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Research Article Physiological Role of Disaccharide Trehalose to Induce Quality and Quantity of *Triticum aestivum* L.

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Abstract

Background and Objective: Saccharides are storage compounds, respiratory substrates and intermediate metabolites in many metabolic processes, hence they play a significant role in plant life. During the winter seasons of 2017/2018 and 2018/2019, a trial was undertaken at the National Research Centre's experimental station in Nubaria, El-Beheira Governorate, Egypt to investigate the effect of trehalose treatments on the quality and quantity of two wheat cultivars grown under sandy soil conditions. **Materials and Methods:** Grains of Sids 12 and Misr 3 cultivars were soaked with trehalose at 5 or 10 mM for 12 hrs and dried at room temperature before sowing. **Results:** According to data, the growth of wheat plants belonging to the Misr 3 cultivar was more adaptable to sandy soil conditions than that of the Sids 12 cultivar. Both levels of trehalose (5 and 10 mM) increased vegetative growth parameters, photosynthetic pigments, grains yield and its components as well as total soluble carbohydrate, total carbohydrate, polysaccharide and proline contents. 10 mM trehalose was a more pronounced treatment than 5 mM trehalose. **Conclusion:** Trehalose treatment at 10 mM was the optimum treatment in improving the quality and quantity of two wheat varieties. The response o Misr 3 cultivar to trehalose treatment at 10 mM was higher than the response of Sids 12 cultivar that reflected on grains yield quality and quantity.

Key words: Wheat, osmoregulator, sandy soil, yield, grains quality, disaccharides, carbohydrate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wheat (Triticum aestivum L.) is one of the main food crops and is cultivated worldwide as a food commodity and strategic commodity. Wheat is the most important source of vegetable protein in the human diet and a good dietary source of natural antioxidants for health promotion¹. In Egypt, the increase in a cultivated area of wheat should be done in the new land due to the limited areas of the Nile Valley and the competition with other crops to overcome the gap between production and consumption. The plant growth under sandy soil conditions is mostly exposed to a combination of environmental stress conditions including low water availability, saline water, saline soil, nutrient deprivation, temperature fluctuations and high irradiances. In this concern, great efforts must be paid to increase plant tolerance to such conditions via selecting tolerant genotypes and/or treating the plants with osmoregulatory as trehalose that play an important role in helping plants to overcome partially the unfavourable conditions and avoid their negative effects on yield quantity and quality.

Saccharides are storage compounds, respiratory substrates and intermediate metabolites in many metabolic processes, hence they play a significant role in plant life²⁻⁵. Additionally, sugars, in millimolar concentrations, manage certain stages of the cell cycle, cell differentiation, cell division, germination, vegetative growth, organ development, flowering, fruit formation and senescence^{6,7}. Sugar-induced signalling interacts with other pathways in plant tissues (such as hormone pathways), resulting in a complex communication and signalling network in plants that precisely regulates plant growth and development^{6,8-10}.

Moreover, soluble sugars are extensively used as osmotic agents to protect plant cells from dehydration and death and to preserve the internal stability of the cells under stress¹¹⁻¹³. Of various sugars associated with inducing stress tolerance, trehalose is a non-reducing disaccharide of glucose, that acts as an effective osmoprotectant or as stress regulating in different plants¹⁴⁻¹⁶. Trehalose is extremely soluble but chemically unreactive due to its non-reducing nature, making it suitable with cellular metabolism even at high doses without disrupting normal metabolism¹⁷⁻¹⁹. Endogenous trehalose production in most plants is insufficient to alleviate stressinduced deleterious effects, so exogenous trehalose elevated the internal level of this osmolyte to reduce the harmful impacts of diverse abiotic stresses^{14,20-24}. Trehalose would be a carbon storage and transport substance that can sustain turgor pressure, preserve macromolecules from the destructive effects of reactive oxygen species (ROS), protect cell membranes from oxidation and stabilize proteins, enzymes and cell structures²⁵⁻²⁷ and protect photosynthetic electron transport chain²⁸. Similarly, trehalose can behave as a signalling molecule, activating enzymatic antioxidants to scavenge ROS and decrease oxidative stress²⁹, as well as acting as an elicitor of genes associated with detoxification and stress response³⁰. The beneficial role of trehalose in alleviating stress is correlated to its ability to promote antioxidant systems, maintain cellular redox balance and enhance photosynthesis^{19,31}. Trehalose treatment (10-30 mM) was found to be very effective in enhancing plant biomass, antioxidative capability, nutrients intake and chlorophyll pigments, compatible solutes (total soluble sugar, trehalose, proline, free amino acids), constituents of carbohydrate content (glucose, sucrose, starch), etc. in different plants e.g., maize¹⁵, radish³² and cowpea³³. In addition, trehalose treatments improved wheat performance by lowering hydrogen peroxide free radicals and increasing antioxidant molecules (phenolics), compatible solutes and membrane integrity³⁴. Furthermore, trehalose is inexpensive, economical and widely available, as well as being readily absorbed up by plants¹⁹.

