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Physiological role of trehalose on enhancing salinity tolerance of wheat plant

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Abstract

Background: Salinity stress is one of the most serious abiotic stress affecting adversely plant growth, various metabolic processes, and crop production. Using different osmoprotectants such as trehalose in alleviating salinity stress adverse effects is very important for plant production. So, the present study investigated the physiological role of trehalose (Tre) in improving wheat tolerance to oxidative stress induced by salt stress.

Results: Salinity stress (6.25 dS/m) caused marked significant decreases in wheat plant growth parameters (shoot height, fresh, and dry weights of the shoot) accompanied by significant increases in lipid peroxidation, hydrogen peroxide contents, and lipoxygenase enzyme (LOX) activity. Osmoprotectant compounds such as glucose, sucrose, trehalose, total soluble sugars (TSS), free amino acids, and proline increased in wheat plants irrigated with saline water compared with unstressed control plant. On the other hand, Tre foliar treatments (10 mM and 50 mM) proved to be effective in enhancing growth parameters and more accumulation of the tested organic solutes of leaves (glucose, sucrose, trehalose, and TSS) of salinity-stressed plants. Meanwhile, trehalose treatments with different levels caused significant decreases in lipid peroxidation, hydrogen peroxide contents, and LOX activity in normally irrigated and salinity-stressed plants. These decreases correlated with significant increases in total phenolic contents as compared with untreated control.

Conclusion: It could be concluded that foliar spray of trehalose was effective in improving wheat performance by reducing hydrogen peroxide free radical and by enhancing antioxidant compounds (phenolics), compatible osmolytes, and membrane stability.

Keywords: Antioxidant enzymes, Glucose, Growth, Osmoprotectants, Phenolics, Trehalose, Wheat

Introduction

In Egypt, *Triticum aestivum* L., wheat plant is the most important strategic food crop, while in the world it is the third most-produced cereal after maize and rice, while in respect to the dietary intake, it is currently considered the second to rice as the main food crop. As a hardy crop, wheat can grow in a wide range of environmental conditions, and this permits large-scale cultivation as well as long-term storage of food. Generally, about 70% of this crop is used for human food, 19% for animal feed and the remaining 11% is used for industrial applications, including biofuels. The importance of wheat is mainly due to the fact that its grains can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pasta, and thus it presents the main source of nutrients to the most of the world population (Abd Allah et al. 2015). Wheat grains nutritional value is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. Moreover, Yu et al. (2002) suggest that wheat may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion.

Resources of fresh water are becoming limited as a result of the competition of human uses and industrial uses. In addition, the type and quantity of dissolved salts caused great variations in quality of saline water. Irrigation of crops with diluted seawater plays an important role in saving freshwater resources and can be used successfully to grow crops under certain conditions (Zeid 2011). Irrigation of plants with saline water increased salt concentrations in soil and thus affect adversely on plant growth and crop yield (Sobhanian et al. 2010). Salt

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stress caused reductions of different crops production these reductions resulted from the increased uptake of salts by roots and the unavoidable consequence of high ion concentrations. Salinity stress caused ion toxicity, osmotic stress, and increased ROS production (Sreenivasulu et al. 2000). Under salinity stress, oxidative damage resulted from the imbalance of ROS production, caused the programmed cell death (Sadak 2016a). Excess of ROS caused by salinity stress triggers phytotoxic reactions such as peroxidation of lipid, mutation of DNA (deoxyribonucleic acid), and degradation of protein (McKersie et al. 1996). Different plants enhance salinity tolerance by decreasing salt content, ion compartmentation, osmotic adjustment, and induction of antioxidant enzymes (Sadak et al. 2015). Salinity stress caused overproduction of different types of osmoprotectants (compatible solutes). Compatible solutes are highly soluble, low molecular weight compounds which are usually non-toxic at high cellular concentrations. Generally, such solutes protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes and proteins (Bohnert and Jensen 1996). Many attempts were done to enhance salinity tolerance of different crops through using osmoprotectant compounds as a presowing seed treatment or exogenous application as foliar treatment on plants at different growth stages. These osmoprotectant compounds include proline, glycinebetaine, or trehalose (Dawood and Sadak 2014). One of these compounds is trehalose (Tre), a non-reducing disaccharide of glucose, which plays an important role as a stress protectant in some plants (Garcia et al. 1997; Duman et al. 2010 and Ali and Ashraf 2011). In addition to being an energy source, the unique physicochemical properties of Tre efficiently stabilize dehydrated enzymes, proteins, and lipid membranes, as well as protect biological structures from damage during desiccation (Fernandez et al. 2010). Trehalose has the added advantage of being a signaling and antioxidant molecule. Trehalose also acts as an elicitor of genes involved in detoxification and stress response (Bae et al. 2005). However, Tre production in most plants is not sufficient to ameliorate stress-induced adverse effects. On the other hand, external Tre application increases the internal level of this osmolyte and has been suggested as an alternative approach to induce stress tolerance (Chen and Murata 2002). Exogenous Tre alleviates the adverse effects of various abiotic stresses including drought in maize, heat, water deficit, and salinity in wheat (Ali and Ashraf 2011; Luo et al. 2010; Ma et al. 2013 and Sadak 2016b).

