The Effects of Drought and Salt Stresses on the Antioxidant System and *PIPs* Gene Expression in *Zea mays* L.

***Akhtar Auobi*[1], *Fatemeh Rahmani*[2 *]**

ABSTRACT

The objective of this study was to investigate the effect of salinity and drought stresses on some physiological and molecular aspects of maize plants (*Zea mays* L. cv.704). The experiment was carried out under growth chamber conditions and stresses were applied by NaCl (200 mM), $CaCl_2$ (40 mM) and PEG 6000 (10%) for 2 periods of 4 and 8 days. In our experiments, a decrease was noticed in guaiacol peroxidase enzyme activity and chlorophyll *a* and *b* under all treatments. In contrast, catalase and ascorbate peroxidase enzymatic activities, malondialdehyde, protein and soluble sugar levels were induced under applied impositions. Semi-quantitative RT-PCR analyses showed significant down or up regulation of *ZmPIPs*, *CKO1*, and *MAPK*s genes expression. The present study demonstrates that drought and salt stresses are associated with oxidative stresses and supports the involvement of the studied genes during these stresses.

Keywords: Antioxidant activities, drought stress, gene expression, *MAPK*, Salinity stress, *ZmPIP*.

INTRODUCTION

Salinity and drought are the major environmental factors which can greatly influence plant growth and productivity. These stresses impose osmotic stress and cause a series of morphological, physiological, biochemical, and molecular changes in plants. To survive these stresses, plants have evolved complex mechanisms to perceive external signals and to manifest adaptive responses (Zhu, 2002).

Drought, salinity and oxidative stress are often interconnected, and may induce similar cellular damage. They are manifested as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell. Drought and salinization are diverse environmental stresses, which often activate similar cell signaling pathways and cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants, and the accumulation of compatible solutes (Wang *et al*., 2003).

The uncontrolled enhancement of oxidative stress could also lead to lipid peroxidation and subsequent leakage of membrane due to damage to membrane and malondialdehyde (MDA) production (Moussa and Abdel-Aziz, 2008). Moreover, accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado *et al*., 2000). Salt and drought stresses were also reported to be responsible for decreased biosynthesis of chlorophyll and inefficiency of photosynthesis (Munns, 2002).

The strategy of plant to enhance tolerance against stresses is mostly based on the manipulation of genes that protect and maintain the function and structure of cellular components (Wang *et al*., 2003). The major intrinsic proteins (MIPs) have been shown to act as aquaporins (AQPs), facilitating the permeation of water across

[1]Urmia Universty, Urmia, Iran.
[2] Biology Department and Institute of Biotechnology, Faculty of Sciences, Urmia University, Urmia, Iran.
f.rahmani@urmia.ac.ir,
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membranes (Johansson *et al*., 2000; Maurel *et al*., 2002; Tyerman *et al*., 2002). Aquaporins are an ancient family of channel proteins which transport water and certain neutral metabolites across biological membranes. The major intrinsic protein members play a role as osmotic or turgor sensors (Hill *et al*., 2004). In nature, the plant encounters a combination of stresses concurrently or at different times. There are reports demonstrating the responsiveness of plant MIPs to drought, low temperature, high salinity, light, pathogens, or hormonal stimuli (Tester and Bacic, 2005).

Cytokinins are hormones that play an essential role in plant growth and development. The *Cytokinine Oxidase 1*(*CKO1*) is expressed in different organs, kernel compartments, and in response to different hormones and stresses (Massonneau *et al*., 2004).

Mitogen activated proteins (MAPKs) are conserved genes in eukaryotic signal transduction which orchestrate responses to extracellular stresses and developmental cues. MAPK activity is controlled by sequential activation of three protein kinases, by which an MAPK kinase (MAPKKK) activates an MAPK kinase (MAPKK) that in turn activates an MAPK (Wang *et al*., 2009b). *Arabidopsis* genome sequence contains 60 genes to encode MAPKKK, 10 genes to encode MAPKK, and 20 genes to encode MAPK (Ichimura *et al*., 2002). The high number of genes for MAPK cascade components indicates that plants rely heavily upon MAPK cascades for signal transduction (Pedley and Martin, 2005). In maize, ZmMPK5 was shown to be involved in ABA-induced antioxidant defense and to enhance the tolerance of plants to drought, salt stress, and oxidative stress (Ding *et al*., 2009; Zhang *et al*., 2014). It has been reported that salt, cold, and drought stresses could create osmotic stress in plants; therefore, the three stresses should induce some common genes (Boudscocq and Laurière, 2005; Zhu, 2002). For example, a MAPK cascade comprising MEKK1, MKK2, MPK4, and/or MPK6 is involved in salt, drought, and cold signaling (Teige *et al*., 2004). These results indicate that MAPKs play important roles in stress tolerance.

Maize (*Zea mays* L.) is one of the most important grown plants in the world. Superior position of maize is due to its very wide and variety utilization (Alahdadi *et al*., 2011; Khodarahmpour, 2011; Bekric and Radosavljevic, 2008). Considering its importance, we aimed to study the effect of drought and salinity stresses on the physiological and molecular responses of three-week old maize plants during two periods of 4 and 8 days.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of maize (*Zea mays* L. cv. 704) were obtained from Agriculture Research Centre, Urmia city, West Azerbijan province, Iran. Seeds were graded and the big uniform shaped ones were selected. Seeds were surface sterilized with 2% sodium hypochlorite for 10min then washed with sterile distilled water three times. Following germination in petridish, seedlings were transferred to the plastic pot in 10cm diameter containing a mixture of sand (30%) and compost (70%). Plant experienced the following growth conditions for 21 days in phytotron: the light regime of 16/8h light/dark with 80% humidity and 22 °C temperature. After 21 days, NaCl (200 mM), $CaCl_2$ (40mM) and PEG 6000 (10%) were applied for 4 and 8 days. Treatments were as follows: **(**1**)**Plants were watered regularly and grown normally until the end of experiment for 4 days (C4), (2**)**plants were watered regularly and grown normally till the end of the experiment for 8 days (C8), (3) plants were watered with 50mM NaCl on the first day, 100mM NaCl on the second day, 150mM NaCl on the third day, and 200mM NaCl on the fourth day, then they were watered with 200mM NaCl for 4 days (NaCl-4), (4) plants were watered with 50mM NaCl on the first day, 100mM NaCl on the second day, 150mM NaCl on the third day, and 200mM NaCl on the fourth day, then they were watered with 200mM NaCl for 8 days (NaCl-8), (5) plants were watered with 10mM $CaCl_2$ on the first day, 20mM $CaCl_2$ on the second day, 30mM $CaCl_2$ on the

