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High temperature induced alterations in plant photosynthetic spectral properties of thylakoids in wheat plants

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Abstract

In this paper an attempt has been made to analyze the effect of high temperature on primary photochemistry of photosynthesis like absorption and fluorescence properties using spectrophotometry and spectrofluorimetry.High temperature affects the pigment and protein interaction as a result primary processes of photochemistry gets hampered in wheat plants.

Introducton

Heat stress is often defined as where temperatures are hot enough for sufficient time that they cause irreversible damage to plant physiological functions and development. The temperature ranges from the near freezing point in arctic zone to 50ºC in the hottest deserts. In addition, plants are subjected to variety of seasonal temperatures due to fluctuations in the diurnal temperatures. Due to this several plant physiological processes are getting affected by temperature and one of such processes is photosynthesis (Bauer,1979; Berry and Bjorkmann, 1980;Hussain *et al.*,2016;Posch *et al.*,2019). In PS II, the main target for high temperature stress is oxygen evolving complex. As a result, there has been loss of Mn ions and extrinsic polypeptides of water oxidation complex (Nash *et al.*, 1985; Enami *et al.*, 1994). It is suggested that the membrane permeability increases during heat treatment, which results in a decrease in the proton gradient formation across the thylakoid membrane and a suppression of the linear electron flow. The changes in the membrane

viscosity could be due to the variation of grana and stroma thylakoids ratio. It was observed that the quantity of grana thylakoids in green leaves is enhanced when exposed to damaging temperatures (Gounaris *et al*, 1983). In the past, PS II was considered a key weak link (Santarius 1975; Berry and Bjorkman, 1980; Enami *et* al., 1994) but damage to PS II only occurs at high temperatures, above 35ºC (Yamane *et al.*, 1998:Bukhov *et al*.,2000).

A large number of thylakoid proteins undergo reversible phosphorylation (Hansson and Vener, 2003) and heat stress is one of the most effective ways of modulating the phosphorylation status of many of them (Vener *et al.,* 2001).Heat induced membrane damage is attributed to lipid hyperfluidity, which alters lipid- protein interactions and subsequently causes protein denaturation (Pali *et al.,* 2003). Membrane fluidity can be regulated by xanthophylls cycle activity. It is suggested that the membrane permeability increases during heat treatment, which results in a decrease in the proton gradient formation across the thylakoid membrane and a suppression of the linear electron flow. The change in the membrane viscosity could be due to the variation of grana and stroma thylakoids ratio. It was observed that the quantity of grana thylakoids in green leaves is enhanced when exposed to damaging temperatures (Gounaris *et al.,* 1983).Studies related to the effect of HT on spectral properties of thylakoids of wheat are scanty.Hence in this study HT effect on absorption and fluorescence properties has been analyzed.

Materials and methods

Healthy seeds of wheat of variety, ***Triticum aestivum*** (Kalyanasona) were obtained from Acharya N.G. Ranga Agricultural University, Hyderabad.The seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes and thoroughly washed with tap and distilled water. The seeds were imbibed for 6 h and then placed on 2 cm thick cotton bed moistened with distilled water. The seedlings were grown in dark for two days and then shifted to light in growth chamber where the photon flux density at the leaf surface was 25 Wm^{-2} (Fig. 10). The relative humidity and temperature were maintained at 60-65% and 25 ± 1ºC respectively. Hoagland solution is supplied at every day intervals which has the following composition.

High temperature treatment

Wheat primary leaves were taken and given high temperature treatment in circulating water bath ranging from 25-50ºC for 20 min to perform *in vivo* conditions. For *in vitro* studies, the thylakoid membranes were first

isolated and HT treatment was given as mentioned above for 10 min.Chlorophyll estimation has been made by following the procedure of Arnon(1949).

Spectral measurements

Absorption Spectra

Absorption spectra of thylakoids were recorded at room temperature (25°C) on Jasco UV-Vis spectrophotometer. The thylakoid membranes were suspended in the reaction buffer (50 mM HEPES-NaOH (pH 7.5), 10 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl). The thylakoid suspension was scanned for the absorption spectra from 400 nm to 750 nm in the visible region(Sabat *et al*.,1990).

