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Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress

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Abstract In the greenhouse experiment, wheat plants (Triticum aestivum L. cv. Giza 168) were treated with 10 mM of maltose and trehalose as foliar spray using Tween 20 as wetting agent at 15, 30 and 45 days post sowing with two times of irrigation at 10 and 20 days intervals. Two samples were taken after 45 and 120 days from planting. At the first sample date, plant height, shoot fresh and dry weights and leaf area were recorded. At harvesting time (the second sample) no. of spikes/plant, no. of spikelets/plant and weight of 1000 grains were taken. Chemical analyses were conducted in leaves at the first sample date for determination of phenolic compounds, flavonoids, amino acids, reducing sugars, total soluble sugars, protein, proline, PAL, POD, ascorbate peroxidase, catalase, PPO and MDA. The obtained results indicated that maltose and trehalose had significant and positive effect on most growth parameters. Opposite trend was found in plant height, no. of spike/plant and weight of 1000 grains by drought treatment. Maltose and trehalose treatments enhanced in the most biochemical components whereas they decreased PAL and catalase activity. Variable trends in amino acids and ascorbate peroxidase were observed by drought. However, the drought has more stimulative effect in most cases than the first time period of irrigation. The results concluded that foliar applications with maltose or trehalose induced water stress tolerance in wheat plants. Maltose treatment gave the best results in most morphological parameters, grains yield and biochemical components than trehalose treatment.

Introduction

During the last 100 years, the misused and uncontrolled use of the world's nature resources has greatly destroyed its vegetation and also led to accumulation of lots of industrial wastes, all of that upturns and changes the ecosystem balance and produces many environmental and climate difficulties as drought

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and soil salinization. Drought certainly is one of the most effective factors of the environmental problems that has a great bad effect on the agricultural production, and greatly affecting crop growth, yield quantity, the variety and the quality of the essential physiological and biochemical processes in plants (Hasanuzzaman et al., 2012). In arid and semi-arid regions, that have Mediterranean climate, wheat crop is usually facing and suffering from drought stress (Mohammadi et al., 2011). Modern evidence has proved that in plants, sugars such as glucose, sucrose, and fructose are not only playing the rule of plant growth but also it affects sugar sensing system that initiates changes in gene expression and then the plant growth (Koch 1996). For example, sugar depletion, upregulates genes for photosynthesis, carbon remobilization and export, and leads to increasing vegetative and shoot growth (Percival and Fraser, 2005). Maltose is the most common sugar in barley (Lindqvist and Asp, 2002). Trehalose is a non-reducing disaccharide of glucose that stabilizes biological structures and the macromolecules as protein and membrane lipids during water deficit (Luo et al., 2010). The increase in osmoregulators Trehalose production in genetically engineered model plants is proved to be better stress tolerance (Wang et al., 2005). Externally applied maltose and trehalose are rapidly accumulated and transported by leaves and root tissues and play important roles as osmoprotectants (Smith and Smith, 1973; Luo et al., 2010). Exposing plants to drought has an effect on the plant-water relations, and decreases the water content in the leaves and whole the plant leading to osmotic stress (Alam et al., 2013). It is usual that plants suffer from the environmental stress. Decrease in the water content conditions causes a reduction in the plant photosynthetic efficiency and stomatal conductance which inhibits Rubisco activity and breaks down energy balance and breaks down the distribution during photosynthesis (Demirevska et al., 2010). As a result, the reactive oxygen species (ROS), superoxide, O_2^-, hydrogen peroxide, and hydroxyl radical, highly accumulate in the plant (Hasanuzzaman et al., 2014). The ROS are major toxic radicals which may destroy biomolecules, including lipids, proteins and DNA (Vranova et al., 2002). In the presence of drought stress, plants improve and speed specific mechanisms to grasp, understand and fast respond to various environmental cues (Demirevska et al., 2010). Enhancing enzymatic and non-enzymatic protection system is a remarkable and significant strategy to proficiently removing ROS as phenolic compounds, flavonoids, amino acids, protein, proline, MDA concentrations and peroxidase (POX), polyphenyl oxidase (PPO), catalase (CAT) and ascorbate peroxidase (APX) activities (Gupta et al., 2009; Hasanuzzaman et al., 2012). Organic compatible solutes such as maltose and trehalose have a great effect on improving drought tolerance in wheat plants (Farooq et al., 2010; Nawaz and Ashraf, 2010).

