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Exogenous Application of Trehalose Improves the Physiological Status of Wheat cv. Giza 168 grown under Stress

Hanaa E. Ahmed, Maimona A. Kord, H.A. Youssef* and Ebtesam A. qaid
Department of Botany, Faculty of Science, University of Cairo, Giza 12613, Egypt

RESPONSE of *Triticum aestivum* (cv. Giza 168) to drought stress and the application, of the inevitable protective effect of exogenous application of trehalose was investigated. Drought reduced the plant growth parameters (dry weights, area of leaves) and photosynthetic pigments. Conversely, drought caused an increase in the levels of proline, lipid peroxidation, peroxidases and endogenous trehalose. The levels of endogenous IAA and GA_3 were decreased in drought-stressed plants, however the level of ABA was increased by more than 3-fold of control plants. Specific activity of trehalose -6-phosphate synthase, which is one of the two enzymes that participates in synthesizing trehalose in plants by the production of trehalose-6-phosphate; was increased by 3-fold of control in root of the drought-stressed plants. On the other hand, the specific activity of trehalase was drastically decreased. Pre-treatment of wheat plant with 40 mM trehalose -by irrigation- improved the above mentioned morphological and physiological parameters to retained them almost near the control. Thus, pre-treatment with 40 mM trehalose alleviated the harmful effect of drought stress on the test wheat cultivar.

Keywords: *Triticum aestivum*, Drought stress, Trehalose, Trehalose-6-phosphate synthase, Trehalase, Proline, Lipid peroxidation, Electrolyte leakage, Antioxidant Enzymes, Phytohormones.

Abbreviations: DAP (Days after planting), RWC (relative water content), T wt (turgid weight), ROS (reactive oxygen species), GP (guaiacol peroxidase), APX (ascorbic peroxidase), CAT (catalase), Tre (trehalose), EL (electrolyte leakage), Pro (proline), MDA (malondialdehyde), TPS (Trehalose phosphate synthase), T6P (Trehalose-6-phosphate), RH (Relative humidity).

Drought-stress of plants has been and still one of the major research topics. It causes several changes in plant biomass (Chandrasekar *et al.*, 2000; Abdalla and

*Corresponding author: erahmanyoussef@hotmail.com

El-Khoshiban, 2007), photosynthetic pigments (Anjum *et al.,* 2003), and induces alterations in cell membrane properties (Navari-Izzo *et al.,* 1993). These alternations include: Selective permeability, fluidity and viscosity. Drought stress inhibits cell expansion and growth due to lowering the relative water content (RWC) (Li *et al.,* 2011). Moreover, it raised the level of reactive oxygen species (ROS) through enhancing leakage of electrons to oxygen (Sofo *et al.,* 2015). Plants possess complex antioxidant defense system such as guaiacol peroxidase (GP, EC 1.11.1.7), ascorbic peroxidase (Apx, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6). Usually, plants maintained high levels of these antioxidant enzymes that are used to resist the oxidative damage caused by ROS (Apel and Hirt, 2004; Gapinska *et al.,* 2008 and Habibi, 2012).

Change in endogenous level of different phytohormones, especially (IAA, GA_3 and ABA), is an important factor enabling plants to cope with drought stress. Regarding IAA, it was reported that drought decreased its level in wheat plant (Xie *et al.,* 2003). GA_3 plays a protective role in maize grown seedlings under drought stress (Wang *et al.,* 2008). ABA was proved as an endogenous signal to initiate adaptive responses (Yang *et al.,* 2001 and Domash *et al.,* 2006).

Trehalose (Tre) is α – D glucopyranoside disaccharide, containing two D-glucose molecules bound in a 1, 1 linkage. Tre is synthesized from UDP-glucose and glucose-6-phosphate in a two step process with two enzymes, trehalose-6-phosphate synthase (TPS, EC 2.4.1.15) and trehalose-6-phosphate phosphatase (TPP, EC 3.1.3.12). Trehalose is one of the important osmoprotectant. The interest in trehalose metabolism has been increased due to its role in facing abiotic stresses, with special focus on water deficit stress. Many attempts have been made using genetic approaches to obtain transgenic plants by overexpressing genes that coding for enzymes of the trehalose biosynthesis pathway or down expression of trehalase gene (Almeida *et al.,* 2007).

Exogenous trehalose was successfully used in laboratory and greenhouse experiments to enhance the resistance of plants to abiotic stresses such as in maize (Zeid, 2009); rice (Nounjan *et al.,* 2012) and *Brassica sp*. (Alam *et al.,* 2014). Spraying plants with Tre improved seed composition and increased antioxidant activity, incorporated with high level of flavonoids (Khan *et al.,* 2012).

Wheat is a major food crop in many countries. There are three species of wheat; each of them has commercial importance; *Triticum aestivium, T. durum* and *T. compactum* (Klein and Klein, 1988). Wheat is grown all over the world in rainfall of 30- 113 cm; the productivity of wheat is highly affected by drought and insufficient irrigation. In Egypt, it is one of the most important crops. According to FAO (2009), 1.2 million hectares are cultivated with wheat, with 9 million tons productivity.

As the demand of water increases in North Africa in this era, the agricultural researchers are aiming to improve plants to cope the limitation in water in order

to maintain plant growth and productivity. In this investigation, we study the role of exogenous trehalose on physiological parameters and antioxidant enzymes activities in the Egyptian wheat (cv. Giza 168) grown under drought stress.

