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RESEARCH ARTICLE

ALLEVIATING SALT STRESS IN TOMATO PLANTS THROUGH HYDROGEN PEROXIDE PRIMING: DIFFERENTIATIONS OF ANTIOXIDANT ENZYME ACTIVITIES AND GENE EXPRESSION PATTERNS

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Abstract

Plants, being sessile organisms, rely on their antioxidant systems to respond to the stress factors induced by both abiotic and biotic stresses in their environment. Among abiotic stress factors, salinity and alkalinity pose the most significant challenges to plant growth. To counteract these stresses, plants activate various signalling pathways to enhance their stress tolerance. While a wide range of pesticides, including insecticides and herbicides, are employed to protect agricultural crops from biotic agents, there exists no established practice for fortifying their defence mechanisms against abiotic stresses. This study delves into the effect of H_2O_2 pre-treatment on mitigating salt stress in tomato seedlings. Four experimental groups were established: control, H_2O_2, Salt, and Salt+H_2O_2. The study evaluated changes in chlorophyll content, malondialdehyde (MDA) accumulation, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzyme activities and expressions. The results revealed that priming treatment led to increased chlorophyll levels and reduced MDA accumulation compared to the group only exposed to salt stress. Additionally, the activation of stress-related enzymes was significantly higher in the priming group compared to the group only exposed to salt stress. Expression levels exhibited a statistically significant increase compared to the control group; however, CAT and APX expression levels were found to be lower than those in the the group only exposed to salt stress. These findings suggest that H_2O_2 priming can enhance plant stress tolerance. Priming can serve as a highly effective tool to alleviate stress in plants; however, the type, concentration, and exposure time of the priming agent are crucial factors in regulating the priming effect.

Keywords

Abiotic Stress,
Hydrogen Peroxide Priming,
Antioxidant Enzymes, Stress Tolerance,
Chlorophyll Content

Time Scale of Article

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1. INTRODUCTION

Within the realm of agricultural production, the arsenal of preventative and lethal chemical agents employed for combating biotic stressors has achieved an advanced stage of development. Nevertheless, recent years have borne witness to an escalated research focus on enhancing plant resilience to abiotic stressors, encompassing conditions such as drought, heat, cold, and salinity [1, 2]. In this context, the

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concept of priming has emerged as a more intricate and efficacious modality for fortifying plant response mechanisms vis-à-vis environmental challenges, transcending traditional paradigms. Priming, particularly when executed through the application of diverse compounds during the seed or seedling stages, elicits a controlled and mild stress response within the plant. This subtle stress imprint, when subsequently confronted with both biotic and abiotic adversities, engenders a heightened state of tolerance and adaptability [3]. The ensuing adaptive responses encompass an augmentation of the plant's antioxidant capacity, fine-tuning of enzymatic antioxidant expression levels, regulation of cellular turgor, synthesis of osmoprotectants, and the meticulous control of stomatal dynamics [4, 5]. It is noteworthy that various priming methodologies, including hydropriming, osmopriming, hormone-priming, and redox-priming, engender analogous mechanisms, which are effectively harnessed in combatting an array of distinct stressors encountered by the plant [6].

In sum, the contemporary scientific exploration into priming strategies has unveiled a promising avenue for the augmentation of plant stress resilience, showcasing the potential to revolutionize the agricultural landscape by equipping crops with a heightened capacity to navigate the multifarious challenges posed by abiotic stress conditions.

The uncontrolled drilling of wells for irrigation purposes depletes underground freshwater resources, increases the salt content of irrigation water, and indirectly causes plants to be exposed to excessive salt. In later stages, it causes a loss of productive agricultural land due to excessive soil salinization. In addition, irregularities in rainfall regimes due to global climate change are another important factor affecting soil salinization. Because many economically critical agricultural plants do not have salinity tolerance, plant growth and crop yield are also negatively affected [7].

In the context of plants subjected to various abiotic stressors, encompassing salinity, drought, cold, heat, and heavy metal exposure, a conspicuous escalation in the levels of reactive oxygen species (ROS) becomes a pivotal event, precipitating redox disequilibrium and oxidative stress. The deleterious consequences of elevated ROS concentrations are profound, inducing extensive damage to essential cellular components, including proteins, DNA, and lipids, thereby instigating a cascade of events leading to metabolic dysfunction and, ultimately, the demise of the plant organism [8].

To address these challenges, extensive research has been conducted to elucidate plant responses to abiotic stressors, with a particular focus on alterations in the activities of antioxidant enzymes. Notably, Duman and Öztürk (2010) subjected *Mentha aquatica* to varying concentrations and durations of nickel exposure, observing a pronounced enhancement in the activities of crucial antioxidant enzymes, including SOD, CAT, and APX [9]. Furthermore, Soydam-Aydın and colleagues (2015) conducted experiments involving the imposition of copper and zinc stress on tomato and aubergine plants, revealing a significant upregulation in the expression levels of CAT and APX enzymes, further underscoring the pivotal role of antioxidant enzymes in the plant's response to abiotic stressors [10].

