Anatolian Journal of Botany
9(1): 20-29 (2025)
Research article *doi:10.30616/ajb.1599595*

Lipoic acid confers osmotic stress tolerance to maize seedlings by upregulating the enzymes of antioxidant defense and glyoxalase systems

Asiye SEZGİN MUSLU[1*], Rabiye TERZİ[2]
[1]*Karadeniz Technical University, Science Faculty, Department of Biology, 61080, Trabzon, Türkiye*
**asiyeszgn@outlook.com, [2]rabiyeterzi@yahoo.com.tr*

Received : 11.12.2024
Accepted : 02.01.2025
Online : 15.02.2025

Lipoik asit, antioksidan savunma ve glioksalaz sistemlerinin enzimlerini artırarak mısır fidelerine ozmotik stres toleransı kazandırır

Abstract: Exogenous application of lipoic acid (LA), which is a special antioxidant substance, alleviates abiotic stress damage in plants. However, the mechanism of action of LA in stress tolerance is still not fully understood. Here, the effect of exogenous LA on the coordination of antioxidant defense and glyoxalase systems to alleviate osmotic stress damage was investigated. LA (12µM) was applied to the roots of 21-day-old seedlings in Hoagland nutrient solution for 8 hours and then the seedlings were exposed to 10% polyethylene glycol (PEG_{6000}) for 3 days. Seedlings grown in Hoagland nutrient solution for 28 days were used as the control group. Exogenous LA under osmotic stress was found to increase the fresh and dry weights of the leaves, leaf relative water content, and non-enzymatic compounds such as ascorbate and glutathione (GSH) while significantly decreasing the contents of hydrogen peroxide (H_2O_2) and methylglyoxal (MG). LA application also increased some antioxidant enzyme activities such as ascorbate peroxidase (1.2-fold), glutathione peroxidase (1.3-fold), glutathione reductase (1.4-fold), and monodehydroascorbate reductase (1.8-fold). LA significantly induced the relative expression levels of the genes coding the antioxidant enzymes. Furthermore, LA stimulated the enzyme activities of the glyoxalase system (glyoxalase I (Gly I) (1.3-fold) and glyoxalase II (Gly II) (1.1-fold). Additionally, the relative expression levels of the *Gly I* and *Gly II* genes were consistent with the findings of the Gly I and Gly II activities. Moreover, exogenous LA induced the expression level of *Gly II* (1.4-fold) more than that of *Gly I* (1.3-fold). As a result, LA mitigates osmotic stress damage in maize by enhancing the activity of antioxidant and glyoxalase systems, enabling the rapid removal of reactive oxygen species and the toxic compound MG, thereby providing a protective mechanism. Further investigation of the effects of LA on crops exposed to abiotic stresses will contribute to improve the stress tolerance and increase agricultural yields.

Key words: Lipoic acid, antioxidant system, glyoxalase system, osmotic stress, maize, gene expression

Özet: Özel bir antioksidan madde olan lipoik asidin (LA) ekzojen uygulaması, bitkilerdeki abiyotik stres hasarını hafifletir. Bununla beraber, LA'nın stres toleransındaki etki mekanizması henüz tam olarak anlaşılamamıştır. Bu çalışmada, osmotik stres hasarını hafifletmek için antioksidan savunma ve glioksalaz sistemlerinin koordinasyonuna exojen LA uygulamasının etkisi incelenmiştir. 21 günlük fidelerin köklerine Hoagland besin solüsyonunda 8 saat süreyle LA (12 µM) uygulandı ve ardından fideler 3 gün süreyle %10'luk polietilen glikole (PEG_{6000}) maruz bırakıldı. Kontrol grubu olarak 28 gün boyunca Hoagland besin solüsyonunda yetiştirilen fideler kullanıldı. Osmotik stres altında eksojen LA'nın, yaprakların taze ve kuru ağırlıklarını, yaprakların bağıl su içeriğini ve askorbat ve glutatyon (GSH) gibi enzimatik olmayan bileşenleri artırdığı, ayrıca hidrojen peroksit (H_2O_2) ve metilglioksal (MG) içeriklerini azalttığı bulunmuştur. LA uygulaması, askorbat peroksidaz (1.2 kat), glutatyon peroksidaz (1.3 kat), glutatyon redüktaz (1.4 kat) ve monodehidroaskorbat redüktaz (1.8 kat) gibi bazı antioksidan enzim aktivitelerini de artırmıştır. LA, antioksidan enzimlerin kodlayan genlerin bağıl ekspresyon seviyelerini de önemli derecede indüklemiştir. Ayrıca, LA, glioksalaz sistemi enzim aktivitelerini (glioksalaz I (Gly I) (1.3 kat) ve glioksalaz II (Gly II) (1.1 kat)) artırmıştır. Ayrıca, *Gly I* ve *Gly II* genlerinin bağıl ekspresyon seviyeleri, Gly I ve Gly II aktiviteleri ile tutarlıdır. Dahası, eksojen LA, *Gly I*'ye (1.3 kat) kıyasla *Gly II*'nin (1.4 kat) ekspresyon seviyesini daha fazla indüklemiştir. Sonuç olarak, LA, antioksidan ve glioksalaz sistemlerinin aktivitesini artırarak mısırdaki osmotik stres hasarını hafifletir, reaktif oksijen türlerinin ve toksik bileşik MG'nin hızla uzaklaştırılmasını sağlayarak koruyucu bir mekanizma sunar. LA'nın abiyotik streslere maruz kalan ürünler üzerindeki etkilerinin daha fazla araştırılması, stres toleransının iyileştirilmesine ve tarımsal verimin artırılmasına katkı sağlayacaktır.

Anahtar Kelimeler: Lipoik asit, antioksidan sistem, glioksalaz sistem, osmotik stres, mısır, gen ekspresyonu

Citation: Sezgin Muslu A, Terzi R (2025). Lipoic acid confers osmotic stress tolerance to maize seedlings by upregulating the enzymes of antioxidant defense and glyoxalase systems. Anatolian Journal of Botany 9(1): 20-29.

1. Introduction

Drought is one of the most unpredictable and uncontrolled environmental stresses and causes many devastating effects on plants (Anjum et al., 2017). Among all other abiotic stresses, drought stress is considered one of the serious threats limiting plant production, growth, and productivity (Junaid et al., 2023). Damage caused by stress factors varies depending on the type of plant, its tolerance and its adaptation ability (Kadıoğlu et al., 2011). In response to stress factors, plants have developed complex physiological and chemical strategies to adapt to sudden environmental changes. Some physiological, biochemical and molecular changes also occur during drought stress (Krasensky and Jonak, 2012).

It is well-established that one of the primary mechanisms plants use to tolerate stress is their antioxidant system, which protects them from the damaging effects of reactive oxygen species (ROS). This system contains a variety of

enzymatic and non-enzymatic antioxidants that scavenge ROS. The enzymatic antioxidant defense system includes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), all of which are part of the ascorbate-glutathione cycle. It comprises enzymes like MDHAR and DHAR, as well as non-enzymatic substances such AsA, GSH, tocopherol, carotenoids, and phenolics (Sharma et al., 2012). Many studies have revealed that tolerance to osmotic stress in some agricultural plants is connected with enhanced antioxidant capability (Sezgin et al., 2019; Altansambar et al., 2024; Sezgin Muslu, 2024).