Materials and Methods

Plant seeds

Wheat seeds (*Triticum aestivum* cv. Giza 168) were purchased from Agriculture Research Center, Giza, Egypt.

Greenhouse experiment

A greenhouse experiment was conducted in the winter 2013/2014. Forty eight plastic pots (12 cm in diameter) were filled with 500 g of sterilized perlite and vermiculate (1:1, w/w). Seeds were surface sterilized with 20% sodium hypochloride (v/v) for 20 min, and thoroughly rinsed with sterilized distilled water. Fifteen seeds were sown in each pot. The pots were arranged in the greenhouse at 25 ±3°C with 16/8 h light and dark photocycle (5000 Lux) at 70% RH. Seedlings were grown up to 10 days irrigated with sterilized tap water to reach 70% field capacity. Pots were thinned; leave only five homogenous seedlings in each pot and divided into two groups. Plants in the first group were irrigated with the half strength of Hoagland solution. Plants in the second group were irrigated with half strength of Hoagland solution contained 40 mM Tre. The water field capacity of soil remained at 70% after 20 days from planting. Each group was divided into two sets. In the first set, plants were grown under the previously explained irrigation conditions; control with or without 40 mM Tre. In the second set, plants were subjected to drought by withholding irrigation during expanded third leaf; drought stress with or without 40 mM Tre.

Samples were collected from 34 day old plants (with four fully expanded leaves). 34 days represent 20 DAP and 14 days either at normal or drought conditions. By the end of the experiment, we have 12 pots for each treatment. Plants of the first three pots were used for the determination of dry weight and photosynthetic pigments. The second three pots were used for determination of leaf area, the percentage of relative water content (RWC). The third three pots were used for the determination of electrolyte leakage (EL), proline (Pro) content and lipid peroxidation by measuring malondialdehyde (MDA). The fourth three pots were used for the determination of antioxidant enzymes, TPS and trehalase activities. Trehalose content and endogenous hormones level were also determined.

RWC

Relative water content (RWC) was measured according to Barrs and Weatherley (1962). RWC was calculated by the following equation: RWC % = (F wt – D wt) / (T wt - D wt) /100.

Determination of photosynthetic pigments

The method described by Metzner *et al.*, (1965) were used to determine the photosynthetic pigments and expressed as mg g^{-1}FW.

Determination of Pro content

Proline (Pro) content was determined in shoot and root according to Bates (1973). The concentration of Pro was determined from a standard curve; the results were expressed as mg g^{-1} FW.

Determination of EL

Electrolyte leakage (EL) of tissue was measured according to the method described by Gilley and Fletcher (1997), using conductance meter (Model CD – 4301, Lutron). EL was calculated as a ratio of the conductivity before and after boiling.

Determination of MDA level

Lipid peroxidation was estimated according to method of Dhindsa and Matowe (1981). Absorbance was measured at 532 and 600 nm. The MDA level was calculated according to molar extinction coefficient of 155 mM cm^{-1}.

Extraction of antioxidant enzymes

Samples of 0.5 g of shoot or root were homogenized in 4 ml ice-cold extraction buffer (100 mM K-phosphate pH 7.8, 60 mg PVP). The homogenate was centrifuged at 12000 g for 15 min at 4 °C. The supernatant was used for determination of activity of GP, APX and CAT. The specific activity was calculated after estimation of total soluble proteins according to lowry *et al.* (1951).

Assay of antioxidant enzymes

Activity of GP was determined according to the method of Velikova *et al.,* (2000). The reaction mixture of 3 ml contained 50 mM K-phosphate buffer PH 7, 0.2% guaiacol (w/v) and 0.04 ml enzyme extract was prepared. The absorbance at 470 nm was measured 5 min after the addition of 3 mM H_2O_2. The activity of GP was calculated as mM of guaiacol reduced using the extinction coefficient of 26.6 mm^{-1} cm^{-1}. The specific activity was calculated as mM guaiacol reduced min^{-1} mg^{-1} protein.

For APX assay, the method described by Nakano and Asada (1980) was used. The reaction mixture of 3 ml contained 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1 ml enzyme extract. The reaction was initiated by adding 1.5 mM H_2O_2. The absorbance of the reaction mixture was measured at 290 nm. The specific activity of APX was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1} and expressed as mM ascorbate oxidized min^{-1} mg protein.

For CAT assay, the method described by Velikova *et al.* (2000) was used.

3 ml reaction mixture contained 10 mM k- phosphate buffer pH7, 0.1 ml enzymes extract and 0.035% H_2O_2. The activity of CAT was calculated based on the decline in the absorbance at 240 nm as the decomposition of H_2O_2.

The activity was calculated using the extinction coefficient of 40 mM^{-1} cm^{-1} and represented as mM H_2O_2 reduced min^{-1} mg^{-1} protein.