third day, and 40mM $CaCl_2$ on the fourth day, then they were watered with 40mM $CaCl_2$ for 4 days ($CaCl_2$-4), (6) plants were watered with 10mM $CaCl_2$ on the first day, 20mM $CaCl_2$ on the second day, 30mM $CaCl_2$ on the third day, and 40mM $CaCl_2$ on the fourth day, then they were watered with 40mM $CaCl_2$ for 8 days ($CaCl_2$-8), (7) plants were watered with PEG6000 (10%) for 4 days (PEG-4), and (8) plants were watered with PEG6000 (10%) for 8 days (PEG-8). The young leaves were harvested after the end of the treatments from each plant and transferred to liquid nitrogen for further analyses.

Preparation of extracts

Shoot and root samples (0.5 g) were homogenized in a mortar and pestle with 3ml ice-cold extraction buffer (0.05 M Tris-HCL buffer, pH 7.5, 3mM $MgCl_2$, 1mM EDTA) with the addition of 2mM ascorbate in the case of APX assay. The homogenate was centrifuged at 5000g for 30min at 4°C, and then the supernatant was filtered through paper. The supernatant fraction was used as a crude extract for the assay of enzyme activity. All operations were carried out at 4°C (Kang and Saltiveit, 2002).

Catalase (CAT) assay

Catalase activity was determined by monitoring the disappearance of H_2O_2 at 240nm according to the method of Aebi (1983). The reaction mixture contained 2.5ml of 50mM K-phosphate buffer (pH 7.0), 1mM EDTA, 0.2 ml H_2O_2 1% and 0.3ml enzyme extract (E = 39.4 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX) assay

Ascorbate peroxidase activity was determined by monitoring a decrease in absorbance at 290nm due to ascorbate oxidation. The assay mixture contained 2.5ml of 50mM potassium phosphate buffer (pH 7.0) 0.1mM EDTA, 1mM ascorbate sodium, 1.2mM H_2O_2 1% and 0.1ml of enzyme extract. The activity was calculated using the extinction coefficient (E = 2.8 mM^{-1} cm^{-1}) (Nakano and Asada, 1981).

Guaiacol peroxidase (GPX) assay

Activity of guaiacol peroxidase was measured by following the change of absorption at 420nm due to guaiacol oxidation. The activity was assayed for 2min in a reaction solution containing 2.5ml of 50mM potassium phosphate buffer (pH 7.0), 1ml guaiacol 1%, 1ml H_2O_2 1%, and 0.3ml of enzyme extract (Updhyaya *et al*.,1985). The enzyme activity was calculated using the extinction coefficient (E = 26.6 mM^{-1} cm^{-1}).

Lipid peroxidation assay

Lipid peroxidation in leaf tissue was determined by measuring MDA, a major thiobarbituric acid reactive species (TBARS) and a product of lipid peroxidation (Badawi *et al*., 2004).

Total protein measurement

The protein content of *B. napus* varieties was measured quantitatively using the method given by Lowry *et al*. (1951).

Soluble sugar measurement

To determine the soluble sugar, phenol-sulfuric method was used. Dry shoot and root samples (100mg) were placed into a test tube. Then 10ml of 70% ethanol was added and placed at 5°C to release soluble sugars for one week. After one week, the samples were centrifuged for 20min and then 1ml of the clear solution removed. Then, 1ml of phenol and 3ml of sulfuric acid were added. The tubes were left for 1h so that their color and appearance can be stabilized instead of extracts, 1ml distill water was used for control. Absorbance was read at 580nm using a standard curve of soluble sugars andsugar levels in shoots. Roots were determined in control and treatments. Finally, the amount of soluble sugars was calculated depending on $mg.g^{-1}$ dry.wt (Dubois *et al*., 1951).

Pigment content assay

Chlorophyll *a*, chlorophyll *b*, and carotenoid content assays were performed according to Dere *et al*. (1998). The supernatant was separated and the absorbance was read at 400-700nm on a spectrophotometer. It was recorded that Chlorophyll *a* showed the maximum absorbance at 662nm, chlorophyll *b* at 646nm, and total carotenoid at 470nm.The amount of these pigments was

calculated according to these formulas:

$Chl_a = 11.75\ A_{662} - 2.350\ A_{645}$

$Chl_b = 18.61\ A_{645} - 3.960\ A_{662}$

$C_{X+C} = 1000\ A_{470} - 2.270\ Chl_a - 81.4\ Chl_b / 227$

Extraction of RNA from Plant Tissue and RT-PCR Reaction

At the end of each treatment, various parts of the seedlings were frozen in liquid nitrogen for RNA isolation. Approximately 100mg of leaf tissue was sampled and placed into 1.5ml eppendorf tube and immediately transferred into liquid nitrogen. Plant materials were grounded to powder by mortar and pestle. Total RNA was extracted according to the method described for Trizol. In addition, 500µl of Trizol was added to the frozen plant tissue and sample vortexes for a few seconds to homogenize. A further 500µl of Trizol was added to the sample. Following vortexing for 15sec, samples were incubated at room temperature (RT) for 5min to permit dissociation of nucleoprotein complexes. Furthermore, 200µl of chloroform was added and shaken by hand for 15sec. The sample was centrifuged for 15min at 4°C. After centrifugation (12000 x g), the aqueous phase was transferred to an eppendorf containing 500µl of isopropyl alcohol for precipitation. This step was followed by 15min incubation on ice. Centrifugation at 4°C for 10min pelleted the RNA. The RNA pellet was washed by the addition of 1ml 70% ethanol and centrifuged for five minutes at 7500rcf. The pellet was air-dried and dissolved in 44µl of RNase free H_2O. RNA concentration was quantified by spectrophotometer for each sample.