Like ROS, methylglyoxal (MG), a reactive oxidizing compound, increases under abiotic stress, which can damage the ultrastructural components of the cell, cause mutation, and finally cause programmed cell death (Hasanuzzaman et al., 2017). MG-detoxifying enzymes are divided into two groups: GSH-dependent and GSH-independent. In the GSH-dependent glyoxalase pathway, MG is detoxified to the nontoxic compound by two sequential reactions catalyzed by glyoxalase I (Gly I) and glyoxalase II (Gly II). In the first step, MG is converted to S-D-lactoylglutathione (SLG) using GSH, and in the final step, Gly II converts the GSH into the system and hydrolyzes SLG to form D-lactate. Thus, MG detoxification of plants ensures the maintenance of GSH homeostasis. In the GSH-independent pathway, MG is irreversibly converted to D-lactate by glyoxalase III (Gly III) in a single step without using GSH (Singh and Dhaka, 2016). The antioxidant system in cooperation with the glyoxalase system is known to increase abiotic stress tolerance in plants (Nahar et al., 2015a). Studies showing the regulatory role of exogenous applications on antioxidant and glyoxalase systems to induce oxidative stress tolerance are still insufficient. Some studies were showing that applications of some antioxidant compounds increased tolerance to some stress factors by stimulating antioxidant and glyoxalase systems (Hossain et al., 2010; Nahar et al., 2015b, c). The activities of these two systems could change depending on the type of plants under different stress factors (Hasanuzzaman et al., 2019; Zaid et al., 2019; Sezgin Muslu and Kadıoğlu, 2021).

Maize, one of the plants with C4 metabolism, is a grain product grown all over the World (Farhad et al., 2009). It grows in the Mediterranean and Black Sea Regions and encounters abiotic stresses during the growth period (Tanyolaç et al., 2007). Researchers reported that water deficiency negatively affected germination and seedling growth in maize (Mohammadkhani and Heidari, 2008). To maintain the economic value of maize, it is necessary to develop genotypes that can tolerate stress or to apply some chemical substances with protective properties externally. Ensuring stress tolerance by applying chemicals to plants has a low cost (Hamdia and Shaddad, 2010), and antioxidants as chemicals are particularly important (Ashraf and Foolad, 2007). One of the antioxidant compounds that play a role in regulating the redox state of plants is lipoic acid (LA).

Unlike other antioxidant substances, LA, a unique short-chain fatty acid with two sulfur atoms, has strong antioxidant properties in both reduced (dihydrolipoic acid, DHLA) and oxidized forms (Sudesh et al., 2002). DHLA is a more effective antioxidant than oxidized lipoic acid and plays a role in directly scavenging ROS such as superoxide, hydroperoxyl and hydroxyl radicals (Navari-Izzo et al., 2002). It has been determined that LA, which plays a role in respiration and indirectly in carbon fixation and nitrogen assimilation in plants, increases glutathione, the most important water-soluble antioxidant (Taylor et al., 2004). It has recently been revealed that different plants can resist some stress factors (drought, salt, alkaline, and cadmium) with LA application (Elkelish et al., 2021; Youssef et al., 2021; Ramadan et al., 2022; Yadav et al., 2005; Daler and Kaya 2024). On the other hand, studies on maize under osmotic stress have shown that exogenous LA stimulates photosystem II activity and enhances water-deficit tolerance by modulating osmoprotectant metabolism (Sezgin et al., 2019; Saruhan Güler et al., 2021). In addition, it was reported that LA stimulated some antioxidant enzyme activities and their expression levels in the maize seedlings under osmotic stress (Terzi et al., 2018; Gümrükçü Şimşek et al., 2024). As far as we know, no reports exist on how LA stimulates the glyoxalase system in plants exposed to stress factors. We aimed to determine which of these systems (antioxidant and glyoxalase) LA has a greater effect. Therefore, in the current study, it was hypothesized that LA regulates the activity of the enzymes of the two systems and thus provides coordination of the antioxidant and glyoxalase systems. Therefore, the present study was designed to evaluate the role of LA in the coordination of antioxidant defense and glyoxalase systems. This is the first study to demonstrate that LA alleviates osmotic stress-induced oxidative damage in maize seedlings by stimulating the antioxidant and glyoxalase defense systems.

2. Materials and Method

2.1. Growing of plants, LA and stress applications

Maize (*Zea mays* L.) seeds were used in our study. For surface sterilization, the seeds were treated with 0.1% $HgCl_2$ for 3 min and then washed three or four times with sterilized distilled water**.** The seeds were grown in a hydroponic medium containing Hoagland nutrient solution (Hoagland and Arnon, 1950) in a growth chamber with light intensity (400-430 µmol $m^{-2}s^{-1}$), temperature (18-20 ^{o}C) and humidity (50-70%). LA (12µM) (Sezgin et al., 2019) was applied to the roots of 21-day-old seedlings in Hoagland nutrient solution for 8 hours. Then, the plants were exposed to osmotic stress and provided with 10% polyethyleneglycol (PEG_{6000}) for 72 hours. The osmotic stress applied to the plants was gradually increased. They were exposed to 3% PEG medium for 1 day, 6% PEG medium for 3 days and 10% PEG medium for 3 days, respectively (Gümrükçü Şimşek et al., 2024). In this current study, experimental groups were designed in four different ways: seedlings grown in Hoagland nutrient solution for 28 days (Control); 21-day-old seedlings exposed to osmotic stress ((3% PEG (1 day), 6% PEG (3 days) 10% PEG (3 days)) (PEG); after LA pretreatment for 8 hours, the seedlings were kept in Hoagland nutrient solution for 7 days (LA); after LA pretreatment for 8 hours, the seedlings exposed to the osmotic stress mentioned above (LA+PEG). After the applications, the leaves were harvested. Leaf fresh and dry weights were measured, and leaf relative water content analyses were completed immediately after sampling**.**

2.2. Determination of fresh and dry leaf weights

Leaf fresh weights (FW) of maize plants were weighed and recorded in 5 replicates from each experimental group. The leaves, whose fresh weights were calculated, were dried in an oven at 75°C for 48 hours, and the dry weights (DW) of the leaves were calculated.

2.3. Leaf relative water content (LRWC)

Determination of LRWC was made according to Castillo (1996). After measuring the fresh weight of the leaves of the plants, their turgid weights were taken by soaking them in water overnight. Then, LRWC was determined by keeping them in an oven set at 80 ºC, recording their dry weights and substituting them in the formula below.

Leaf RWC (%) = (Fresh weight-Dry weight/Turgid weight-Dry weight) x100

2.4. Detection of the presence of H_2O_2 by 3,3′ - Diaminobenzidine (DAB) staining method

To quantify H_2O_2, the DAB staining procedure was modified by Daudi and O'brien (2012). The leaves of the samples were treated with DAB made with Tween 20 and sodium phosphate buffer (pH 7.0). The leaves were then inserted in test tubes and incubated on a laboratory shaker at 90 rpm. After incubation, the leaves were boiled in ethanol, acetic acid, and glycerol in a water bath at 95 °C for 15 min. The leaves were placed in a fresh bleach solution for 30 min before being inspected and photographed for colour changes.

2.5. Determination of MG content

Leaf samples (0.1 g) were homogenized in perchloric acid and then centrifuged at 16,000 xg for 15 min. Before using this supernatant for the analysis, it was neutralized by immersing it in a saturated potassium carbonate solution at ambient temperature for 15 min before centrifuging again at 16,000 xg for 15 min. The supernatant was treated with 1 mL of 1,2-diaminobenzene and perchloric acid. The derivatized MG was measured at 335 nm after 25 min (Yadav et al., 2005).

