EXPLOITATION OF ALTERED SPECTRAL PROPERTIES OF THYLAKOID MEMBRANES AS A PROBE TO MONITOR HIGH TEMPERATURE STRESS

Hemalatha, K and Murthy, S.D.S*

Department of Biochemistry, Sri Venkateswara University, Tirupathi-517502.India.

ABSTRACT: In this communication, the effect of high temperature has been studied on photosynthetic electron transport by using spectral properties of thylakoid membranes as a tool by using maize thylakoid membranes as an experimental system. High temperature (30°C-45°C) treatment specifically caused the suppression of carotenoid absorption without affecting other pigment proteins. The decrease in the chlorophyll a fluorescence is an indication regarding existence of inhibitory site at donor region of photosystem II in maize thylakoid membranes. Pulse amplitude modulated fluorescence kinetics measurement clearly demonstrated that alterations in light harvesting complex of photosystem II through Fo rise in spectrum.

Key words: Fluorescence, High temperature, Maize primary leaves, Photosystem II

*Corresponding author: Murthy, S.D.S, Department of Biochemistry, Sri Venkateswara University, Tirupathi-517502.India E-mail: Email: sdsmurthy@rediffmail.com

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INTRODUCTION
Photosynthesis, both at intact leaf and organelle levels, is highly sensitive to thermal stress [1, 2]. Elevated temperature inhibits the thylakoid membrane activity more than the activity of other stroma enzymes [3]. Photosystem (PS) II is more sensitive to elevated temperature than PS I [1]. The heat induces loss of PS II activity is largely related to inactivation of oxygen evolving complex [4, 5]. Nevertheless, the PS II activity per unit chlorophyll amount was stimulated in maize chloroplasts that greened at 40°C for 24 and 48 h [6]. PS II inactivation is mainly associated with the loss Mn²⁺ and Cl'ions [7, 5 and 8]. Elevated temperature treatment stimulates the PS I catalyzed electron transport [9, 10 and 11]. The stimulation of the PS I activity is a consequence of temperature mediated uncoupling of thylakoids [12, 13]. However, uncoupler mediated PS I electron transport studies on temperature stressed thylakoids have indicated that this stimulation is not fully related to the thylakoid uncoupling of phosphorylation but also rises due to some structural alteration(s) of thylakoid membrane which affect the oxidizing side of PS I [10, 14].
As heat induced changes affect differentially the PS II and the PS I functions, we have investigated the effect of elevated temperature on chlorophyll a fluorescence emission and kinetics in thylakoid membranes of maize primary leaves.

**MATERIALS AND METHODS**

Maize (Zea mays) seeds were obtained from Acharya N.G Ranga agriculture college, Tirupati. After raising the seedlings in dark they were provided with Hoagland solution and the seedlings were raised upto 8th day and primary leaves were obtained for experiment purpose. The well germinated seedlings containing petriplates of 8th day old plants were selected and to high temperature stress (30-45°C) at influence rate of 20Wm² for 15 min.

Absorption spectra of thylakoid membranes were recorded at room (25 o) on a Hitachi 557 spectrophotometer. Thylakoid membranes were suspended in a medium 50mM HEPES-NaOH (pH 7.5) 100mM sucrose, 2mM MgCl₂ and 5mM KCl the spectra’s were normalized at 700 nm to give the same absorption and recorded from 400-700 nm. Fluorescence emission spectra of thylakoids were recorded in absence of 10µM DCMU at 25°C using Hitachi MPE4 spectrofluorimeter. Thylakoid membrane equivalent to by 5µg of Chl/ml were suspended in a buffer containing 50mM MgCl₂ and 5mM KCl. The samples were excited at 440 nm in a slit width of 5 nm. the emission collected from 600 to 750 nm. Pulse amplitude modulated Chl a fluorescence measurements were based on a new pulse modulation measuring priciple. The sample is excited repetitively at a frequency of 1.6 or 100 KHz by 1µ sec light pulses from a light emitting-diode (LED). The pulsed fluorescence signal received by the photodiode is selectively amplified and by a newly developed selective window amplifier. The intensity of weak modulated light was 1mWm⁻² with a modulation frequency of 100 KHz and the intensity of red actinic light (> 680 nm) was 60 Wm⁻². Cell suspension equivalent to 8 µg of Chl was used for kinetic measurements.

**RESULTS AND DISCUSSION**

In this research article an attempt has been made to exploit the fluorescence technique as a tool. To characterize the alterations in the maize primary leaves under high temperature. To achieve this objective, 8 day old seedling maize were subjected to high temperature stress (30°-45°C) for 15 min in dark and then thylakoid membrane have been isolated and used for the spectral measurements. Table-1 shows the absorption ratio of different pigments related to photosynthesis. High temperature treatment caused slight decrease in the absorption ratio of chlorophyll a soret band and chlorophyll a absorption at red region of spectra. Surprisingly the ratio of absorption between carotenoids and chlorophyll drastically decrease to 0.88 to 0.72. The ratio between Chl b and Chl a didn’t change much unlike the case of carotenoids. Similar reports were earlier made by (Ramanaiah et al., 2003) in the case of cyanobacterium, Spirulina platensis [15]. Since absorbance is related to fluorescence, Chl a fluorescence of thylakoids was measured by exciting the sample with 440 nm light. In control sample a peak at 677 nm was prominent as shown in Table-2. High temperature induced decrease in the fluorescence intensity and shifted the peak from 677 nm to 673 nm. At 45°C of high temperature treatment, fluorescence intensity was decreased by 41%. This loss in fluorescence indicates the existence of inhibitory site at donor region of PS II. Earlier similar reports were made by Sudheer et al., (2011) in barley thylakoids under UV-B radiation stress [16]. To verify the susceptibility of LHC, high temperature effect was studied on Pulse amplitude modulated fluorescence kinetics (Fig-1). The excitation of the sample with low light intensity cause the fluorescence to reach a point called initial fluorescence (F₀) and the further excitation of the sample of strong light caused the fluorescence to reach at a point called maximum fluorescence (Fₘ). The difference between Fₘ and F₀ is known as variable fluorescence (Fᵥ). The height of Fᵥ indicates the extent of photo chemistry in PS II. High temperature (40°C) caused increase in F₀ value and 60% loss in Fᵥ. This loss in the Fᵥ indirectly explains the decrease in PS II activity. Rise in F₀ clearly depicts the alterations in LHC of PS II. Earlier similar observations were made by Murthy et al., (1990) in the case of cyanobacterium, Spirulina platensis under high temperature stress [17]. Thus Chl a can be used as a probe to identify the alterations in PS II photochemistry in higher plants.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Absorption ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>440/680</td>
</tr>
<tr>
<td>30</td>
<td>1.11</td>
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<td>35</td>
<td>1.09</td>
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<td>40</td>
<td>1.05</td>
</tr>
<tr>
<td>45</td>
<td>1.03</td>
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Table -2: Effect of high temperature on Chl a fluorescence emission spectra of maize thylakoid membranes

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Chl a fluorescence Intensity (rel. units)</th>
<th>Peak Position (nm)</th>
<th>Percentage of loss</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>45</td>
<td>40</td>
<td>673</td>
<td>41</td>
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</table>

Fig-1: PAM fluorescence kinetics of Chl a fluorescence of maize thylakoids.

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REFERENCES


