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Seed priming with hydrogen peroxide enhances tolerance to salt stress of hydroponic lettuce[1]

Condicionamento de sementes com peróxido de hidrogênio melhora a tolerância da alface hidropônica ao estresse salino

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HIGHLIGHTS:
Seed priming with H_2O_2 increase the salt tolerance in lettuce plants.
The H_2O_2 priming improves water status and increase the chlorophylls concentration.
The dose of 0.1 H_2O_2 for 12 hours is recommended to decrease the negative effects of salt stress in lettuce plants.

ABSTRACT: Brackish waters has been increasingly used in hydroponic systems for the cultivation of vegetables. However, its use can cause significant losses in crop production. Therefore, new alternatives to enhance the tolerance of plants to salt stress are being studied, including seed priming with hydrogen peroxide (H_2O_2). Thus, this study aimed to assess the seed priming with H_2O_2 at different periods of exposure for enhancing the production, water status and pigments concentration of crisp lettuce grown under salt stress. The experiment was carried out under protected conditions, in a completely randomized design, with four replicates. The plants were cultivated in a floating hydroponic system, containing nutrient solution. Five treatments were tested: control (absence of H_2O_2 and absence of NaCl); salt control (absence of H_2O_2 and presence of 100 mM NaCl); 0.1 mM H_2O_2 (12 hours) + 100 mM NaCl; 0.1 mM H_2O_2 (24 hours) + 100 mM NaCl, and 0.1 mM H_2O_2 (36 hours) + 100 mM NaCl. In general, salinity reduced the height, production of the fresh and dry mass of the shoot, relative water content, and chlorophylls concentration of lettuce plants. However, the application of 0.1 mM H_2O_2 for 12 and 36 hours on the seeds, enhanced the growth, water status, and chlorophylls concentration of the plants. Seed priming with H_2O_2 at a 0.1 mM concentration for 12 hours can be recommended to increase tolerance of lettuce plants grown in a hydroponic system under salt stress.

Key words: *Lactuca sativa* L., H_2O_2, salinity, growth, hydroponics

RESUMO: As águas salobras vêm sendo cada vez mais utilizadas em sistemas hidropônicos para o cultivo de hortaliças. Entretanto, seu uso pode provocar prejuízos significativos na produção das culturas. Assim, este trabalho teve como objetivo avaliar o condicionamento fisiológico de sementes com H_2O_2 em diferentes períodos de exposição para melhorar a produção, estado hídrico e teor de pigmentos de alface crespa cultivada sob estresse salino. O experimento foi conduzido em condições protegidas, em delineamento inteiramente casualizado, com quatro repetições. As plantas foram cultivadas em sistema hidropônico do tipo floating, contendo solução nutritiva de Furlani. Foram testados cinco tratamentos: controle (ausência de H_2O_2 e ausência de NaCl); controle salino (ausência de H_2O_2 e presença de 100 mM NaCl); 0,1 mM H_2O_2 (12 horas) + 100 mM NaCl; 0,1 mM H_2O_2 (24 horas) + 100 mM NaCl e 0,1 mM H_2O_2 (36 horas) + 100 mM NaCl. Em geral, a salinidade reduziu a altura, a produção de massa fresca e seca da parte aérea, o teor relativo de água e o teor de clorofilas das plantas de alface. No entanto, a aplicação de 0,1 mM de H_2O_2 por 12 e 36 horas na semente melhorou o crescimento, o estado hídrico e o teor de clorofila das plantas. O condicionamento de sementes com H_2O_2 na concentração de 0,1 mM por 12 horas pode ser recomendado para aumentar a tolerância de plantas de alface cultivadas em sistema hidropônico sob estresse salino.

Palavras-chave: *Lactuca sativa* L., H_2O_2, salinidade, crescimento, hidroponia

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Introduction

The production of leafy vegetables is one of the main sources of income for small farmers in the Northeast region of Brazil. Among the leafy greens, lettuce is the main cultivated plant. However, in conventional cultivation, several limitations related to environmental conditions have been reducing crop production in these locations. One of the main limitations is the scarcity of freshwater, since in these areas the source of water available for agriculture is usually brackish water (Lacerda et al., 2021; Tavares Filho et al., 2022).

