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DIFFERENTIAL EFFECT OF UV-B RADIATION ON PHOTOSYNTHETIC ELECTRON TRANSPORT OF THE CYANOBACTERIUM *SPIRULINA PLATENSIS*

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ABSTRACT

In the present study an attempt has been made to study the effect of Ultraviolet-B (UV-B) radiation (0.7-2.8 Wm^{-2}) for different time intervals of (15-60 min) on the electron transport activities (Whole chain electron transport activity, photosystem II catalysed electron transport activity, photosystem I catalysed electron transport activity) and lipid peroxidation in the cyanobacterium, *Spirulina platensis.* To know the inhibition in the electron transport activity various types of donor - acceptor systems are used. Our results clearly demonstrated that there is an inhibition in whole chain electron transport activity, photosystem II is more susceptible to UV-B radiation than that of photosystem I and also alterations in the membrane is cleared by the lipid peroxidation.

KEYWORDS: Electron transport, Photosystem, Lipid peroxidation, *Spirulina platensis.*

INTRODUCTION

Ultraviolet (UV) radiation is one of the serious issues since past few decades due to industrialization and environmental pollution. The ultraviolet region of the electromagnetic spectrum coming from sun is subdivided into three bands termed UV-A (315- 400nm), UV-B (280-315nm) and UV-C (200-280 nm). Due to the depletion of ozone layer by the harmful pollutants results in the entry of these harmful UV rays to the earth surface[1] (Crutzen,1992). These harmful UV rays reaching the earth surface effects the production of algae and

photosynthetic macrophytes as they can easily absorb UV light by biomolecules such as nucleic acids and proteins.[2], 2005;[3]. Cyanobacteria which is representing an important group of algae that is playing a key rle in the present global ecology especially in the marine system. These are oxygenic photosynthetic prokaryotes whose photosynthetic apparatus shows resemblance to those of higher plants[4]. Plants and cyanobacteria contain similar photosystem (PS) II and PS I reaction center complex.

Cyanobacteria do not consist of chloroplast but contain naked membrane system inside the cell, known as the photosynthetic membrane (or thylakoid membrane), which contains most of the proteins required for the light reactions. The proteins required for the fixation and reduction of CO_2 is located outside the photosynthetic membrane in the surrounding aqueous phase. The thylakoid proteins are arranged into four membrane- protein complexes to perform photosynthetic electron transport. They are PS II complex, Cyt b_6 *f* complex, PS I complex and ATP synthase.[5,6,7] Various factors like morphology, growth, survival, cell differentiation, pigmentation, motility, N_2 metabolism, phycobiliproteins, composition of protein, DNA, CO_2 uptake are severely affected by the harmful UV radiation when absorbed by cyanobacteria.[8,9] UV-B has been shown to interrupt the flow of electrons at multiple sites. PS II is the membrane protein complex found complex found in oxygenic photosynthetic organisms (higher plants, green algae and cyanobacteria), which harnesses light energy to split H_2O into O_2, protons and electrons.[10,11,12] PS II Is surrounded by its light harvesting antenna which is comprised of the inner minor antenna complex (built by CP24, CP26 and CP29, encoded by the genes LHC b 4, 5 and 6) and the outer major antenna complex LHC II.[13,14] PS II is more susceptible than PS I by the effect of UV-B radiation. The three major kinds of membrane lipids are phospho-lipids, glycolipids, and cholesterol. The thylakoid membrane is unique in plant cell in having a high proportion of glyceroglycolipids and a high proportion of phospholipids. The glycerolipids are monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) and sulpholipids (SQDG) and they account for 40-50%, 20-30% and 5-10%, respectively. Due to the effect of UV-B radiation the membrane lipid layer will be damaged. UV-B is able to cause the lipid peroxidation of the thylakoid lipids by means of increase in the free radical formation.[15,16]

MATERIALS AND METHODS

Polarographic measurements of O_2 evolution or uptake activities in Clark-type oxygen electrode.

SPECTRAL MEASUREMENTS

Absorption spectra of intact cells

After harvesting the cells, the pellets were suspended in 3 ml of reaction buffer. The absorption spectra of intact cell suspension were taken by using a Hitachi-557 double beam spectrophotometer. At 750 nm the absorption of the cell suspension was adjusted to give approximately the same reading. The 77K absorption spectra were monitored using the low temperature accessory in a Hitachi-557 spectrophotometer. For 77K spectral measurements, the samples were prepared in 70 % glycerol in buffer. Cells equivalent to 6 µg of Chl were used in absorption measurements.