First-strand cDNA was synthesized in a 12µl reaction system (Fermentas) containing 1µl oligo dT and 11µl total RNA (1µg) at 65°C for 5min followed by the addition of 2µl dNTP, 1µl M-MLV reverse transcriptase (Fermentas), 1µl RNase inhibitor and 4µl reaction buffer at 42°C for 1 hour and 70°C for 5min. PCRs were conducted in 25µl volumes, containing 12.5µl of PCR master mix (Fermentas), 0.75 µM of each of the primer and 2µl cDNA. The reactions were initiated by 94°C for 5min, followed by 30 cycles of: 94°C 1min, 60°C 1min, and 72°C 1min and a final extension at 72°C for 5min. The intensity of PCR amplified bands was visualized under UV and measured using Image J 1.41 software. The primers are listed in Table 1. The *GAPDH* was used as a reference gene. RT-PCR was performed for two independent experiments with three replicates.

Statistical analysis

The experiment was conducted by a completely randomized design with three replications for sampling. Statistical analysis was performed using SPSS 19 program. The data represent means calculated from three replicates. The one-way analysis of variance (ANOVA) was used to compare the effect of these stresses and the statistical significance was set at $p < 0.05$.

RESULTS

Physiology and biochemistry

CAT activity significantly increased during experimental period in $CaCl_2$-4 (4.7 fold), PEG-4 (6.5 fold), NaCl-8 (5.3 fold), $CaCl_2$-8(6.8 fold), and PEG-8 (4.1 fold) plants compared to corresponding controls (Fig.1A). The induction of CAT occurred in a time-dependent manner in case of NaCl and $CaCl_2$. However, maize plants acted differently under PEG as we detected more induction under PEG-4 treatment than PEG-8. The NaCl-4 stress did not affect leaf CAT activity in *Zea mays* leaves.

APX activity remarkably increased in maize leaves as a result of $CaCl_2$-4 (1.9 fold) NaCl-8 (1.8 fold), $CaCl_2$-8 (2.5 fold), and PEG-8 (2.4 fold) compared to their controls. The enzyme activity slightly decreased in NaCl-4 although it was not significant (Fig.1B).

Leaf GPX activity significantly decreased in $CaCl_2$-4 (28%), PEG-4 (12%), NaCl-8 (13%), $CaCl_2$-8 (26%), and PEG-8 (33%) plants compared to corresponding controls (Fig.1C).

Lipid peroxidation, measured as MDA content, is given in Fig. 2A. The MDA content increased in the

leaves of NaCl-4 (1.38 fold), $CaCl_2$-4 (1.17 fold), PEG (1.5 fold) compared to C4. The CaCl2-4 and PEG induced MDA level to 1.1 and 1.2 fold, respectively (Fig. 2A).

Analyses of variance showed a wide range and highly significant effects of NaCl (200mM) and PEG (10%) on total protein content (data not shown). This parameter was reduced in NaCl-4(88%) and PEG-4(80%) plants compared to C4 (Fig. 2B). However, the amount of protein was increased in NaCl-8, $CaCl_2$-8 and PEG-8 by 2.1, 1.7 and 1.7 fold, respectively.

The leaf sugar content increased approximately 1.1, 1.3, 1.2, 1.1, and 1.3 fold in $CaCl_2$-4, PEG-4, NaCl-8, $CaCl_2$-8, and PEG-8 when compared to control plants, while it remained unaffected in NaCl-4 plants (Fig.2C).

Our results show that the chlorophyll *a* content significantly decreased in NaCl-4, NaCl-8, $CaCl_2$-8, and PEG-8 and reached to approximately 76%, 74%, 83%, and 90% of corresponding controls (Fig. 3). The content of chlorophyll *b* was less variable than chlorophyll *a* under different exposures. However $CaCl_2$-8 and PEG-8 significantly affected the contents of chlorophyll *b* by 8% and 14% reduction compared to control. The carotenoid content significantly decreased in NaCl-4(14%) and PEG-8 (18%), and remained unaffected under the rest of impositions compared to their controls.

Gene expression

In the present study, the transcriptional response of *ZmPIP1-1* gene was different at the two stages of exposures, with down regulation at day 4 and no change at day 8 (Figs 4A, 4B, 5A). However, its expression was more significant in $CaCl_2$ (40mM) in comparison to the others.

Comparing the *ZmPIP2-1* gene expression levels between controls and treatments indicated down regulation under NaCl-4 (32%), $CaCl_2$-4 (82%), PEG-4(79%), NaCl-8 (53%) $CaCl_2$-8 (92%), and PEG-8 (74%) compared to control (Figs 4A, 4B, 5B). These observations show the effect of salinity and drought stresses on *ZmPIP2-1* gene expression (Figs 4B, 5B).

Transcript abundance pattern of *ZmPIP2-4* revealed significant down regulation under NaCl-4 (41%), $CaCl_2$-4 (69%), PEG-4 (75%), NaCl-8 (75%), and $CaCl_2$-8 (45%). PEG-8 plants did not show any change in expression (Figs 4A, 4B, 5 C).

In our study, *CKO1* gene responded to NaCl with down regulation at day 4 (38%) (Figs 4A, 4B, 5D), while it responded with up regulation at day 8 (1.5 fold). However, $CaCl_2$-4 and PEG-4 enhanced its expression to 2.1 and 1.33 fold compared to C4 plants, respectively. The $CaCl_2$-8 and PEG-8 did not exert any effect on *CKO1* transcript level.

