

## BRIEF COMMUNICATION

This is an open access article distributed under the terms of the Creative Commons BY-NC-ND Licence

## Application of sucrose modulates the expressions of genes involved in proline and polyamine metabolism in maize seedlings exposed to drought

C. ALTUNTAŞ<sup>1</sup>, A. SEZGİN<sup>1</sup>, M. DEMİRALAY<sup>2</sup>, R. TERZİ<sup>1\*</sup>, A. SAĞLAM<sup>3</sup>, and A. KADIOĞLU<sup>1</sup>

Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Turkey<sup>1</sup>

Artvin Coruh University, Faculty of Forestry, Department of Forest Engineering, 0800, Artvin, Turkey<sup>2</sup>

Karadeniz Technical University, Department of Molecular Biology and Genetics, 61080 Trabzon, Turkey<sup>3</sup>

### Abstract

Sucrose, proline, and polyamines are compatible solutes accumulating in plant tissues and increasing cellular osmolarity under environmental stresses. These compatible solutes and hydrogen peroxide can function as signaling molecules in plants. There has been very little evidence how the supply of sucrose changes the biosynthesis of compatible solutes. This study aimed to assess the cross-talk among sucrose, H<sub>2</sub>O<sub>2</sub>, and compatible solutes on the expression of genes encoding key enzymes in the pathways of proline and polyamine metabolism in drought stressed maize seedlings. Drought stress (induced by polyethylene glycol solution) increased the expressions of genes encoding pyrroline-5-carboxylate synthetase (*P5CS*), arginine decarboxylase (*ADC*), and S-adenosylmethionine decarboxylase (*SAMDC*), while decreased proline dehydrogenase (*ProDH*), diamine oxidase (*DAO*), and polyamine oxidase (*PAO*) expressions. Addition of sucrose to the stressed seedlings increased the *P5CS*, *ADC* and *SAMDC* expressions more than drought stress alone and reduced more the *ProDH*, *DAO*, and *PAO* expressions. Moreover, exogenous sucrose increased leaf water potential and the content of proline, polyamines, and total soluble sugars, whereas decreased H<sub>2</sub>O<sub>2</sub> content and membrane damages under the drought stress conditions. Consequently, exogenous sucrose contributed to the preservation of water status and the amelioration of damage in maize seedlings under the drought stress.

*Additional key words:* arginine decarboxylase, diamine oxidase, hydrogen peroxide, polyamine oxidase, proline dehydrogenase, pyrroline-5-carboxylate synthetase, S-adenosylmethionine decarboxylase.

Drought, salinity, high temperature, and chilling are the important abiotic stresses limiting biomass production of plants (Ashraf and Foolad 2007). Abiotic stresses can stimulate a wide variety of plant responses such as alteration of cellular metabolism and regulation of gene expression. Plants alter their metabolism in various ways, including production of compatible solutes, stabilizing proteins and cellular structures, and antioxidants to remove the excess of reactive oxygen species (ROS) (Krasensky

and Jonak 2012). While a high ROS content leads to a redox imbalance and oxidative stress, freely diffusible and relatively long-living H<sub>2</sub>O<sub>2</sub> can also act as a central player in signal transduction pathways and activate multiple acclimation responses (Hossain *et al.* 2015). Compatible solutes, such as sugars, proline, glycine betaine, and polyamines facilitate the maintenance of pressure potential and provide an effective stress tolerance in plants (Singh *et al.* 2015).

Submitted 3 April 2018, last revision 28 September 2018, accepted 18 October 2018.

*Abbreviations:* ADC - arginine decarboxylase; DAO - diamine oxidase; g<sub>s</sub> - stomatal conductance; MDA - malondialdehyde; P5CR - pyrroline-5-carboxylate reductase; P5CS - pyrroline-5-carboxylate synthetase; PAO - polyamine oxidase; PEG - polyethylene glycol; ProDH - proline dehydrogenase; Put - putrescine; ROS - reactive oxygen species; RT-PCR - reverse transcription polymerase chain reaction; SAMDC - S-adenosylmethionine decarboxylase; Spd - spermidine; Spm - spermine; TBA - thiobarbituric acid; TCA - trichloroacetic acid; Ψ<sub>leaf</sub> - leaf water potential.

*Acknowledgements:* This work was supported by the Turkish National Science Foundation (Project No: 111T511). We thank Assoc. Prof. Dr. Ahmet Yaşar (Faculty of Pharmacy, Karadeniz Technical University) for his assistance with analysis of HPLC. The authors contributed equally to this work.

