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# Exploitation of Chlorophyll a Fluorescence as a Tool to Monitor the UV-B Radiation Stress Induced Alterations in Photosystem II Photochemistry of Barley Primary Leaves

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**Abstract:** In this investigation an attempt has been made to study the effect UV-B radiation induced alterations in the thylakoid membranes isolated for UV-B (2-6 $Wm^{-2}$) exposed plants. Spectral measurements clearly demonstrated that chlorophyll *a* pigment proteins are main targets for UV-B exposure, Chlorophyll *a* fluorescence measurements at both room and low temperature (77 K) gave clear indication that light harvesting complex II is main target for the action of UV-B stress. The chlorophyll *a* fluorescence kinetics clearly demonstrated that light harvesting complex II is highly susceptible to UV-B radiation as evidenced from the increase of $F_0$ value in the fluorescence kinetics of treated sample.

**Abbreviations:** Chl-Chlorophyll; PS-Photosystem; UV-B radiation-Ultra violet-B radiation

**Key words:** Absorption · Barley primary leaves · Energy transfer · Fluorescence · UV-B radiation

## INTRODUCTION

Depletion of ozone layer by different chemicals and environment pollutants leads enhanced levels of UV-B radiation on earth surface [1]. This ozone layer is useful to absorb UV-B radiation and protect the plants from its damage. It also affects the important plant biological process called photosynthesis, which determine in the plant productivity and biomass production [2-4]. UV-B radiation is known to affect the photosynthesis in preferential manner [3]. The electron transport activity catalyzed by PS II is very much sensitive to UV-B radiation [5]. UV-B induced damage in PS II has been monitor by using spectroscopic technique is called chlorophyll (Chl) fluorescence [6]. The UV-B effect was characterized by using Chl *a* fluorescence using intact leaves [7]. UV-B causes the change in the rate of degradation and turnover of $D_1$ polypeptide of PS II [8]. UV-B also causes the functional disconnection between light harvesting complex and reaction centre of PS II [9]. In cyanobacteria UV-B radiation affects the energy transfer between phycocyanin and chlorophyll *a* [10-12]. Studies related to the exploitation of Chl *a* fluorescence as a tool to assays the photochemistry of PS II in barley leaves under the influence of UV-B radiation are scanty. Therefore an attempt has been made to study the effect of UV-B radiation on spectral properties and energy transfer in barley thylakoid membranes.

## MATERIALS AND METHODS

Barley (*Hardeum vulgare*) seeds were obtained from Acharya N.G Ranga agriculture college, Bapatla. After raising the seedlings in dark they were provided with Hoagland solution and the seedlings were raised upto on 8$^{th}$ day and primary leaves were obtained for experiment purpose. The well germinated seedlings containing petriplates of 8$^{th}$ day old plants were selected and exposed to UV-B radiation at influence rate of different intensities ranging from 2-6 $Wm^{-2}$ (Obtained from Philips TL 20 type 05 type in the spectral range of 280-320 nm and with a peak at 312 nm) for 10 to 30 min.

Absorption spectra of thylakoid membranes were recorded at room (25°C) on a Hitachi 557 spectrophotometer. Thylakoid membranes were suspended in a medium containing 50 mM HEPES-NaOH (pH 7.5) 100 mM. sucrose, 2mM $MgCl_2$ and 5mM KCl the spectras were normalized at 750 nm to give the same absorption and recorded from 400 to 750 nm. Fluorescence emission spectra of thylakoids were recorded in absence

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and in presence of 10 µM DCMU at 25 °C using Hitachi *MPE4* spectrofluoremeter. Thylakoid membrane equivalent to 2 µg of Chl/ml were suspended in a buffer containing 50 mM HEPES-NaOH (pH 7.5) 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl. The samples were excited at 440 nm is a slit width of 5 min. The emission was collected from 640 to 740 nm via a 5 nm slit for width of 5 nm. The emission spectral measurements at low temperature (77 K), the samples were prepared by mixing equal volumes of thylakoid suspension and 60 % glycerol. Samples were taken in to micro capillaries tubes and frozen quickly in a specially constructed Dewar flask which fits in the corvette position of Hitachi *MPE4* spectrofluoremeter. The samples were excited at 432 nm and emission was collected from 650 nm to 750 nm. The slit widths of excitation and emission monochromators were 8 and 2 nm respectively.

## RESULTS AND DISCUSSION

To study the effect of UV-B radiation on absorption properties, thylakoids have been isolated from UV-B treatment given barley primary leaves (Fig 1). UV-B treatment caused decrease in absorption capacity of Chl *a* under both red as well as blue region of the spectrum. In addition it caused decrease in the carotenoid absorption capacity. Since absorption is related to the Chl fluorescence and PS II photochemistry, thylakoids have been excited with 440 nm light. In fluorescence emisswion spectra, an emission peak emanating light from PS II Chl is observed (Fig 2). The exposure of UV-B light caused 23% decrease in fluorescence intensity of treated thylakoid membranes. According to literature it is clear that if there is a decrease in Chl fluorescence it indicates the alterations at the level of oxidizing side of PS II [13]. Similarly if Chl fluorescence increase is observed when compared with control, it indicates the damage at PS II reducing side. Therefore results showed that there is an alteration at WOC as evidensed from Chl fluorescence kinetics. Similar observations have been made by other workers in higher plant system radish seedlings [14].

