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Short Communication

Trehalose mitigates heat stress induced damages in wheat seedlings

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Heat stress causes multifarious, and often adverse, alterations in plant growth, development, physiological function, and yield (Hasanuzzaman *et al.*, 2012). Heat stress adversely affects organisms by producing reactive oxygen species (ROS), causing membrane integrity loss, damaging proteins, DNA and lipids, and potentially disrupting cell function (Mittler *et al.*, 2004).

Plants continuously struggle for survival under various environmental stress conditions. To mitigate and repair the damage initiated by ROS species produced during stress and to protect the cell from injury under stress condition, it is important that ROS should be scavenged by antioxidant system (Srivalli *et al.*, 2003). Plants have enzymatic and non-enzymatic antioxidant system to alleviate the damage from ROS. Antioxidant enzymes include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX) and catalase (CAT) and non-enzymatic antioxidant compounds viz. ascorbate, glutathione, flavonoids, carotenoids and -tocopherol play important role in removal of toxic ROS (Ma *et al.*, 2008). Exogenous applications of protectants such as osmoprotectants, phytohormones,signaling molecules and trace elements, etc. have shown beneficial effects on plants growing under heat stress. They interact with the membranes, protein complexes or enzymes and protect them by scavenging reactive oxygen species or due to the growth promoting and antioxidant activities of these compounds (Garg *et al.*, 2002, Hasanuzzaman *et al.*, 2013).

Trehalose (-D-glucopyranosyl-[1,1]- -D-glucopyranoside) is a non-reducing disaccharide of glucose, which plays an important physiological role as a compatible solute in a large number of plants. During heat stress, trehalose treatment protects the ultrastructure of chloroplasts and some polypeptides in thylakoid membranes and also improves the photosynthetic capacity of thylakoids. It has been reported that trehalose can stabilize proteins and protects the biological membranes from lipid peroxidation efficiently in microorganisms under stress (Sebollela *et al.*, 2004). Reduction in carbohydrate accumulation under high temperature conditions may result from the imbalance between photosynthesis and respiration (Huang *et al.,* 2000). Trehalose is degraded to glucose, resulting in a higher extracellular and consequently intracellular glucose concentration. Engineering plants to synthesize these compounds may be an alternative way of developing thermotolerance in important crop plants and represents a potentially important area of research on thermotolerance. Keeping this in view the present study was planned to study the effect of trehalose treatment on antioxidative system in wheat seedlings under heat stress.

Seeds of wheat (*Triticum aestivum* L.) cultivars WH-711 (heat sensitive) and WH 730 (heat-tolerant) were surface sterilized and grown in Hogland solution at 25 °C with 15-h photoperiod and irradiance of 250 µmol m^{-2} s^{-1}, and then exposed to 35 °C. Trehalose treatment was given on the 10th day of germination, by supplementing solution with 1 and 2mM trehalose. While Hoagland solution without trehalose consisted of control. Sampling was done on 15th day of germination. The leaves of seedlings were analysed for further study.

MTS was measured according to method of Ibrahim and Quick (2001).

Extraction conditions were standardized with respect to type, molarity and pH of buffer, concentration of stabilizing agents and other constituents of extraction medium to achieve maximum extraction of enzymes. All the steps of extraction were carried out at 0-4°C. Extraction medium for APX, CAT and Lipoxygenase (LOX) consisted of 0.1 M phosphate buffer (pH 7.5) containing 5% (*w/v*) polyvinyl pyrrolidine (PVPP), 1 mM EDTA, and 10 mM β-mercaptoethanol as described by Chawla *et al.* (2013). Method of Nakano and Asada (1981) was employed to assay APX. Peroxidase and CAT activities were assayed at 37°C as described by Shannon *et al.* (1966) and Sinha (1972) respectively. Lipoxygenase was assayed by the method of Catherine *et al.* (1998).

Preparation of extract For the extraction of MDA and H_2O_2, 1.0 g seedlings each from control and stressed plants were taken and ground in 5 ml of chilled 0.8 N $HClO_4$ and centrifuged at 10,000 rpm for 25 min. The clear supernatant was decanted carefully and was used for further estimation. The lipid peroxidation was measured in terms of malondialdehyde (MDA) contents, a product of lipid peroxidation, according to the method of Heath and Packer (1968). H_2O_2 was estimated by the method of Sinha (1972).

For the extraction of Glucose and trehalose One hundred mg of leaf tissue was macerated in chilled pestle and mortar in the presence of 0.5 ml 0.1 M phosphate buffer (pH 7.5). The homogenate was centrifuged at 10,000 x g for 20 min at 4°C. The supernatant was carefully decanted and used for estimation of glucose and trehalose. Glucose was estimated by method of Bergmeyer and Bernt (1974) and Trehalose was estimated by method of Tourinhodos Santos *et al.* (1994).

