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RESEARCH PAPER

H_2O_2 and NO mitigate salt stress by regulating antioxidant enzymes in two genotypes of eggplant (*Solanum melongena* L.)

Fahriye Öcal Özdamar[1]*, Gökçen Baysal Furtana[1], Rukiye Tıpırdamaz[2], Hayri Duman[1]

[1]Biology Department, Science Faculty, Gazi University, 06500 Ankara, Turkey
[2]Biology Department, Science Faculty, Hacettepe University, 06800 Ankara, Turkey

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Corresponding Author
Tel.: +90 228 214 1251
E-mail: fahriyeocal@gazi.edu.tr

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Abstract

H_2O_2 and NO are the key molecules of plant signaling and perception. In this study, we aimed at the antioxidant capacity of foliar-applied eggplant genotypes which shows different responses to salinity (Artvin: salt-sensitive; Mardin: salt-tolerant). For this purpose, H_2O_2 and NO donor (SNP) were sprayed on the leaves of the seedlings for 2 days and then exposed to 100 mM NaCl for 10 days. The amount of Malondialdehyde (MDA), which increased with salt application and is the most important indicator of lipid peroxidation, decreased significantly with individual or combined pretreatments of H_2O_2 and NO donors. SOD and CAT enzyme activities are affected by foliar spraying of donors. While CAT enzyme activity increased significantly with salt application in both genotypes, it showed a significant increase again with individual or combined application of donors. SOD enzyme activity, on the other hand, showed a minor increase in both genotypes with the application of salt stress, while it was significantly increased with the application of donors individually or together.

Introduction

H_2O_2 and NO are biologically active molecules involved in the signaling pathways in plants (Uchida et al., 2002; Azevedo-Neto et al., 2005; Hung et al., 2005; Li et al., 2011; Wahid et al., 2022). Both molecules show a dose-dependent manner, at the high concentrations they have deleterious effects on the plant body, and at the low concentrations play an important role as "signaling molecules" (Gechev & Hille, 2005; Quan et al., 2008). Especially under stress conditions, these molecules play important roles in inducing acclimation (Hayat et al., 2013).

Among the environmental stresses, salt stress is one of the most important factor that limits yield productivity and food security. High salt concentrations cause osmotic, ionic, and oxidative stresses that affect plant metabolism negatively (Munns & Tester, 2008). Plants have developed different mechanisms to cope with these multiple stress factors, the most important of which is activating the plant's antioxidant systems. Possible mechanisms of the positive effects of externally applied H_2O_2 and NO to plants on salt tolerance have been investigated in several studies (Uchida et al., 2002; Tanou et al., 2009a; Tanou et al., 2009b, Qiao et al., 2009; Gohari et al., 2020; Hasanuzzaman et al., 2018; Niu & Liao, 2016; Hajihashemi & Pavla, 2020). Thus, the negative effects of salt stress were eliminated and the salt tolerance was increased.

Under normal respiratory conditions, the production of ROS takes place due to the leakage of electrons to oxygen. Under stress conditions, this process intensifies and excess ROS production takes place. Plants possess various antioxidant systems keeping ROS at low levels but if ROS production

exceeds the capacity of antioxidant systems, then ROS becomes deleterious, causing damage to proteins, lipids, and nucleic acids (Gupta & Igamberdiev, 2015).

In this study, under salt stress conditions, the effects of pretreatment of H_2O_2 and NO on the antioxidant metabolism of eggplant were investigated.

Materials and Methods

Plant material

The eggplant (*Solanum melongena* L.) seeds of the Artvin (susceptible) and Mardin (tolerant) genotypes (Yaşar, 2003) were obtained and grown in a climate chamber under controlled conditions until 4-5 leaf stage. All studies covering the germination and growth cycle were carried out under controlled conditions in the "Digi-Tech Growth Chamber PG34-3" climate chamber. The temperature was set to 25 °C and the humidity was 60-70%.

Pretreatments

After the plants reached a 4-5 leave-stage, 50 days after seed sowing, their leaves were sprayed with chemicals (H_2O_2 and NO) every 6 hours for 48 hours. Accordingly, a total of 5 applications were composed. These; are Control, Salt, H_2O_2 (Hydrogen peroxide, Sigma-Aldrich, 1.08600) SNP (Sodium nitroprusside dihydrate, Sigma-Aldrich, puriss. p.a., ACS reagent, reag. Ph. Eur., ≥99%), H_2O_2 +SNP. The leaves of the seedlings were harvested, submerged in liquid nitrogen, and stored in a refrigerator at -80ºC for analysis. The independent sampling process was made with 3 repetitions.