Hydroponic cultivation has been a widely recommended technique for increasing water use efficiency (Verdoliva et al., 2021). In addition, the use of brackish or saline water in hydroponics has shown positive results when compared to conventional soil cultivation (Verdoliva et al., 2021).

In agricultural crops, salinity reduces water absorption capacity, negatively affecting water status and significantly impairing crop growth (Silva et al., 2020). Additionally, several studies have also reported a decrease in the concentration of chlorophylls a and b (Chl a and Chl b) in plants under saline conditions (Silva et al., 2022). Chlorophylls are pigments that play a key role in light harvesting during the photosynthesis process (Gong et al., 2018). On the other hand, carotenoids are isoprenoids that, in addition to helping to capture light, are also directly related to the photoprotection mechanism in plants under stress conditions (Swapnil et al., 2021).

In recent years, several studies have been carried out in the search for viable alternatives that act directly on enhancing the tolerance of crops to salinity. Among these alternatives is the use of chemical priming, for instance with hydrogen peroxide (H_2O_2) (Khan et al., 2018). H_2O_2 can act as a signaling molecule by triggering various metabolic reactions and increasing the ability of plants to withstand hostile conditions, such as salt stress and others (Khan et al., 2018).

However, as H_2O_2 is considered a reactive oxygen species (ROS), its inappropriate use can cause negative effects and damage to cell membranes (Ransy et al., 2020). Thus, several studies have been carried out to identify the best way to apply H_2O_2 priming in plants.

Considering the need to enhance the tolerance of plants to salt stress, this study aimed to assess the seed priming with H_2O_2 at different times of exposure for enhancing the tolerance of lettuce plants to salt stress.

Material and Methods

The experiment was conducted in a greenhouse with ceiling height of 3 m, a width of 7 m and length of 28 m, in the experimental area of the Universidade Federal do Recôncavo da Bahia (UFRB), in the municipality of Cruz das Almas, Bahia, Brazil (12º 40' 19" S, 39º 6' 23" W), mean altitude of 220 m. The climate is classified as tropical hot and humid (Af) according to Köppen's classification (Alvares et al., 2013). During the experimental period, the averages of minimum temperature, maximum temperature, and relative humidity were 21.0, 31.1 °C, and 87.4%, respectively.

The experiment was carried out in a completely randomized design with four replicates, with a total of five treatments. Pelleted lettuce seeds ('Jade') were germinated in phenolic foam under dark conditions at 25 °C. Seed priming with H_2O_2 was established by soaking the seeds with 0.1 mM of H_2O_2 and keeping them under these conditions for different periods of exposure (12, 24, and 36 hours). The treatments with 0.1 mM of H_2O_2 for 12 and 24 hours were subjected to washing with water to eliminate H_2O_2 and were kept under this condition until completing the total period of 36 hours. Two seed lots irrigated only with water for 36 hours were also included, forming the treatments: Control (absence of H_2O_2 and absence of NaCl) and salt control (absence of H_2O_2 and presence of 100 mM NaCl). The doses of NaCl and H_2O_2 were selected based on the reduction of fresh and dry mass reported in previous studies (Silva et al. 2019a,b).

After soaking the seeds (36 hours), the seedlings were transferred to a greenhouse, where they remained for 14 days, irrigated with a nutrient solution (Furlani, 1999) at half-strength + 100 mM NaCl, except in the control treatment. Subsequently, the seedlings were transferred to polyethylene pots with 15 L capacity, and cultivated in a Deep Water Culture (DWC) system, containing complete nutrient solution + 100 mM NaCl, except for the control treatment plants (absence of NaCl). The plants remained under these conditions for 20 days, when data and the plant material were collected. The pH values of the nutrient solution oscillated within the range recommended for hydroponic cultivation, 5.5 to 6.5. The aeration system consisted of an air compressor with a flow rate of 18,000 L h^{-1} (Resun, GF-180), activated by an analog timer every 3 hours and kept on for a period of 0.25 h.