\* Corresponding author; e-mail: [rabiaterzi@ktu.edu.tr](mailto:rabiaterzi@ktu.edu.tr)

In addition to meeting carbon requirement and energy for plant growth, soluble sugars can also act as primary messengers and regulate signals that control the transcription of genes related to the stress tolerance (Lastdrager *et al.* 2014). The most common soluble sugars in cells are sucrose, glucose, and fructose (Taiz and Zeiger 2006, Gao *et al.* 2018). Sucrose also affects plant growth and development (*e.g.*, regulation of flowering, differentiation of vascular tissues, and development of storage organs; Tognetti *et al.* 2013). A limited number of studies have indicated that supply of sucrose promoted biosynthesis of other compatible solutes in plants exposed to environmental stresses (Khalid *et al.* 2010, Cao *et al.* 2015). Some researchers observed the induced expressions of a variety of genes when sucrose is applied at low concentrations under water limited conditions (Solfanelli *et al.* 2006).

Proline is well known osmolyte accumulating in plants in response to abiotic stresses. It is synthesized from glutamate *via* two enzymes, pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) in chloroplasts (Verbruggen and Hermans 2008). Proline accumulation under drought stress is related to an increasing expression of the *P5CS* gene and a decreasing expression of the proline dehydrogenase (*ProDH*) gene (Miller *et al.* 2005). In addition to the osmolyte role of proline, it is considered to act as a ROS scavenger, a regulator of cellular redox potential, a molecular chaperone stabilizing the structure of proteins, or a component of signal transduction pathways that regulate stress-responsive genes (Szabados and Saviouré 2010). Also, proline application was found to be an effective approach for enhancing abiotic stress tolerance (Moustakas *et al.* 2011).

The most abundant polyamines are the diamine putrescine (Put), the triamine spermidine (Spd), and the tetramine spermine (Spm). Polyamines are also involved in a complex signaling system and have key roles in the regulation of the drought tolerance (Pál *et al.* 2015). Biosynthetic pathways of polyamines are decarboxylation of arginine or ornithine, catalyzed by ornithine decarboxylase or arginine decarboxylase (ADC) to form Put. Spermidine and Spm are synthesized from Put by the addition of an aminopropyl transferred from decarboxylated S-adenosylmethionine, which is produced by S-adenosyl-methionine decarboxylase (SAMDC) (Liu *et al.* 2015). Diamine oxidase (DAO) catalyzes Put degradation, whereas polyamine oxidase (PAO) oxidizes Spd and Spm (Flores and Filner 1985). Most of the polyamines biosynthetic genes are up-regulated by abiotic stresses in spite of 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). However, there are not enough reports about the effects of exogenous sucrose on proline and polyamine metabolism under abiotic stresses.

We aimed to determine cross-talks among exogenous

sucrose, H<sub>2</sub>O<sub>2</sub>, proline, and polyamines in maize seedlings exposed to drought stress. We tested some physiological (leaf water potential and stomatal conductance), biochemical (lipid peroxidation, H<sub>2</sub>O<sub>2</sub>, proline, polyamines, and total soluble sugars), and molecular (expression of genes involved in proline and polyamine metabolism) parameters. We hypothesized that exogenous sucrose can protect water status and ameliorate damages caused by drought stress.

The seeds of *Zea mays* L. (cv. Akpınar) were provided from Karadeniz Agricultural Research Institute. The seeds were germinated in pots containing peat soil at a plant growth chamber (a temperature of 25 ± 2 °C, a relative humidity of 60 ± 5 %, a photosynthetic photon flux density, of 400 µmol m<sup>-2</sup> s<sup>-1</sup>, and a 16-h photoperiod). The seedlings were grown under these conditions also during all treatments. Irrigation was done three times a week. When the third leaves were fully expanded, all shoots were cut, and to minimize the water deficit, they were kept in tubes with distilled water for 1 h. After that, the shoots were subjected to the following treatments: 1) distilled water (control), 2) polyethylene glycol 6 000 (PEG) solution (20 g dm<sup>-3</sup>, an osmotic potential of -0.5 MPa), 3) 0.1 M sucrose (SUC; a similar concentration to that used by Sulmon *et al.* 2004; an osmotic potential of -2.5 × 10<sup>-4</sup> MPa), and 4) 0.1 mM sucrose plus PEG (SUC+PEG) (-0.5 MPa). The experiment was arranged in a completely randomized design with nine replicates per treatment. After 12 h, leaf samples were immediately frozen in liquid nitrogen and stored at -20 °C until analyses.

