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Exogenous hydrogen peroxide enhances the response of corn (*Zea mays* L.) plants to drought stress

Hamid Valizade[1], Saeid Navabpour*[1], Ali Dehestani[2], Pooyan Mehrabanjoubani[3]

[1.] Department of Plant Breeding and Biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[2.] Genetics and Agricultural Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.
[3.] Department of Basic Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

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Correspondence
Dr. Saeid Navabpour
s.navabpour@gau.ac.ir

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Abstract: Drought stress is a significant factor limiting crop growth and production. In this experiment, the effect of hydrogen peroxide (H_2O_2) application on water-stressed corn plants was investigated using various biochemical and molecular methods. Corn seedlings grown in hydroponic culture were treated with 2 mM H_2O_2 and subsequently exposed to water stress using polyethylene glycol 6000 at three levels: 0, -2 bar, and -4 bar. The results showed that drought stress significantly altered all of the studied traits. With an increase in stress levels, the activity of the catalase enzyme was decreased, and the highest drop, 50%, occurred eight days after stress. It was revealed that catalase activity increased by up to 18% on the second day after the stress, but it decreased significantly over time. The indigenous accumulation of H_2O_2 increased significantly in the -4 bar treatment four days after stress, while it was reduced by 50% on the eighth day post-stress. It was revealed that H_2O_2 application increased *PAO* gene expression 1.7 fold compared to the control plants. Its expression was decreased by 35% at -4 bar in control plants, while H_2O_2 treatment increased its expression by 2.8 times. These results indicate that H_2O_2 application enhanced tolerance to drought stress in corn plants.

Keywords: antioxidant enzymes, drought stress, hydrogen peroxide, maize.

Introduction

Corn is a vital crop used for both human consumption and livestock feed. Similar to other plants, corn is affected by many abiotic stresses throughout its growth period. Among these stresses, water scarcity stands out as one of the most important environmental factors strongly impacting corn production. Insufficient water during the initial growth phase diminishes the survival rate of seedlings, elevates abortion rates, and ultimately leads to a decrease in yield (Farooq et al., 2009). To overcome the problems caused by drought stress, plants deploy several strategies, including escape, tolerance, and resistance mechanisms. Many of these responses result from the expression of inducible genes during physiological, biochemical, and molecular defense mechanisms. For instance, the physiological processes involved in biotic (Ramezani et al., 2017) and abiotic (Mahdavian et al., 2021) defense responses. Different genetic engineering approaches have been employed for more than four decades to improve plant tolerance to different biotic and abiotic stresses (Dehestani et al., 2010; Dolatabadi et al., 2014). To achieve this goal, studying physiological responses and the diverse expressions of genes is crucial for comprehending the intricate physiological mechanisms involved in responding to drought stress.

Corn seedlings subjected to drought stress conditions exhibit various physiological responses, including reduced cell expansion, leaf curling, reduced CO_2 exchange, diminished chlorophyll content and photosynthetic efficiency (Mittler, 2006). Gonçalves et al. (2019) observed a decrease in net photosynthesis rate, relative growth rate, leaf area index, grain yield, and harvest index due to drought stress. Chen et al. (2015) demonstrated in their study that drought stress damages both the recipient and receptor components of photosystem II, including the photosystem reaction center II, and the photosystem receptor part I which eventually reduces the efficiency of electron transfer and photosynthesis.

Numerous studies have been conducted to identify genes associated with metabolic and regulatory enzymes, as well as photosynthesis in response to drought stress. In an experiment, Wang et al. (2018) investigated the effects of drought stress on the physiological responses and gene expression of corn seedlings. They observed that drought stress strongly affected water content, leaf size, and photosynthetic parameters, resulting in a substantial decrease in seedling growth. They also observed that several genes with different expression patterns under stress conditions, influencing both photosynthetic and hormone biosynthesis systems. These genes with distinct expressions have the potential to be utilized in enhancing and refining drought-tolerant corn lines. When plants are subjected to stress, the plant's antioxidant system is activated. This activation involves an increase in the activity of the catalase enzyme, which serves as the primary defense barrier against the assault of oxygen radicals. In this way, the system resists the damage caused by drought stress (Maiti and Satya, 2014). As long as the plant can control the amount of superoxide produced in the plant, this process continues. To achieve this, non-enzymatic antioxidants collaborate with scavenging enzymes like catalases and peroxidases to eliminate hydrogen peroxide (H_2O_2). Therefore, knowledge of antioxidant genes and enzyme activities at a specific level of drought tolerance can be beneficial. Studies indicate a correlation between drought tolerance and antioxidant defense capacity (Farooq et al., 2009).

Tolerance inducers are biological or chemical factors that trigger various defense responses during stress, leading to notable changes in physiologic (Barzegargolchini et al., 2017), antioxidative (Moradi et al., 2016), and metabolic (Ramezani et al., 2017) functions. The foliar application of H_2O_2 results in an increase in photosynthetic pigments, water content, and lipid oxidation rate, coupled with a reduction in cellular H_2O_2. Simultaneously, it enhances the activity of antioxidant enzymes such as catalase, superoxide dismutase and ascorbate peroxidase, glutathione reductase, and malondialdehyde (MDA). This increased tolerance to drought stress is attributed to the modulation of the antioxidant system (He and Gao, 2009; Guler and Pehlivan, 2016). In a study investigating the foliar application of H_2O_2 to alleviate the effects of drought stress in soybean, Ishibashi et al. (2011) demonstrated that the application of this substance reduced the wilting of

leaves and increased the rate of net photosynthesis and stomatal conductance in the leaves. This research aims to investigate the effect of the externally applied H_2O_2 on drought stress, and the biochemical and molecular responses of corn plants.

