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Scientific Article

Growth and solute accumulation in collard greens after pre-treatment with H_2O_2 under salt stress

Crescimento e acúmulo de solutos em couve-folha após pré-tratamento com H_2O_2 sob estresse salino

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ABSTRACT - Hydrogen peroxide (H_2O_2) acts as a signaling molecule inducing increased plant tolerance to stress conditions. The objective of the present study was to evaluate the effect of pre-treatment with H_2O_2 as a possible attenuator of salt stress on the production of biomass and organic and inorganic solutes in collard greens under salt stress. The plants were grown under hydroponic conditions in a greenhouse. The experimental design used was completely randomized with eight replications with the combination of three times of exposure to H_2O_2 (12, 24, and 36 h) and four levels of H_2O_2 in the pre-treatment solution (0.1, 1.0, 10, and 100 µM). Two control treatments were added, one without the presence of NaCl and another with the presence of 100 mM NaCl, both without pre-treatment with H_2O_2. After 60 days of assay the plants were collected and subsequently analyzed. Salinity affected collard green biomass production regardless of the concentration and time of exposure to H_2O_2. Salinity reduced biomass production in collard greens and there was an increase in Na^+ and Cl^-, while K^+ decreased, but pre-treatment with H_2O_2 proved to be effective in increasing the levels of organic solutes, highlighting the importance of H_2O_2 in the plant's adaptive response.

Keywords: *Brassica oleracea*. Salinity. ROS. Biomass production.

RESUMO - O peróxido de hidrogênio (H_2O_2) atua como uma molécula de sinalização, estimulando o aumento da tolerância das plantas a condições de estresse. O presente estudo teve como objetivo avaliar o efeito do pré-tratamento com H_2O_2 como possível atenuante do estresse salino na produção de biomassa e solutos orgânicos e inorgânicos nas folhas de couve-folha. As plantas foram cultivadas em condições hidropônicas em casa de vegetação. O delineamento experimental utilizado foi inteiramente casualizado, com oito repetições, tendo os tratamentos a combinação de três tempos de exposição ao H_2O_2 (12, 24 e 36 horas) e quatro níveis de H_2O_2 na solução de pré-tratamento (0,1; 1,0; 10; 100 µM). Foram adicionados dois tratamentos controle, um sem a presença de NaCl e outro com a presença de 100 mM de NaCl, ambos sem o pré-tratamento com H_2O_2, totalizando 14 tratamentos. Aos 60 dias de ensaio, as plantas foram coletadas e posteriormente analisadas. A salinidade reduziu a produção de biomassa na couve-folha e houve aumento de Na^+ e Cl^- e diminuição de K^+, mas o pré-tratamento com H_2O_2 mostrou-se eficaz ao aumentar os teores de compostos orgânicos evidenciando a importância do H_2O_2 na resposta adaptativa da planta.

Palavras-chave: *Brassica oleracea.* Salinidade. EROs. Produção de biomassa.

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INTRODUCTION

Brassica oleracea L. var. Acephala, commonly known as collard greens, is a plant belonging to the *Brassicaceae* family, which includes a variety of vegetables such as cauliflower, broccoli, and cabbage. The leaves of this species are highly valued for their nutritional properties, culinary versatility, and health benefits. They are rich in vitamin A, vitamin C, antioxidants, fiber, and minerals such as iron, calcium, and potassium, which help in the prevention of various diseases (IULIANELLI et al., 2021).

Leafy vegetables are widely grown in hydroponic systems, especially in the NFT type (Nutrient Film Technique), with increase in cultivation area in recent years (NOBOA et al., 2019). Many farmers have adopted this cultivation method in major production regions due to the following advantages: earlier harvests, higher productivity compared to conventional farming, efficient use of water and fertilizers, absence of weed competition, reduced impact of adverse weather conditions, lower incidence of phytosanitary issues, and better commercial quality, as the plants are cleaner (PURQUERIO et al., 2018).

However, in arid and semi-arid regions, the use of hydroponics may present some limitations, mainly due to the predominance of saline water (COVA et al., 2016). The use of low-quality water in hydroponic cultivation can reduce crop growth and yield due to osmotic and ionic effects that induce morphological,

biochemical, and physiological changes (AZEVEDO NETO; SILVA, 2015).

In this context, hydrogen peroxide (H_2O_2) emerges as an alternative to mitigate the effects of salt stress on plants. Characterized as a reactive oxygen species (ROS), at low or moderate concentrations, H_2O_2 can act as a secondary messenger, signaling responses within cells against various types of stress (AZEVEDO NETO; SILVA, 2015).

Several researchers have used H_2O_2 as a strategy to increase salinity tolerance in various crops under hydroponic conditions (SILVA et al., 2020; DANTAS et al., 2022; SILVA et al., 2023b,c). Silva et al. (2020) observed that H_2O_2 treatment reduced Na^+ and Cl^- absorption in sunflower leaves. Dantas et al. (2022) reported that the application of 20 µM of H_2O_2 increased the total fruit weight and basal fruit diameter of zucchini when the plants were grown in a nutrient solution with an electrical conductivity of 2.1 dS m^{-1}. Silva et al. (2023c) found that priming lettuce seeds with 0.1 mM of H_2O_2 for 12 h increased shoot fresh mass by 94% and priming for 36 h increased shoot dry mass by 215%.