A leaf water potential ( $\Psi_{\text{leaf}}$ ) was measured according to Savage and Cass (1984) with a thermocouple psychrometer (*PSYPRO C-52*, *Wescor*, Logan, USA) at 27 ± 1 °C calibrated by NaCl solutions (0.1, 0.2, 0.5, and 1.0 M) of known water potentials. The discs (a 6-mm diameter) were cut from the leaves of three plants and sealed in a C-52 sample chamber. The samples were equilibrated for 60 min, and then the data were recorded. The osmotic potential of a 0.1 mM sucrose solution at 25 °C was calculated by a formula:  $\Psi_s = -cRT$ , where *c* is the molar concentration, *R* is the universal gas constant, and *T* is the absolute temperature. To prepare a -0.5 MPa PEG solution, 20 g of PEG 6000 was dissolved in 1 dm<sup>3</sup> of distilled water according to Mohammadkhani and Heidari (2008). Stomatal conductance (*g<sub>s</sub>*) was monitored by an *AP4* dynamic diffusion porometer (*Delta T Devices*, Cambridge, UK) after calibration with a standard calibration plate following the manufacturer's instructions.

Malondialdehyde (MDA) content was measured following the Heath and Packer (1968) method. Samples of fresh leaves (0.1 g) were homogenized with 0.1 % (m/v) trichloroacetic acid (TCA) and centrifuged at 15 000 *g* for 5 min. The supernatant (1 cm<sup>3</sup>) was added to a reaction mixture containing 20 % (m/v) TCA with 0.5 % (m/v) thiobarbituric acid. The mixture was incubated at 95 °C for 30 min and quickly cooled in an ice-bath. Content of MDA

was calculated from the difference of absorbances at 532 and 600 nm using a UV-visible spectrophotometer (*Thermo Scientific*, Bellefonte, USA).

Content of H<sub>2</sub>O<sub>2</sub> was measured according to the method of Velikova *et al.* (2000). Samples of fresh leaves (0.1 g) were homogenized in 1 % (m/v) TCA combined with activated charcoal at 4 °C. The homogenate was centrifuged at 15 000 g for 15 min, and then 1 cm<sup>3</sup> of the supernatant was added to 0.5 cm<sup>3</sup> of 10 mM potassium phosphate buffer (pH 7.0) and 0.75 cm<sup>3</sup> of 1 M KI. Hydrogen peroxide content was measured at 390 nm and calculated from a standard curve.

For soluble sugar content, samples of dry leaves (0.2 g) were homogenized at 80 °C with 5 cm<sup>3</sup> of 70 % (v/v) ethanol. Then, 0.9 cm<sup>3</sup> of distilled water was added to 0.1 cm<sup>3</sup> of the supernatant. After that, 5 % (m/v) phenol and 96 % (m/v) sulphuric acid were added to this mixture. Sugar content in this mixture was determined using the Dubois (1956) method.

Proline content was measured according to Bates (1973). Dry leaves (0.2 g) were homogenized in sulfosalicylic acid (10 cm<sup>3</sup>, 3 %, m/v) and then centrifuged at 6 000 g for 10 min. The supernatant (1 cm<sup>3</sup>) was added to 1 cm<sup>3</sup> of glacial acetic acid and 1 cm<sup>3</sup> of ninhydrin, and the mixture was incubated at 100 °C for 1 h. To stop reaction, the samples were cooled in an ice-bath, and then toluene was added and absorbance of the reaction mixture was measured at 520 nm.

An HPLC-based method was used to determine polyamine content according to Ben-Gigirey *et al.* (1998). Polyamines from leaf samples (5 g) were extracted twice with 0.4 M perchloric acid (10 cm<sup>3</sup>). For derivatization, 0.2 cm<sup>3</sup> of 3M NaOH, 0.3 cm<sup>3</sup> of 2 M NaHCO<sub>3</sub> and 2 cm<sup>3</sup> of dansyl chloride (10 mg cm<sup>-3</sup> in acetone) were added to the samples or polyamine standard solutions. The mixtures were incubated at 40 °C for 45 min and 0.1 cm<sup>3</sup> of 25 % (m/v) NH<sub>4</sub>OH was added to stop the reaction. Then, acetonitrile was put in the mixtures to arrange the total volume to 5 cm<sup>3</sup>, and after 30 min incubation at room temperature, they were filtered through a 0.45 µm syringe filter (*Woongki Science Co.*, Daejeon, Korea). Chromatographic separations were performed by an HPLC unit (*LC 20 AT/Prominence, Shimadzu*, Kyoto, Japan) with a C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) (*Supelco*, Bellefonte, USA). A mobile phase included 0.1 M ammonium acetate and 19 M acetonitrile at a flow rate of 1 cm<sup>3</sup> min<sup>-1</sup> with a gradient elution program for 35 min. An injection volume was 0.01 cm<sup>3</sup>. A flow rate was 1 cm<sup>3</sup> min<sup>-1</sup> for 35 min.