Over the course of several years, reactive oxygen species (ROS) have been traditionally perceived as deleterious byproducts of cellular metabolism. However, contemporary perspectives on these molecular entities have undergone a profound shift, wherein ROS are now recognized as pivotal molecules that facilitate plant adaptation to diverse environmental conditions. Among the ROS, the " H_2O_2" radical has garnered substantial scientific attention due to its distinctive attributes, including reduced intrinsic toxicity in comparison to other oxygen radicals, facile cellular membrane permeability on account of its molecular dimensions, and an extended half-life of approximately 1 millisecond [11, 12]. The multifaceted roles of H_2O_2 are manifold, spanning participation in protein, carbohydrate, and lipid metabolism, mediation of signal transduction, orchestration of transcriptional regulation for responsive genes, and central involvement in various other metabolic activities, thereby underscoring the complexity of its functional significance [13].

H_2O_2 priming is capable of engendering a controlled oxidative stress response, thereby activating a redox-dependent signalling network. This stress-induced priming effect culminates in the production of latent defensive proteins, including enzymes and transcription factors, which serve to quench reactive oxygen species, consequently catalysing an amplified stress response in the primed plant [14].

Notably, salt stress instigates oxidative bursts within plants, and the application of low concentrations of H_2O_2 as a priming agent has been demonstrated to augment salt tolerance by serving as a signalling molecule capable of governing the expression of stress-responsive genes. Wahid et al. (2007) conducted a study that revealed wheat seedlings derived from seeds subjected to H_2O_2 priming and subsequently exposed to salt stress exhibited significantly lower levels of H_2O_2 production compared to seedlings from non-primed seeds exposed to salt stress [15]. Furthermore, Ashraf and colleagues (2015) investigated the advantageous effects of exogenous H_2O_2 on drought stress tolerance in maize. Maize seedlings were pretreated with varying concentrations of H_2O_2 and subsequently cultivated under drought stress conditions, wherein seeds primed with 140 mM H_2O_2 exhibited heightened germination percentages. Drought-induced alterations included a marked decrease in photosynthetic pigments, accompanied by elevated levels of endogenous H_2O_2, lipid peroxidation, as well as augmented activities of CAT, SOD, and peroxidase (POX), underscoring the role of H_2O_2 in mitigating the consequences of drought stress [16].

The principal aim of the present study is to delineate alterations in total chlorophyll content, MDA accumulation, as well as the activities of antioxidant enzymes SOD, CAT, and APX, alongside the examination of gene expression levels in tomato seedling leaves primed with H_2O_2 and subsequently subjected to salt stress. This inquiry endeavours to elucidate the role of H_2O_2 priming in bolstering abiotic stress tolerance, contributing to our comprehension of the intricate mechanisms underpinning plant resilience in challenging environmental conditions.

2. MATERIALS AND METHODS

2.1. Plant Material and Experimental Conditions

The experimental study was conducted within the growth chamber of the Stress Biology Laboratory at Nevsehir Haci Bektas Veli University, Department of Molecular Biology and Genetics. The controlled environmental conditions encompassed a temperature of 28°C, relative humidity maintained at 58%, and a photoperiod of 16 hours of light followed by 8 hours of darkness (16/8 h).

Tomato seeds underwent sterilization through immersion in a 0.7% sodium hypochlorite solution, followed by germination in a perlite-soil mixture. Germinated seedlings were subsequently transplanted into a hydroponic medium comprised of Hoagland's solution and were continuously aerated to ensure optimal growth conditions.

After eight days in hydroponic medium, seedlings were subjected to two distinct treatments: either distilled water (control) or a 10 mM H_2O_2 solution containing 0.025% Tween 20 detergent (to reduce surface tension and facilitate penetration). Application was administered via foliar spraying, with each plant receiving 15 ml of either H_2O_2 or distilled water. The initial treatment was conducted at 6:30 a.m, followed by a second application after 24 hours. Subsequently, after 48 hours from the initial spraying, the seedlings were subjected to salt stress, administered as an 80 mM NaCl treatment. To mitigate the risk of osmotic shock, the salt concentration was incrementally increased by 40 mM per day.

The experimental treatments were categorized as follows: Distilled water spray without salt stress (water/control), H_2O_2 solution spray without salt stress (H_2O_2/control), Distilled water spray under salt stress (water/salt-stressed) H_2O_2 solution spraying with salt stress (H_2O_2/salt-stressed).

To evaluate the effects of these treatments, samples were systematically collected at three distinct time points: 24 hours, 72 hours, and 120 hours following the induction of saline stress. Collected samples were promptly stored in a refrigeration unit at -80°C to preserve their integrity until further analysis. All measurements and analyses were performed with a minimum of three biological replicates to ensure robust statistical assessment.