A commonly observed response to the use of H_2O_2 is an improvement in osmotic and ionic homeostasis, leading to greater water uptake and a reduction in the accumulation of toxic ions and nutritional imbalances. These homeostatic mechanisms involve the compartmentalization and accumulation of organic and inorganic solutes and play a key role in maintaining plant growth in saline environments (AZEVEDO NETO; SILVA, 2015).

Thus, the objective of this study was to evaluate the effect of H_2O_2 as a potential mitigator of salt stress effects on biomass production and on the foliar concentrations of inorganic and organic solutes in collard greens grown in a hydroponic system.

MATERIALS AND METHODS

The experiment was conducted from January to July 2023 at the facilities of the Graduate Program in Agricultural Engineering of the Federal University of Recôncavo da Bahia, in the municipality of Cruz das Almas, BA, Brazil, located at 12°40'19" S, 39°06'23" W, at an altitude of 220 m. According to Köppen's classification, the climate is hot and humid (Af) (ALVARES et al., 2013). During the experimental period, the average temperature and relative humidity recorded at the Embrapa Cassava and Fruit Meteorological Station near the greenhouse were 26 °C and 68%, respectively (INMET, 2023).

Seedlings were produced from seeds of collard greens of the Georgia cultivar (*Brassica oleracea* var. Acephala), due to its status as a traditional cultivar with wide adaptability. Sowing was carried out in phenolic foam, with three seeds placed per cell, irrigated daily with public-supply water until the seedlings reached a height of 2 to 3 cm. Nine days after sowing (DAS), all phenolic foam cells were separated and transferred to a nursery using a Nutrient Film Technique (NFT) system constructed with corrugated PVC roofing sheets. During the nursery phase, the seedlings were irrigated with half-strength nutrient solution according to Furlani (1998). At 13 DAS, the seedlings were thinned to maintain only one plant per cell.

The experimental design used was completely randomized, with 14 treatments and eight replicates. The treatments consisted of two controls: non-saline (C) and saline (SC). In addition to these, combinations of four doses of H_2O_2 (0.1; 1.0; 10; 100 µM) with three exposure times to H_2O_2 (12, 24, and 36 h) were tested, totaling 14 treatments, as shown in Table 1.

Table 1. Description of the treatments applied to the collard greens plants used in the experiment.

Treatment	H_2O_2 Dose	Exposure Time to H_2O_2	Cultivation under NaCl
T1	Absence	Absence	Absence
T2	Absence	Absence	100 mM NaCl
T3	0.1 µM H_2O_2	12 h	100 mM NaCl
T4	0.1 µM H_2O_2	24 h	100 mM NaCl
T5	0.1 µM H_2O_2	36 h	100 mM NaCl
T6	1 µM H_2O_2	12 h	100 mM NaCl
T7	1 µM H_2O_2	24 h	100 mM NaCl
T8	1 µM H_2O_2	36 h	100 mM NaCl
T9	10 µM H_2O_2	12 h	100 mM NaCl
T10	10 µM H_2O_2	24 h	100 mM NaCl
T11	10 µM H_2O_2	36 h	100 mM NaCl
T12	100 µM H_2O_2	12 h	100 mM NaCl
T13	100 µM H_2O_2	24 h	100 mM NaCl
T14	100 µM H_2O_2	36 h	100 mM NaCl

The seedlings were transferred to plastic trays where they received the respective pre-treatments with H_2O_2, while the seedlings designated for the two control treatments (non-saline - C and saline – SC) were maintained only with half-strength nutrient solution. After the appropriate pre-treatment time, at 25 DAS all plants were simultaneously transferred to the final cultivation channels in the NFT system. The non-saline control plants were irrigated with the full-strength nutrient solution of Furlani (1998), while the saline control plants and the others (pre-treated with H_2O_2) were supplied

with the same nutrient solution supplemented with 100 mM NaCl.

The pH values of the nutrient solutions were measured daily and maintained between 5.5 and 6.0 by adding 1.0 M sulfuric acid (H_2SO_4) or 1.0 M potassium hydroxide (KOH). The solution levels were replenished daily with spublic-upply water, and the solutions were renewed every six days until the material was harvested.

At the end of the experimental period (60 DAS), the plants were harvested to count the number of leaves (NL) and determine leaf fresh mass (LFM), stem fresh mass (SFM), and shoot fresh mass (ShFM). Total leaf area (LA) was measured using 20 discs taken from five leaves, following the methodology described by Tavares-Júnior et al. (2002). During harvest, samples of approximately 1.0 g of fresh mass from the first fully expanded leaf were frozen in liquid nitrogen, lyophilized, ground, and stored in a freezer (-25 °C) for the determination of organic solute contents.

The remaining plant material was transferred to a forced-air circulation oven at 65 °C and, after 72 h, leaf dry mass (LDM), stem dry mass (SDM), and shoot dry mass (ShDM) were determined. After drying, the leaves were ground using a mortar and pestle for the determination of inorganic solute contents.