The goal of this study was to explore how trehalose affected the performance of two wheat cultivars grown under sandy soil conditions.

MATERIALS AND METHODS

Study area: During the winter seasons of 2017/2018 and 2018/2019, a field experiment was conducted at the National Research Centre's experimental station in Nubaria, El-Behera Governorate, Egypt.

Methodology: The experimental design was a split block design with four replicates. Wheat grains were soaked with trehalose at 5 or 10 mM for 12 hrs, subsequently dried at room temperature. Then, wheat grains were sown in the middle of November in both seasons in rows 3.5 m long and the distance between rows was 20 cm apart, plot area was 10.5 m² (3.0 m in width and 3.5 m in length). The soil texture of the experimental site was sandy soil and had the following characteristics: Sand 85.3%, silt 10.7%, clay 4%, pH 7.84, CaCo₃, 1.0%, EC 3.95 dS m⁻¹ and the available total N and K were 8.0, 3.0 and 19.8 ppm, respectively at 30 cm depth according to the method described by Chapman and Pratt³⁵.

According to the Agricultural Research Centre in Giza, Egypt, appropriate agricultural processes for cultivating wheat grain were used. Plant samples were collected 60 days following sowing for measuring various growth characteristics (plant height, number of branches and leaves/plant as well as plant fresh and dry weight). Photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids) in fresh leaf tissues were determined as the procedure described by Moran³⁶.

The following characteristics were recorded on the random selection of plants in each treatment at harvest time: Spike length, spike weight, grains number/plant, 100 grains weight and grains yield/fadan (kg). The yielded grains were cleaned and used for the determination of soluble sugars, total carbohydrates and proline contents. Total carbohydrate and soluble sugar content were determined using the colourimetric method described by DuBois *et al.*³⁷. Polysaccharides were calculated by subtracting soluble sugar from total carbohydrates. Proline was estimated according to Bates *et al.*³⁸.

Statistical analysis: Data were statistically analyzed using the least significant difference at a 5% level of probability according to Snedecor and Cochran³⁹. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \le 0.05$).

RESULTS

Vegetative growth parameters: Table 1 show that wheat plants belonging to Misr 3 cultivars were characterized by significant increases in all vegetative growth parameters (plant height, number of branches and leaves/pant, fresh and dry weight plant) than those of Sids 12 cultivar. Trehalose treatments at 5 and 10 mM significantly increased most of the

investigated parameters relative to control. The enhancement effect of trehalose treatment was increased by increasing its level.

Regarding the interaction between wheat cultivars and trehalose treatments, it was noted that trehalose treatment at 10mM significantly increased all vegetative growth parameters of the two cultivars. Since it caused significant increases in dry weight by 28.04% in Sids 12 and 86.53% in Misr 3 cultivars as compared to corresponding controls. The response Misr 3 cultivar to trehalose treatment at 10 mM was higher than the response of Sids 12 cultivar.

Photosynthetic pigments: Table 2 show that fresh leaf tissues of Misr 3 cultivar were characterized by higher photosynthetic pigments than Sids 12 cultivar. Trehalose treatments at both levels significantly increased total photosynthetic pigments relative to control. The enhancement effect of trehalose treatments on photosynthetic pigments was increased by increasing its level.

Regarding the interaction between wheat cultivars and trehalose treatments, it was noted that trehalose treatment at 10 mM increased total photosynthetic pigments by 2.91 and 15.41% in fresh leaf tissues of Sids 12 and Misr 3 cultivars respectively as compared to corresponding controls. The response Misr 3 cultivar to trehalose treatment at 10 mM was higher than the response of Sids 12 cultivar.

