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RELATIVE LEAF WATER CONTENT AND WATER SATURATION DEFICIT IN WHEAT (*TRITICUM AESTIVUM* L.) SEEDLINGS UNDER HEAT STRESS AS AFFECTED BY TREHALOSE APPLICATION

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ABSTRACT

Heat stress due to increased temperature is an agricultural problem in many areas in the world. It affects the wide spectrum of both biochemical and physiological responses within the plant cells. Relative Leaf Water Content (RLWC) and Water Saturation Deficit (WSD) was evaluated under control ($25\pm 2^\circ\text{C}$), heat stress ($35\pm 2^\circ\text{C}$ and $40\pm 2^\circ\text{C}$) and interactive effect of heat stress and trehalose was studied in wheat (*Triticum aestivum* L.) genotypes viz. HD2967, C306, PBW621, PBW343, PBW175 and PBW590. Trehalose at concentration of 1mM and 1.5mM was applied followed by heat stress of $35\pm 2^\circ\text{C}$ (moderate) and $40\pm 2^\circ\text{C}$ (severe) for 4 and 8 hours. Heat stress reduced the RLWC and increased the WSD of studied wheat genotypes. The duration of heat stress for 8 hours had more adverse effect on tested parameters. Trehalose application ameliorated the adverse effect of heat stress to some extent. Trehalose application @1.5mM concentration was found more effective as compared with 1mM concentration tested presently.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cereal of choice and main food for people all over the world (Rauf *et al* 2007). India, the second largest producer of wheat (*Triticum aestivum* L.) in the world has greatest success stories of Green Revolution and has made rapid progress in food grain production during second half of the 20th century (Dhillon *et al* 2010). Wheat is a premier cereal crop of Punjab, cultivated on 35.12 lac hectares in 2012-13 with production of 165.91 lac tonnes. Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than maize or rice, the other major cereals. Heat stress cause an array of morpho-anatomical, physiological and biochemical changes in wheat and other plants, which affect plant growth and development and drastically reduces both yield and quality of wheat (Wardlaw *et al* 2002, Altenbach *et al* 2003, Dupont *et al* 2006).

Biochemical and physiological responses within the plant cells are affected by heat stress (Sikder and Paul 2010). High temperature regime induces activity of antioxidant enzyme compared to control temperature (Badawi *et al* 2007). Heat shock proteins (HSPs) are synthesized to cope up with the heat stress and thus provide protection and repair to cellular damage caused by heat. Development of heat tolerance is correlated with HSP synthesis. Mutants defective in HSP synthesis are not able to acquire thermotolerance (Mitra and Bhatia 2008). Trehalose is a soluble, non-reducing disaccharide of glucose.

Three isomers exist: α , α -trehalose, α , β -trehalose and β , β -trehalose. Of these, only α , α -trehalose (1-O- (α -D-glucopyranosyl) α -glucopyranoside) is found in biological material. It is present in a large variety of organisms and can serve as reserve of carbohydrate and as a protectant in response to different stress conditions. Trehalose is known to protect membranes and macromolecules. Its accumulation allowing plants to tolerate stress, including heat-shock.

Therefore, a primary aim of this study is to determine whether trehalose helps to sustain the RLWC and tends to decrease WSD of wheat seedlings when exposed to heat stress conditions.

MATERIAL AND METHODS

Six genotypes of wheat (*Triticum aestivum* L.) viz. HD 2967, C306, PBW621, PBW590, PBW343 and PBW175 were obtained from Department of Plant Breeding and Genetics (PAU) and used for studies of morpho-physiological parameters under control and different stress conditions.

With a view of evaluate the effect of heat stress on above mentioned parameters, only healthy seeds of six genotypes of wheat were used in experiments. Seeds were surface sterilized with 0.1 per cent mercury chloride for 2-3 min. to avoid any fungal infection during seed germination. Petri dishes were sterilized in oven at 100°C for 1 hour. Ordinary blotting papers were used in Petri dishes and were autoclaved before use.

