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Research article

Regulatory role of methylglyoxal in osmotic stress adaptation: Insights into the antioxidant defense, glyoxalase system, and osmoprotectant metabolism in maize seedlings

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Metilglioksalın ozmotik stres adaptasyonundaki düzenleyici rolü: Mısır fidelerinde antioksidan savunma, glioksalaz sistemi ve ozmoprotektan metabolizmasına ilişkin görüşler

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Abstract: Methylglyoxal (MG) functions in plants as both a cytotoxic byproduct and a signaling molecule that regulates responses to abiotic stress. To investigate its dose-dependent effects, maize seedlings were subjected to polyethylene glycol (PEG)-induced osmotic stress combined with different MG concentrations (5, 10, 25, and 35 mM). Measurements included relative water content, oxidative stress markers, proline, polyamines, and antioxidant enzyme activities (SOD, CAT, APX, GPX), as well as glyoxalase (Gly I, Gly II) enzyme activities, along with the expression of related genes. Results showed that low MG levels, particularly 10 mM, significantly improved RWC, enhanced antioxidant and glyoxalase activities, upregulated genes involved in antioxidant defense and osmoprotectant biosynthesis, while downregulating genes related to proline and polyamine catabolism. These changes reduced oxidative damage and promoted the accumulation of osmoprotectants. By contrast, higher MG doses increased oxidative stress, MG accumulation, and reduced stress tolerance. Overall, MG exhibited a concentration-dependent dual role in osmotic stress tolerance: acting as a beneficial signaling molecule at low levels but exerting toxicity at higher concentrations. This study highlights MG's regulatory role in stress adaptation and provides a theoretical basis for its potential application in improving drought resilience in crops.

Key words: Methylglyoxal, osmotic stress, *Zea mays*, polyamine, antioxidant and glyoxalase system

Özet: Metilglioksal (MG), bitkilerde hem sitotoksik bir yan ürün hem de abiyotik strese verilen yanıtları düzenleyen bir sinyal molekülü olarak işlev görür. Doza bağlı etkilerini araştırmak için mısır fideleri, farklı MG konsantrasyonları (5, 10, 25 ve 35 mM) ile birleştirilmiş polietilen glikol (PEG) kaynaklı ozmotik strese tabi tutuldu. Ölçümler, nisbi su içeriği, oksidatif stres belirteçleri, prolin, poliaminler ve antioksidan enzim aktiviteleri (SOD, CAT, APX, GPX) ile glioksalaz (Gly I, Gly II) enzim aktivitelerinin yanı sıra ilgili genlerin ekspresyonunu da içeriyordu. Sonuçlar, özellikle 10 mM olan düşük MG seviyelerinin, RWC'yi önemli ölçüde iyileştirdiğini, antioksidan ve glioksalaz aktivitelerini artırdığını, antioksidan savunma ve ozmoprotektan biyosentezinde yer alan genleri yukarı düzenlerken, prolin ve poliamin katabolizmasıyla ilgili genleri aşağı düzenlediğini gösterdi. Bu değişiklikler oksidatif hasarı azalttı ve ozmoprotektanların birikimini destekledi. Buna karşılık, daha yüksek MG dozları oksidatif stresi ve MG birikimini artırmış ve stres toleransını azaltmıştır. Genel olarak, MG, ozmotik stres toleransında konsantrasyona bağlı ikili bir rol sergilemiştir: düşük seviyelerde faydalı bir sinyal molekülü görevi görürken, daha yüksek konsantrasyonlarda toksisite yaratmaktadır. Bu çalışma, MG'nin stres adaptasyonundaki düzenleyici rolünü vurgulamakta ve bitkilerde kuraklığa dayanıklılığı artırmadaki potansiyel uygulaması için teorik bir temel sunmaktadır.

Anahtar Kelimeler: Metilglioksal, ozmotik stres, *Zea mays*, poliamin, antioksidan ve glioksalaz sistemi

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1. Introduction

Drought stress is one of the most critical environmental factors limiting plant growth and productivity worldwide. It triggers extensive physiological and metabolic changes, disrupting normal growth and development and ultimately leading to stunted growth and yield losses. To cope with such stress, plants accumulate various metabolites, including soluble sugars, proline, and polyamines. These metabolites function as osmolytes, antioxidants, and scavengers, thereby maintaining cellular homeostasis and enhancing tolerance (Ozturk et al., 2021).

Proline accumulation is one of the most well-documented plant responses to abiotic stress. It contributes to osmotic adjustment, ROS scavenging, and stress tolerance (Pál et al., 2018). Proline is synthesized mainly via the glutamate and ornithine pathways, where pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) play key roles (Sekhar et al., 2007). Its degradation, in contrast, is mediated by proline dehydrogenase (ProDH) (Kishor et al., 2005). Similarly, polyamines (PAs) such as putrescine, spermidine, and spermine are another important group of osmotic compounds. They contribute to drought tolerance through osmotic adjustment, membrane stability, and free radical scavenging (Fariduddin et al., 2014). Their biosynthesis is driven by ornithine decarboxylase (ODC), arginine decarboxylase (ADC), and S-adenosylmethionine decarboxylase (SAMDC), while their catabolism is regulated by diamine oxidase (CuAO) and polyamine oxidase (PAO) (Sun et al., 2020).

Alongside osmolyte accumulation, drought stress is associated with the overproduction of reactive oxygen species (ROS), which cause oxidative damage to membranes, proteins, and nucleic acids (Seleiman et al., 2021). This oxidative injury critically impairs energy production and key biochemical processes in plants. Through prolonged natural selection, plants have evolved various defense and adaptation mechanisms to cope with drought stress. Malondialdehyde (MDA), a by-product of lipid peroxidation in cell membranes, serves as an indicator of the extent of lipid peroxidation and the plant's response to stress conditions (Zhang et al., 2021). In this study, lipid peroxidation was evaluated using the TBARS assay, which provides an indirect estimation of MDA levels. To counteract oxidative damage, plants activate a robust antioxidative defense system involving several key enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR). This network plays a critical role under drought conditions by efficiently scavenging ROS and maintaining cellular homeostasis (Hasanuzzaman et al., 2020; Kolupaev et al., 2024)