Fresh samples of the youngest and fully expanded pair of leaves were collected for the evaluation of variables related to leaf water status and pigment concentration using 10 discs of 0.80 cm in diameter for each evaluation.

The relative water content (RWC), water saturation deficit (WSD), leaf succulence (SUC), and sclerophylly index (SI) were determined according to Silva et al. (2020). Leaf discs were sampled and immediately weighed to obtain the fresh mass (FM). Next, the discs were immersed in Petri dishes containing distilled water, for 24 hours at 25 °C, in the dark, to determine the turgid mass (TM). Finally, the leaf discs were dried in an oven at 75 °C for 48 hours to determine the dry mass (DM). RWC, WSD, SUC, and SI were calculated using the following equations:

$$RWC(\%) = \frac{(FM - DM)}{(TM - DM)} \times 100$$

$$WSD(\%) = \frac{(TM - FM)}{(TM - DM)} \times 100$$

$$SUC(\text{mg } H_2O \text{ cm}^{-2}) = \frac{(FM - DM)}{LA}$$

$$SI(\text{mg cm}^{-2}) = \frac{DM}{LA}$$

where: LA - leaf area of the 10 leaf discs (0.80 cm diameter).

Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) concentrations were determined by spectrophotometry at 664.1, 648.6, and 470 nm, in 95% ethanol extract, according to the methodology described by Lichtenthaler & Buschmann (2001), using the following equations:

$$\text{Chl a}\left(\mu g\ mL^{-1}\right) = 13.36 \times A_{664.1} - 5.19 \times A_{648.6}$$

$$\text{Chl b}\left(\mu g\ mL^{-1}\right) = 27.43 \times A_{648.6} - 8.12 \times A_{664.1}$$

$$\text{Car}\left(\mu g\ mL^{-1}\right) = \frac{\left(1000 \times A_{470} - 2.13 \times \text{Chl a} - 97.64 \times \text{Chl b}\right)}{209}$$

From the data of Chl a and Chl b, the concentrations of Chl a + b and Chl a/Chl b and (Chl a + Chl b)/Car ratios were calculated.

At the end of the experiment, 35 days after sowing (DAS), the plant height (cm) was measured using a graduated ruler, and then the plants were harvested to determine the shoot fresh mass (ShFM). Then, the material was dried in an oven at 65 °C for 72 hours (until a constant mass was obtained), and then the plants were weighed on a precision (0.001 g) balance to quantify the shoot dry mass (ShDM).

The data were first tested for normality (Shapiro-Wilk test) and then subjected to analysis of variance (ANOVA), using the F test ($p \leq 0.05$). The means were compared by Tukey's test ($p \leq 0.05$), using the statistical software Sisvar (Ferreira, 2019).

Results and Discussion

Significant effect ($p \leq 0.01$) of treatments was observed for all variables analyzed (Table 1). Salinity negatively affected plant height, shoot fresh mass, and shoot dry mass variables. However, in general, seed priming with H_2O_2 for 12 and 36 hours minimized the harmful effects caused by salinity (Figure 1).

Table 1. Summary of analysis of variance for the variables analyzed in lettuce plants treated with H_2O_2 at different periods of exposure and under salt stress

Variables	P-value	CV (%)
Height (cm)	**	11.62
ShFM (g per plant)	**	13.29
ShDM (g per plant)	**	14.84
RWC (%)	**	3.38
WSD (%)	**	10.08
SI (mg DM cm^{-2})	**	7.76
SUC (mg H_2O cm^{-2})	**	3.82
Chl a (mg g^{-1} DM)	**	7.67
Chl b (mg g^{-1} DM)	**	12.96
Chl a + Chl b (mg g^{-1} DM)	**	6.82
Car (mg g^{-1} DM)	**	10.48
Chl a / Chl b ratio	**	7.70
(Chl a +Chl b) / Car ratio	**	16.02

