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UV-B RADIATION INDUCED ALTERATIONS IN PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF THE THYLAKOID MEMBRANES OF BARLEY PRIMARY LEAVES

S. D. SUDHEER, M. AYYA RAJU AND S. D. S. MURTHY*
Department of Biochemistry, Sri Venkateswra University,
Tirupati – 517 502, Andhra Pradesh, INDIA.
E-mail: sdsmurthy@rediffmail.com

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***Corresponding author**

ABSTRACT
Ultraviolet- B radiation induced changes in the photochemical activities of the thylakoid membranes isolated from barley primary leaves have been characterized by using polarographic studies through oxygen electrode. UV- B radiation (2 - 8 Wm^{-2}) caused inhibition in whole chain electron transport as well as photosystem II catalyzed electron transport activities in an intensity dependent manner. Almost 50% inhibition was noticed in the above catalyzed electron transport activities after the exposure of 4 Wm^{-2} for 30 min. The reason for the loss PS II activity could be alteration at the level of water oxidation complex. Contrary to the above, PS I catalyzed electron transport less sensitive to UV-B radiation (2 - 8 Wm^{-2}) and even at 8 Wm^{-2} only 20% loss was observed. But the measurement of intersystem electron transport mediated by DQH_2 showed that intersystem electron transport carriers like plastoquinone is sensitive to UV–B radiation and 40% loss was observed at 8 Wm^{-2} of UV-B. Thus UV-B radiation exerts multiple effects on photosynthetic electron transport based on the intensity of exposure in barley leaves.

INTRODUCTION

Depletion of stratospheric ozone by different environmental pollutants leads to the enhanced levels of UV –B radiation on earth surface (Smith *et al.,* 1992). This UV-B radiation affects the important plant biological process *i.e.* photosynthesis which determines the plant productivity and biomass production. (Tevini *et al.,* 1981;Bornman,1989; Murthy and Rajagopal, 1995; Bouchard *et al.*, 2006). UV-B is known to influence the photosynthetic process at multiple sites. Between two photosystems, photosystem (PS) II has been found to be highly susceptible to UV-B radiation in spinach (Renger *et al.,* 1989) Vigna (Noorudeen and Kulandaivelu, 1982). Even at higher levels of UV-B radiation, it causes the partial inhibition of PS I catalyzed electron transport (Iwanzik *et al*., 1983). Vass *et al*., (2000) reported that the reason for the altered PS II photochemistry is alterations at tyrosine of D_1 polypeptide. In this investigation an attempt has been made to identify the alterations in photosynthetic electron transport in different segments using oxygen electrode and varying electron donors and accepts in barley thylakoid membranes.

MATERIALS AND METHODS

Healthy seeds of barley (*Hordeum vulgare*) were used to raise seedlings and they were grown under the light intensity (15 Wm^{-2}) at 25 $\pm$1ºC. The nutrient solution of Hoagland was provided along with water for every two days to barley seedlings. The well germinated seedlings of eighth day old were exposed to UV-B radiation ranging from 2-8 Wm^{-2} for 30 min. and proceeded for isolation of thylakoid membranes using the procedure of Saha and Good (1970). Photochemical activities were measured polarographycally using Clark type oxygen electrode at saturating intensities of white light (450 Wm^{-2}) by the following the method of Sabat *et al.,* (1989). The 2 mL reaction mixture of whole chain electron transport contained 50 mM HEPES-NaOH (pH 7.5) buffer 0.5 mM Methyl viologen (MV) and 1mM sodium azide and thylakoid membranes equivalent to 30 μg of Chl. The PS II catalyzed electron transport assay mixture contained the above reaction buffer, 0.5 mM pBQ and thylakoid membranes equal to 30 μg of Chl. PSI reaction mixture comprises of 5mM ascorbate, 0.5 mM MV, 1.0 mM sodium azide, 0.1 mM DCPIP; 10 μM DCMU and thylakoid membranes equivalent to 30 μg of Chl. Intersystem electron transport assay mixture contained 5 mM ascorbate, diuroquinone, 10 μM DCMU, 0.5 mM MV and 1 mM sodium azide thylakoid membranes equivalent to that 30 μg of Chl.

