



Exogenously-applied Salicylic Acid and Ascorbic Acid Modulate some Physiological Traits and Antioxidative Defense System in *Zea mays* L. Seedlings under Drought Stress

Naglaa Loutfy^{(1)#}, M.M. Azooz⁽¹⁾, Mona F. Abou Alhamd⁽¹⁾

⁽¹⁾Botany and Microbiology Department, Faculty of Science, South Valley University, Qena, Egypt.



THIS WORK was undertaken to evaluate the effects of salicylic acid (SA) (0.0 and 0.5 mM) and ascorbic acid (AsA) (0.0 and 100 ppm) on enzymes activity, soluble sugars, and some physiological traits of maize seedlings (*Zea mays* L.) under drought stress using PEG-6000. In general, under drought conditions, significant reduction in plant biomass and photosynthetic pigments was detected. On the other hand, soluble sugars (glucose, fructose and sucrose), soluble proteins, antioxidant enzymes activity [catalase (CAT), ascorbate peroxidase (APOX) and superoxide dismutase (SOD)], glutathione (GSH), proline and malondialdehyde (MDA) contents were increased significantly under drought as compared to control. Shoots of tested plants were more affected by drought stress than roots. Seed presoaking in AsA or SA solutions resulted in massive increase in growth parameters, chlorophyll contents, osmoprotectants (soluble sugars, free amino acids and soluble proteins), antioxidant enzymes activity [ascorbate peroxidase (APOX) and superoxide dismutase (SOD)] and non-enzymatic antioxidants [carotenoids, and glutathione (GSH)] content as compared to control. Conversely, proline, catalase (CAT) and malondialdehyde (MDA) content were decreased significantly. The present study established that, both salicylic acid and ascorbic acid alleviate drought stress in maize plants which could attribute to the increased in osmotic solutes and antioxidative capacity of maize plants.

Keywords: Ascorbic acid, Antioxidants, Drought stress, Salicylic acid.

Introduction

Maize (*Zea mays* L.) is one of the important grown cereal crops in Egypt, plays an essential role and is used in both human and animal feeding. It is cultivated in tropical humidity and subtropical area (Harris et al., 2007). Abiotic environmental conditions, such as drought stress, are critical elements towards restricting the crop efficiency having adverse effect on yield capability of plant. Drought is one of the most detrimental abiotic stresses across the world which is seriously hampering the productivity of agricultural crops (Nazar et al., 2015; Hegab, 2016). Flexas & Medrano (2002) reported that limitation of plant growth by drought is mainly due to the decrease of plant carbon balance, which

is dependent on photosynthesis. When plants are exposed to abiotic stresses, reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and singlet oxygen (1O_2) are produced (Almeselmani et al., 2006). These activated oxygen damages protein, membrane lipid and nucleic acid cellular constituents (Foyer et al., 1994). Plants have an antioxidant system to alleviate the harmful effect caused by ROS (Del Rio et al., 2002). Drought is known to alter a variety of physiological and biochemical processes from photosynthesis to protein synthesis and accumulation of solutes (Mafakheri et al., 2011). Plant cells accumulate various compounds, such as sugars, proline, glycerol, antioxidants, glycine betaine and secondary metabolites, as an adaptive response to

#Corresponding author email: naglaaloutfy@yahoo.com

Received 25/11/2019; Accepted 3/3/2020

DOI: 10.21608/ejbo.2020.20077.1400

Edited by: Prof. Dr. Wedad Kasim, Faculty of Science, Tanta University, Tanta, Egypt.

©2020 National Information and Documentation Center (NIDOC)

water stress (Halimeh et al., 2013; Waseem et al., 2015; Kosar et al., 2015). Number of enzymatic antioxidants [catalase, superoxide dismutase, ascorbate peroxidase and peroxidase] and non-enzymatic antioxidants [vitamin C, glutathione and tocopherols] are produced in plants due to abiotic stresses that protect plants against ROS-produced oxidative agents (Mittler, 2002; Ashraf, 2009; Nazi et al., 2016).

As a powerful signaling molecule in plants, salicylic acid (SA) is involved in protection mechanisms through modifiable physiological and biochemical functions and has varied effects on tolerance of abiotic and biotic stresses elements (Gunes et al., 2007; Nazar et al., 2011; Kang et al., 2013; Mahmoud, 2017; Morsi et al., 2018). The application of exogenous SA enhanced the growth and photosynthetic speed of crops under water stress (Hussein et al., 2007; Loutfy et al., 2012) and increased stomatal conductance and photosynthetic activity under drought stress (Habibi, 2012). Additionally, it has been found that plants treated with SA generally shown better resistance to drought stress (Al-Hakimi & Hamada, 2001). Palma et al. (2013) stated that SA and its associated compounds are important elements of redox balance modulation and cause a rise in total glutathione content. Ascorbic acid, as one of the most important plant cell antioxidant, is synthesized in mitochondria and transported to other cell compartments (Loutfy et al., 2019). Ascorbic acid has an antioxidant property that decreases oxygen radical injury resulting from drought pressure (Rosales et al., 2006). Therefore, it plays an essential role in photosynthesis process and decreases the negative effect of environmental stresses on plant growth through increase the defense mechanisms against oxidative stress (Azooz & Al-Fredan, 2009; El-Awadi et al., 2014). Under stress conditions, it is also active component in plant metabolism where it increases the availability of water and nutrient (Barakat, 2003; Khan et al., 2011). It was reported that salicylic and ascorbic acid alleviate damage effects of drought (Mekki et al., 2015; Kareem et al., 2017; Penella et al., 2017).

The aim of this experiment was to determine whether both SA and AsA could efficiently amend the injurious effects of drought stress on the growth of maize plants, as well as comparing between the role of each of them in the alleviation of drought stress.

