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# UV-B RADIATION INDUCED ALTERATIONS IN THE PHOTOSYNTHETIC ELECTRON TRANSPORT PROCESSES OF PHOTOSYNTHESIS IN MAIZE SEEDLINGS

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## ABSTRACT

Our planet earth is surrounded by a thin layer of ozone which acts as a shield to protect the planet from harmful UV light (Mackenzie *et al.,* 2003). Increased pollutants cause the depletion of ozone layer and results in the leakage of harmful UV radiation on to the surface of the earth. Since plants and terrestrial organisms depend on light for their survival, they become the targets for UV radiation which results in the damage. Several workers have made attempts to the effect of UV radiation on both plants under *in vitro* and *in vivo* conditions to know the damage occurred due to UVR. Hence in this thesis an attempt has been made by using maze seedlings as experimental system to completely understand the targets of damage. Therefore the 8$^{th}$ day old maize seedlings have been selected and treated with the UV-B radiation at different intervals of time (20-80 min) are measured the photosynthetic electron transport activities. The effect of UV-B radiation was studied on photosynthetic electron transport activities and LPO studies..The UV B treatment gradually caused the enhancement in the lipid peroxidation where 81% enhancement in MDA formation. Inhibition of PS II catalyzed electron transport activities could be due to alterations of oxidizing side of D1/D2 proteins or reducing side of PS II.

**Keywords : Electron transport ; Photosystam; Maize seedlings.**

# INTRODUCTION

Ultraviolet-B radiation have been studied during the last thirty years in small growth cabinets, growth chambers and green houses or in the field supplementing white light or ambient solar UV radiation with artificial UV-B or attenuating (or even excluding) the UV-B from the solar light. Present studies reveals that UV-B acts on macromolecules and molecules of biological importance, perturbing the processes in which they are involved as described below (McKenzie *et al.,*2003). DNA is one of the most notable targets of UV. Irradiation in both the UV-B and UV-C regions results in a multitude of DNA photo products (Mac Kerness *et al.,* 1998), which may cause mutations during replication ( Buma *et al.,*2003). DNA protein cross links, and DNA strand breaks and deletion or insertion of base pairs can also be induced by UV exposure (Smith,1989; Cannon *et al.,* 1995; Kumar *et al.,* 2004). UV induced damage to DNA has been studied in detail in human beings, mammals, fungi and cyanobacteria (Malloy *et al.,* 1997; Buma *et al.,* 2003; Wulff *et al.,* 2008). 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 (Anderson and Styring, 1991; Scmidth *et al.,*2010; Yunsheng *et al.,* 2011).

There is general consensus that UV-B radiation influences primarily PS II, there are many different reports on possible targets (Imre Vass., 2011). Different techniques were used to reveal the possible target sites of UV-B radiation such as fluorescence induction, flash-induced absorption changes, and measurement of $O_2$ evolution. However it seems to be well established that the redox components of PS II are affected by UV-B to some degree. From previous experiments it has been assumed that UV-B acts on either reaction centre itself, producing dissipative sinks for excitation energy, which quenches the variable fluorescence and/ or the reducing site of PS II (George *et al.,* 2011). Recent comparative studies indicated the water oxidizing complex as the most UV-sensitive part of PS II. Since the Mn cluster of water oxidation seems to be the most fragile component of the electron transport chain, UV-B absorption by the protein matrix or other redox components may lead to conformational change and inactivation of the Mn cluster. Most observations support the notion that UV-B preferentially inactivates the water oxidizing complex with additional effects on the $Q_A$ and $Q_B$ acceptors, as well as on the Tyr-Z and Tyr-D donors (Tystjarvi, 2008).

Maize is directly used for human consumption. In industry it is used for synthesis of organic compounds such as starches, acids and alcohols. Maize also used for production of ethanol and petroleum-based fuels. India occupies fifth place in the world maize production following by US, Brazil, China and

Mexico. Nutritionally maize contains 60-68% starch and 7-8% proteins, essential amino acids and B-complex vitamins like B1, riboflavin, folate, pantothenic acid. The objectives are To analyze the alterations in photosynthetic electron transport activities like whole chain, PS II, PS I and lipid peroxidation in maize plants.