Materials and Methods

Plant growth and treatment

The seeds of a single cross hybrid "704" corn plant were grown under hydroponic conditions using Hoagland's solution in a greenhouse with 16 hours of light at a temperature of 25 ˚C and 8 hours of darkness. After germination and establishment of seedlings, foliar application of H_2O_2 (2 mM) was administered at 21 days of age. Subsequently, at 28 days of age, drought stress was applied using polyethylene glycol 6000 at three different levels (0, -2 bar, - 4 bar). After the application of drought treatment, leaf samples were collected on day 28 at the 4-leaf stage, with stress duration (period) of 1, 2, 4, and 8 days. This experiment was carried out with three replications in a factorial experiment designed with a completely randomized (CRD) layout. Leaf samples were placed in liquid nitrogen immediately and transferred at -80 ˚C for further molecular and enzyme analysis. The data were subjected to the analysis of variance using SPSS-19 software. Means were statistically compared by Duncan test at $P < 0.01$ level.

Measurement of metabolites

Hydrogen peroxide

Leaf H_2O_2 content was measured using 0.1% trichloroacetic acid solution. Thus, 0.2 grams of powdered leaves were mixed with 1800 microliters of TCA solution in liquid nitrogen. After 12000g centrifugation, the liquid phase was used to measure H_2O_2. To 0.5 ml of liquid phase prepared above, we add one ml of 1M KI and 0.5 ml of 10 mM phosphate buffer (pH=7). The sample was placed in the dark at room temperature for 15 minutes and then the absorbance was read at 390 nm. H_2O_2 standard in concentrations of 100 to 250 nanomoles was used to draw the standard curve (R2=0.98, Y=0.4349X+ 0.0771), (Alexieva et al., 2001).

Measurement of total phenolic contents

About 0.2 gr of powdered leaves were combined with 1800 microliters of pure methanol. Then it was placed in an ultrasonic bath for 30 minutes. After centrifugation (10 minutes – 1000 g), the liquid phase was used to measure the amount of phenolic compounds. 100 microliters of methanol extract were combined with 200 microliters of 10% folin reagent, and then 800 microliters of sodium carbonate were added to it and left at room temperature for 3 hours. The absorbance was read at 765 nm, and the amount of total phenolic compounds was calculated based on gallic acid standard (concentrations 0 to 10 µg/ml, Y=0.0197X + 0.0019, R2=0.98) (Singleton and Rossi, 1965).

Antioxidant enzyme activity assay

Preparation of enzyme extract and estimation of protein content

Powdered leaves (0.1 gr) were homogenized in liquid nitrogen with 1800 microliters of cold potassium phosphate buffer (pH=7.5) and subsequently centrifuged for 20 minutes at 12,000 g at 4 °C. Then the liquid phase was removed and kept in a freezer at -20 °C for further studies. The amount of extracted protein was estimated using the method of Bradford. For this purpose, 40 microliters of enzyme extract were combined with 960 microliters of Bradford's reagent and after 20 minutes, the absorption of the samples was recorded at a wavelength of 530 nm. A standard curve was drawn using Bovine Serum Albumin (BSA) (Y=0.019 X + 0.027, R2=0.98). This parameter was used to correct the amount of enzyme activity.

Catalase enzyme activity

Catalase enzyme activity was assessed by recording the decrease in absorbance at 240 nm (Aebi, 1984). The reaction components included 2.450 ml of 50 mM phosphate buffer (pH=7), 0.5 ml of 7.5 mM H_2O_2, and 50 microliters of enzyme extract. After adding H_2O_2, the reaction started and the 240 nm decrease in absorbance was estimated in 3 minutes (every 30 seconds). Catalase enzyme activity was calculated by the method of Bergmayer (1983).

RNA extraction, cDNA, and RT-PCR

Total RNA was extracted from leaf samples by Threezol (Riragene, Iran), and RNA quality was determined by spectrophotometry and agarose gel electrophoresis. cDNA was made using the SinnaClone kit. For this purpose, first, 5 microliters of RNA treated with Dnase1 was mixed with 1

microliter of primer oligo(dt), 1 microliter of10 mM dNTP mix and 3 microliters of DEPC water, and placed at 70 °C for 5 minutes. Then it was placed on ice for 2 minutes. Four microliters of 5X buffer, one microliter of MMuLV enzyme, one microliter of RNase inhibitor, and four microliters of DEPC treated water were added to the above solution and placed at 42 °C for 50 minutes. Then it was placed at a temperature of 85 ˚C for 5 minutes. Finally, the reaction product was kept in a freezer at -20 °C.