Stress-related signaling events in plants are regulated not only by ROS (Terzi et al., 2014) but also by other signaling molecules, such as reactive nitrogen species (RNS), and reactive carbonyl species (RCS) (Hossain et al., 2015; Fancy et al., 2017; Mittler, 2017). Recent studies have shown that RCS, primarily generated through ROS-driven lipid peroxidation, can play a role in signal transduction by interacting with other signaling compounds (Biswas and Mano, 2015; Islam et al., 2016). Among these, MG, a specific type of RCS, has recently been identified as a signaling molecule in plants (Hoque et al., 2016). MG is a by-product generated during the metabolism of carbohydrates, fatty acids, and proteins within the cytosol, mitochondria, and chloroplasts (Kaur et al., 2016; Shimakawa et al., 2018). Under abiotic stress, MG levels grow, causing damage to the cell's ultrastructural components, mutation, and, ultimately, programmed cell death. MG-detoxifying enzymes are divided into two categories: GSH-dependent and GSH-independent. The GSH-dependent glyoxalase pathway detoxifies MG to a harmless molecule by two consecutive processes mediated by glyoxalase I (Gly I) and glyoxalase II (Gly II). In the first phase, GSH transforms MG to S-D-lactoylglutathione (SLG), and in the final step, Gly II introduces GSH into the system and hydrolyzes SLG to produce D-lactate. Thus, MG detoxification in plants maintains GSH balance. In the GSH-independent process, glyoxalase III (Gly III) converts MG to D-lactate in a single step without using GSH (Zheng et al., 2024). During normal growth conditions, MG concentration varies in different plant species, and it can increase 2- to 6-fold in response to salinity, drought, and cold stress conditions (Li et al. 2017; Shumilina et al., 2019). Excessive accumulation of MG leads to toxic effects, impairing cellular function and proliferation. MG also disrupts the structure and stability of amino acids and nucleic acids, resulting in the formation of advanced glycation end-products (Shumilina et al., 2019). To mitigate the toxicity of MG, plants have evolved an efficient glyoxalase system for detoxification, involving two key enzymes: glyoxalase I (Gly I) and glyoxalase II (Gly II). Interestingly, MG also exhibits a dual role in plants. While high concentrations are toxic, low concentrations act as signaling molecules that influence stress responses. Recent evidence indicates that MG pretreatment can enhance heat tolerance in maize seedlings and improve frost hardiness in wheat (Wang et al., 2019; Majláth et al., 2020). Despite many reports detailing plant tolerance mechanisms to osmotic stress, the specific responses induced by MG pretreatment under such stress conditions are not still obvious. In particular, little is known about the limited effects of MG treatment on osmolyte accumulation, especially proline (Li et al., 2017) and its negligible impact on polyamine content and the expression of genes related to proline and polyamine metabolism under osmotic stress. To address this gap, the present study aimed to investigate whether MG treatment enhances osmotic stress tolerance in maize seedlings, with particular attention to changes in key metabolites such as proline and polyamines, and the activation of antioxidant and glyoxalase defense systems. We hypothesize that exogenous MG application modulates maize seedling responses to osmotic stress by enhancing tolerance through the activation of antioxidant and glyoxalase systems and the regulation of osmoprotectant metabolism.

2. Materials and Method

2.1. Plant growth and treatments

Maize (*Zea mays* L.) seeds were cultivated hydroponically in Hoagland nutrient solution (Hoagland and Arnon, 1950) inside a controlled growth chamber maintained at 18–20 °C with 50–70% relative humidity and a light intensity of 400–430 µmol m^{-2} s^{-1}. After 22 days of growth, the plants were subjected to foliar application of methylglyoxal (MG) at concentrations of 0, 5, 10, 25, and 35 mM (Sigma-Aldrich, CAS No: 78-98-8), following the procedure of Majláth et al. (2020) and Lin et al. (2023). Each plant received 10 mL of solution containing Tween-20 (0.1% (v/v), BioShop, CAS No: 56-40-6) as a surfactant. The foliar treatments were applied twice at three-day intervals under the same environmental conditions. After MG application, osmotic stress was induced by exposing 26-day-old seedlings to 10% polyethylene glycol (PEG 6000) dissolved in nutrient solution for 72 h (Gumrukcu Simsek et al., 2024). Upon completion of the treatments, leaf samples were harvested and immediately stored at –80 °C in an ultra-low freezer for subsequent physiological, biochemical, and molecular analyses.

2.2. Leaf relative water content (LRWC)

The RWC of leaves was determined as described by Castillo (1996). Fresh leaf weight was first recorded, after which samples were immersed in distilled water overnight to obtain their turgid weight. The samples were then oven-dried at 80 °C to a constant weight, and dry weight was recorded. LRWC (%) was calculated using the following equation:

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

2.3. Determination of thiobarbituric acid-reactive substances (TBARS)

TBARS content in 0.1 g of fresh leaf tissue was evaluated according to Heath and Packer (1968). Samples were homogenized in 1.8 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 15 000 × g for 5 min. One milliliter of the supernatant was mixed with 4 mL of 0.5%

thiobarbituric acid prepared in 20% TCA. The mixture was heated at 95 °C for 30 min, rapidly cooled in an ice bath, and centrifuged at 10 000 × g for 10 min. Absorbance readings were recorded at 532 nm and 600 nm, and TBARS concentration was computed accordingly.

2.4. Determination of hydrogen peroxide (H_2O_2) content

Hydrogen peroxide content was quantified following Velikova et al. (2000). Leaf samples were ground with activated charcoal in TCA and centrifuged; 1 mL of the supernatant was mixed with 10 mM potassium phosphate buffer and 1 M potassium iodide. The absorbance of the resulting solution was measured at 390 nm.

2.5. Determination of methylglyoxal (MG) content

The quantification of MG was performed as described by Yadav et al. (2005). Leaf tissues (0.1 g) were homogenized in perchloric acid and centrifuged at 16 000 × g for 15 min. The resulting supernatant was neutralized with saturated potassium carbonate at room temperature for 15 min, followed by a second centrifugation under the same conditions. The final supernatant was reacted with 1 mL of 1,2-diaminobenzene in perchloric acid, and the derivatized MG concentration was determined spectrophotometrically at 335 nm after 25 min.

2.6. Osmolyte content assays

Proline determination was performed according to Carillo and Gibon (2011). Fresh tissue (0.1 g) was homogenized in ethanol:water (40:60, v/v), incubated overnight at 4 °C, and centrifuged at 15 000 × g for 10 min. One milliliter of supernatant was mixed with a reaction solution containing 1% (w/v) ninhydrin in 60% acetic acid and 20% ethanol. The samples were incubated in a water bath at 100 °C for 1 h, cooled to room temperature, and absorbance was measured at 520 nm.

Polyamine quantification was carried out via high-performance liquid chromatography (HPLC) according to Ben-Gigirey et al. (1998). Approximately 5 g of leaf tissue was homogenized in 10 mL of 0.4 M perchloric acid, centrifuged at 3000 × g for 12 min at 4 °C, and the supernatant was adjusted to a final volume of 25 mL. One milliliter of the extract was derivatized with dansyl chloride, filtered through a 0.45 μm syringe filter, and analyzed using a Shimadzu LC 2030C 3D Plus HPLC system (Japan) equipped with a UV–VIS detector. Separation was achieved on a Purospher STAR RP-18 end-capped column (5 μm, 250 × 4 mm) under a 35-min gradient elution with ammonium acetate and acetonitrile at 1 mL min^{-1}. Injection volume was 10 μL, and the derivatized polyamines were detected at 254 nm.

2.7. Determination of antioxidant enzyme activities

Fresh leaves (0.1 g) were homogenized in extraction buffer (K_2HPO_4, pH 7.0; EDTA; PVPP; and ascorbate for APX), and the supernatant obtained after centrifugation was used for enzyme and protein analyses (Sezgin Muslu & Kadıoğlu, 2025). Protein content was measured using the Bradford method (Bradford, 1976).