Table 1 exemplifies that membrane thermostability decreased under heat stress. The higher reduction was observed in WH-711(49.06%), a thermo-sensitive genotype in comparison to thermo-tolerant i.e., WH-730 (32.77%) at 35°C. Membrane thermostability increased under control and stressed conditions with trehalose treatment. It increase from 68.76 to 68.84 and 69.89 units with 1 and 2mM trehalose treatment respectively in control seedlings. In stressed seedlings it increases from 46.23 to 50.09 and 67.76 units with 1 and 2mM trehalose treatment respectively in stressed seedling.

The loss of membrane thermo-stability was recorded under heat stress conditions whereas improved thermo-stability was found in trehalose treated seedlings. Membrane thermo-stability demonstrated that the structural integrity of the thylakoid membrane remained intact under high temperatures.

Table 1. Effect of heat stress and trehalose treatment on membrane thermo stability (%) in leaves of heat tolerant and heat sensitive genotypes of wheat seedlings

Treatments	Membrane thermo stability (%)	
	Genotypes	
	WH-730 (Heat-tolerant)	WH-711 (Heat-sensitive)
25°C (Control)	68.76	59.89
35°C(Stressed)	46.23 (-32.77)	30.51 (-49.06)
25°C, 1mM	68.84 (0.12)	67.22 (12.24)
35°C, 1mM	50.09 (-27.15)	49.16 (-17.92)
25°C, 2mM	69.89 (1.64)	68.14 (13.78)
35°C, 2mM	67.76 (-1.45)	58.78 (-1.85)
CD at 5%	Genotypes = 2.12	Treatments = 3.67
	Genotypes x Treatments = 5.19	

Values in parenthesis indicates per cent change over control

The results presented in Fig. 1A and B demonstrate that MDA and H_2O_2 increased under heat stress conditions when compared with control. WH-711 suffered greater damage to cellular membranes due to lipid peroxidation as indicated by higher accumulation of H_2O_2 and malondialdelyde content. Lower content of H_2O_2 and MDA in both the cultivars was observed during trehalose treatment. On treatment with trehalose, H_2O_2 content reduced. At 35°C, it decreased from 66.21 to 9.66 % in WH-730 and from 72.92 to 14.58 % in WH-711 with increasing trehalose from 1mM to 2mM. Similar trend has been observed for MDA content. Parallel increase in MDA and H_2O_2 content observed under high temperature stress conditions in both the cultivars with significant higher increase in sensitive cultivar indicates that higher production of reactive oxygen species in sensitive cultivar leads to more disruption of membrane integrity. Our results are in agreement with those of Sairam *et*

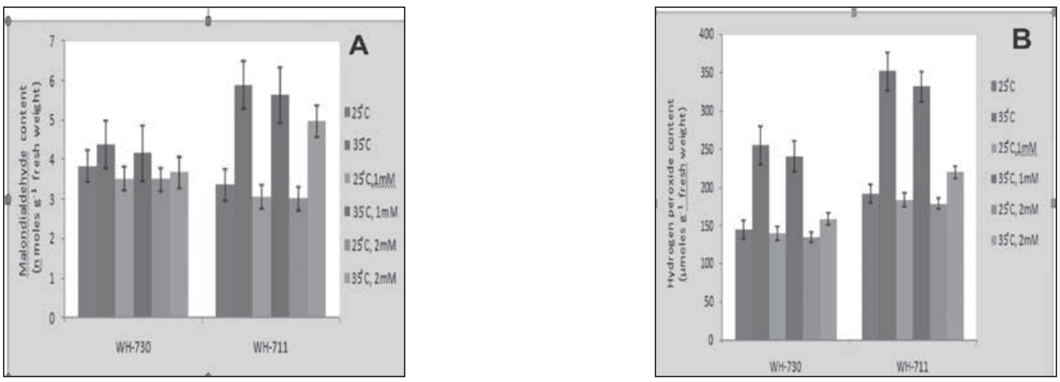

Fig 1. Effect of heat stress and trehalose treatment on malondialdehyde (nmoles g^{-1} fresh weight) and Hydrogen peroxide content (μmoles g^{-1} fresh weight) in leaves of heat tolerant and heat sensitive genotypes of wheat seedlings (A and B)

al. (2000) and Almeselmani *et al.* (2006), who reported higher increase in amount of electrolyte leakage with the increase in heat stress in the heat -sensitive cultivar as compared to tolerant cultivar of rice and wheat. Trehalose treatment lowers H_2O_2 indicating that Trehalose could directly scavenge H_2O_2 (Luo *et al.*, 2008). Therefore, the decrease in ROS by trehalose pretreatment should most likely be attributed to its ability to scavenge ROS, in addition to its possible protective role for membranes and macromolecules.