Fluorescence emission spectra

Jasco spectroflourimeter was used to record the fluorescence emission spectra of thylakoids in absence and presence of 10 μM DCMU at 25°C. Thylakoid membranes equivalent to 5 pg of Chllml were suspended in reaction buffer (50 mM Hepes-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl) and the samples were exited with 440 nm light beam via a slit width of 5 nm. The emission was collected from 600-750 nm in the visible region(Mohanty *et al*,1989).

Results

According to literature, spectral properties of photosynthetic pigments like absorption and fluorescence are very much related with the primary processes of photosynthesis like electron transport. Since an inhibition has been observed under HT, this could be due to alterations at the level of either LHC or reaction center. To confirm this, an attempt has been made by isolating thylakoid membranes from HT treated leaves. Analysis of absorption and fluorescence spectra of thylakoid membranes provide information about photosynthetic pigments involvement in the photosynthetic process and in photo chemical reactions. Hence the absorption spectra of thylakoid membranes of control and HT treated samples were measured by scanning the spectra from 400 to 700 nm. The peak at 679 nm indicates the presence of Chl *a* and the peak at 440 nm indicates the absorption peak of Chl *a* at soret region of the spectrum and the shoulder at 650 nm indicates the presence of Chl *b* and another shoulder at 480 nm indicates the presence of carotenoids and xanthophylls. HT treatment mainly caused the decrease in carotenoid region in addition to chlorophyll peaks. Table 1 shows the effect of

HT on the absorption ratios of Chl (679/440), carotenoid ratio (679/480), Chi *a* to (679/650). The increase in temperature gradually caused an enhancement in the ratio from 0.75 to 0.9 in chlorophyll ratios whereas the ratio between 679/480 has gone up from 1.05 to 1.21 due to treatment of HT from 25ºC to 45ºC. The rise in the temperature 25ºC to 45ºC changed the absorption spectra of Chl *a* to Chl *b* from 2.31 to 1.72.

Table 1: Effect of HT on absorption properties of thylakoid membranes isolated from wheat primary leaves.

Temperature (ºC)	Absorption ratios		
	679/440	679/480	679/650
25	0.75	1.05	2.01
30	0.78	1.12	1.93
35	0.82	1.16	1.81
40	0.90	1.21	1.72

Thus, it is clear that Chl *a* is the main target to HT when compared to other photosynthetic pigments. Since the absorption properties of thylakoid membranes is related to the fluorescence, an attempt has been made to study the HT effect on chlorophyll fluorescence emission spectra as well as Chl *a* fluorescence kinetics. Up to now, several investigators have used fluorescence kinetics as a tool to study the photochemical reactions in the thylakoid membranes (Papageorgiou, 1975; Singhal *et al.*, 1981; Fork and Mohanty, 1986). Upon excitation of' thylakoid membranes with 440 nm light beam, an emission spectrum with a peak at 686 nm emanating from Chl *a* was prominent in the spectra. HT treatment at 40ºC caused decrease in the fluorescence emission intensity without causing any shift in the peak position. Table 2 shows the temperature dependent effect on Chl *a* fluorescence of wheat thylakoids. The rise in the temperature from 25ºC to 40ºC caused 37% loss in the fluorescence emission intensity. According to earlier studies, it is clear that the loss of fluorescence intensity demonstrates the presence of inhibitory site at donor region of the water oxidation complex. Therefore, HT is mainly affecting either the cofactors or polypeptides of WOC in the thylakoid membranes. As a result, the loss in the fluorescence intensity was noticed. To verify the existence of another inhibitory site near acceptor region, attempts were made to measure the Chl *a* fluorescence emission spectra in the presence and absence of diuron. It is known as an electron transport inhibitor which binds to Q_B protein of thylakoid membranes and impairs the PS II photochemistry.

Table 2: Effect of HT on room temperature chlorophyll a fluorescence of wheat thylakoid membranes.

Temperature (ºC)	Fluorescence intensity (rel. units)	Peak position, nm	Percent loss
25	65	685	0
30	56	684	14
35	49	680	25
40	41	677	37

After measuring the spectra. the ratios both in the presence and absence of diuron against temperature are mentioned in the above Table 3. The treatment of HT caused the decrease in the ratio from 1.6 to 1.08. This decrease in the ratio indicates the impairment of PS II by HT.