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Twenty seeds were sown in each Petri-dish lined with circular blotting paper and incubated at 25±2°C temperature. On seventh DAS trehalose (1mM and 1.5mM) application was given followed by heat stress, incubated at 35°C and 40°C, for 4 and 8 hrs. Controlled Petri-dishes were placed in an incubator in which temperature was maintained at 25°C.

Relative leaf water content (RLWC) and water saturation deficit (WSD) was calculated as per Weatherley (1950)

Shoot pieces of equal size were cut and immediately weighed to obtain fresh weight and then saturated by submerging in distilled water in petri dishes. After 6 hr, pieces were removed. Surface water was blotted off without putting any pressure and weighed to obtain saturated weight. After drying at 70°C for 48 hr dry weight was determined from these data following parameters were calculated.

$$RLWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}} \times 100$$

$$WSD = \frac{\text{Saturated weight} - \text{Fresh weight}}{\text{Saturated weight} - \text{Dry weight}} \times 100$$

RESULTS AND DISCUSSION

Relative leaf water content (RLWC)

High values of RLWC stand for high ability of plant to absorb water. RLWC significantly decreased with an increase in heat stress in wheat genotypes studied presently (Table 1 and 2). Under controlled conditions C306 showed more RLWC followed by PBW621 and HD2967. Whereas under control as well as under stress conditions PBW175 and PBW590 had low RLWC as compared to other genotypes. Even under moderate (35±2°C) and severe (40±2°C) heat stress the C306, HD2967 and PBW621 genotypes performed better than others.

In all the genotypes it was recorded that the application of trehalose results in increase in RLWC both controlled as well as in heat stressed conditions. The application of 1.5 mM of trehalose showed more positive results as compared to 1mM of trehalose under moderate as well as severe heat stress conditions.

The higher relative water content help the tolerant genotypes to performed physio-biochemical processes more efficiently under high temperature conditions (Gupta and Gupta 2005). Evaporative demand exhibits near exponential increase in day-time temperatures and can results in high transpiration rates and low plant water potentials (Hall 2001).

Water saturation deficit (WSD)

WSD significantly increased with increase in levels of imposed heat stress (Table 3 and 4). The WSD recorded highest in PBW175 followed by PBW590 genotype and least WSD was recorded in PBW621 and C306 genotypes. It was recorded in all the six selected genotypes that the value of WSD showed increase with increase in temperature. The highest WSD was observed in all the genotypes when they were stressed under the severe (40±2°C) heat for 8hrs as compared to 25±2°C and moderate heat stress.

The water saturation deficit recorded less in all the genotypes after the application of trehalose. Perhaps, it was due to property of trehalose that it maintains the membrane stability (Colaco et al 1995), tends to maintain the water content of leaf i.e RLWC, thus tends to decrease the WSD.

Table 1 Effect of trehalose on Relative Leaf Water Content (%) of wheat genotypes under heat stress (4hrs) of 35±2°C and 40±2°C.

Treatments	Genotypes					
	HD2967	PBW175	C306	PBW343	PBW621	PBW590
T1-Control at 25°C	67.07	56.06	71.92	62.96	67.16	59.03
T2-T1+(tre-1mM)	67.08	56.08	71.96	62.96	67.18	59.06
T3-T1+(tre-1.5mM)	67.09	56.09	71.98	62.98	67.20	59.09
T4- at 35°C	62.03	52.08	67.83	60.61	63.18	55.03
T5-T4+(tre-1mM)	62.18	52.09	67.84	60.62	63.13	55.06
T6-T4+(tre-1.5mM)	62.19	52.12	67.85	60.64	63.21	55.18
T7- at 40°C	59.06	50.03	61.08	58.03	60.63	52.61
T8-T7+(tre-1mM)	59.07	50.00	61.03	58.03	60.63	52.58
T9-T7+(tre-1.5mM)	59.08	50.02	61.08	58.04	60.63	52.64
CD 5%	V=0.0212, T=0.0259, V×T=0.0636					

Analysed by analysis of variance (ANOVA).

Table 2 Effect of trehalose on Relative Leaf Water Content (%) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C.