2.2. Determination of Total Chlorophyll and Lipid Peroxidation

Plant samples (0.1 g) were crushed in 100 ml of 80% acetone. The filtrate was passed through filter paper and the total chlorotic content was calculated according to Witham et al. [17].
mg kl.a/g tissue= [12.7 (D630)-2.69 (D450)]. (V/1000.A)

MDA was measured using thiobarbituric acid reactive substances (TBARS), according to the method described by Heath and Packer [18]. The concentrations of lipid peroxides were calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and were expressed as nmol TBARS g^{-1} protein.

2.3. Measurements Antioxidant Enzyme Activities

Enzyme activities were measured according to Chen and Zhang [19].

2.4. Crude Protein/Enzyme Extract

0.5 g of tomato seedling leaves were pulverized with liquid nitrogen in a mortar and pestle. Then 100 mM 6 ml PBS buffer was added and homogenised. The homogenate was distributed into four different 1.5 ml centrifuge tubes and centrifuged at 10000 g for 20 min at +4°C.

The protein concentration of the resulting solution is determined by the Warburg-Christian formula: (protein) (mg/ml) = 1,55xA280-0,76xA260. For the reliability of further studies, this value should be below 2. In case of excess, dilution with PBS buffer will be performed. The supernatant is taken and stored in a -80 ^{o}C degree freezer for further analysis.

2.5. Determination of Superoxide Dismutase (SOD) Activity

A reaction solution containing 100 mM PBS (pH 7.8), 1 mM EDTA-2Na, 130 mM Methionine, 750 µM NBT, and 20 µM Riboflovin was prepared. Then 50 µl of crude protein extract was added to each 1 ml of reaction solution. In addition, 2 control tubes with PBS instead of 50 µl of crude enzyme solution are prepared. The samples are to be analysed and one of the control tubes is exposed to 4000 lux light for 10-15 minutes. The other control tube is kept in the dark without exposure to light. At 560 nm, the tube kept in the dark is taken as a reference.

SOD total activity (unit: u/mg protein) = [(Ack-As) x V]/ (0.5 x Ack x Vt)/Cp
ACk absorbance of the control tube kept in the light, as absorbance of the tube with sample, V: total volume of crude protein, Vt. Volume of crude protein used in the test tube, Cp: concentration of crude protein extraction (mg/ml)

2.6. Determination of Catalase (CAT) Activity

Add 77.5 µl of 30% H_2O_2 into 50 ml of 10 mM PBS buffer to obtain the reaction solution. Then 50 µl of crude protein extract was added to the cuvette and 1 ml of reaction solution was added. Measurements at 240 nm are taken every 15 seconds. Instead of the enzyme extract, 50 µl of 100 ml PBS buffer is used as a reference reading.

CAT activity (unit: u/mg protein) = ∆A240 x (V/Vt)/ (0.1 x t)/Cp

∆A240: absorbance change at 240 nm every 15 s, V: total crude protein extraction volume, Vt: volume of crude enzyme used in the test tube, t: reaction time (min), Cp: crude protein concentration (mg/ml).

2.7. Determination of Ascorbate Peroxidase Activity

A reaction mixture containing 50 mM potassium phosphate buffer (pH 7), 0.5 mM ascorbate, 0.5 mM H_2O_2, and 10 µl of crude protein was prepared in a cold medium. The prepared mixture was read at 290 nm wavelength for 3 minutes using a quartz quartet. The extinction coefficient of 2.8 mM^{-1} cm^{-1} for reduced ascorbate will be used to calculate the enzyme activity.

2.8. RNA Isolation cDNA Synthesis and qRt-PCR Analysis

Transgenbiotech EasyPure® plant RNA kit was used for RNA isolation from leaves. The amounts of RNA obtained from all samples were determined by nanodrop. Then, the total RNA amount of the samples to be used in cDNA synthesis was adjusted to 40 ng. EasyScript First strand cDNA synthesis kit was used to synthesize cDNA. Bioneer AccuPower® RT-PCR PreMix was used for real-time reactions. The DNA fragment was amplified for 35 cycles using the following thermal conditions: denaturing the DNA template at 94 °C for 30 s, primer annealing at 5 °C below primer Tm for 15 s, and DNA synthesis at 72 °C for 1 min. The primer sequence was taken from SolGenomik Network. The primer list is given in Table 1. The data obtained from Real-Time PCR were analysed using the $2^{-\Delta\Delta CT}$ method [20]

Table 1. Primer pairs used in real-time PCR

Name	Accession Number	Forward	Reverse
Actin7	Solyc03g078400	GGGATGGAGAAGTTTGGTGGTGG	CTTCGACCAAGGGATGGTGTAGC
Cu/Zn-SOD	Solyc11g066390	TCACCACAACCAGCACTACCA	AGTGACAACCCCCTCAACATTAG
CAT1	Solyc12g094620	CGCATACGACACCCCTTTC	CGGAGAAAATCAGCACAAGTAAG
APX	Solyc06g005160	TCTGAATTGGGATTTGCTGA	CGTCTAACGTAGCTGCCAAA

2.9. Statistical Analysis

One-way ANOVA and post hoc Tukey tests were performed to determine the differences between the groups and the significance level of the difference ($p<0.05$). All calculations were performed with the SPSS 22.0 software package.