Our RT-PCR analysis showed up regulation of *ZmMAPK6* under NaCl-4 (1.5 fold), $CaCl_2$-4 (1.2 fold), NaCl-8(1.38 fold), and PEG-8 (1.09 fold) conditions (Figs 4A, 4B, 5E). Exposing 21-day old maize plants significantly reduced mRNA level under $CaCl_2$-8 (37%) and PEG-4(0.88%) treatments compared to their controls.

The *ZmMAPK7* expression was down-regulated by NaCl-4 (50%), NaCl-8 (40%), and $CaCl_2$-8(14%), while $CaCl_2$-4, PEG-4, and PEG-8 showed significant up regulation of 1.5, 1.7, and 1.6 fold on its mRNA synthesis, respectively (Figs 4A, 4B, 5F).

DISCUSSION

In our assay, CAT activity significantly increased under both experimental periods. High activity of CAT has been shown in *Catharanthus roseus* (Abdul Jaleel *et al*., 2007), alfalfa (Wang *et al*., 2009a) and peanut (Akcay *et al*., 2010) under various environmental stresses.

APX scavenges peroxidase by converting ascorbic acid to dehydroascorbate (Ozkur *et al*., 2009). In this study, higher activity for APX was detected under most treatments, suggesting a more effective H_2O_2 removal.

In this study, GPX enzyme acted differently. Imposition of all treatments resulted in reducing its enzymatic activity with 8 days NaCl and PEG, causing more reduction than a shorter period of 4 days. The GPX has been reported as non-specific peroxidase and it plays a key role in roots (Azevedo Neto *et al*., 2006).

Salt stress is known to result in extensive lipid peroxidation, which has often been used as indicator of drought (Moussa and Abdel-Aziz, 2008) and salt (Hernandez and Alamansa, 2002) induced oxidative damage to membranes. The MDA content appeared in a higher level under most of our impositions. We also detected coordination between APX activity and MDA level under 4 day's impositions. Increase in MDA level may result in an increase in membrane permeability or loss of membrane integrity leading to an increase in solute leakage.

To survive under stress, plants accumulate proteins that protect cells from stress effects (Wang *et al*., 2003). In our experiment, we detected higher protein level in leaves of 8 days stress exposed plants than controls. A higher content of soluble proteins has also been noticed in salt tolerant than in salt sensitive cultivars of plants, such as barley, sunflower, finger millet, and rice (Ashraf and Harris, 2004).

Soluble sugars play an essential role in plant metabolism as products of hydrolytic processes, substrates in biosynthesis processes, energy production, sugar sensing, and signaling systems (Gibson, 2005). Exposing 21-day old maize plants to applied stresses elevated the level of soluble sugar content. The rise in concentration of sugars and reserve polysaccharides could be due to impaired carbohydrate utilization (Munns and Termaat, 1986).

The leaf is a very important photosynthetic organ, in which green pigment chlorophyll transform light energy into the potential energy of assimilates. The results from our experiment show that salinity causes a significant reduction in chlorophyll *a* which is in agreement with some previous studies on different crops, e.g. sunflower (Ashraf and Sultana, 2000) and wheat (El-Hendawy *et al*., 2005). A decrease in chlorophyll contents implies a lowered capacity for light harvesting. Moreover, ROS production is mainly driven by energy absorption in the photosynthetic apparatus, which might be avoided by degradation of pigments (Herbinger *et al*., 2002).

Plasma membrane intrinsic proteins belonging to the aquaporin family have been reported to function in water transport in many plant species (Katsuhara *et al*., 2002; Tester and Bacic, 2005). The expression patterns of aquaporin genes have been monitored in *Arabidopsis*, which shows that PIP transcripts are generally down-regulated upon gradual drought stress in leaves, with the exceptions of *AtPIP1;4* and *AtPIP2;5*, which are up-regulated (Alexandersson *et al*., 2005). The PIP1-type protein in *Zea mays* functions as a water channel by forming a heterotetramer with several ZmPIP2-type proteins (Fetter *et al*., 2004). Aquaporins modulate the water flow across the membranes; hence, they are important in processes such as stomatal opening, phloem loading, pulvinar movement, etc. (Chuanfeng, 2005).

In accordance with our finding, imposition of salinity (NaCl 150 mM) for 4 days also increased *ZmPIP1-1* gene expression in the roots of three-week old maize plants (Marulanda *et al*., 2010). However, the NaCl (200 mM) has been reported to repress the expression of several *ZmPIPs*, including *ZmPIP1-1* after 24h incubation under salt stress (Alexandersson *et al*., 2005). The induction of *ZmPIP1-1* at day 4 could be due to the acquisition of compatible solutes which will eventually increase the cellular pathway of water uptake. The ABA independent pathway was reported to be, at least, responsible for the activation of salt induced *ZmPIP1-1* (Chuanfeng, 2005). The lower mRNA level of *ZmPIP1-1* at longer exposures indicates that maize plants manifested a new water balance. Previous studies indicate that the expression of aquaporin genes is affected by the varieties, severity of stressors, duration of treatment and species, and developmental stage of plants (Chuanfeng, 2005).

The existence of discrepancy between transcriptional and protein level of aquaporins has been also reported, showing these two levels of gene expression not necessarily correlated. Katsuhura *et al.* (2002) showed the repression of *ZmPIP2-1* mRNA by NaCl (200mM) after 24 and 48 hours; however, its protein level had only

reduced after 48 hours.

The NaCl (200 mM) has been reported to repress the expression of several *ZmPIPs*, including *ZmPIP2-4* after 24h incubation under salt stress (Alexandersson *et al*., 2005). In our experiment, the mRNA level of this gene was also repressed at day 4 under NaCl imposition. The induction of *ZmPIP 2-4* has been shown to be ABA dependent (Chuanfeng, 2005).