## MATERIALS AND METHODS

Healthy seeds of maize (*Zea mays .L*) were obtained from Regional Agricultural research station, Tirupati, India. The Chl and carotenoid concentration was measured in supernatant after dilution to a total volume of 15 ml by following the method of (Arnon 1949). Thylakoid membranes have been isolated by following the procedure of (Sabat *et al*.,1986). Whole chain electron transport assay ($H_2O \rightarrow MV$) activity was measured as $O_2$ consumption by using MV as an electron acceptor using thethylakoid membranes. The 2 ml reaction mixture contains reaction buffer [50 mM HEPES-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl], 0.5 mM MV, 1.0 mM Na-azide and thylakoid membranes equivalent to 40 µg of Chl by following the procedure of (Sabat *et al* 1986). PS II catalyzed electron transport assay ($H_2O \rightarrow p$-BQ) activity was measured as $O_2$ evolution using the thylakoid membranes. The 2 ml reaction mixture contains reaction buffer [50 mM HEPES-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl], 0.5 mM freshly prepared *p*-BQ and thylakoid membranes equivalent to 40 µg of Chl by following the procedure of Sabat *et al* (1986). PS I catalyzed electron transport assay was measured as $O_2$ consumption using the thylakoid membranes.The 2 ml reaction mixture contains reaction buffer [50 mM HEPES-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl], 0.1 mM 2,6- dichlorophenol indophenol (DCPIP), 0.5 mM MV, 5 mM ascorbate, 1 mM sodium azide, 10 µM DCMU and thylakoid membranes equivalent to 40 µg of Chl by following the procedure of (Sabat *et al* 1986). Lipid peroxidation has been measured according to the method of (Heath and Packer 1968). Fresh leaf was homogenized in 3 ml 1% (w/v) TCA at 4 $^0$C. The homogenate was centrifuged at 20,000 x g for 15 min. To the 0.5 ml of the above supernatant 3 ml of TBA-TCA reagent (0.5% (v/v) TBA in 20% TCA) was added. The mixture was incubated at 95 $^0$C in a shaking water bath for 50 min, and the reaction was stopped by cooling the tubes in an ice water bath. The samples were centrifuged at 9,000 x g for 10 min, and the absorbance of the supernatant was read at 532 nm, and the value for nonspecific absorption at 600 nm was subtracted. The concentration of malondialdehyde (MDA) was calculated using the absorption coefficient of 155 $mM^{-1}cm^{-1}$. The amount of MDA was expressed as n moles MDA /mg protein

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## RESULTS

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 analyze the impact of UV-B radiation(2 $Wm^{-2}$) on the morphological changes and photosynthetic electron transport of the maize thylakoid membranes. To verify the above preposition, after exposed to different time intervals of UV-B radiation (20-100 min). After giving the treatment initially, whole chain electron transport activity ($H_2O$ →MV) was measured using MV as terminal electron acceptor. Control cells exhibited the whole chain electron transport activity equal to that of 193 µmoles of $O_2$ consumed $mg^{-1}$ Chl $h^{-1}$(Table-1).The increase in the duration of UV-B((2 $Wm^{-2}$) exposure from 20-80 min under conditions stirred caused a time dependent inhibition in whole chain electron transport activity. Further increase in the exposure caused additional loss of PS II catalysed electron transport activity to 78% at the end of 80 min of exposure (Table 2).

