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ORIGINAL ARTICLE

Comparative analysis of Photosynthesis activity in some plants grown under Enhance solar UVB (280-320nm) Radiation

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ABSTRACT

In the experiment conducted in field condition, the seedlings a few days old subjected to enhanced UVB radiation for several weeks. The photosynthetic activity and chlorophyll a fluorescence were analysed and the variable/maximum fluorescence was calculated. A significant effect of UVB on PS II activity was observed on Carrot, Beetroot Radish and onion plants. In radish, no negative effect was found. Under the UVB radiation the PSII activity was increased approx.50% as compared to other plants. However, the UVB radiation did not affect or slightly affect the PSI activity and Fluorescence parameter. The result shows the natural UVB radiation in the tropical region may significantly contribute to the reduction of photosynthetic activity, but which depended on the plant genotype.

Key words: *UVB, PSII activity,Fluoresence, carrot,onion, beetroot and radish,*

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INTRODUCTION

Stratospheric ozone depletion, resulting in increased intensity of UV-B radiation at the Earth's surface. Ambient solar UV-B is thought to be an important ecological limiting factor [1] and even a small increase in solar UV-B can have significant consequences for a variety of organisms. Elevated levels of UV-B have a potential to alter the productivity and reproductive success of individual species, and therefore can have effect throughout ecosystems.

The effects of enhanced UV-B radiation on plants have been widely studied, showing that UV-B radiation can have the deleterious effect on plants i.e. damage to DNA, alterations in transpiration, photosynthesis and respiration potential, growth, development and morphology [2,3,4,5]. The intensity of the effects depends on the species and on the balance between potential damage and the induction of protective mechanisms [7,8]. Consequently, natural systems can be affected by changes in plant phenology, biomass and seed production, plant consumption by herbivores, diseases and changes in species composition [9]. Crops have been shown to be even more sensitive because they have been subjected to long-term breeding [10].

PSII is the most sensitive component of the thylakoid membrane of photosynthetic apparatus to UV-B radiation [11,12,13]. The direct effects of enhanced UV-B radiation in the sensitive plants include impairment of PSII and to a less extent PSI, Rubisco activity, decreased CO_2 fixation and O_2 evolution [14,15,16].The effects of UV-B radiation are not evenly distributed between the two photosystems. Based on a variety of experiments, a general consensus has arrived that UV-B has little or no effect on PSI as compared to PSII. It was demonstrated that addition of artificial donor couple to PSI in UV-B treated chloroplasts, restored all the functions [17] Different techniques were used to reveal the possible target sites of UV-radiation such as Chl fluorescence induction kinetics, flash- induced absorption changes and polarography [18,19,]. From previous studies it has been assumed that UV-B sink of excitation energy that quenches the variable fluorescence or the reducing site of PSII [21]. However, the quinone electron acceptors [22], the redox tyrosine (Tyr-Z, Tyr-D) were considered as the primary target of UV-B action.

Recent studies confirm earlier suggestions that the water oxidizing complex seems to be most UV-B sensitive part of PSII. The light absorption by the manganese cluster in the oxygen-evolving complex of PSII causes primary photo damage, whereas excess light absorbed by light-harvesting complexes acts to cause inhibition of the PSII repair process chiefly through the generation of reactive oxygen species[23].Chlorophyll fluorescence not only allows us to study the different functional levels of photosynthesis indirectly but also is a useful method to study the effects of environmental stress on plants, since photosynthesis is often reduced in plants experiencing adverse conditions.[24].

The aim of this research was to compare the effects of UV-B on different plants with underground storage organs grown in the field and determine the photosynthesis and electron transport activity in response mechanisms to the UV-B radiation.

MATERIALS AND METHODS

Plant materials

Certified seeds of *Daucus carota* L (Early nanties), *Raphanus sativus* L.(Pusa chetki), *Allium cepa* L. (Nasik N-53) and *Beta vulgaris* L. (Hy-Pronto) obtained from the Agriculture Department, Madurai were sown in experimental plots in the Madurai Kamaraj University Botanical Garden. One set of plants was grown under ambient solar radiation and other under 20% UVB enhanced solar radiation.

Plant growth and UV-B treatment

The seeds were soaked overnight, in running water. Separate soil beds were prepared for control (ambient) and UV-B treatment and seeds were sown in these experimental plots. The plants were watered regularly and care was taken to avoid microbial or pest infection during the experimental period. Plants with the first foliage leaf stage were used for UV-B treatment. UV-B treatment was given to these plants for 4 hours daily from 10 am to 2 pm. Treatment was continued under ambient solar radiation and 20% UV-B enhanced solar radiation supplemented by a Philips TL40W/12 sunlamp (Gloelampenfabrieken, Holland). The first formed leaves were collected at different time periods and all the physiological and biochemical analyses were carried out.