2.6. Determination of changes in antioxidant capacity

Fresh leaves (0.1 g) were extracted in 1.8 mL extraction buffer (50 mM K_2HPO_4 (pH 7.0), 1 mM EDTA, 1% PVPP, and ascorbate for APX). The extract was centrifuged, and the supernatant was used to assess enzyme activity and protein content. Protein determination was carried out spectrophotometrically by Bradford (1976). GPX activity by measuring a 100 mM potassium phosphate buffer (pH 7.0), 0.1mM EDTA, 5 mM guaiacol, 20 mM H_2O_2, and enzyme extract (50 µL) in a 1 mL total volume at 470 nm for 1 min (Urbanek et al., 1991). The APX activity was assessed using Nakano and Asada's (1981) method, which measured the decrease at 290 nm of a 1 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 250 µM AsA, 5 mM H_2O_2, and enzyme extract (20 µL). GR activity was evaluated using Foyer and Halliwell's (1976) method, which involved adding enzyme extract (50 µL) to a combination of 50 mM Tris-HCl (pH 7.8), 1 mM GSSG, and 200 µL 0.25 mM NADPH in 200 µL 0.5 mM EDTA. It was assessed by a decrease in oxidation at 340 nm over 5 min. MDHAR was computed by measuring at 340 nm the 1 mL reaction mixture created by adding 50 mM potassium phosphate buffer (pH 7.8), 150 µM NADH, and 500 µM AsA to the sample extract (100 µL). The measured values were extrapolated from data collected without ascorbate oxidase (Hossain et al., 1984).

To assess DHAR activity, 0.1 g of fresh leaves were homogenized in an extraction buffer (1.8 mL) comprising 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 2 mM EDTA, and 1 mM $MgCl_2$). The extract was centrifuged at 15,000 xg for 10 min. The enzyme activity was measured by measuring 1 mL of a mixture containing 50 mM K_2HPO_4/KH_2PO_4 (pH 6.5), 0.5 mM DHA, 1 mM GSH, and enzyme extract (100 µL), as described by Hossain and Asada (1984). DHAR activity was evaluated by measuring the rise in absorbance at 265 nm.

Fresh leaf samples (0.1 g) were homogenized with m-phosphoric acid. The extract was centrifuged at 15,000 xg for 10 min and then added to a reaction medium containing citrate-phosphate buffer (pH 6.2). AsA content was determined using the protocol of Liso et al. (1984).

To determine GSH concentration, plant leaf samples (0.1 g) were extracted in metaphosphoric acid with EDTA. The extract was centrifuged at 15,000 xg for 15 min, and the supernatant was utilized to measure GSH content. It was determined using the "glutathione assay kit" (Cayman Chemical) protocol described by Tietze (1969).

2.7. Determination of the changes in activities of glyoxalase system enzymes

To extract enzymes, leaf samples (0.1 g) were homogenized in a potassium phosphate buffer (pH 7.0) containing KCl, AsA, β-mercaptoethanol, and glycerol were centrifuged at 13,000 xg for 10 min, and the supernatants were utilized to assess the activity. Gly I activity was assessed by Hasanuzzaman et al. (2011). The reaction began with the addition of MG, and the rise at 240 nm in 1 min was recorded. Gly II activity was determined by measuring GSH production at 412 nm for 1 min, as described by Principato et al. (1987).

2.8. Real-Time PCR Analyzes

Fresh leaf samples were thoroughly shredded with a tissue disintegrator. Then, the RNA isolation kit protocol was applied to obtain total RNA. The amount and purity of the RNA samples were determined using a nanodrop. Using the cDNA transcription kit (Applied Biosystems), cDNA was obtained from the total RNA samples that had been isolated. To determine the gene expressions of the samples, analyses were completed on the CFX Connect Real-Time PCR System device, using cDNA results and iTaq Universal SYBR Green Supermix (Bio-Rad) solution according to the manufacturer's instructions. With a final volume of 20 µL, the reactions are follows: (10 µL Supermix (2X), 1 µL forward and reverse primers (10 µM), 1 µL cDNA, and 7 µL nuclease-free water). The Real-Time PCR process steps were modified according to iTaq Universal SYBR Green Supermix' instructions as follows: an initial step of 1 min at 95°C, followed by 44 cycles of 15 sec at 95°C, 30 sec at 60°C, and 30 sec at 72°C. A melt curve was generated with 0.5°C increments from 65°C to 95°C. A melting curve analysis was performed at the end of each PCR reaction to confirm the presence of a single peak, ensuring the absence of primer dimers and non-specific product formation. Primers belonging to the genes shown in Table 1 were used to determine the expression

levels of genes belonging to enzymes responsible for antioxidant defense and glyoxalase system (Zhao et al., 2017; Talaat et al., 2022). The data obtained from the analysis were normalized to the *β-Actin* reference gene and presented as relative gene expression using the $2^{-\Delta\Delta CT}$ method, following the protocol outlined by Bookout and Mangelsdorf (2003). Each biological replication was analyzed as 3 technical replicates and the mean technical error was accepted as 0.5 (±1) Cq values. The Cq values of these genes were examined using the Bio-Rad CFX Manager Software (version 3.1).

2.9. Statistical Analysis

The analyses were done in triplicate. The results of the experiments are presented as mean ± standard deviations. One-way ANOVA was used for statistical studies in SPSS (version IBM 23). P< 0.05 was considered statistically significant. Relative gene expression during qRT-PCR analysis was examined using Bio-Rad CFX Manager 3.1.

3. Results

3.1. Leaf dry and fresh weight, and LRWC

LA application increased leaf fresh weight under both stressful and non-stressful conditions. Moreover, it was determined that LA application did not statistically change leaf dry weight and LRWC under non-stressful conditions. However, under stressful conditions, LA significantly increased leaf dry weight and LRWC (Fig. 1a-c).

3.2. H_2O_2 and MG contents

As illustrated in Figure 2a, the brown speck indicated the existence of H_2O_2. Brown spots on PEG-treated leaves significantly increased as compared to controls. However, under a non-stressful situation, LA-treated seedlings had fewer brown patches than the control treatment. Moreover, LA-treated leaves showed fewer brown spots under non-stress conditions than PEG-treated leaves.

Table 1 Primer sequences of genes whose expression levels were determined

Target gene	Sequences 5'-3'
Ascorbate peroxidase (APX)	F: GCCTTCTTCAGCTCCCAAGT R: TGCAAAAGACCACATGCAGC
Glutathione peroxidase (GPX)	F: CGCTATGCTCCAACCACTTC R: GCTCTCAGAGCAATGTTCATACAG
Glutathione reductase (GR)	F: ATG GTG GGA CTT GCG TGA TA R: GCA TCA ACT AGA CTG CCT GC
Monodehydroascorbate reductase, (MDHAR)	F: CTG TAA AGG CGA TCA AGG GC R: ACC TTG CCG TCC TTA ATC CA
Dehydroascorbate reductase (DHAR)	F: GCT GAT CTC TCT CTG GGT CC R: GCG CCA TCC AGC AAT TAC AT
Glyoxalase I (Gly I)	F: TGAGGCAGTTGATCTGGCG R: CCCGAGTCTTCACTGTAGTTCC
Glyoxalase II (Gly II)	F: CACATGGATGTTTGCTGGTC R: CGTGCATCATCAAAATGGTC
β-Actin	F: ACCAGTTGTTCGCCCACTAG R:GAAGATCACCCTGTGCTGCT