Materials and Methods

Plant material and growth conditions

Maize (*Zea mays* L.) was obtained from the Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Seeds sterilized with sodium hypochlorite solution (5%) for five minutes, washed thoroughly with distilled water, the seeds were divided into three groups. The first group was soaked in distilled water (control) for 12hrs, the second group was soaked in 0.5mM SA solution for 12hrs and the third group was soaked in 100ppm AsA for 12hrs before grown in each petri dish on single layer of Whatman filter paper. Seeds germinated in Hoagland's solution under lab conditions for two weeks ($28 \pm 2^\circ\text{C}/20 \pm 2^\circ\text{C}$ day-night and a relative humidity of $80 \pm 5\%$) and all solutions were renewed every two days. Hoagland's solution was diluted to 0.5 strength and the PH maintained at 5.5. The nutrient solution contains different concentrations of polyethylene glycol 6000 (0.0, -0.4MPa) in the presence and absence of 0.5 mM salicylic acid or 100ppm ascorbic acid. Preliminary experiments were used to determine the recommended concentration of SA, AsA or PEG concentration. Osmotic potentials of PEG 6000 were calculated as described by Michael & Kaufman (1973). The main growth experiments consisted of the following treatments: 1) well-watered without SA or AsA (control), 2) well-watered with SA or AsA (SA and AsA treatments), 3) water stress (as PEG 6000) without SA or AsA (drought treatment), 4) water stress (as PEG 6000) with SA or AsA. These experiments were conducted for 15 days. Triplicates samples were used for each treatment.

Determination of growth parameters

The harvested plant seedlings were divided into roots and shoots and the fresh weight (FW) of each sample was determined. The harvested plant's organs were quickly frozen and stored at -30°C for biochemical analyses. Some parts of the samples were rapidly dried in an oven at 80°C to estimate constant weight for determination of dry weight (DW).

Determination of photosynthetic pigments

According to Metzner et al. (1965), chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and carotenoids were determined spectrophotometry at the wavelengths of 663, 647 and 470nm.

Analysis of soluble sugars by high performance liquid chromatography

Total soluble sugars (glucose, fructose and sucrose) were analyzed according to the method of Karkacier et al. (2003). The samples (20 μ l) were injected into a carbohydrate analysis column (Shodex NH2P-50 4E, 250mm x 4.6mm i.d., Showa Denko, Tokyo, Japan) connected to a high performance liquid chromatography (HPLC) pump (L-7000, Hitachi, Tokyo) at a flow rate of 0.5ml min⁻¹. Oligosaccharides and monosaccharides eluted from the column were quantified with a refractive index (RI) detector (L7490, Hitachi) equipped with a chromatographic-data processor (D-2500, Hitachi).

Determination of organic solutes

According to the method of Bradford (1976), soluble protein content was determined. Proline was determined according to the procedures described by Bates et al. (1973). Total free amino acids were extracted from plant tissues and determined (Moore & Stein, 1948).

Assays of antioxidant enzymes activity

About 500mg of plant tissue was homogenized in 1ml K-phosphate buffer pH.7, containing 0.1mM Na₂EDTA and 1% of PVP and then centrifuged at 10,000rpm at 4°C for 20min. The supernatant was used for enzyme assay. Catalase activity was determined following Aebi (1984). Peroxidase (POX) activity was determined according to MacAdam et al. (1992). Superoxide dismutase activity was determined using the technique mentioned by Beauchamp & Fridovich (1971).

Determination of total glutathione (GSH)

Estimation of GSH was carried out according to the procedure of Beutler (1963). Total glutathione concentration was estimated by monitoring the formation of 5-thio-2-nitrobenzoic acid, which is proportional to GSH at 412nm against reagent controls.

Lipid peroxidation

According to the method of Heath & Packer (1968), the extent of lipid peroxidation was estimated by quantifying the malondialdehyde (MDA) content of shoot and root

Statistical analysis

The data were statistically analyzed by one-

way ANOVA analysis of variance using SPSS program (Snedecor & Cochran, 1980). Values in the figures indicate the mean values \pm SD based on three independent determinations (n= 3) and the Least Significant Difference (LSD) was used to test the differences between treatments; and $P \leq 0.05$ was considered statistically significant.

Results

Growth parameters

Several growth parameters including shoot and root fresh weights and shoot and root dry weights of maize plants were determined (Fig. 1). The studied parameters were decreased under the effect of drought, compared to control. Salicylic acid and ascorbic acid significantly increased the fresh weight of roots (38.54% with SA and 59.37% with AsA) and shoots (23.80% with SA and 28.91 % with AsA) under drought stress, compared by drought-stressed seedlings. Also, dry weight of both roots (14.28% with SA and 21.42% with AsA) and shoots (21.05% with SA and 26.31% with AsA) under drought stress significantly increased, compared with drought stressed seedlings. Treatments with salicylic acid and ascorbic acid lighten the damaging effect produced with drought stress.

Drought stress induced a noticeable decrease in photosynthetic pigments (Chl. *a*, Chl. *b* and Carot.) in maize seedlings compared to control (Fig. 2). Photosynthetic pigments, Fig. 2 showed that there was observable increase in photosynthetic pigments (Chl. *a*, Chl. *b* and Carot.) in SA and AsA treated seedlings under drought stress compared with drought- stressed seedlings. The increase in chl. *a* was 80.20% with SA and 92.67% with AsA. However, the increase in chl. *b* and carotenoids was about 17% and 20%, respectively, in both SA and AsA.

Biochemical characteristics

As shown in Fig. 3, drought stress, SA or AsA stimulated the accumulation of soluble sugars (glucose, fructose and sucrose) in maize seedlings. However, this accumulation was higher in the drought stressed samples treated with SA or AsA than in the drought-stressed ones. The content of soluble sugars in the stressed-AsA treated roots increased by 20.23% for glucose, 11.28% for fructose and 60.23% for sucrose higher than drought treatment.