Lipid peroxidation

Lipid peroxidation (LPO) has been measured according to the method of Heath and packer[17]. The malondialdehyde (MDA) calculations were made by using the extinction coefficient 155 mM^{-1} cm^{-1}. The amount of MDA was expressed as n moles of MDA per mg protein.

RESULTS AND DISCUSSION

Depletion in the ozone layer causes the entry of UV-B radiation to the earth surface both in aquatic and terrestrial bodies. Therefore, aquatic and terrestrial organisms respond in a different way to the UV-B radiation which leads to the damage in their physiology. In this investigation a study has been made to analyse the impact of UV-B radiation on the photosynthetic electron transport and lipid peroxidation in the cyanobacterium, *Spirulina platensis.* Studies are made on photosynthetic electron transport activities under the influence of different intensities of UV-B radiation. To verify the above preposition, intact cells of *Spirulina platensis* were exposed to different intensities of UV-B radiation (0.7-2.8 Wm^{-2}). After giving the treatment whole chain electron transport activity ($H_2O \rightarrow MV$) was measured by using methyl viologen (MV) as terminal electron acceptor. The control sample exhibited a high rate of O_2 consumption in the activity of whole chain electron transport. The exposure of different intensities of UV-B radiation (0.7-2.8 Wm^{-2}) under continuous stirring caused inhibition in whole chain electron transport activity. Almost 49% inhibition was observed at 2.1 Wm^{-2} further increase in the treatment of UV-B leads to increase in the

percentage of inhibition (Fig 1a). To confirm the above result at time duration additionally time dependent effect of 2.1 Wm^{-2} UV-B radiation treatments is given for different time intervals (15- 60 min) were studied. The control sample exhibited a high rate of O_2 consumption in the activity of Whole chain electron transport. The treatment of cells to 2.1 Wm^{-2} for 15 min showed decrease in the rate of O_2 consumption about 32% inhibition in whole chain electron transport activity. Increase in the time duration to 30 min exhibited decrease in the rate of O_2 consumption about 50% inhibition in the whole chain electron transport activity. Further increase in the time duration to 45 min and 60 min exhibited maximum decrease in the rate of O_2 consumption of almost about 65% and 76% inhibition in the whole chain electron transport activity (Fig: 1b). This inhibition in whole chain electron transport could be due to alterations at two levels *i.e*. inhibition at PS II level or at PS I level has been earlier discovered by several workers.[18]

From the above results it is clear that alterations occurred at the level of photosystems. To identify the target photosystem an attempt has been made to measure the partial electron transport reactions between PS II and PS I. Since parabenzoquinone (pBQ) is known to accept the electrons from PQ pool.[19] It has been employed as an accepter of PS II to measure Hill reaction. The control sample exhibited a high rate of O_2 evolved in PS II catalysed electron transport activity. The exposure of different intensities of UV-B radiation (0.7 - 2.8 Wm^{-2}) under continuous stirring caused almost upto 47% - 70% inhibition in PS II catalysed electron transport activity (Fig 2). The reason for the inhibition in the PS II catalysed electron transport activity could be due to the alterations at the level of oxidizing site or changes in the reducing side of PS II. UV-B is able to cause loss in PS II catalysed electron transport by inactivation of electron transport carriers and suppress energy transfer between the LHC and RC.[20,21,22]

Unlike the situation with the whole chain and PS II catalyzed electron transport, PS I mediated reactions could not be assayed in the intact cells of *Spirulina* as reduced Dichlorophenolindophenol (DCPIP)/ tetramethyl phenyl durene (TMPD)/ diaminodurene (DAD) did not readily enter into the intact cells. Therefore, thylakoid membrane fragments have to be prepared to study the effect of different intensities of UV-B radiation on PS I catalysed electron transport activity. These prepared thylakoid membranes are measured for the absorption spectra to obtain a spectral graph in visible region *i.e* 400- 700 nm (Fig 3). The absorption characteristics of control cells, the obtained spectral graph show different peaks. The