3. RESULTS

In this study, tomato plants were divided into four different groups: control (C), H_2O_2 (H), salt (S), and H_2O_2+salt (HS), and collected at various sampling times. The changes in total chlorophyll content, lipid peroxidation, SOD, CAT, APX enzyme activity, and gene expression levels in plant leaves were studied. When the change in total chlorophyll content was analysed, it was found that there was no significant difference between the C and H groups. However, there was a significant difference between the S and HS groups. The amount of chlorophyll in the HS group was higher than that in the S group was (Figure 1. A).

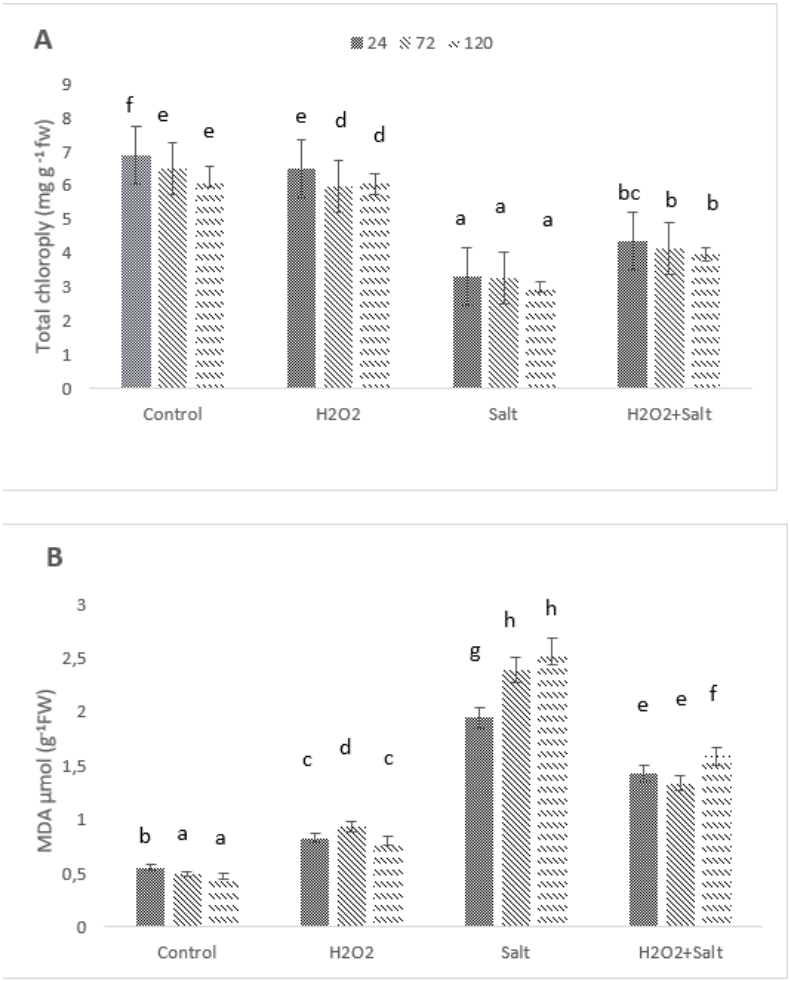

Figure 1. Effect of H_2O_2 pretreatment on total chlorophyll and MDA concentrations in tomato seedlings exposed to salt stress for different periods. (A) Total chlorophyll and (B) MDA levels. Bars indicate a standard error; different letters indicate statistical difference ($p<0.05$)

The first target of stress-induced reactive oxygen species is lipid peroxidation of the cell membrane. MDA produced as a result of peroxidation provides an idea of the stress status of the plant. In this study, the H group was statistically different from the C group, and the highest MDA accumulation occurred in the S group at 72 and 120 h. In addition, MDA accumulation in all HS groups was lower than that in the S group (Figure 1 B).

When antioxidant enzyme activities were examined, upward changes occurred in enzyme activities depending on the application time and group, compared to the control. SOD enzyme activity reached the highest value at 72 h and 120 h of H_2O_2+ salt treatment. Compared to the control, a statistically significant increase was observed in all treatment groups (Figure 2 A).