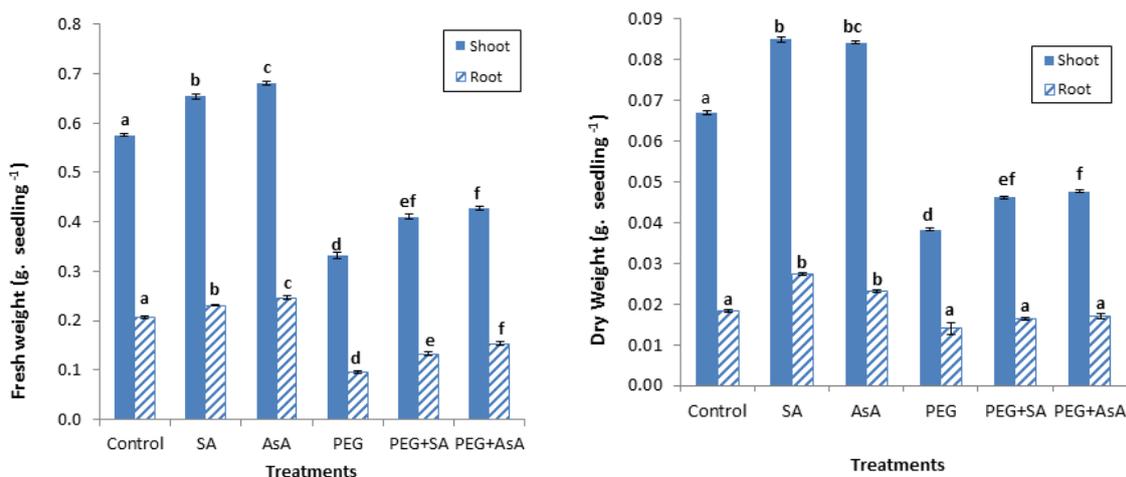


Fig. 1. Fresh weight and dry weight of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates (n= 3), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

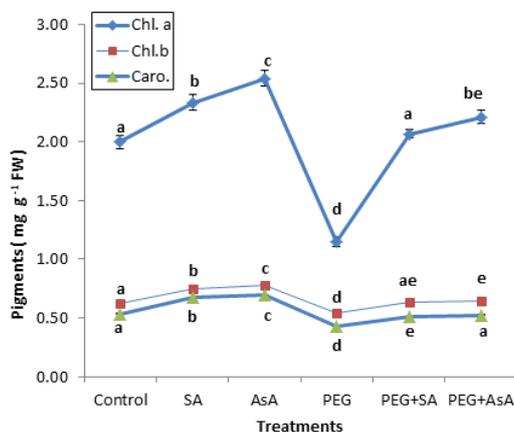


Fig. 2. The content of Chl. a, Chl. b and carotenoids of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates (n= 3), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

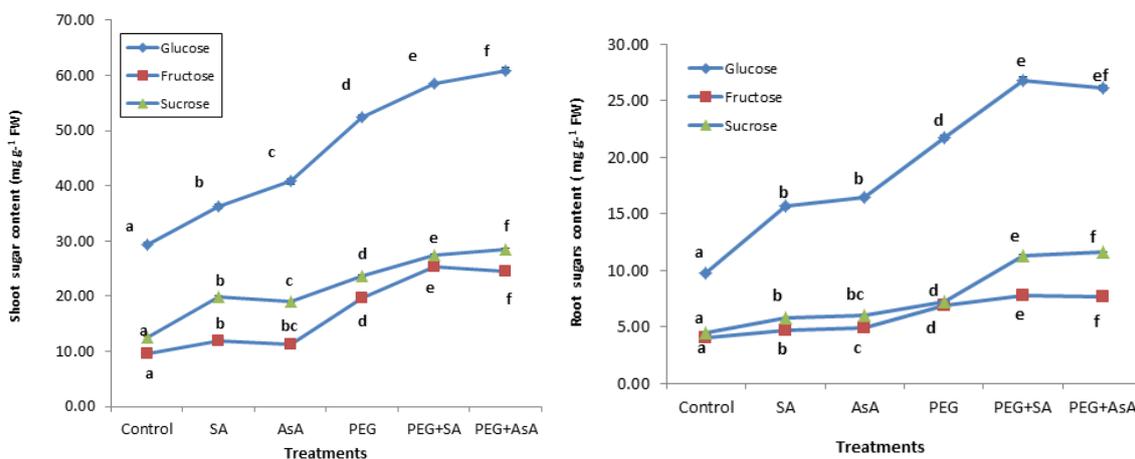


Fig. 3. The content of glucose, fructose and sucrose of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates (n= 3), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

The data presented in Figs. 4 and 5 showed that, drought increased soluble proteins, total free amino acids content and proline accumulation in maize seedlings, compared to control. The increase of soluble proteins in maize roots and shoots was 77.24% and 60.37%, respectively as compared with control. While the increase of total free amino acids content in maize shoots was 10.53%, as compared with control. On the other hand, the increase in proline content in maize roots and shoots was 3.5 -fold and 1.5-fold, respectively, higher than control (Fig. 4). SA and AsA increased soluble proteins in the drought stressed-roots and shoots while total free amino acids was increased only in roots, as compared with the drought-stressed plants. Soluble proteins had maximum increase

(31.84%) in the drought stressed-roots treated. Conversely, in drought stressed-shoots, SA and AsA decreased total free amino acids contents by about 4% with SA and 24% with AsA, as compared with drought stressed-plants. Also, proline accumulation was highly significantly reduced in maize roots and shoots treated with SA and AsA under drought stress (about 50% less than drought stressed-seedlings).

Figure 5 showed that glutathione (GSH) accumulation under drought treatment reached about 100% increase higher than those of the control in maize roots and shoots. However, each of SA and AsA significantly increased glutathione in roots and shoots of the drought-stressed plants.

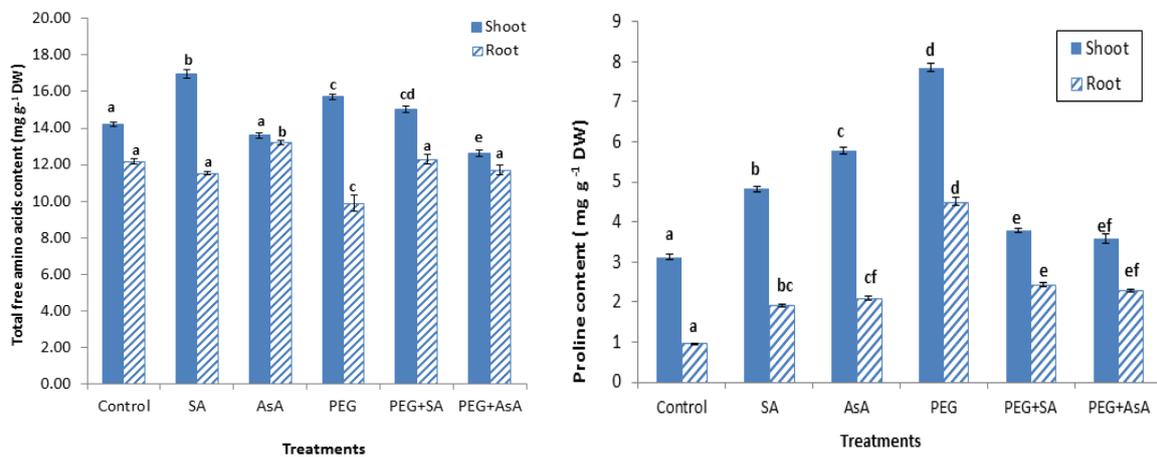


Fig. 4. Total free amino acids and proline in root and shoot of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates (n= 3), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