Total RNA was extracted from maize leaves (0.1 g) with an RNeasy plant mini kit (*Qiagen*, Düsseldorf Germany). Concentration of RNA was measured at 260 nm using a *Nanodrop 2000* spectrophotometer (*Thermo Scientific*, Waltham, USA). The cDNA was synthesized from total RNA (1 µg) using a high-capacity cDNA reverse transcription kit (*Applied Biosystems*, Waltham, USA) according to the manufacturer's protocols. Plates for

PCR (*BioRad*, Hercules, USA) having 96 wells were used. A quantitative PCR *Supermix* (4 mm<sup>3</sup>; *Solis Biodyne*, Tartu, Estonia), 1 mm<sup>3</sup> of reverse and 1 mm<sup>3</sup> of forward primers, 1 mm<sup>3</sup> of a cDNA sample, and 13 mm<sup>3</sup> of nuclease free water were added per well. The analysis was performed on a *CFX Connect* real time PCR system (*BioRad*) based on a real time quantitative PCR protocol was modified according to *Solis Biodyne* instructions; at 95 °C for 12 min, 45 repeats of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. A melt curve was held at 0.5 °C increments from 60 °C to 95 °C. Results were normalized according to the *actin1* gene and relative gene expressions were presented. Forward primers of *P5CS*, *ProDH*, *ADC*, *SAMDC*, *PAO*, *DAO* and *actin1* genes were:

5'-AACATCTTGCCCTCTGGGTG-3',  
5'-TCAGCAAGTACCTGCCGTAC-3',  
5'-GACATCACCTGCGACAGTGA-3',  
5'-GGAGGCGTGAAGAAGTTCCA-3',  
5'-CGCTACGAATACGACCAGCT-3',  
5'-ACAGCAAGTCCGAGAAGTGG-3', and  
5'-GAAGATCACCTGTGCTGCT-3',

whereas their reverse primers were:

5'-CCATTGCCACTTCGAACTGC-3',  
5'-ACCCTCCTCACCAACTCCTT-3',  
5'-TTATCAGGAAGCAGCAGGCC-3',  
5'-TGGGCGCAGTTGATGAGAAT-3',  
5'-TGTACCACAGCACGATGTCC-3',  
5'-ACCAGTTGTTGCCCACTAG-3', respectively.

All experiments were carried out in triplicate with three biological replicates. All physiological data and gene expressions were processed with one-way analysis of variance ( $\alpha = 0.05$ ) using the *SPSS v. 15.0* software for *Microsoft Windows* (*SPSS Inc.*, Chicago, USA). Mean differences were determined with the Duncan multiple comparison test at  $\alpha = 0.05$ . Relative gene expressions were analyzed with the *Bio-Rad CFX Manager 3.1*.

We found that PEG treatment decreased  $\Psi_{leaf}$  and  $g_s$  in comparison to the control. Treatment SUC+PEG enhanced  $\Psi_{leaf}$  and  $g_s$  in comparison to PEG treatment but the values of  $\Psi_{leaf}$  and  $g_s$  were lower in SUC seedlings in comparison to the control (Table 1).

There was no significant difference between the control and SUC treatments in MDA content, but SUC treatment increased H<sub>2</sub>O<sub>2</sub> content. Content of MDA and H<sub>2</sub>O<sub>2</sub> were lower after SUC+PEG treatment in comparison to PEG treatment (Table 1). Treatment SUC increased content of soluble sugar, proline, Put, Spd, and Spm as compared to the control. Their content increased in SUC+PEG treated seedlings more as compared with PEG treatment. Interestingly, Spd, Spm, and polyamines content of SUC treated seedlings were higher as compared to PEG treated seedlings. Moreover, Spd and Spm content after SUC treatment was even higher in comparison with SUC+PEG treatment but content of polyamines was lower (Table 1).

The expression of the *P5CS* gene was 1.5-fold up-

regulated in SUC treated seedlings in comparison to the control. Likewise, the expression of the *P5CS* gene in the SUC+PEG group was up-regulated 1.2-fold in comparison to the PEG group. In contrast, *ProDH* gene expression was 1.6-fold down-regulated in SUC treated seedlings as compared with the control. Treatment SUC+PEG 1.5-fold down-regulated the relative expression of the *ProDH* gene under the drought stress (Table 1).

Treatment SUC up-regulated *ADC* and *SAMDC* gene expressions 1.8- and 1.3-fold, respectively, in comparison to the control. When compared to PEG treatment, SUC+PEG treatment up-regulated the relative expressions of *ADC* and *SAMDC* genes 1.4- and 2.3-fold, respectively. Treatment SUC up-regulated the expressions of *PAO* and *DAO* genes 1.4- and 1.1-fold in comparison to the control, respectively. Treatment SUC+PEG down-regulated *PAO* and *DAO* gene expressions 4.2- and 1.4-fold in comparison

to PEG treatment, respectively. (Table 1).