Measurement of radiation

A Li-Cor Li-188B quantum/radiometer (Li-Cor., Inc., USA) with suitable photodetector was used to measure all the visible and photosynthetically active radiation. Radiation below 400 nm was determined by an IL 700 radiometer with a SEE 400 photodiode detector (International Light Inc., USA)

Estimation of pigments

Chlorophylls

Pigments were extracted in 80% acetone and the amount of total Chl, Chl a, Chl b and carotenoid was quantified using the formulae of Wellburn and Lichtenthaler (25).

Chlorophyll a (mg/l): (12.21 x A663) – (2.81 x A646)
Chlorophyll b (mg/l): (20.13 x A646) – (5.03 x A663)
Total Chlorophyll (mg/l): 7.18 x A663 + 17.32 x A646

PSII electron transport

Oxygen evolution ($H_2O \rightarrow BQ$)

PSII mediated O_2 evolution in the presence of BQ as electron acceptor was continuously monitored at 25^{o}C in an electrode set up same as described above. The reaction mixture in a total volume of 1.0 ml contained 50 mM Na/K phosphate, pH 6.5, 5 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose, 0.5 mM BQ and chloroplasts equivalent to 20 µg Chl.

PSI electron transport ($DCPIPH_2 \rightarrow MV$)

PSI mediated electron transport from the artificial electron donor (reduced DCPIP) was assayed as O_2 uptake. The reaction mixture in a final volume of 1 ml contained 20 mM Na/K phosphate buffer (pH 6.5), 5 mM NH_4Cl, 0.1 mM NaN_2, 2 mM sodium ascorbate, 2 µM DCMU, 50 µM DCPIP, 1 mM MV and chloroplasts equivalent to 20 µg Chl.

FLUORESCENCE TRANSIENTS

Chl a fluorescence induction was followed in intact leaves after excitation with broad band blue light (400-520 nm, Corning CS4-96). The irradiance at the sample surface was 100 W.m^{-2}.The photomultiplier (Hamamatzu, R376) placed at 90˚ to the excitation beam, was protected by an interference filter (λ max 690 nm, half band width 12 nm Schott, Germany). The signal from the photomultiplier was displayed on a Iwatsu model SS-5802 digital oscilloscope (Iwatsu Corp., Japan).

RESULT

Photosynthetic electron transport system

The changes in PSI and PSII were measured in plant grown under different conditions and were expressed on unit chlorophyll basis. The efficiency of photosynthetic activity was determined using an oxygen electrode.

PSII activity

The rate of PSII mediated electron transport activity was measured as O_2 evolution in the presence of BQ as the electron acceptor. Except radish, all other plant species studied showed an increase in the rate of PSII activity up to the full growth period and a large decrease during the early senescing stage (Fig. 1).