Therefore, this study was conducted to investigate the physiological effects of exogenous application of trehalose on various growth parameters, physiological parameters, and antioxidant defense system of wheat plants grown under salinity stress.

Materials and methods

Plant material and growth conditions

A pot experiment was conducted at the screen of National Research Centre, Cairo, Egypt, during two successive seasons of 2014/2015 and 2015/2016. Wheat grains, Giza 168 cultivar were obtained from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Wheat grains were selected for uniformity, then washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min, and thoroughly washed again with distilled water and left to dry at room temperature for approx. 1 h. Ten uniform air-dried wheat grains were sown in pots 30 mm depth, each filled with about 7.0 kg clay soil mixed with sandy soil in a proportion of 3:1(*v*:*v*), respectively, in order to reduce compaction and improve drainage. Before planting calcium superphosphate (15.5% P_2O_5) and potassium sulfate (48% K_2O) in the rate of 3.0 and 1.50 g/pot were added, respectively. Ammonium sulfate (20.5% N) in the rate of 6.86 g/pot was added in two equal doses, the first one was added after 2 weeks from sowing and the second 2 weeks later. At 45 and 60 days after planting, trehalose foliar treatment was added, consisted of three levels of trehalose, namely, 0 mM (control), 10 mM, and 50 mM considered as Tre0, Tre1, and Tre2 respectively. The experiment was arranged in a complete randomized design with two levels of seawater (S0 and S1). Four replicates were used. Seawater was dissolved in fresh water, and the plants were watered with an equal volume of 0.23 dS/m and 6.25 dS/m. One week after the second foliar spray of trehalose (treatments S0 and S2, respectively). The concentration of EC, pH, cations, and anions of irrigation water and soil used were determined according to Chapman and Pratt 1978 and shown in Table 1.

At 75 days after sowing, samples were taken to study some growth parameters like plant height (cm), no. of leaves/plant, fresh and dry weight of shoot/plant (g), photosynthetic pigments of leaves, carbohydrate constituents such as (glucose, sucrose, trehalose, total soluble sugars. and starch), some osmoprotectants as proline, total free amino acid, phenolic contents as well as hydrogen peroxide content (H_2O_2), lipid peroxidation, and lipoxygenase enzyme (LOX).

Biochemical analysis

Determination of photosynthetic pigments

Chlorophyll a, chlorophyll b, and carotenoid concentrations of wheat plants were estimated using the method of Moran (1982). Fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded (for chlorophyll a, b, and

Table 1 EC, pH, and concentrations of cations and anions of irrigation water and soil used in pots

	EC dS/m	pH	Cations meq^{-1}				Anions meq^{-1}			
			Ca^{2+}	Mg^{2+0}	Na^{+}	K^{+}	HCO^{3-}	CO^{3-}	SO_4^{2-}	Cl^{-}
Soil										
Sandy	0.17	8.34	2.65	2.54	1.38	0.21	1.12	0.00	4.25	0.76
Clay	1.43	7.68	5.68	1.87	5.86	0.33	1.52	0.00	6.79	5.53
Water										
Tap water	0.23	7.34	1.01	0.52	2.42	0.21	0.11	0.00	1.32	2.67
Sea water	51.25	7.76	43.17	15.16	454.98	1.52	6.09	0.00	76.98	432.34

carotenoids) at 662, 645 nm, and 470 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/g FW.