** - Significant at $p \leq 0,01$ by F test; Height - Height of plants; ShFM - Shoot fresh mass; ShDM - Shoot dry mass; RWC - Relative water content; WSD - Water saturation deficit; SI - Sclerophylly index; SUC - Leaf succulence; Chl a - Chlorophyll a concentration; Chl b - Chlorophyll b concentration; Chl a + Chl b - Chlorophylls a + b concentration; Car - Carotenoids concentration; Chl a / Chl b Ratio - Chlorophyll a / chlorophyll b ratio and (Chl a + Chl b) / Car ratio - Chlorophylls a + b concentration/ carotenoids ratio

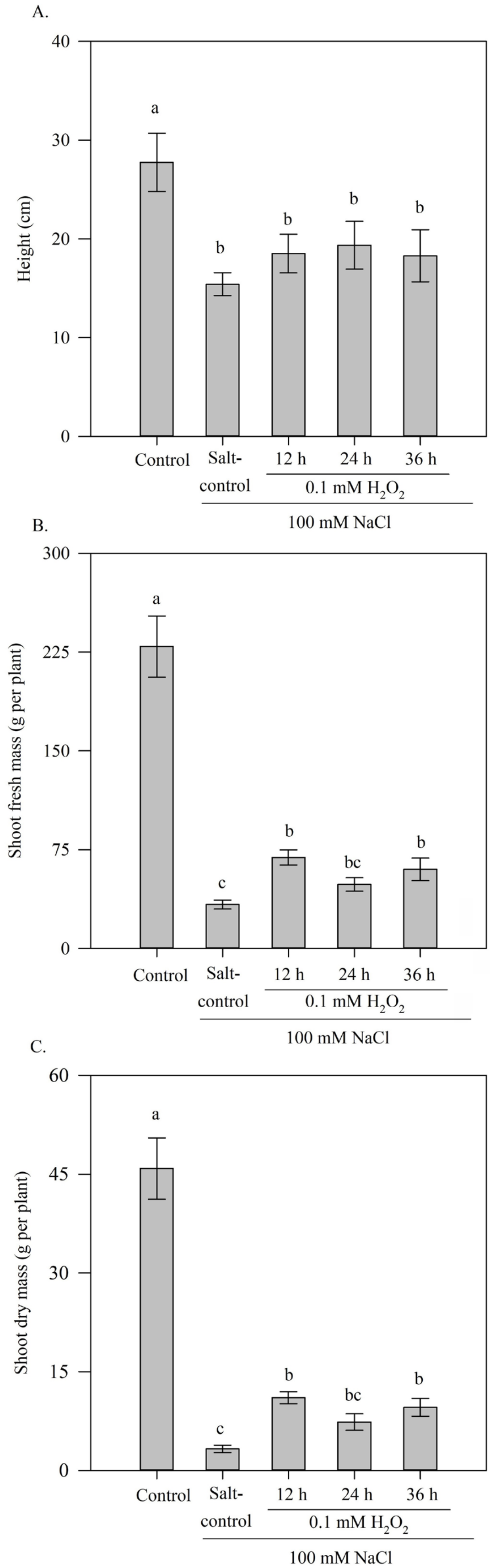

Figure 1. Effect of salt stress and different period of exposure to seed priming with H_2O_2 on height (A), shoot fresh mass (B), and shoot dry mass (C) in lettuce plants grown in a hydroponic system. Treatments: Control (absence of H_2O_2 and absence of NaCl) and Salt control (absence of H_2O_2 and presence of 100 mM NaCl), and 0.1 mM of H_2O_2 for 12, 24, and 36 hours in presence of 100 mM NaCl