Extracts for the determination of sodium (Na^+), potassium (K^+), and chloride (Cl^-) were prepared according to Jones Junior (2001), with minor modifications. In test tubes, 0.1 g of plant material and 10 mL of deionized water were added. The tubes were heated at 100 °C in a water bath for 1 hour with agitation every 15 minutes. Na^+ and K^+ contents were determined in the extract by flame photometry. Chloride (Cl^-) content was determined by spectrophotometry at 460 nm (GAINES; PARKER; GASCHO, 1984).

To obtain the extract for the determination of organic solutes, 0.1 g of lyophilized leaf tissue was homogenized with 5 mL of 0.1 M potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA. The homogenate was filtered through fine mesh nylon fabric and centrifuged at 10,000 × g for 10 minutes. The supernatant was stored in a freezer and used for the determination of soluble carbohydrates, free amino acids, and soluble proteins.

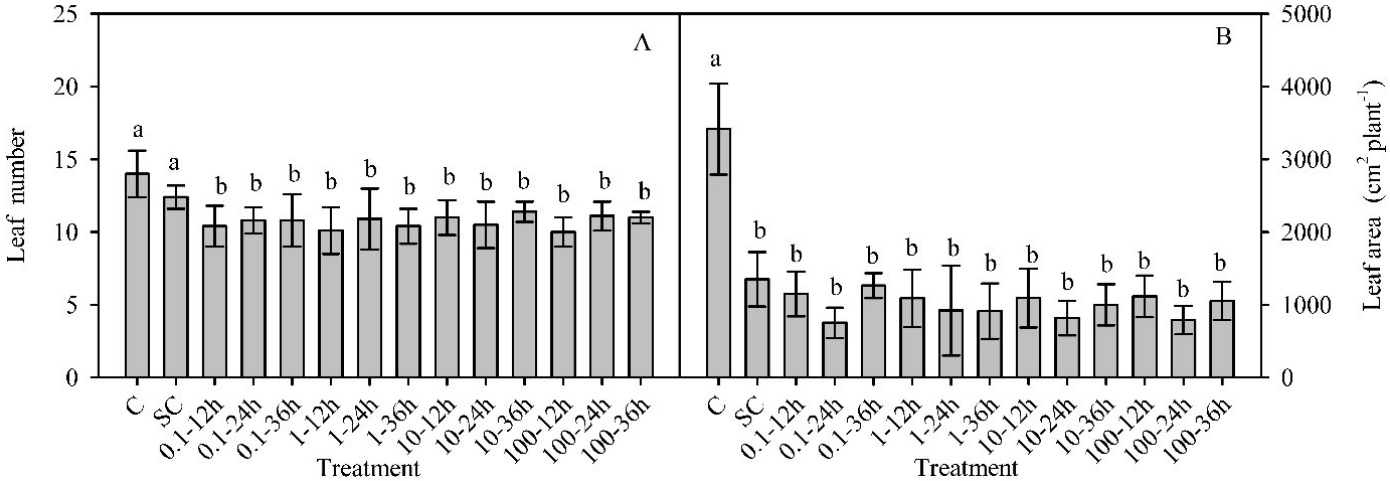
The determination of soluble carbohydrate content (CH) was performed at 490 nm using the phenol-sulfuric acid method (DUBOIS et al., 1956); free amino acids (AA) were measured at 570 nm using the ninhydrin method (YEMM; COCKING, 1955); and soluble proteins (SP) were determined at 595 nm by the protein dye-binding method (BRADFORD, 1976), using bovine serum albumin as the standard.

The data were subjected to the F-test of analysis of variance (ANOVA), and means were compared by the Scott-Knott test at a 0.05 probability level. For Principal Component Analysis (PCA), the packages corrr, ggcorrplot, FactoMineR, dplyr, and factoextra were used. All analyses were performed with the aid of R software (R DEVELOPMENT CORE TEAM, 2022).