Exogenous sucrose alleviates damages by various abiotic stresses by inducing the antioxidant system in *Arabidopsis thaliana* and *Cucumis sativus* (Qiu *et al.* 2014, Cao *et al.* 2015). However, according to the best of our knowledge, we worked on the cross-talking among sucrose, H<sub>2</sub>O<sub>2</sub>, proline, and polyamine in plants under drought stress for the first time. Our results show that  $\Psi_{leaf}$  and  $g_s$  decreased, while MDA content increased in PEG seedlings in comparison to the control. However, sucrose application enhanced values of  $\Psi_{leaf}$  and  $g_s$  and decreased MDA content. Similarly,  $\Psi_{leaf}$  and  $g_s$  increase after application of sucrose in *Commelina benghalensis* and *Cucumis sativus* exposed to drought (Reddy and Das 1986, Cao *et al.* 2015), and Cao *et al.* (2015) also reported less membrane damage in cucumber leaves.

Table 1. Effect of application of 0.1 M sucrose (SUC) and 20 g dm<sup>-3</sup> polyethylene glycol (PEG) for 12 h on  $\Psi_{leaf}$ ,  $g_s$ , content of MDA, H<sub>2</sub>O<sub>2</sub>, soluble sugar, proline, and polyamines, and relative expressions of *P5CS*, *ProDH*, *ADC*, *SAMDC*, *PAO*, and *DAO* genes. Means  $\pm$  SDs of three replicates. Different letters indicate significant differences ( $P < 0.05$ ).

Parameters	Control	SUC	PEG	SUC+PEG
Leaf water potential ( $\Psi_{leaf}$ ) [-MPa]	0.53 $\pm$ 0.03d	0.74 $\pm$ 0.02c	1.27 $\pm$ 0.02a	0.83 $\pm$ 0.007b
Stomatal conductance ( $g_s$ ) [mmol m <sup>-2</sup> s <sup>-1</sup> ]	4.33 $\pm$ 0.007a	3.95 $\pm$ 0.05b	2.32 $\pm$ 0.10c	3.86 $\pm$ 0.025b
Malondialdehyde (MDA) [nmol g <sup>-1</sup> (d.m.)]	0.62 $\pm$ 0.01c	0.63 $\pm$ 0.01c	1.37 $\pm$ 0.01a	1.25 $\pm$ 0.01b
H <sub>2</sub> O <sub>2</sub> [ $\mu$ mol g <sup>-1</sup> (d.m.)]	32.40 $\pm$ 0.50d	34.30 $\pm$ 0.80c	51.50 $\pm$ 1.6a	37.70 $\pm$ 0.50b
Soluble sugar [mg g <sup>-1</sup> (d.m.)]	0.90 $\pm$ 0.006d	2.68 $\pm$ 0.024b	1.04 $\pm$ 0.003c	3.01 $\pm$ 0.016a
Proline [ $\mu$ g g <sup>-1</sup> (d.m.)]	0.54 $\pm$ 0.04d	1.50 $\pm$ 0.08b	1.05 $\pm$ 0.03c	2.14 $\pm$ 0.05a
Putrescine [ $\mu$ g g <sup>-1</sup> (d.m.)]	2.33 $\pm$ 0.07d	5.26 $\pm$ 0.04c	8.08 $\pm$ 0.07b	14.27 $\pm$ 0.06a
Spermidine [ $\mu$ g g <sup>-1</sup> (d.m.)]	0.44 $\pm$ 0.04d	2.69 $\pm$ 0.10a	1.10 $\pm$ 0.04b	1.20 $\pm$ 0.02c
Spermine [ $\mu$ g g <sup>-1</sup> (d.m.)]	0.85 $\pm$ 0.03d	4.37 $\pm$ 0.05a	1.48 $\pm$ 0.03c	3.12 $\pm$ 0.04b
Polyamines [ $\mu$ g g <sup>-1</sup> (d.m.)]	3.60 $\pm$ 0.14d	12.32 $\pm$ 0.20b	10.60 $\pm$ 0.14c	18.50 $\pm$ 0.10a
Pyrroline-5-carboxylate synthetase ( <i>P5CS</i> )	1.00 $\pm$ 0.10d	2.23 $\pm$ 0.05b	1.64 $\pm$ 0.10c	5.70 $\pm$ 0.08a
Proline dehydrogenase ( <i>ProDH</i> )	1.00 $\pm$ 0.05a	0.60 $\pm$ 0.01c	0.78 $\pm$ 0.03b	0.53 $\pm$ 0.04d
Arginine decarboxylase ( <i>ADC</i> )	1.00 $\pm$ 0.10d	1.80 $\pm$ 0.20c	2.63 $\pm$ 0.20b	3.66 $\pm$ 0.20a
S-adenosylmethionine decarboxylase ( <i>SAMDC</i> )	1.00 $\pm$ 0.15d	1.37 $\pm$ 0.10c	2.71 $\pm$ 0.20b	6.34 $\pm$ 0.50a
Polyamine oxidase ( <i>PAO</i> )	1.00 $\pm$ 0.10b	1.47 $\pm$ 0.09a	0.80 $\pm$ 0.08c	0.19 $\pm$ 0.01d
Diamine oxidase ( <i>DAO</i> )	1.00 $\pm$ 0.04a	1.02 $\pm$ 0.04a	0.80 $\pm$ 0.05b	0.54 $\pm$ 0.01c