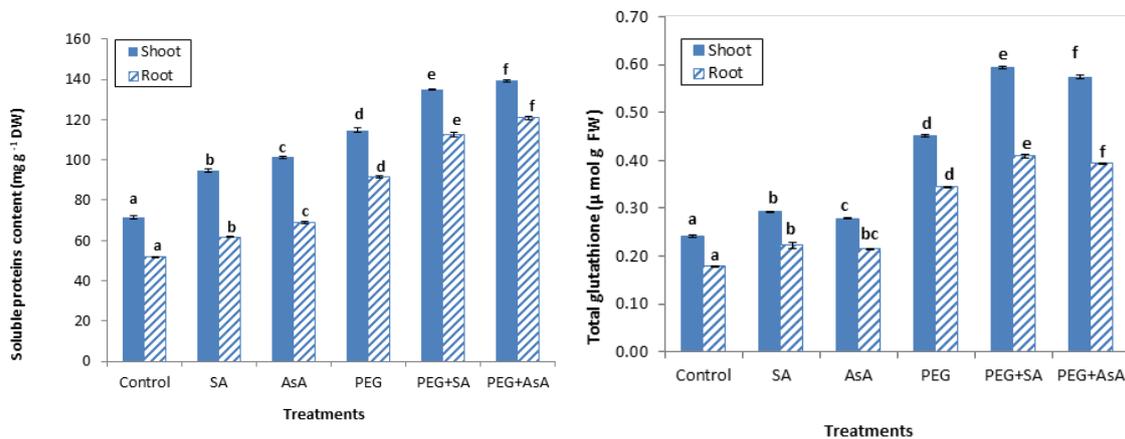


Fig. 4. Soluble proteins and total glutathione in root and shoot of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates (n= 3), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

The data present in Figs. 6 and 7 indicated that there was a significant increase in APOX and SOD activity under the stressed-SA and stressed-AsA treatments, compared to drought-stressed seedlings. APOX activity increased under drought stress by 34% with AsA-treated roots and 38% in case of SA-treated roots, compared with those under drought stress. While the increase in shoots under drought stress was 30 and 32% with SA- and AsA-treated seedlings, as compared to drought-stressed seedlings. Also, SOD activity increased under drought stress by 31 and 35% with SA- and AsA-treated roots, respectively, compared with those under drought stress. While the increase of SOD activity in maize shoots was 8 and 13% with AsA and SA, respectively, as compared with shoots under drought stress. On the contrary, CAT activity decreased under drought stress by 29 and 33% with SA- and AsA-treated roots, respectively,

as compared with those under drought stress. On the other hand, the decrease of CAT activity in maize shoots was 10 and 16% with SA and AsA, respectively, as compared with shoots under drought stress. In general, CAT, APOX and SOD activities increased under drought stress, as compared with control.

Malondialdehyde (MDA) content highly significantly increased in the drought-stressed-roots, as compared with control. The increase in roots was 80.37% higher than control, while in the drought-stressed-shoots, it reached 2.5-fold higher than control. On the other hand, MDA content significantly decreased in the drought-stressed-roots and shoots treated with SA or AsA, as compared with the drought-stressed seedlings and it was about 35% with SA and 40% with AsA (Fig. 6).

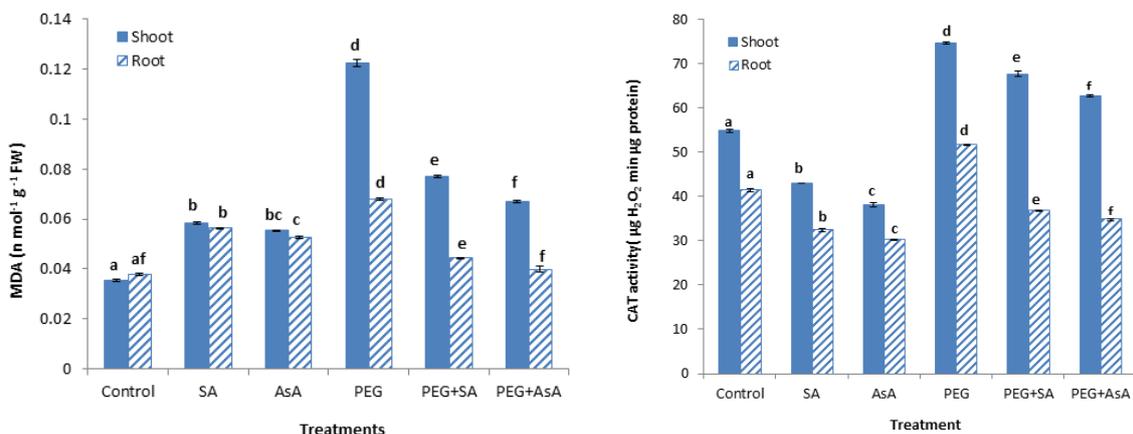


Fig. 6. MDA content and catalase activity in root and shoot of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates ($n=3$), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