The inhibition in the PS II catalyzed electron transport activity could be due to alterations at the level of oxidizing site or changes at D1, D2 proteins or changes in the reducing side of PS II as has been suggested earlier by others (Renger et al., 1989; Wilson et al., 1993; Rajagopal and Murthy, 1996). When compare to the whole chain electron transport and PS II electron transport, PS I catalyzed reactions could be assayed in thylakoids with different reduced DCPIP/TMPD/DAD did not readily enter in to thylakoids. Therefore, we have

**Table 1: Effect of UV-B radiation on whole chain electron transport ($H_2O$ → MV) on the thylakoids of maize seedlings**

| UV-B exposure, min | Whole chain electron transport activity ($H_2O$ → MV) µ moles of $O_2$ ↓ mg $Chl^{-1}$ $h^{-1}$ | Percentage inhibition |
|---|---|---|

| | | |
|---|---|---|
| Control | 193 ± 15 | 0 |
| 20 | 130 ± 11 | 33 |
| 40 | 93± 7 | 52 |
| 60 | 52± 4 | 74 |
| 80 | 37±3 | 81 |

| **UV-B exposure, min** | **PS II electron transport activity ($H_2O \rightarrow$ *p*-BQ) µ moles of $O_2\uparrow$mg $Chl^{-1}$ $h^{-1}$** | **Percentage inhibition** |
|---|---|---|
| Control | 314 ± 24 | 0 |
| 20 | 240 ± 23 | 24 |
| 40 | 164± 16 | 48 |
| 60 | 118± 11 | 63 |
| 80 | 62 ± 4 | 79 |

**Table 2: Effect of UV-B radiation on PSII catalyzed electron transport ($H_2O \rightarrow$ *p*-BQ) on the thylakoids of maize seedlings**

prepared the thylakoid membrane fragments to study theeffect of UV-B radiation on PS I catalyzed electron transport. These fragments did not evolve $O_2$ with pBQ as a Hill oxidant. Hence we have characterized them by assaying PS I activity with different donor systems as shown in (Table 3). Reduced DCPIP/DAD//TMPD acts as donors in mediating the MV photoreduction by PS I. The rates were coinciding with the rates of chloroplasts thylakoid membranes and with reduced DCPIP the rates are quite high when compared to other two donors. The PS I Catalysed electron transport indicated thet only 18% inhibition was noticed (Table 4). Thylakoid membranes contain MGDG, DGDG, sulpholipids and phospholipids. These lipids are necessary for package of LHC units in the photosystems of thylakoid membranes (Kurisu *et* al., 2000).All the stress factors have ability to influence the organization of lipids with different polypeptides of two photosystems. For this purpose, the plants were given UV B treatment and the lipid peroxidation was measured. In control sample, the

lipid peroxidation is equal to 44 n moles MDA formed per mg protein.The UV B treatment gradually caused the enhancement in the lipid peroxidation where 81% enhancement in MDA formation.

## DISSCUSSION

UV radiation is one of the serious issues since past few decades due to industrialization. Increase in the industrialization results in the increase in anthropogenically important atmospheric pollutants such as chlorofluorocarbons (CFCs), halocarbons, chloroform (MCF) and dioxins (NOx). Considerable amounts of natural production of reactive nitrogen species (RNS) such as nitric oxide ($NO^-$), peroxynitrate ($ONOO^-$) and nitrous oxide ($N_2O$) from unpolluted aquatic and terrestrial ecosystems also contribute to the depletion of ozone layer (Kramlich and Linak, 1994).

**Table 3: PS I catalyzed electron transport activity mediated by various donor systems in the thylakoid membranes of maize.**

| 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 | 433 ± 44 |
| DAD + TMPD → MV | 507 ± 51 |

**Table 4: Effect of UV-B radiation on PSI catalyzed electron transport ($DCPIPH_2 \rightarrow$ MV) in the thylakoid membranes of maize.**

| UV-B exposure, min | PS I electron transport activity $DCPIPH_2 \rightarrow$ MV μ moles of $O_2\downarrow$mg $Chl^{-1}$ $h^{-1}$ | Percentage inhibition |
|---|---|---|
| Control | 372 ± 25 | 0 |

| | | |
|---|---|---|
| 20 | 361 ± 24 | 3 |
| 40 | 343± 23 | 8 |
| 60 | 331± 22 | 12 |
| 80 | 316 ±21 | 16 |

