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UV-B RADIATION INDUCED CHANGES IN PHOTOSYSTEM II PHOTOCHEMISTRY OF THE THYLAKOID MEMBRANES FROM PRIMARY LEAVES OF BARLEY

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ABSTRACT
Exposure of UV-B radiation (25-100 μ moles) induced inhibition in both whole chain electron transport as well as PS II photochemistry in a intensity dependent manner. The probable site of inhibition in PS II catalyzed electron transport could be water oxidation complex. To confirm this, an artificial donor, Diphenylcarbazide has been used and methyl viologen is employed as an acceptor. The above electron transport measurements clearly demonstrated that water oxidation complex is main target for the action of UV-B radiation.

INTRODUCTION

UV-B radiation exerts multiple effects on photosynthetic electron transport at different sites (Bornman, 1989). Between two photo systems (PS), PS II is more sensitive and highly vulnerable to UV-B radiation in various higher plant systems (Iwanzik *et al.,* 1983; Renger *et al.,* 1989; Melis *et al.,* 1992; Jensen *et al.,* 1996). Similar observations have been also reported in cyanobacteria indicating PS II is main target (Nedunchezhian *et al.,* 1996; Kolli *et al.,* 1998; Praveen kumar *et al.,* 2010). However the studies related to the molecular mechanism of action of UV-B radiation on PS II photochemistry in higher plant system are scanty. Therefore in these investigations an attempt has been made to study the effect of UV-B radiation (25-100 μ moles) on PS II photochemistry by measuring the PS II catalyzed electron transport activity in the thylakoids isolated from barley leaves by using polarographic measurements.

MATERIALS AND METHODS

Barley (*Hordeum vulgare*) seedlings were raised in petriplates under continuous white light (160 μ moles m^{-2} s^{-1}) at 25ºC. Hoagland solution was supplied at 4 day intervals to the seedlings. 8-day-old seedlings were exposed to different doses of UV-B radiation (80 μ moles m^{-2} s^{-1}) for 60 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 Sabat *et al.,* (1986) with slight modifications. The assay mixture for whole chain electron transport activity contained 0.5mM MV (Methyl viologen) and 1mM sodium azide in three ml of the 25μM HEPES reaction buffer (pH 7.8). For PS II mediated oxygen evolution, the reaction mixture consisted of 0.5μM pBQ in three ml reaction buffer. PS I catalyzed assay mixture contained 0.1 μM DCPIP (2, 6- Dicholorophenol-indophenol), 2 μM azide, 1 μM MV and 5 μM DCMU. The whole chain electron transport assay mediated by DPC as electron donor contains 25 mM HEPES buffer (pH 7.5) 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

After giving the different doses of UV-B radiation (25-100 μM) to barley plants primary leaves have been collected from control and treated samples and thylakoid membranes have been isolated and proceeded for measurement of photochemical activities mediated by different electron transport donors, acceptors and inhibitors.

Methyl viologen is known to accept electrons from A_0 in photosynthetic electron transport chain (Trebst, 1974). Control thylakoids with out UV-B treatment showed high rate of oxygen consumption (205 μ moles). Increase in the UV-B radiation

Table 1: Effect of UV-B radiation on whole chain electron transport activity of the thylakoids isolated from control and UV-B treated barley primary leaves. Other details were mentioned in Material and Methods

UV-B radiation(μ moles m^{-2} s^{-1})	Whole chain electron transport activity $H_2O \rightarrow MV$ μ moles of $O_2\downarrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	205 ± 18	0
25	167 ± 15	19
50	108 ± 9	48
75	75 ± 6	64
100	52 ± 6	75

Table 2: UV-B radiation induced alterations in PS II catalyzed electron transport activity of the barley thylakoid membranes. Other details were mentioned in Material and Methods

UV-B radiation (μ moles m^{-2} s^{-1})	PS II catalyzed electron transport activity $H_2O \rightarrow pBQ$ μ moles of $O_2\uparrow mg^{-1}$ Chl h^{-1}	Percentage loss
Control	276± 26	0
25	185 ± 17	36
50	121 ± 11	57
75	91 ± 8	68
100	61 ± 6	78

Table 3: UV-B radiation mediated alterations in photosystem I catalyzed electron transport activity of the thylakoid membranes isolated from barley primary leaves. Other details were mentioned in Material and Methods

UV-B radiation (μ moles m^{-2} s^{-1})	PS I catalyzed electron transport activity $DCPIPH_2 \rightarrow MV$ μ moles of $O_2\downarrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	384 ± 32	0
25	361 ± 31	6
50	355 ± 28	8
75	321 ± 24	17
100	315 ± 22	18

Table 4: Effect of UV-B radiation on whole chain electron transport activity (DPC-MV) of the thylakoids isolated from barley primary leaves. Other details were mentioned in Material and Methods

UV-B radiation (μ moles m^{-2} s^{-1})	Whole chain electron transport activity $DPC \rightarrow MV$ μ moles of $O_2\downarrow mg^{-1}$ Chl h^{-1}	Percentage loss
Control	167 ± 15	0
25	158 ± 14	6
50	147 ± 13	12
75	142 ± 11	15
100	139 ± 12	17

from 25-100 μM brought dose dependent inhibition in whole chain electron transport (Table 1). Almost 50 μ moles of UV-B radiation caused 48% inhibition and further rise brought the inhibition to 75%. The possible reason for the loss of whole chain electron transport could be either alterations at water oxidation complex of PS II or at oxidizing side of PS I has been suggested by Friso *et al.,* (1995). To verify the above proportions UV-B effect has been studied on PS II catalyzed electron transport using PBC as Hill acceptor. Control thylakoids exhibited a rate of oxygen evolution equal to 276 μ moles (Table 2). The activity is dropped by 56% with 50 μ moles of UV-B radiation and the inhibition is dependant on the dose. The possible reason for the loss of PS II activity could be due to either at water oxidation complex or alterations in D_1 or D_2 polypeptides (Renger *et al.,* 1989; Rajagopal, 1999).

To know the site of action in PS I the electron transport activity has been measured in barley thylakoids after exposing to UV-B radiation (Table 3). The increase in the UV-B radiation to hundred (100μm) caused only 18% loss in the electron transport activity. The possible reason for the observed marginal inhibition in PS I catalyzed electron transport activity could be alteration at the level of either at reaction centre or at the reducing side of PS I catalyzed electron transport (Rajagopal,1999).

To identify the exact target in PS II catalyzed electron transport DPC has been selected as substitute for the water and the whole chain electron transport activity was measured using DPC as donor and methyl viologen as acceptor. From the literature it is clear that DPC can donate electrons when water oxidation complex is not functioning. Table 4 demonstrates the effect of UV-B on whole chain electron transport mediated by DPC. At high dose 100 μM of UV-B radiated only marginal inhibition (17%) was noticed indicating that oxygen evolving complex is the main target for UV-B action. Thus the inhibition observed in PS II catalyzed electron transport is mainly due to the damage caused by UV-B radiation to water oxidation complex in the thylakoids of barley.

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