For further confirmation of the presence of the target site at reducing side, Chl fluorescence has been measured in the presence and absence of dicron. In control sample the ratio between the presence and absence of dicron is found to be 1.5 (Fig 2). The increase in the UV-B radiation treatment brought the decrease in the ratio from 1.5 to 1.1. This again confirms the existance of additionl site of inhibition near reducing side of PS II. Similar observations have been reported under the influence of high temperature in wheat plants [15].

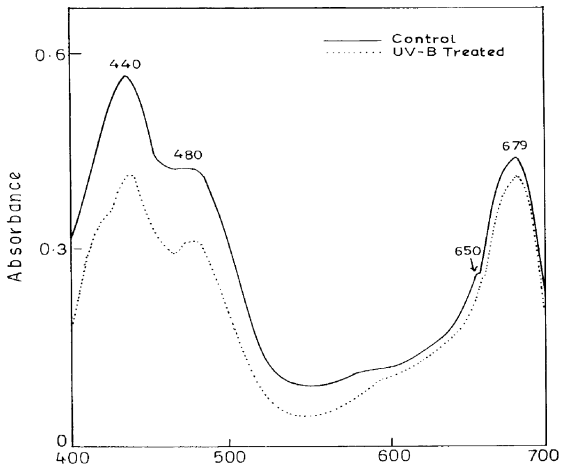


Fig. 1: Absorption spectra of thylakoid membranes isolatwed from control and UV-B treated barley primary leaves.

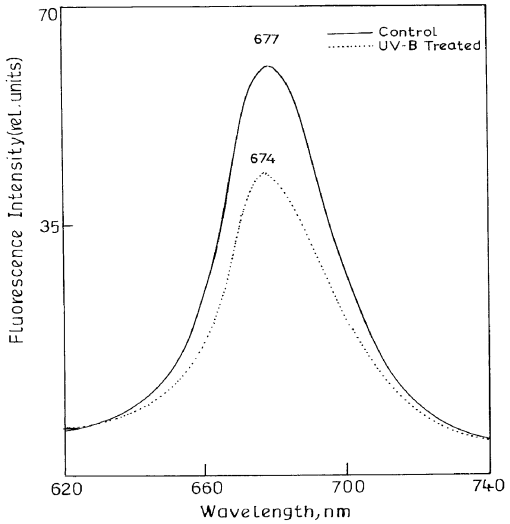


Fig. 2: Room temperature chlorophyll fluorescence emission spectra of thylakoid membranes isolated from control and UV-B treated barley primary leaves

To rule out the effect of UV-B on energy distribution between two photosystems, we have measured the Chl fluorescence emission spectra at low temperature (77K) by exciting with 440 nm light (Fig 3). To measure the Chl fluorescence the thylakoid membranes have been frozen at 77 K in the presence of liquid nitrogen and excited with 440 nm light beam. In control fluorescence emission spectra three peaks were clearly resolved. The peak at 679 nm is due to the presence of LHC II of PS II and peak at 695 is due to the reaction centre Chl. These two peaks compared to CP 43 and CP47 Chl of PS II (Fig 3).

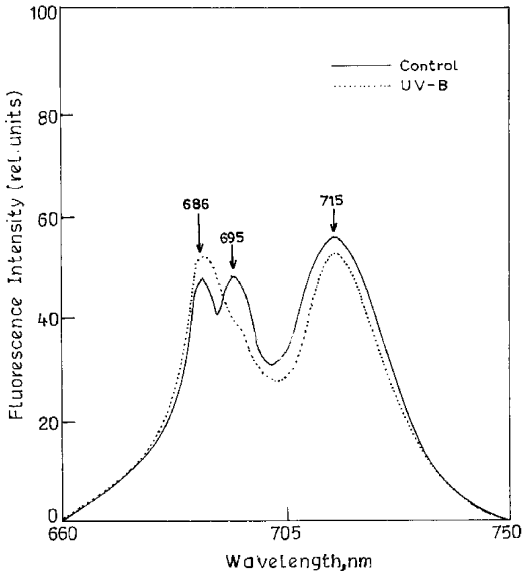


Fig. 3: Low temperature (77 K) chlorophyll fluorescence emission spectra of thylakoid membranes isolated from control and UV-B treated barley primary leaves.

The peak at 728 nm is contributed by the PS I Chl proteins [16]. This peak is observed from PS I only at 77 K. The UV-B treatment caused the complete loss of 695 nm peak indicating the damage on the Chl protein of PS II mostly CP 47. Thus UV-B, specifically alters the PS II reaction centre and causes damage to PS II photochemistry. The similar observation in the cyanobacterium *Synechococus* 6301 under the influence of low concentrations of mercury was made by [17]. These fluorescence measurements clearly show the targets for UV-B action in thylakoid membrne and the fluorescence can be used as a tool to identify the inhibitory site during the stress induction.

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