Grains yield and yield attributes: Table 3 show that wheat plants belonging to Misr 3 cultivars were characterized by significant increases in grains yield and its components as compared with those of Sids 12 cultivar. Trehalose treatments at two levels significantly increased grains yield and its

Table 1: Impact of disaccharide trehalose on ve	egetative growth parameters of wheat	t plants grown under sandy soil conditions

Cultivars	Trehalose	Plant height (cm)	Number of leaves	Number of branches	Plant fresh weight (g)	Plant dry weight (g)
Effect of cultivars						
Sids 12 cultivar		71.66 ^b	6.77 ^b	1.44ª	6.24 ^b	2.15 ^b
Misr 3 cultivar		96.77ª	9.44ª	1.88ª	12.23ª	3.69ª
Effect of trehalose	2					
Control		77.16 ^b	6.66°	1.33 ^b	7.96 ^b	2.08 ^b
Trehalose (5 mM)		85.50ª	8.00 ^b	1.83ª	8.86 ^b	3.12ª
Trehalose (10 mM)		90.00ª	9.66ª	1.82ª	10.92ª	3.55ª
Effect of cultivars	×trehalose					
Sids 12	Control	61.33 ^d	5.00 ^d	1.00 ^b	5.14 ^d	1.71 ^e
	Trehalose (5 mM)	75.00 ^c	7.00 ^c	1.65ªb	6.32 ^{cd}	2.21 ^d
	Trehalose (10 mM)	78.66 ^c	8.33 ^{bc}	1.66 ^{ab}	7.35°	2.53°
Misr 3	Control	93.00 ^b	8.30 ^{bc}	1.67ª	10.79 ^b	2.45°
	Trehalose (5 mM)	96.00 ^b	9.00 ^b	2.00ª	11.40 ^b	4.04 ^b
	Trehalose (10 mM)	101.33ª	11.00ª	2.00ª	14.49ª	4.57ª

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \le 0.05$)

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Cultivars	Trehalose	Chlorophyll A	Chlorophyll B	Carotenoid	Total photosynthetic pigment
Effect of cultivars					
Sids 12 cultivar		1.92ª	0.55ª	0.44ª	2.62ª
Misr 3 cultivar		2.04ª	0.55ª	0.40ª	2.77ª
Effect of trehalose					
Control		1.91 ^b	0.52°	0.41 ^b	2.60 ^c
Trehalose (5 mM)		1.97 ^b	0.54 ^b	0.44ª	2.69 ^b
Trehalose (10 mM)		2.06ª	0.60ª	0.43ª	2.84ª
Effect of cultivars×tre	halose				
Sids 12	Control	1.85 ^b	0.59 ^b	0.44 ^{ab}	2.64 ^b
	Trehalose (5 mM)	2.06 ^{ab}	0.56 ^{ab}	0.47ª	2.80 ^{ab}
	Trehalose (10 mM)	1.86 ^b	0.58ª	0.42 ^{ab}	2.61 ^b
Misr 3	Control	1.97 ^ь	0.51 ^b	0.39 ^b	2.67 ^b
	Trehalose (5 mM)	1.88 ^b	0.51 ^b	0.40 ^b	2.57 ^b
	Trehalose (10 mM)	2.25ª	0.62ª	0.43 ^{ab}	3.08ª

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \le 0.05$)

Table 3: Impact of disaccharide trehalose on grains yield and its components of wheat plant grown under sandy soil conditions