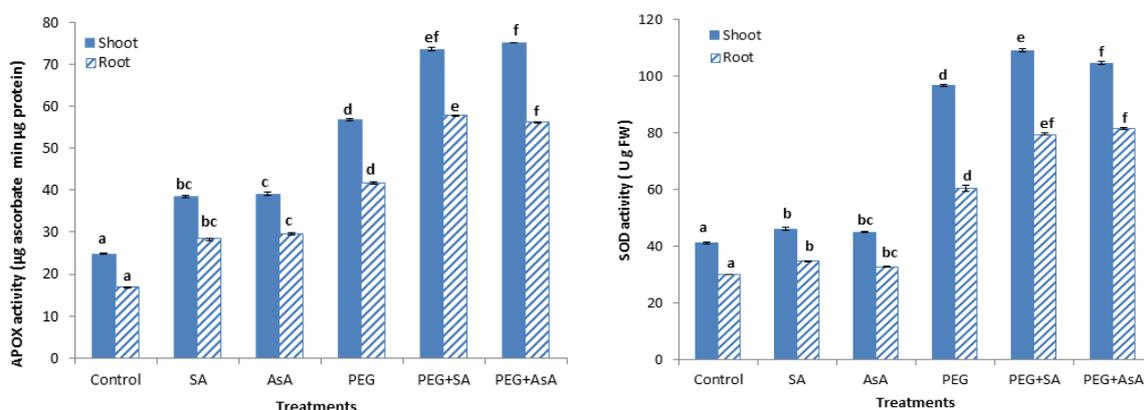


Fig. 7. Ascorbate peroxidase activity and sodium desmutase activity in root and shoot of *Zea mays* L. in response to water stress (PEG), salicylic acid (SA) and ascorbic acid (AsA) [Vertical bars represent \pm SD of three replicates ($n=3$), bars carrying different letters are significantly different at $P \leq 0.05$ between the control, PEG, SA and AsA treated-plants].

Discussion

Salicylic acid and ascorbic acid influence the metabolism of plant reactions and modified several changes. These changes are accounted for as adaptabilities which increase the tolerance of plants against the environmental influences (Metwally et al., 2003). SA is a significant signal molecule for modifying plant responses to drought stress which contributes in the regulation of physiological processes (Loutfy et al., 2012; Kabiri et al., 2014). Vitamin C (AsA) is an antioxidant and, in association with other constituents of the antioxidant system, protects plants against oxidative injury resulting from aerobic metabolism, photosynthesis, a range of pollutants and protecting the cells against the damaging effects of the free radicals through preventing its production (Azooz et al., 2013; Kaya, 2017).

Plant growth is severely limited under drought stress as a result of a number of changes in the physio-biochemical processes (Kosar et al., 2015). In the results of this research, drought stress highly significantly decreased the growth of maize seedlings. The reduction in growth may be attributed to drought stress induced disorders in chlorophyll biosynthesis, transpiration rate, photosynthesis rate, stomatal regulations, uptake of essential nutrient, signal transduction pathways, oxidative protection system, (Nazi et al., 2016). Salicylic acid and ascorbic acid enhanced growth under water stress in maize seedlings. SA has growth stimulating properties, since it increases the rate of cell division within the apical meristem of seedling roots, causing growth acceleration (Shakirova et al., 2003; Chen et al., 2014). El-Khamissi et al. (2018) found that pre-treatment of Fenugreek seeds with AsA significantly increased the tolerance of seeds to drought stress. In this study, drought stress caused a decrease in photosynthetic pigments but SA and AsA increased these pigments under these drought stress conditions. Reduction of photosynthetic pigments under drought stress might be due to degradation of chloroplast structure and photosynthetic apparatus, chlorophyll photooxidation, damage of chlorophyll substrate, inhibition of chlorophyll biosynthesis, and the enhancement of chlorophyllase activity (Kabiri et al., 2014). The decrease in carotenoids content under drought stress might be associated to the degradation of β -carotene (Sultana et al., 1999).

SA has role in the improvement of chlorophyll and carotenoid biosynthesis, photosynthetic rate, carboxylase activity of Rubisco (Hayat & Ahmad, 2007; Nazar et al., 2015). Ascorbic acid as an antioxidant has the ability to alleviate the harmful effects of drought stress on plants by counteracting injurious oxidants which have been reported to damage plant membranes such as the thylakoid membranes of chloroplasts (Malik & Ashraf, 2012).

Drought stress, SA or AsA stimulated the accumulation of soluble sugars (glucose, fructose and sucrose) in tested maize seedlings. Soluble sugars accumulated in plants for osmotic adjustment in response to drought stress resulting in the protection of macromolecules and DNA structures (Juan et al., 2005). Salicylic acid treatment under water stress in plants had earlier described to increase the points of different osmolytes for stress tolerance (Farooq et al., 2009; Sharma et al., 2017).

The presented data revealed that, drought stress either singly or combined with SA or AsA increased soluble proteins, total free amino acid and proline accumulation in maize seedlings, while proline increased only with stress. It is well established that high accumulation of osmoprotectants including proline, GB and amino acids can be used as selection criteria for drought tolerance in different species (Fahmy & Ouf, 1999, Ashraf & Foolad, 2007; Kamran et al., 2009). Yazdanpanah et al. (2011) reported that the greatest amount of proline is related to the plants exposed to severe drought in combination ascorbic and salicylic. Hao et al. (2012) have shown that the increased drought stress tolerance and improved photosynthetic rate in *Cucumis sativus* were the results of the increased proline accumulation and inhibition of the membrane lipid peroxidation.

The obtained results revealed that the tested maize seedlings treated with SA and AsA may have used the soluble sugars, soluble proteins and total free amino acids as osmoprotectants to alleviate the harmful effects of drought.

The present results showed that CAT, APOX and SOD activity and GSH content greatly increased under treatment with drought, compared with control. However there was a significant increase in APOX and SOD activity

and GSH content under stressed-SA and stressed-AsA treatments, compared to drought stressed seedlings. Water stress induces the production of reactive oxygen species (ROS), for example superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), causing oxidative injuries, which could be associated with limitation of root growth due to water stress (Xu et al., 2015). Plants improve non-enzymatic antioxidants such as AsA and GSH and the enzymatic scavenging systems such as SOD, CAT, POD, APOX to detoxifying ROS (Noctor & Foyer, 1998; Mittler et al., 2004). There are data supporting the claim that SA and AsA increased the antioxidant enzymes activities, such as SOD and antioxidant materials ex. GSH which in turn defend plants against ROS generation and membrane damage, or may affect the synthesis of other substances having a defensive influence on plants under stress (Khan et al., 2011; Chen et al., 2014). Increased activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR and catalase (CAT) observed in SA treated soybean plants under control and stressed conditions at vegetative stage (Sharma et al., 2018). As a product of lipid peroxidation, MDA can reflect the degree of membrane lipid peroxidation (Upadhyaya et al., 2007). ROS, such as H_2O_2 , OH, and $O_2^{\cdot-}$ can destroy normal metabolism through oxidative damage to lipids, proteins and DNA (Farooq et al., 2009). Previous studies reported that the levels of MDA significantly increased in response to drought stress (Farooq et al., 2016; Cao et al., 2017). Increasing the lipids peroxidation reflected the increasing the oxidative stress (Meirs et al., 1992). Yazdanpanah et al. (2011) and Omidi et al. (2018) reported that the amount of MDA was increased under drought stressed plants, they explained that, osmotic stress causes alterations in membrane lipid composition and properties at a cellular level. Salicylic acid (SA) and scorbic acid (AsA) protected maize plant against oxidative stress by reducing the lipids peroxidation through their influence on the protective mechanism of enzymes (Ganesam & Thomson, 2001; Davis, 2005). Similarly, and in support of the present results, Yazdanpanah et al. (2011) have found a significant decrease in the concentration of MDA in the drought stressed plants in response to SA pretreatment.