Cytokinin oxidase irreversibly degrades cytokinins by cleaving the N6-side chain from the adenine/adenosine moiety. The enzyme thought to play a key role in the regulation of cytokinin levels in plants (Massonneau *et al*. 2004). The *CKO1* expression has been shown to be induced by cytokinins. Hence, the observed change in transcriptional level of *CKO1* under our experimental condition could be explained by alteration in cytokinin level under the above mentioned stresses.

In cell signaling, protein phosphorylation is involved in osmotic stress adaptation. MAPKs are kinases that phosphorylate a variety of substrates, including transcription factors, transcription regulators, splicing factors, and other protein kinases (Mishra *et al*., 2006). They are involved in normal cell metabolism such as physiological, developmental and hormonal responses to biotic and abiotic stresses, e.g. pathogen infection, wounding, low temperature, drought, hyper- and hypo-osmolarity, high salinity, mechanical stress, metals, and ROS (Pitzschke and Hirt, 2009). Under our experimental conditions, the expression of *ZmMPK6* was positively affected by $CaCl_2$-4, NaCl-8, and PEG-8. MAPKs play important roles in Ca^{2+} signal transduction. Exposing two-week old maize roots to $CaCl_2$ significantly up-regulated *ZmMPK6* at 5h-treatment (Wu *et al*., 2011), which is in line with the result of the present study. Our RT-PCR analysis also revealed up-regulation of this gene after 4 days of exposure to $CaCl_2$. However, $CaCl_2$-8 lowered its transcriptional level compared to control plants.

The expression of *ZmMPK7* was either down regulated or unaffected under applied exposures. In accordance with our finding, Wu *et al.* (2011) demonstrated down regulation of *ZmMAPK7* at 5 hours $CaCl_2$ treatment. However, *ZmMAPK6* was more affected under our studied conditions than *ZmMAPK7*. The alterations in mRNA abundance of both studied genes indicate their involvement in signal transduction induced by applied impositions.

CONCLUSION

Based on the results of this experiment, it can be concluded that increase in antioxidant activities, MDA, protein, and soluble sugar levels under salt and drought conditions will induce tolerance in maize plants. Semi-quantitative RT-PCR analysis of *ZmPIPs*, *CKO1*, and *MAPKs* genes expression in maize also showed differential response and significant down or up regulation under applied adverse conditions. Further studies should be carried out to investigate the mechanism by which plant gene expression is controlled by abiotic stresses.

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COMPETING INTEREST

The authors have declared that there are no competing interests.

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Table 1: Primers used in semi-quantitative RT-PCR experiment

Genes	Primer sequence	Reference
ZmPIP1-1	F: 5'-AGGTCTTAAAGGAGCCGATG -3′ R: 5'-TGAACTCTTAAAGCTTGACTCG -3′	Chuanfeng 2005
ZmPIP2-1	F: 5′-CTTGGCTGTGGAGCAATG -3′ R: 5′-CGGAATAATCCTTCAGAGT -3′	Chuanfeng 2005
ZmPIP2-4	F: 5′-GCACCGTTCCGATTCTGT -3′ R: 5'-GTGATCGGATAAAAACTCACG -3′	Chuanfeng 2005
ZmMPK6	F: 5′-TATGCCAAGGGATCTACAGG-3′ R: 5′-GACCACCACGACTATCAACG	Wu et al 2011
ZmMPK7	F: 5'-TCGAGGAGCTTATGGGATAGTTT -3' R: 5'-GTCTATGGATGTGCCGTAGTTGT -3'	Wu et al 2011
CKO1	F: 5'- GGTGCACGGCGAGGAGGT -3' R: 5'- CGTCCCACATGGATTTGTTGAG -3'	Massonneau et al 2004
GAPDH	F: 5′-TTGCCATCAATGACCCCTTCA-3′ R: 5′-CGCCCCACTTGATTTTGGA-3′	Wu et al 2011