MDA Analysis

The amount of Malondialdehyde (MDA) in leaf tissues was measured based on the work done by (Lutts et al., 1996). According to this method; a fresh leaf sample, trichloroacetic acid (TCA) was added and homogenized by crushing in a mortar. The homogenate was centrifuged and thiobarbituric acid (TBA) was added. The mixture, which was kept in a water bath, was read at 532 and 600 nm in the spectrophotometer, and the results were obtained.

CAT Analysis

Catalase activity (CAT) was measured (Cakmak & Marschner, 1992) based on the degradation rate of H_2O_2 at 240 nm (E=39.4 mM cm^{-1}).

SOD Analysis.

Superoxide dismutase (SOD) activity was measured by the method of reduction of NBT (nitro blue tetrazolium chloride) by O_2^- under light (Giannopolis & Ries, 1977).

Statistical Analysis

The experiment was set up according to a random plot design and was carried out in 3 replications. The obtained numerical data were evaluated with the GraphPad Prism 8. program. First of all, the data belonging to both genotypes were evaluated separately and whether the changes within each genotype were significant or not was examined by analysis of variance (One-way-ANOVA), and the significance of the differences between the applications was checked with the Duncan test ($p< 0.05$). Then, the mean values of all replications were also evaluated with the t-test, so the importance of the difference between genotypes was checked.

Results

Effects of H_2O_2 and SNP pre-treatments on Malondialdehyde (MDA) Amount

100mM NaCl treatment significantly increased the lipid peroxidation in both genotypes ($p< 0.05$). H_2O_2 pretreatment slightly reduced the MDA amount in the Artvin genotype, but had no change in Mardin genotype, SNP-pretreatment did not affect the MDA amount in Artvin genotype compared to the salt-stressed group but reduced the MDA amount in Mardin genotype. The combined H_2O_2+NO application group significantly reduced the MDA amount in both genotypes compared to the salt-stressed group (Figure 1).

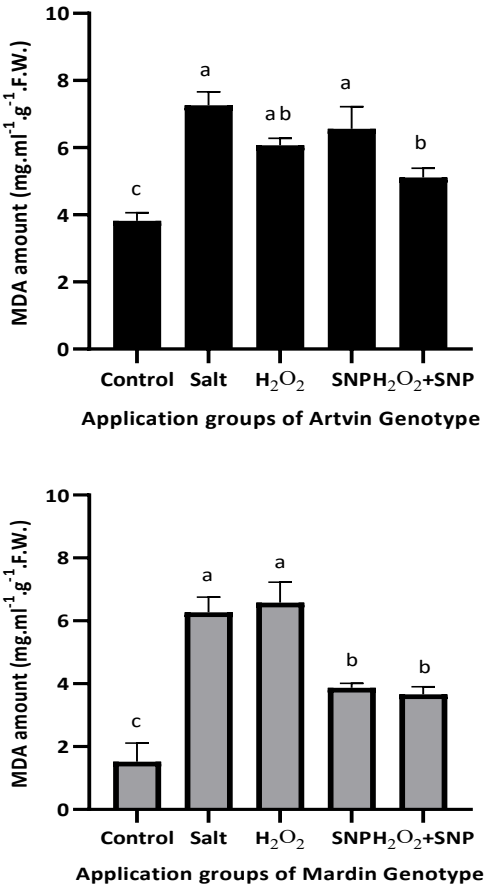

Figure 1. MDA amount in eggplant genotypes.

Effects of H_2O_2 and NO applications on CAT Enzyme Activity

Salt stress significantly increased CAT activity in both genotypes ($p< 0.05$). The H_2O_2 application significantly increased the CAT Activity ($p< 0.05$). SNP pre-treatment increased the CAT activity compared to the salt-stressed group. H_2O_2+SNP pretreated seedlings were significantly increased in both genotypes compared to the salt-stressed group (Figure 2).