Conclusion

It can be concluded that treating maize plant with

SA and AsA improved the drought tolerance of *Zea mays* L. plants by enhancing photosynthetic pigments, osmoprotectants contents and antioxidant system, which may be responsible for increase plant fresh and dry weights. They can be effectively used to protect *Zea mays* from the damaging effects of drought stress during the early stages of growth.

References

- Aebi, H. (1984) Catalase in vitro. *Methods Enzymol.* **105**, 121-126.
- Al-Hakimi, A.M.A., Hamada, A.M. (2001) Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. *Biologica Plantarum*, **44**, 253-261.
- Almeselmani, M., Deshmukh, P.S., Sairam, R.K., Kushwaha, S.R., Singh, T.P. (2006) Protective role of antioxidant enzymes under high temperature stress. *Plant Science*, **171**, 382-388.
- Ashraf, M. (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*, **27**, 84-93.
- Ashraf, M., Foolad, M.R. (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, **59**, 206-216.
- Azooz, M.M., Al-Fredan, M.A. (2009) The inductive role of vitamin C and its mode of application on growth, water status antioxidant enzyme activities and protein patterns of *Vicia faba* L. cv. hassawi grown under seawater irrigation. *American Journal of Plant Physiology*, **4**(1), 38-51.
- Azooz, M.M., Alzahrani, A.M., Youssef, M.M. (2013) The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (*Vicia faba* L.). *Australian Journal of Crop Science*, **7**, 2091-2100.
- Barakat, H. (2003) Interactive effects of salinity and certain vitamins on gene expression and cell division. *International Journal of Agriculture and Biology*, **3**, 219-225.
- Bates, L.S., Waldern, R.P., Tear, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*, **39**, 205-207.

- Beauchamp, C., Fridovich, I. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, **44**, 276-287.
- Beutler, E., Duron, O., Kelly, B.M. (1963) Improved method for determination of blood glutathione. *Journal of Laboratory and Clinical Medicine*, **61**, 882-888.
- Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248-254.
- Cao, Y., Luo, Q., Tian, Y., Meng, F. (2017) Physiological and proteomic analyses of the drought stress response in *Amygdalus Mira* (Koehne) Yü et Lu roots. *BMC Plant Biology*, **17**, 53.
- Chen, Z.L., Li, X.M., Zhang, L.H. (2014) Effect of salicylic acid pretreatment on drought stress responses of *Zoysia japonica*. *Russian Journal of Plant Physiology*, **61**, 619-625.
- Davis, P.J. (2005) "*Plant Hormones Biosynthesis, Signal Transduction, Action*". Springer. Chapter 22.
- Del Rio, L.A., Corpas, F.J., Sandalio, L.M., Palma, J.M., Gomez, M., Barroso, J.B. (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. *Journal of Experimental Botany*, **53**, 1255-1272.
- El-Awadi, M.E., El-Lethy, S.R., El-Rokiek, K.G. (2014) Effect of the two antioxidants; Glutathione and ascorbic acid on vegetative growth, yield and some biochemical changes in two wheat cultivars. *Journal of Plant Sciences*, **2**, 215-221.
- El-khamissi, H.A.Z., El-Hamamsy, S.M.A., Behairy, R.T. (2018) Mitigation of drought stress on fenugreek seedlings by application of ascorbic acid. *Journal of Biological Chemistry*, **13**, 343-358.
- Fahmy, G.M., Ouf, S.A. (1999) Significance of microclimate, transpiration and surface temperature on phylloplane mycoflora of green and senescing leaves of *Zygophyllum album* L. *Arid Environment*, **41**(3), 257-276.
- Farooq, F.K., Mohammad, H.S., Ahmad, E. (2016) Changes in some physiological and osmotic parameters of several pistachio genotypes under drought stress. *Scientia Horticulturae*, **198**, 44-51.
- Farooq, M., Basra, S.M.A., Wahid, A., Ahmad, N., Saleem, B.A. (2009) Improving the drought tolerance in rice (*Oryza sativa* L.) by exogenous application of salicylic acid. *Journal of Agronomy & Crop Science*, **195**, 237-246.
- Flexas, J., Medrano, H. (2002) Drought-inhibition of photosynthesis in C3 plants: Stomatal and non-stomatal limitation revisited. *Annals of Botany*, **89**, 183-189.
- Foyer, C.H., Maud, L., Kunert, K.J. (1994) Photooxidative stress in plants. *Physiologia Plantarum*, **92**, 696-717.
- Ganesam, V., Thomas, G. (2001) Salicylic acid response in rice: Influence of salicylic acid on H₂O₂ accumulation and oxidative stress. *Plant Science*, **160**, 1095-1106.
- Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G., Cicek, N. (2007) Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *Journal of Plant Physiology*, **164**, 728-736.
- Habibi, G. (2012) Exogenous salicylic acid alleviates oxidative damage of barley plants under drought stress. *Acta Biologica Szegediensis*, **56**, 57-63.
- Halimeh, R., Mahlagh, G., Maryam, P., Pazoki, A. (2013) Effect of drought interactions with ascorbate on some biochemical parameters and antioxidant enzymes activities in *Dracocephalum moldavica* L. *Middle East Journal of Scientific Reserach*, **13**, 522-531.
- Hao, J.H., Yi, Y., Shang, Q.M., Dong, C.J., Zhang, Z.G. (2012) Effects of exogenous salicylic acid on membrane lipid peroxidation and photosynthetic characteristics of *Cucumis sativus* seedlings under drought stress. *Ying Yong Sheng Tai Xue Bao*, **23**, 717-723.
- Harris, A., Rashid, G., Miaj, M., Arif, M., Shah, H. (2007) On-farm seed priming with zinc sulphate solution-A cost-effective way to increase the maize yields of resources-poor farmers. *Field Crops Research*, **110**, 119-127.
- Hayat, S., Ahmad, A. (2007) "*Salicylic Acid: A Plant*

