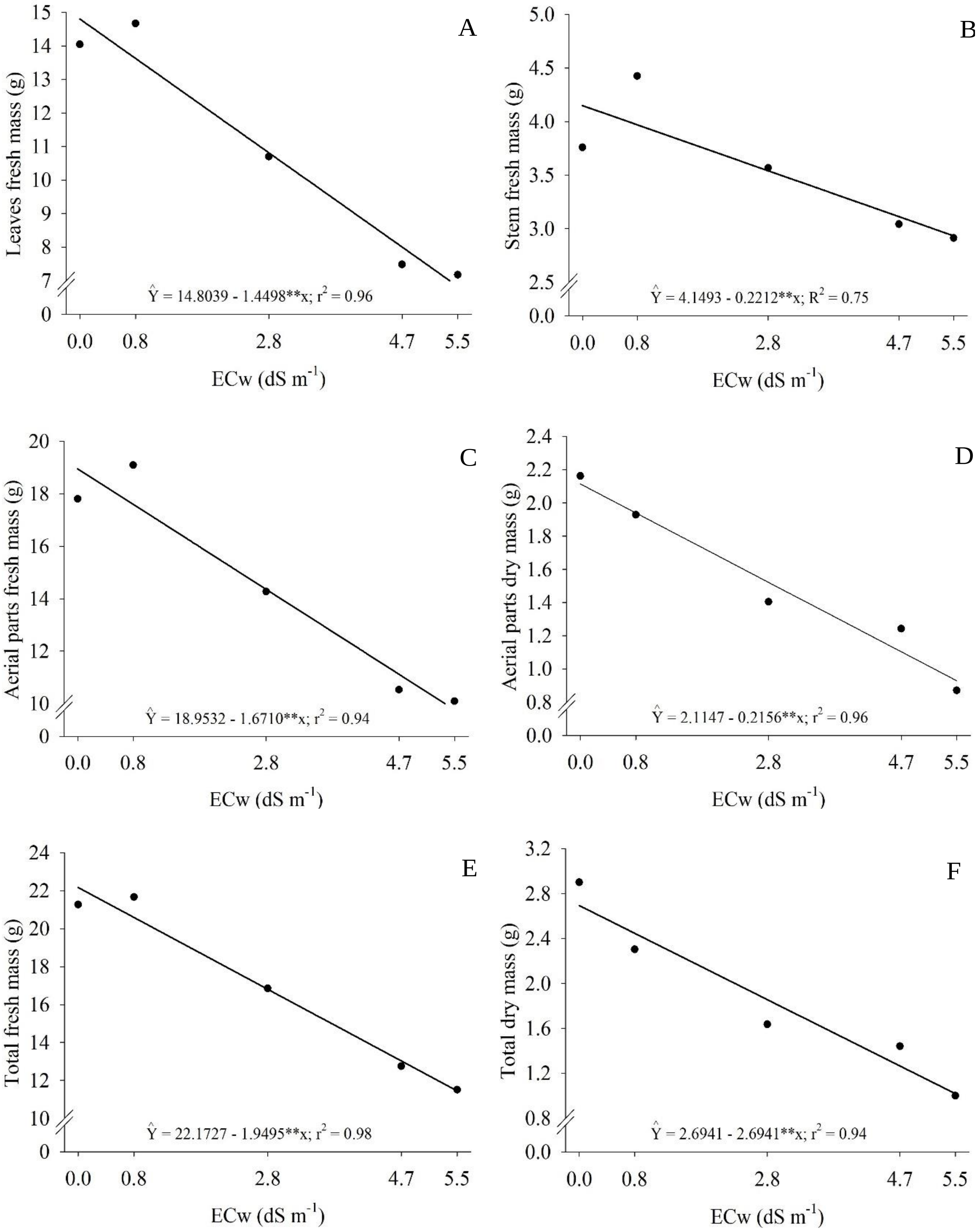

Figure 2. Leaf fresh mass (A), stem fresh mass (B), shoot fresh mass (C), shoot dry mass (D), total fresh mass (E), and total dry mass (F) of purple basil submitted to salinity.