ABI-step one plus real-time PCR machine was used to check gene expression. Each 12-microliter reaction contained 6 microliters of SYBR green master mixs (Amplicon), 15 micromoles of each primer, and 2 microliters of diluted cDNA (five-fold), and nuclease-free water. The *PAO* gene was designed using prime software and NCBI database data (Table 1). The *GAPDH* gene was used as a reference gene.

Results

The overall results demonstrated significant effects of the treatments, namely drought stress and exogenous application of H_2O_2, on the studied traits (Table 2).

Catalase activity

The amount of catalase enzyme activity did not show significant changes on the first day after drought stress. However, on the second day, it significantly decreased under -4 bar stress compared to the control. In addition, it was decreased at -2 and -4 bar levels. Upon eighth-day stress period, it showed a decrease of 18% at -4 bar stress level. The most substantial decrease in the amount of catalase enzyme activity, compared to the initial control, occurred at -4 bar stress on the eighth day following the stress, reaching less than half of the initial level (Figure 1-a). The use of H_2O_2 on the first day resulted in a decrease in the amount of catalase enzyme, but on the second day, we saw a significant increase in the enzyme level, and on the eighth day, a 35% decrease in the amount of catalase activity was observed (Figure 1-b). With the increase of drought stress, the amount of catalase enzyme decreases, and in the stress of -4 bar, we saw a decrease in the activity of catalase enzyme by about 16%, and the use of peroxide inducer increased the amount of enzyme at the beginning, and there was a significant decrease in the activity of catalase enzyme on the eighth day stress period (Figure 1-c).

Hydrogen peroxide assay

On the first and second days after drought stress, the amount of H_2O_2 decreases at the level of -4 bar. However, over the four-day drought stress period, we observed an increase in the amount of H_2O_2 at the -4 bar level and a decrease at the -2 bar level. On the eighth day after the stress, we again observed a significant decrease in the amount of H_2O_2 at the -4 bar level (Figure 2-a). By foliar application with H_2O_2 inducer on the second and fourth days of the stress, the amount of internal H_2O_2 was increased. But on the eighth day period, compared to the first day, its quantity was reduced by 50% (Figure 2-b). The foliar application of H_2O_2 at the stress of -2 bar level resulted in a 20%increase in the H_2O_2 concentration (Figure 2-c).

Table 1. Primer sequences used for the real-time PCR.

Gene name	Forward Sequence	Reverse Sequence
GAPDH	CCCTTCATCACCACGGACTA	CTCACCCCACGGGATTTC
Polyamine oxidase (*PAO*)	GCAAGTACCATGTCCAGGG	CGAGGGAACATGGCTGTCA

Table2. Analysis of variance for the effect of treatments on studied traits.

S.O.V	df	CAT	Phenol	H_2O_2	Protein
Treat(T)	1	0.001**	0.009*	0.529**	0.009^{ns}
Stress(S)	2	0.001**	0.002^{ns}	2.018**	0.001^{ns}
Period (P)	3	0.009**	0.047**	6.797**	0.002^{ns}
T×S	2	0.001^{ns}	0.018**	1.453**	0.145**
T×P	3	0.003**	0.015**	0.479**	0.012**
S×P	6	0.001**	0.01**	0.846**	0.012*6
T×S×P	6	0.002**	0.012**	1.101**	0.005^{ns}
Error	48	0.001	0.001	0.07	0.002
% CV		8.65	13.81	11.32	10.23

*, ** Significant at the 5% and 1% levels of probability respectively, and ns (non-significant)