RESULTS AND DISCUSSION

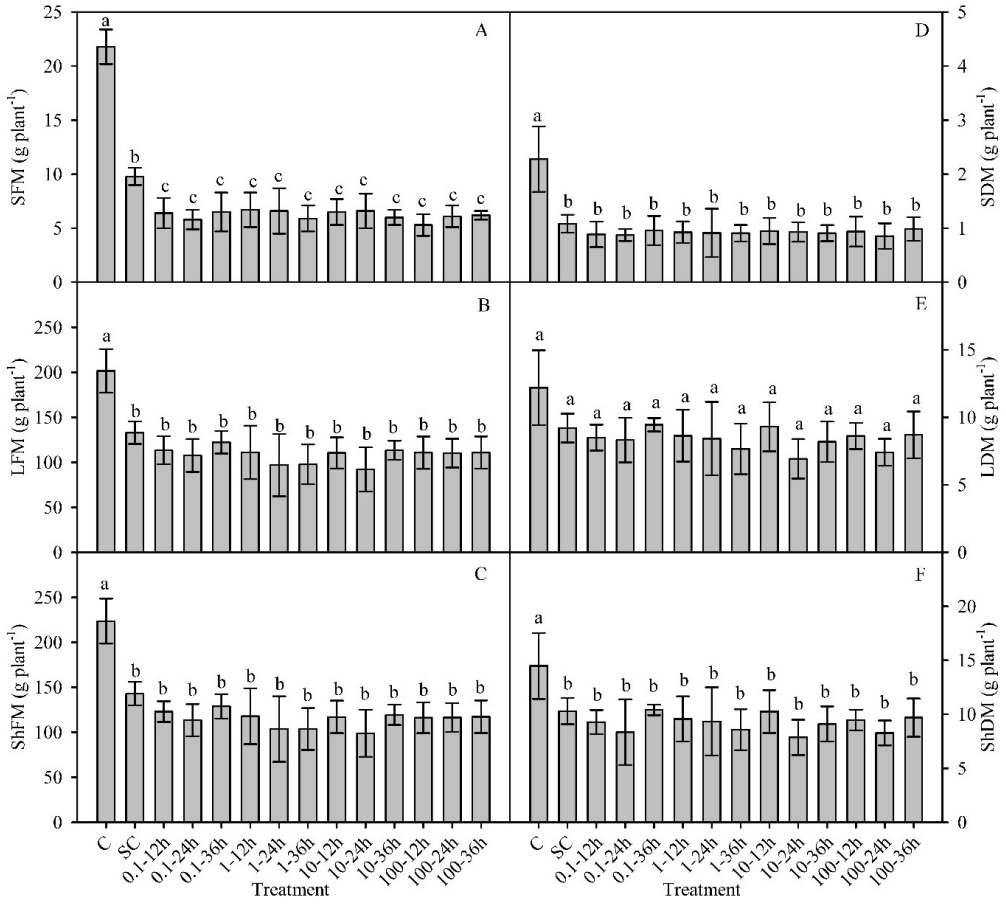
At 35 days after treatments (60 DAS), there was a significant difference between the applied treatments ($p \leq 0.01$) for all variables analyzed, except for leaf dry mass (LDM) (Figures 1 and 2).

The highest number of leaves (NL) was observed in the non-saline control (C) and saline control (SC) treatments. However, there was no significant difference between these two treatments, with an average of 13 leaves per plant. For the treatments with H_2O_2 application, the NL decreased by an average of 23% compared to the controls (C and SC) (Figure 1A). Thus, for this variable, the H_2O_2 pre-treatment did not mitigate the effects of salinity.



C - Non-saline control – absence of H_2O_2 and absence of NaCl; SC - Saline Control absence of H_2O_2 + 100 mM NaCl; 0.1, 1, 10, and 100 µM are the concentrations of H_2O_2; 12, 24, and 36 are the exposure times (in hours) to H_2O_2; and 100 mM is the concentration of NaCl in the saline nutrient solution.

Figure 1. Number of leaves - NL (A) and leaf area - LA (B) of collard greens plants cultivated for 35 days in an NFT hydroponic system, as a function of the treatments applied.



C - Non-saline control (absence of H_2O_2 and absence of NaCl); SC - Saline Control (absence of H_2O_2 + 100 mM NaCl); 0.1, 1, 10, and 100 µM are the concentrations of H_2O_2; 12, 24, and 36 are the exposure times (in hours) to H_2O_2; and 100 mM is the concentration of NaCl in the saline nutrient solution.

Figure 2. Stem - SFM(A), leaf - LFM (B), and shoot - ShFM (C) fresh mass and stem - SDM (D), leaf - LDM (E), and shoot - ShDM (F) dry mass of collard greens plants grown for 35 days in an NFT hydroponic system, according to the treatments applied.

In Figure 1B, it can be seen that the leaf area (LA) values of plants under saline treatments did not differ among themselves and were about 71% smaller than those of plants in the non-saline control (C) treatment. However, salinity did not cause other impacts related to product quality, such as leaf burn or chlorosis. The results also indicate that the effect of salinity on LA was much more pronounced than on NL (COSTA et al., 2020; SILVA et al., 2023a), which may affect consumer acceptance of the product. This occurs because consumers are usually attracted by the appearance of the leaves, especially their size. However, producers compensate for the reduction in leaf area by increasing the number of leaves in the commercial bunch. Among the visual characteristics noticed by consumers of leafy vegetables, Silva et al. (2024) reported that salinity affected quality by causing leaf burn in chicory (*Cichorium intybus* L.), reducing leaf size in lettuce (*Lactuca sativa* L.), and altering the coloration and size of leaves in endive (*Cichorium endivia* L.).

In Figure 2, it can be observed that salinity significantly reduced the variables stem, leaf, and shoot fresh and dry mass, except for leaf dry mass (LDM), which showed no significant difference among treatments, with an average of 8.68 g plant^{-1} (Figure 2E).

When comparing treatments under saline conditions, the application of H_2O_2 resulted in an average 36% reduction in stem fresh mass (SFM) compared to the saline control (SC) (Figure 2A). For the other variables analyzed - leaf fresh mass (LFM), shoot fresh mass (ShFM), stem dry mass (SDM), and shoot dry mass (ShDM) - no significant differences were observed between SC and the H_2O_2 treatments, regardless of dose or exposure time (Figures 2B, 2C, 2D, and 2F).