The highest chlorophyll *a* index (26.6 FCI) was noted at ECw of 3.8 dS m^{-1}, followed by decreases with the rising ECw, representing a 15.65% increase compared to the control (Figure 3A). The highest chlorophyll *b* index (6.25 FCI) was recorded at ECw of 4.0 dS m^{-1}, decreasing with higher salinity levels, representing a 25% increase compared to the ECw of 0 dS m^{-1} (Figure 3B). The maximum total chlorophyll index (32.8 FCI) was observed at ECw of 3.8 dS m^{-1}, indicating a 17.14% increase compared to the control (Figure 3C). The elevation of ECw enhanced the indexes of chlorophylls *a*, *b*, and total in basil plants, reaching peaks at doses of 3.8, 4.0, and 3.8 dS m^{-1}, respectively, followed by reductions at higher levels. This suggests that basil plants tolerate salinity stress up to 4.0 dS m^{-1}.

The physiological changes induced by the stress enable plants to resist and/or tolerate salinity stress (Pandolfi et al., 2012). The rise in chlorophyll content may preserve the proper functioning of photosynthesis, as chlorophyll biosynthesis tends to increase under conditions of moderate salinity stress (Shah et al., 2017). Therefore, the results of this study may indicate that the genotype employed is tolerant to NaCl, as an elevated chlorophyll content under salinity stress can serve as a biochemical indicator of tolerance to salinity (Akrami and Arzani, 2018).

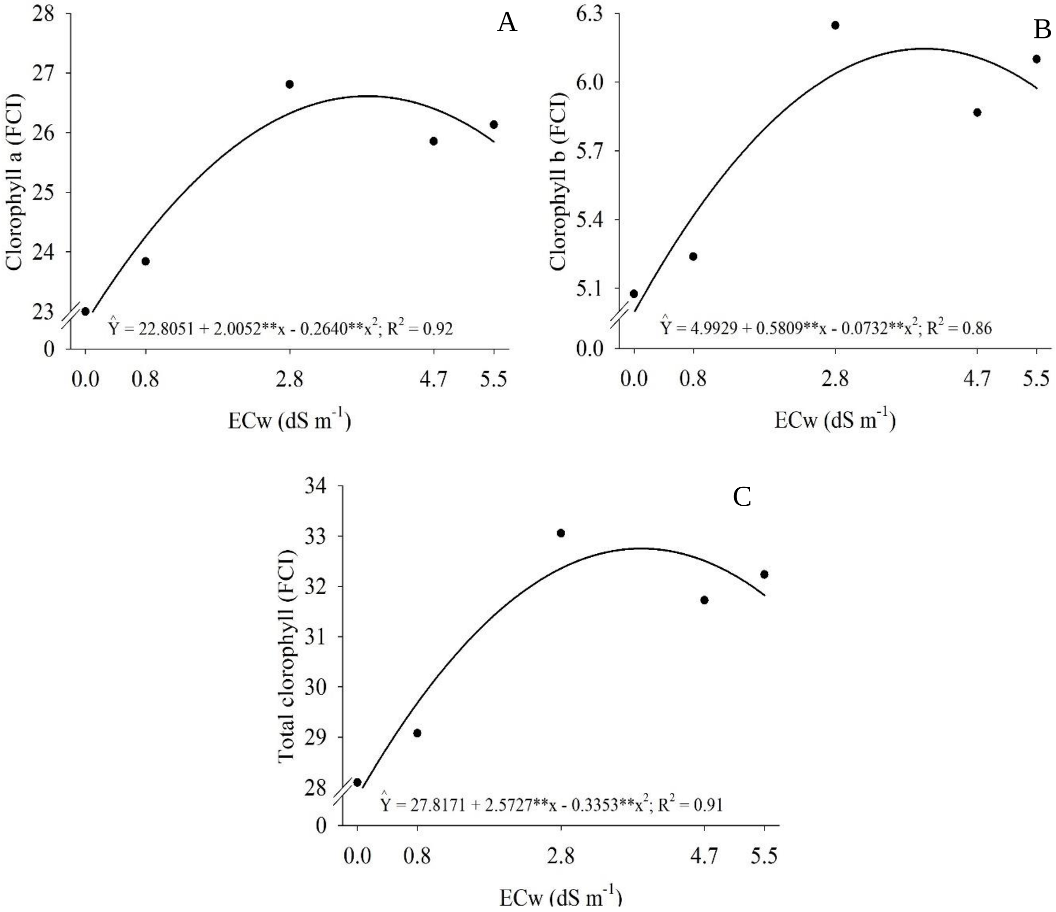

Figure 3. Chlorophyll *a* index (A), chlorophyll *b* index (B), and total chlorophyll index (C) of purple basil submitted to salinity.