Extraction and assay of TPS

Plant extract was prepared by homogenizing 0.2 g of tissue in a mortar with 33% PVP and 2 mL of 50 mM Tris-HCl buffer (pH 7.5) containing 2.5 mM $MgCl_2$, 100 mM NaCl and 10 mM β-mercaptoethanol (López *et al.*, 2009). TPS activity was assayed at 37 °C by a colorimetric method using 5 mM of UDPG and G-6-P as substrates according to published protocol (Chaudhuri *et al.*, 2008). The assay mixture contained 50 mM Tris–HCl buffer, pH 8.5, 10 mM $MnCl_2$ and 1 µg heparin salt. HCl was added at the end of incubation to a final concentration of 100 mM and the tubes were heated at 100 °C for 10 min. NaOH was next added to a final concentration of 150 mM. Tubes were again heated similarly at 100 °C for 10 min. Trehalose-6-phosphate was estimated by anthrone against standard. Unit of enzyme activity (U) was expressed as µM of T6P synthesized min^{-1} under the assay conditions.

Extraction and assay of trehalase

Enzyme extract was prepared according to the method reported by Můller *et al.*(1992).

Trehalase activity was measured by estimating the glucose produced by hydrolysis of trehalose with the glucose oxidase-peroxidase kit (Spainreact), as described by Bergmeyer and Bernt, (1974). The reaction mixture contained 100 mM trehalose, 50 mM sodium citrate buffer (pH 5.5) and 0.25 ml crude extract in a final volume of 1.5 ml. After incubation at 37 °C for 30 min, the reaction was stopped by boiling for 3 min and then the reaction mixture was centrifuged (10 min, 5 000g). For the analysis, 10 µl of the supernatant was mixed with 1 mL of glucose oxidase-peroxidase kit solution. The mixture was incubated at 37 °C for 15 min. The absorbance of the sample was measured at 470 nm. The activity of trehalase was calculated according to the following equation: A sample × standard conc. × 0.0555/ A_{470} standard × 2

where: A= difference between optical density of sample before and after addition of substrate. A_{470} = Optical density of commercial standard at 470 nm. Concentration of standard=100 mg dL^{-1}. Conversion Factor = 0.0555µmol mL^{-1}. Dividing by 2 = Hydrolysis of 1 mole of trehalose produce 2 moles of glucose.

One unit (nkat) of trehalase activity is defined as the amount of enzyme that hydrolysis nM trehalose per second at pH 5.5. The specific activity was calculated and represented as nkat mg^{-1} protein.

Trehalose content

Trehalose content was determined using HPLC according to Cizmarik *et al.* (2004). Trehalose content was determined using Hewlett Packard HP 1090 liquid chromatographic column (Thermo, APS-2 Hypersil, 1000 3mm, 3mM). The flow rate was 0.8 mL min^{-1}. The mobile phase was acetonitrile: H_2O_2 (85: 15). The elute was detected by diode Array Detector (DAD) at 192 nm. The trehalose content was estimated by comparing the chromatogram with that of different concentrations of authentic trehalose.

Extraction and determination of endogenous hormones

Endogenous IAA, GA and ABA were extracted according to Topçuoğlu, and Ünyayar (1995), and were estimated using gas chromatography according to Du and Xu (2000). The gas chromatographic conditions were as follows: FID detector; HP-1 capillary column, 5 m x 0.53 mm x 2.65 microns film; the column temperature, 220°C the injector temperature, 250°C the detector temperature, 280°C carrier gas, 3.5 ml min^{-1} N_2; internal standard n-docosane. All components and internal standard were separated in 8 min. The detection limit of IAA, ABA and GA were 0.16, 0.08 and 0.48 mg/L respectively. The relative standard deviations were 2.2%, 1.7% and 2.8% respectively. The linear range were 0.16-80 mg/L(r = 0.9986), 0.08-40 mg/L (r = 0.9993) and 0.48-240 mg/L (r = 0.9991) respectively. The average recoveries were (88.4 +/- 2.4%)%, (92.2 +/- 1.2)% and (91.8 +/- 1.8)% respectively.

Statistical analysis

Results are the mean of three measurements for each treatment. All obtained data were subjected to ANOVA and the mean differences were compared by a Duncan's multiple range test (DMRT) using SPSS software var. 10. Differences at $P \leq 0.05$ were significant (Steel and Torrie, 1980).

Results

Effects of Tre treatment without or with drought stress on some growth parameters

As shown in Table 1, treatment with 40 mM Tre had almost no effect on RWC of wheat plants. When plants were exposed to drought, RWC decreased to 74% of control. Combination of Tre treatment and drought stress significantly elevated RWC of the plants to 85% of control.

TABLE 1. Mean values of the relative water content (%), dry wt of shoot (mg/plant), dry wt of root (mg/plant) and leaf area (cm^2/plant) of *Triticum aestivum L.* (cv. Giza 168) at 34 DAP untreated or treated with 40 mM trehalose under normal condition (control) or drought stress.

Treatments	Parameters			
	RWC	**Dry wt of shoot**	**Dry wt of root**	**Area of leaves**
Control	93.83 ± 0.43 c (100 %)	52.17 ±1.89 c (100 %)	14.92 ± 0.51 c (100 %)	20.81 ±0.39 c (100%)
+Ttre	91.93 ±1.66 c (98 %)	55.77 ±1.02 d (107 %)	14.35 ± 0.58 c (96 %)	19.92 ± 0.55 c (96 %)
Drought	69.38 ±2.49 a (74 %)	34.57 ± 1.11 a (63 %)	9.18 ± 0.37 a (62 %)	12.43 ± 0.76 a (60 %)
Drought+Tre	79.46 ± 1.45b (85 %)	39.25 ± 1.50 b (75 %)	11.97 ± 0.39 b (80 %)	15.53 ± 0.85 b (75 %)

From Photo 1, it is clear that, treatment with Tre had no effect on growth of wheat plant (cv. Giza 168). The growth was retarded in the drought stressed plants, however the effect of drought was diminished when plants were pretreated with 40 mM Tre. As shown in Table 1 treatment with Tre increased slightly the dry weight of shoot, but had no effect on the dry weight of root. Drought stress decreased the dry weights to about 62% of control. Exogenous application of Tre in combination with drought significantly increased the dry weights of shoot to 75% and to 80 % in root as compared with controls. With regard to the area of leaves, treatment with Tre has no effect on the area of leaves. Drought resulted in drop of the area of leaves to 60% of control, which increased to 75% when stressed plants were treated with Tre.