SOD activity was assayed following Beauchamp and Fridovich (1971) by exposing the reaction mixture to white light and recording absorbance at 560 nm. CAT activity was determined according to Aebi (1983) by monitoring H_2O_2 decomposition at 240 nm. APX activity was measured as described by Nakano and Asada (1981) based on the decrease in absorbance at 290 nm. GPX activity was evaluated following Urbanek et al. (1991) by tracking absorbance at 470 nm.

2.8. Determination of glyoxalase system enzyme activities

Enzymes of the glyoxalase pathway were extracted by homogenizing 0.1 g of leaf tissue in potassium phosphate buffer (pH 7.0) containing KCl, ascorbate, β-mercaptoethanol, and glycerol. The homogenate was centrifuged at 13 000 × g for 10 min, and the supernatant was used for enzyme assays. Gly I activity was determined according to Hasanuzzaman et al. (2011) by initiating the reaction with MG and recording the increase in absorbance at 240 nm for 1 min. Gly II activity was assayed by measuring the formation of GSH at 412 nm for 1 min, following Principato et al. (1987).

2.9. Quantitative Real-Time PCR (qRT-PCR) analyses

Total RNA was extracted from freshly ground leaf tissues using a commercial RNA isolation kit according to the manufacturer's protocol. RNA quality and quantity were determined using a Nanodrop spectrophotometer. cDNA was synthesized from total RNA using the cDNA transcription kit (Applied Biosystems). qRT-PCR analyses were conducted on a CFX Connect Real-Time PCR System using iTaq Universal SYBR Green Supermix and gene-specific primers. The primers used for quantifying expression levels of antioxidant enzymes, glyoxalase system components, and genes involved in proline and polyamine metabolism were listed in Table 1. Relative expression levels were normalized to the *β-Actin* reference gene, and fold changes were calculated using the $2^{-\Delta\Delta CT}$ method (Bookout and Mangelsdorf, 2003).

Data analysis and statistics

All experiments were carried out in triplicate, and the results are reported as mean ± standard error. Statistical analyses were conducted using one-way ANOVA in SPSS (IBM v23), with significance set at $P < 0.05$. Relative qRT-PCR gene expression levels were analyzed using Bio-Rad CFX Manager 3.1 software

3. Results

3.1. Physiological and oxidative stress markers in response to MG under PEG stress

PEG treatment decreased the leaf RWC compared to the untreated control seedlings. Furthermore, compared with the PEG group, leaf RWC was found to increase in 5 and 10 mM MG-applied plants under PEG stress, while it was found to decrease in 25 and 35 mM MG-applied plants. Additionally, the highest increase was found in seedlings treated with 10 mM MG under PEG stress, while the highest decrease was determined in seedlings treated with 35 mM MG (Fig. 1a).

The TBARS level content of plants subject to PEG stress was greater than that of untreated control seedlings. Also, compared with the PEG group, the TBARS level was found to decrease in 5 and 10 mM MG-applied plants under PEG stress, while it was found to increase in 25 and 35 mM MG-applied plants. Additionally, the highest decrease was found in seedlings treated with 10 mM MG under PEG

Table 1. The sequences of specific primers of genes used for qRT-PCR

Gene	Sequences 5'-3'	Accession no	References
B-Actin 1(ACT1)	F: ACCAGTTGTTCGCCCACTAG R: GAAGATCACCCTGTGCTGCT	XM_008656735.3	Gümrükçü Şimşek et al., 2024
Superoxide dismutase (SOD)	F: TTGTTGCAAATGCTGAGGGC R: AGGCAAGGATGTAACAGCGT	NM_001114655.1	Gümrükçü Şimşek et al., 2024
Catalase 1(CAT1)	F: TGCTTTCTGCCCAGCGATTA R: CACTTCTCACGACAGCCTGT	NM_001254879.2	Gümrükçü Şimşek et al., 2024
Ascorbate peroxidase 1(APX1)	F: GCCTTCTTCAGCTCCCAAGT R: TGCAAAAGACCACATGCAGC	NM_001159274.2	Gümrükçü Şimşek et al., 2024
Glutathione peroxidase (GPX)	F: CGCTATGCTCCAACCACTTC R: GCTCTCAGAGCAATGTTCATACAG	GRMZM5G884600	Zhao et al., 2017
Glyoxalase I (Gly I)	F: TGAGGCAGTTGATCTGGCG R: CCCGAGTCTTCACTGTAGTTCC	NC_050105.1	Sezgin Muslu and Terzi, 2025
Glyoxalase II (Gly II)	F: CACATGGATGTTTGCTGGTC R: CGTGCATCATCAAAATGGTC	NM_001153376	Sezgin Muslu and Terzi, 2025
Pyrroline 5-carboxylate synthase (P5CS1)	F: AACATCTTGCCCTCTGGGTG R: CCATTGCCACTTCGAACTGC	DQ864376.1	Sezgin Muslu et al., 2022
Proline dehydrogenase (ProDH1)	F: TCAGCAAGTACCTGCCGTAC R: ACCCTCCTCACCAACTCCTT	NM_001154105.1	Sezgin Muslu et al., 2022
Arginine decarboxylase (ADC)	F: GACATCACCTGCGACAGTGA R: GAACAGGTTGTGCTTGCCAG	EU968980.1	Sezgin Muslu et al., 2022
Polyamine oxidase (PAO1)	F: CGCTACGAATACGACCAGCT R: TGGGCGCAGTTGATGAGAAT	NM_001111636.1	Sezgin Muslu et al., 2022

stress, while the highest increase was determined in seedlings treated with 35 mM MG (Fig. 1b).

PEG-stressed plants had higher H_2O_2 levels than untreated control seedlings. Furthermore, when compared to the PEG group, the H_2O_2 levels in 5 and 10 mM MG-applied plants decreased under PEG stress, whereas they increased in 25 and 35 mM MG-applied plants. Furthermore, during PEG stress, seedlings treated with 10 mM MG showed the greatest decline, while seedlings treated with 35 mM MG had the greatest rise (Fig. 1c).

MG levels were greater in PEG-stressed plants than in untreated control seedlings. Additionally, during PEG stress, the MG levels in plants treated with 5 and 10 mM MG dropped in comparison to the PEG group, but they rose in plants treated with 25 and 35 mM MG. Additionally, seedlings treated with 10 mM MG exhibited the biggest decrease during PEG stress, whereas seedlings treated with 35 mM MG exhibited the greatest increase (Fig.1d).

3.2. Changes in antioxidant capacity in response to MG under PEG stress

PEG stress significantly increased SOD activity compared to the control without MG application. However, SOD activity was found to increase gradually with increasing MG concentrations under PEG stress compared to the control without PEG stress (Fig. 2a). While the increased CAT and APX activities with PEG stress compared to the control were further increased with the application of 10, 25, and 35 mM MG, no statistically meaningful difference was detected in the CAT and APX activities of the seedlings treated with 5 mM MG and PEG alone (Fig. 2b,c). It was found that GPX activity increased more with different concentrations of MG applied under PEG stress compared to the PEG group. However, no statistically significant difference was found between the GPX activities of seedlings treated with 25 and 35 mM MG (Fig.2d).