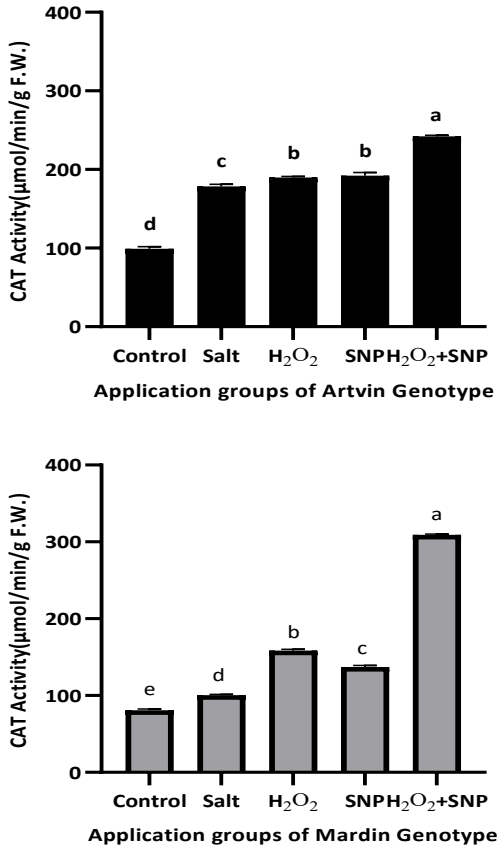

Figure 2. CAT enzyme activity in eggplant genotypes

Effects of H_2O_2 and NO applications on SOD Enzyme Activity

In the Artvin genotype, there was no significant alteration in SOD activity in the H_2O_2 pretreatment group, but SNP pre-treatment markedly increased the SOD activity ($p< 0.05$). H_2O_2+SNP group had the highest value of SOD-enzyme activity in the Artvin genotype.

In Mardin genotype, salt stress increased the SOD activity ($p< 0.05$). While H_2O_2 pretreatment significantly increased the SOD activity, SNP pretreatment significantly decreased the SOD activity compared to the salt-stressed group ($p< 0.05$). H_2O_2 combined with the SNP application group significantly increased the SOD activity (Figure 3).

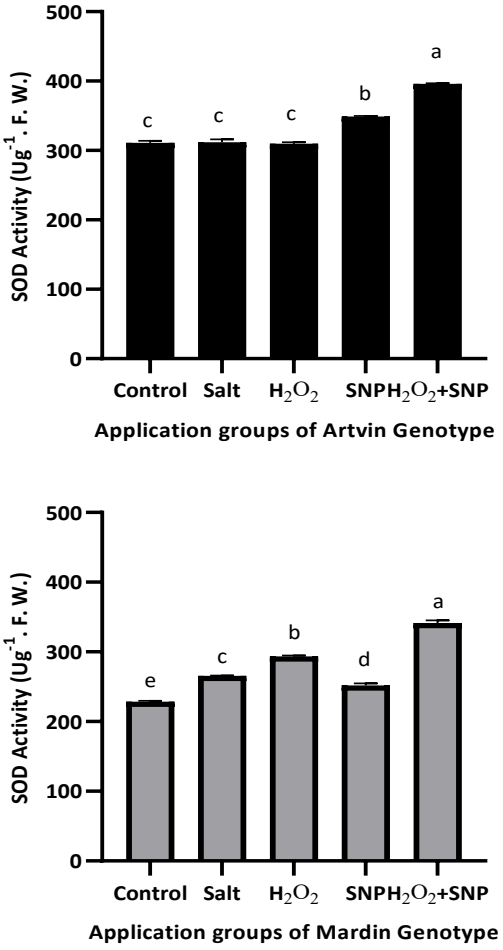

Figure 3. SOD enzyme activity in eggplant genotype

Discussion

Ion toxicity and osmotic stress resulting from salinity inhibit the plant photosystem, leading to excessive ROS production (Hasanuzzaman et al., 2017; Hajihashemi & Pavla, 2020; Wahid et al., 2022). It is known that the increased amount of ROS in the cell increases the amount of MDA by causing lipid peroxidation (Mishra & Choudhuri, 1999). In our study, salt treatment significantly increased the MDA amount in both genotypes. Alone or together both pretreatments decreased the MDA amount but these applications had never as few as the Control group (Figure 1).

Similar to our results, externally applied H_2O_2 in corn (Chen & Li, 2002), mung bean (Saleh, 2007), wheat (Li et al., 2008), barley (Kim et al., 2013), and Panax ginseng (Sathiyaraj et al., 2014) has been reported to prevent the increase in MDA amount by reducing electrolyte leakage, which increases with stress. In cucumber (Hasanuzzaman et al., 2017), soybean (Güler & Pehlivan, 2016), and canola (Gao et al., 2010), H_2O_2 application did not cause any change in the amount of MDA.