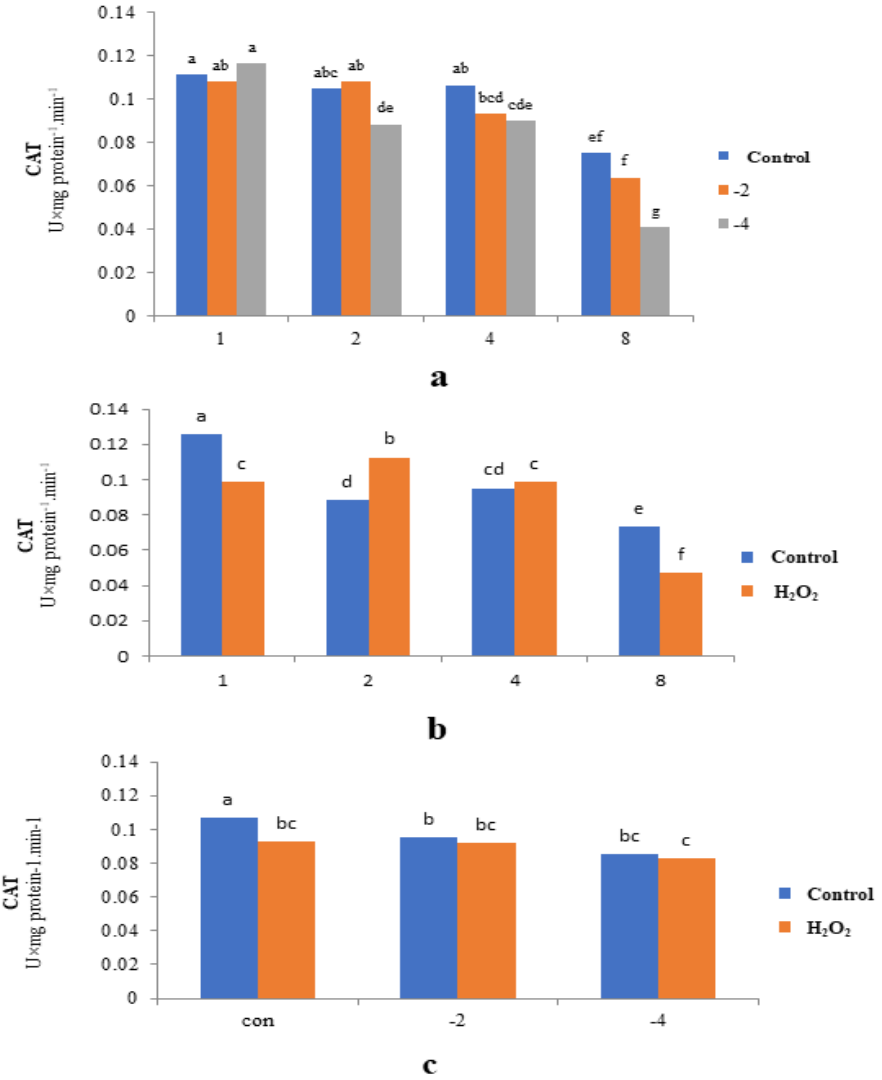

Figure 1. Effects of drought stress (3 levels) at four periods (a), inducer and periods (b), and inducer and drought stress on catalase activity (c).

Total phenolics content

On the first day, there was no change in the phenol content. However, on the second day, a significant increase in the amount of phenol was observed at the stress of -2 bar level. From the fourth day, the phenol content was increased by 14% and 26%, at the -2 and -4 bar levels, respectively. On the eighth day after stress, the amount of phenol was reduced again at the level of -4 bar (Figure 3-a). Using H_2O_2 on the first days after stress increased the amount of phenol. But from the 8th day after stress, the amount of phenol decreased (Figure 3-b). Application of H_2O_2 at -2 bar stress level caused a 20% increase in the amount of phenol. In the first days after the stress, the application of H_2O_2 at the level of -2 bar caused a 20% increase in the amount of phenol, and with the continuation of the drought stress and on the eighth day after the stress, we saw a 15% decrease in the amount of phenol (Figure 3-c).

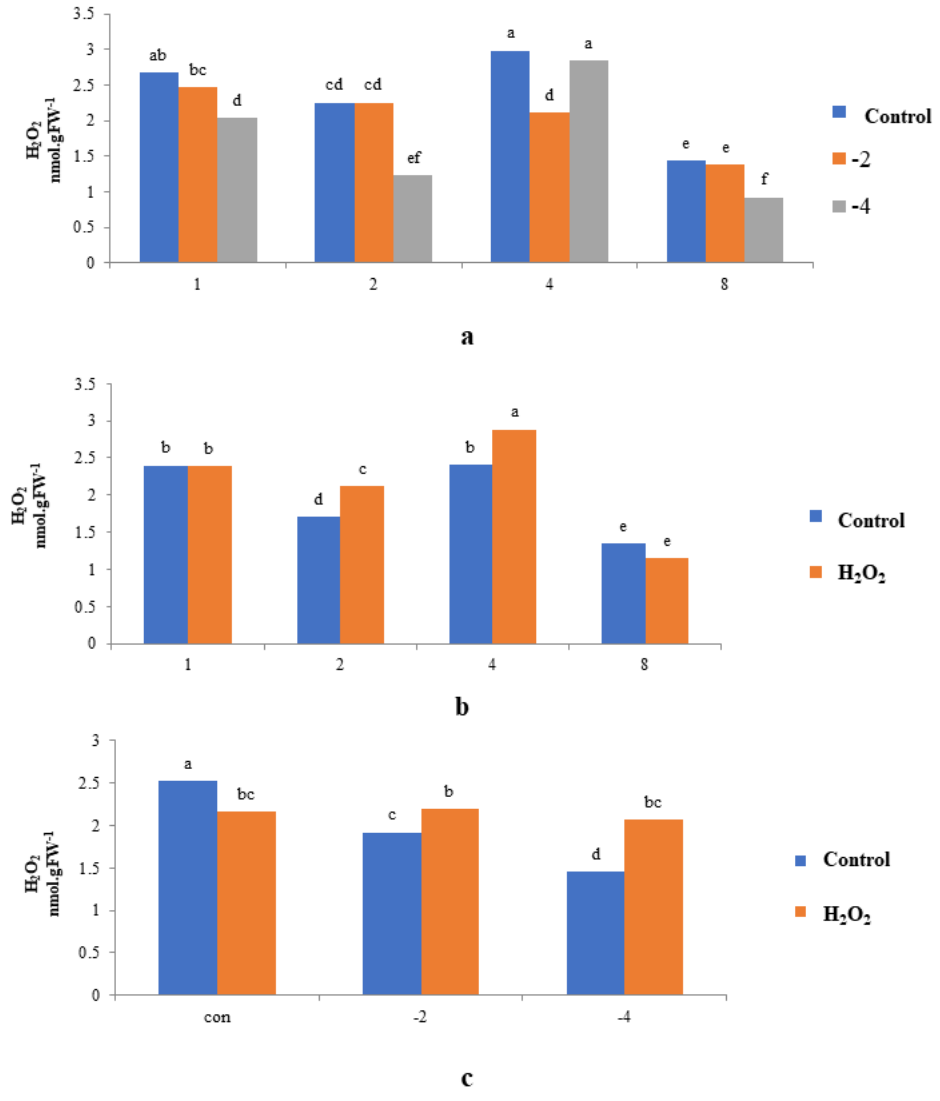

Figure 2. Effects of drought stress (3 levels) at four periods (a), inducer and period (b,) and inducer and drought stress on H_2O_2 content (c).