The independent effect of foliar fertilization with nitrogen had a notable impact on the leaf fresh mass, stem fresh mass, total fresh and dry mass, chlorophyll *a* index, and total chlorophyll (Figure 4). The maximum values of leaf fresh mass (11.5 g) and total fresh mass (17.8 g) were achieved at nitrogen doses of 200 and 193 mg L^{-1}, respectively. Subsequently, there were reductions with the increase in nitrogen doses. This signifies an increase of 17.7% and 13.4%, respectively, compared to the control (0 mg L^{-1} of N) (Figure 4A and 4C).

Foliar fertilization with nitrogen in basil plants led to an increase, up to a certain dose, in the leaf fresh mass, total fresh mass, chlorophyll *a*, and total chlorophyll while negatively impacting the stem fresh mass and total dry mass. This highlights that the effect of nitrogen can vary depending on the specific variable studied. For the leaf fresh mass and total fresh mass, there was an increase up to the dose of 200 mg L^{-1} of nitrogen, followed by a decrease with higher doses. This pattern suggests that nitrogen can enhance phytomass up to a certain threshold. However, an excess of nitrogen exerts a harmful effect, decreasing the variables under investigation (Miranda et al., 2015; Acosta-Motos et al., 2017).

The linear decrease in stem fresh mass and total dry mass might be attributed to the reduction in O_2 assimilation resulting from increased nitrogen doses. O_2 is crucial for energy production in cellular pathways, and a decrease in its assimilation by the roots can lead to an anaerobic environment, rendering root system respiration impossible under conditions of anoxia or hypoxia (Taiz et al., 2017). Consequently, nitrogen use diminishes with the escalating applied dose, surpassing the crops' demand (Neumann et al., 2017). Conversely, nitrogen application can induce biochemical and physiological changes in plants, such as alterations in intracellular pH and hormone metabolism, influencing overall plant metabolism and potentially hindering optimal nitrogen assimilation (Li et al., 2014).

Increases in chlorophyll *a* and total indexes were observed starting from the dose of 200 mg L^{-1} of nitrogen. This is attributed to nitrogen's multifaceted role in plant metabolism, serving structural functions in the composition of amino acids, proteins, enzymes, RNA, DNA, ATP, chlorophyll, and other molecules (Huang, 2018). Moreover, the elevation in nitrogen levels facilitates greater CO_2 assimilation by plants, enhancing chlorophyll concentration, efficiency, and the content of essential ions such as Ca^{2+} and Mg^{2+} in the leaf mesophyll (Sá et al., 2015). This stimulation of the plant system results in increased dynamics of mineral nutrient transport (Huang, 2018).