Compared to the non-saline control (C), the reductions caused by salinity in LFM, ShFM, SDM, and ShDM were on average 45%, 48%, 59%, and 36%, respectively.

The results of the present study are consistent with the literature, as reported by Costa et al. (2020), who highlighted the adverse effects of salinity on shoot dry mass (SDM) production, with a reduction of 6.14% per unit increase in electrical conductivity of the solution (ECsol) when it varied from 1.94 to 7.01 dS m^{-1}, compromising cauliflower plant production. Silva et al. (2023a) observed reductions of 8.64% and 7.91% per unit increase in salinity when ECsol varied from 1.95 to 7.98 dS m^{-1} in the stem dry mass of cauliflower grown in a hydroponic system under conditions similar to those of the present study, during the winter-spring and spring -summer seasons, respectively.

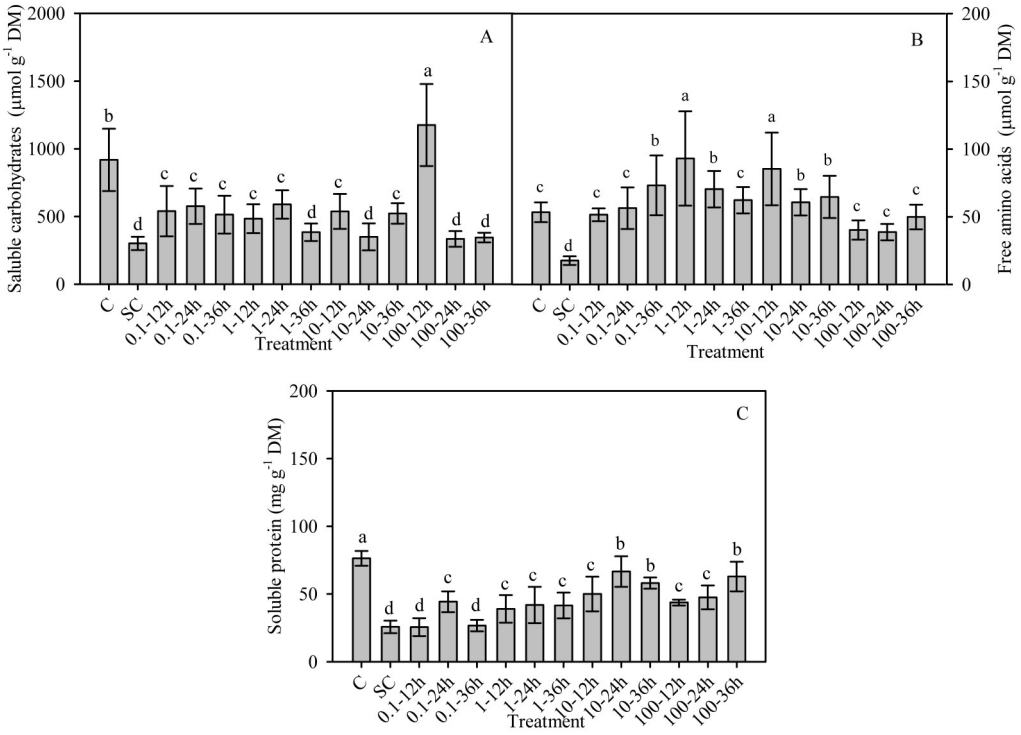
Salt stress induces various disturbances in physiological and biochemical processes, resulting in reduced growth and production of plants (SILVA et al., 2019, 2023b). For the variables analyzed (leaf fresh mass, shoot fresh mass, stem dry mass, leaf dry mass, shoot dry mass), regardless of the time and dose of H_2O_2 exposure, no significant differences

were observed compared to plants under the saline control treatment (SC). Thus, the results indicate that H_2O_2 did not mitigate the effect of salinity on all biomass variables of collard greens plants.

These results may be attributed to variations in plant responses to different application methods. Additionally, it is important to highlight that the method used for applying H_2O_2 via the nutrient solution may have interacted with other components of the nutrient solution, reducing its efficacy and/or causing complex interactions between salt stress and peroxide treatments in the metabolic and physiological pathways of the plants.

Results like these suggest that the pre-treatment with H_2O_2 via nutrient solution did not mitigate the negative impacts induced by salt stress. Some authors have observed that prior application of H_2O_2 reduced the harmful effects of salt stress on plant growth. Silva et al. (2023b) found that in the cultivation of coriander grown from seeds pre-treated with H_2O_2, regardless of concentration, under saline conditions, plant tolerance to salt stress was significantly increased. Therefore, the method of application and the crop species are factors that influence the results, indicating the need for further studies.

Salinity reduced soluble carbohydrate (CH), free amino acid (AA), and soluble protein (SP) contents by 67%, 67%, and 66%, respectively, in collard greens under the saline control treatment (SC) compared to the control treatment (Figure 3). However, the levels of organic solutes in collard green plants pre-treated with H_2O_2, regardless of dose and exposure time, were higher than those in the SC treatment.