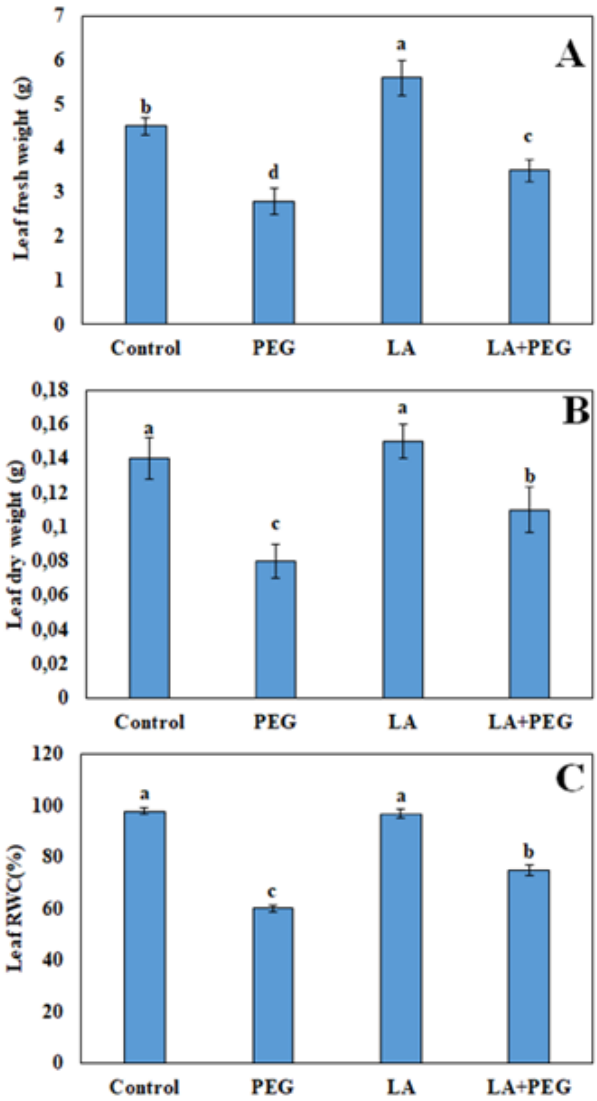

Figure 1. Effects of LA on Leaf dry weight (a), Leaf fresh weight (b), and Leaf RWC (c) under osmotic stress. The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments.

MG content increased 1.2-fold in PEG-treated seedlings compared to the control group. Moreover, MG content in LA-treated leaves decreased by 1.07-fold compared to the control group, while MG content in LA+PEG-treated leaves decreased by 1.13-fold compared to PEG-treated leaves. (Fig. 2b).

3.3. Antioxidant capacity

Under non-stressed conditions, exogenous LA increased the antioxidant system enzymes activities (except GPX and MDHAR) and antioxidant compounds. Under PEG stress, exogenous LA induced the antioxidant system enzyme activities (except DHAR) (Figure 3a-d). There was a statistically significant difference in the APX activity between control and LA groups. The APX activity of LA-treated leaves was 1.3-fold higher than the control leaves. In addition, the activity of the LA+PEG treatment was 1.2-fold higher than the PEG-treated leaves (Figure 3a). Under PEG-stressed conditions, LA significantly increased the GPX activity (1.3-fold) compared to the PEG treatment. However, there was no difference in the GPX activity

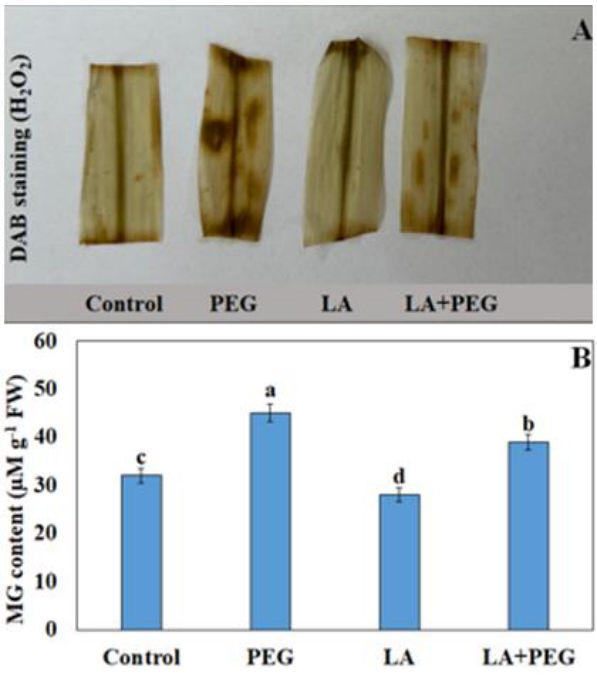

Figure 2. Effects of LA on DAB staining (H_2O_2) (a), MG content (b) under osmotic stress. The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments.

between the control and LA treatments (Figure 3a). There was a statistical difference in the GR activity between PEG and LA+PEG groups. The GR activity of the LA+PEG-treated leaves was 1.4-fold higher than the PEG alone. The LA application resulted in a 1.5-fold increase in GR activity compared to the control (Figure 3b). In addition, the MDHAR activity of the LA+PEG-treated leaves was 1.8-fold higher than the PEG treatment. However, there was no difference in the MDHAR activity between the control and LA treatments (Figure 3c). There was a statistically significant difference in the DHAR activity between control and LA groups. The LA application resulted in a 1.5-fold increase in DHAR activity compared to the control. However, there was no difference in the DHAR activity between the PEG and LA+PEG treatments (Figure 3d).

There was a statistical difference in the AsA content between control and PEG groups The PEG treatment resulted in a 2.5-fold increase in AsA content compared to the control. AsA content in the LA-treated leaves was 1.17-fold higher than the control. The LA+PEG application caused a 1.2-fold increase in AsA content compared to the PEG group (Figure 4a). The GSH content of the LA+PEG group was higher than all groups. Interestingly, the content increased 2.5-fold in the PEG-treated leaves and 1.3-fold in LA-treated leaves compared to the control. Similarly, under PEG-induced osmotic stress, exogenous LA stimulated the GSH content compared to the PEG treatment, with a 1.1-fold increase in the LA+PEG-treated leaves (Figure 4b).

3.4. Glyoxalase system enzymes

Exogenous LA under non-stressful and stressful conditions caused to significantly increase in the glyoxalase system enzymes (Gly I and Gly II) activities. Gly I activities in the PEG and LA-treated leaves were 4.0- and 3.0-fold higher than the control, respectively. The Gly I activity increased 1.3-fold in the LA+PEG treatment compared to the PEG treatment. The Gly II activity significantly increased 4.8-fold in the PEG treatment and 3.1-fold in the LA treatment compared to the control. Moreover, the activity was 1.1-fold higher in LA+PEG treatment than in the PEG treatment (Figure 5).