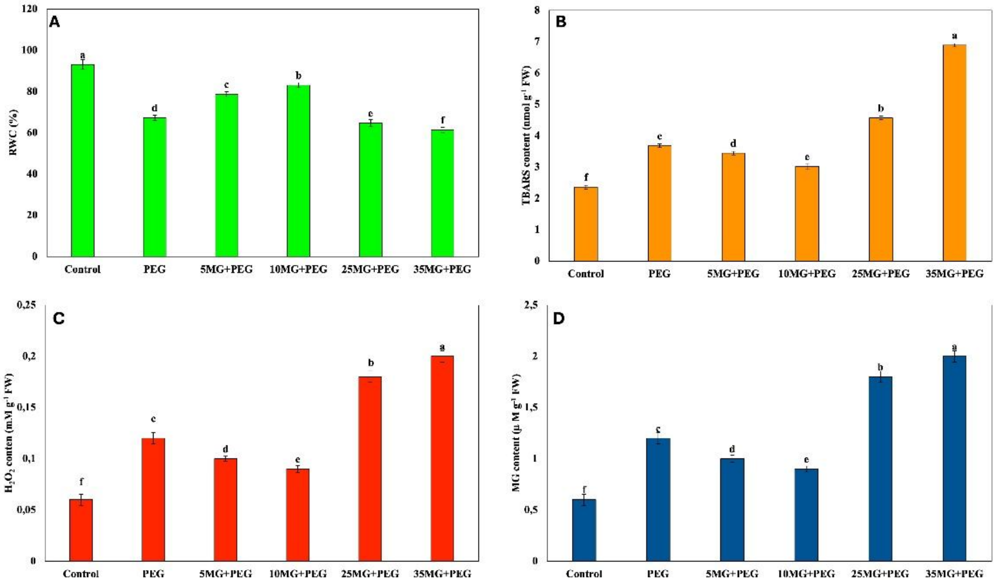

Figure 1. The effects of MG treatments on RWC (**a**), TBARS (**b**), H_2O_2 (**c**), and MG (**d**) content in maize seedlings under drought stress. Bars, s.d. Different letters indicate significant differences between treatments at $P < 0.05$

With respect to the expression of antioxidant enzymes-related genes, the results showed that, compared to the control group, the expression levels of *SOD*, *CAT1*, *APX1*, and *GPX* genes were upregulated under osmotic stress (Figure 3). *SOD* gene expression was upregulated under osmotic stress following MG treatment at various concentrations, compared to osmotic stress alone. Nonetheless, no significant difference was observed between the 5 and 10 mM treatments, or among the higher concentrations (25 and 35 mM) (Fig. 3a). The expression of the *CAT* gene was upregulated at all MG concentrations. However, no statistically significant differences were observed between 5 and 25 mM. The 10 mM treatment resulted in a greater increase than 5 mM, and the highest expression level was recorded at 35 mM (Fig. 3b). Moreover, the relative expression level of the *APX* gene increased progressively with rising MG concentrations; however, no statistically significant difference was observed between the 25 and 35 mM treatments (Fig. 3c). *GPX* gene expression increased progressively with MG treatment, and the highest expression level was observed at 35 mM. While the expression levels at 5 and 10 mM were similar, 25 mM induced a moderately higher expression (Fig. 3d).

3.3. Changes in the glyoxalase system in response to MG under PEG stress

Compared to the control, activities of Gly I and Gly II were significantly increased in PEG-treated seedlings. Similarly, MG pretreatment at 5, 10, 25, and 35 mM under PEG-induced stress further enhanced the activities of these enzymes. However, the 5 and 10 mM treatments did not differ significantly from each other, nor did the 25 and 35 mM treatments (Fig. 4a,b).

As for the expression profiles of glyoxalase system enzyme genes, the results demonstrated that PEG treatment led to an upregulation of *Gly I* and *Gly II* gene expression relative

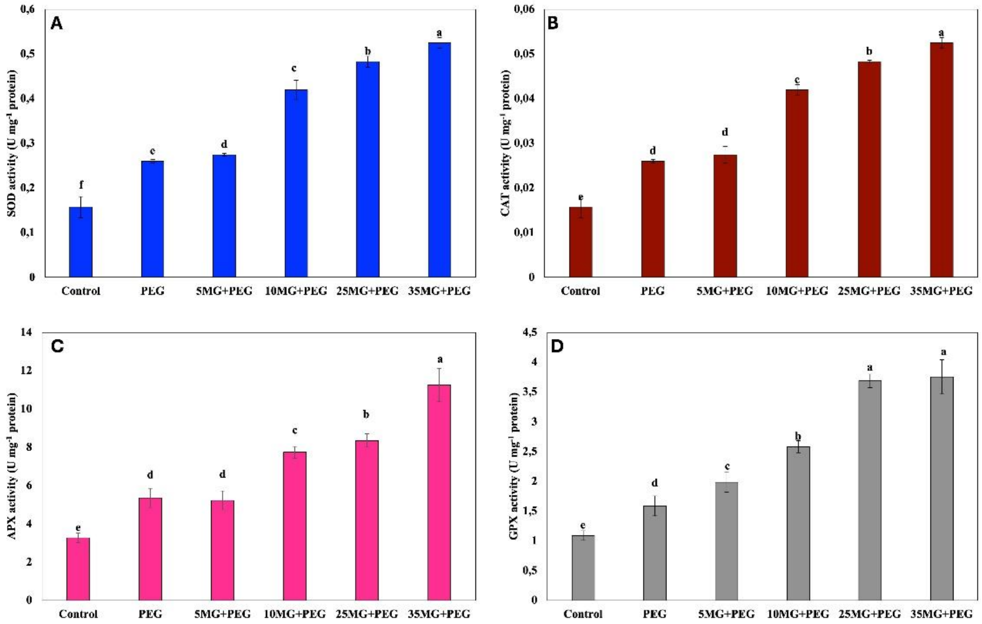

Figure 2. The effects of MG treatments on SOD (**a**), CAT (**b**), APX (**c**), and GPX (**d**) activities in maize seedlings under drought stress. Bars, s.d. Different letters indicate significant differences between treatments at $P < 0.05$.

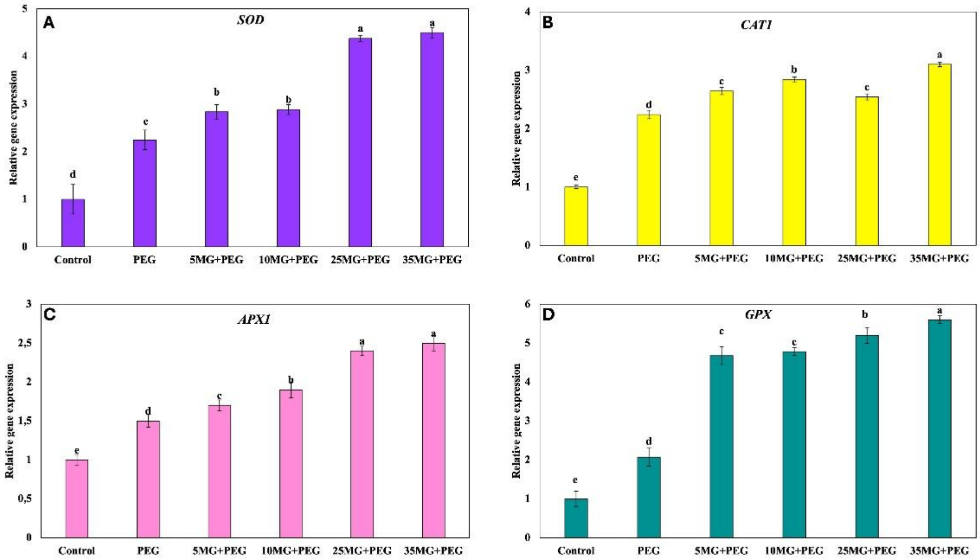

Figure 3. The effects of MG treatments on the expression levels of *SOD* (**a**), *CAT1* (**b**), *APX1* (**c**), and *GPX* (**d**) genes in maize seedlings under drought stress. Bars, s.d. Different letters indicate significant differences between treatments at $P < 0.05$.

