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Chlorophyll and carotenoid content of wheat (*Triticum aestivum* L.) seedlings under heat stress as affected by trehalose application

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Abstract: Presently, chlorophyll and carotenoid contents were evaluated under control (25±2°C), heat stress (35±2°C and 40±2°C) and interactive effect of heat stress and trehalose in six wheat (*Triticum aestivum* L.) genotypes (HD2967, PBW175, C306, PBW343, PBW621 and PBW590). Trehalose an osmoprotectant, at concentration of 1mM and 1.5mM was applied at 7 days after sowing (DAS) followed by heat stress of 35±2°C (moderate) and 40±2°C (severe) on 8DAS for 4 and8 hours. As chloroplast thylakoid membranes, are highly vulnerable to heat stress, the chlorophyll content decreased with increased temperature stress in all selected genotypes. Heat stress significantly reduced ($P< 0.05$) the carotenoid content in all genotypes. Severe heat stress (8 hours) more adversely affected these mentioned parameters. The application of Trehalose @ 1.5mM as compared with 1mM concentration was found more effective to ameliorate the adverse effect of heat stress on chlorophyll and carotenoid contents to sustain photosynthetic process.

Keywords: Heat stress, Photosynthetic pigments, Trehalose, Wheat

INTRODUCTION

All over the world wheat i.e. *Triticum aestivum* L. is an necessary cereal crop. In the form of various products wheat is used as staple food. Global wheat supplies are predicted remain commodious in 2016/17 marketing season. Although below the 2015 record, world wheat production in 2016 is to outstrip utilization for the fourth consecutive season, boosting world stocks to a 15 year high. World wheat utilization is seen to decline slightly mostly because of reduced feed use (FAO 2016). It has been found that wheat contain the higher value of vegetable protein in the food of human beings, as it contains higher content of proteins and amino acids than the other major cereals like maize rice (NPCS board, 2012).

As heat stress or temperature stress is described as increase in temperature up to a threshold level for a particular interval of time that cause the unrecoverable harm to the plant or crop growth and development. A sudden rise in temperature basically above 10-15°C is consider heat shock or heat stress (Wahid *et al.,* 2007).

Due to massive rise in the population the requirement or desire for wheat has been also increased than the previous times, but its yield or harvest decreased due to the environmental stress. Extreme or late temperature stress during the grain filling period of normal as well as delayed planted wheat is the large abiotic stress that severely reducing wheat yield (Khan *et al.,* 2007). Unstable or stable heat stress causes various changes in morpho-anatomical, biochemical and physiological modifications in wheat, that severely affect the crop growth and development. Heat stress violently decreased both quantity and quality of wheat (Wardlaw *et al.,* 2002, Altenbach *et al.,* 2003, Dupont *et al.,* 2006).

Trehalose is a resolveable, 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 -Dglucopyranosyl) α -glucopyranosi de) is found in biological material. It is present in a huge variety of organisms and can serve as reserve of carbohydrate and as a protectant in response to different environmental stress factors. Trehalose is known to protect biological membranes and macromolecules. Its stockpilling has been implicated in permit crops to indulge stress, including heat-stress. Trehalose does protect against desiccation in certain specialized resurrection plants. Gomez *et al.,* 2011 described the discovery of trehalose metabolism in the recent years has pointed out the importance of trehalose biosynthesis in stress responses in plants. Therefore, a primary aim and the significance of this work was to determine the role of trehalose, whether exogenous application of trehalose helps to protect and maintain the chlorophyll and carotenoid content from the destructive effect of heat stress when wheat seedlings exposed to different (35±2°C and 40±2°C) levels of heat stress.

MATERIALS AND METHODS

Plant material: Six genotypes of wheat (*T. aestivum*

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L.) viz. HD 2967, C306, PBW621, PBW590, PBW343 and PBW175 were obtained from Department of Plant Breeding and Genetics (PAU) and used for studies related to chlorophyll and carotenoid content under control and different heat stress levels. Statistical Analysis: Analysis of variance (ANOVA), critical difference at 5% level of significance (P< 0.05%) was used for the data analysis.

With a view of assess the effect of heat stress on above stated 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 kind of mycosis during seed germination. Petri plates were sterilized in oven at 100°C for 1 hour. Ordinary blotting papers were used in Petri dishes and were autoclaved before use. 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 8 hrs. Controlled Petri-dishes were placed in an BOD in which heat was maintained at 25°C.

T1- control at 25°C, T2-T1+(tre-1mM), T3-T1+(tre-1.5mM), T4- at 35°C, T5-T4+(tre-1mM), T6-T4+(tre-1.5mM),T7- at 40°C, T8-T7+(tre-1mM), T9-T7+(tre-1.5mM)

Chlorophyll content and carotenoid content: Chlorophyll content and carotenoid contentwas determined by method as followed by Hiscox and Israelstam (1979).

