STUDIES ON SHORT TERM EFFECT OF UV-B RADIATIONON PHOTO SYNTHETIC ELECTRON TRANSPORT ACTIVITIES IN THE RICE (ORYZASATIVA) PRIMARY LEAVES.

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Abstract: Ultraviolet-B (UV-B) radiation induced changes in the photochemical activities of the thy lakoid membranes isolated from Oryza sativa primary leaves have been characterized by using polarographic studies through oxygen electrode. UV-B radiation (8-12Wm-2) caused inhibition in whole chain electron (WCE)transport as well as photosystem IIcatalyzed electron transport activities in an intensity dependent manner. Almost50% inhibition was noticed in the above catalyzed electron transport activities after the exposure of 8Wm-2 UV-B radiation for30min.There as on for the loss PSII activity could be altered changes at the level of water oxidation complex (WOC). However, PSI catalyzed electron transport is less sensitive to UV-Bradiation (8-12Wm-2) and even at 12Wm-2only 16%losswas detected. Thus UV-Bradiation exerts multiple effects on photosynthetic electron transport based on the intensity and time of exposure in rice (Oryza sativa) leaves.

Keywords: Oryza sativa leaves, Electron transport activities, Thylakoid membranes,d UV-Bradiation.

Introduction: On physiochemical basis, the energy role of UV-B is of insignificant value. However, on the basis of its photo biological effects, it is extremely essential to the earth's biosphere. Since plants and terrestrial photosynthetic organisms in natural environment are necessarily exposed for long time periods to UV-B, it is often a source of significant stress to them [1], [2]. Analysis and assessment of more than 300 plant species have been accepted out and characterized the UV-B effects on photosynthesis results about 50% have been considered sensitive, 20-30% moderate sensitive and the rest insensitive to UV-B radiation [3]. In several sensitive plant species (e.g. wheat, rice, maize, rye), reduced leaf areas and/or stem growth was observed [4]. Photosynthesis is one of the mainly considered processes under UV-B accompanied essentially by growth experiments [5]. Even though there are varieties of UV-B targets in plants, it seems that the photosynthetic apparatus is among the main action sites of UV-B harm drastically contributes to the overall UV-B damage [6]. Proteins, photosynthetic pigments and unsaturated fattyacids of galactolipids, all present in the bilayered structure of the thylakoid membrane, may be UV-B targets due to their electronic absorption in the UV-B region [7], [8]. Yet, it is essential to discriminate involving direct damage, e.g. by absorption of high energy UV-radiation causing damages the molecules itself and indirect damage by reactive oxygen species (ROS) formed during the destruction. For example, ROS oxidize polyunsaturated fatty acids and generate reactive fatty acid peroxides, which further react with synthetic pigments[9].Moreover, it was found in green leaves that ROS may down-regulated the expression of photosynthetic genes [10]. Thus, it is not clear whether reductions in photosynthetic pigments as often found under high UV-B irradiances [11] are due to direct destruction or to biosynthetic defects following DNA damage. Direct damage to unsaturated membrane lipids was concluded from the formation of malondialdehyde[12], [13]. The investigations on short term effect of UV-B radiation on the primary process of photosynthesis in the main food yielding crop Oryza sativa are scanty. So in this regard the investigations are made on this main crop (Oryza) of Andhra Pradesh.

Materials and Methods: Rice (Oryza sativa) seedlings were raised in petriplates under continuous white light (160 μ moles m^{-2} s^{-1}) at 25ºC. Hoagland solution was supplied at 8 day intervals to the seedlings. 10 day-old seedlings were exposed to different doses of UV-B radiation (8-12Wm^{-2}) for 10-30 min. After the treatment primary leaves of both control and UV-B treated seedlings were sampled for thylakoid membranes isolation and assay of photochemical activities. The thylakoids were used for measurement of photochemical activities by following the procedure of [14] with slight modifications. The assay mixture for WCE transport activity consists of 0.5mM MV (Methyl viologen) and 1mM sodium azide in three ml of the 25μM HEPES reaction buffer (pH 7.6). For PS II mediated oxygen evolution, the reaction mixture contain of 0.5μM pBQ in 3 ml reaction buffer. PS I catalyzed assay mixture consists of 0.1 μM DCPIP (2, 6- Dicholorophenol-indophenol), 2 μMazide, 1 μM MV and 5 μM DCMU. The WCE transport assay mediated by DPC as electron donor contains 25 mM HEPES buffer (pH 7.3) 10 mM magnesium chloride and 5 mM potassium chloride in addition to 0.2 mM DPC, 1 mM sodium azide and 0.5 mM methyl viologen.