Cultivars	Trehalose	Spike length (cm)	Spike weight (g)	Grains number/plant	100 grains weight (g)	Grains yield (kg/feddan)
Effect of cultivars						
Sids 12 cultivar		9.54 ^b	2.75 ^b	53.88 ^b	4.64 ^b	1247.9 ^b
Misr 3 cultivar		11.23ª	4.57ª	65.00ª	5.29ª	1421.5ª
Effect of trehalose						
Control		8.64 ^c	2.16 ^c	42.50 ^c	4.58°	1221.2 ^c
Trehalose (5 mM)		10.65 ^b	4.04 ^b	62.83 ^b	5.03 ^b	1356.2 ^b
Trehalose (10 mM)		11.88ª	4.78ª	73.00 ^a	5.29ª	1426.7ª
Effect of cultivars ×	trehalose					
Sids 12	Control	7.74 ^e	1.89ª	39.66 ^d	4.32 ^e	1176.4 ^f
	Trehalose (5 mM)	10.06 ^{cd}	2.94 ^{cd}	58.33°	4.66 ^d	1245.1 ^e
	Trehalose (10 mM)	10.83 ^{bc}	3.40 ^c	63.66 ^{bc}	4.95°	1322.3°
Misr 3	Control	9.53 ^d	2.43 ^b	45.33 ^d	4.83 ^c	1265.9 ^d
	Trehalose (5 mM)	11.23 ^b	5.13 ^b	67.33 ^b	5.40 ^b	1467.4 ^b
	Trehalose (10 mM)	12.93ª	6.16ª	82.33ª	5.63ª	1531.2ª

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \le 0.05$)

Table 4: Impact of disaccharide trehalose on some chemical composition of the yielded grains of wheat plant grown under sandy soil conditions							
Cultivars	Trehalose	Total soluble sugars (%)	Total carbohydrates (%)	Total polysaccharides (%)	Proline (mg/g)		
Effect of cultivars							
Sids 12 cultivar		3.90 ^b	65.48 ^b	61.57 ^b	1.13ª		
Misr 3 cultivar		4.18ª	70.20ª	66.01ª	1.10ª		
Effect of trehalose							
Control		3.16 ^c	66.95 ^b	63.78ª	1.23ª		
Trehalose (5 mM)		4.18 ^b	67.88ª	63.69ª	1.12 ^b		
Trehalose (10 mM)		4.79ª	68.69ª	63.90ª	0.99°		
Effect of cultivars×treh	alose						
Sids 12	Control	3.12 ^d	63.79 ^d	60.66 ^d	1.27ª		
	Trehalose (5 mM)	4.04 ^c	65.39°	61.34 ^d	1.16 ^{ab}		
	Trehalose (10 mM)	4.53 ^b	67.25 ^b	62.71°	0.96 ^b		
Misr 3	Control	3.20 ^d	70.11ª	66.90ª	1.18 ^{ab}		
	Trehalose (5 mM)	4.32 ^{bc}	70.36ª	66.04 ^{ab}	1.08 ^{ab}		
	Trehalose (10 mM)	5.03ª	70.13ª	65.09 ^b	1.03 ^{ab}		

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \le 0.05$)

attributes and the increases concomitant with increasing of trehalose levels.

Regarding the interaction between wheat cultivars and trehalose treatments, it was obvious that two applied treatments significantly increased spike length, spike weight, grains number/plant,100 grains weight and grains yield (kg/feddan) of both cultivars under investigation. Trehalose treatment at 5 mM significantly increased the grains yield of Sids 12 cultivar by 5.84% and those of Misr 3 cultivars by 15.91% relative to corresponding controls. Likewise, the grains yield of Sids 12 and Misr 3 cultivars were significantly increased by 10 mM trehalose by 12.38 and 20.94%,

respectively relative to corresponding controls. The response Misr 3 cultivar to trehalose treatment at 10 mM was higher than the response of Sids 12 cultivar.

Chemical composition of the yielded grains: Table 4 show that Misr 3 cultivar was characterized by a significant increase in total soluble carbohydrate, total carbohydrate and polysaccharide contents relative to those of the Sids 12 cultivar. Proline content shows non-significant differences between the two cultivars. Both levels of trehalose significantly increased total soluble carbohydrate, total carbohydrate and polysaccharide contents. These increases are accompanied by increasing trehalose levels.

Regarding the interaction between wheat cultivars and trehalose treatments, it was obvious that 5 mM and 10 mM trehalose increased total soluble carbohydrate, total carbohydrate, polysaccharide and proline contents of both cultivars. 10 mM trehalose treatment was more pronounced than 5 mM trehalose.