C - Non-saline control (absence of H_2O_2 and absence of NaCl); SC - Saline Control (absence of H_2O_2 + 100 mM NaCl); 0.1, 1, 10, and 100 µM are the concentrations of H_2O_2; 12, 24, and 36 are the exposure times (in hours) to H_2O_2; and 100 mM is the concentration of NaCl in the saline nutrient solution.

Figure 3. Contents of soluble carbohydrates – CH (A), free amino acids – AA (B), and soluble proteins – SP (C) of collard greens plants cultivated for 35 days after treatment in an NFT system, according to the treatments applied.

In most treatments with H_2O_2 application, the levels of soluble carbohydrates (CH) were higher than in the saline control (SC), with particular emphasis on the 100 µM 12 h treatment, whose content was 28% and 289% higher than those observed in the control and saline control, respectively (Figure 3A). The increase in soluble carbohydrate levels in plants under salt stress is a commonly observed acclimation mechanism under these conditions (SILVA et al., 2020). According to these authors, this biochemical response may be associated with the plants' ability to adjust the osmotic potential in the cytosol, revealing itself as an effective mechanism of tolerance to adverse conditions.

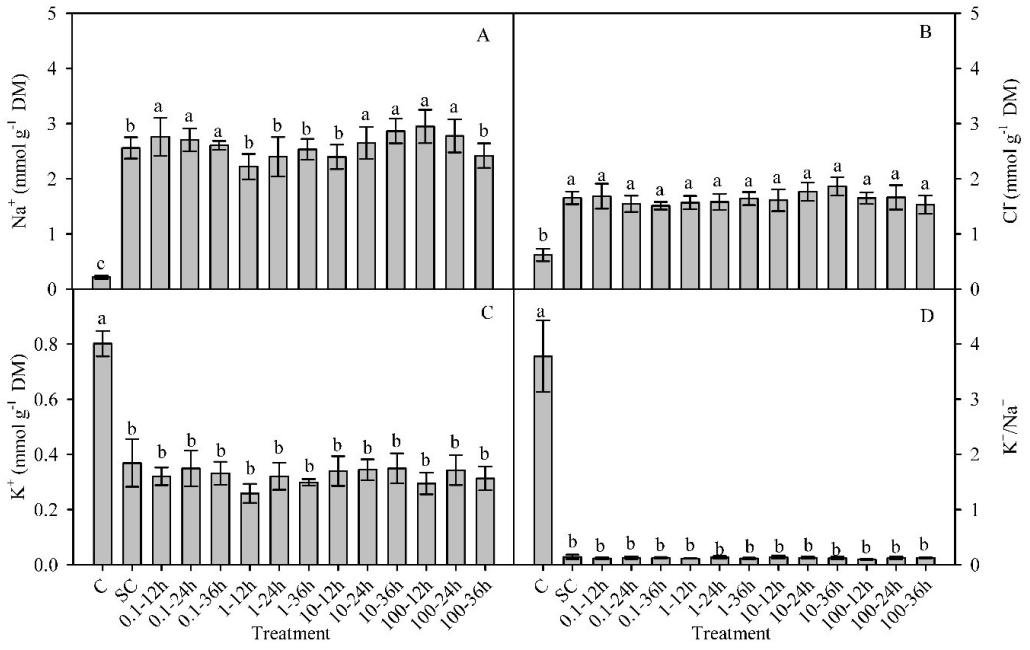
The levels of free amino acids (AA) in collard green leaves decreased in the saline control treatment (SC). In contrast, treatments with H_2O_2 application showed higher AA levels than the SC treatment (Figure 3B). It is also noteworthy that in half of the treatments with H_2O_2 application, the AA levels were higher than the control (C), with the most pronounced increases observed in the 1 µM–12 h and 10 µM–12 h treatments (on average 67%).

The results for soluble proteins (SP) were similar to those for CH. Overall, the pre-treatment with H_2O_2 increased SP levels compared to the SC treatment (Figure 3C); however, these increases were particularly evident in the 10 µM–24 h (159%), 10 µM - 36 h (126%), and 100 µM - 36 h (145%) treatments. Similar results were reported by Ó et al. (2021), who highlighted that under high salinity conditions, mini watermelon plants may reduce SP concentration.

Under different abiotic stresses (including salinity), it is common to observe changes in free amino acid (AA) and soluble protein (SP) contents as a result of alterations in the rates of biosynthesis and degradation of these compounds (BATISTA-SILVA et al., 2019; Ó et al., 2021). Thus, variations in AA levels can affect the biosynthesis of proteins and/or secondary metabolites. Therefore, the observation that AA and SP decreased in the saline control treatment (SC) suggests that the reduction in SP induced by NaCl was the result of decreased protein synthesis due to the reduction in AA content. However, in plants pre-treated with H_2O_2, the decrease in SP was less pronounced, suggesting that the higher AA content in these plants mitigated the deleterious effects of salinity on SP biosynthesis.