The highest CAT activity was detected after 120 h of salt stress. CAT activity was found to be significantly higher than the control group in the H_2O_2+Salt treated groups at different time points, but lower than that in the salt-only treated group ($p<0.05$) (Figure 2 B).
When APX enzyme activity was analysed, the highest activity was detected after 120 and 72 h of H_2O_2+ salt treatment. Although the activity was higher than that of the control at the concentration where only salt was applied, it was lower than that of the H_2O_2+salt treatment (Figure 2. C).

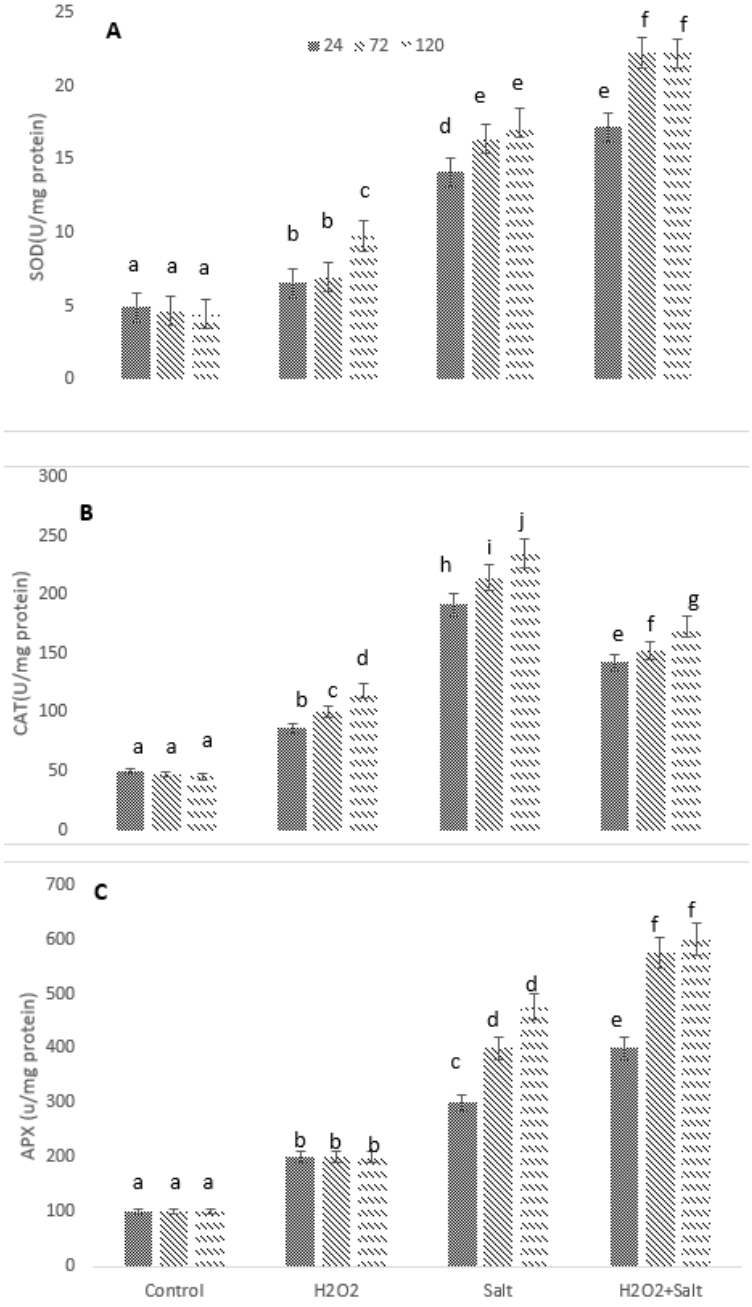

Figure 2. Effect of H_2O_2 pretreatment on antioxidant enzyme activities in tomato seedlings exposed to salt stress for different periods. A. SOD, B. CAT, and C. APX. Bars indicate a standard error; different letters indicate statistical difference ($p<0.05$).

Changes in antioxidant gene expression were evaluated according to the expression of the actin7 gene used as a housekeeping gene.

When SOD expression was analysed, the highest expression level was found at 72 h and 120 h H_2O_2+salt concentrations, which were 4 fold higher than that of the control group. In addition, it was determined that the expression level was statistically different from that of the control group under other application conditions (Figure 3A).

There was a statistically significant increase in CAT gene expression in the group treated with H_2O_2 alone compared to the control. The highest CAT expression was detected after 120 h of salt application, which was 3 fold higher than that in the control. Expression levels in the H_2O_2+salt application group were higher than those in the control and H_2O_2 groups but lower than those in the salt-only group ($p<0.05$) (Figure 3-B).

When APX gene expression was analysed, similar to CAT, the highest expression levels were observed only in the salt treatment group, whereas the expression level in the H_2O_2+salt treatment group was statistically lower than that in the salt treatment group and higher than that in the control group ($P<0.05$) (Figure 3-C).