- Hormone. Springer*", Netherlands.
- Heath, R.L., Packer, L. (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125**, 189-198.
- Hegab, M.M. (2016) Physiological and biochemical responses of *medicago truncatula* to drought stress. *Egyptian Journal of Botany*, **56**(3), 895-912.
- Hussein, M.M., Balbaa, L.K., Gaballah, M.S. (2007) Salicylic acid and salinity effects on growth of maize plants. *Research Journal of Agriculture and Biological Sciences*, **3**, 321-328.
- Juan, M., Rivero, R.M., Romero, L., Ruiz, J.M. (2005) Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Environmental and Experimental Botany*, **54**, 193-201.
- Kabiri, R., Nasibi, F., Farahbakhsh, H. (2014) Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. *Plant Protect Science*, **50**, 43-51
- Kamran, M., Shahbaz, M., Ashraf, M., Akram, N.A. (2009) Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as pre-sowing seed treatment. *Pakistan Journal of Botany*, **41**, 621-632.
- Kang, G.Z., Li, G.Z., Liu, G.Q., Xu, W., Peng, X.Q., Wang, C.Y., Zhu, Y.J., Guo, T.C. (2013) Exogenous salicylic acid enhances wheat drought tolerance by influence on the expression of genes related to ascorbate-glutathione cycle. *Biologia Plantarum*, **57**, 718-724.
- Kareem, F., Rihan, H., Fuller, M.P. (2017) The Effect of exogenous applications of salicylic acid and molybdenum on the tolerance of drought in wheat. *Agricultural Research and Technology*, **9**(4), 1-9.
- Karkacier, M., Ebras, M., Uslu, M.K., Aksu, M. (2003) Comparison of different extraction and detection methods for sugars using amino-bonded phase HPLC. *Journal of Chromatographic Science*, **41**, 331-333.
- Kaya, A. (2017) Exogenous ascorbic acid improves defense responses of sunflower (*Helianthus annuus*) exposed to multiple stresses. *Acta Biologica Hungarica*, **68**, 290-299.
- Khan, T.A., Mazid, M., Mohammad, F. (2011) A review of ascorbic acid potentialities against oxidative stress induced in plants. *Journal of Agrobiology*, **28**, 97-111.
- Kosar, F., Akram, N.A., Ashraf, M. (2015) Exogenously applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. *South African Journal of Botany*, **96**, 71-77.
- Loutfy, N., El-Tayeb, M.A., Hassanen, A.M., Moustafa, M.F.M., Sakuma, Y., Inouhe, M. (2012) Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum* L.). *Journal of Plant Research*, **125**, 173-184.
- Loutfy, N., Azooz, M.M., Hassanein, A.M., Bassiony, A. (2019) Potassium synergize the positive effect of ascorbic acid on some morpho-physiological parameters of salt stressed faba bean cultivars. *Egyptian Journal of Botany*, **59**(3), 735-751.
- MacAdams, J.W., Nelson, C.J., Sharp, R.E. (1992) Peroxidase activity in the leaf elongation zone of tall fescue. *Plant Physiol.* **99**, 872-878.
- Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y. (2011) Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. *Australian Journal of Crop Science*, **5**, 1255-1260.
- Morsi, M., Abdelmigid, H., Aljoudi, N. (2018) Exogenous salicylic acid ameliorates the adverse effects of salt stress on antioxidant system in *Rosmarinus officinalis* L. *Egyptian Journal of Botany*, **58**(2), 249-263. doi: 10.21608/ejbo.2018.1772.1124
- Malik, S., Ashraf, M. (2012) Exogenous application of ascorbic acid stimulates growth and photosynthesis of wheat (*Triticum aestivum* L.) under drought. *Soil and Environment*, **31**, 72-77.
- Mahmoud, M. (2017) Ameliorative effect of salicylic acid on growth, minerals and nitrogenous