Total protein content

The protein content did not change significantly on the first days of stress. However, on the eighth day following drought stress at -2 and -4 bar, an increase of 18.5% and 21.5% was observed, respectively (Figure 4-a). The application of H_2O_2 on the first days after drought stress did not have any significant changes in the amount of protein. But on the eighth day period of stress, the protein concentration increased by 17% (Figure 4-b). In non-stressed conditions, the use of H_2O_2 led to a decrease in the amount of protein, but in drought stress of -4 bar, it increased by 31% (Figure 4-c).

PAO gene expression:

The expression of the *PAO* gene was increased 1.7 fold compared to the control under normal conditions with the use of a H_2O_2 inducer. Applying -4 bar stress during four-day stress period, decreased the expression level in the control sample by 35%, and the treatment with H_2O_2 stimulus at this stress level increased the expression level of this gene by 2.8 fold. The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used as an internal control gene (Figure 4-d).

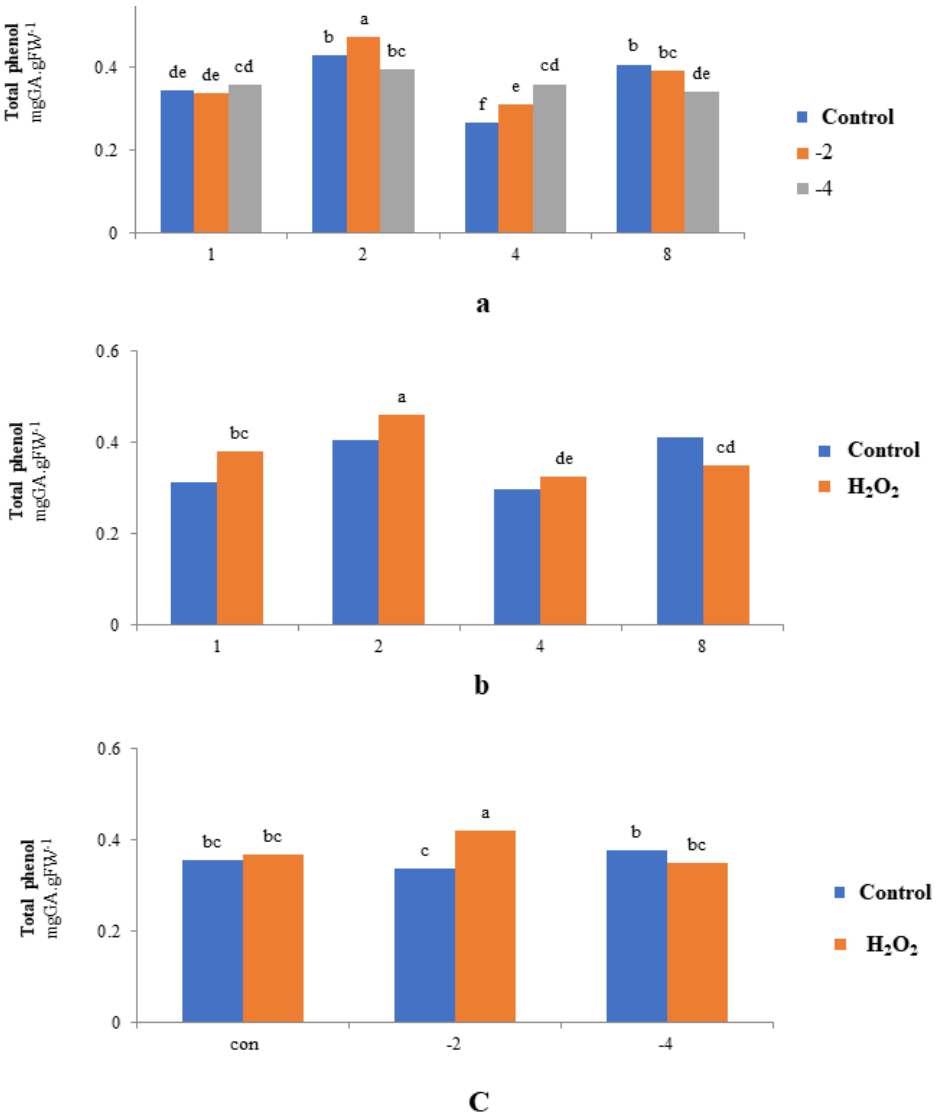

Figure 3. Effects of drought stress (3 levels) at four periods (a), inducer and period (b) and inducer and drought stress on total phenolic content (c).