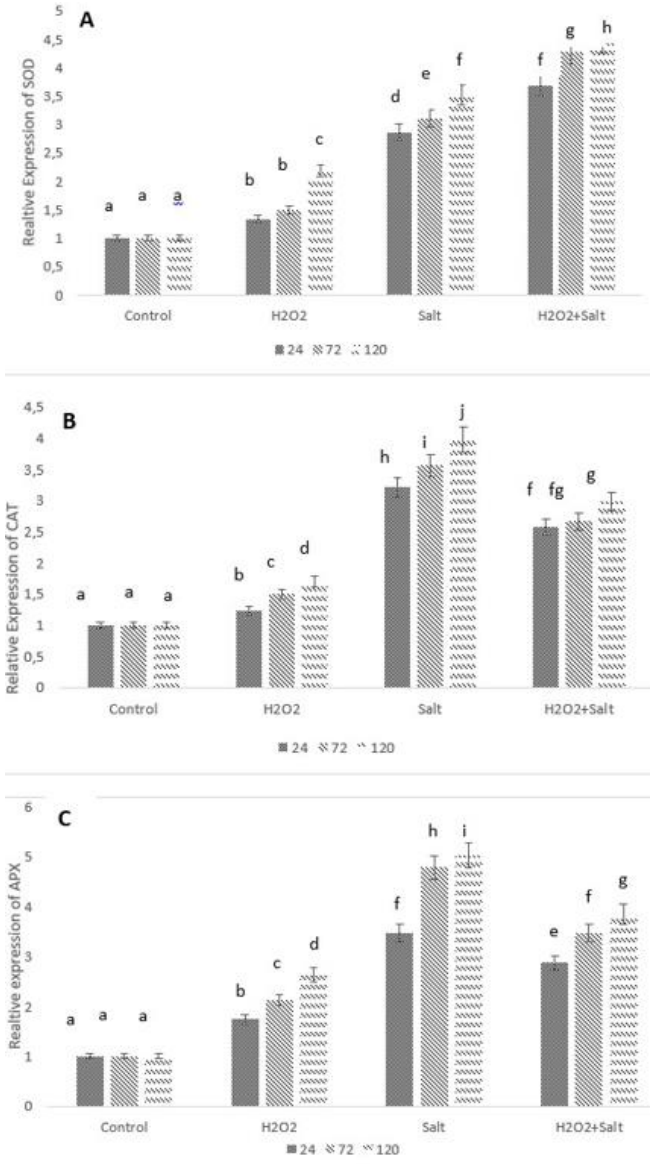

Figure 3. Effect of H_2O_2 pre-treatments on antioxidant enzyme expression in tomato seedlings exposed to salt stress for different periods. A. SOD, B. CAT, and C. APX. Bars indicate a standard error; different letters indicate statistical difference ($p<0.05$)

4. DISCUSSION

Salinity represents a significant environmental factor that poses challenges for plants. The strategies developed to mitigate the effects of salinity stress are pivotal for enhancing plant tolerance and crop productivity. In the current study, tomato seedlings were subjected to a pre-treatment with H_2O_2 prior to exposure to salt stress, revealing the establishment of a more effective defence mechanism against this form of stress.

The quantification of changes in chlorophy ll concentration is recognized as a reliable indicator for evaluating stress tolerance in plants. In this investigation, the concentration of chlorophyll observed in the HS treatment group exceeded that in the salt treatment group. Salt stress, by its nature, leads to chlorophyll damage due to increased sodium content, consistent with findings from other studies [21]. Notably, similar studies on various plant species have also reported a significant reduction in chlorophyll content under salt stress, aligning with the observations in the current study [22, 23] In related research, Yao et al. explored the exogenous application of H_2O_2 to mitigate salt stress in buckwheat seedlings and noted that 5 mM H_2O_2 pre-treatments resulted in higher total chlorophyll content than the stress group, with no significant difference between the 10 mM H_2O_2 pre-treatments and the stress group. In accordance with Yao et al. in this study we figure out that; the HS stress group exhibited higher chlorophyll content, with the specific H_2O_2 concentration critical for improvement depending on plant species and concentration. In this study, a significant improvement in chlorophyll levels was observed with 10 mM H_2O_2 [24]. Furthermore, Asgher et al. investigated the effects of H_2O_2 pre-treatments in response to arsenic stress in rice and found that chlorophyll content decreased by 14.8% upon arsenic exposure compared to the control. Notably, H_2O_2 treatment resulted in a 29.6% increase in total chlorophyll content compared to that in the control group, aligning with findings similar to the current study [25]. These findings underscore the potential of H_2O_2 pre-treatment as an effective strategy to enhance stress tolerance in plants, particularly in the context of salinity stress, and emphasize the importance of species-specific responses and optimal H_2O_2 concentrations in achieving these benefits.