The photosynthetic pigments from the wheat leaves by placing the 100g of fresh leaves used in the photosynthesis and reflectance measurements in 5ml of the dimethyl sulfoxide (DMSO) and extracting for 12 h in the dark. The concentration of the extracted pigments was calculated by recording the absorbance values at 665,645 and 480 nm.

Chl a = 12.19 (OD 665)-3.45(OD 645) x V/1000xW

Chl b= 21.99(OD 645)-5.32(OD665) x V/1000xW

Total Chl = 20.2(OD 480) + 8.02(OD665) x V/1000 x W

Carotenoids=(OD480)+0.114(OD665)– 0.638(OD645)

where, OD_{663} = OD at 663 nm; OD_{645} = OD at 645 nm; OD_{480}= OD at 480 nm;V = Total volume of solution made; W = Weight of sample (g) taken

The chlorophyll and carotenoid contents were expressed as mg chl g^{-1} fresh weight.

RESULTS AND DISCUSSION

Chlorophyll content: During the present study six genotypes of wheat (*T.aestivum* L.) were exposed to varying levels of heat stress (35±2°C and 40±2°C) under laboratory conditions (Table 1-6)) and contents of chlorophyll a, b and total were estimated. The chl a content was in range of 0.598 to 0.646 and chl b in range of 0.312 to 0.432 in control. The contents of total chlorophyll varied significantly (P< 0.05) all treatments in all the wheat genotypes studied presently and was recorded more in PBW621 and HD2967 genotypes and least in PBW590. Under severe heat stress i.e. 40±2°C for 8 hours duration chlorophyll a value was recorded more in PBW621 genotype and chl b was more in HD2967 and PBW621 genotype. These results were similar to that of Gautam *et al.* (2016) as heat stress decreased the chlorophyll a content in the durum wheat genotypes. Khan *et al.* (2015) also observed that heat stress significantly reduced chl a and chl b contents of wheat genotypes similar to that of Ahmed and Hassan (2011) and Kumar *et al.* (2012).Presently, it has been also recorded that in all the studied genotypes the chlorophyll content decreased with increased temperature stress. Shirdelmoghanloo (2016) also observed that there was positive correlation between heat stress and chlorophyll degradation or chlorophyll loss. Chloroplast thylakoid membranes, are highly vulnerable to heat stress. High temperatures led not only to disintegration of the lipid bilayer (Losa *et al.,* 2004), but also to damage of the oxygen- evolving complex of photosystem 2 (Komayama *et al.,* 2007). There is an inverse correlation between growth temperature and membrane saturation level. Changes in lipid-protein interactions are thought to play a major role in heat-induced increase in

Table 1. Effect of trehalose on chlorophyll a (mg gm^{-1} fresh weight) 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	0.646	0.628	0.643	0.632	0.646	0.598
T2-T1+(tre-1mM)	0.647	0.638	0.644	0.636	0.646	0.598
T3-T1+(tre-1.5mM)	0.649	0.639	0.649	0.639	0.649	0.599
T4- at 35°C	0.632	0.622	0.628	0.628	0.633	0.596
T5-T4+(tre-1mM)	0.634	0.626	0.629	0.629	0.638	0.596
T6-T4+(tre-1.5mM)	0.638	0.631	0.631	0.630	0.642	0.599
T7- at 40°C	0.618	0.598	0.611	0.599	0.617	0.566
T8-T7+(tre-1mM)	0.619	0.598	0.613	0.614	0.619	0.572
T9-T7+(tre-1.5mM)	0.626	0.600	0.614	0.615	0.621	0.578
CD 5%	V=0.077, T=0.094, V×T=0.232					

Table 2. Effect of trehalose on chlorophyll a (mg gm^{-1} fresh weight) 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	0.641	0.622	0.632	0.631	0.645	0.598
T2-T1+(tre-1mM)	0.646	0.626	0.638	0.636	0.654	0.602
T3-T1+(tre-1.5mM)	0.451	0.631	0.642	0.636	0.661	0.636
T4- at 35°C	0.590	0.546	0.588	0.566	0.600	0.532
T5-T4+(tre-1mM)	0.596	0.546	0.591	0.566	0.616	0.538
T6-T4+(tre-1.5mM)	0.598	0.548	0.596	0.571	0.617	0.542
T7- at 40°C	0.538	0.510	0.536	0.518	0.586	0.506
T8-T7+(tre-1mM)	0.548	0.516	0.568	0.532	0.588	0.516
T9-T7+(tre-1.5mM)	0.551	0.518	0.572	0.549	0.589	0.526
CD 5%	V=0.042, T=0.051, V×T=0.126					