Determination of hydrogen peroxide

Hydrogen peroxide content was determined using the method Velikova et al. (2000) in which fresh samples of leaf tissue (100 mg) was extracted with 5 ml of 0.1% trichloroacetic acid (TAC) and centrifuged at 12000 g for 15 min. Then, 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM phosphate buffer (pH = 7) and 1 ml of 1 M potassium iodide. The absorbance was determined at 390 nm. The amount of H_2O_2, read using the extinction coefficient 0.28 μm^{-1} $cm^{-1}$and expressed as nmol g^{-1} FW.

Determination of lipid peroxidation

The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges et al. (1999). A sample (200 mg) was homogenized in 10 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000×*g* for 10 min to 2.0 ml aliquot of the supernatant and 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath and centrifuged at 10000×*g* for 10 min the absorbance of the supernatant was recorded at 532 nm by spectrophotometer (Shimadzu). The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 $nmol^{-1}$ cm^{-1} and expressed as nmol (MDA) g^{-1} fresh weight.

Lipoxygenase activity

Enzyme extracts were collected following the method described by Chen and Wang (2006). Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8000 rpm and 4-C for 15 min. The supernatant was used immediately to determine the activities of enzymes. LOX (EC 1.13.11.12) activity was estimated according to the method of Doderer et al. (1992) by monitoring the increase in absorbance at 234 nm using linoleic acid as a substrate. The activity was calculated using the extinction coefficient (25 mM^{-1} cm^{-1}) and expressed as units (1 nmol of substrate oxidized per minute) per milligram protein.

Determination of glucose

Glucose estimations were performed using a modification of O-toluidine procedure (Feteris 1965). Sugars were extracted by overnight submersion of dry tissue in 80% (*v*/*v*) ethanol at 25 °C with periodic shaking. A 1.0-ml aliquot of the alcoholic extract was heated with 5.0 ml O-toluidine reagent (60 ml O-toluidine and 2.0 g thiourea made to 1 L with glacial acetic acid) for 15 min at 97 °C. Absorbance was measured at 630 nm.

Determination of sucrose

Sucrose contents were determined by first degrading reactive sugars present in 0.1 ml extracts with 0.1 ml 5.4 N KOH at 97 °C for 10 min (Handel 1968). Three milliliters of freshly prepared anthrone reagent was then added to the cooled reaction product, and the mixture was heated at 97 °C for 5 min, cooled, and read at 620 nm.

Determination of trehalose

Trehalose content in wheat leaves was determined following the method described by Li et al. (2014) with some modifications. The leaves (1.0 g) were homogenized in 5 mL of 80% (*v*/*v*) hot ethanol and centrifuged at 11,500×*g* for 20 min. The supernatants were dried at 80 °C followed by resuspension in 5 mL distilled water. The solution (100 μL) was mixed with 150 μL 0.2 N H_2SO_4 and boiled at 100 °C for 10 min to hydrolyze any sucrose or glucose-1-phosphate and then chilled on ice. NaOH (0.6 N, 150 μL) was added to the above mixture and boiled for 10 min to destroy reducing sugars, and then chilled again. To the above mixture, 2.0 mL of anthrone reagent (0.2 g anthrone per 100 ml of 95% H_2SO_4) was added and boiled for 10 min to develop a color, and then chilled again. The absorbance was recorded at 630 nm, and trehalose concentration was calculated as micromoles per gram FW using a standard curve developed with commercial trehalose.

Determination of total soluble sugars

Total soluble sugars (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (*v*/*v*) ethanol at 25 °C with periodic shaking and centrifuged at 600×*g*. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme et al. 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for 10 min and reading the cooled samples at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss (Yemm and Willis 1954).