Hydrogen peroxide acts as a signal to induce a range of molecular, biochemical, and physiological responses, and mediates cross-talk between signaling pathways (Neill *et al.* 2002). We observed that SUC treatment increased H<sub>2</sub>O<sub>2</sub> content compared to the control. Conversely, exogenous sucrose decreases H<sub>2</sub>O<sub>2</sub> content in cucumber (Cao *et al.* 2014, 2015). Here, we can write that sucrose application probably triggered an increase in H<sub>2</sub>O<sub>2</sub> content in maize seedlings. However, H<sub>2</sub>O<sub>2</sub> content was lower in SUC+PEG treated seedlings in comparison with those treated only with PEG maybe due to an increased content of proline, polyamines and soluble sugars with antioxidant properties. Indeed, proline is not only an osmolyte but also an antioxidant and a signal molecule during abiotic stresses (Hayat *et al.* 2012). Polyamines and sugars can also scavenge ROS and preserve the membrane and

cellular integrity (Roychoudhury *et al.* 2015). Cao *et al.* (2015) showed that sucrose application decreases H<sub>2</sub>O<sub>2</sub> content by increasing activities of guaiacol peroxidase and glutathione reductase in water-stressed cucumber leaves.

Treatments SUC and SUC+PEG increased content of soluble sugar, proline, Put, Spd, and Spm in maize seedlings. We infer that the applied sucrose could be easily taken-up by detached shoots and transported to leaves, and thus the content of soluble sugar could increase. Cao *et al.* (2015) reported similar data for increased endogenous sucrose, fructose, glucose, and proline in cucumber leaves under water stress. We can suppose that application of sucrose might induce a signaling network leading to osmolyte accumulation and so maintenance of pressure potential under stress. Content of Put was more increased than content of Spd and Spm. Do *et al.* (2014) have

asserted that only one of the three polyamine types showed a significant increase in rice cultivars under salt stress. Interestingly, as compared to PEG seedlings, content of Spd, Spm, and polyamines in SUC seedlings was high, and content of Spd and Spm in SUC+PEG seedlings was lower than in SUC seedlings. We think that Spd and Spm could be converted to Put. Indeed, some studies showed that Spm is converted backward to Spd and then Spd to Put (Kamada-Nobusada *et al.* 2008, Moschou *et al.* 2008). On the other hand, the effects of exogenous polyamines on the content of sucrose and compatible compounds were reported in plants under water stress (Saruhan *et al.* 2006, Li *et al.* 2015).

Further, we observed that *P5CS* gene expression was up-regulated, while *ProDH* gene expression was down-regulated by exogenous sucrose, which was in agreement with proline content. Therefore, drought tolerance induced by exogenous sucrose may be related to up-regulated *P5CS* expression and down-regulated *ProDH* expression. We also determined relative expressions of some genes concerning polyamines metabolism under drought stress. As compared to the control, SUC treatment up-regulated

the expressions of *ADC*, *SAMDC*, *PAO*, and *DAO* genes. Treatment SUC+PEG significantly increased *ADC* and *SAMDC* expressions but markedly down-regulated *PAO* and *DAO* expressions as compared to PEG treatment. So, as a new finding, we determined that SUC treatment can influence polyamines metabolism with a probable interaction between sucrose and polyamines. We can conclude that exogenous sucrose alleviated the effects of the drought stress also by up-regulating expressions of *ADC* and *SAMDC* genes and down-regulating expressions of *PAO* and *DAO* genes.

As a conclusion, our results point out that there may be the interactions among osmolytes and H<sub>2</sub>O<sub>2</sub>, which modulate the genes involved in proline and polyamine metabolisms to stimulate drought tolerance. The application of sucrose may alleviate damages from drought because it is transported to leaves where it serves as a source of energy and a substrate for other syntheses. Further studies are necessary to highlight the alleviating role of sucrose and the cross-talk between sucrose and interacting signal molecules under abiotic stress conditions.