3.5. The levels of antioxidant enzyme gene expression

The PEG treatment resulted in a significant increase in the relative expression of the *APX* gene by 1.5-fold over the control. Under non-stressful conditions, the LA treatment contributed to the significant upregulation in the expression of the *APX* gene by 1.2-fold in comparison to the control. However, there was no difference in the *APX* gene expression between the PEG and LA-combined PEG treatments (Figure 6a). The PEG treatment significantly up-regulated the expression of the *GPX* by 2.1-fold, while the

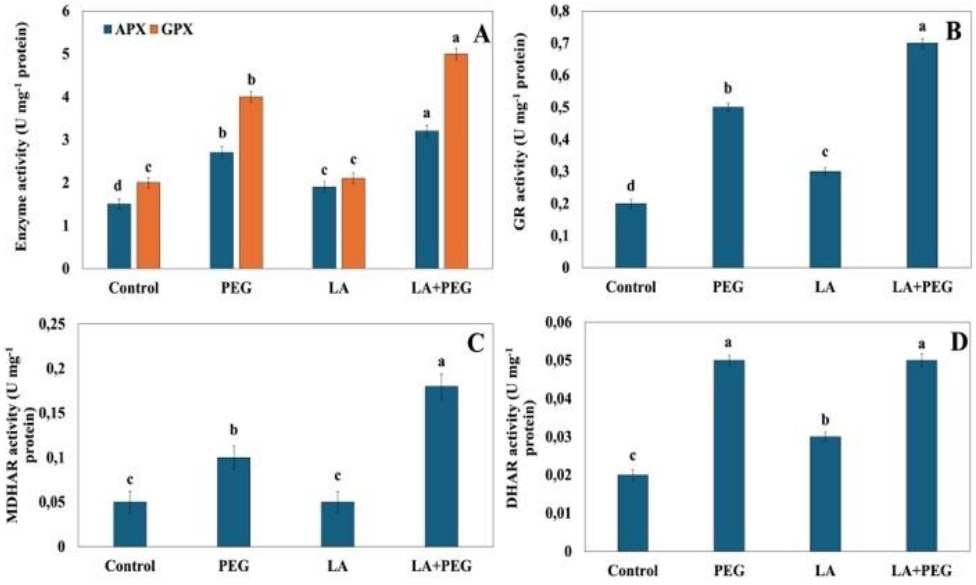

Figure 3. Effects of LA on antioxidant enzyme activities under osmotic stress: APX and GPX activities (a), GR activity (b), MDHAR activity (c), and DHAR activity (d). The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments.

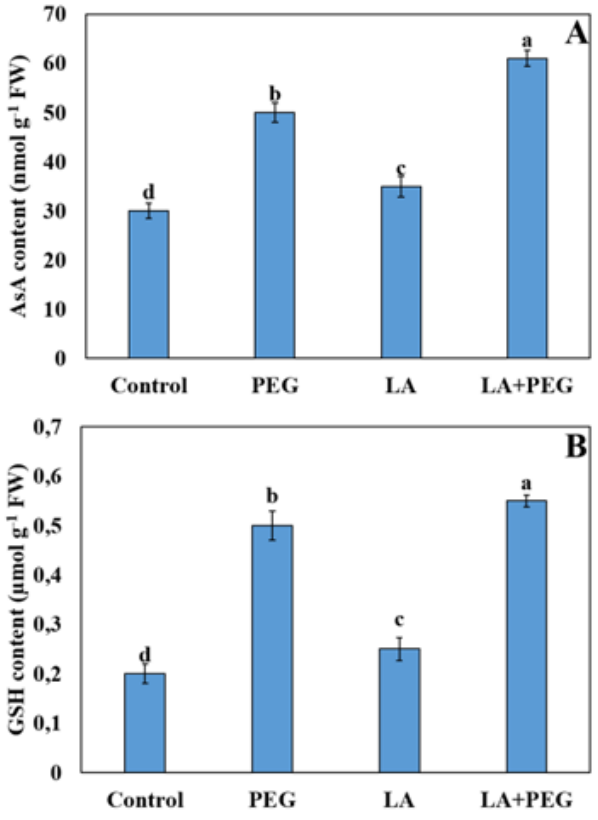

Figure 4. Effects of LA on antioxidant compounds under osmotic stress: AsA content (a), GSH content (b). The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments

LA-combined PEG treatment up-regulated this gene by 2.3-fold compared to the PEG treatment. However, there was no statistical difference in the *GPX* gene expression between the control and LA treatments (Figure 6b). The PEG treatment up-regulated *GR* expression more than the control. The *GR* expression level increased 1.2-fold in the

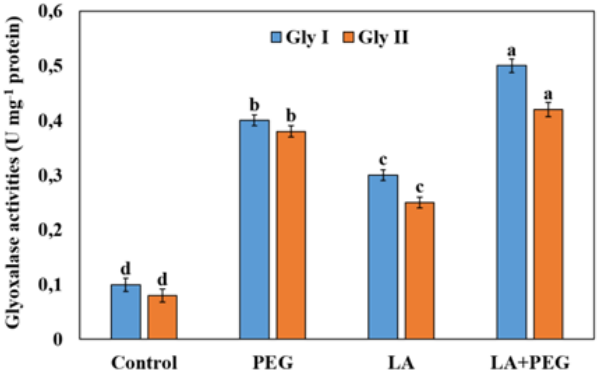

Figure 5. Effects of LA on glyoxalase system enzyme activities under osmotic stress: Gly I activity (a), Gly II activity (b). The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments.

LA-treated leaves compared to the control. Under PEG stress conditions, exogenous LA stimulated the gene expression of *GR* by 1.14-fold compared to the PEG alone (Figure 6c). The *MDHAR* gene expression level increased under PEG-induced osmotic stress compared to the control. The *MDHAR* gene was up-regulated 1.3-fold in the LA-treated leaves compared to the control. The gene expression increased 1.2-fold in the LA+PEG treatment compared to the PEG alone (Figure 6d). Exogenous LA under the non-stressed conditions contributed to the up-regulation in the expression of the *DHAR* gene by 1.2-fold in comparison to the control, while exogenous LA combined with PEG treatment up-regulated this gene by 1.1-fold over the PEG alone (Figure 6d).

3.6. The levels of gene expression of glyoxalase system enzymes

PEG-induced osmotic stress resulted in a significant increase in the relative expression of the *Gly I* gene by 2.5-fold over the control. The LA treatment contributed to a

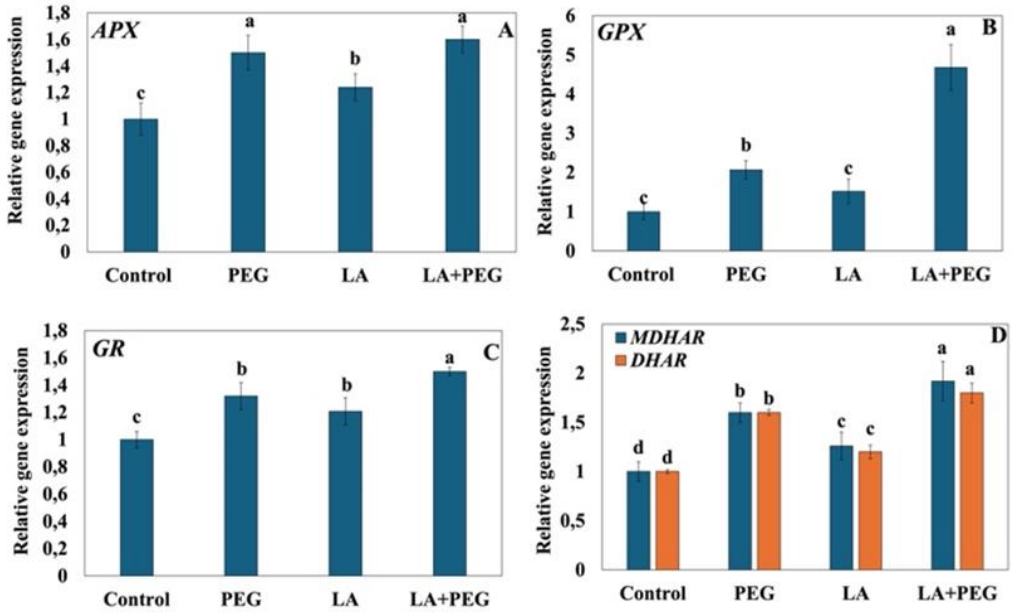

Figure 6. Effects of LA on the relative gene expressions of antioxidant enzymes under osmotic stress: *APX* gene (a), *GPX* gene (b), *GR* gene (c), *MDHAR* and *DHAR* genes. The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments.