Determination of starch

For starch measurement, the insoluble fraction remaining after ethanolic extraction of soluble sugars was resuspended in 2 mL of 2.5 M NaOH and boiled for 5 min. After cooling the solution, pH was adjusted to pH 4.5 with 2 M HCl, and the resulting gelatinized starch was hydrolyzed 10 min at 50 °C with buffered (0.1 M sodium acetate buffer, pH 4.5). After this process, starch was measured as reducing sugars by Nelson's method (Nelson 1944) and expressed in maltose equivalents.

Determination of free amino acids

Free amino acid content was extracted according to the method described by Vartainan et al. (1992). Free amino acid was determined with the ninhydrin reagent method (Yemm and Cocking 1955). One milliliter acetate buffer (pH 5.4) and 1.0 ml chromogenic agent were added to 1.0 ml free amino acid extraction. The mixture was heated in a boiling water bath for 15 min. After being cooled in tap water, 3-ml ethanol (60% *v*/*v*) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocololourimeter VEB Carl Zeiss.

Determination of proline

Proline was assayed according to the method described by Bates et al. (1973). Two milliliters of proline extract, 2.0 ml of acid ninhydrin, and 2.0 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Determination of phenolics

A known weight of the fresh samples was taken and extracted with 85% cold methanol (*v*/*v*) for three times at 0 °C. The combined extracts were collected and made up to a known volume with cold methanol. And then, 0.5 ml of the extraction was added to 0.5-ml Folin reagent, shaken, and allowed to stand for 3 min. Then 1 ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60 min. The optical density was determined at wavelength of 725 nm using spectrophotometer as described by Danil and George (1972).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) for a randomized complete block design, after testing for homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Statistically significant differences between means were compared at $p \leq 0.05$ using Duncan's multiple range test and presented with the standard errors.

Result

Changes in growth parameters

Irrigation of wheat plants with diluted seawater (6.25 dS/m EC) caused significant decreases in growth criteria (plant height, leaves number/plant, fresh, and dry weight of shoot/plant) relative to control plants except, relative water contents (RWC) the decreases were insignificant (Table 2). On the other hand, trehalose foliar treatments proved to be effective in enhancing shoot length, leaves number/plant, fresh, and dry weights of shoot under unstressed and salinity-stressed plants compared to untreated plants (Table 2). It was noted that Tre2 was more effective than Tre1 treatment at unstressed and

Table 2 Effect of trehalose at 0 mM (Tre 0), 10 mM (Tre 1), and 50 mM (Tre 2) on growth parameters of wheat plants irrigated with tap water (S0) or diluted sea water at 6.25 dS/m (S1). Results are means of two successive seasons

Salinity (dS/m)	Trehalose conc (mM)	Plant height (cm)	Leaves no/plant	Fresh wt/plant (g)	Dry wt/plant (g)	RWC%
S0	Tre0	47.67	4.33	5.17	1.30	74.85
	Tre1	52.33	5.33	6.83	1.66	75.69
	Tre2	56.66	6.33	7.99	1.93	75.84
S1	Tre0	29.33	3.33	3.07	0.85	72.31
	Tre1	35.33	4.67	4.24	1.09	74.29
	Tre2	38.67	5.00	5.32	1.36	74.43
LCD at 5%		4.087	0.524	0.675	0.274	4.23

Table 3 Effect of trehalose at 0 mM (Tre 0), 10 mM (Tre 1), and 50 mM (Tre 2) on photosynthetic pigments (mg/g fresh wt) of wheat plants irrigated with tap water (S0) or diluted sea water at 6.25 dS/m (S1). Results are means of two successive seasons

Salinity	Trehalose conc (mM)	Chlo a	Chlo b	Chl a + b	Carotenoids	Total pig
S0	Tre0	1.128	0.465	1.593	0.269	1.862
	Tre1	1.302	0.536	1.838	0.323	2.161
	Tre2	1.365	0.629	1.994	0.381	2.375
S1	Tre0	0.858	0.335	1.193	0.214	1.407
	Tre1	1.035	0.428	1.463	0.252	1.715
	Tre2	1.169	0.537	1.709	0.317	2.023
LCD at 5%		0.135	0.032	0.254	0.034	0.354

salinity-stressed conditions as it caused significant increases in different studied growth parameters.