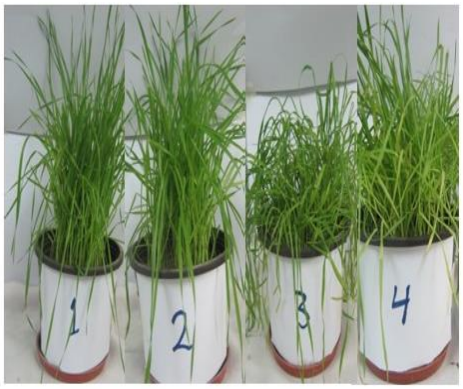

Photo 1: ***Triticum aestivum L.*** **(cv.Giza 168) at 34 DAP untreated or treated with 40 mM trehalose under normal condition (control) or drought stress**
1- Control.
2- Plants treated with 40 mM Tre.
3- Drought-stressed plants.
4- Plants treated with 40 mM Tre and grew under drought.

It is clear from Table 2 that treatment with Tre increased slightly the level of Chl a, but had no effect on the level of Chl b or carotenoids.

Exposure of wheat plants to drought greatly decreased the photosynthetic pigments. However, pretreatment with Tre considerably alleviated the drought effect on the photosynthetic pigments.

Pro content

Figure 1 represented the Pro content of shoot or root of the wheat cultivar (cv. Giza 168). It is clear that, Tre treatment had slight effect on the Pro content. Drought stress increased Pro content in shoot or root of the plant, however pre-treatment with Tre decreased it as compared with the drought-stressed plants.

TABLE 2. Mean values of the photosynthetic pigments (□g / FW) of *Triticum aestivum L.* (cv. Giza 168) at 34 DAP untreated or treated with 40 mM trehalose under normal condition (control) or drought stress.

Treatments	Photosynthetic pigments		
	Chl a	Chl b	Carotenoids
Control	1.026 ±0.028 c (100 %)	0.404 ±0.017 c (100 %)	0.127 ± 0.006 c (100 %)
+Ttre	1.098 ±0.010 d (107 %)	0.389 ±0.015 c (96 %)	0.132 ± 0.01 c (104 %)
Drought	0.784 ±0.02 a (76 %)	0.280 ± 0.02 a (69 %)	0.083±0.003 a (65 %)
Drought +Tre	0.959 ± 0.029 b (94 %)	0.351 ± 0.020 b (87 %)	0.104 ± 0.005 b (82 %)

DAP: days after planting, + Tre: Plants treated with 40 mM trehalose, Values are means of three replicates (5 plants each) ±SD, Mean values followed by the same letters within each column are not significantly different at ≤ 0.05 level. Values between parentheses are calculated as % of the corresponding control.

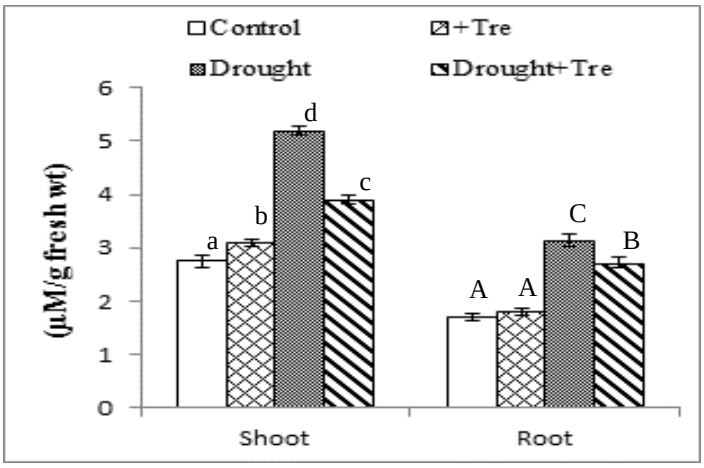

Fig. 1. Proline content (µM/g FW) of shoot and root of *Triticum aestivum L.* (cv.Giza 168) at 34 DAP.

Lipid peroxidation

The level of lipid peroxidation as indicated by MDA content in shoot or root of wheat cultivar was illustrated in Fig. 2. This Figure shows that, the level of MDA was higher in shoot than in root, and Tre application had no effect on this level. Drought stress increased the level of MDA of shoot or root of the plants; however obvious decline in this level was obtained when plants pretreated with 40 mM Tre and grew under drought stress.