Material and Methods

Pot experiments were carried out under field conditions at Agricultural Botany Department, Faculty of Agriculture, Ain Shams University. Grains of wheat (*Triticum aestivum* L.) cultivar Giza 168 were kindly obtained from the Crop Research Institute, ARC, Ministry of Agriculture, Egypt. Fifteen grains were directly sown on 15 November 2014, in plastic pots (40 cm in diameter) filled with clay/sand (2:1 v/v) soil. Germinated seeds were thinned to five uniform seedlings per pot after two weeks of sowing. Two interval periods of irrigation; 10 and 20 days, and 10 mM of maltose or trehalose were applied as foliar application, compared with control (untreated plants). The plants were sprayed 15, 30 and 45 days after sowing. Fertilization was performed according to the recommendation of Ministry of Agriculture, as follows: calcium super phosphate (13.5% P_2O_5) was added before sowing; ammonium nitrate (33.5% N) and potassium sulfate (48% K_2O) were added in two equal doses at first and third irrigation, at rates of 2 g/pot and 0.5 g/pot, respectively. Nitrogen, phosphorus and potassium fertilizes were added as per recommendation of Ministry of Agriculture.

Four replicates for each treatment were grown in a complete randomized design. Three plants were randomly taken from each treatment for the biochemical analysis after the third spray. Three plants were randomly selected after 45 days from planting for growth measurements, i.e. plant height (cm), shoot fresh weight (g), shoot dry weight (g), flag leaf area (cm^2). At grain maturation stage, number of spikes/plant, number of spikelets/plant and weight of 1000 grains (g) were measured.

Biochemical analyses

Free amino acids and total soluble sugars were extracted from fresh leaves according to Ackerson (1981) by using 80% ethanol at 70 °C. Free amino acids were determined colorimetrically by using ninhydrin solution according to Jayeraman (1985) using glycine as a standard. Reducing sugars were determined colorimetrically by using 3,5-dinitrosalicylic acid solution according to Miller (1959) using glucose as a standard. Total soluble sugars were determined in the previous extract by adding 5 ml HCl (2N) to 15 ml of sugar extract and heated in a water bath at 60 °C for 30 min. The solution was cooled, neutralized and made the total volume 50 ml with distilled water. Total soluble sugars were determined colorimetrically by using 3,5 dinitrosalicylic acid solution according to Miller (1959).

Proline concentration was determined using ninhydrin colorimetric methods of Bates et al. (1973). Phenolic compounds and total flavonoids were extracted by macerated 0.5 g of fresh leaves in 10 ml 80% ethanol for at least 24 h at 5 °C and repeated three times. The collected extracts were completed to 50 ml using 80% ethanol. Phenolic compounds were determined by the method of Folin-Ciocalteu as described by Shahidi and Naczk (1995) using gallic acid as a standard. Total flavonoids concentration was determined by the aluminum chloride colorimetric assay according to Marinova et al. (2005) using quercetin as a standard.

Soluble protein concentration was estimated to calculate specific activity of enzymes. Protein concentrations were quantified in the crude extract by the method of Bradford (1976) using bovine serum albumin as a standard. All determinations were expressed as mg/100 g fresh weight (f.wt.).

The level of lipid peroxidation was measured by determination of malondialdehyde (MDA) in plant tissues as described by Heath and Packer (1968). The MDA concentration was calculated using an extinction coefficient of 155 $mM^{-1}\,cm^{-1}$. MDA concentration was expressed as μmol MDA/g f.wt.