Changes in photosynthetic pigments

Table 3 shows that chlorophyll (Chl. a, b), total chlorophyll, carotenoids, and total pigment contents of wheat leaves were significantly decreased by irrigation with diluted seawater (S1) (6.25 dS/m) as compared with control plants (S0). The percentages of decreases were 23.94%, 27.96%, 25.11%, 20.45%, and 24.43% in chlorophyll a, chlorophyll b, Chlorophyll a + b, carotenoids, and total pigments, respectively. Different concentrations of trehalose foliar treatment to wheat plant significantly increased photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls, carotenoids, and total pigments) as compared to that of untreated plants in normally irrigated and salinity-stressed plants. Data clearly show the gradual increases of photosynthetic pigments with increasing trehalose concentrations.

Changes in hydrogen peroxide

The level of hydrogen peroxide (H_2O_2) upon salinity stress are presented in (Table 4), H_2O_2 level sharply increased in wheat leaves. The H_2O_2 level increased by 79.39% in the S1 salinity stress plants compared with the control plants (S0). Trehalose foliar treatment markedly decreased the level of H_2O_2 in the normally irrigated plants and salinity-stressed plants compared with their corresponding controls.

Table 4 Effect of trehalose at 0 mM (Tre 0), 10 mM (Tre 1), and 50 mM (Tre 2) on H_2O_2, MDA contents (nmol/g FW), and LOX (U/min/g FW) of wheat plants irrigated with tap water (S0) or diluted sea water at 6.25 dS/m (S1). Results are means of two successive seasons

Salinity	Trehalose conc (mM)	H_2O_2 (nmol/g FW)	MDA	LOX (U/min/g FW)
S0	Tre0	21.85	32.52	12.52
	Tre1	16.52	29.42	11.54
	Tre2	15.52	25.65	10.98
S1	Tre0	41.84	45.87	21.87
	Tre1	32.42	38.65	16.75
	Tre2	26.85	34.54	13.84
LCD at 5%		6.153	6.082	3.254

Changes in lipid peroxidation

The effect of trehalose foliar treatment on wheat plants grown under salinity stress is presented in (Table 4). Salinity stress induced oxidative damage to the cell membrane, assessed by increase in lipid peroxidation in the tested wheat plant (Table 4) as compared with unstressed control plants. Meanwhile, gradual decreases in lipid peroxidation in wheat leaves by trehalose foliar treatment with different concentrations (Tre1 and Tre2) as compared with their corresponding controls in normal and stressed plants.

Changes in lipoxygenase enzyme activity

Lipoxygenase activity in wheat leaves was increased by 75.68% under salinity stress (S1) compared to non-stressed control plants (S0) Table 4. In non-stressed plants, trehalose treatment had no significant effect on LOX activity. Regarding trehalose treatment combined with salinity treatment (Table 4), LOX enzyme activity decreased gradually and significantly as compared with their corresponding control.

Changes in carbohydrate constituents

Different carbohydrate constituents of wheat leaves as glucose, sucrose, trehalose, TSS, and starch contents treated with trehalose with different concentrations Tre 0, Tre1, and Tre2 grown under normal and salinity stress are presented in Table 5. Data clearly show that irrigation of wheat plant with diluted seawater (6.25 d/m) increased significantly the glucose, sucrose, trehalose, total soluble sugars, and starch contents of leaves as compared with those irrigated with tap water (control plants). More accumulation of the tested organic solutes of leaves of the trehalose-treated plant in both normally irrigated and salinity-stressed wheat plants.

Table 5 Effect of trehalose at 0 mM (Tre 0), 10 mM (Tre 1), and 50 mM (Tre 2) on carbohydrate constituents of wheat plants irrigated with tap water (S0) or diluted sea water at 6.25 dS/m (S1). Results are means of two successive seasons

Salinity	Trehalose conc (mM)	Glucose (mg/g FW)	Sucrose	Trehalose (μmole/g FW)	TSS (mg/g FW)	Starch
S0	Tre0	1.98	0.89	212.35	3.24	42.52
	Tre1	2.14	1.68	232.52	4.12	51.52
	Tre2	2.65	1.95	275.23	4.68	59.36
S1	Tre0	2.23	1.24	334.52	4.25	53.65
	Tre1	2.58	1.89	368.25	5.12	61.52
	Tre2	2.95	2.24	425.35	6.24	73.52
LCD at 5%		0.153	0.082	25.254	0.024	78.354