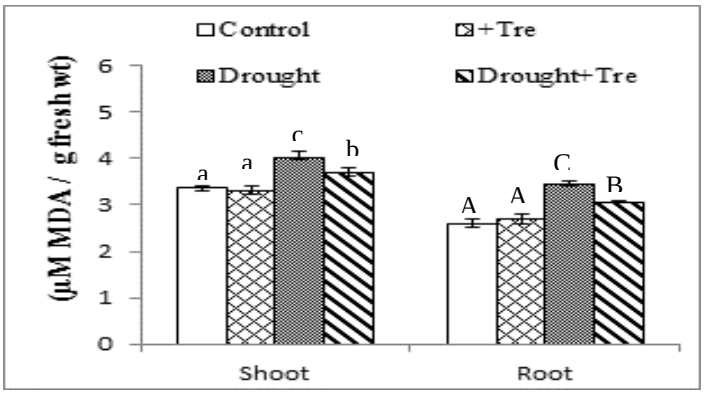

Fig. 2. Lipid peroxidation (µM MDA/g FW) of shoot and root of *Triticum aestivum L.* (cv.Giza 168) at 34 DAP.

+Tre: Plants were treated with 40 mM trehalose; The same letters written by the same case are not significantly different at ≤ 0.05 level. Bars indicate ±SD.

EL

The EL in shoot and root of wheat cultivar, Giza 168 was illustrated in Fig. 3. From this Figure it is clear that, Tre had slight effect on EL. Drought stress caused damage in the cell membranes of all samples. This damage was reflected by the increase of percentage of EL. Combination between Tre application and drought, significantly decreased EL almost near the control level.

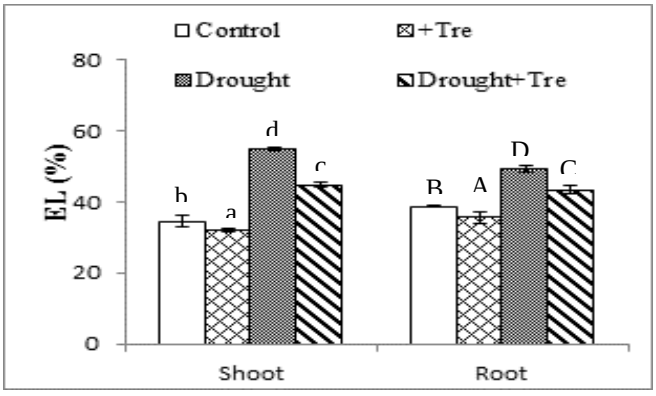

Fig. 3. Percentage of electrolyte leakage (EL) of shoot and root of *Triticum aestivum L.* (cv.Giza 168) at 34 DAP.

+Tre: Plants were treated with 40 mM trehalose; The same letters written by the same case are not significantly different at ≤ 0.05 level. Bars indicate ±SD.

Specific activities of some antioxidant enzymes

Figure 4 shows the specific activities of GP, APX and CAT in shoot and root of wheat plants. From this Figure it is clear that, treatment with Tre caused significant elevation in the specific activity of GP in shoot but not in root. Drought resulted in sharp increase in the specific activity of GP in shoot or root. Combination of Tre and drought stress significantly decreased the specific activity of GP in both shoot and root of wheat plants as compared with drought-stressed plants.

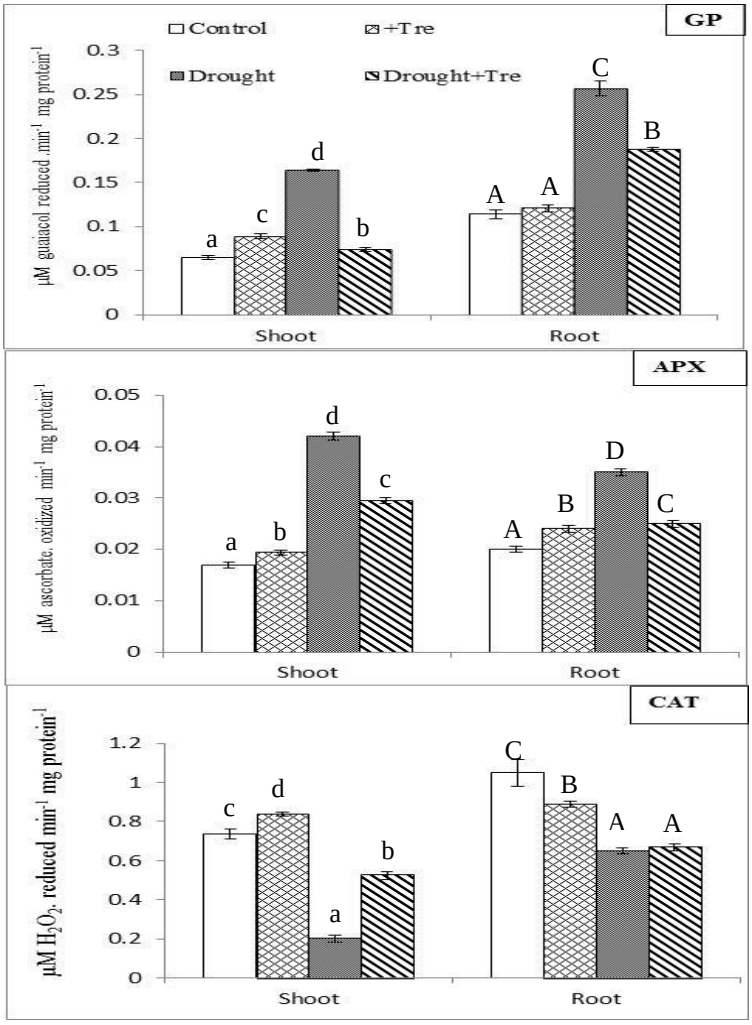

Fig. 4. Specific activities of GP, APX and CAT as unit/mg protein of shoot and root of *Triticum aestivum L.* (cv.Giza 168) at 34 DAP.