The salt control treatment showed decreases in height, ShFM, and ShDM on order of 44, 85, and 93%, respectively, compared to the control treatment (Figure 1). The evaluation of plant height showed that the treatments primed with H_2O_2 (regardless of the period of exposure) showed no difference compared to the salt control treatment. However, seed priming with H_2O_2 for 12 and 36 hours increased ShFM and ShDM by an average of 94 and 215%, respectively, compared to the salt control treatment, whereas for the plant height no significant differences were observed in plants under salt control and pretreatment of seeds with H_2O_2 (Figures 1A and B). Several studies show that under salt stress conditions, plants have a strong reduction in growth and production (Shin et al., 2020; Santos et al., 2021). However, some studies show that seed priming with H_2O_2 can attenuate the negative effects caused by salinity, significantly increasing the tolerance of plants to salt stress (Silva et al., 2019a; Araújo et al., 2021; Silva et al., 2022).

The salt control treatment showed a 23% reduction in

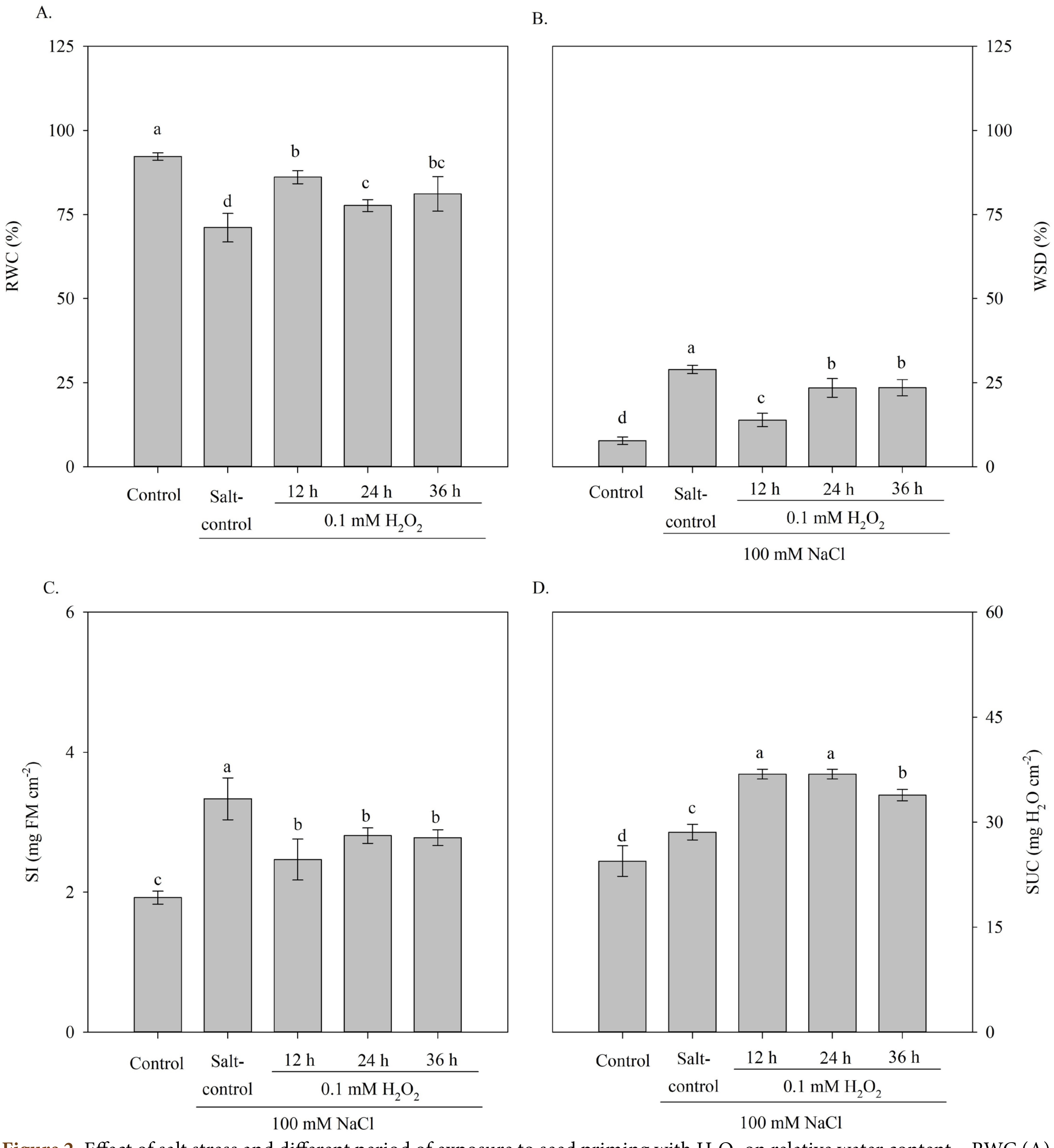

Figure 2. Effect of salt stress and different period of exposure to seed priming with H_2O_2 on relative water content – RWC (A), water saturation deficit – WSD (B), sclerophylly index – SI (C), and leaf succulence – SUC (D) in lettuce plants grown in a hydroponic system. Treatments: Control (absence of H_2O_2 and absence of NaCl) and Salt control (absence of H_2O_2 and presence of 100 mM NaCl), and 0.1 mM of H_2O_2 for 12, 24, and 36 hours in presence of 100 mM NaCl

RWC compared to the control treatment (Figure 2A). On the other hand, the priming of seeds with H_2O_2 increased the RWC, when compared to the salt control treatment, especially when seeds were primed with H_2O_2 for 12 hours, representing an increase of 21% compared to the salt control treatment (Figure 2A).