DISCUSSION

The differences in two wheat cultivars (Table 1-4) may be explained according to El-Sarag and Ismaeil⁴⁰ who mentioned that wheat cultivars were significantly different in plant height, spike length, number of spikes, number of grains per spike and grain weight per spike. In addition, Abd El-Razek and El-Sheshtawy.⁴¹ established that grain, straw and biological yields and their components were significantly differed owing to cultivar differences. Further, there were significant differences in all the growth characters of three wheat cultivars as reported by Abd-Rabboh and Koriem⁴². Dawood et al.43 stated that three wheat cultivars differed in their growth, yield and yield components. According to Dawood et al.44, wheat plants belonging to Misr 3 cultivar and grown under sandy soil conditions was more adaptable than that of Sids 12 cultivar and showed significant differences in most of the growth and yield characters.

The increases in different vegetative growth parameters (Table 1) may be attributed to the effect of compatible osmolytes that act as a safeguard of the structure of many biomolecules and membranes and operate as free-radical scavengers to protect DNA from the destructive effects of Reactive Oxygen Species (ROS)⁴⁵. In addition, sugars not only participate in the metabolism of carbon and energy in cells but serve as an important function in plant development and stress tolerance as a signal molecule⁴⁶. Likewise, trehalose-induced plant growth improvement via activation of oxidative

defence system to suppress the levels of reactive oxidants, maintenance of turgor potential and nutritional balance under stress conditions^{23,47}.

The increases in photosynthetic pigments (Table 2) may be attributed to the enhancement of the photosynthetic performance achieved by the sugar signalling mechanism^{48,49}. In addition, trehalose treatment played a significant role in preserving the macromolecules (membrane lipids and proteins), regulation of carbon metabolism and stabilization of biological structures thereby helping the plant to sustain photosynthesis under normal and/or stressed conditions^{23,50}.

Regarding the stimulatory effect of trehalose on grains yield as shown in Table 3, Yuan *et al.*⁵¹ found that osmoregulatory reduced fruit abscission due to its role in lowering ethylene production, resulting in an increase in the number of fruits and seeds per silique and consequently increased seed yield per plant. Additionally, osmoregulators may boost photosynthetic pigments, resulting in higher dry matter accumulation and seed yield⁵². These results were in good harmony with that obtained previously^{33,34,53}.

The enhancement effect of trehalose on soluble sugar and carbohydrate content of the yielded grains (Table 4) may be attributed to the role of trehalose in regulating sugar accumulation, distribution and metabolism by inducing the up-regulation of sugar transporter genes^{13,54}. Soluble sugars may serve as ROS scavengers and assist in turgor maintenance and stabilization of cellular membranes^{34,55}. In addition, exogenous application of trehalose induced accumulation of starch in Arabidopsis, by increasing the activity of ADP-glucose pyrophosphorylase, a major enzyme controlling starch synthesis^{30,56} and also by inhibition of starch degradation⁵⁷.

Regarding the increment proline level due to trehalose treatment, Hasegawa *et al.*⁵⁸ stated that accumulation of proline content plays an important role in osmotic adjustment, stability and protection of proteins, enzymes and membranes⁴⁵. Moreover, proline can preserve membrane structure by decreasing lipid oxidation through scavenging free radicals and maintaining cellular redox potential⁴⁵.

Regarding the trehalose effect, Zeid⁴⁹ stated that soaking maize grains with10 mM trehalose alleviated the undesirable effects of salinity stress on the metabolic activity of maize seedlings via increasing photosynthetic pigments, nucleic acids content and organic solutes e.g., sugars, soluble proteins and proline content. Moreover, Ibrahim and Abdellatif²⁸ stated that trehalose treatment (10 mM) enhanced water stress resistance of wheat plants through increasing total soluble sugar, proline and free amino acids.

CONCLUSION

The growth of wheat plants belonging to the Misr 3 cultivar was more adaptable to sandy soil conditions than that of the Sids 12 cultivar. Both levels of trehalose (5 and 10 mM) increased vegetative growth parameters, photosynthetic pigments, grains yield and its components as well as total soluble carbohydrate, total carbohydrate, polysaccharide and proline contents. 10 mM trehalose was a more pronounced treatment than 5 mM trehalose.

SIGNIFICANCE STATEMENT

This study discovers that trehalose treatment at 10 mM was the optimum treatment in improving the quality and quantity of two wheat varieties. The response Misr 3 cultivar to trehalose treatment at 10 mM was higher than the response of Sids 12 cultivar that reflected on grains yield quality and quantity.

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