peak at 679 nm is due to absorption of Chl *a*, peak at 622 nm is due to absorption of PC of PBS, a hump at 485 nm is due to absorption of carotenoids and a peak at 436 nm is due to presence of Chl *a*. These membrane fragments did not evolve oxygen with pBQ as Hill oxidant. The electron transport catalyzed by PS I with reduced DCPIP, TMPD and DAD were measured (Table: 1). The DCPIP supported PS I activity was observed to be 415 µ moles of oxygen consumption. The rates were considering with the rates of chloroplast thylakoid membranes and with reduced DCPIP the rates are quite high when compared to other two donors. After giving the treatment of different intensities of UV-B radiation studies were made on PS I catalysed electron transport activities. The control sample exhibited a high rate of O_2 consumption in PS I catalyzed electron transport activity. At 0.7 Wm^{-2} intensity of UV-B radiation showed decrease in the rate of O_2 consumption almost 3% inhibition was noticed in PS I catalyzed electron transport activity. Further raise in the intensities to 2.1 and 2.8 Wm^{-2} exhibited decrease in the rate of O_2 consumption and almost 11% and 15% of inhibition in the PS I catalyzed electron transport activity (Fig:4). Marginal inhibition in PS I catalysed electron transport activity is observed. From this it is clear that PS I is getting less effected when compared to that of PS II under the given stress. Between two photosystems, PS II is more susceptible than PS I. To identify the possible target site in PS II a study has to be made to analyse the effect of UV-B (2.1 Wm^{-2}) at both light saturating (420 Wm^{-2}) and light limiting (12 Wm^{-2}) conditions (Table: 2). The inhibition in PS II activity was more at light saturating conditions (48%) than at light limiting conditions (36%). The inhibition at light limiting conditions of UV-B treated sample could be the alterations in the light harvesting complex pigment proteins of this cyanobacterium. The possible reason for the enhancement of inhibition at light saturating conditions by UV-B radiation in Hill reaction could be due to the presence of additional inhibitory site near PS II.

The changes under stress in the membrane organisation can also affect the functional aspects of photosynthetic electron transport chain besides PS II. Therefore, an attempt was made to study the effect of different intensities of UV-B radiation (0.7 Wm^{-2} - 2.8 Wm^{-2} for 30 min) on thylakoid membrane organisation. Lipids are rich in olefinic bonds and thus are the primary targets for oxidative reactions. Lipid peroxidation has been shown to increase under UV-B stress conditions. Hydroperoxides and Malondialdehyde (MDA) were often considered as indicators of membrane damage. The LPO was measured in terms of MDA formation in control thylakoids 46 n moles of MDA/mg of protein was observed (Fig: 5). Whereas in treated cells of UV-B radiation 2.1 Wm^{-2} for 30 min showed the increase in the MDA levels to 72 n moles of MDA/mg of protein. That is, it caused an enhancement of almost 57% in

lipid peroxidation. Thus alteration in energy transfer and change in LPO is responsible for altered PS II photochemistry in intact cells of *Spirulina*. UV-B is able to cause the lipid peroxidation of the thylakoid lipids by means of increase in the free radical formation.[15,16]

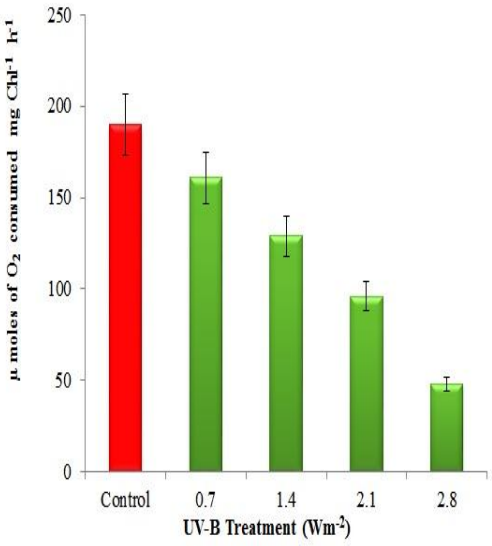

Fig : 1a Effect of different intensities of UV-B radiation (0.7- 2.8 Wm^{-2}) on Whole chain electron transport assay ($H_20 \rightarrow MV$) in the cyanobacterium,*Spirulina platensis*.