Treatments	Genotypes					
	HD2967	PBW175	C306	PBW343	PBW621	PBW590
T1-Control at 25°C	67.04	56.00	71.86	62.90	67.18	59.05
T2-T1+(tre-1mM)	67.05	56.01	71.84	62.84	67.19	59.06
T3-T1+(tre-1.5mM)	67.06	56.02	71.86	62.86	67.20	59.07
T4- at 35°C	60.07	50.07	67.81	58.03	59.06	53.03
T5-T4+(tre-1mM)	60.07	50.08	67.81	58.04	59.05	53.02
T6-T4+(tre-1.5mM)	60.08	50.09	67.82	58.06	59.07	53.04
T7- at 40°C	57.07	48.03	60.01	52.81	52.61	52.01
T8-T7+(tre-1mM)	57.08	48.04	60.01	52.81	52.62	52.03
T9-T7+(tre-1.5mM)	57.09	48.08	60.03	52.82	52.68	51.04
CD 5%	V=0.0183, T=0.0225, V×T=0.0551					

Analysed by analysis of variance (ANOVA).

Table 3 Effect of trehalose on Water Saturation Deficit (%) of wheat genotypes under heat stress (4hrs) of 35±2°C and 40±2°C

Treatments	Genotypes					
	HD2967	PBW175	C306	PBW343	PBW621	PBW590
T1-Control at 25°C	15.08	16.21	15.06	15.18	15.06	15.98
T2-T1+(tre-1mM)	15.08	16.20	15.02	15.16	15.05	15.96
T3-T1+(tre-1.5mM)	15.07	16.18	15.02	15.13	15.04	15.92
T4- at 35°C	16.18	16.16	16.21	16.14	16.12	16.22
T5-T4+(tre-1mM)	16.18	16.15	16.20	16.11	16.12	16.21
T6-T4+(tre-1.5mM)	16.17	16.15	16.20	16.10	16.10	16.20
T7- at 40°C	17.06	17.81	17.03	17.21	17.21	17.31
T8-T7+(tre-1mM)	17.04	17.60	17.03	17.11	17.20	17.30
T9-T7+(tre-1.5mM)	17.02	17.60	17.00	17.10	17.20	17.30
CD 5%	V=0.0265, 0.0324, V×T=0.0795					

Analysed by analysis of variance (ANOVA).

Table 4 Effect of trehalose on Water Saturation Deficit (%) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C

Treatments	Genotypes					
	HD2967	PBW175	C306	PBW343	PBW621	PBW590
T1-Control at 25°C	15.04	16.10	15.02	15.21	15.03	15.62
T2-T1+(tre-1mM)	15.04	16.05	15.01	15.20	15.03	15.60
T3-T1+(tre-1.5mM)	15.03	16.09	15.01	15.20	15.02	15.61
T4- at 35°C	16.21	16.32	16.33	16.36	16.62	16.66
T5-T4+(tre-1mM)	16.20	16.31	16.32	16.32	16.61	16.61
T6-T4+(tre-1.5mM)	16.19	16.30	16.30	16.32	16.60	16.60
T7- at 40°C	17.15	17.86	17.61	17.33	17.13	17.45
T8-T7+(tre-1mM)	17.15	17.83	17.60	17.32	17.13	17.45
T9-T7+(tre-1.5mM)	17.14	17.80	17.58	17.30	17.12	17.42
CD 5%	V=0.056, T=0.068, V×T=0.168					

Analysed by analysis of variance (ANOVA).

CONCLUSION

Genotypes showed more RLWC under controlled condition. Whereas, PBW175 and PBW590 showed low RLWC as compared to other genotypes under stress conditions. At moderate and severe heat stress the PBW621 genotype performed better than others. The application of trehalose resulted in increased RLWC both controlled as well as in heat stress conditions. WSD significantly increased with increase in level of imposed heat stress. The WSD recorded highest in PBW175 and least in HD2967 and PBW621 genotypes. WSD recorded less in all the genotypes after the application of trehalose. Present study reported that exogenous application of trehalose partially ameliorated adverse effect of heat stress by sustaining the RLWC and reducing the WSD of wheat seedlings under heat stress.

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