Enzymes assay

Leaves were homogenized with potassium phosphate buffer (100 mM, pH = 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (PVP) (W/V) at 4 °C. The extraction ratio was 4 ml buffer for each one gram of plant materials. Homogenate was centrifuged at 15,000*g* for 15 min at 4 °C. Supernatant was considered as enzyme crude extract and used to measure the activities of guaiacol peroxidase (POD), catalase, polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and ascorbate peroxidase using spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). Peroxidase, POD (E.C 1.11.1.7) activity in enzyme crude extract was determined as described by Hammer Schmidt et al. (1982). The POD activity was calculated by measuring the absorbance changes at 470 nm per min Unit of enzyme (IU) equal 0.01 $\Delta OD\ min^{-1}$. Catalase (CAT) (EC 1.11.1.6) activity was determined according to Cakmak et al. (1993). The activity was calculated from extinction coefficient ($\varepsilon = 40\ mM^{-1}\ cm^{-1}$) for H_2O_2. One unit of enzyme activity was defined as the decomposition of 1 µmol of H_2O_2 per minute at 240 nm. Polyphenol oxidase (PPO) (EC 1.14.18.1) activity was measured according to Benjamin and Montgomery (1973). One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per min at 420 nm. Phenylalanine ammonia lyase (PAL) (EC 4.3.1.5) activity was quantified by the method of Beaudoin-Eagan and Thorp (1985). One unit of enzyme activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 per hour at 290 nm. Ascorbate peroxidase (APX) (E.C 1.11.1.11) was measured according to Nakano and Asada (1981). One unit of enzyme activity was defined as the amount of enzyme required for oxidation of 1 µmol of ascorbate per minute and caused a decrease in absorbance at 290 nm. The enzymes activities were expressed as unit/mg protein.

Data of all experiments were analyzed by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat. Significance between means was tested by "F" test and the value of LSD ($p = 0.05$) was calculated (Snedecor and Cochran, 1982).

Results and discussion

Growth parameters

Data in Tables 1 and 2 indicate that maltose and trehalose treatments caused significant increase in all plant growth parameters except trehalose treatment in no. of spikes/plant and no. of spikelets/plant when compared with control.

Shoot fresh weight, plant height and flag leaf area have more values in plants treated with maltose (10 mM) than those treated with trehalose (9.43 and 6.89 g, 74.5 and 62.33 cm, 8.51 and 8.02 cm^2) respectively (Table 1), whereas plant dry weight was higher in plants treated with trehalose (1.60 and 1.48 g). According to the time of irrigation no significant differences were noticed in all plant yield parameters in Table 1 except plant height. The highest value of plant height (67.22 cm) was recorded by the 10 days intervals between irrigation.

Generally, all sugar treatments had promotion effect on growth parameters in Table 2. Maltose treatment gave the highest significant values of no. of spikes/plant and number of spikelets/plant except for trehalose treatment which showed more stimulation effect. As for the effect of time of water irrigation on no. of spikes/plant and weight of 1000 grain, the minority was due to the 20 days intervals between irrigation.

In general, the best results of plant growth parameters were recorded by wheat plants treated with maltose (10 mM) and irrigated every 10 days than that treated with trehalose (10 mM) or irrigated every 20 days. Our results were in agreement with Alam et al. (2014) who found that drought stress on Brassica decreased fresh and dry weight, whereas combination of trehalose with drought stress enhanced seedlings fresh and dry weight. Drought causes osmotic stress and inhibits cell expansion and division which markedly influence plant growth (Mahmood et al., 2012). Moreover Percival and Fraser (2005) reported that application of maltose on *Betula pendula Roth* increased shoot and root dry weight in 1999 trial. The results were in harmony with the results of Kilic and Yagbasanlar (2010) who investigated that drought stress on *Triticum turgidum* ssp. *durum* decreased plant height, number of spikes, grain yield and 1000 grain weight. Yang et al. (2007) reported that maltose level is modulated in response to drought. Improved photosynthetic performance can be achieved by sugar signaling mechanism and trehalose metabolism through its interaction with sugar-signaling pathways that can enhance photosynthetic capacity (Paul et al., 2001; Zeid, 2009).

Biochemical changes

Data presented in Table 3 show the effect of foliar application of maltose and trehalose at 10 mM under water stress on phenolic compounds, flavonoids and free amino acids. It is clearly shown from this table that, trehalose treatment significant increased these components as compared with the control. As for time of water irrigation, the superiority was due to the second one while the reverse was true with phenolic compounds.