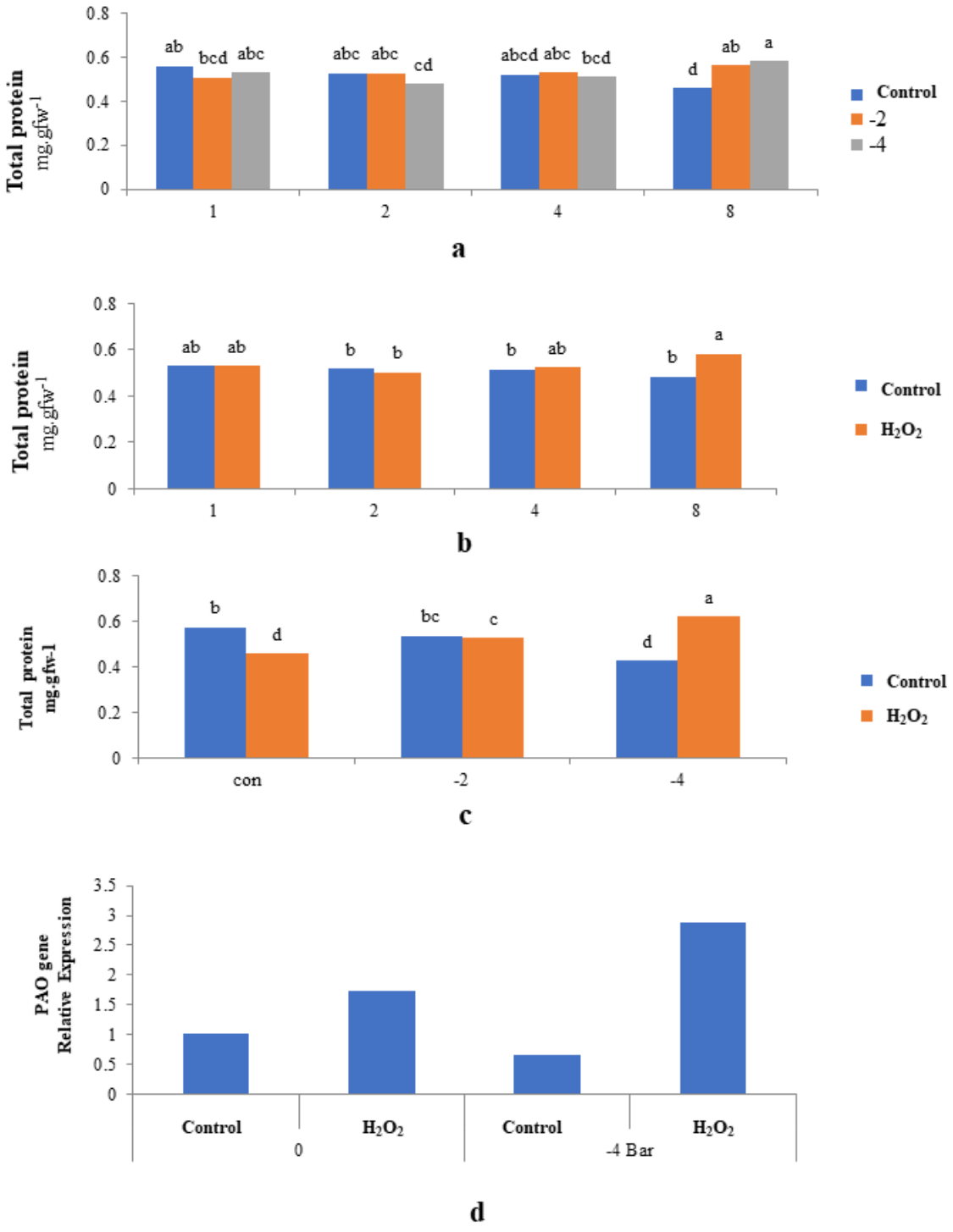

Figure 4. Effects of drought stress (3 levels) at four periods (a), inducer and period (b) and inducer and drought stress on total phenolic content (c), and effects of drought stress and inducer on *PAO* gene expression (d).

4. Discussion

Drought is critical environmental stress, especially prevalent in arid and semi-arid regions worldwide, significantly impacting plant yield. Drought stress disrupts physiological and biochemical processes, posing challenges to crop production. The response to drought stress is a highly intricate process. Indeed, drought stress influences plants from various aspects and manifests at different morphological, biochemical, and molecular levels. This includes impeding growth, the accumulation of compatible organic substances, and alterations in the expression of stress-responsive genes. Drought stress results in the generation of large amounts of reactive oxygen species (ROS), leading to oxidative stress (Hussain et al., 2018). Drought stress also leads to alteration in metabolic processes, steering them towards biosynthesis of the secondary metabolites. Consequently, there will be an upsurge in the biosynthesis of important bioactive compounds such as polyphenols, terpenoids, and alkaloids. To alleviate the damage caused by ROS, plants deploy antioxidant mechanisms that encompass non-enzymatic components, polyphenols, ascorbate, and glutathione, carotenoids, as well as enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, polyphenol oxidase, and glutathione reductase. The activity of both enzymatic and non-enzymatic antioxidants is crucial in scavenging ROS. This will enhance the plant's ability to tolerate stress (Agarwal and Pandey, 2004).

Antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD)play a protective role in safeguarding the photosynthetic systems of plants exposed to environmental stress (Cavalcanti et al., 2004). Catalase enzyme destroys H_2O_2 produced in photorespiration pathways inside peroxisomes (Mittler, 2002). Catalase is an antioxidant enzyme that halts the chain reactions of free radicals and protects plants against oxidative stress by eliminating H_2O_2 (Rukmini et al., 2004). In the present study, a significant decrease in catalase enzyme activity was observed concomitant with the increase in drought levels. The most substantial decrease occurred at the -4 bar level, specifically on 8 days post- stress, where the activity was halved. The elevation in the amount of H_2O_2-type ROS under -4 bar stress conditions, coupled with the persistence of these conditions, resulted in the reduced activity of antioxidant enzymes including catalase.

The H_2O_2 foliar application led to an increase in the amount of catalase enzyme by up to 18% on the second day following stress. However, its concentration was decreased significantly with ongoing drought stress particularly by the eighth day. Similar results were reported in drought stress (Ahmed et al., 2022) and salinity stress (Abdel Latef et al., 2019). Catalase is one of the iron-containing proteins and it is activated in plant cells when the amount of h2o2 in the environment is high. Catalase plays an important role in removing H_2O_2 in peroxisome. Antioxidant enzymes such as catalase play an effective role in tolerance to drought stress (Hameed et al., 2013). Catalase enzyme, as a compatible osmolyte and anti-ROS enzyme, while protecting macromolecules and cell membranes, neutralizes the damage caused by ROS caused by drought stress (Hameed et al., 2013).

Reactive oxygen species (ROS) are produced in various plant cellular organelles including chloroplast, mitochondria, and peroxisomes under normal conditions as part of the processes involved in the electron transport chain of respiration and photosynthesis. Disturbance in oxidant-antioxidant balance and growth conditions in plants increases the cellular concentration of ROS (O_2, H_2O_2, and OH). Among these, H_2O_2 acts as a stress signal transmitter due to its relative stability compared to other ROS and its diffusion capacity through the membrane and intercellular space and is involved in various physiological functions in plants (Anjum et al., 2022). During evolution, plants develop an effective ROS removal system that includes a set of enzymatic and non-enzymatic antioxidants to control excess ROS generated in cells (Singh et al., 2020). Treatment with H_2O_2 helps in stress tolerance by starting the ROS removal mechanism in different plants (Dikilitas et al., 2020). Ahmed et al. (2022) stated during an experiment that drought stress increases the concentration of internal H_2O_2 and spraying with H_2O_2 reduces its concentration, which effectively reduces the effects of oxidative stress. In this experiment, we saw a 22% increase in the amount of internal H_2O_2 at -4 bar stress and on

the fourth day after the stress, and with the continuation of the stress on the eighth day, we again had a decrease in the amount of internal H_2O_2 at the level of -4 bar. The external application of H_2O_2 caused a 32% decrease in the amount of internal h2o2 on the eighth day after stress. H_2O_2 foliar application at the level of -2 bar increased the concentration of internal H_2O_2 by 20%, which is due to the low intensity of stress and the activation of the internal messenger system. Similar results were reported by Guler and Pehlivan (2016) in soybean, in mustard under stress (Alam et al., 2013), and in wheat under stress (Abdel Latef et al., 2019). H_2O_2 serves as a messenger molecule that plays multifaceted functions at different levels in plants. This molecule, in collaboration with hormones and other messenger molecules, regulates the metabolism of plants, thereby contributing in stress tolerance (Smirnoff and Arnaud, 2019).

In an experiment, the effect of different stresses including drought, salinity, cold, and heat was investigated on four wheat cultivars by Kamal et al. In this experiment, on the 8th day after drought stress at -2 and -4 bar levels, the amount of total protein increased by 18.5% and 21.5%, respectively. The use of H_2O_2 under stress conditions of -4 increased the amount of protein by 31%. Drought stress leads to the alteration of metabolic processes towards biosynthesis activities. Therefore, the biosynthesis of proteins increases (Agarwal and Pandey, 2004).

Phenolic compounds, encompassing flavonoids and tannins, are the most abundant secondary metabolites that assume diverse molecular and biochemical functions within plants. They contribute to essential processes, including the scavenging of free radicals, mediation of auxin transport, participation in signaling pathways, and bolstering plant defense mechanisms (Soleimani et al., 2022). These multifaceted roles underscore the significance of phenolic compounds in orchestrating various physiological and protective aspects vital for the overall well-being and resilience of plants (Kiani et al., 2021; Soleimani et al., 2022). In the current study, an elevation in phenol levels was observed on the second day following exposure to stress level of -2 bar. By the fourth day, under stresses of -2 and -4 bar, the phenol content demonstrated an increase of 14% and 16%, respectively. Additionally, the foliar application of H_2O_2 foliar application on the first and second days after stress contributed to the augmentation of phenol levels. On the eighth day under a stress level of -4 bar, the amount of phenol decreased by 15%. Desoky et al. (2021) stated that increasing the levels of drought stress led to an increase in proline, phenol, and flavonoid content; thereby the highest amount was observed in the drought stress of -10 bar. Increasing the concentration of phenol is effective in eliminating free radicals and inhibiting lipid peroxidation (Blasco et al., 2013). In many studies, a positive correlation between phenolic content and antioxidant activity has been reported, and some of the mechanisms of antioxidant activity of these compounds are inactivating lipid free radicals and preventing the decomposition of hydroperoxides into free radicals, as well as their ability to chelate metal ions (Banerjee and Roychoudhury, 2019). Researchers stated that the tolerance of some plants against environmental stresses including drought, can be related to the accumulation of phenolic compounds in these plants. One of the important features of phenolic compounds is the antioxidant property that is related to the hydroxyl groups in their molecular structure (Halliwell and Gutteridge, 2015; Sehar et al., 2021).