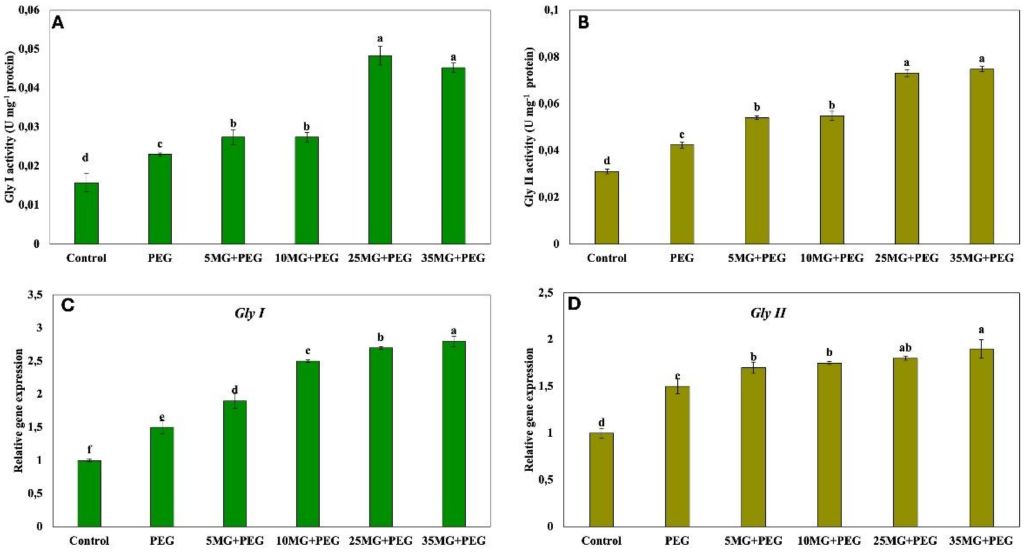

Figure 4. The effects of MG treatments on the glyoxalase system in maize seedlings under drought stress: Gly I (**a**) and Gly II (**b**) activities; and the expression levels of *Gly I* (**c**) and *Gly II* (**d**) genes. Bars, s.d. Different letters indicate significant differences between treatments at $P < 0.05$.

to the control. While *Gly I* expression increased progressively with rising MG concentrations, *Gly II* expression did not show significant variation across different MG treatments (Fig. 4c,d).

3.4. Changes in proline and polyamine metabolism in response to MG under PEG stress

Proline content was found to increase in PEG-treated seedlings compared to the control, and this increase was further enhanced by rising MG concentrations. However, the 25 and 35 mM treatments showed no statistically significant variation (Fig. 5a).

The expression levels of *P5CS1* and *ProDH1* genes are presented in Figure 5b and c. In addition, the expression level of the proline biosynthesis gene *P5CS1* progressively increased with 5, 10, 25, and 35 mM MG treatments under PEG-induced stress (Fig. 5b). In contrast, the expression of the proline degradation gene *ProDH1* was found to decrease compared to PEG treatment alone (Fig. 5c).

The levels of Put, Spd, and Spm were quantified, and all three polyamines increased in PEG-treated seedlings relative to the control (Fig. 6a). Put content also rose under MG treatments; however, no significant differences were detected between the lower concentrations (5 and 10 mM) or between the higher concentrations (25 and 35 mM). Moreover, Spd content was elevated under PEG stress with MG applications compared to PEG treatment alone, but no statistically significant difference was found between the 25 and 35 mM treatments. In contrast, Spm content did not change significantly with 5 and 10 mM MG treatments under PEG stress compared to PEG alone, whereas a significant increase was observed at 35 mM MG relative to PEG treatment (Fig. 6a).

In the PEG-treated group, the expression of the *ADC* gene increased 1.6-fold compared to the control. Furthermore, the expression levels of the *ADC* gene in seedlings subjected to 5, 10, 25, and 35 mM MG + PEG stress were 1.20, 1.38, 1.56, and 1.62 times higher than those under PEG treatment, respectively (Fig. 6b). The expression of the *PAO1* gene was reduced 1.6-fold in the PEG-treated group compared to the control. Under PEG stress, treatment with 5 and 10 mM MG further decreased *PAO1* expression by 1.2-fold relative to the PEG group, with no statistically significant difference observed between these two

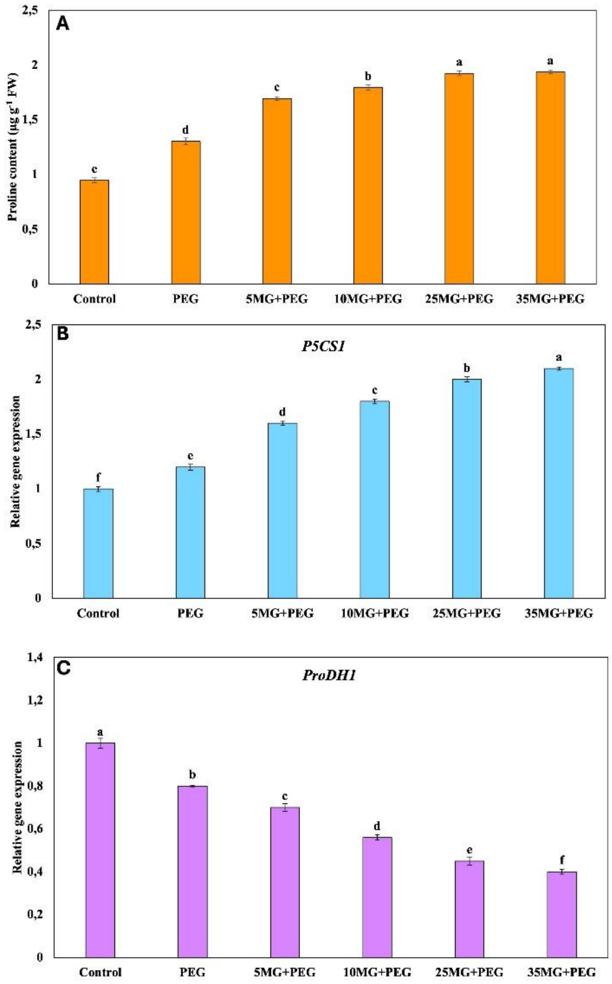

Figure 5. The effects of MG treatments on the proline metabolism in maize seedlings under drought stress: proline content (**a**), and the expression levels of *P5CS1* (**b**) and *ProDH1* (**c**) genes. Bars, s.d. Different letters indicate significant differences between treatments at $P < 0.05$.