- compounds of *Vicia faba* L. plants under salt stress. *Egyptian Journal of Botany*, **57**(1), 11-29. doi: 10.21608/ejbo.2017.212.1003
- Meirs, S., Philosophadas, S., Aharoni, N. (1992) Ethylene increased accumulation of fluorescent lipid-per oxidation products detected during parsley by a newly developed method. *Journal of the American Society for Horticultural Science*, **117**, 128-132.
- Mekki, B.E., Hussien, H., Salem, H. (2015) Role of glutathione, ascorbic acid and α -Tocopherol in alleviation of drought stress in cotton plants. *International Journal of ChemTech Research*, **8**, 1573-1581.
- Metwally, A., Finkemeier, I., Georgi, M., Dietz, K.J. (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiology*, **132**, 272-281.
- Metzner, H., Rau, H., Senger, H. (1965) Untersuchungen zur Synchronisierbarkeit einzelner-pigmentmangle-mutanten von chlorella. *Planta*, **65**, 186-194.
- Michael, B.E., Kaufman, M.R. (1973) The osmotic potential of polyethyleneglycol-6000. *Plant Physiol*, **51**, 914-916.
- Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, **7**, 405-410.
- Mittler, R., Vanderauwera, S., Gollery M., Van Breusegem, F. (2004) Reactive oxygen gene network of plants. *Trends in Plant Science*, **9**, 490-498.
- Moore, S., Stein, W.H. (1948). Photometric nin-hydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry*, **176**, 367-388.
- Nazar, R., Iqbal, N., Syeed, S., Khan, N.A. (2011) Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *Journal of Plant Physiology*, **168**, 807-815.
- Nazar, R., Umar, S., Kh, N.A., Sareer, O. (2015) Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *South African Journal of Botany*, **98**, 84-94.
- Nazi, H., Akrami, N.A., Ashraf, M. (2016) Impact of ascorbic acid on growth and some physiological attributes of cucumber (*Cucumis sativus*) plants under water-deficit condition. *Pakistan Journal of Botany*, **48**, 877-883.
- Noctor, G., Foyer, C.H. (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Biology*, **49**, 249-279.
- Omidi, J.F., Shoja, M.H., Sariri, R. (2018) Effect of water-deficit stress on secondary metabolites of *Melissa officinalis* L.: Role of exogenous salicylic acid. *Caspian J. Environ. Sci.* **16**, 121-134.
- Palma, F., López-Gómez, M., Tejera, N.A., Liuch, C. (2013) Salicylic acid improves the salinity tolerance of *Medicago sativa* in symbiosis with *Sinorhizobium meliloti* by preventing nitrogen fixation inhibition. *Plant Science*, **208**, 75-82.
- Penella, C., Calatayud, A., Melgar, J.C. (2017) Ascorbic acid alleviates water stress in young peach trees and improves their performance after rewatering. *Front Plant Science*, **8**, 16-27.
- Rosales, M.A., Ruiz, J.M., Hernandez, J., Soriano, T., Castilla, N., Romero, L. (2006) Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation. *Journal of the Science of Food and Agriculture*, **86**, 1545-1551.
- Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova M.V., Fathutdinova R.A., Fathutdinova, D.R. (2003) Changes in hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science*, **164**, 317-322.
- Sharma, M., Gupta, S.K., Majumder, B., Maurya, V.K., Deeba, F., Alam, A., Pandey, V. (2017) Salicylic acid mediated growth, physiological and proteomic responses in two wheat varieties under drought stress. *Journal of Proteomics*, **163**, 28-51.
- Sharma, M., Gupta, S.K., Majumder, B., Maurya, V.K., Deeb, F., Alam, F., Pandey, V. (2018) Proteomics unravel the regulating role of salicylic acid in soybean under yield limiting drought stress. *Plant Physiology and Biochemistry*, **130**, 529-541.
- Snedecor, G.W., Cochran, W.G. (1980) "Statistical

- Methods*", 7th ed. Iowa State University Press, Ames, Iowa, USA.
- Sultana, N., Ikeda, T., Itoh, R. (1999) Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environmental and Experimental Botany*, **42**, 211-220.
- Upadhyaya, H., Khan, M.P., Panda, S.K. (2007) Hydrogen peroxide induces oxidative stress in detached leaves of *Oryza sativa* L. *Gen. Appl. Plant Physiology*, **33**, 83-95.
- Waseem, H., Bano, R., Khatak, B., Hussain, I., Yousaf, M., David, U. (2015) Temperature sensitivity and soil organic carbon pools decomposition under different moisture regimes: Effect on total microbial and enzymatic activity. *Clean, Soil, Air; Water*, **43**, 391-398.
- Xu, Y., Xu, Q., Huang, B. (2015) Ascorbic acid mitigation of water stress inhibition of root growth in association with oxidative defense in tall fescue (*Festuca arundinacea* Schreb.). *Front Plant Science*, **6**, 807.
- Yazdanpanah, S., Baghizadeh, A., Abbasi, F. (2011) The interaction between drought stress and salicylic and ascorbic acids on some biochemical characteristics of *Satureja hortensis*. *African Journal of Agricultural Research*, **6**, 798-807.

إضافة حمض الساليسيليك وحمض الأسكوربيك يعمل على تحسين بعض الصفات الفسيولوجية ونظام مضاد الأكسدة في بادرات الذرة الشامية تحت تأثير اجهاد الجفاف

نجلاء لطفى⁽¹⁾، محمد محجوب عزوز⁽¹⁾، منى فوزى أبو الحمد⁽²⁾

⁽¹⁾ قسم النبات والميكروبيولوجي - كلية العلوم - جامعة جنوب الوادي - قنا - مصر، ⁽²⁾ قسم الأحياء - كلية العلوم - جامعة الطائف - المملكة العربية السعودية.

أجريت هذه الدراسة لتقييم تأثير كل من حمض الساليسيليك (0.0, 0.5 مللى مول) وحمض الاسكوربيك (0.0, 100 ميكرومول) على نشاط بعض الانزيمات والسكريات الذائبة وبعض الأنشطة الفسيولوجية لبادرات نبات الذرة الشامية النامية تحت اجهاد الجفاف باستخدام البولى ايثيلين جليكول. وقد أسفرت الدراسة عن النتائج التالية:

أدى نمو بادرات الذرة الشامية تحت تأثير اجهاد الجفاف إلى انخفاض شديد في الوزن الغض والجاف وكذلك المحتوى الصبغى. في حين تسبب اجهاد الجفاف في رفع مستويات السكريات الذائبة (جلوكوز، فركتوز، سكروز) والبروتينات الذائبة، وأنشطة الأنزيمات المضادة للأكسدة ممثلة في السوبر اوكسيد ديسميوتيز (SOD)، الأسكوربيت بير أوكسيديز (APOX)، الكاتاليز (CAT) والجلوتاثيون (GSH)، فوق أكسدة الدهون مثل المالون داي الدهايد (MDA) مقارنة بالنباتات النامية في الظروف الطبيعية.

أدى نقع البذور في حمض الساليسيلك وحمض الأسكوربيك إلى تحسن ملحوظ في معدلات النمو والمحتوى الصبغى وزيادة في محتوى السكريات الذائبة والأحماض الامينية الكلية، البروتينات الذائبة ونشاط كل من انزيم السوبر اوكسيد ديسميوتيز والأسكوربيت بيروكسيديز ومحتوى البرولين والجلوتاثيون مقارنة بالنباتات النامية تحت الظروف الطبيعية. أدى نقع البذور في كل من حمض الساليسيلك وحمض الأسكوربيك وانمائها تحت تأثير اجهاد الجفاف إلى نقص ملحوظ في محتوى كل من الحمض الأميني البرولين والمالون داي الدهايد ونشاط انزيم الكاتاليز. أوضحت الدراسة الحالية أن كل من حمض الساليسيلك وحمض الأسكوربيك يخفف من التأثير الضار لإجهاد الجفاف من خلال زيادة نشاط النظام المضاد للأكسدة قيد الدراسة لبادرات نبات الذرة.