+Tre: Plants were treated with 40 mM trehalose. The same letters written by the same case are not significantly different at ≤ 0.05 level.

Treatment with Tre had slight effect on the specific activity of APX in shoot and root of wheat cultivar. When plants grew under drought stress a great increase in specific activity of APX was observed. However, pre-treatment with 40 mM Tre significantly decreased the specific activity of APX as compared with drought-stressed plants.

High values of specific activity of CAT were recorded in control as well as in Tre- treated plants. In drought-stressed plants, sharp decrease in the specific activity of CAT was observed, particularly, in shoot than in root. Such decrease was considerably recovered in shoot by pre-treatment of Tre.

Specific activities of TPS and trehalase and endogenous Tre

Trehalose- phosphate synthase (TPS) showed low specific activity in shoot and root of the wheat plant, which is slightly raised when plants were treated with 40 mM Tre (Table 3). In drought-stressed plants, sharp increase in the specific activity of TPS was recorded and this increase was more pronounced in root than in shoot. Combination of drought stress and Tre treatment decreased the specific activity in both shoot and root as compared with drought-stressed plants.

With regard to specific activity of trehalase, it showed sharp decrease in all treatments as compared to control. The decrease was more pronounced in root than in shoot.

It is clear from Table 3 that, the wheat plant organs contained minute amount of Tre, which increased when plants were irrigated with 40 mM Tre. Exposing plants to drought stress increased the level of Tre in shoot or root over the control. Combination of Tre treatment and drought stress further raised the level of endogenous Tre over both Tre treatment and drought-stressed plants.

Levels of endogenous hormones

Table 4 represented the endogenous levels of IAA, GA_3 and ABA in the wheat cultivar (cv. Giza 168). Treatment of 40 mM Tre raised the level of IAA and GA_3 in plants, but had no effect on the level of ABA. Drought stress decreased drastically the level of IAA and GA_3, but sharply increased the level of ABA. Combination of Tre treatment and drought resulted in incline in the level of IAA and GA_3, and decline in level of ABA as compared with drought-stressed plants.

TABLE 3. Specific activity of TPS (µM T6P synthesized/min/ mg protein) and trehalase (nkat/mg protein), and trehalose content (mg / g dry weight) of *Triticum aestivum L.* (cv.Giza 168) at 34 DAP under drought stress untreated or treated with 40 mM Tre.

Treatments	Shoot		
	TPS	**Trehalase**	**Trehalose**
Control	0.08 ± 0.002 a (100 %)	0.31 ± 0.009 d (100 %)	0.30 ± 0.01 a (100 %)
+Ttre	0.095 ± 0.001 c (118.8 %)	0.13 ± 0.006 c (40.6 %)	1.06 ± 0.05 c (355.7 %)
Drought	0.13 ± 0.001 d (162.5 %)	0.11 ± 0.051 b (36.8 %)	0.54 ± 0.006 b (179.5 %)
Drought +Tre	0.09 ± 0.003 b (112.5%)	0.06 ± 0.021 a (18.4%)	1.70 ± 0.06 d (571.8%)
Treatments	**Root**		
	TPS	**Trehalase**	**Trehalose**
Control	0.065 ± 0.03 a (100 %)	0.40 ± 0.01 c (100 %)	0.33 ± 0.014 a (100 %)
+Ttre	0.08 ± 0.02 b (123.1 %)	0.06 ± 0.003 b (14.1 %)	1.03 ± 0.04 c (316.9 %)
Drought	0.20 ± 0.04 d (333.3 %)	0.03 ± 0.001 a (7.9 %)	0.69 ± 0.005 b (211.1 %)
Drought +Tre	0.145 ± 0.01 c (241.6 %)	0.04 ± 0.002 a (8.70 %)	1.52 ± 0.019 d (468.6 %)

Values are mean of three replicates (five plants each) ±SD.
Mean values followed by the same letters within each column are not significant different at ≤0.05 level.
Values between parentheses represent the percentage of control.

TABLE 4. Contents of IAA, GA_3 and ABA (ng /g fresh wt) of *Triticum aestivum L.* (cv.Giza 168) at 34 DAP untreated or treated with 40 mM trehalose under normal condition (control) or drought stress.

Treatments	Hormones		
	IAA	**GA_3**	**ABA**
Control	60.29 ± 2.09 c (100 %)	48.56± 0.87 c (100 %)	54.73 ± 1.83 a (100 %)
+Ttre	79.9 ± 2.13 d (133 %)	55.52 ± 2.10 d (114 %)	52.65 ± 1.76 a (96 %)
Drought	33.62 ± 0.80 a (56 %)	31.00 ± 0.52 a (64%)	189.3 1 ± 5.60 c (346%)
Drought +Tre	49.16 ± 0.82 b (82 %)	39.71 ± 0.92 b (82%)	127.18 ± 3.98 b (232%)

Values are mean of three replicates (five plants each) ±SD
Mean values followed by the same letters within each column are not significantly different at ≤0.05 level.
Values between parentheses represent the percentage of control.