Lipoxygenase (LOX) activity decreased with the increase in trehalose concentreation in both the genotypes (Table 2). Tolerant cultivar, WH-730 showed lowest activity (20.34 units) at 2mM trehalose as compared to sensitive cultivar (WH-711) at 25°C whereas under stressed conditions LOX activity remained higher (36.26 % in WH-730 and 60.60 % in WH-711) in comparison to control. Our results supported by Bae *et al.* (2005) who also reported that when seedlings pretreated with trehalose and were exposed to heat, they exhibited less ROS production,

Table 2. Effect of heat stress and trehalose treatment on lipoxygenase activity, ascorbate peroxidase activity, Catalase activity, Peroxidase activity in leaves of heat tolerant and heat sensitive genotypes of wheat seedlings

Treatments	Lipoxygenase activity (units g^{-1} fresh weight)		Ascorbate peroxidase activity (units g^{-1} fresh weight)		Catalase activity (units g-1 fresh weight)		Peroxidase activity (units g-1 fresh weight)	
	Genotypes		Genotypes		Genotypes		Genotypes	
	WH-730 (Heat-tolerant)	WH-711 (Heat-sensitive)	WH-730 (Heat-tolerant)	WH-711 (Heat-sensitive)	WH-730 (Heat-tolerant)	WH-711 (Heat-sensitive)	WH-730 (Heat-tolerant)	WH-711 (Heat-sensitive)
25°C (Control)	25.29	27.31	10.04	7.36	1.31	1.20	6.32	6.16
35°C(Stressed)	34.46	43.86	17.72	11.30	2.61	2.14	9.29	8.00
	(36.26)	(60.60)	(76.49)	(53.53)	(99.24)	(78.33)	(46.99)	(29.87)
25°C, 1mM	22.79	26.96	9.91	7.26	1.29	1.19	6.24	6.05
	(-9.89)	(9.70)	(-1.29)	(-1.36)	(-1.53)	(0.83)	(-1.27)	(-1.79)
35°C, 1mM	31.48	38.4	12.49	10.56	2.04	1.72	7.42	7.32
	(24.48)	(40.61)	(24.40)	(43.48)	(55.73)	(43.33)	(17.41)	(18.83)
25°C, 2mM	20.34	25.69	9.31	7.15	1.26	1.18	6.19	5.94
	(-19.57)	(-5.93)	(-7.27)	(-2.85)	(-3.82)	(1.67)	(-2.06)	(-3.57)
35°C, 2mM	27.67	33.78	9.22	7.76	1.76	1.32	7.01	6.84
	(9.41)	(23.69)	(9.31)	(7.15)	(34.35)	(10.00)	(10.92)	(11.04)
CD at 5%	Genotypes =1.74	Treatments =3.02	Genotypes = 0.84	Treatments = 1.47	Genotypes = 0.19	Treatments = 0.34	Genotypes = N.S	Treatments = 0.97
	Genotypes x Treatments = N.S		Genotypes x Treatments = 2.06		Genotypes x Treatments = N.S		Genotypes x Treatments = N.S	

Values in parenthesis indicates per cent change over control

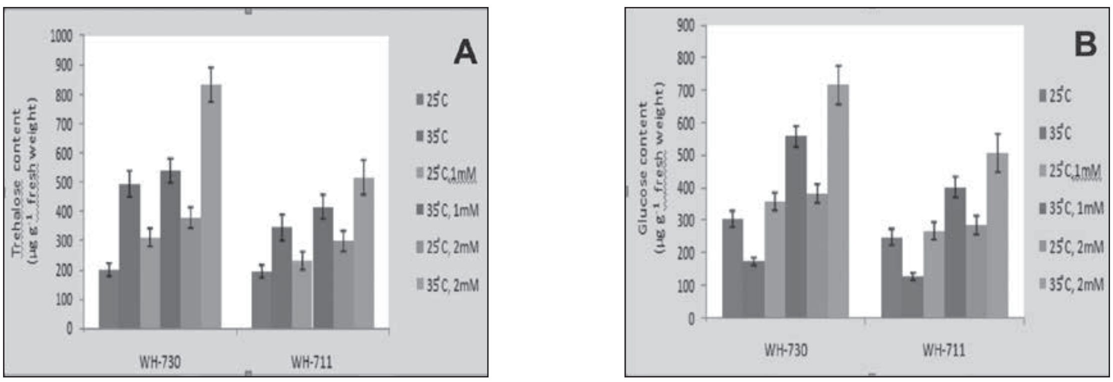

Fig 2. Effect of heat stress and trehalose treatment on trehalose and glucose content (µg g^{-1} fresh weight) in leaves of heat tolerant and heat sensitive genotypes of wheat seedlings (A and B)

lower LOX activity and MDA content, which indicated that trehalose pre-treatment might alleviate the damage caused by LOX-mediated lipid peroxidation. This lower LOX activity caused by trehalose pretreatment during periods of heat might be associated with a decrease in LOX synthesis. Trehalose could directly scavenge H_2O_2 in the same wheat cultivar (Luo *et al.*, 2010).