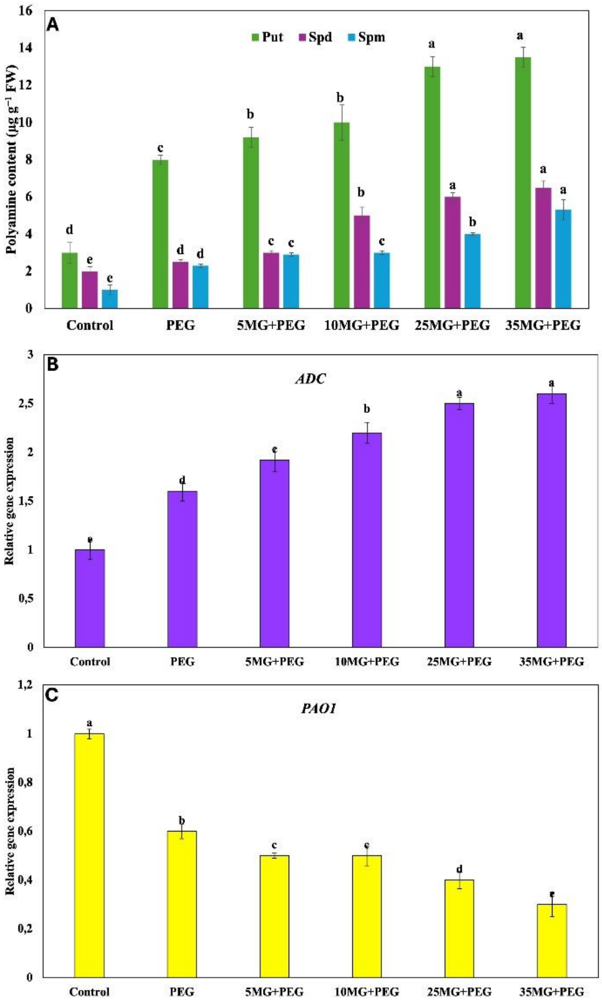

Figure 6. The effects of MG treatments on the polyamine metabolism in maize seedlings under drought stress: polyamine content (**a**), and the expression levels of *ADC* (**b**) and *PAO1* (**c**) genes. Bars, s.d. Different letters indicate significant differences between treatments at $P < 0.05$

concentrations. In contrast, 25 mM and 35 mM MG treatments led to a 1.5-fold and 2-fold reduction, respectively, compared to PEG (Fig. 6c).

4. Discussions

Drought is one of the most critical abiotic stressors limiting global crop productivity, as it reduces water availability, disturbs cellular homeostasis, and induces oxidative and osmotic stress responses in plants. Enhancing drought tolerance is therefore crucial for sustaining crop yield under changing climatic conditions (Ozturk et al., 2021). Methylglyoxal (MG) has recently emerged as a stress-related signaling molecule that modulates gene expression, redox regulation, and osmotic balance, thereby influencing plant stress responses (Hoque et al., 2016). While excessive MG accumulation is cytotoxic, evidence suggests that low concentrations act as signals to activate defense mechanisms. In this study, we demonstrated the concentration-dependent effects of exogenous MG on maize seedlings under PEG-induced osmotic stress, highlighting its dual role as both a signaling molecule and a cytotoxic compound, while also revealing its influence on antioxidant and glyoxalase systems as well as gene expression related to proline and polyamine metabolism. In this study, we demonstrated the concentration-dependent effects of exogenous MG on maize seedlings under PEG-induced osmotic stress, highlighting its dual role as both a signaling molecule and a cytotoxic compound, while also revealing its influence on antioxidant and glyoxalase systems as well as gene expression related to proline and polyamine metabolism. Specifically, in the present study, we investigated the dose-dependent effects of exogenous MG (5, 10, 25, and 35 mM) on maize seedlings exposed to PEG-induced osmotic stress.

Physiological parameters such as relative water content (RWC) and biochemical markers including TBARS and H_2O_2 confirmed this dose-dependent effect. In our study, PEG stress significantly reduced leaf RWC, indicating impaired water balance. In contrast, low MG concentrations (particularly 5 and 10 mM) significantly alleviated PEG-induced water loss, suggesting that moderate levels of MG activate osmoprotective mechanisms. This effect has previously been reported to be related to MG's ability to increase proline and polyamine accumulation (Szabados and Savouré, 2010; Mostofa et al., 2014). Our findings also indicate that MG is not merely a general stress signal but also plays a role in shaping cellular osmoprotection and redox balance by directly affecting proline and polyamine metabolism. Indeed, a study conducted by Lin et al. (2023) on maize under drought stress also revealed that exogenously applied MG increased leaf RWC, thereby improving water retention capacity and stress tolerance. The researchers reported that MG application delayed leaf senescence and increased photosynthetic efficiency, supporting our findings that MG at low concentrations is an important regulator in maintaining water balance.

Additionally, our study found that MG at low concentrations (5 and 10 mM) reduced TBARS and H_2O_2 levels in drought-stressed maize seedlings, indicating that moderate MG doses can mitigate oxidative damage. By contrast, high MG concentrations (25 and 35 mM) exacerbated water loss and were accompanied by elevated TBARS and H_2O_2 levels, reflecting intensified membrane lipid peroxidation and oxidative stress. This dose-dependent duality of MG action parallels previous reports. For example, in *Salix matsudana*, Guo et al. (2023) demonstrated that MG application decreased ROS accumulation and TBARS content, improved growth, and restored antioxidant enzyme activities and glutathione metabolism—key components of cellular detoxification under stress. Taken together, these findings indicate that while low MG concentrations enhance antioxidative capacity and act as a protective signal, excessive MG can disrupt cellular redox balance and thereby aggravate oxidative damage.

Overall, the collective evidence reinforces the central role of the glyoxalase system in regulating MG homeostasis and thereby shaping plant stress tolerance. As previously established, enhanced activities and expression levels of Gly I and Gly II promote efficient MG detoxification, contributing directly to improved stress resilience (Zheng et al., 2024). The present findings further demonstrate that endogenous MG dynamics exhibit a clear threshold-dependent pattern: low exogenous MG concentrations activate the glyoxalase pathway, lowering MG accumulation and preventing cellular damage through the conversion of MG into less toxic intermediates (Kaur et al., 2016; Hoque et al., 2016). In contrast, high MG concentrations exceed the detoxification capacity—likely due to enzyme saturation or glutathione limitation—resulting in pronounced MG buildup, suppressed glyoxalase function, and enhanced ROS generation and lipid peroxidation (Zheng et al., 2024). Taken together, these observations highlight that the glyoxalase system operates efficiently only within a certain MG load, beyond which its protective function collapses. Thus, MG acts as both a beneficial signaling molecule and a harmful cytotoxin depending on its intracellular level, and the glyoxalase pathway serves as the key determinant that governs this balance.