Reactive oxygen species (ROS) serve as initiators of stress responses in plant cells, where their first interaction occurs with the cell membrane, causing lipid peroxidation. MDA, a product of lipid peroxidation, is a primary indicator of stress in plants. In this study, a notable increase in MDA concentration in leaves was observed solely in response to salt exposure. Intriguingly, a decrease in MDA concentration was detected in the HS stress group, suggesting that the addition of H_2O_2 induces a protective mechanism against oxidative damage, aligning with findings from Bagheri et al. (2019).

Priming treatments using various organic and inorganic compounds have proven effective in reducing MDA concentration in response to stress. For instance, Jiang et al. found that the exogenous application of salicylic acid reduced MDA levels in response to arsenic stress in rice plants [26]. Similarly, certain plant extracts, such as Cupressus, have been employed as priming agents to enhance stress tolerance, leading to decreased MDA concentrations compared to stress-only treatments [27]. Consistent with these studies, our findings demonstrated a decrease in MDA levels in the pre-treated group, further emphasizing the efficacy of priming in mitigating oxidative damage.

Moreover, studies like that of Silva et al. (2021) have indicated that H_2O_2 pretreatment can lead to a reduction in MDA levels in plants exposed to salt stress, suggesting that H_2O_2 may prevent MDA accumulation by activating the antioxidant system early through its signalling effect. These results coincide with this study, which found that exogenous H_2O_2 application reduced lipid peroxidation and preserved leaf chloroplast structure, as evidenced by both changes in chlorophyll content and MDA accumulation [28]

Priming, especially during the early stages of seed or seedling development, has garnered significant interest for enhancing plant tolerance to abiotic stresses. [27, 29].

Among the array of priming agents, H_2O_2 stands out as a particularly intriguing choice due to its dual nature [30]. Despite its classification as a potentially toxic molecule, H_2O_2 has gained recognition in recent years as a key signalling molecule in oxidative stress responses due to its high permeability and relatively long half-life in cellular membranes [31]. SOD activity is vital in determining a plant's response to stress, as it represents the front line of defence against oxidative stress. This study demonstrated an increase in SOD activity in the H_2O_2-treated salt stress group compared to the control group, with the highest activity observed in the group subjected to combined H_2O_2 and salt application. Similar findings were reported by Gomez et al, who noted an increase in SOD enzyme activity under salinity stress [32]. In another study, the effects of H_2O_2 priming against temperature stress were explored, revealing that SOD activity increased independently of the stress factor. This suggests that H_2O_2 pre-treatments can mitigate lipid peroxidation by modulating the relative levels of superoxide radicals (O_2^-) and H_2O_2 via SOD under stress conditions [33]. These results are in line with the current study, which also suggests that H_2O_2 priming can enhance SOD activity and reduce lipid peroxidation.

Additionally, Asgher et al. reported that SOD and APX activity increased primarily in the H_2O_2 treatment group, with the highest increase occurring when H_2O_2 and arsenic were applied together, underscoring the potential of H_2O_2 application at low concentrations to enhance plant tolerance to subsequent arsenic stress [25].

In plant cells exposed to abiotic stress, the glutathione-ascorbate cycle plays a crucial role in detoxifying H_2O_2, primarily by converting it to H_2O through APX isoenzymes. APX is pivotal in plant tolerance to salinity and alkaline stress, as well as in inhibiting reactive oxygen species (ROS). The regulation of H_2O_2 detoxification through ascorbic acid (AsA) homeostasis in different cellular components contributes to intercellular ROS regulation. Notably, H_2O_2 priming can act as a signalling molecule, triggering the up-regulation of antioxidant defence systems in plants, including the activation of enzymes like APX, responsible for H_2O_2 detoxification [34]. Consistent with this study, that APX activity was higher in the priming group than in other groups.

CAT and APX activities play a crucial role in mitigating the detrimental effects of H_2O_2 to counteract metabolic damage. Elevated CAT activity is closely associated with an enhanced ability to withstand salinity stress, as it facilitates the detoxification of H_2O_2. Notably, Afrin et al. conducted a study in which they explored the impact of H_2O_2 priming on rice plants subjected to freezing stress, reporting a notable increase in CAT activity following the application of H_2O_2 [35]. In this study, the highest levels of CAT activity were detected in the group exposed to salt-induced stress. It is important to acknowledge that variations in CAT activity are contingent upon a myriad of factors, including the developmental stage of the plant, its metabolic status, as well as the duration and intensity of the stress. Furthermore, the work by Yao et al. is relevant, where they noted that the application of 5 nmol of H_2O_2 combined with salt during priming resulted in the highest CAT activity, while 10 nmol of H_2O_2 applied with salt led to a decrease in activity, aligning it with the levels observed in the group subjected to salt stress alone [24]. These dynamics in enzyme activities observed in our study may indeed be correlated with the findings of Yao and colleagues.