Table 3. Effect of trehalose on chlorophyll b (mg gm^{-1} fresh weight) 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	0.415	0.404	0.413	0.405	0.432	0.312
T2-T1+(tre-1mM)	0.416	0.406	0.414	0.406	0.433	0.318
T3-T1+(tre-1.5mM)	0.417	0.406	0.415	0.406	0.439	0.332
T4- at 35°C	0.400	0.359	0.398	0.398	0.406	0.312
T5-T4+(tre-1mM)	0.400	0.386	0.399	0.398	0.406	0.313
T6-T4+(tre-1.5mM)	0.401	0.400	0.399	0.398	0.408	0.313
T7- at 40°C	0.358	0.315	0.354	0.341	0.370	0.306
T8-T7+(tre-1mM)	0.358	0.318	0.359	0.342	0.381	0.309
T9-T7+(tre-1.5mM)	0.359	0.335	0.360	0.346	0.385	0.311
CD 5%	V=0.042, T=0.052, V×T=0.127					

Table 4. Effect of trehalose on chlorophyll b (mg gm^{-1} fresh weight) 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	0.418	0.402	0.410	0.406	0.432	0.330
T2-T1+(tre-1mM)	0.418	0.412	0.411	0.407	0.433	0.330
T3-T1+(tre-1.5mM)	0.419	0.413	0.412	0.407	0.433	0.331
T4- at 35°C	0.361	0.316	0.343	0.342	0.370	0.296
T5-T4+(tre-1mM)	0.376	0.318	0.346	0.348	0.371	0.303
T6-T4+(tre-1.5mM)	0.376	0.349	0.348	0.356	0.372	0.326
T7- at 40°C	0.306	0.296	0.298	0.296	0.306	0.217
T8-T7+(tre-1mM)	0.307	0.297	0.298	0.296	0.316	0.218
T9-T7+(tre-1.5mM)	0.307	0.297	0.299	0.297	0.317	0.276
CD 5%	V=0.0023, T=0.0029, V×T=0.0071					

the fluidity of the thylakoid membranes (Larkindale *et al.,* 2004).

In the present study it was also recorded that the application of trehalose increased the chlorophyll content in all genotypes. The application of higher concentration (1.5mM) of trehalose showed more increase in chlorophyll content as compare to 1mM of trehalose. Similar finding of Benaroudj *et al.,* 2001 also pointed out that trehalose accumulation during heat stress protects the cells and cellular proteins of *Saccharomyces cerevisiae* from damage by oxygen radicals. During heat stress, trehalose pretreatment protects the ultrastructure of chloroplasts some polypeptides in thylakoid membranes, and also improves the photosynthetic capacity of thylakoids , which indicates a protective role of trehalose or its metabolite for the thylakoid membrane.

Carotenoid content: Heat stress decreased the carotenoid content in all the selected wheat genotypes (Table 7 and 8). The carotenoid content recorded between 0.0291 to0.0298. The maximum carotenoid content was recorded in HD2967 and minimum was observed in PBW175 genotype. In all the genotypes it was recorded that the carotenoid content showed maximum value at 25±2°C as compared to moderate and severe heat stress. It was found all the genotypes showed increase in carotenoid content with the application of Trehalose. The concentration of 1mM of Trehalose showed increase in PBW621 and PBW590 under control conditions, whereas the concentration of 1.5mM of trehalose showed increase in carotenoid content in all the selected genotypes both in control and heat stressed conditions. The heat stress of 40±2°C (severe heat

Table 5. Effect of trehalose on total chlorophyll (mg gm^{-1} fresh weight) 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	1.055	1.036	1.049	1.049	1.059	0.938
T2-T1+(tre-1mM)	1.056	1.037	1.049	1.049	1.059	0.941
T3-T1+(tre-1.5mM)	1.058	1.038	1.049	1.051	1.069	0.943
T4- at 35°C	0.916	0.906	0.930	0.936	0.940	0.836
T5-T4+(tre-1mM)	0.917	0.906	0.939	0.936	0.941	0.838
T6-T4+(tre-1.5mM)	0.918	0.907	0.942	0.937	0.942	0.839
T7- at 40°C	0.736	0.846	0.746	0.741	0.726	0.696
T8-T7+(tre-1mM)	0.736	0.847	0.746	0.742	0.727	0.697
T9-T7+(tre-1.5mM)	0.737	0.848	0.747	0.743	0.727	0.699
CD 5%	V=0.0114, T= 0.0140, V×T=0.0344					