*Vertical bars indicate means ±SD; (n=3)

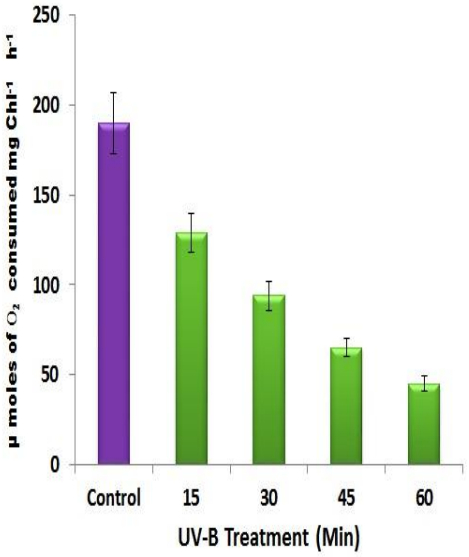

Fig : 1b Effect of different time intervals of UV-B radiation (2.1 Wm^{-2}) exposure (15-60 min) on whole chain electron transport assay ($H_20 \rightarrow MV$) in the cyanobacterium, *Spirulina platensis*.

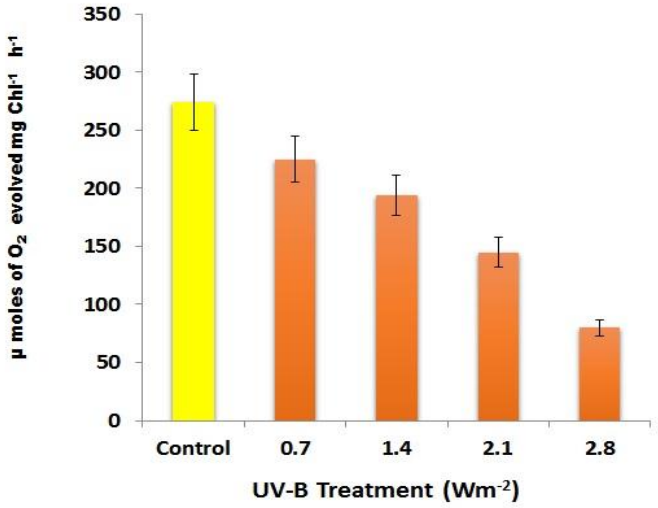

Fig : 2 Effect of different intensities of UV-B radiation (0.7- 2.8 Wm^{-2}) on PS II catalysed electron transport assay ($H_20 \rightarrow pBQ$) in the cyanobacterium,*Spirulina platensis*.

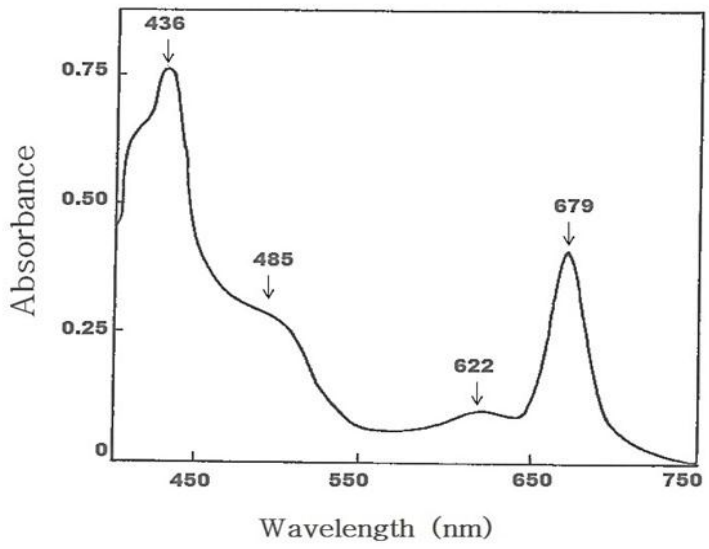

Fig: 3 Absorption spectra of thylakoid membranes isolated from *Spirulina platensis*.