The effect of different sugars treatments under two time of irrigation in Table 3, revealed that maltose and trehalose treatments after 20 days of irrigation significantly increased phenolic compounds, flavonoids and free amino acids as compared with the first time of irrigation.

The obtained results were in harmony with the finding of Aldesuquy and Ghanem (2015), drought stress on wheat cultivars elevated total phenols and flavonoids, also trehalose increased total phenols and flavonoids. Flavonoid is one of the largest classes of plant phenolics participated in plant defense system (Harborne and Williams, 2000). Phenolic compounds are stress induced metabolites accumulated in plant under water deficit stress. These compounds involve in scavenging of reactive oxygen species (ROS) through the antioxidative enzymes which use polyphenols as substrates (Sgherri et al., 2003). Generally, increase in phenolic and flavonoid compounds by trehalose treatments due to that trehalose was a signaling molecule which induces plants to activate non enzymatic antioxidants synthesis for scavenging ROS to reduce water stress.

Regarding reducing and total soluble sugars (Table 4), an increase in these parameters was obtained by maltose and trehalose treatments. Meanwhile, a positive correlation was noticed between times of irrigation. The effect of times of irrigation on reducing and total soluble sugars, revealed that the 20 days intervals of irrigation significantly increased these parameters.

Table 1 Effect of foliar spraying with maltose and trehalose on plant fresh weight, dry weight, shoot height and flag leaf area of wheat plants under water stress.

Treat	Shoot f.wt. (g)		Mean	Shoot d.wt. (g)		Mean	Shoot height (cm)		Mean	Flag leaf area (cm^2)		Mean
	Ir 1	Ir 2		Ir 1	Ir 2		Ir 1	Ir 2		Ir1	Ir2	
Control	5.18	3.24	4.21	1.08	0.69	0.885	56.43	46.2	51.32c	4.72	3.28	4.00
Maltose	10.17	8.70	9.43	1.53	1.42b	1.48	74.73	74.26	74.5a	8.66	8.36	8.51
Trehalose	8.54	5.25	6.89	1.61	1.59	1.60	70.5	54.17	62.33b	8.36	7.70	8.02
Mean	7.96	5.73		1.41	1.24		67.22	58.21		7.15	6.54	
LSD treat	1.61			0.590			5.672			1.34		
LSD Ir	n.s			n.s.			7.120			n.s.		
LSD Ir_x treat	n.s.			n.s.			8.022			n.s.		

Ir 1: irrigation after 10 days intervals and Ir 2: irrigation after 20 days intervals.

Table 2 Effect of foliar spraying with maltose and trehalose on no. of spikes/plant and no. of spikelets/plant and weight of 1000 grain of wheat plants under water stress.

Treatments	No. of spikes/plant		Mean	No. of spikelets/plant		Mean	Weight of 1000 grain (g)		Mean
	Ir 1	Ir 2		Ir 1	Ir 2		Ir1	Ir2	
Control	5.33	3.33	4.33	13.67	13.33	13.5	43.92	39.55	41.73
Maltose	7	6	6.5	15.67	14.33	15	50.78	43.56	47.17
Trehalose	6.33	5.67	6	15	13.67	14.33	48.57	48.25	48.41
Mean	6.22	5		14.78	13.78		47.75	43.79	
LSD treat	1.789			0.89			4.76		
LSD Ir	0.48			n.s.			3.63		
LSI$r_x$treat	2.53			n.s.			6.74		

Table 3 Effect of foliar spraying with maltose and trehalose on phenolic compounds, flavonoids and free amino acids concentrations (mg/100 g f.wt.) of wheat leaves under water stress.

Treatments	Phenolic compounds		Mean	Flavonoids		Mean	Free amino acids		Mean
	Ir 1	Ir 2		Ir 1	Ir 2		Ir 1	Ir 2	
Control	156	198	177	131	155	143	291	354	322.5
Maltose	143	217	180	205	263	234	222	417	319.5
Trehalose	174	235	204.5	158	172	165	302d	397	349.5
Mean	157.67	216.67		164.67	196.67		271.67	389.33	
LSD treat	1.537			1.087			1.215		
LSD Ir	2.353			3.312			1.434		
LSD Ir_xtreat	2.17			1.54			1.719		

These results agree with Ibrahim (2001) who found that water stress on *Mentha piperita* increased total soluble sugars and free amino acids. The increase in total soluble sugars caused a decrease in cellular osmotic potential resulting in lowered water potential and enhanced water uptake (Lambers et al., 1998).