The salinity significantly altered the water status of the plants, and WSD in the salt control treatment was 274% higher compared to the control treatment (Figure 2B), whereas in the treatment with the application of H_2O_2 for 12 hours, these values were about 52% lower compared to the salt control treatment (Figure 2B). The water status of leaves is an important factor for the maintenance of several processes related to plant metabolism. Decreased water content in the leaf can lead to a decrease in osmotic potential and cell pressure, causing turgor-induced stomatal closure, consequently reducing CO_2 absorption and photosynthesis (Buckley, 2019; Ratzmann et al., 2019).

For SI, the salt control treatment showed values 73% higher than the control treatment, while, even under saline conditions, the treatments consisting of seeds primed with H_2O_2 showed average values 19% lower when compared to the salt control treatment but significantly higher than the control plants (Figure 2C). The SUC values in the salt control treatment were about 17% higher compared to the control treatment. However, in plants primed with H_2O_2, this increase was even more expressive, with average values of 47 and 26% compared to the control and salt control treatments, respectively (Figure 2D).

The evaluation of SI and SUC in plants under stress is related to a mechanism of increase in leaf thickness, with the objective of allocating and maintaining the water level in the plant tissue (Mantovani, 1999). Cova et al. (2016) also reported an increase in SUC and SI in noni plants under salt stress conditions. However, in our study, the increase in SUC in plants primed with H_2O_2 may have occurred as an acclimation mechanism to maintain the water supply and dilute the toxic ions present in the leaf tissue (Silva et al., 2019a).

When optimally applied (concentration and exposure time), H_2O_2 acts as a metabolic signal inducing an increase in the expression of genes related to antioxidant activity, consuming excess ROS, and consequently decreasing the negative effects caused by salt (Khan et al., 2018; Silva et al., 2020; Silva et al., 2021).

Compared to the control treatment, the H_2O_2 primed treatments increased Chl a, Chl b, and Chl a + b concentrations by 28, 69, and 40% (0.1 mM H_2O_2 12 hours + 100 mM NaCl), 23, 45, and 23% (0.1 mM H_2O_2 24 hours + 100 mM NaCl), and 37, 122 and 53% (0.1 mM H_2O_2 36 hours + 100 mM NaCl) (Figures 3A, B, and C).