2.0-fold upregulation in the expression of the *Gly I* gene compared to the control, while the LA+PEG treatment up-regulated this gene by 1.2-fold compared to the PEG treatment. The relative expression level of the *Gly II* gene showed a similar trend to that of the *Gly I* gene expression. The PEG treatment significantly up-regulated the expression of *Gly II* by 2.7-fold, while the LA treatment up-regulated this gene by 1.7-fold compared to the control. The LA treatment under osmotic stress up-regulated the expression of the *Gly II* gene by 1.4-fold in comparison with the PEG treatment (Figure 7).

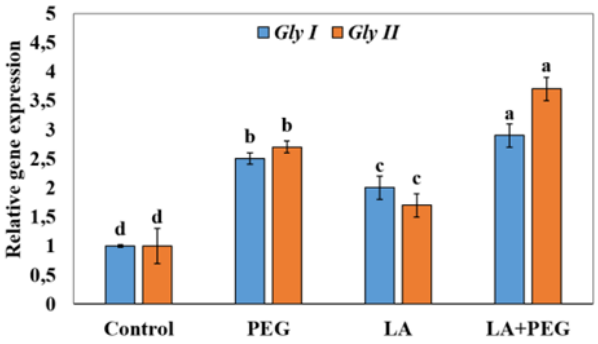

Figure 7. Effects of LA on the relative expression levels of some genes involved in the glyoxalase system under osmotic stress: *Gly I* gene (a), *Gly II* gene. The vertical bars reflect the standard deviations of three replicated means. At P <0.05, different letters denote significant differences among all treatments.

4. Discussions

It was suggested that one of the parameters that change in the plant under stress conditions and indicate the degree of water deficiency in the plant is the RWC (Flower and Ludlow, 1986). For this reason, in our study, the change in LRWC was determined to assess whether the plants were exposed to stress. It was determined that the LRWC decreased under stress conditions and exogenous LA alleviated the decrease in LRWC value. A similar study on maize reported that RWC decreased in the leaves exposed to drought (Goodarzian Ghahfarokhi et al., 2015). In the current study, the decrease in LRWC value in the LA-treated leaves was less than that of LA-untreated leaves, indicating that LA can protect plants against water loss. As another indicator of stress, leaf dry weight analyses were performed. LA treatment reduced dry weight loss in maize under PEG-induced osmotic stress. In previous investigations, LA improved water loss in maize leaves under osmotic stress (Sezgin et al., 2019; Saruhan Güler et al., 2021). Our present data showed that maintaining the efficacy of the antioxidant and glyoxalase system could reduce dry matter loss in maize during osmotic stress.

Under non-stressed conditions, various metabolic processes lead to ROS production. The production of toxic oxygen derivatives increases in response to abiotic or biotic stress (Mohammadkhani and Heidari, 2008). Under osmotic stress, the interaction between LA and ROS levels is not well known. In the current study, PEG-treated seedlings had the greatest H_2O_2 level indicating that endogenous ROS synthesis outpaced the ability of cellular antioxidant defense system to remove ROS (Liu et al., 2010). The rise in H_2O_2 levels induced oxidative stress, leading to enhanced membrane damage. However, LA application significantly reduced endogenous ROS levels by increasing enzymatic and non-enzymatic antioxidants such as AsA and GSH to enhance oxidative stress tolerance under both stress and control conditions. Our results on AsA and GSH content were consistent with the findings of Gorcek and Erdal (2015), who researched the effect of LA on salt-stressed wheat seedlings. The decrease in H_2O_2 content after LA application may be due to its ability to scavenge ROS as an antioxidant. Plants have efficient ROS-scavenging systems that keep them safe from harmful oxidative reactions. Antioxidant enzymes and chemicals play an important role in defensive systems.

Turk et al. (2018) found that applying LA dramatically lowered SOD, GPX, APX, CAT, and GR activities in wheat subjected to heavy metal stress. In our study, LA treatment increased APX, GPX, GR, MDHAR, and DHAR activities in seedlings under osmotic stress. Our findings confirmed previous research on salinity-stressed canola, wheat, and sorghum (Görçek and Erdal, 2015; Youssef et al., 2021; Khan et al., 2022). The decrease of H_2O_2 in seedlings treated with LA under both stressful and non-stressful conditions can be the outcome of increased APX activity compared to the control and PEG treatments. Similarly, another study showed that LA may have protective and antimutagenic effects against oxidants (especially H_2O_2) in sorghum plants through its antioxidant activity (Youssef et al., 2021). The application of LA+PEG significantly increased the activities of APX (1.2-fold) and GPX (1.3-fold) enzymes, which play a role in H_2O_2 scavenging, and GR (1.4-fold) and MDHAR (1.8-fold), compared to PEG treatment. GR is another important antioxidant enzyme that is vital in maintaining cellular redox balance (Dwivedi et al., 2020). In our study, maize seedlings subjected to osmotic stress exhibited a significant increase (1.4-fold) in GR activity after treatment with LA. This increase in GR activity promoted the reduction of glutathione disulfide to glutathione (GSH), indicating that GSH could be crucial for regulating redox balance and protecting cells from ROS-induced damage.

Apart from the enzyme activities, the relative expression levels of *APX*, *GPX*, *GR*, *MDHAR*, and *DHAR* genes were determined in LA-treated seedlings under unstressed and stressed conditions. The current study also determined that increased transcripts of many genes encoding antioxidant enzymes reflect genetic regulations. Moreover, it has been reported that increasing the activities of SOD, APX, and CAT caused by the application of LA, the expressions of their related genes were up-regulated in maize seedlings under osmotic stress (Gümrükçü Şimşek et al., 2024). For the first time, this study determined that LA treatment caused a significant increase of 2.3-fold in GPX gene expression under stress conditions. Similarly, we also found that GR and MDHAR enzyme activities showed notable increases of 1.4-fold and 1.8-fold, respectively.

Increased GR activity may facilitate the recycling of GSSG to GSH (Foyer and Halliwel, 1976). GSH and AsA levels increased in plants exposed to water loss (Ansari et al., 2017). D'Amico et al. (2004) discovered that AsA content increased but GSH content decreased in wheat watered with sea water over a 21-day growth period. Görcek and Erdal (2015) and Türk et al. (2018) found that exogenous LA boosted AsA and GSH levels in wheat after abiotic stress.

Our findings demonstrated that under stressful and non-stressful conditions, LA increased the accumulation of AsA and GSH, improving ROS scavenging effectiveness and reducing oxidative damage caused by ROS. The data supported that LA may operate as a signalling molecule, boosting antioxidant enzyme activity in osmotically stressed-maize seedlings.