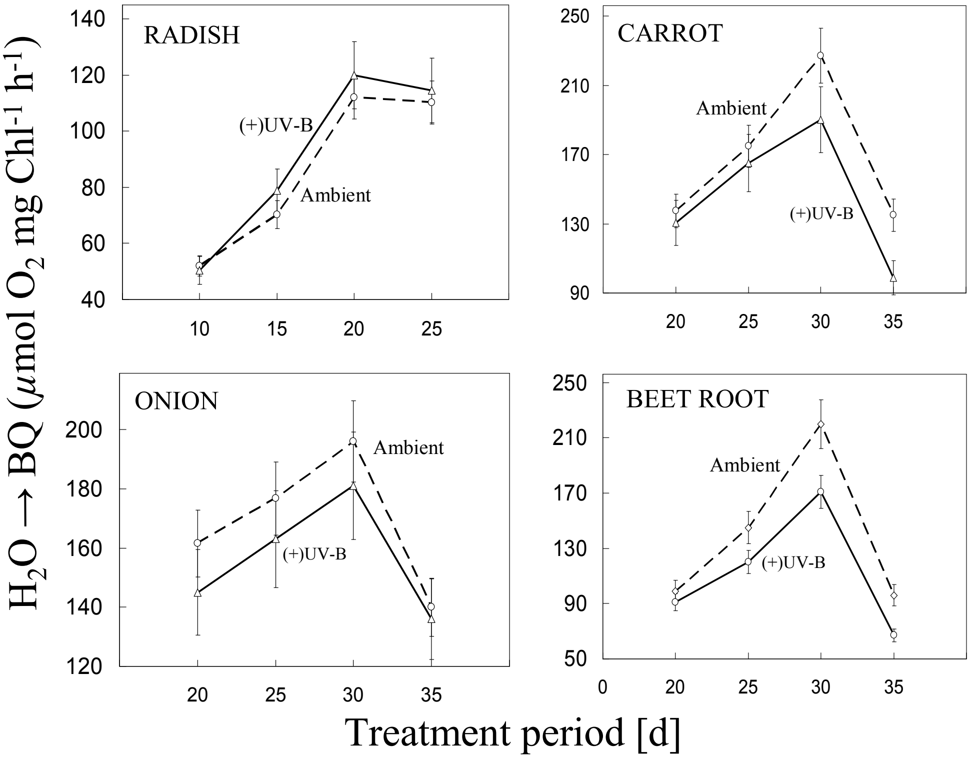

Fig. 1. Changes in the PSII mediated ($H_2O \rightarrow BQ$) electron transport activity in Chloroplasts isolated from radish, carrot, onion and beet root plants grown under ambient and enhanced UV-B radiation. The values represent an average of 5 independent measurements and are significant at ±5% level.

In these plants, UV-B enhanced radiation treatment caused significant decreases in PSII activity. In contrast to these plants, radish showed a marginally increased rate at all the stages of plant growth under UV-B enhanced radiation. Apart from this, the loss of PSII activity at the early senescing stage was small.

PSI activity

The PSI activity was measured as the rate of O_2 uptake in the presence of reduced DCPIP and MV as the terminal electron acceptor. In all the plants studied here the changes in PSI activity differed only marginally in plants grown under ambient and UV-B enhanced radiation (Fig.2). As observed for the growth parameters radish plants, under UV-B enhanced radiation, maintained slightly higher level of PSI activity over the respective ambient light grown plants at different stages of growth.

Changes in chlorophyll fluorescence kinetics

The optimum photosynthetic quantum yield, Fv/Fm, was determined when the plants are in early maturation period. The typical fluorescence induction showed a rapid raise to Fo level followed by a slow one reaching the maximum level, Fm, after above 600 ms. The level of slow variable part (Fv) differed in these four plants species grown under ambient and UV-B enhanced radiation (Fig. 3).

In radish, the fluorescence kinetics in both ambient and UV-B enhanced light grown plants showed no significant change. But in carrot, the level of Fv is smaller in UV-B treated plants than that of ambient light grown plants. Apart from this, rate of Fo → Fm was reduced. Both onion and beet root showed only a marginal difference in plants grown under ambient and UV-B enhanced solar radiation