Physiologically, the synthesis and accumulation of organic solutes, also known as compatible solutes or compatible osmolytes, in plants grown under saline conditions represent an essential acclimation mechanism to preserve cell turgor (AZEVEDO NETO et al., 2020). However, analyzing the results from Figures 2 and 3 together, the observation that increased organic solutes levels in plants pre-treated with H_2O_2 did not induce an increase in biomass production suggests that Georgia collard greens did not use this mechanism as a response to salt stress. Similar results were observed in the selection of sunflower genotypes tolerant to salt stress (AZEVEDO NETO et al., 2020) and in hydroponic mini watermelon under salt stress (Ó et al., 2021).

Overall, it can be observed that the addition of NaCl to the nutrient solution increased foliar levels of Na^+ and Cl^- and decreased K^+ levels and the K^+/Na^+ ratio by averages of +1106%, +164%, -59%, and -97%, respectively, when comparing saline treatments to the control (Figures 4A, 4B, 4C, 4D, respectively).



C -C - Non-saline control – (absence of H_2O_2 and absence of NaCl); SC - Saline Control (absence of H_2O_2 + 100 mM NaCl); 0.1, 1, 10, and 100 µM are the concentrations of H_2O_2; 12, 24, and 36 are the exposure times (in hours) to H_2O_2; and 100 mM is the concentration of NaCl in the saline nutrient solution.

Figure 4. Contents of sodium – Na^+ (A), chloride – Cl^- (B), potassium – K^+ (C), and potassium/sodium ratio – K^+/Na^+ (D) in hydroponically grown collard greens plants cultivated for 35 days after treatment in an NFT system, according to the treatments applied.

In Figure 4A, it can be observed that in half of the treatments with H_2O_2, Na^+ contents were slightly higher (8%) than in the saline control (SC) treatment. For Cl^- and K^+ contents, as well as the K^+/Na^+ ratio, no significant differences were observed between the SC treatment and the other saline treatments, regardless of the dose or duration of the H_2O_2 pre-treatment. Similar results were observed in lettuce under salt stress conditions in different hydroponic systems (COVA et al., 2017).

The results of the present study show a salinity-induced increase in Na^+ and Cl^- contents and a decrease in K^+ contents in collard green leaves, regardless of whether H_2O_2 was applied or not. Similar results were reported by Cova et al. (2016) in noni seedlings cultivated in a hydroponic system for 40 days with saline nutrient solution (electrical conductivity ranging from 2 to 12 dS m^{-1}, through the addition of NaCl.

The accumulation of Na^+ and Cl^- in plant tissues exposed to salt stress is one of the main factors affecting physiological and biochemical processes and, consequently, crop growth and production (AZEVEDO NETO; SILVA, 2015; COVA et al., 2017). The reduction in K^+ content induced by Na^+ is widely reported in the literature (COVA et al., 2016; 2017; SILVA et al., 2020). As reported by Šamec, Linić and Salopek-Sondi (2021), treatment with 200 mM NaCl resulted in a sixfold (83.3%) reduction in the K^+/Na^+ ratio in collard greens leaves. In roots, the authors observed an even more pronounced reduction of 99.8%. This reduction is related to the antagonism between these two ions since the excess of Na^+ ions influences the selectivity of K^+ transporters due to the physicochemical similarities between the ions (MIRANDA et al., 2017). Thus, the results suggest that

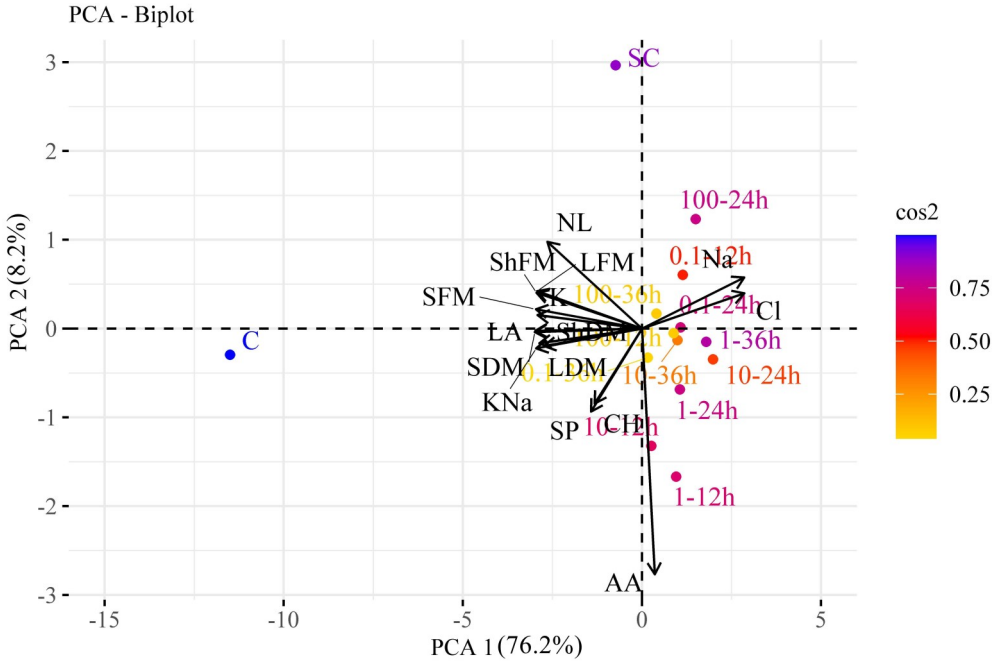
excess Na^+ in the root environment affected K^+ versus Na^+ selectivity in collard green plants.