However, when compared with the salt control treatment, these increases were even more significant: 30, 221, and 61% (0.1 mM H_2O_2 12 hours + 100 mM NaCl), 24, 175 and 42% (0.1 mM H_2O_2 24 hours + 100 mM NaCl), and 38, 321 and 76% (0.1 mM H_2O_2 36 hours + 100 mM NaCl), respectively (Figures 3A, B, and C). Significant increase in chlorophyll concentration in plants primed with H_2O_2 has already been observed in our previous studies (Silva et al., 2020; Silva et al., 2022). This increase can be, at the least in part, explained by role of H_2O_2 priming in protecting the chloroplast ultrastructure under stress conditions and, consequently, improving the efficiency of photosynthetic machinery, which may explain the increased tolerance of plants even under salt stress conditions (Araújo et al., 2021).

In general, carotenoid concentration had higher values in treatments under salt stress, but this increase was less expressive in plants primed with H_2O_2. The salt control treatment had an increase of about 188% in carotenoid concentration compared to the control treatment (Figure 3D). On the other hand, even under saline conditions, the treatments primed with H_2O_2 (12, 24 and 36 hours) showed, respectively, values around 52, 31, and 42% lower when compared to the salt control treatment (Figure 3D). The increase in carotenoid concentration occurs as a photoprotection mechanism of plants under stress conditions. Carotenoids act in the process of energy dissipation in the form of heat, reducing the deleterious effects of salinity on plants (Maslova et al., 2021). Taken together, the results of the shoot dry mass and carotenoid concentration suggest that plants primed with H_2O_2 were less impaired by salinity.

The salt control treatment had a Chl a/Chl b ratio about 89% higher when compared to the control treatment. However, seed priming treatments with H_2O_2 for 12, 24 and 36 hours caused reductions of 59, 32, and 59%, respectively, compared to the salt control treatment (Figure 3E). In contrast, the (Chl a + Chl b)/Car ratio was about 70% lower in the salt control treatment compared to the plants under the control treatment (Figure 3F). On the other hand, plants primed with H_2O_2 (12, 24 and 36 hours) showed, respectively, values of Chl a + Chl b/Car ratio around 242, 144, and 185% higher compared to the salt control treatment, especially for the treatment with H_2O_2 for 12 hours, which had values similar to those of the control treatment, followed by the treatment with H_2O_2 for 36 hours (Figure 3F). The significant increase in the Chl a/Chl b ratio observed in the salt control treatment was basically due to the strong decrease observed in the Chl b concentration. Katayama & Shida (1970) state that, in general, the equilibrium of the Chl a/Chl b ratio is approximately 3:1, and large changes can directly affect the photosynthetic capacity, since Chl a is present in the reaction center of photosystems, while Chl b is present only in the proteins of the light-harvesting complexes (Terashima & Hikosaka, 1995). Some authors associate the signaling role of H_2O_2 with a protective function related to the maintenance of the chloroplast ultrastructure. Araújo et al. (2021) reported that it improves the efficiency of the photosynthetic machinery of maize leaves under salt stress due to the protective role of H_2O_2 in the chloroplast ultrastructure.