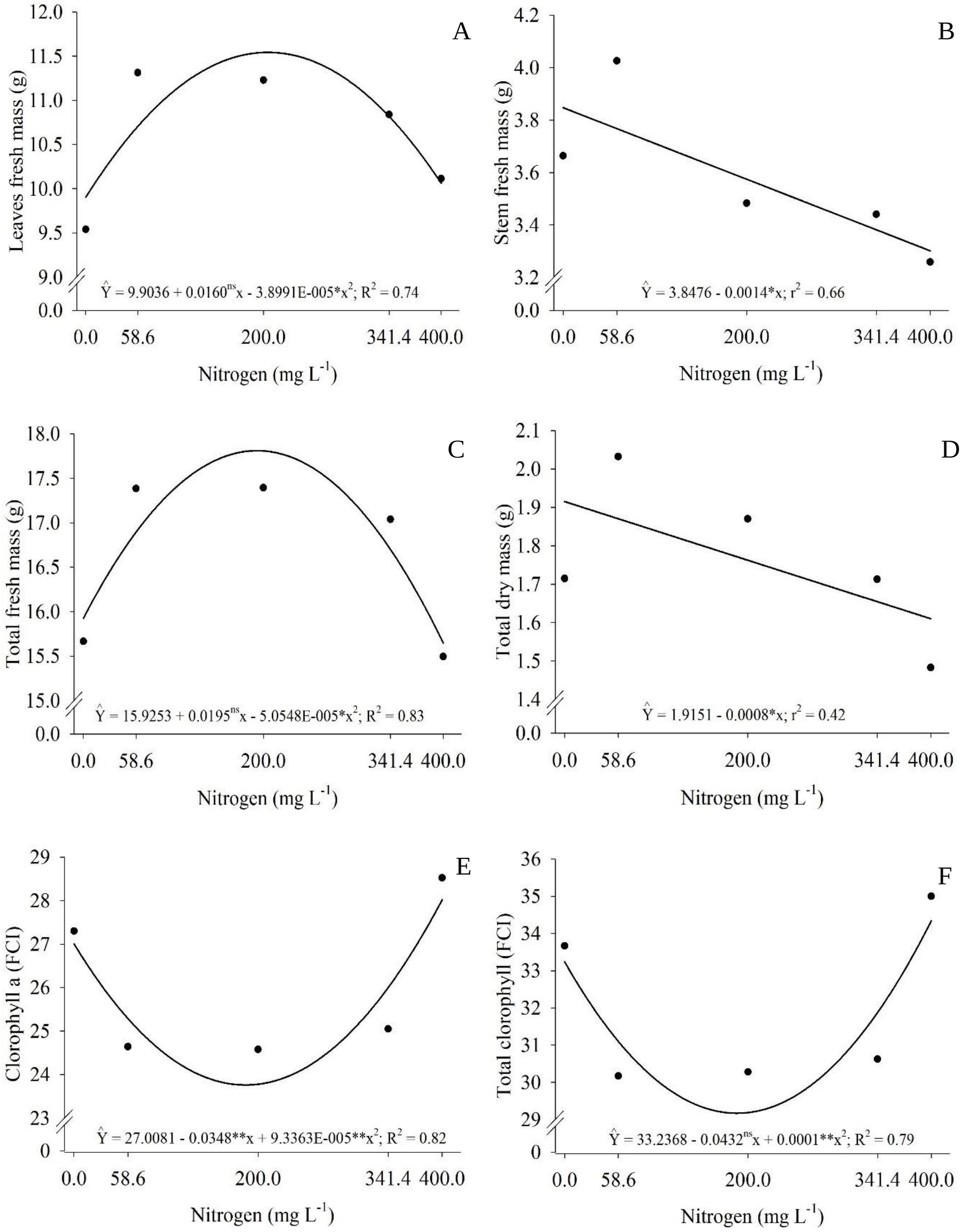

Figure 4. Leaf fresh mass (A), stem fresh mass (B), total fresh mass (C), total dry mass (D), chlorophyll *a* index (E), and total chlorophyll index (F) of purple basil plants according to the foliar fertilization with N.

4. Conclusions

Salt stress negatively affects biomass production and chlorophyll synthesis in basil plants. Nevertheless, applying foliar nitrogen enhances basil plants' tolerance to saline stress, promoting the production of phytomass and photosynthetic pigments.

Authors' Contribution

Antônio Veimar da Silva and Raimundo Nonato Moraes Costa = Investigation, Writing – original draft; Edcarlos Camilo da Silva = Investigation, Writing – original draft; Jackson Silva, Nóbrega and João Everthon da Silva Ribeiro = Conceptualization, Project administration, Supervision, Writing – review & editing; Thiago Jardelino Dias = Funding acquisition, Validation, Supervision, Writing – review & editing; Ana Carolina Bezerra = Funding acquisition, Validation, Writing – review & editing; Adriano Salviano Lopes = Resources, Investigation, Validation; Toshik Iarley da Silva = Methodology, Visualization, Writing – review & editing.

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