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STUDIES ON THE UV-B RADIATION INDUCED OXIDATIVE DAMAGE IN THYLAKOID PHOTOFUNCTIONS AND ANALYSIS OF THE ROLE OF ANTIOXIDANT ENZYMES IN MAIZE PRIMARY LEAVES

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ABSTRACT
In this study an attempt has been made to identify the targets for the action of UV-B radiation and possible protection mechanism in primary leaves of maize. UV- B radiation (2 – 8Wm^{-2}) affected the photosystem II photochemistry (68% loss) and this inhibition is closely related to the extent of lipid peroxidation of thylakoid membranes. In addition there is an enhancement in the activities of antioxidant enzymes like superoxide dismutase, (76% increase) catalase (94% increases) which protects the thylakoid membrane from UV-B radiation induced oxidative damage.

INTRODUCTION

Investigation and examination of more than 300 plant species and cultivars have been carried out and characterized the UV-B effects on photosynthesis results above 50% have been considered sensitive, 20-30% moderate sensitive and the rest insensitive to UV-B radiation (Teramura and Sullivan, 1994). In many sensitive plant species (*e.g.* Wheat, rice, sunflower and cucumber), reduced leaf areas and/or stem growth was observed (Yung-gang *et al*., 2010). Photosynthesis is one of the most studied processes which get affected by UV-B radiation (Qing *et al.,* 2010). Despite the diversity of UV-B targets in plants, it seems that the photosynthetic apparatus is among the main action sites of UV-B damage significantly contributes to the overall UV-B damage (Wulff *et al.*, 2008; Kim *et al*., 2009). However, it is important to distinguish between direct damage, *e.g.* by absorption of high energy UV radiation causing destruction of the molecules itself and indirect damage by reactive oxygen species (ROS) producing during the destruction. For example, ROS oxidize polyunsaturated fatty acids and generate reactive fatty acid peroxides, which further react with synthetic pigments (Pospisil Pavel, 2011). Furthermore, it was found in green leaves that ROS may down-regulated the expression of photosynthetic genes (Mackerness *et al.,* 1999; Kim *et al*., 2009). Direct damage to unsaturated membrane lipids was concluded from the formation of malondialdehyde (Kramer *et al.,* 1991; Gupta *et al*., 2008; Wulff *et al.*, 2008). Studies related to the effect of UV-B damage on PS II photochemistry in relation to lipid peroxidation and anti oxidant protection in higher plant system are scanty. Therefore in this investigation an attempt has been made to study the effect of UV-B on the above aspects by taking maize as experimental system.

MATERIALS AND METHODS

Healthy seeds of maize (*zea mays*) were collected and surface sterilized with 0.1% $HgCl_2$ and seeds were germinated. The seedlings were placed in plastic trays and daily watered with Hoagland nutrient medium, providing fluorescence light. Fully expanded 8th day leaf segments were used for treatment. The maize plants were exposed to UV-B radiation at influence rate of 2 to 6 Wm^{-2} for different intervals (10-30 min). PS II catalyzed electron transport assay ($H_2O \rightarrow pBQ$) activity was measured as O_2 evolution in the thylakoid membranes. Lipid peroxidation has been measured according to the method of Carmak and Horst (1991). Superoxide dismutase activity was assayed by measuring its ability to inhibit the photochemical reaction nitrobluetetrazolium (NBT) using the method of Van Rossum *et al.* (1997). The activity of catalase was estimated by the method of Havir and Mc Hale (1997).

RESULTS AND DISCUSSION

In this study efforts were made to identify the target for UV-B radiation in the thylakoid membranes of maize primary leaves.

Table 1: Effect of UV-B radiation on PS II catalyzed electron transport ($H_2O \rightarrow pBQ$) and lipid peroxidation

UV- B radiation, (Wm^{-2})	PS II catalyzed electron transport $H_2O \rightarrow pBQ$ μ moles of O_2 evolved g Chl^{-1} h^{-1}	Lipid peroxidation n moles MDA mg^{-1} protein
Control	276 ± 26	47 ± 3
2	185 ± 17	54 ± 5
4	121 ± 11	66 ± 5
6	91 ± 8	71 ± 6

For this purpose maize leaves were exposed to different intervals of UV-B radiation (2-8 Wm^{-2}) by placing them in the petri plates. After exposure thylakoids have been isolated to measure the PS II catalyzed electron transport activity by using O_2 electrode. Control thylakoid membranes exhibited the Hill activity equivalent to 223μ moles of O_2 evolved. UV-B treatment gradually caused the increase in the loss of PS II activity and 68% was noticed with 6 Wm^{-2} (Table 1). The possible reason for the loss of PS II activity could be either alteration at water oxidation complex (WOC) or due to changes in the D_1 or D_2 polypeptides as suggested by earlier workers (Noorudeen and Kulandaivelu, 1982; Renger *et al.*, 1989).

Table 2: UV-B radiation induced changes in the enzyme activity of SOD in maize primary leaves. Incubation medium contained in a final volume of 3mL, 50mM potassium phosphate buffer (pH 7.8) 45μM methionine, 5.3μM Riboflavin, 84μM NBT and 20μM potassium cyanide. Reduced NBT was measured spectrophometrically at 600nm after exposure to light for 10min

UV- B radiation, (Wm^{-2})	SOD activityUnits x $10^{-2}g^{-1}$FW	Percentage of increase
Control	65 ± 5.8	0
2	82 ± 7.7	26
4	94 ± 8.9	45
6	99 ± 8.7	52

Table 3: Effect of UV-B radiation on changes in activity of catalase in the maize primary leaves. Buffer contain 50mM phosphate buffer (pH 7.0) 30mM H_2O_2, 340μL of 30% (v/v), H_2O_2 was dissolved in 100mL of phosphate buffer. Decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240nm

UV- B radiation, (Wm^{-2})	CAT activity μ mole H_2O_2 oxi g^{-1}FW	Percentage of increase
Control	13 ± 1.1	0
2	19 ± 1.4	44
4	23 ± 1.9	77
6	25 ± 2.4	94

To identify the alterations in thylakoid membranes lipid peroxidation measurement has been made in relation with PS II activity. In control thylakoids lipid peroxidation equal to 38n moles of MDA formed g^{-1}FW was observed. The treatment of UV-B radiation caused gradual enhancement in lipid peroxidation and at 6 Wm^{-2}, 78% enhancement in lipid peroxidation was noticed (Table 1). Thus enzymes like superoxide dismutase and catalase are known to be involved in scavenging of toxic oxy radicals. To verify the above properties an attempt has been made to measure the SOD enzyme activity. In control samples the activity of the enzyme is equal to 65.2 units/g FW of leaf material. The increase in the exposure of UV-B radiation from 2 to 6 Wm^{-2} caused enhancement in the enzyme activity by 76% (Table 2). This could be due to the induction of enzyme to neutralize the formed super oxide radicals under UV-B stress.To identify the role of another antioxidant defense enzyme catalase, which scavenge free peroxy radicals, the enzyme activity has been measured after giving UV-B treatment to maize leaves (Table 3). The activity of control sample is equal to 11.2 μ moles of H_2O_2 oxidized/g^{-1} FW. The treatment of UV-B caused the increase in the catalase activity and at 6 Wm^{-2} of UV-B radiation 94% increase in the catalase activity was noticed. When the above two enzymes activity induction is compared it is clear that UV-B radiation promotes the generation of peroxy radicals than super oxy radicals in maize plants to protect from the oxidative damage mediated by UV-B radiation.

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