Changes in free amino acids and proline contents

Data presented in Table 6 clearly show the effect of treatments of wheat plants with different concentrations of trehalose under normal and salinity stress. Irrigation of wheat plants with diluted seawater caused significant increases in free amino acids and proline contents of wheat leaves as compared with those plants irrigated with tap water (control). Meanwhile, foliar treatment of different concentrations of trehalose 10 mM and 50 mM (Tre1 or Tre2) decreased significantly and gradually the free amino acids and proline contents.

Changes in phenolic contents

Data presented in Table 6 showed the effect of different concentrations of trehalose treatment on the wheat plant under salinity stress. Salinity stress (S1) caused significant increases in phenolic contents relative to the control plant. Trehalose foliar treatment with different concentrations (Tre1 and Tre2) in normal irrigation caused more significant increases in phenolic content as compared with the control plant. 0.5 mM trehalose was more effective than 0.1 mM trehalose in improving phenolic contents.

Table 6 Effect of trehalose at 0 mM (Tre 0), 10 mM (Tre 1), and 50 mM (Tre 2) on proline, free amino acids, and phenolic contents (mg/g DW) of wheat plants irrigated with tap water (S0) or diluted sea water at 6.25 dS/m (S1). Results are means of two successive seasons

Salinity (dS/m)	Trehalose conc (mM)	Free amino acids	Proline	Phenolic content
S0	Tre0	260.89	51.35	352.52
	Tre1	238.82	42.35	392.52
	Tre2	213.56	31.52	435.53
S1	Tre0	358.73	65.73	424.52
	Tre1	324.39	56.58	528.65
	Tre2	282.24	52.35	575.35
LCD at 5%		12.082	3.153	23.254

Discussion

Osmotic and oxidative stresses resulting from salinity is the first and second phase of toxicity respectively. The plant responds to each of them at different times. Osmoprotectant compounds as proline, glycine betaine, sorbitol, ectoine, and trehalose are produced and accumulate in plant cells under salinity stress to alleviate hyperosmotic stress (Chen and Jiang 2010). Trehalose, has an important physiological role in improving plant tolerance against abiotic stresses in different plants. Trehalose as an osmoprotectant maintains cellular osmotic balance and protects biological structures from damage at desiccation (Garg et al. 2002).

Our results of reduced salinity stress effect on growth parameters of wheat plant are in harmony with Bakhoum and Sadak (2016), Elewa et al. (2017), and Dawood et al. (2019); they stated that growth parameters of sunflower, quinoa, and sunflower plants decreased with salinity and drought stress and attributed these reductions to the metabolic disorders induced by stress and generation of excess ROS. Also, the decrease in shoot height in response to salinity might be due to the decrease in cell elongation, cell turgor, cell volume, and eventually cell growth (Banon et al. 2006). In addition, these reductions in growth parameters might be referred to the effect of high osmotic stress and ion toxicity (Hasanuzzaman et al. 2013) or due to altered cell wall structure induced by stress (Sweet et al. 1990). With regard to trehalose effect, Tre application could alleviate the adverse effects of salinity stress on the wheat plant by improving their growth and physiological attributes. However, water content or growth reduction was restored by exogenous Tre supplementation under salinity stress as evidenced by improved shoot RWC, fresh weight, and dry weight of the plant. Similar findings were documented previously by Tre addition with different plants (Theerakulpisut and Gunnula 2013; Alam et al. 2014; and Sadak 2016b). Trehalose reduced inhibitory effects of salinity stress on growth might be through improving water status of plant tissues, since relative water content of shoot increase (Table 2). Zeid (2009) indicated that trehalose treatment on maize plants improves water retention and plant tolerance through osmoregulation and stomatal closing at stress.