GSH is not only the main compound of the AsA-GSH cycle but also plays an important function in the MG detoxification system, and GSH plays a role as a compound that connects these two systems (antioxidant and glyoxalase). It was reported that the antioxidant defense and glyoxalase system of plant cells work in coordination to alleviate oxidative stress by reducing toxic ROS and MG accumulation (Hasanuzzaman et al., 2019). However, the exact mechanism by which exogenous protective agent applications affect these two defense systems remains unclear. In our study, it has been determined that LA reduced the MG content, which increases under the PEG-induced stress. Also, it has been detected that Gly I and Gly II activities, included in the glyoxalase system that effectively reduce MG content, and the relative expression levels of the *Gly I* and *Gly II* genes were stimulated with LA application. The Gly I and Gly II activities increased 1.3 and 1.1-fold, respectively, in LA+PEG treatment compared to the PEG treatment. Moreover, under non-stressed conditions, LA treatment stimulated *Gly I* (2.0-fold) gene expression more than *Gly II* (1.7-fold). On the contrary, external LA application stimulated *Gly II* (1.4-fold) gene expression under stressed conditions more than *Gly I* (1.2-fold), providing osmotic stress tolerance. As a result, it has been revealed that the LA treatment induced the glyoxalase system, which includes the Gly I and Gly II, can keep the oxidative stress trigger MG under control, where GSH is used by Gly I and recycled after MG detoxification. The glyoxalase system also participates in the AsA-GSH cycle via GSH. Our study determined that increasing the GSH level by LA was effective in alleviating oxidative stress damage in maize seedlings.

In conclusion, under both non-stressful and stressful conditions, exogenous application of LA showed enhanced tolerance to oxidative damage by enhancing ROS and MG detoxification systems. Thus, an interaction relationship between ROS and MG detoxifying systems in maize seedlings treated with LA under osmotic stress was considered. The increased GSH in LA-treated seedlings was a cofactor for the glyoxalase system. As a result, this system not only detoxified MG, but also helped to preserve GSH homeostasis and subsequent ROS detoxification. In our study, we also revealed for the first time which enzymes related to these systems were more affected by exogenous LA. Under non-stress conditions, LA treatment significantly increased the activities of GR (1.5-fold), DHAR (1.5-fold), Gly I (3.0-fold), and Gly II (3.1-fold) enzymes, as well as the relative expression of *Gly I* (2.0-fold) and *Gly II* (1.7-fold) genes. Under stressful conditions, it was revealed that LA application stimulated GR (1.4-fold) and MDHAR (1.8-fold) enzyme activities, as well as the relative gene expressions of *GPX* (2.3-fold) and *Gly II* (1.4-fold), to a greater extent. The current study demonstrated that LA activated these two systems in a coordinated manner under both non-stressful and stressful conditions. The activated antioxidant and glyoxalase defense systems played a significant role in the osmotic stress tolerance of LA-treated maize seedlings through ROS and MG detoxification. It is critical to learn more about the mechanisms that drive osmotic stress tolerance induced by exogenous LA in plants and their responses. The varying responses to LA treatments, especially the significant benefits seen under non-stress conditions and escalated with increased LA concentrations, align with the findings of Navari-Izzo et al. (2002) and Saruhan Guler et al. (2021). These studies emphasize LA's powerful antioxidant properties, which help alleviate oxidative stress caused by osmotic stress and promote plant growth in challenging conditions.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

ASM and RT contributed to the study's conception and design. ASM conducted the experiments. ASM and AK analyzed all data and wrote the manuscript.

Acknowledgements

This study was funded by the Scientific and Technological Research Council of Türkiye (TUBITAK) Grant No 123Z653.

References

Altansambar N, Sezgin Muslu A, Kadıoglu A (2024). The combined application of rutin and silicon alleviates osmotic stress in maize seedlings by triggering accumulation of osmolytes and antioxidants' defense mechanisms. Physiology and Molecular Biology of Plants 30(3): 513-525. https://doi.org/10.1007/s12298-024-01430-z.

Anjum SA, Ashraf U, Zohaib A, Tanveer M, Naeem M, Iftikhar ALI, Tabassum T, Nazir U (2017). Growth and developmental responses of crop plants under drought stress: a review. Zemdirbyste-Agriculture 104: 267-276.

Ansari WA, Atri N, Singh B, Pandey S (2017). Changes in antioxidant enzyme activities and gene expression in two muskmelon genotypes under progressive water stress. Biologia Plantarum 61(2): 333-341. https://doi.org/10.1007/s10535-016-0694-3.

Ashraf MFMR, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59(2): 206-216.

Bookout AL, Mangelsdorf DJ (2003). Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways. Nuclear Receptor Signal 1:1-7. doi:10.1621/nrs.01012.

Bradford MM (1976) A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Castillo FJ (1996). Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia 107: 469-477.

Daler S, Kaya O (2024). Exogenous alpha-lipoic acid treatments reduce the oxidative damage caused by drought stress in two grapevine rootstocks. Physiologia Plantarum 176(4), e14437.

D'Amico ML, Navari-Izzo F, Sgherri C, Izzo R (2004). The role of lipoic acid in the regulation of the redox status of wheat irrigated with 20% sea water. Plant Physiolgy and Biochemistry 42(4): 329-334. doi: 10.1016/j.plaphy.2004.02.009.

Daudi A, O'brien JA (2012). Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves. Bio-Protocol 2(18): e263-e263.

Dwivedi S, Kumar A, Mishra S, Sharma P, Sinam G, Bahadur L, Tripathi RD (2020) Orthosilicic acid (OSA) reduced grain arsenic accumulation and enhanced yield by modulating the level of trace element, antioxidants, and thiols in rice. Environ Sci Pollut Res 27:24025-24038

Elkelish A, El-Mogy MM, Niedbała G, Piekutowska M, Atia MA, Hamada MM, Ibrahim, MF (2021). Roles of exogenous α-lipoic acid and cysteine in mitigation of drought stress and restoration of grain quality in wheat. Plants 10(11): 2318-2343.

Farhad W, Saleem MF, Cheema MA, Hammad HM (2009). Effect of poultry manure levels on the productivity of spring maize (*Zea mays* L.). Journal Animal and Plant Science 19: 122-125.

Flower DJ, Ludlow MM (1986). Contribution of osmotic adjustment to the dehydration tolerance of water stressed pigeonpea (*Cajanus cajan* L.) Millsp. leaves. Plant Cell Environment 9: 33-40.

Foyer CH, Halliwell B (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133: 21-25.

Goodarzian Ghahfarokhi M, Mansurifar S, Taghizadeh-Mehrjardi R, Saeidi M, Jamshidi AM, Ghasemi E (2015). Effects of drought stress and rewatering on antioxidant systems and relative water content in different growth stages of maize (*Zea mays* L.) hybrids. Archives of Agronomy and Soil Science 61(4): 493-506.

Gorcek Z, Erdal S (2015). Lipoic acid mitigates oxidative stress and recovers metabolic distortions in salt-stressed wheat seedlings by modulating ion homeostasis, the osmo-regulator level and antioxidant system. Journal of the Science of Food and Agriculture 95:2811-2817.

Gümrükçü Şimşek SD, Terzi R, Saruhan Güler N (2024). Lipoic acid can provide homeostasis of reactive oxygen species and induce ascorbate and glutathione biosynthesis in osmotic stressed maize. Russian Journal of Plant Physiology 71. https://doi.org/10.1134/S1021443724604373

Hamdia MA, Shaddad MAK (2010). Salt tolerance of crop plants. a review. Journal of Stress Physiology and Biochemistry 6:64-90.

Hasanuzzaman M, Hossain MA, Fujita M (2011). Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. Plant Biotechnology Reports 5: 353-365. https://doi.org/10.1007/s11816-011-0189-9.