Maltose and trehalose treatments significantly increased soluble protein and proline concentrations comparing with control (Table 5). Protein and proline concentrations were increased significantly with increasing the period of irrigation. The same results were found by Balla et al. (2011) on wheat. Compatible solutes such as trehalose and maltose stabilize biological structure, proteins and membrane lipids in different organisms during dehydration and protect photosynthetic electron transport chain (Crowe et al., 1992; Kaplan and Guy, 2004). Organic compatible solutes effectively take part in plant stress tolerance. (Ashraf and Foolad, 2006). Maltose has hydrophilic groups and replacing water molecules with maltose may alleviate the damage caused by drying (Bordat et al., 2004; Lerbret et al., 2005).

Application of maltose or trehalose led to increase proline concentration in plants watered every 20 days comparing with

Table 4 Effect of foliar spraying with maltose and trehalose on reducing sugars and total soluble sugars (TSS) mg/100 g f.wt. of wheat leaves under water stress.

Treatments	Reducing sugars mg l/100 g f.wt.		Mean	Total soluble sugars		Mean
	Ir 1	Ir 2		Ir 1	Ir 2	
Control	244.67	340	292.33	587.33	793.33	690.33
Maltose	274.33	421.33	347.83	731.33	952.67	842
Trehalose	281	347.33	314.17	786.67	964.67	875.67
Mean	266.67	369.55		701.78	903.55	
LSD treat	11.19			7.80		
LSD Ir	4.99			20.57		
LSD Ir_xtreat	15.82			11.04		

Table 5 Effect of foliar spraying with maltose and trehalose on proline and protein concentrations (mg/100 g f.wt.) of wheat leaves under water stress.

Treatments	Protein		Mean	Proline		Mean
	Ir 1	Ir 2		Ir 1	Ir 2	
Control	414.33	806.33	610	14	21	17.5
Maltose	545.67	1052.67	799.17	12	33	22.5
Trehalose	616.33	925	770.67	16	29	22.5
Mean	525.44	928		14	27.67	
LSD treat	10.502			1.33		
LSD Ir	10.42			1.43		
LSD Ir_xtreat	14.85			1.89		

Table 6 Effect of foliar spraying with maltose and trehalose on PAL and POD activities (unit/mg protein) of wheat leaves under water stress.

Treatments	PAL		Mean	POD		Mean
	Ir 1	Ir 2		Ir 1	Ir 2	
Control	555	578	566.5	8154.67	8815	8484.83
Maltose	492	421	456.5	7518.33	10,310	8914.33
Trehalose	414.67	231	322.83	11,648	12577.67	12112.83
Mean	487.22	410		9107	10,567	
LSD treat	4.54			37.92		
LSD Ir	2.66			64.44		
LSD Ir_xtreat	6.42			53.63		

every 10 days. The same results were reported by Alam et al. (2014) who noticed that drought stress on *Brassica* elevated proline content. Also, Abdellatif and Yasmin (2012) investigated an accumulation of proline as biochemical mechanism of plant osmotic adjustment under water stress. Ashraf and Foolad (2006) reported that proline increased in maize under influence of water deficit.

Activity of PAL was significantly decreased in plants irrigated every 20 days as compared to 10 days. Maltose and trehalose treatments (Table 6) significantly decreased PAL activity. The reverse was true in POD activity. The activity of POD significantly increased with application of trehalose and maltose. The same results were reported by Aldesuquy and Ghanem (2015) drought stress on wheat cultivars elevated PAL and POD activities, and also trehalose increased POD and PAL activities while PPO activity showed non significant decrease. Trehalose was effective in repairing the negative effects of drought. Hence, POD is major enzyme in H_2O_2 elimination in plant (Srivastava et al., 2010). Moreover, these enzymes activity increasing might offer considerable protection against the water stress as an index of the stress tolerance. Trehalose acts as a signaling molecule under abiotic stresses which induce ROS production in plant that sends signal to activate enzymatic antioxidants for scavenge ROS in order to reduce oxidative stress (Fernandez et al., 2010). PAL is a key enzyme in phenylalanine conversion to trans cinnamic acid, the first step in biosynthesis of anthocyanin. PAL was induced by drought (Guo and Wang, 2009).