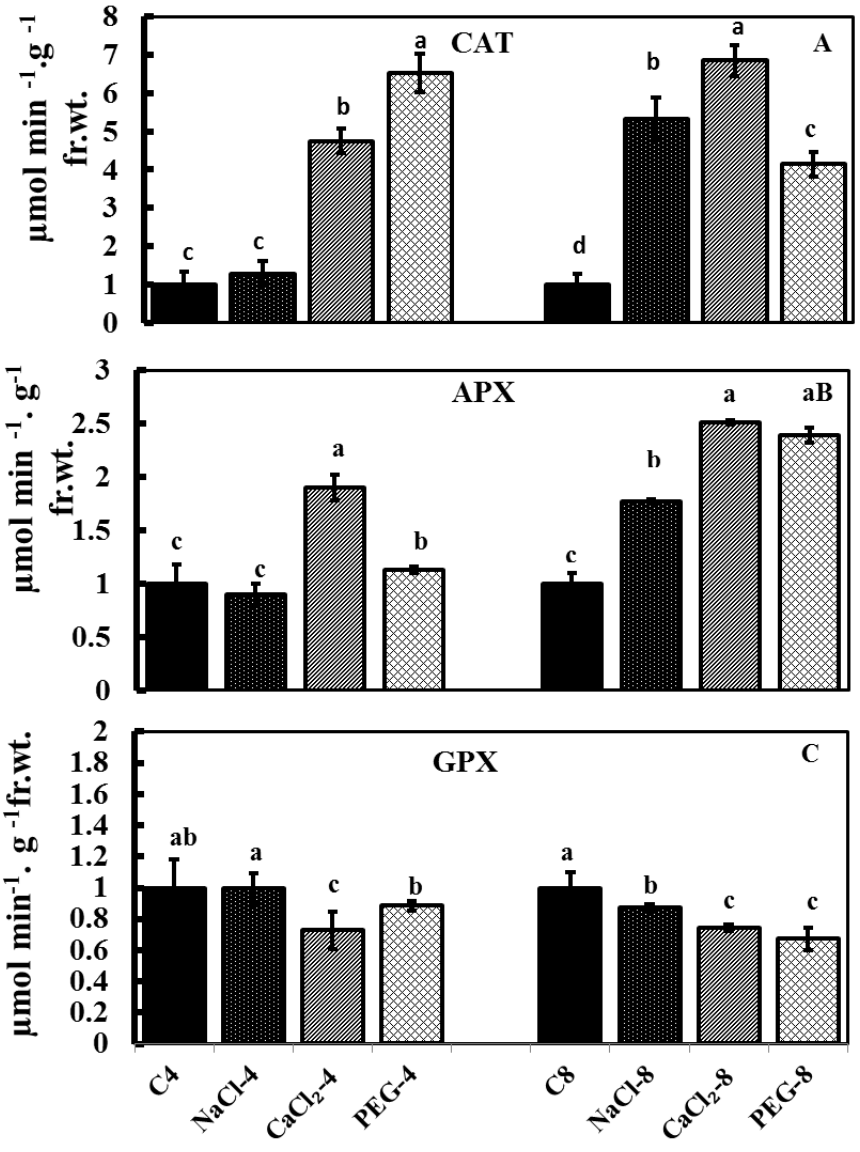

Figure 1: **Effect of NaCl (200 mM), $CaCl_2$ (40 mM) and PEG (10%) on A: CAT, B: APX and C: GPX (µmol mg^{-1} fr.wt.min^{-1}) in *Zea mays* cv.704 plants. C4: plants with 4 days normal watering, NaCl-4: plants with 4 days NaCl watering, $CaCl_2$-4: plants with 4 days CaCl2 watering, PEG-4: C4: plants with 4 days PEG watering, C8: plants with 8 days normal watering, NaCl-8: plants with 8 days NaCl watering, $CaCl_2$-8: plants with 8 days $CaCl_2$ watering, PEG-8: plants with 8 days PEG watering. All the values followed by the same letter in each column are not statistically different at the $p < 0.05$ probability level.**

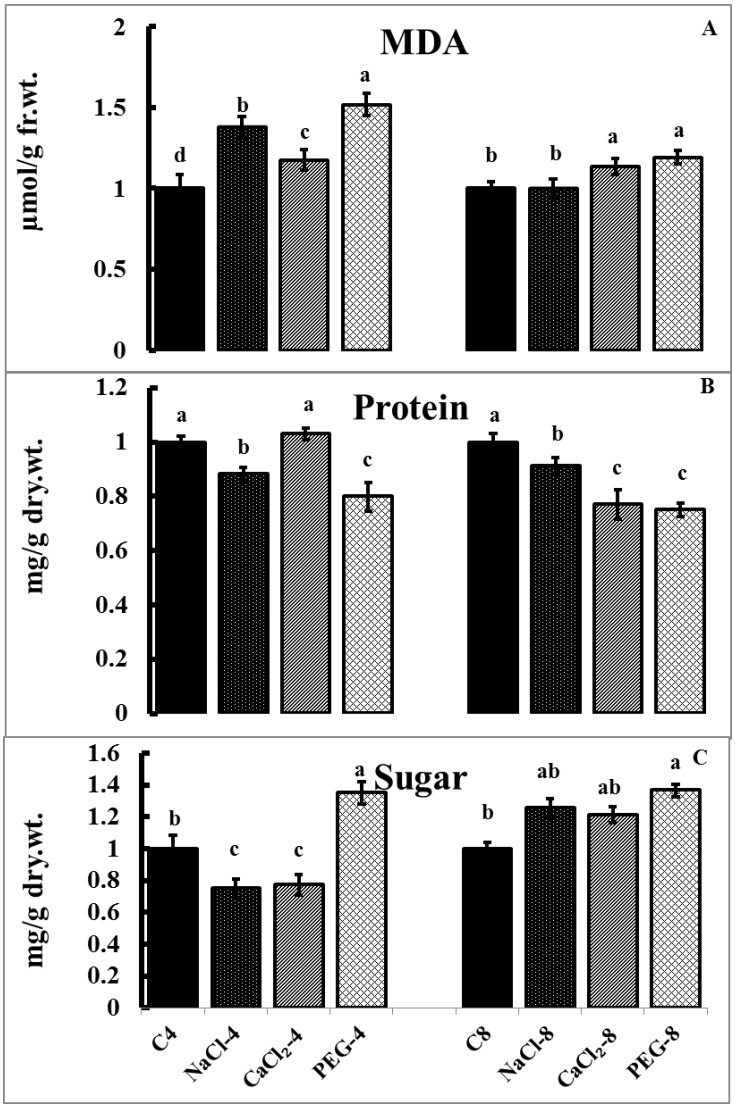

Figure 2: Effect of NaCl (200mM), CaCl2 (40 mM) and PEG (10%) on A; MDA, B: protein and C: sugar contents (µmol g-1 fr. wt.) in Zea mays cv.704 plants. C4: plants with 4 days normal watering, NaCl-4: plants with 4 days NaCl watering, CaCl2-4: plants with 4 days CaCl2 watering, PEG-4: C4: plants with 4 days PEG watering, C8: plants with 8 days normal watering, NaCl-8: plants with 8 days NaCl watering, CaCl2-8: plants with 8 days CaCl2 watering, PEG-8: plants with 8 days PEG watering. All the values followed by the same letter in each column are not statistically different at the $p < 0.05$ probability level.