RESULTS AND DISCUSSION

MV is known to accept the electrons for A_o photosynthetic electron transport chain (Trebst, 1974). Control thylakoids with out UV-B treatment exhibited a high rate of oxygen consumption (182 μmoles of $O_2\downarrow$mg Chl^{-1} h^{-1}). Increase in the UV-B treatment from 2 -8 Wm^{-2} brought enhancement in the inhibition of whole chain electron transport. Almost 50% loss was noticed after 4 Wm^{-2} of UV-B treatment (Table 1). The reasons for the loss of whole chain electron transport is either alteration at the level of PS II (Strid and Poora, 1992; Rajagopal

Table 1: Effect of UV B radiation on whole chain electron transport activity of thylakoids isolated from control and UV-B treated barley primary leaves. Other details were mentioned in "Materials and methods". The 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
2	144 ± 11	21
4	104 ± 9	43
6	64 ± 5	65
8	44 ± 3	76

Table 2: UV B radiation induced changes in PS II catalyzed electron transport activity of the thylakoid membranes isolated from control and UV-B treated barley primary leaves. Other details were mentioned in "Materials and methods". The 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
2	167 ± 14	25
4	109 ± 9	51
6	74 ± 6	67
8	51 ± 4	77

Table 3: Altered PS I catalyzed electron transport activity of the thylakoid membranes isolated from control and UV-B treated barley primary leaves under the influence of UV-B radiation. Other details were mentioned in "Materials and methods". The 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
2	307 ± 27	6
4	294 ± 28	10
6	275 ± 25	16
8	262 ± 24	20

Table 4: Effect of UV B radiation on Intersystem catalyzed electron transport activity of thylakoid membranes isolated from control and UV-B treated barley primary leaves. Other details were mentioned in "Materials and methods". The SD is not more than 10%

UV B radiation Wm^{-2}	Intersystem catalyzed electron transport activity $DQH_2 \rightarrow MV$ μmoles of $O_2 \downarrow$ mg Chl $^{-1}$ h^{-1}	Percent ion loss
Control	152 ± 13	0
2	138 ± 10	9
4	114 ± 9	25
6	96 ± 7	37
8	88 ± 6	42

et al, 2000) or at PS I (Prasad *et al*., 2005). Since UV-B radiation inhibited the whole chain electron transport, to identify the target photosystem, PS II catalyzed electron transport has been measured under the influence of UV-B. UV-B treatment caused gradual increase inhibition of PS II and at 4 Wm^{-2} the PS II activity got inhibited by 50%. The possible reason for the loss of PS II activity could either due to alterations at water oxidation complex (WOC) or due to changes at D_1 or D_2 polypeptide or due to modification at the level of reducing side of PS II (Renger *et al*., 1989; Friso *et al*., 1995; Vass *et al*., 2002; Bouchard *et al*., 2006). To rule out the susceptibility PS I catalyzed electron transport activity, DCPIP + Asc mediated PS I electron transport assay has been made in both control and UV-B treated samples. Only marginal inhibition 20% in PS II activity was observed due to UV-B exposure in thylakoids of barley primary leaves. These results are in agreement with the observations of Prasad *et al*., (2005). To identify the presence of target site in intersystem electron transport chain, the activity has been measured by using reduced diuroquinone as electron donor and MV as electron acceptor. Control thylakoid membranes exhibited the activity equal to 152 μmoles of oxygen consumed (Table 4). UV-B treatment (2-8 Wm^{-2}) caused 42% inhibition in electron transport activity indicating the presence of susceptible carrier in intersystem electron transport chain *i.e.* plastoquinone in the thylakoids of barley primary leaves. Thus UV-B radiation exerts multiple effects on the photosynthetic electron transport chain of photosynthesis depending on the dose applied.

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