Table 3: Effect of HT on room temperature Chl fluorescence in the presence (10 µM DCMU at 25ºC) and absence of DCMU.

Temperature (ºC)	Fluorescence intensity (rel.units)		Ratio +/-
	F685 (-DCMU)	F685 (+DCMU)	
Control	62 ± 3	99 ± 7	1.6
25	58 ± 4	80 ± 6	1.4
30	50 ± 3	60 ± 5	1.2
35	40 ± 2	45 ± 4	1.1
40	35 ± 2	38 ± 3	1.08

Discussion

Temperature is one of the ecological factors which influence the plant growth and development. The temperature ranges from near freezing point in arctic zone to 50ºC in the hottest deserts. In addition, due to revolution of industries mainly CO_2 is getting accumulated in the atmosphere. This accumulation of CO_2 causes greenhouse effect and raises the temperature in the atmosphere. Often plants are getting exposed or subjected to variety of seasonal temperatures due to fluctuations in the diurnal temperatures. However, some of the plants are able to grow in the high temperature by synthesizing important and new polypeptides which act as molecular chaperones. They provide thermotolerance to the organisms to overcome the stress conditions in the environment. Before discussing the temperature tolerance, it is important to know the specific alterations induced by high temperature in the selected plant system.

Up to now majority of the studies were coming out in higher plants by isolating thylakoid membranes (Thomas *et al.,* 1986). Further, most of the studies were carried out under in *vitro* conditions regarding the HT effect. Therefore, critical studies are required under *in vivo* as well as *in vitro* conditions to have an integrated approach of the problem. The above studies can provide information to identify the specific targets of action as a response to HT in the primary process of photosynthesis.

Since, wheat *(Triticum aestivum)* is rich in proteins and related essential amino acids, the above plant system has been taken as an experimental material for the present study. Even from literature also it is clear that the above wheat species is highly susceptible to the high temperature. Based on the above facts the variety 'Kalyanasona' has been selected as experimental material and HT effects were studied both under in *vitro* and *in vivo* conditions. Since, primary reactions of photosynthesis determine the plant productivity, the effect of HT was studied on the above variety of wheat system. This variety has been consumed by northern part of India as staple food. Therefore, in the present study HT effect has been studied in the photosynthetic electron transport machinery of wheat plants of 8 days old by subjecting them for 20 min to HT ranging from 25 – 50ºC. To confirm the alterations in the LHC II attempts were made to study the effect of HT on spectral properties of different pigment proteins. From the spectral properties it is clear that Chl *a* is getting more affected by HT than that of Chl *b* (Fig. 1). Since absorption properties are related to fluorescence and energy transfer, a study has been under taken to characterize the effect of HT on fluorescence spectral properties. At room temperature, Chl *a* fluorescence mainly comes from PS II (Papageorgiou, 1975; Singhal *et al.*, 1981; Fork and Mohanty, 1986). HT caused gradual decrease in the fluorescence intensity by 37% (Table 2). Any decrease in the Chl *a* fluorescence emission at room temperature could be due to existence of inhibitory site at WOC level. To verify the presence of additional inhibitory site at reducing side, attempts were made to measure Chl *a* fluorescence both in the presence and absence of diuron (Table 3). Diuron is an inhibitor which impairs the electron flow at the reducing side of PS II (Trebst, 1974). HT treatment induced the change of ratio in Chl fluorescence in the presence and absence of DCMU from 1.6 to 1.08. This change of ratio clearly indicates the existence of another inhibitory site at reducing side of PS II near PQ. To identify the alterations in the LHC II, Chl *a* fluorescence kinetic measurements were made using PAM fluorimeter. The temperature treatment from 30°C to 40°C caused increase in the F_o value and 50% loss in F_v value. The increase in the F_o value is a clear indicator of alterations of LHC in PS II. The loss of F_v clearly shows the

inhibition of PS II catalyzed electron transport. Similar results have been earlier reported by Campbell *et a1* (1988) showing the damage of LHC II in PS II.

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