Table 6. Effect of trehalose on total chlorophyll (mg gm^{-1} fresh weight) 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	1.046	1.038	1.042	1.038	1.056	0.941
T2-T1+(tre-1mM)	1.049	1.038	1.041	1.038	1.057	0.941
T3-T1+(tre-1.5mM)	1.049	1.039	1.047	1.038	1.058	0.942
T4- at 35°C	0.841	0.846	0.846	0.900	0.896	0.806
T5-T4+(tre-1mM)	0.842	0.847	0.847	0.918	0.897	0.807
T6-T4+(tre-1.5mM)	0.843	0.848	0.848	0.939	0.899	0.808
T7- at 40°C	0.696	0.636	0.626	0.641	0.730	0.616
T8-T7+(tre-1mM)	0.697	0.637	0.627	0.642	0.734	0.616
T9-T7+(tre-1.5mM)	0.698	0.638	0.628	0.643	0.736	0.617
CD 5%	V=0.0152, T=0.0187, V×T=0.0458					

Table 7. Effect of trehalose on carotenoid (mg gm^{-1} fresh weight) 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	0.0298	0.0291	0.0295	0.0296	0.0293	0.0294
T2-T1+(tre-1mM)	0.0298	0.0291	0.0295	0.0296	0.0294	0.0295
T3-T1+(tre-1.5mM)	0.0299	0.0293	0.0296	0.0297	0.0296	0.0297
T4- at 35°C	0.0280	0.0282	0.0276	0.0278	0.0279	0.0276
T5-T4+(tre-1mM)	0.0281	0.0281	0.0283	0.0278	0.0281	0.0283
T6-T4+(tre-1.5mM)	0.0282	0.0282	0.0281	0.0279	0.0282	0.0284
T7- at 40°C	0.0253	0.0255	0.0251	0.0248	0.0264	0.0265
T8-T7+(tre-1mM)	0.0271	0.0269	0.0264	0.0262	0.0265	0.0266
T9-T7+(tre-1.5mM)	0.0272	0.0270	0.0265	0.0262	0.0266	0.0268
CD 5%	V=0.00278, T=0.00341, V×T=0.00835					

Table 8. Effect of trehalose on carotenoid (mg gm^{-1} fresh weight) 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	0.0296	0.0293	0.0294	0.0295	0.0294	0.0295
T2-T1+(tre-1mM)	0.0296	0.0293	0.0294	0.0295	0.0294	0.0296
T3-T1+(tre-1.5mM)	0.0297	0.0295	0.0295	0.0296	0.0295	0.0297
T4- at 35°C	0.0261	0.0262	0.0263	0.0270	0.0271	0.0268
T5-T4+(tre-1mM)	0.0261	0.0261	0.0263	0.0271	0.0272	0.0266
T6-T4+(tre-1.5mM)	0.0262	0.0262	0.0264	0.0272	0.0273	0.0267
T7- at 40°C	0.0223	0.0225	0.0221	0.0218	0.0221	0.0218
T8-T7+(tre-1mM)	0.0223	0.0225	0.0222	0.0221	0.0221	0.0220
T9-T7+(tre-1.5mM)	0.0224	0.0226	0.0223	0.0221	0.0222	0.0228
CD 5%	V=0.00493, T=0.00604, V×T=0.01479					

stress) for longer duration i.e. 8 hrs showed maximum carotenoid content decrease in all the genotypes.

The carotenoid content in leaf blade, awn and lemma were much lower after heat stress treatment than the control (Xu Xiao-Ling *et al.,* 2001). In response to heat stress, chloroplasts in the mesophyll cells of grape plants became round in shape, the stroma lamellae became swollen and the content of vacuoles formed clumps, whilst the cristae were disrupted and mitochondria became empty (Zhang *et al.,* 2005).

Conclusion

Presently, as the six wheat genotypes were subjected to moderate (35±2°C) and severe (40±2°C) heat stress conditions. It has been observed in all the genotypes that the heat stress resulted in loss of photosynthetic pigments. More chlorophyll and carotenoid loss occurred under the severe heat stress conditions as compared to control and moderate stress. The genotype HD2967 showed high chlorophyll content even under the severe heat stress for 8 hours of duration. On the other hand PBW 175 followed by HD2967 showed higher carotenoid content under severe heat stress for 8 hours duration. Now in the treatments with the exogenously applied trehalose, there were increase in chlorophyll (a, b and total) and carotenoid contents as the trehalose known to protect the biological membranes from degradation by stabilizing them. Thus, the present findings demonstrated that exogenous application of trehalose to wheat has considerable potential for maintenance of thyllakoid membrane stability (mechanism unknown) in order to maintain the chlorophyll and carotenoid content in all the six studied wheat seedlings growing under heat stress conditions.

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