Trehalose content: increased significantly in both the cultivars of wheat i.e. WH-730 and WH-711 by trehalose treatment in control and stressed seedlings. Increase was higher in case of WH-730 reflecting its tolerant property. At 35ºC, increase was 101.25 and 239.23% in WH-730 and 76.75 and 164.01% in WH-711 by 1 and 2mM trehalose treatment, respectively over the control. The higher concentration of trehalose under heat stress is an indicator of protection from oxidative damage Exposure of *Saccharomyces cerevisiae* to a mild heat shock (38 ºC) induced trehalose accumulation and markedly increased the viability of the cells upon exposure to an H_2O_2-generating system, showing trehalose accumulation in stressed cells plays a major role in protecting cellular constituents from oxidative damage by acting as a free radical scavenger (Benaroudj *et al.*, 2001). Exogenous trehalose treatment has been shown to up-regulate a specific combination of genes known from biotic stress responses, and trehalose induces gene expression responses related to ROS and secondary metabolism activation (Almeida *et al.*, 2005).

At 35ºC, glucose content decreased in both the cultivars. The decline was more in sensitive cultivar. However, glucose content was significantly higher under control and stressed conditions in trehalose treated seedlings. At 1mM trehalose concentration, increase was 83.42% and 47.78% in WH-730 and WH-711 respectively, whereas with 2mM trehalose, increase was 135.40% and 104.05% in WH-730 and WH-711, respectively. Trehalose supplied exogenously might be transported into the cytosol by an unknown transporter or it enters the cytosol without the help of any transporter (Von et al., 2006). Trehalose is degraded to glucose, resulting in a higher extracellular and consequently intracellular glucose concentration. Liu and Huang (2000) reported that that high carbohydrate availability, particularly glucose and sucrose, during heat stress was an important physiological trait associated with heat-stress tolerance in creeping bentgrass. In plants, sugar signals are generated by photosynthesis and carbon metabolism in source and sink tissues to modulate growth, development and stress responses (Rolland *et al.*, 2006).

Activity of Ascorbate peroxidase was significantly increased under heat stress conditions and the increase was higher in WH-730 (76.49%) as compared to WH-711 (53.53%). At 35ºC, 1 and 2mM trehalose treatment decreased, the activity of APX by 24.40 and 9.31% in WH-730 and by 43.48 and 7.15 % in WH-711, respectively. In the present study, an increase in APX activity under heat stress is in agreement with the observations of Mittler and Zilinskas (1994) who also reported that an increase in transcript level of APX under heat stress. Increase in APX under stressed conditions may be due to up regulation of ascorbate-glutathione cycle in response to high temperature stress (Ma *et al.*, 2008). Shima *et al.* (2007) observed that very low concentrations of trehalose can improve drought resistance implies a signalling or regulatory role than merely an osmoprotective function.

Catalase activity increased under heat stress in both the cultivars. The increase at 35ºC over control was 99.24% WH-730 as compare to 78.33 % in WH-711. Catalase activity of both cultivars decreased with the increasing concentration of trehalose from 1 to 2mM, but remained higher in comparison to control seedlings, whereas in control conditions significant reduction was obsrerved in trehalose treated seedling.

Peroxidase activity increased by 46.99 % in WH-730 as compared to 29.87 % in WH-711 at 35ºC. POX activity decreased under trehalose treatment (10.92 % in WH-730 and 11.04 % in WH-711 with 2mM trehalose but it was significantly higher in comparison to untreated stressed seedlings. In the present investigation catalase (CAT) and peroxidase (POX) showed the similar trend. There is evidence from various studies that the activities of enzymes involved in scavenging ROS are altered by environmental stresses (Sairam *et al.*, 2005). Trehalose markedly decreased the H_2O_2 under heat stress probably by acting as an antioxidant by itself, since trehalose cannot protect APX and CAT (two enzymes which eliminate H_2O_2) under heat stress (Luo *et al.*, 2008). It could thus be concluded that trehalose, an osmoprotectant enhanced the antioxidant ability and protected seedlings against oxidative stress, thereby improving membrane thermo stability under heat stress condition.

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