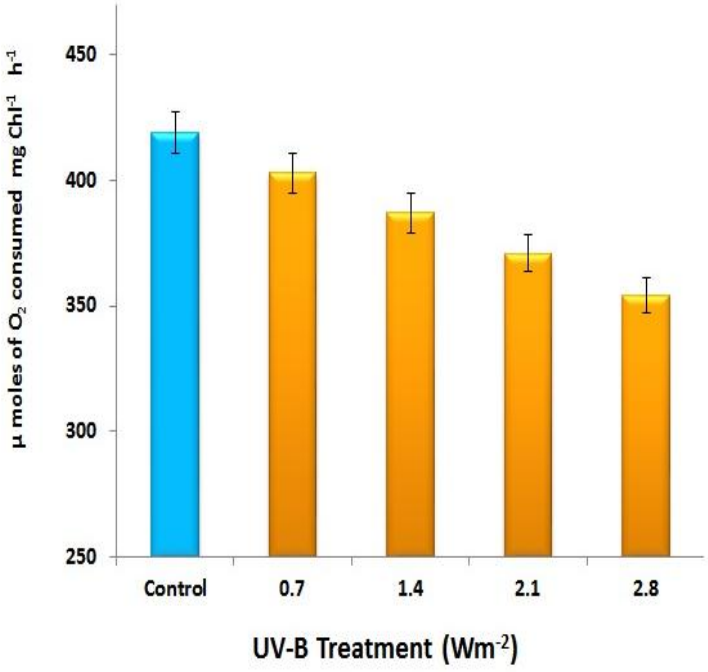

Fig : 4 Effect of different intensities of UV-B radiation (0.7 - 2.8 Wm^{-2}) on PS I catalysed electron transport assay ($DCPIPH_2 \rightarrow MV$) in the cyanobacterium, *Spirulina platensis*.

Table : 1 PS I catalyzed electron transport activity mediated by various donor systems in the thylakoid membranes of *Spirulina platensis*.

Assay	Photosystem I catalyzed electron transport activity ($DCPIPH_2 \rightarrow MV$) µ moles of O_2 consumed mg Chl/h
ASC + DCPIP →MV	415 ± 40
ASC +TMPD → MV	430 ± 43
DAD + TMPD → MV	509 ± 51

Table : 2 Effect of different illuminated light intensities on UV-B induced PS II catalyzed electron transport activity inhibition in the cyanobacterium *Spirulina platensis*. Other details were mentioned in the materials and method.

Light intensity (Wm^{-2})	PS II activity (µ mol O_2 evolved mg Chl^{-1} h^{-1}) ($H_2O \rightarrow pBQ$)		% inhibition
	Control	UV-B Treated (2.1 Wm^{-2})	
12	42 ± 4	27 ± 2	36
110	67 ± 6	40 ± 3	40
220	132 ± 11	74 ± 6	44
420	265 ± 23	138 ± 12	48

CONCLUSIONS

The effect of UV-B radiation was studied on photosynthetic electron transport activities, energy transfer studies, thylakoid membrane organisation After performing the work the following conclusions were drawn.

Among the tested UV-B radiation 2.1 Wm^{-2} for 30 min seems to be the potent to cause inhibition in whole chain and PS II catalysed electron transport in intact cells of *Spirulina*. Between two photosystems, PS II catalysed electron transport is more susceptible to UV-B than that of PS I. UV-B radiation causes alterations in the energy transfer of light from PC to Chl *a* in intact cells. Lipid peroxidation measurement of thylakoid membranes under UV-B

radiation clearly demonstrated that increase in the lipid peroxidation leads to the damage in the membrane.