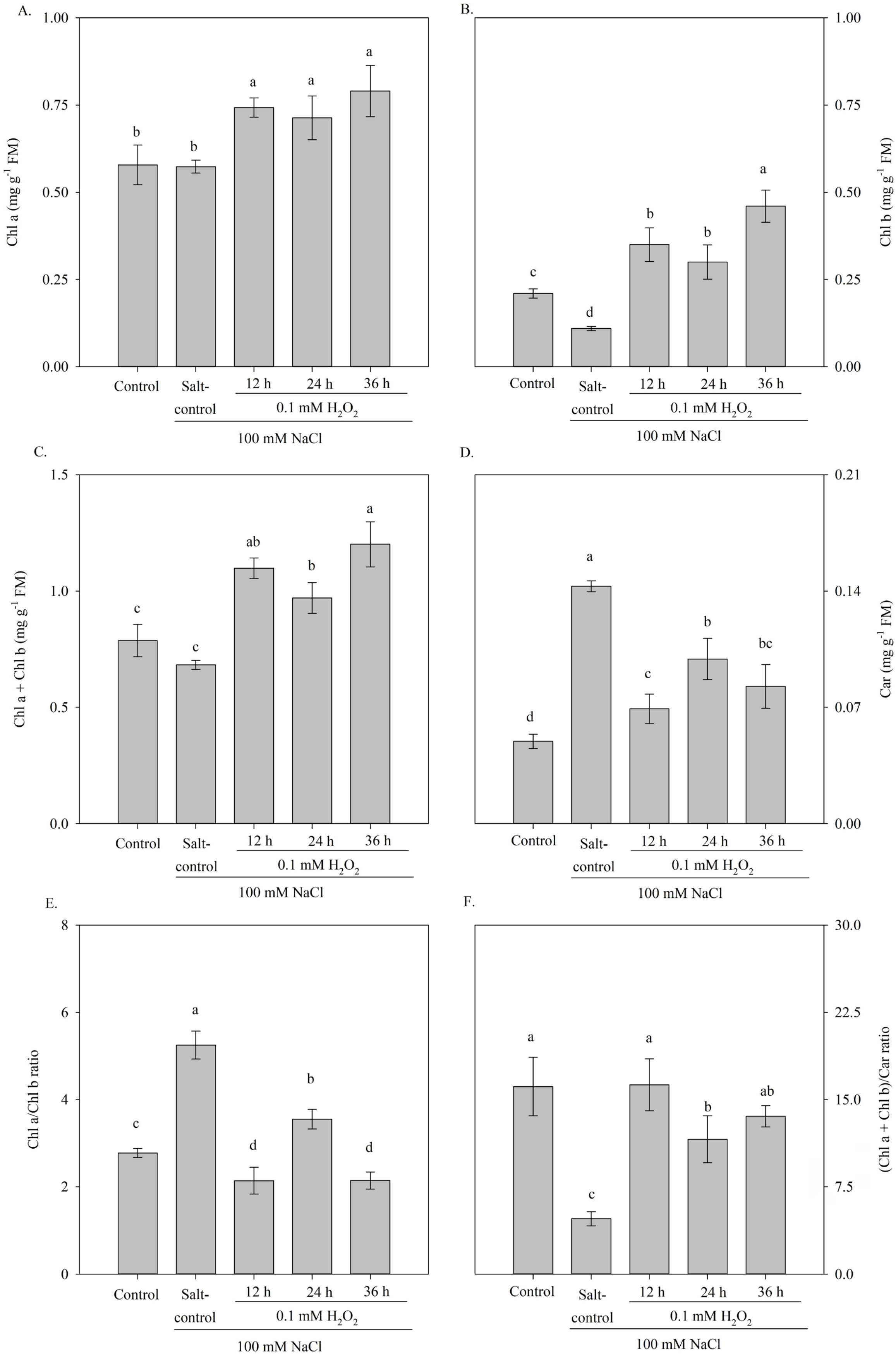

Figure 3. Effect of salt stress and different period of exposure to seed priming with H_2O_2 on chlorophyll a concentration – Chl a (A), chlorophyll b concentration – Chl b (B), chlorophylls a + b concentration – Chl a + Chl b (C), carotenoids concentration – Car (D), chlorophyll a / chlorophyll b ratio – Chl a / Chl b ratio (E), and chlorophylls a + b concentration/carotenoids ratio – (Chl a + Chl b) / Car ratio (F) in lettuce plants grown in a hydroponic system. Treatments: Control (absence of H_2O_2 and absence of NaCl) and Salt control (absence of H_2O_2 and presence of 100 mM NaCl), and 0.1 mM of H_2O_2 for 12, 24, and 36 hours in presence of 100 mM NaCl

Conclusions

1. In general, salinity reduced the height, fresh and dry mass of the shoot, relative water content, and chlorophylls concentration of lettuce plants. However, the application of 0.1 mM H_2O_2 for 12 and 36 hours on the seeds enhanced the growth, water status, and chlorophylls concentration of the plants.

2. Seed priming with H_2O_2 at 0.1 mM concentration for 12 hours can be recommended to increase tolerance of lettuce plants grown in a hydroponic system under salt stress.

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