In this context, our results indicate disturbances induced by NaCl in the ionic homeostasis of collard greens, given that the K^+/Na^+ ratio (in mmol g^{-1}) in all saline treatments averaged 0.126. Moreover, the K^+/Na^+ ratio can be used as a nutritional indicator of salt stress for Georgia collard greens, as also recommended by Šamec, Linić and Salopek-Sondi (2021) for different species of the Brassicaceae family, even before visible damage appears in plants.

Considering the biomass and organic and inorganic solute results together, they contrast with those reported by other authors (SILVA et al., 2020; SILVA et al., 2023c in different crops, indicating that H_2O_2 pre-treatment did not mitigate the deleterious effects of Na^+ and Cl^- ions on growth and ionic homeostasis in collard greens. Studies using H_2O_2 as a seed pre-treatment in sunflower, at doses of 10 e 100 mM H_2O_2 (12 h), 1 mM H_2O_2 (24 h), and 0.1 mM H_2O_2 (36 h), were effective in mitigating the negative effects of salinity, promoting better osmotic balance and enhancing plant growth (SILVA et al., 2020). However, the exposure time and H_2O_2 concentration affected Na^+ contents but did not significantly alter Cl^- and K^+ ion accumulation or the K^+/Na^+ ratio in collard greens plants.

Principal Component Analysis (PCA) was applied after confirming that the variables under study exhibited significant correlations (Figure 5). The first and second principal components explain 84.4% of the total data variation, allowing a comprehensive analysis of the multiple characteristics associated with the different treatments. It is observed that the non-saline control (C) is positioned in the opposite direction of the other treatments with H_2O_2, regardless of concentration and exposure time. This indicates that the pretreatment with hydrogen peroxide in the nutrient solution did not alleviate the deleterious effects of salinity.

The control treatment (C) was positioned separately, distant from the cluster formed by the other treatments, demonstrating its strong influence on the biometric variables analyzed and, on the K^+/Na^+ ratio, as expected. Among the organic solutes, the PCA showed that the presence of salt begins to modulate the variables soluble proteins (SP) and soluble carbohydrates (CH), bringing them closer to the general behavior of the saline treatments. Free amino acids (AA) can be used as possible biochemical indicators of salinity tolerance in collard greens plants under the experimental conditions used. Regarding AA contents, it is observed that the treatments 1 µM-12 h and 10 µM-12 h were the most correlated with increases in this variable, as also observed in Figure 3. For inorganic solutes, salinity correlated positively with Na^+ and Cl^- concentrations, reinforcing their role in ionic stress in plants. Thus, the PCA results suggest that pretreatment with H_2O_2 applied in the nutrient solution may be a promising strategy to mitigate the adverse effects of salinity by promoting biochemical and ionic adjustments as tolerance mechanisms. Additionally, the treatments provide biochemical indicators useful for monitoring plants grown under salt stress conditions.



NL – number of leaves, LA – leaf area (cm^2), LFM – leaf fresh mass (g), SFM – stem fresh mass (g), ShFM – shoot fresh mass (g), LDM – leaf dry mass (g), SDM – stem dry mass (g), ShDM – shoot dry mass (g), CH – soluble carbohydrates (µmol g^{-1} DM), AA – free amino acids (µmol g^{-1} DM), SP – soluble proteins (mg g^{-1} DM), Na^+ – sodium (mmol g^{-1} DM), Cl^- – chloride (mmol g^{-1} DM), K^+ – potassium (mmol g^{-1} DM), and K^+/Na^+ - sodium/potassium ratio. C - Non-saline control (absence of H_2O_2 and absence of NaCl); SC - Saline control (absence of H_2O_2 + 100 mM NaCl); 0.1, 1, 10, and 100 µM refer to the concentrations of H_2O_2; 12, 24, and 36 refer to the exposure times (in hours) to H_2O_2; and 100 mM is the concentration of NaCl in the saline nutrient solution.

Figure 5. Principal component analysis and clustering of the variables analyzed in hydroponically grown collard green plants cultivated for 35 days after treatment in an NFT system, according to the treatments applied.

CONCLUSIONS

Salinity (100 mM NaCl) affects biomass production, increases Na^+ and Cl^- contents, but reduces K^+ content, and the K^+/Na^+ ratio in collard green plants, regardless of the concentration and exposure time to H_2O_2 under hydroponic NFT cultivation. Salt stress decreased the levels of soluble carbohydrates, free amino acids, and soluble proteins in collard greens. However, pre-treatment of seedlings with H_2O_2 increased these organic solutes, especially free amino acids.

Pre-treating collard green seedlings with H_2O_2 in the nutrient solution may be a promising strategy to mitigate the adverse effects of salinity by promoting adjustments in ionic and osmotic homeostasis as mechanisms of salinity tolerance.

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