Discussion

Effects of Tre treatment without or with drought stress on some growth parameters

It is clear from Table 1, that the growth parameters namely, the dry weights of shoot and root as well as, the area of plant leaves showed slight or no effect when plants were treated with Tre. Exposing plants to drought stress decreased the previously mentioned growth parameters to about 60% of the control. Combination of Tre treatment and drought significantly raised the values of these growth parameters to 75%-80% of the control. In this context, many researchers suggested that, Tre acts as an osmoprotectant maintains osmotic pressure in the cell and stabilizes the dehydrated enzymes, proteins and membranes (Garg *et al.,* 2002; Theerakulpisut and Gunnula, 2012 and Alam *et al.,* 2014). The improvement of growth due to application of Tre combined with drought may be due to metabolization of T6P to usable sugars resulting in improved growth during stress conditions.

In this investigation, drought decreased the levels of Chl a, b and carotenoids. Combination of Tre treatment and drought improved the levels of photosynthetic pigments in the leaves of the wheat cultivar (Table 2). Generally, the calculated percentage of improvement ranged between 15- 20 % in all cases. Numerous studies reported that inhibition of photosynthesis due to drought were attributed to damages of photosynthetic pigments that in turn due to oxidation of pigments and impaired pigment biosynthesis (Anjum *et al.,* 2011 and Alam *et al.,* 2014).

Pro content

The role of Pro in alleviating the harmful effect of drought stress is well recognized. Pro has remarkable roles in osmotic adjustment, stress signal transduction, and as an antioxidant compound. In the present work, the level of Pro increased under drought stress in the wheat cultivar. Pre-treatment of Tre prior to drought stress decreased the level of Pro, but still above the value of control. In this connection, similar profounds were reported by Alam *et al.* (2014) on seedling of *Brassica*; Nounjan *et al.* (2012) on rice; Ali and Ashraf (2011) on maize.

Lipid peroxidation

Induction of oxidative stress is a well recognized effect of drought. Level of MDA indicates the oxidative stress in the plants under investigation. In this study, treatment with Tre has no effect on the level of MDA; however drought stress increased the level of MDA. Combination of both Tre and drought stress decreased the level of MDA to be near the control (Fig. 2). Similar response was reported on *Barssica juncea* (Alam *et al.,* 2013); *Allium sativum* (Bideshki and Arvin, 2013).

EL

Electrolyte leakage reflects the effect of certain stress on the plasma membrane. In this study, there was gradual increase in electrolyte leakage of

shoot and root of the wheat cultivar exposed to drought stress (Fig. 3). However, this increase disappeared when wheat plant was treated with Tre and grew under drought stress.

Specific activities of some antioxidant enzymes

Drought stress resulted in high production of ROS that disturb the regular metabolic process in cells through oxidative damage to pigments, lipids and proteins (Ashraf, 2009). In order to avoid the harmful effect of ROS, plants evolve an effect scavenging system composed of enzymatic antioxidant such as GP, APX and CAT.

In this study, the specific activity of GP and APX increased slightly in shoot and root of the wheat cultivar, as a result of Tre application (Fig. 4). Drought stress resulted in sharp increase of specific activity of GP and APX in shoot and root of the plants. In this context, exogenous application of Tre enhanced APX activity under Cd stress in *Lemna gibba* (Duman *et al.,* 2011) and under salt stress in *Oryza sativa* (Nounjan *et al.,* 2012).

In our study, Tre application with drought stress resulted in decreasing the specific activities of GP and APX in shoot and root of the wheat cultivar but the values still higher than the corresponding controls (Fig. 4).

In this study, application of Tre increased the specific activity of CAT in shoot, but decreased it in root. Drought stress resulted in a considerable decline in the specific activity of CAT in both shoot and root of the plant. Combination of Tre and drought resulted in a considerable incline in the specific activity of CAT in shoot only. CAT is one of the most effective antioxidant enzymes; it has the highest turnover rates among all enzymes (Garg and Manchanda, 2009). In this connection, several reports mentioned the changes in specific activity of CAT in drought stressed plants such as *Zea mays* (Ali and Ashraf, 2011); *Brassica spp*. (Alam *et al.,* 2014) and *Lemna gibba* (Duman *et al.,* 2011).

Specific activities of TPS and trehalase and level of endogenous Tre

The enzyme TPS is responsible of the production of T6P in the biosynthetic pathway of trehalose biosynthesis. In higher plants, it is possible that, the major role of trehalose pathway is to control metabolite regulator. This regulatory function is performed at least in plants by T6P, which is considered as a signal in metabolic events. In the present work, the specific activity of TPS slightly increased with Tre treatment. When plants exposed to drought stress, specific activity of TPS showed sharp increase in the shoot and root of plants. It amounted to about 1.5 fold in shoot and more than 3 folds in root (Table 3). This indicates the high production of the intermediate signal (T6P). Pre-treatment of Tre to drought-stressed plants sustained the value of specific activity of TPS at a high level. In the present study, T6P together with trehalose seemed to alleviate the harmful effects of drought stress in wheat plants.

The specific activity of trehalase in shoot and root of wheat plant (cv. Giza 168) is represented in Table 3. It is clear that, there is a significant decrease in specific activity of trehalase in Tre treatment either alone or combined with drought stress.