Polyamines (spermidine and spermine) are involved in a complex messenger network and play an essential role in stress tolerance (Pal et al., 2015). Polyamine oxidase (PAO) oxidizes spermidine (Spd) and spermine (Spm) (Flores and Filner, 1985). Most of the polyamine biosynthetic genes are up-regulated by abiotic stresses despite a difference in timing and the degree of induction (Liu et al., 2011; Wang et al., 2011). The application of polyamines can modulate drought responses (Li et al., 2014; Ebeed et al., 2017). In this experiment, the expression level of the *PAO* gene in the control sample decreased by 35% on the fourth day of -4 bar stress, whereas the exogenous application of H_2O_2 increased the expression by 2.8-fold. As a result, the expression of *PAO* gene increases with H_2O_2 spraying.

Conclusion

The results of the analysis indicate that drought stress significantly altered the amount of internal

H_2O_2, catalase enzyme, phenolic compounds, and protein content. Additionally, this stress condition induced oxidative damage and an increase in ROS. The expression level of the *PAO* gene experienced a 35% decrease under -4 bar drought conditions. Treatment with H_2O_2 stimulus at this stress level increased the expression level of this gene by 2.8 times, thereby mitigating the negative effects of drought stress. As a result, foliar application of a low dose of H_2O_2, taking into account its role in signaling and the regulation of gene expression, yielded significant effects on the levels of investigated compounds. Ultimately, this approach is anticipated to reduce the damage caused by drought stress.

Supplementary Materials

No supplementary material is available for this article.

Author Contributions

AD designed research; HV performed research and analyzed the data; SN, PM, and AD interpreted the data and were major contributors in writing the manuscript and revising it critically; All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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کاربرد پراکسید هیدروژن موجب افزایش واکنش گیاه ذرت (*Zea mays* L.) به تنش خشکی

حمید ولی زاده[۱]، سعید نواب پور[۲]*، علی دهستانی[۲]، پویان مهربان[۳]

[۱].گروه اصلاح نباتات و بیوتکنولوژی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران
[۲].مؤسسه ژنتیک و بیوتکنولوژی کشاورزی طبرستان، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران
[۳].گروه علوم پایه، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

چکیده: تنش خشکی عامل مهمی است که رشد و تولید محصول زراعی را محدود می کند. در این مطالعه، اثر کاربرد پراکسید هیدروژن (H_2O_2) بر روی گیاهان ذرت تحت تنش آبی با استفاده از روش‌های مختلف بیوشیمیایی و مولکولی مورد بررسی قرار گرفت. بوته‌های ذرت رشد یافته در کشت هیدروپونیک با ۲ میلی‌مولار H_2O_2 تیمار شدند و متعاقباً با استفاده از پلی اتیلن گلیکول ۶۰۰۰ در سه سطح ۰، ۲- و ۴- بار در معرض تنش آبی قرار گرفتند. نتایج نشان داد که تنش خشکی به طور معنی‌داری تمامی صفات مورد مطالعه را تغییر داد. با افزایش سطح استرس، فعالیت آنزیم کاتالاز کاهش یافت و بیشترین افت به میزان ۵۰ درصد هشت روز پس از استرس مشاهده گردید. فعالیت آنزیم کاتالاز در روز دوم پس از آغاز تنش تا ۱۸ درصد افزایش یافت، اما با گذشت زمان به طور قابل توجهی کاهش یافت. تجمع داخلی H_2O_2 در بافت‌های گیاهی بطور قابل توجهی در تیمار ۴- بار چهار روز پس از استرس افزایش یافت، در حالی که در روز هشتم پس از استرس ۵۰ درصد کاهش یافت. نتایج نشان داد که کاربرد پراکسید هیدروژن بیان ژن *PAO* را ۱/۷ برابر نسبت به گیاهان شاهد افزایش داد. بطوری که بیان آن در سطح تنش بار ۴- به میزان ۳۵ درصد در گیاهان شاهد کاهش یافت، در حالی که تیمار H_2O_2 بیان آن را ۲/۸ برابر افزایش داد. این نتایج نشان دهنده افزایش تحمل به تنش خشکی در گیاهان ذرت تحت تاثیر تیمار H_2O_2 می‌باشد.

کلمات کلیدی: آنزیم‌های آنتی اکسیدان، تنش خشکی، پراکسید هیدروژن، ذرت.

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دانشگاه صنعتی اصفهان

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نویسنده مسئول
دکتر سعید نواب پور
s.navabpour@gau.ac.ir

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