Chlorophyll (Chl. a, b, and a + b), carotenoids, and total pigment contents of wheat leaves were significantly decreased by salinity stress (Table 3). The reduced effect of salinity on photosynthesis was attributed by oxidation of pigments which damages photosynthetic pigments and impaired pigment biosynthesis (Rady et al. 2015). These reduced effects of salinity might be due to the synthesis of proteolytic enzymes as chlorophyllase that is responsible for the degradation of chlorophyll and/or photosynthetic apparatus damaging (Sadak and Ahmed 2016). The

reduction in growth criteria as a result of reducing the anabolic processes by the influence of salinity stress could be attributed to the limiting effect of salinity-induced stress on the chlorophyll content and photosynthetic activity. Additionally, Santos (2004) stated that these reductions in stressed wheat leaves were mainly due to the decrease of ALA (5-aminolinolic acid) synthesis. This acid is a precursor of protochlorophyllide, which converts to chlorophyll when exposed to light. On the other hand, trehalose treatment exhibited a positive effect on the photosynthetic pigments (Chlorophyll (Chl. a, b, a + b), carotenoids, and total pigment content in salinity-stressed and unstressed leaves. Trehalose may preserve the stability of the chloroplast envelope and maintain the osmotic potential of the chloroplast. Interaction of salinity and Tre improved photosynthetic pigment levels in the studied plant (Table 3) which is corroborated with the results of previous studies with *Lemna gibba* L. under cadmium stress (Duman et al. 2010). Also, Theerakulpisut and Gunnula (2013) and Abdelgawad et al. (2014) stated that using trehalose treatment enhanced photosynthetic pigments of rice plant under salinity and drought stresses respectively.

Generally, salinity stress causes oxidative stress via increasing production of ROS. A potential biomarker of salt-induced oxidative damage to the cell membrane is the increased ROS level which provokes lipid peroxidation thus leading to electrolyte leakage, permeability of membrane loss, and malfunctioning of membrane proteins and ion channels (Nedjimi 2014). As shown in Table 4, wheat plants responded to irrigation of dilute seawater stress by inducing marked increases in H_2O_2 concomitantly with increased MDA contents, indicating an evident oxidative burst in the leaf tissues. Similar results were obtained by Mishra et al. (2013), Mostafa et al. (2015), and Orabi and Sadak (2015) on different plant species. Salinity stress caused modification in lipid matrix of plasma membrane and changes in physical organization of membrane. In addition, it caused marked increases in MDA and H_2O_2 levels in different plant species including rapeseed and mustard may be due to inadequate induction of antioxidant system as mentioned by Hossain et al. (2013). In addition, increased LOX activity might have contributed to the lipid peroxidation of membrane lipids and thereby significantly participate in the oxidative damage in salt-stressed plant. Increased LOX activity is responsible for the oxidation of polyunsaturated fatty acids and thus enhances lipid peroxidation under stress conditions (Sánchez-Rodríguez et al. 2012 and Alam et al. 2014). However, trehalose foliar treatment prior to salinity stress resulted in lower H_2O_2 production and reduced MDA content. These results are in agreement with Nounjan and Theerakulpisut (2012); Ma et al. (2013); Liu et al. (2013); and Sadak et al. (2019); they confirmed that exogenous application of Tre was effective in reducing oxidative stress of different plants in different abiotic stresses. These reductions might be achieved through trehalose-mediated direct ROS scavenging, membrane stabilizing, or modulating the antioxidative mechanism involved in eliminating ROS.