It was observed that MDA amounts remained

close to the control group in plants where H_2O_2 and SNP were applied together. Thus, it can be said that H_2O_2 and SNP act in the direction of reducing the lipid peroxidation occurring in the cells by controlling the sudden increases in MDA that occurred in the salt-treated group. Similar to our results, in rice (Uchida et al., 2002) and citrus fruits (Tanou et al., 2009a), externally applied H_2O_2+SNP decreased the amount of MDA and this was achieved by reducing the amount of ROS in the cell by stimulating the antioxidant enzyme activity has been reported.

In various studies, it has been reported that CAT activity either increases (Baysal Furtana & Tıpırdamaz, 2010) or does not change (Nohar et al., 2015) or decreases (Hasanuzzaman & Fujita, 2012) in plants grown under salt stress conditions. In our study, it was determined that CAT activity increased in plants treated with H_2O_2 alone compared to the only salt-applied group. This increase was found higher and more significant in Mardin genotype. Similar to our study, it has been reported by de (Azevedo Neto et al., 2005) and (Gechev et al., 2002) that H_2O_2 applied through leaf increased CAT activity in corn and tobacco. In a different study, it has been reported that the increase in CAT enzyme activity in corn plants treated with H_2O_2 occurs by a complex mechanism including CAT gene regulation (Gondim et al., 2012). In addition, unlike other enzymes that sweep H_2O_2, it has been reported by (Mhamdi et al., 2010) that CAT has more affinity for H_2O_2. In addition, CAT transcripts have been reported by researchers to increase in plants treated with H_2O_2 (Gondim et al., 2012; Mhamdi et al., 2010). In our study, salt stress significantly increased CAT activity in both genotypes. The pretreatments applied alone or together increased the enzyme activity in both genotypes compared to the salt-stressed group (Figure 2). In plants treated with H_2O_2 +SNP, CAT activity reached the highest value among all groups in both genotypes.

In plants treated with NO donor SNP, SOD activity increased in Artvin genotype compared to Control and salt-stressed groups, while it increased compared to the control group in Mardin genotype and decreased compared to the salt-stressed group. NO is thought to be a molecule that regulates ROS metabolism through stimulation of the cellular antioxidant system in stress tolerance. Similar to our results, externally applied NO donor SNP has been reported to increase CAT and SOD activity (Fan et al., 2007). The SOD enzyme is a powerful antioxidant enzyme whose primary activity causes the change of $O_2^{\bullet-}$ reagent to H_2O_2 and O_2 (Fridovich, 1986). In our study, under the salt-stressed conditions, SOD enzyme activity did not show a significant increase in the susceptible Artvin genotype, however, increased significantly in the tolerant Mardin genotype. In plants treated with H_2O_2, SOD activity increased in both genotypes compared to Control and only-salt applied groups. H_2O_2+SNP application group significantly increased the SOD activity.

In recent studies, it has been shown that the increase in antioxidant enzyme activity in plants with H_2O_2 and NO pre-treatment is due to the increase in the expression of the genes encoding these enzymes by H_2O_2 and NO (Beligni & Lamattina, 2001; Neill et al., 2002; de Pinto et al., 2006; Zhang et al., 2007).

Conclusion

In this study, the possible effects of $H_2O_2$and NO pretreatment under salt stress on two genotypes of eggplant were studied. 100mM NaCl-stress significantly increased MDA content and CAT activity in both genotypes. H_2O_2 pretreatment slightly reduced the lipid peroxidation in the Artvin genotype and significantly increased the CAT Activity in two genotypes.

H_2O_2 combined with the NO application group significantly reduced the MDA amount in both genotypes compared to the salt-stressed group, this pretreatment also increased the CAT and SOD activity in both genotypes compared the salt-stressed group. H_2O_2 combined with the SNP application group significantly increased the SOD activity in two genotypes.

Author contributions

FOO: Investigation, data curation, visualization, writing and reviewing; **HD and RT:** supervision, conceptualization, administration and reviewing, **GBF** helped to design the analysis and reviewed the paper. All authors have read and agreed to the published version of the manuscript.

Credit

This work was based on the Ph.D thesis studies of the first author Fahriye Öcal Özdamar.

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