The antioxidant defense system was markedly influenced by MG treatment, with all concentrations enhancing both the activities and transcript levels of SOD, CAT, APX, and GPX, resulting in reduced ROS accumulation and improved redox homeostasis. This suggests that MG at moderate levels may act as a priming agent, activating antioxidant machinery to counter oxidative stress. Similar stress-induced activation of antioxidant enzymes has been reported in other systems; for instance, cadmium stress increased both the activities and relative gene expression of several antioxidant enzymes in *Salix matsudana* (Guo et al., 2023). However, despite transcriptional upregulation of antioxidant genes under 25 and 35 mM MG, excessive ROS and TBARS accumulation persisted, indicating that antioxidant defenses were overwhelmed. This discrepancy indicates that the antioxidant capacity was likely exceeded under severe carbonyl and oxidative stress, leading to cellular damage. High MG levels may disrupt antioxidant enzyme function either by depleting cofactors (e.g., AsA, GSH), inducing enzyme inactivation, or promoting protein carbonylation. These results align with earlier studies showing that under intense stress conditions, ROS-scavenging systems become overwhelmed, despite transcriptional activation (Hasanuzzaman et al., 2012). These findings suggest that while low-dose MG enhances antioxidant defense and maintains redox homeostasis under PEG stress, high-dose MG acts beyond the adaptive threshold, leading to oxidative damage despite transcriptional activation of antioxidant genes. This highlights a threshold-dependent dual role of MG, consistent with its previously reported functions as both a signaling molecule and a cytotoxic agent in plant stress responses (Lin et al., 2023; Bless et al., 2023).

In addition to antioxidant and glyoxalase systems, osmoprotectants such as proline and polyamines played a significant role. Consistent with previous findings in wheat under salt stress (Li et al., 2017), our results demonstrated that MG pretreatment enhances proline accumulation in maize seedlings under osmotic stress, highlighting its role as a signaling molecule in modulating osmolyte levels. Proline accumulation in plants is a well-documented response to osmotic and carbonyl stress, often increasing under drought or other abiotic stresses. However, research indicates that proline biosynthesis and accumulation can reach a saturation point at higher stress levels, such as elevated methylglyoxal (MG) concentrations, where further increases in MG do not significantly raise proline levels. This plateau effect suggests that the metabolic pathways responsible for proline synthesis may have a maximum activation capacity under severe or chronic stress conditions, leading to stabilization of proline content despite increasing stress intensity (Kijowska-Oberc et al., 2023; Spormann et al., 2023). Studies on drought stress in woody plants also support a nonlinear relationship between stress duration and proline accumulation, implying an optimum level beyond which proline does not increase further (Kijowska-Oberc et al., 2023). The saturation phenomenon aligns with findings that proline transport and metabolism can be saturable processes, as seen in other biological systems (Spormann et al., 2023). Overall, proline accumulation serves as a protective mechanism but is limited by physiological and biochemical constraints at high stress levels (Raza et al., 2023). Moreover, in our study, genes involved in the proline pathway were investigated for the first time, revealing that MG treatments upregulated the relative expression of *P5CS1*, responsible for proline biosynthesis, while downregulating the expression of *ProDH1*, which is involved in proline degradation. Shifting metabolic activity toward proline accumulation helps the cell sustain osmotic homeostasis, counteract ROS, and protect membrane structure (Ghosh et al., 2021). This solidifies the signaling role of MG through proline metabolism and mechanistically explains its effect in stress tolerance. Conversely, higher MG doses impaired this balance, with reduced proline accumulation, consistent with excessive oxidative stress. Polyamine metabolism showed a similar pattern. PEG stress induced putrescine, spermidine, and spermine accumulation, which was further modulated by MG. All MG concentrations, the *ADC* gene was strongly upregulated, suggesting enhanced polyamine biosynthesis that contributes to osmoprotection and stress signaling. By contrast, *PAO1* expression was significantly downregulated in PEG alone and decreased further with MG treatments, particularly at 25 and 35 mM, reflecting a possible reduction in catabolic H_2O_2 generation under moderate MG but exacerbated polyamine degradation at higher MG levels (Alcázar et al., 2010). These findings highlight that MG influences both biosynthesis and catabolism of polyamines in a concentration-dependent manner, thereby shaping redox and osmotic responses. The effect of MG on these two metabolic pathways is critical in explaining its “signaling or toxic” dilemma.

In conclusion, this study demonstrates that MG exerts a concentration-dependent dual role in maize seedlings under PEG-induced osmotic stress. Low concentrations (5–10 mM) act as a signaling cue that improves water retention, enhances antioxidant and glyoxalase activities, and promotes proline and polyamine biosynthesis, thereby conferring stress tolerance. In contrast, high concentrations (25–35 mM) induce MG accumulation, ROS overproduction, and lipid peroxidation, overwhelming detoxification systems and impairing osmotic adjustment. These results provide novel insights into the threshold-dependent function of MG, suggesting that fine-tuning its levels may represent a potential strategy for improving plant resilience to drought stress.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors’ Contribution

ASM and SDGŞ contributed to the study’s conception and design. ASM and SDGŞ conducted the experiments and analyzed all data and ASM wrote the manuscript.

References

Aebi H (1983). Catalase in vitro. In: Boyer PD (ed.). Methods in enzymology, Vol. 105. Academic Press, pp. 121-126.

Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Tiburcio AF (2010). Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. Planta 231(6): 1237-1249.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44(1): 276-287.

Ben-Gigirey B, De Sousa JMV, Villa TG, Barros-Velazquez J (1998). Changes in biogenic amines and microbiological analysis in albacore (Thunnus alalunga) muscle during frozen storage. Journal of Food Protection 61(5): 608-615.

Biswas MS, Mano JI (2015). Lipid peroxide-derived short-chain carbonyls mediate hydrogen peroxide-induced and salt-induced programmed cell death in plants. Plant Physiology 168(3): 885–898.

Bless Y, Ndlovu L, Gcanga E, Niekerk L, Nkomo M, Bakare O, Mulaudzi T, Klein A, Gokul, Keyster M (2023). Methylglyoxal improves zirconium stress tolerance in *Raphanus sativus* seedling shoots by restricting zirconium uptake, reducing oxidative damage, and upregulating glyoxalase I. Scientific Reports 13(1): 13618.

Bookout AL, Mangelsdorf DJ (2003). Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways. Nuclear Receptor Signaling 1(1): 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(1-2): 248-254.

Carillo P, Gibon Y (2011). Protocol: extraction and determination of proline. PrometheusWiki: 1-5

Castillo FJ (1996). Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia 107(4): 469-477.

Fancy NN, Bahlmann AK, Loake GJ (2017). Nitric oxide function in plant abiotic stress. Plant, Cell & Environment 40(4): 462-472.

Fariduddin Q, Mir BA, Yusuf M, Ahmad A (2014). 24-Epibrassinolide and/or putrescine trigger physiological and biochemical responses for the salt stress mitigation in *Cucumis sativus* L. Photosynthetica 52(3): 464-474.

Ghosh U, Islam M, Siddiqui M, Cao X, Khan M (2021). Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. Plant biology 4(2):227-239.

Gumrukcu Simsek SD, Terzi R, Guler NS (2024). Lipoic acid can maintain stimulation of the antioxidant system at lower reactive oxygen species, ascorbate and glutathione levels in osmotic stressed maize. Russian Journal of Plant Physiology 71(3): 63-73.