Different carbohydrate constituents of wheat leaves were treated with trehalose with different concentrations and grown under normal or salinity stress (Table 5). Data show accumulation of different carbohydrates constituents as glucose sucrose, trehalose, TSS, and starch contents. Although accumulation of high soluble sugar levels has also been demonstrated in shoots of different plant species under salinity stress conditions (Rady et al. 2015; Bakhoum and Sadak 2016; and Tawfik et al. 2017). Increased levels of soluble sugars in salinity-stressed plants may help in turgor upkeeping and cellular membrane stabilization (Hosseini et al. 2014). These increases of different soluble sugars are the response of plants to stress has been widely reported despite the decrease in net CO_2 assimilation rate (Murakeozy et al. 2003). In addition, soluble sugars may act as ROS scavengers so improve membrane stabilization (Hosseini et al. 2014). During plant development, trehalose could play an important role in regulating carbohydrate allocation, as Tre acting as soluble sugars in wheat leaves and its function is an osmoprotectant under salinity-induced stresses. The present study shows that in wheat leaves, trehalose accumulation is concomitant with increasing sucrose level under normal and salinity stress (Table 5). Garg et al. (2007), Ma et al. (2013), and Sadak (2016a) confirmed our obtained results. With regard to the indirect effect of Tre on carbohydrate metabolism, this effect may be via conversion and utilization of other sugars and interfering with photosynthetic capacity (Ranwala and Miller 2009). The improving effect of trehalose may be due to its physicochemical properties that stabilize dehydrated enzymes, proteins, and lipid membranes, as well as protect structures from damage during desiccation (Fernandez et al. 2010). Data in Table 5 show that starch content increased in wheat plant irrigated with tap water or diluted sea water-stressed plant compared with untreated plants. Bae et al. (2005) reported that in *Arabidopsis thaliana* seedlings, starch was threefold greater in trehalose-treated plants than in the control. Accumulation of starch in wheat leaves may be a protective mechanism as it is an important component of plant cells and its increases in leaves as a reserve form of carbon (Mishra and Prakash 2010). The increases in starch by trehalose treatment might be due to increased activity of ADP-glucose pyrophosphorylase (AGPase), a major enzyme-controlling starch synthesis in Arabidopsis (Bae et al. 2005).

Accumulation of compatible osmolytes in high concentrations causes osmotic adjustment in plants under

drought stress (Jagesh et al. 2010). Proline metabolic accumulation of different plants under salinity stress usually correlates with enhanced plant tolerance to different abiotic stress. In the present work, salinity stress caused significant increases in free amino acids and proline contents whereas decreased by trehalose application (Table 6). These increased levels of free amino acids and proline were obtained in different plant under abiotic stress (Mostafa et al. 2015, Sadak 2016a, 2016b, and Tawfik et al. 2017). They concluded that these compounds have an important role in enhancing tolerance of plant cells to various abiotic stress via increasing osmotic pressure in the cytoplasm and increasing relative water contents essential for plant growth and different metabolic processes. Proline has vital roles in osmotic adjustment (Hasegawa et al. 2000), stabilization, and protection of enzymes, proteins, and membranes (Ashraf and Foolad 2007) from damaging effects of osmotic stresses, also reduction of oxidation of lipid membranes (Demiral and Türkan 2004). Trehalose addition with salinity stress reduced free amino acids and proline levels in wheat plant (Table 6). These results are corroborated to previous studies (Alam et al. 2014 and Abdallah et al. 2016). Prevention of extra proline biosynthesis due to exogenous trehalose addition under salinity stress suggests that trehalose prevented wheat seedlings from adverse effects of salinity stress by other means so that the studied wheat seedlings did not need to increase proline levels further.

Table 6 shows that salinity stress and/or trehalose foliar treatments enhanced phenolic content of wheat leaves. This increase in phenol contents in different plants under osmotic stress have been reported earlier (Rady et al. 2011 and Dawood and Sadak 2014). These increases may be due to total phenols role that play a significant mechanism in the regulation of plant metabolic processes and consequently overall plant growth (Abd Allah et al. 2015). Moreover, phenols act as a substrate for many antioxidant enzymes; so, it mitigates salinity stress injuries (Bakry et al. 2012). Another mechanism underlying the antioxidative properties of phenolic compounds is the ability of phenols to decrease membrane fluidity. In addition, salinity induced disturbances in the metabolic process leading to an increase in the synthesis of phenolic compounds. Phenolic compounds play an important role as antioxidants in scavenging free radicals arising from their high reactivity as hydrogen or electron donors to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions (Huang et al. 2005). The beneficial effects of trehalose may result from its signaling function, through the induction of different metabolic pathways and stimulate the production of various substances, preferably operating under stress (Alam et al. 2014).

Conclusion

Finally, we can conclude that trehalose is an osmoprotectant that can enhance salinity stress tolerance of wheat plant through increasing some osmoprotectant compounds such as glucose, trehalose TSS, proline, and free amino acids whereas, decreasing H_2O_2, MDA contents, and LOX enzyme activity.

Abbreviation

LOX: Lipoxygenase enzyme; Tre: Trehalose; TSS: Total soluble sugars

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