Regarding the level of endogenous trehalose, a high increase was observed in shoot and root of the wheat cultivar, due to Tre application (Table 3). The level of trehalose was inclined in response to drought stress as compared to control. Combination of Tre application with drought stress resulted in increasing endogenous Tre over the control or the drought-stressed plants. One of the adaptive mechanism to deal with drought is to maintain turgor pressure by the production of osmolytes, such as trehalose that can also provide secondary protective effects such as protecting structure of proteins from unfolding and so preventing the denaturation of proteins (Ford *et al.,* 2011). Additionally, T6P (the intermediate compound in trehalose biosyntheses pathway) is now confirmed to act as a sensor for available sucrose (Hanaa *et al.,* 2013), therefore directly effecting the type of response to different stresses. (Delorge *et al.,* 2014), reported that T6P and/or Tre or their biosynthetic enzymes are participating in a complex network with other crucial plant hormones to improve plant growth and development and this participation becomes more clear and important when plants grow under stress conditions.

Levels of endogenous hormones

Plants respond to drought through various physiological and biochemical changes, including changes of the endogenous phytohormone levels especially, IAA, GA_3 and ABA. The contents of these hormones in leaves of the wheat cultivar were represented in Table 4. Exogenous application of Tre increased the endogenous levels of IAA and GA_3; however the level of ABA remained unchanged. Under drought stress, the levels of endogenous IAA and GA_3 were significantly decreased, but ABA was sharply increased. Exogenous application of Tre in combination with drought retained the level of the endogenous IAA and GA_3 near that of control and kept ABA at a higher level. The level of endogenous ABA in plants treated with Tre and grown under water deficient reached double fold of the control in wheat cultivar (Table 4).

It was reported that drought stress resulted in a decrease in IAA content in corn (Wang *et al.,* 2008) in wheat leaves (Xie *et al.,* 2003).

GA_3 was able to improve plant growth under osmotic stress. In the present study, the level of GA_3 is slightly affected by exogenous application of Tre. However, its level is drastically declined under drought stress; then inclined in case of the combination of Tre application and drought. The same trend was reported in maize plants subjected to drought stress (Wang *et al.,* 2008). GA_3 and IAA usually promote growth, so when level of GA_3 and IAA decreased the growth will retard.

ABA acts as an endogenous signal to initiate adaptive responses toward different abiotic stresses; especially drought stress. The adaptation includes alteration of gene expression and stomatal closure (Seki *et al.,* 2002). The accumulation of ABA under drought stress was reported in rice (Yang *et al.,* 2001); cucumber (Pustovoitova *et al.,* 2004) and bean (Domash *et al.,* 2006). These results were greatly correlated with our results. As the increase in ABA level reached to more than 3 fold in the stressed wheat cultivar (Table 3).

Conclusion

In conclusion, treatment with Tre had almost slight or no effect on growth parameters, EL, proline content and peroxidases (GP and APX).

Drought stress resulted in obvious drastic effects in growth parameters. However proline, peroxidases, ABA, trehalose content showed remarkable increase.

Treatment with Tre in combination with drought could help in facing the drought stress. This was evident in improving the growth of shoot and root, maintaining the level of endogenous trehalose, and manipulating the level of phytohormones. It seems that, Tre together with its intermediate T6P, which was produced by TPS, could alleviate the harmful effects of drought.

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تحسين الحالة الفسيولوجية لنبات القمح (صنف جيزة١٦٨) - النامي تحت إجهاد الجفاف- بإستخدام سكر التريهالوز

هناء البدوى احمد- ميمونة عبدالعزيز كرد- الحسينى عبدالرحمن يوسف- ابتسام احمد عبده قائد
قسم النبات- كلية العلوم- جامعة القاهرة- مصر.

يتناول هذا البحث دراسة المعاملة بسكر التريهالوز على تخفيف إجهاد الجفاف على نبات القمح (صنف جيزة ١٦٨). و قد تسبب إجهاد الجفاف فى نقص معدلات نمو النبات ممثلة فى نقص الوزن الجاف و الوزن الطازج و مساحة الاوراق و أصباغ البناء الضوئي. كما تسبب إجهاد الجفاف فى زيادة البرولين و اكسدة الدهون و نفاذية الايونات خلال الاغشية، مما يدل على تلفها و نشاط الانزيمات المضادة للأكسدة (البيروكسيديز و الاسكوربات بيروكسيديز)، و كذلك محتوى التريهالوز الداخلي.

و قد انخفض محتوى الهرمونات النباتية (اندول حمض الاسيتك وحمض الجبرالين) فى النباتات المجهدة بالجفاف و التى سجلت زيادة كبيرة (٣ اضعاف) من حمض الابسيسك) مقارنة بنباتات المقارنة (المعامل الضابط) . كما زاد بدرجة كبيرة (٣ اضعاف) نشاط انزيم تريهالوز- فوسفات- سينثيز، كما انخفض النشاط النوعي لانزيم التريهاليز.
و عند المعاملة المسبقة بسكر التريهالوز (باضافة ٤٠ مللى مولار لماء الري) حدث تحسن ايجابي فى المعايير المورفولوجية و الفسيولوجية للنبات. برهن هذا البحث على أن المعاملة بسكر التريهالوز و مركب T6P يعملان على تخفيف الاثار الضارة للجفاف على نبات القمح.