REFERENCES

1. Crutzen, P.J., Ultraviolet on the increase. *Nature,* 1992; 356: 104-105.
2. Häder, D. P., and Sinha, R. P. Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact. *Mutation Res. Fund. Mol. Mech. Mutagenesis,* 2005; 571: 221-233.
3. Fernanda Pessoa, M. Harmful effects of UV-radiation in algae and aquatic macrophytes-A review. *Emir. J. Food Agri,* 2012; 24: 510-526.
4. Fromme, P and Grotjohann, I. Structure of cyanobacterial photosystems I and II. In : Bioenergetic Processes of Cyanobacteria , pp. 285-335. Springer Netherlands, 2011.
5. Croce, R., and van Amerongen, H. Light-harvesting and structural organization of photosystem II: from individual complexes to thylakoid membrane. *J. Photochem.Photobiol,* 2011; 104: 142-153.
6. Liberton, M., Page, L. E., O'Dell, W. B., O'Neill, H., Mamontov, E., Urban, V. S., and Pakrasi, H. B. Organization and flexibility of cyanobacterial thylakoid membranes examined by neutron scattering. *J. Biol Chem.,* 2013; 288: 3632-3640.
7. Carraretto, L., Formentin, E., Teardo, E., Checchetto, V., Tomizioli, M., Morosinotto, T., and Szabó, I. A thylakoid-located two-pore K+ channel controls photosynthetic light utilization in plants. *Sci.,* 2013; 342: 114-118.
8. Gao, K., Yu, H., Brown, M.T., Solar PAR and UV radiation affects the physiology and morphology of the cyanobacterium *Anabaena* Sp. PCC 7120. *J. Photochem. Photobiol,* 2007; 89: 117-124.
9. Sinha, R.P., Hader, D.-P., Life under solar UV radiation in aquatic organisms. *Adv. Space Res.,* 2002; 30: 1547-1556.
10. Anderson, B. and Styring, S. Phtosystem II: Molecular organization, function and acclimation. *Curt. Top.Bioenerg,* 1991; 16: 1-81.
11. Scmidth, E.C. Nunes, B.G., Maraschin, M. and Bouzon, Z.L. Effect of ultraviolet-B radiation on growth, photosynthetic pigments and cell biology of Kappphycus alvarezii (Rhodophyta, Gigartinales) macroalgae brown strain. *Photosynthetica,* 2010; 48: 161-172.

12. Yunsheng Lou,. Ren, L., Li, Z., Cheng, H., and Zhang, T. Effect of elevated ultraviolet-B radiation on microbial biomass carbon and nitrogen in barley rhizosphere soil. *Water, Air, and Soil Pollution*, 2011; 219: 501-506.
13. Susann, S., Latowski, D., Jemioła-Rzeminska, M., Dawood, A., Wilhelm, C., Strzałka, K., and Goss, R. Regulation of LHC II aggregation by different thylakoid membrane lipids. *Biochim. Biophys. Acta*, 2011; 1807: 326-335.
14. Kolyo Dankov, G., Dobrikova, A. G., Ughy, B., Bogos, B., Gombos, Z., and Apostolova, E. L. LHC II organization and thylakoid lipids affect the sensitivity of the photosynthetic apparatus to high-light treatment. *Plant. Physiol. Biochem*, 2011; 49: 629-635.
15. Vass, I., Szilard, A. and Sicora, C. Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus - In: Handbook of Photosynthesis (Pessarakli, M. edt). 2nd Ed, 2005; 827-833, Taylor and Francis, Boca Raton – London –New York – Singapore.
16. Gupta, R., Bhadauriya, P., Chauhan, V.S, and Bisen, P.S. Impact of UV- B radiation on thylakoid membrane and fatty acid profile of *Spirulina platensis*. *Curr. Microbiol*, 2008; 56: 156-161.
17. Heath, R.L. and Packer, L. Photoperoxidation in isolated chloroplast .I. Stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*, 1968; 125: 189-198.
18. Kulandaivelu,G., Geetha,V and Periyanan, S. Inhibition of energy transfer reactions in cyanobacteria by different ultraviolet radiation. In: Photosynthesis- Molecular Biology and Bioenergetics. (Singhal, G.S., Barber,J., Delley, R.A., Govindjee, Haselkorn, R and Mohanty, P.eds), 1989; 305-313, Narosa publishing House, New Delhi.
19. Trebst, A. Energy conservation in photosynthetic electron transport of chloroplasts. *Ann. Rev. Plant Physiol*, 1974; 25: 423-458.
20. Rajagopal, S., Murthy, S. D. S. and Mohanty, P. Effect of UV-B radiation on intact cells of the cyanobacterium, Spirulina platensis: characterization of the alterations in thylakoid membrances. *J. Photochem. Photobiol. Biol*. 1998; 54: 61-66.
21. Tevini, M., Plant responses to ultraviolet radiation stress. In: *Chlorophyll a Fluorescence a Signature of Photosynthesis* (Papageorgiou, G.C., Govindjee, eds.), 2004; 605-621, Springer, Dordrecht.
22. Lukins, P. B., Rehman, S., Stevens, G. B., and George, D. Time- resolved spectroscopic fluorescence imaging, transient absorption and vibrational spectroscopy of intact and photo-inhibited photosynthetic tissue. *Luminescence*, 2005; 20: 143-151.