Hasanuzzaman M, Nahar K, Hossain MS, Mahmud JA, Rahman A, Inafuku M, Oku H, Fujita M (2017). Coordinated actions of glyoxalase and antioxidant defense systems in conferring abiotic stress tolerance in plants. International Journal of Molecular Science 18: 200-228. doi: 10.3390/ijms18010200.

Hasanuzzaman M, Bhuyan MB, Anee TI, Parvin K, Nahar K, Mahmud JA, Fujita M (2019). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants 8(9): 384-434. https://doi.org/10.3390/antiox8090384.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. Circ Calif Agric Exp Stn 347(2nd edit):32

Hossain MA, Nakano Y, Asada K (1984). Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiology 25:385-95.

Hossain MA, Asada K (1984). Purification of dehydroascorbate reductase from spinach and its characterization as a thiol enzyme. Plant Cell Physiology 25: 85-92.

Hossain, MA, Hasanuzzaman M, Fujita M (2010). Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiology and Molecular Biology of Plants 16: 259-272.

Junaid MD, Öztürk ZN, Gökçe AF (2023). Drought stress effects on morphophysiological and quality characteristics of commercial carrot cultivars. Turkish Journal of Botany 47(2): 111-126.

Kadıoğlu A, Saruhan N, Sağlam A, Terzi R, Acet T (2011). Exogenous salicylic acid alleviates effects of long term drought stress and delays leaf rolling by inducing antioxidant system. Plant Growth Regulation 64: 27-37.

Khan R, Ma X, Hussain Q, Asim M, Iqbal A, Ren X, Shah S, Shi Y (2022). Application of 2, 4-epibrassinolide improves drought tolerance in tobacco through physiological and biochemical mechanisms. Biology 11(8):1192-1213. doi: 10.3390/biology11081192.

Krasensky J, Jonak C (2012). Drought, salt and temperatures tress-induced metabolic rearrangements and regulatory networks. The Journal of Experimental Botany 63:1593-1608.

Liso R, Calabrese G, Bitonti MB, Arrigoni O (1984). Relationship between ascorbic acid and cell division. Experimental Cell Research 150:314-320.

Liu X, Williams, CE, Nemacheck JA, Wang H, Subramanyam S, Zheng C, Chen MS (2010). Reactive oxygen species are involved in plant defense against a gall midge. Plant physiology 152(2): 985-999.

Mohammadkhani N, Heidari R (2008). Water stress induced by polyethylene glycol 6000 and sodium chloride in two corn cultivars. Pakistan Journal of Biological Sciences 11(1): 92-97.

Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015a). Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methylglyoxal detoxification system. Environment Experimental Botany 112: 44- 54.

Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015b). Glutathione-induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. AoB PLANTS 7: plv069. https://doi.org/10.1093/aobpla/plv069

Nahar K, Hasanuzzaman M, Alam MM, Fujita MJBP (2015c). Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. Biologia plantarum 59: 745-756.

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology 22(5): 867-880.

Navari-Izzo F, Quartacci MF, Sgherri C (2002). Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. Plant Physiology and Biochemistry 40(6-8): 463-470.

Principato GB, Rosi G, Talesa V, Giovannini E, Uotila L (1987) . Purification and characterization of two forms of glyoxalase II from the liver and brain of wistar rats. Biochimica et Biophysica Acta 911: 349-355.

Ramadan KM, Alharbi MM, Alenzi AM, El-Beltagi HS, Darwish DBE, Aldaej MI, Ibrahim MF (2022). Alpha lipoic acid as a protective mediator for regulating the defensive responses of wheat plants against sodic alkaline stress: physiological, biochemical and molecular aspects Plants 11(6): 787-803. https://doi.org/10.3390/plants11060787.

Saruhan Guler N, Ozturk K, Sezgin A, Altuntas C, Kadioglu A, Terzi R (2021). Alpha lipoic acid application promotes water-deficit tolerance by modulating osmoprotectant metabolism-related genes in maize. Russian Journal of Plant Physiology 68(6): 1152-1160.

Sezgin A, Altuntaş C, Demiralay M, Cinemre S, Terzi R (2019). Exogenous alpha lipoic acid can stimulate photosystem II activity and the gene expressions of carbon fixation and chlorophyll metabolism enzymes in maize seedlings under drought. Journal of Plant Physiology 232: 65-73.

Sezgin Muslu A, Kadıoğlu A (2021). The antioxidant defense and glyoxalase systems contribute to the thermotolerance of *Heliotropium thermophilum*. Functional Plant Biology 48(12): 1241-1253.

Sezgin Muslu A (2024). Improving salt stress tolerance in *Zea mays* L. by modulating osmolytes accumulation and antioxidant capacity with Rutin. Anatolian Journal of Botany, 8(1): 21-29.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2011: 1-26. https://doi.org/10.1155/2012/217037.

Singh P, Dhaka N (2016). Glyoxalase system and salinity stress in plants. In Managing salt tolerance in plants: molecular and genomic perspectives (pp. 173-185). London: CRC Press.

Sudesh V, Linda L, Pawan S (2002). Nutrition and Hypertension. Journal Nutrition Research 22: 111-123.

Talaat NB, Ibrahim AS, Shawky BT (2022). Enhancement of the expression of ZmBZR1 and ZmBES1 regulatory genes and antioxidant defense genes triggers water stress mitigation in maize (*Zea mays* L.) plants treated with 24-epibrassinolide in combination with spermine. Agronomy 12(10): 2517.

Tanyolac D, Ekmekçi Y, Ünalan Ş (2007). Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. Chemosphere 67(1): 89-98.

Taylor NL, Day DA, Millar AH (2004). Targets of stress-induced oxidative damage in plant mitochondria and their impact on cell carbon/nitrogen metabolis. Journal of Experimental Botany 55: 1-10.

Terzi R, Saruhan GN, Güven FG, Kadioglu A (2018). Alpha lipoic acid treatment induces the antioxidant system and ameliorates lipid peroxidation in maize seedlings under osmotic stress. Archives of Biological Sciences 70(3): 503-511.

Tietze F (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Analytical Biochemistry 27(3): 502-522.

Turk H, Erdal S, Karayel U, Dumlupinar R (2018). Attenuation of lead toxicity by promotion of tolerance mechanism in wheat roots by lipoic acid. Cereal Research Communications 46: 424-435. https://doi.org/10.1556/0806.46.2018.020

Urbanek H, Kuzniak-Gebarowska E, Herka K (1991). Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Physiologia Plantarum 13(1): 43-50.

Yadav SK, Singla-Pareek SL, Ray M, Reddy MK, Sopory SK (2005). Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. Biochemical and Biophysical Research Communications 337: 61-67.

Youssef MH, Raafat A, El-Yazied AA, Selim S, Azab E, Khojah E, El Nahhas N, Ibrahim MFM (2021). Exogenous application of alpha-lipoic acid mitigates salt-induced oxidative damage in sorghum plants through regulation growth, leaf pigments, ionic homeostasis, antioxidant enzymes, and expression of salt stress responsive genes, Plants (Basel) 10(11): 2519-2535. doi: 10.3390/plants10112519.

Zaid A, Mohammad F, Wani SH, Siddique KM (2019). Salicylic acid enhances nickel stress tolerance by up-regulating antioxidant defense and glyoxalase systems in mustard plants. Ecotoxicology and Environmental Safety 180: 575-587.

Zhao L, Hu Q, Huang Y, Keller AA (2017). Response at genetic, metabolic, and physiological levels of maize (*Zea mays*) exposed to a Cu $(OH)_2$ nanopesticide. ACS Sustainable Chemistry & Engineering 5(9): 8294-8301.