Guo L, Ling L, Wang X, Chen Y, Wang Y, Li J (2023). Exogenous hydrogen sulfide and methylglyoxal alleviate cadmium-induced oxidative stress in *Salix matsudana* Koidz by regulating glutathione metabolism. BMC Plant Biology 23: 73.

Hasanuzzaman M, Bhuyan MB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fotopoulos V (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants 9(8): 681.

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(4): 353-365.

Hasanuzzaman M, Nahar K, Alam MM, Fujita M (2012). Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. Australian Journal of Crop Science 6(8): 1314-1323.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125(1): 189-198.

Hoque TS, Hossain MA, Mostofa MG, Burritt DJ, Fujita M, Tran LSP (2016). Methylglyoxal: An emerging signaling molecule in plant abiotic stress responses and tolerance. Frontiers in Plant Science 7: 1341.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil (2nd ed., Circular No. 347). California: California Agricultural Experiment Station.

Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Tran LSP (2015). Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. Frontiers in Plant Science 6: 420.

Islam MM, Ye W, Matsushima D, Munemasa S, Okuma E, Nakamura Y, Murata Y (2016). Reactive carbonyl species mediate ABA signaling in guard cells. Plant and Cell Physiology 57(12): 2552-2563.

Kaur C, Sharma S, Singla-Pareek SL, Sopory SK (2016). Methylglyoxal detoxification in plants: Role of glyoxalase pathway. Indian Journal of Plant Physiology 21(4): 377-390.

Kijowska-Oberc J, Dylewski Ł, Ratajczak E (2023). Proline concentrations in seedlings of woody plants change with drought stress duration and are mediated by seed characteristics: a meta-analysis. Scientific Reports 13: 15157.

Kishor PK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KS, Sreenivasulu N (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science 88(3): 424-438.

Kolupaev Y, Shakhov IV, Kokorev AI, Relina LI, Dyachenko AI, Dmitriev AP (2024). Gamma-aminobutyric acid induction of triticale protective systems under drought, salt stress or a combination of the two. Turkish Journal of Botany 48(5): 235-248.

Li ZG, Duan XQ, Min X, Zhou ZH (2017). Methylglyoxal as a novel signal molecule induces the salt tolerance of wheat by regulating the glyoxalase system, the antioxidant system, and osmolytes. Protoplasma 254(5): 1995-2006.

Lin YH, Jin YK, Chen ZY, Xiao ZD, Shen S, Zhou SL (2023). Exogenous methylglyoxal ameliorates source strength and retrieves yield loss under drought stress during grain filling in maize. Journal of Plant Growth Regulation 42(6): 3934-3946.

Majláth I, Éva C, Tajti J, Khalil R, Elsayed N, Darko E, Janda T (2020). Exogenous methylglyoxal enhances the reactive aldehyde detoxification capability and frost-hardiness of wheat. Plant Physiology and Biochemistry 149: 75-85.

Mittler R (2017). ROS are good. Trends in Plant Science 22(1): 11–19.

Mostofa MG, Seraj ZI, Fujita M (2014). Exogenous sodium nitroprusside and glutathione alleviate copper toxicity by reducing copper uptake and oxidative damage in rice (*Oryza sativa* L.) seedlings. Protoplasma 251(6): 1373-1386.

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

Ozturk M, Turkyilmaz Unal B, García-Caparrós P, Khursheed A, Gul A, Hasanuzzaman M (2021). Osmoregulation and its actions during drought stress in plants. Physiologia Plantarum 172(2): 1321-1335.

Pál M, Tajti J, Szalai G, Peeva V, Végh B, Janda T (2018). Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. Scientific Reports 8(1): 12839.

Principato GB, Rosi G, Talesa V, Giovanni 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 (BBA) – Protein Structure and Molecular Enzymology 911(3): 349-355.

Raza A, Charagh S, Abbas S, Hassan M, Saeed F, Haider S, Sharif R, Anand A, Corpas F, Jin W, Varshney R (2023). Assessment of proline function in higher plants under extreme temperatures. Plant biology 25(3):379-395.

Szabados L, Savouré A (2010). Proline: a multifunctional amino acid. Trends in plant science 15(2): 89-97.

Sekhar PN, Amrutha RN, Sangam S, Verma DPS, Kishor PK (2007). Biochemical characterization, homology modeling and docking studies of ornithine δ-aminotransferase-An important enzyme in proline biosynthesis of plants. Journal of Molecular Graphics and Modelling 26(4): 709–719.

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Battaglia ML (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10(2): 259.

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.

Sezgin Muslu A, Kadıoğlu A (2025). *Heliotropium hirsutissimum* from geothermal areas: Evidence of thermal adaptation. Protoplasma 1-20.

Sezgin Muslu A, Sağlam A, Kadıoğlu A (2022). Paraquat applied under osmotic stress interferes with proline and polyamine metabolisms in *Zea mays* L. Cereal Research Communications 50(4): 965-972.

Shimakawa G, Ifuku K, Suzuki Y, Makino A, Ishizaki K, Fukayama H, Miyake C (2018). Responses of the chloroplast glyoxalase system to high CO_2 concentrations. Bioscience, Biotechnology, and Biochemistry 82(12): 2072-2083.

Shumilina J, Kusnetsova A, Tsarev A, Janse van Rensburg HC, Medvedev S, Demidchik V, Frolov A (2019). Glycation of plant proteins: Regulatory roles and interplay with sugar signalling? International Journal of Molecular Sciences 20(9): 2366.

Spormann S, Nadais P, Sousa F, Pinto M, Martins M, Sousa B, Fidalgo F, Soares C (2023). Accumulation of Proline in Plants under Contaminated Soils—Are We on the Same Page?. Antioxidants 12(3):666.

Sun X, Li X, Zhu J, Huang N, Bian X, Li H, Han L (2020). Polyamines and ethylene metabolism during cold acclimation in zoysiagrass (*Zoysia japonica* Steud.). Acta Physiologiae Plantarum 42(8): 138.

Terzi R, Kadioglu A, Kalaycioglu E, Saglam A (2014). Hydrogen peroxide pretreatment induces osmotic stress tolerance by influencing osmolyte and abscisic acid levels in maize leaves. Journal of Plant Interactions 9(1): 559565.

Urbanek H, Kuźniak-Gębarowska E, Herka K (1991). Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Physiologiae Plantarum 13: 4350.

Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Science 151(1): 5966.

Wang Y, Ye XY, Qiu XM, Li ZG (2019). Methylglyoxal triggers heat tolerance in maize seedlings by driving AsA–GSH cycle and reactive oxygen species-/methylglyoxal-scavenging system. Plant Physiology and Biochemistry 138: 9199.

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(1): 6167.

Zhang Y, Luan Q, Jiang J, Li Y (2021). Prediction and utilization of malondialdehyde in exotic pine under drought stress using near-infrared spectroscopy. Frontiers in Plant Science 12: 735275.

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): 82948301.

Zheng Q, Xin J, Zhao C, Tian R (2024). Role of methylglyoxal and glyoxalase in the regulation of plant response to heavy metal stress. Plant Cell Reports 43(4): 103.