Antioxidant enzyme activation and expression represent distinct aspects of the antioxidant defence system in plants. Enzyme activation pertains to the conversion of an enzyme from an inactive form to its active state, while enzyme expression levels refer to the amount of mRNA or protein produced by a gene encoding an antioxidant enzyme. Both aspects are vital for maintaining ROS balance in plant cells and enhancing plant tolerance to abiotic stress conditions, and post-transcriptional processing can contribute to the divergence between enzyme activation and expression [24]. The variations in activity observed in our study may be attributed to factors like the specific concentration of H_2O_2 applied for priming and the salt concentration and duration.

This study focused on the multifaceted role of H_2O_2 in inducing stress-related genes and stress responses in plants. Studies like the one by Yao et al. have shown that exogenous application of H_2O_2 can significantly increase the expression of stress-related genes and related enzyme genes, with these changes in gene expression paralleling other physiological and biochemical alterations [24]. Moreover, numerous research findings have indicated that priming applications can indeed enhance the expression of stress-related genes [36, 37].

However, it's interesting to note that, while stress-related gene expression levels increased as a result of priming compared to the control group, this increase did not necessarily follow the same pattern as the activation of antioxidant enzymes. Specifically, when examining CAT activity and CAT and APX expression levels, the expression levels did not reach the highest levels of activity and expression, contrasting with what's commonly reported in the literature.

It's important to recognize that the effectiveness and outcomes of priming treatments can vary depending on a multitude of factors, including the specific concentration of H_2O_2 applied, the type of stress (in this case, salt concentration and exposure duration), and the timing of the applications. The discrepancies between our study and some of the literature may indeed be attributed to these variations.

Although an increase in the expression level was detected in our study compared to the control group, it did not show a pattern such as enzyme activation. When CAT activity and CAT and APX expression levels were considered, the expression levels did not reach the highest levels of activity and expression, unlike in the literature. Si et al. examined the effect of H_2O_2 pre-treatment on freezing tolerance caused by mechanical injury and found that there was no significant effect, unlike other studies on freezing stress. This situation was associated with the temperature in the experimental setup that they set up for the cold stress. [38] Similarly, the reason why our study differs from the literature may be the amount of H_2O_2 applied for priming and the salt concentration and durations.

Antioxidant enzyme activation and expression are two different aspects of the antioxidant defense system in plants. Antioxidant enzyme activation refers to the process of converting the inactive form of an enzyme into its active form, whereas antioxidant enzyme expression levels refer to the amount of mRNA or protein produced by a gene encoding an antioxidant enzyme. Both are important for maintaining the balance of ROS in plant cells and enhancing the tolerance of plants to abiotic stress conditions. This may be because of post-transcriptional processing after expression [39, 40].

5. CONCLUSIONS

In conclusion, this comprehensive study provides valuable insights into the potential of H_2O_2 priming as a powerful tool for enhancing stress tolerance in tomato seedlings, offering a promising avenue for addressing the challenges posed by abiotic stressors, particularly salinity.

The evaluation of chlorophyll content revealed that H_2O_2 priming, particularly at a 10 mM concentration, significantly improved stress tolerance. This enhancement may result from the ability of H_2O_2 to activate a protective mechanism against oxidative stress, as evidenced by the decreased accumulation of MDA, a marker of oxidative damage, in the H_2O_2+salt group. Antioxidant enzyme activities, including SOD, CAT, and APX, exhibited dynamic responses to the treatments. SOD activity peaked at 72 and 120 hours in the H_2O_2+salt group, while CAT activity was highest in the salt-stressed group. APX activity was significantly enhanced in the H_2O_2+salt treatment. These findings underscore the role of H_2O_2 priming in regulating antioxidant enzyme activities for enhanced stress tolerance. Moreover, gene expression analyses illuminated the complex interplay between H_2O_2 priming and stress responses. Stress-related gene expression levels increased due to priming, although not always in tandem with enzyme activities, highlighting the intricate nature of plant stress responses. This study emphasizes the multifaceted dynamics of plant adaptation to environmental stressors, influenced by factors such as

priming agent concentration, type, and timing, as well as the specific stress conditions. These findings open up new possibilities for advancing agricultural strategies to bolster crop resilience against abiotic stresses. In the face of increasing environmental challenges, our research underscores the importance of developing innovative approaches to enhance plant stress tolerance, offering a promising path to improve crop yields and sustainability in agriculture. Further investigation into the nuances of priming conditions and their interaction with specific stress factors is warranted to unlock the full potential of this promising technique.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Musa Kar: Conceptualization, Project administration, Investigation, Writing, and editing– original draft **Gökhan Gökpınar**: Investigation, Writing – review & editing, **Özlem Doğan** Investigation, Writing – review & editing

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