**Table 5: Effect of UV-B radiation on the lipid peroxidation of thylakoid membranes. Thylakoid membranes equivalent to 15 µg/ml of Chl *a* were used for the estimation of MDA levels.**

| Temperature (°C) | Lipid peroxidation n moles MDA $mg^{-1}$ protein | Percent Enhancement |
|---|---|---|
| 25 | 44 ± 4.1 | 0 |
| 30 | 50 ± 4.8 | 13 |
| 35 | 69 ± 5.3 | 56 |
| 40 | 80 ± 7.2 | 81 |
| 45 | 84 ± 7.3 | 91 |

These pollutants are being responsible for the depletion ozone layer in the stratosphere that helps in screening of UVR (Singh *et al.,* 2010b). Effect of UVR on growth and survival is severe and leads to complete killing of the organisms within 120-180 min exposure (Tyagi *et al.,*1992; Priyadarshini and Rath, 2012). The resistance capacity and sensitivity is different to the different species organisms. Photosynthetic pigments like chlorophylls, phycobilins, carotenoids and xanthophylls which are present in the photosynthetic apparatus are also destroyed by UV- radiation (De Oliveira *et al.,*2014). The consequences of reduced synthesis of chlorophyll pigment complex which is encoded by the *cab* gene family decreases in the amount of pigment content due to UVR (Jordan *et al.,*1994). A special type of light harvesting complex is present in cyanobacteria

called phycobiliproteins which consists of open chain tetra pyrole pigments can also be destroyed by UVR (Pandey *et al.,* 1997).

Maize is the most widely distributed crops of the world. It occupies is the third place in world production following wheat and rice. More than 70 countries produce maize. It is important cereal in many developed and developing countries of the world. Maize is mainly used for animal feed and industrial raw materials, mainly used for food and industry. Its growth habit and breeding system have made it an extremely useful plant for scientific study. In this investigation a study has been made to analyze the impact of UV-B radiation on the morphological changes and photosynthetic electron transport of maize plants. After giving the treatment initially whole chain electron transport activity ($H_2O$ →MV) was measured using MV as terminal electron acceptor. Control cells exhibited the whole chain electron transport activity equal to that of 193 µmoles of $O_2$ consumed $mg^{-1}$ Chl $h^{-1}$(Table-1).

The increase in the duration of UV-B exposure from 20-80 min under conditions stirred caused a time dependent inhibition in whole chain electron transport activity. Further increase in the incubation period to 80 min caused an enhancement in the inhibition to 81%. The possible reason for the loss of whole chain electron transport could be due to alterations either at the level of PS II or PS I or intersystem electron transport carrier level. To prove this, time dependent effect of UV-B was studied using pBQ as Hill acceptor. UV-B radiation was able to induce almost 50% inhibition in Hill activity after 40 min of incubation only.

Further increase in the exposure caused additional loss of PS II catalysed electron transport activity to 78% at the end of 80 min of exposure (Table 2). The inhibition in the PS II catalyzed electron transport activity could be due to alterations at the level of oxidizing site or changes at D1, D2 proteins or changes in the reducing side of PS II as has been suggested earlier by others (Wilson et al., 1993; Rajagopal and Murthy, 1996). When compare to the whole chain electron transport and PS II electron transport, PS I catalyzed reactions could be also assayed in thylakoids of maize as reduced DCPIP readily enter in to intact cells. Reduced DCPIP acts as donors in mediating the MV photoreduction by PS I. The rates were coinciding with the rates of chloroplasts thylakoid membranes and with reduced DCPIP the rates are quite high when compared to other two donors. After giving the UV-B treatment we have studied the effect of UV-B on PS I catalyzed electron transport activities. The electron transport measurements indicated marginal inhibition in PS I activity was noticed (Table 3).

All the stress factors have ability to influence the organization of lipids with different polypeptides of two photosystems.Therefore, an attempt has b een made toverify whether the HT mediated alterations of electron transport are relatedto the lipid changes or not. The lipid peroxidation has been measured interms of MDA formation. For this purpose, the plants were given UV B treatment and the lipid peroxidation was measured. In control sample, the lipid peroxidation is equal to 44 n moles MDA formed per mg protein.The UV B treatment gradually caused the enhancement in the lipid peroxidation where 81% enhancement in MDA formation.

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