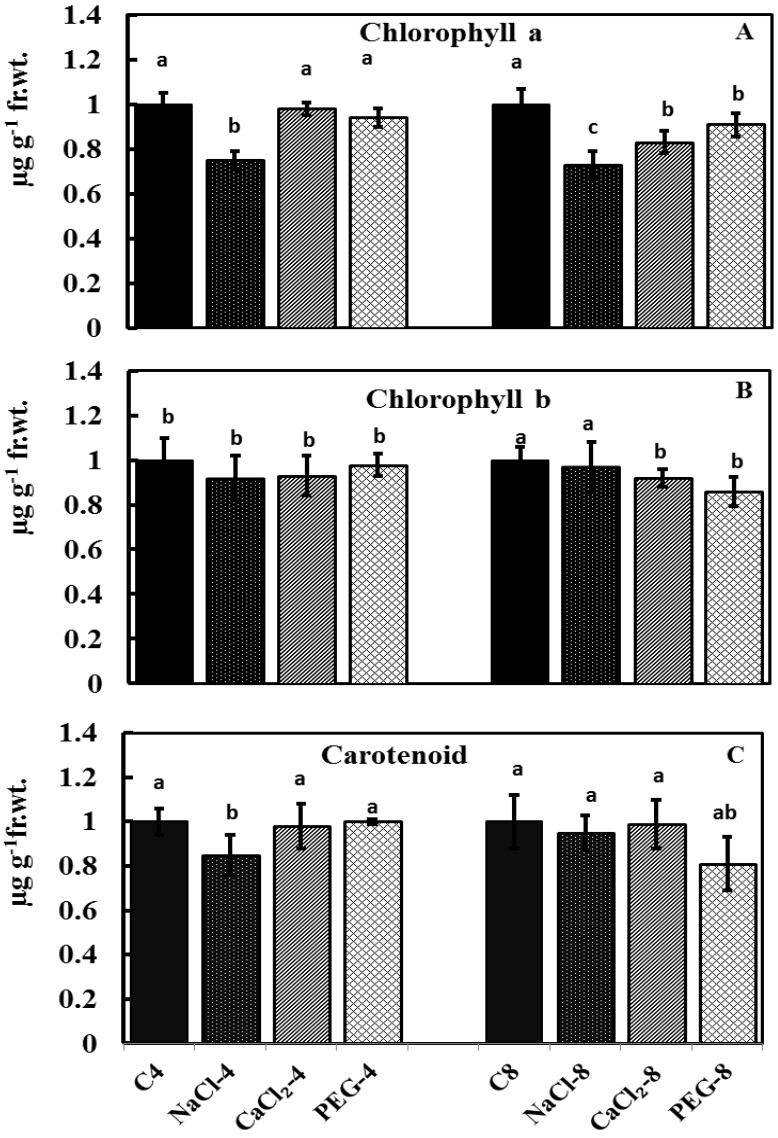

Figure 3: Effect of NaCl (200 mM), $CaCl_2$ (40 mM) and PEG (10%) on A: chlorophyll a, B: chlorophyll b and C: carotenoid contents (µg/g fr. wt.) in *Zea mays* cv.704. plants. C4: plants with 4 days normal watering, NaCl-4: plants with 4 days NaCl watering, CaCl2-4: plants with 4 days CaCl2 watering, PEG-4: C4: plants with 4 days PEG watering, C8: plants with 8 days normal watering, NaCl-8: plants with 8 days NaCl watering, CaCl2-8: plants with 8 days CaCl2 watering, PEG-8: plants with 8 days PEG watering. All the values followed by the same letter in each column are not statistically different at the $p < 0.05$ probability level.