Trehalose treatment gave significantly increase in ascorbate peroxidase (APX) activity (51.67 unit/mg protein) as compared to the control (Table 7). The same results were reported by Alam et al. (2014) who noticed that the combination of trehalose with drought stress increased ascorbate peroxidase in *Brassica*. On the other hand, no significant differences were

Table 7 Effect of foliar spraying with maltose and trehalose on ascorbate peroxidase and catalase activities (unit/mg protein) of wheat leaves under water stress.

Treat	Ascorbate peroxidase		Mean	Catalase		Mean
	Ir 1	Ir 2		Ir 1	Ir 2	
Control	37	42.67	39.83	1978	2453.33	2215
Maltose	49.33	37.67	43.5	968.33	1594	1281
Trehalose	40.33	63	51.67	1144	390.67	767.33
Mean	42.22	47.78		1363	1479.33	
LSD treat	9.18			14.12		
LSD Ir	5.64			25.55		
LSD Ir_xtreat	13			19.97		

Table 8 Effect of foliar spraying with maltose and trehalose on PPO activity (unit/mg protein) and MDA concentrations (n mol) of wheat plants under water stress.

Treat	PPO		Mean	MDA		Mean
	Ir 1	Ir 2		Ir 1	Ir 2	
Control	6.67	11	8.83	0.922×10^{-3}bc	1.553×10^{-3}	1.24×10^{-3}
Maltose	18	13	15.5	0.59×10^{-3}cd	0.63×10^{-3}	0.61×10^{-3}
Trehalose	13	24.67	18.83	1.12×10^{-3}b	0.53×10^{-3}	0.83×10^{-3}
Mean	12.55	16.22		0.88×10^{-3}a	0.90×10^{-3}	
LSD treat	3.75			0.26×10^{-3}		
LSD Ir	4.30			0.40×10^{-3}		
LSD Ir_xtreat	5.31			0.364×10^{-3}		

noticed in ascorbate peroxidase activity by maltose treatment and periods of irrigation. Maltose and trehalose decreased catalase activity as compared with control (Table 7). Significant increase in catalase activity in plant irrigated every 20 days in comparison with plant irrigated every 10 days. Ren et al. (2016) investigate that significant upregulation in ascorbate peroxidase (APX) and catalase (CAT) for drought resistance *Cerasus humilis* seedling exposed to water stress. These results suggested that CAT and APX involved in ascorbate and glutathione cycle. The decomposition of H_2O_2 was generated by superoxide dismutase in different cellular organelles. The maintenance of CAT activity in leaves of drought stressed plants likely allowed the removal of photorespiratory H_2O_2 produced when plants are subjected to water deficit (Sofa et al., 2015).

Maltose and trehalose treatments increased PPO activity. Application of sugars significantly increased the concentration of MDA in both treatments (Table 8). The highest increment in MDA concentration was shown in trehalose treatment.

The same results were reported by Alam et al. (2014) who noticed that drought stress on *Brassica* elevated MDA and the combination of trehalose with drought stress decreased MDA content. MDA concentration was used as an indicator to lipid peroxidation which led to membrane fluidity and increased membrane permeability (Liu et al., 2011). Trehalose was used as an osmoprotectant cellular osmotic balance. It stabilizes dehydrated enzymes, proteins and lipid membrane efficiently. It protects biological structures from damage of water leakage (Garg and Manchanda, 2009).

In Ali and Ashraf (2011) water stress decreased plant biomass production in maize, whereas elevated MDA content and the antioxidant enzymes activities (POD & CAT). Also, foliar application of trehalose elevated plant biomass production, phenolic compounds and antioxidant enzymes.

These results concluded that foliar applications with maltose or trehalose induced water stress tolerance in wheat plants. Sugars are water soluble, non-toxic and environmental safe. Maltose gave the best results in most biochemical and morphological parameters and grains yield than trehalose treatments especially low price of maltose.

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