Results and Discussion: Agricultural activities and

widespread industrialization is accountable for the alterations in the distribution of stratospheric ozone layer in atmosphere. This ozone layer absorbs the UV-radiation and protects the living organisms on earth surface from harmful effects. Chlorofluorocarbons due to their chemical reactions are the main reason for the thinning of ozone layer and leads to the worse levels of UV-B radiation on earth surface. Along with overload CO_2 concentrations and water vapour, this UV-B radiation contributes for the global warming which in turn affects the plant, animal and microbial life on our planet. Photosynthesis is basic and necessary process on which our earth depends. This UV-B radiation can influence the primary processes of photosynthesis in a differential manner [15], [16].In this exploration a study has been made to investigate the effects of UV-B radiation (8-12 Wm^{-2}) on photosynthetic electron transport of the thylakoid membranes of rice primary leaves. For this purpose rice leaves were exposed to UV-B radiation as mentioned above for different time intervals by placing them in petriplates. After exposure to UV-B radiation, thylakoids have been isolated to measure the photosynthetic electron transport activities using oxygen electrode. Methyl viologen (MV) is known to accept the electrons from A_o in photosynthetic electron transport chain [17]. Hence, the whole chain electron transport activity has been calculated in thylakoid membranes using MV as terminal electron acceptor ($H_2O \rightarrow MV$). Control thylakoids devoid of UV-B treatment exhibited a high rate of O_2 consumption (182 µ moles of $O_2 \downarrow$ mg $Chl^{-1}h^{-1}$). Increase in the UV-B exposure from 8-12 Wm^{-2} brought improvement in the inhibition. Almost 43% loss was noticed at 12 Wm^{-2} and further raise in UV-B exhibited 65% of inhibition in whole chain electron transport activity (Table 1).

Table 1: Effect of UV-B radiation on whole chain electron transport assay($H_2O \rightarrow MV$) in the thylakoid membranes of rice primary leaves. The values are average of three separate experiments and SD is not more than 10%.

UV B radiation, Wm^{-2}	**Whole chain electron transport activity $H_2O \rightarrow MV$ µmoles of $O_2 \downarrow$ mg $Chl^{-1} h^{-1}$**	**Percent Inhibition**
Control	182 ± 19	0
8	144 ± 11	21
10	104 ± 9	43
12	64 ± 5	65

The possible reason for the loss of whole chain electron (WCE) activity could be either alterations at the level of PS II or at PS I or both. The UV-B radiation induced inhibition of WCE is also time dependant (Table 2).

Table 2: Time dependent effect of UV-B radiation on whole chain electron transport assay($H_2O \rightarrow MV$) in the thylakoid membranes of rice primary leaves. The values are average of three separate experiments and SD is not more than 10%.

Time of exposure Min.	**Whole chain electron transport activity H2O → MV µmoles of O2 ↓ mg $Chl^{-1} h^{-1}$**	**Percent Inhibition**
Control	185 ± 16	0
10	157 ± 13	15
20	136 ± 11	26
30	83 ± 6	55

To establish this, 8 Wm^{-2} of UV-B radiation was selected and Oryza sativa plants were exposed to different time intervals (10-30 min). Only after 30min of exposure, UV-B (Wm^{-2}) is able to induce 55% of inhibition in whole chain electron transport. Thus UV-B radiation induced inhibition is only dose dependant but also time dependant. To identify the target photosystem an attempt has been made to measure the partial electron transport reactions between PS II or PS I. Since parabenzoquinone (pBQ) is known to accept the electrons from PQ pool [17], [18].It has been employed as an acceptor of PS II to measure Hill reaction. Control thylakoid membranes exhibited a higher rate of O_2 evolution activity (223 µ moles of $O_2 \uparrow$ mg $Chl^{-1}h^{-1}$). UV-B treatment gradually caused the increase in the loss of PS II activity and 67% was noticed with 12 Wm^{-2} of UV-B radiation (Table 3).

Table 3: Effect of UV-B radiation on PS II assay($H_2O \rightarrow pBQ$) in the thylakoid membranes of rice primary leaves. The values are average of three separate experiments and SD is not more than 10%.

UV B radiation, Wm^{-2}	**PS II catalyzed electron transport activity $H_2O \rightarrow pBQ$ µmoles of $O_2 \uparrow$ mg $Chl^{-1} h^{-1}$**	**Percent ion loss**
Control	223 ± 21	0
8	167 ± 14	25
10	109 ± 9	51
12	74 ± 6	67

The possible reason for the loss of PS II activity could be either due to alterations at oxygen evolving complex (OEC) or due to changes in D_1 or D_2 polypeptides or action as reducing side of PS II. Artificial donors like Asc + DCPIP is known to donate electrons to PS I near $Cytb_6$ f. To establish the susceptibility of PS I, an attempt has been made to

measure PS I activity using artificial donor Asc + DCPIP as donor and MV as acceptor (Table 4).

Table 4: Effect of UV-B radiation on PS I assay($DCPIPH_2 \rightarrow MV$) in the thylakoid membranes of rice primary leaves. The values are average of three separate experiments and SD is not more than 10%.

UV B radiation, Wm^{-2}	**PS I catalyzed electron transport activity $DCPIPH_2 \rightarrow MV$ µmoles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}**	**Percent ion loss**
Control	327 ± 28	0
8	307 ± 27	6
10	294 ± 28	10
12	275 ± 25	16

Control thylakoid membranes exhibited a high rate of O_2 consumption of PS I activity equal to 327 µ moles of $O_2 \downarrow$ mg $Chl^{-1}h^{-1}$. The increase in the intensity of UV-B treatment for 8-12 Wm^{-2} caused enhancement in the inhibitory percent from 6 to 16%. The possible reason for the loss of marginal inhibition in PS I catalyzed electron transport would be due to alterations at the level of P_{700} or at reducing side of PS I catalyzed electron transport. This clearly demonstrates that there is a site interrupting electron transport and susceptible for UV-B action in rice thylakoid membranes.

Conclusion: UV-B radiation (8-12 Wm^{-2}) inhibits the WCE ($H_2O \rightarrow MV$) and PS II catalyzed electron transport in a dose dependent manner. Between two photosystems, PS II catalyzed electron transport is more sensitive to UV-B radiation when compared to that of PS I.The reason for the loss of PS II photochemistry may be due to altered changes at oxidizing side of PS II.

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