## References

- Ashraf, M., Foolad, M.R.: Roles of glycine betaine and proline in improving plant abiotic stress resistance. - *Environ. exp. Bot.* **59**: 206-216, 2007.
- Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - *Plant Soil* **39**: 205-207, 1973.
- Ben-Gigirey, B., De Sousa, J.M.V.B., Villa, T.G., Barros-Velazquez, J.: Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. - *J. Food Prot.* **61**: 608-615, 1998.
- Cao, Y.Y., Yanga, M.T., Lia, X., Zhou, Z.Q., Wang, X.J., Baia, J.G.: Exogenous sucrose increases chilling tolerance in cucumber seedlings by modulating antioxidant enzyme activity and regulating proline and soluble sugar contents. - *Sci. Hort.* **179**: 67-77, 2014.
- Cao, Y.Y., Yang, M.T., Chen, S.Y., Zhou, Z.Q., Li, X., Wang, X.J., Bai, J.G.: Exogenous sucrose influences antioxidant enzyme activities and reduces lipid peroxidation in water-stressed cucumber leaves. - *Biol. Plant.* **59**: 147-153, 2015.
- Do, P.T., Drechsel, O., Heyer, A.G., Hincha, D.K., Zuther, E.: Changes in free polyamine levels, expression of polyamine biosynthesis genes, and performance of rice cultivars under salt stress: a comparison with responses to drought. - *Front. Plant Sci.* **5**: 182, 2014.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. - *Anal. Chem.* **28**: 350-356, 1956.
- Ebeed, H.T., Hassan, N.M., Aljarani, A.M.: Exogenous applications of polyamines modulate drought responses in wheat through osmolytes accumulation, increasing free polyamine levels and regulation of polyamine biosynthetic genes. - *Plant Physiol. Biochem.* **118**: 438-448, 2017.
- Flores, H.E., Filner, P.: Polyamine catabolism in higher plants: characterization of pyrroline dehydrogenase. - *Plant Growth Regul.* **3**: 277-291, 1985.
- Gao, L., Zhao, S., Lu, X., He, N., Zhu, H., Dou, J., Liu W.: Comparative transcriptome analysis reveals key genes potentially related to soluble sugar and organic acid accumulation in watermelon. - *Plos ONE* **13**: 1-22, 2018.
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A.: Role of proline under changing environments. - *Plant Signal Behav.* **7**: 1456-1466, 2012.
- Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplast. I. kinetics and stoichiometry of fatty acid peroxidation. - *Arch. Biochem. Biophys.* **125**: 189-198, 1968.
- Hossain, M.A., Bhattacharjee, S., Armin, S.M., Qian, P., Xin, W., Li, H.Y., Burritt, D.J., Fujita, M., Tran, L.S.: Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. - *Front Plant Sci.* **6**: 2-19, 2015.
- Kamada-Nobusada, T., Hayashi, M., Fukazawa, M., Sakakibara, H., Nishimura, M.: A putative peroxisomal polyamine oxidase, AtPAO4, is involved in polyamine catabolism in *Arabidopsis thaliana*. - *Plant Cell Physiol.* **49**: 1272-1282, 2008.
- Khalid, A., Jaime, A., Da Teixeira, S., Weiming, C.: Water deficit and polyethylene glycol<sub>6000</sub> affects morphological and biochemical characters of *Pelargonium odoratissimum* (L.). - *Sci. Hort.* **125**: 159-166, 2010.
- Krasensky, J., Jonak, C.: Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. - *J. exp. Bot.* **63**: 1593-1608, 2012.
- Lastdrager, J., Hanson, J., Smeekens, S.: Sugar signals and the control of plant growth and development. - *J. exp. Bot.* **65**: 799-807, 2014.
- Li, Z., Jing, W., Peng, Y., Zhang, X.Q., Ma, X., Huang, L.K.,