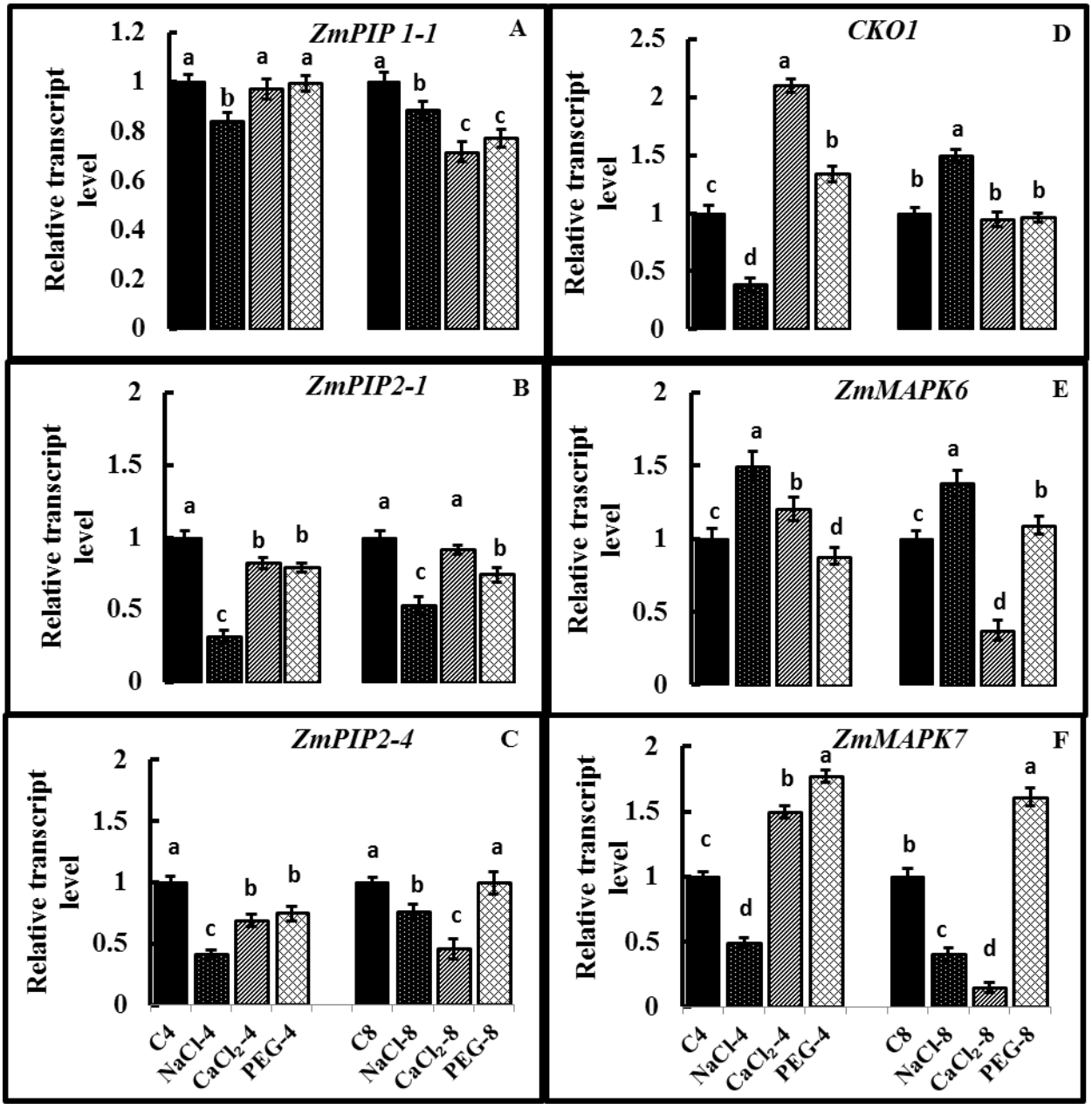

Figure 4: Gel graph of *ZmPIPs*, *CKO1*and *ZmMPKs* genes expression in maize leaves. Maize plants exposed to various stresses for A) 4 and B) 8 days and their mRNA levels analyzed by RT-PCR.

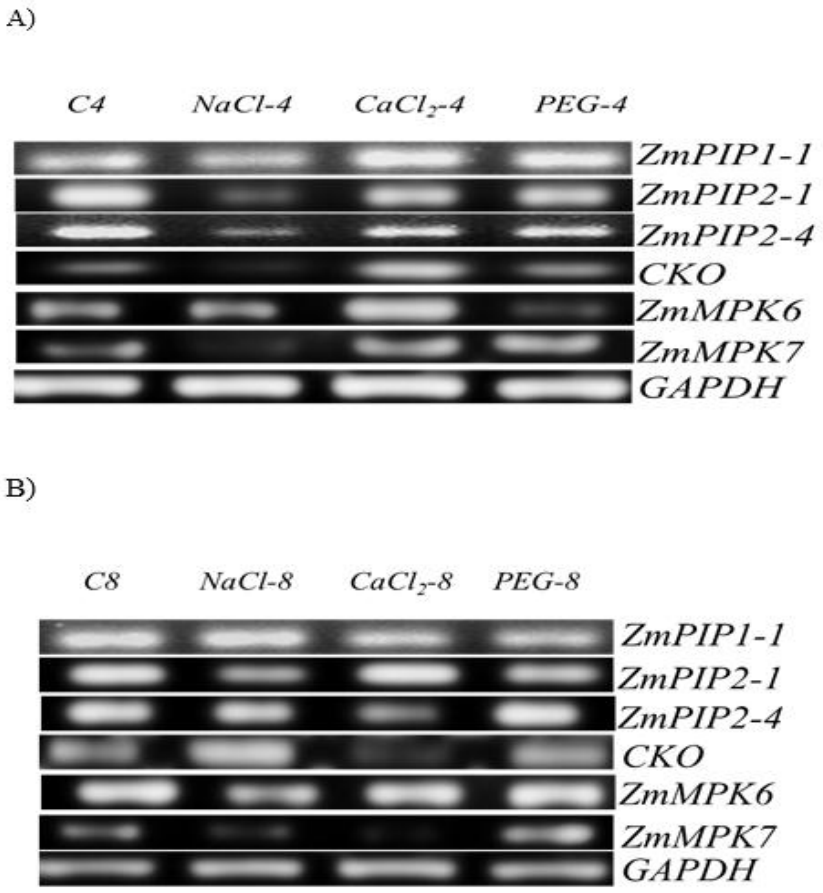

Figure 5: Relative transcript levels of A) *ZmPIP1-1,* B) *ZmPIP2-1,* C) *ZmPIP2-4,* D) *CKO1,* E) *ZmMAPK6 and* F) *ZmMAPK7* using semi-quantitative RT-PCR in Zea mays leaves. The 21 day old maize plants were exposed to non-treatment (C), NaCl (200 mM), CaCl2 (40 mM) and PEG 6000(10%) for duration of 4 and 8 days. Values were compared with GAPDH gene and experiments carried out with three replications. Values were normalized to control. Different letters show significant differences.

تأثيرات الجفاف والملوحة على مضادات الأكسدة في التعبير الجيني *Zea mays* L. *PIPs*

أختر ايوبي[1]، فاطمة رحماني[2]

ملخص

تهدف هذه الدراسة إلى التعرف على تأثير ضغوط الملوحة والجفاف على بعض النواحي الفسيولوجية والجزيئية لنباتات الذرة (الشامية cv.704). وقد أجريت التجربة في غرفة مخصصة للنمو وتم تطبيق الضغوط NaCl (200 mM)، $CaCl_2$ (40 mM) و PEG 6000 (10)٪ على مدار فترتين من 4 و 8 أيام. وقد لوحظ انخفاض في نشاط انزيم GPX ولوحظ وجود الكلوروفيل a و b في جميع العلاجات. في المقابل، اظهرت النتائج حفز النشاطات الأنزيمية الكاتالازية والأسكوربات البيروكسيديز ، malondialdehyde والبروتين ومستويات السكر القابل للذوبان في إطار التطبيقات المعمول بها. أظهرت تحاليل RT-PCR شبه الكمية تباينًا قليلا أو كبيرا في الجينات ZmPIPs و CKO1 و MAPKs. وتوضح الدراسة الحالية أن الضغط الناتج عن الجفاف والملح يرتبط مع الضغط التأكسدي ويدعم فكرة إدخال الجينات المدروسة خلال تطبيق هذه الضغوط.

الكلمات الدالة: الأنشطة المضادة للأكسدة، الإجهاد ،الجفاف، التعبير الجيني، MAPK، ضغوط الملوحة. ZmPIP.

[1] فسيولوجيا النبات، جامعة أورميا، أورميا، إيران. f.rahmani@urmia.ac.ir .

[2] قسم الأحياء ومعهد التكنولوجيا الحيوية، كلية العلوم، جامعة أورميا، أورميا، إيران.

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