- Yan, Y.H.: Spermine alleviates drought stress in white clover with different resistance by influencing carbohydrate metabolism and dehydrins synthesis. - *Plos ONE* **10**:1-16, 2015.
- Li, Z., Peng, Y., Zhang, X.Q., Ma, X., Huang, L.K., Yan, Y.H.: Exogenous spermidine improves seed germination of white clover under water stress *via* involvement in starch metabolism, antioxidant defenses and relevant gene expression. - *Molecules* **19**: 18003-18024, 2014.
- Liu, J.H., Nakajima, I., Moriguchi, T.: Effects of salt and osmotic stresses on free polyamine content and expression of polyamine biosynthetic genes in *Vitis vinifera*. - *Biol. Plant.* **55**: 340–344, 2011.
- Liu, J.H., Wang, W., Wu, H., Gong, X., Moriguchi, T.: Polyamines function in stress tolerance: from synthesis to regulation. - *Front. Plant Sci.* **6**: 196-206, 2015.
- Miller, G, Stein, H., Honig, A., Kapulnik, Y., Zilberstein, A.: Responsive modes of *Medicago sativa* proline dehydrogenase genes during salt stress and recovery dictate free proline accumulation. - *Planta* **222**: 70-79, 2005.
- Mohammadkhani, N., Heidari, R.: Water stress induced by polyethylene glycol 6000 and sodium chloride in two maize cultivars. - *Pak. J. biol. Sci.* **11**: 92-97, 2008.
- Moschou, P.N., Paschalidis, K.A., Roubelakis-Angelakis, K.A.: Plant polyamine catabolism. The state of the art. - *Plant Signal Behav.* **3**: 1061-1066, 2008.
- Moustakas, M., Sperdouli, I., Kouna, T.: Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. - *Plant Growth. Regul.* **65**: 315-325, 2011.
- Neill, S.J., Desikan, R., Hancock, J.: Hydrogen peroxide signaling. - *Curr. Opin. Plant Biol.* **5**: 388-395, 2002.
- Pál, M., Szalai, G., Janda, T.: Speculation: polyamines are important in abiotic stress signaling. - *Plant Sci.* **237**:16-23, 2015.
- Qiu, Z.B., Wang, Y.F., Zhu, A.J., Peng, F.L., Wang, L.S.: Exogenous sucrose can enhance tolerance of *Arabidopsis thaliana* seedlings to salt stress. - *Biol. Plant.* **58**: 611-617, 2014.
- Reddy, A., Das, V.S.R.: Stomatal movement and sucrose uptake by guard cell protoplasts of *Commelina benghalensis* L. - *Plant Cell Physiol.* **27**: 1565-1567, 1986.
- Roychoudhury, A., Banerjee, A., Lahiri, V.: Metabolic and molecular-genetic regulation of proline signaling and its cross-talk with major effectors mediates abiotic stress tolerance in plants. - *Turk. J. Bot.* **39**: 887-910, 2015.
- Saruhan, N., Turgut-Terzi, R., Kadioglu, A.: The effects of exogenous polyamines on some biochemical changes during drought stress in *Ctenanthe setosa* (Rosc.) Eichler. - *Acta biol. hung.* **57**: 221-229, 2006.
- Savage, M.J., Cass, A.: Psychrometric field measurement of water potential changes following leaf excision. - *Plant Physiol.* **74**: 96-98, 1984.
- Singh, M., Kumar, J., Singh, S., Singh V.P., Prasad, S.M.: Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. - *Rev. environ. Sci. Biotech.* **14**: 407-427, 2015.
- Solfanelli, C., Poggi, A., Loreti, E., Alpi, A., Perata, P.: Sucrose-specific induction of the anthocyanin biosynthetic pathway in *Arabidopsis*. - *Plant Physiol.* **140**: 637-646, 2006.
- Sulmon, C., Gouesbet, G., Couée, I., Amrani, A.E.: Sugar induced tolerance to atrazine in *Arabidopsis* seedlings: interacting effects of atrazine and soluble sugars on psbA mRNA and D1 protein levels. - *Plant Sci.* **167**: 913-923, 2004.
- Szabados, L., Savouré, A.: Proline: a multifunctional amino acid. - *Trends Plant Sci.* **15**: 89-97, 2010.
- Taiz, L., Zeiger, E.: *Plant Physiology*. 4th Ed. - Sinauer Associates Inc., Sunderland 2006.
- Tognetti, J.A., Pontis, H.G., Martinez-Noel, G.M.: Sucrose signaling in plants: a world yet to be explored. - *Plant Signal Behav.* **8**: 1-10, 2013.
- Velikova, V., Yordanov, I., Edreva, A.: Oxidative stress and some antioxidant systems in acid rain treated bean plants protective role of exogenous polyamines. - *Plant Sci.* **151**: 59-66, 2000.
- Verbruggen, N., Hermans, C.: Proline accumulation in plants: a review. - *Amino Acids* **35**:753-759, 2008.
- Wang, J., Sun, P.P., Chen, C.L., Wang, Y., Fu, X.Z., Liu, J.H.: An arginine decarboxylase gene PtADC from *Poncirus trifoliata* confers abiotic stress tolerance and promotes primary root growth in *Arabidopsis*. - *J. exp. Bot.* **62**: 2899-2914, 2011.