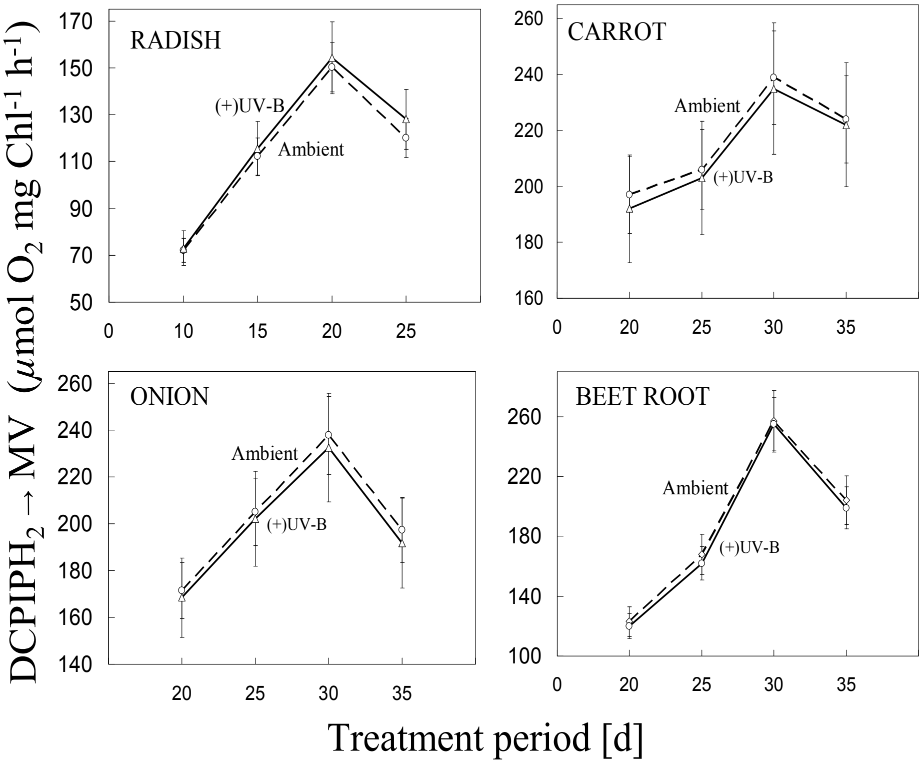

Fig. 2 . Changes in the PSI mediated (DCPIPH$_2$→MV) electron transport activity in chloroplasts isolated from radish, carrot onion and beet root plants grown under ambient and enhanced UV-B radiation. The values represent an average of 5 independent measurements and are significant at ±5% level

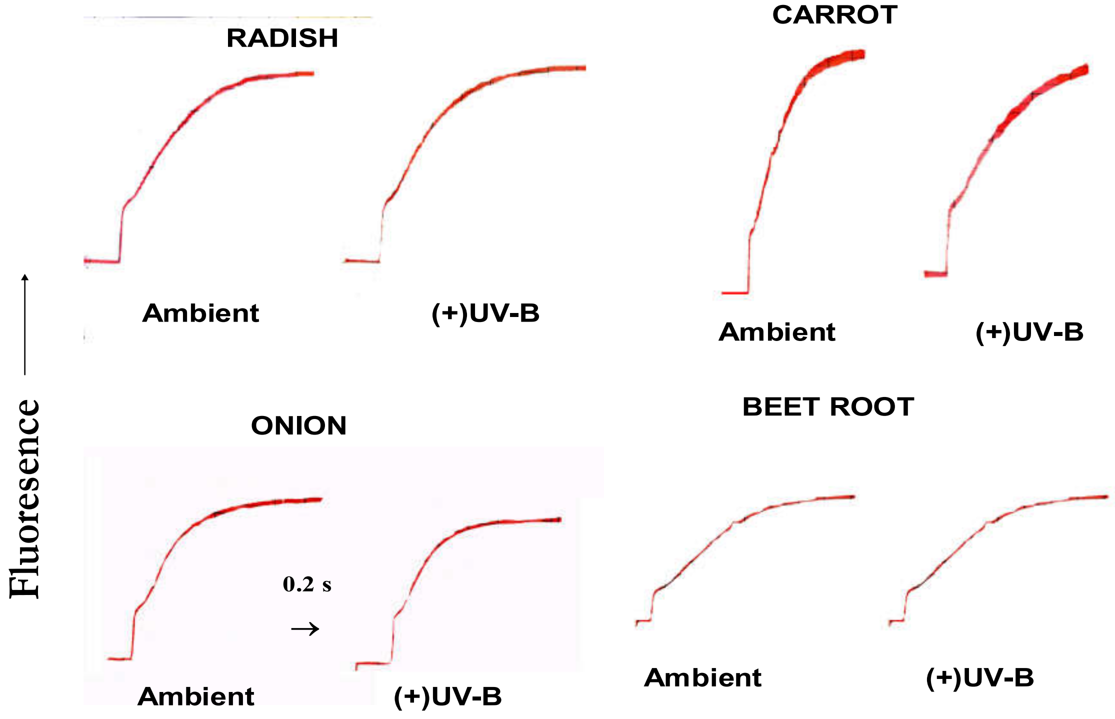

Fig. 3. Typical fast fluorescence induction kinetics of radish, carrot, onion and Beetroot plants grown under ambient and enhanced UV-B radiation. The Leaves were incubated in the dark for 10 min prior to excitation.

DISCUSSION

Changes in photosynthetic electron transport

Photosynthetic activity response to UV-B radiation depends on crop species, cultivars and experimental conditions. In the present investigation, UV-B radiation was found to increase the PSII activity in radish and this may be due to the structural reorganisation of the PSII complex which had resulted in the stabilisation of the PSII activity in UV-B enhanced light grown plant. On the other hand, a significant decrease in PSII activity was observed in carrot, onion and beetroot plants grown under enhanced UV-B radiation. The loss in PSII activity in ambient light grown plants indicates that the primary target site of UV-B resides at PSII. It has been reported that UV-B primarily attacks the reaction centre components of PSII and water oxidising complex [26,27]. The loss of PSII activity could also be due to the loss of extrinsic proteins of the PSII complex [28, 29]. Direct action of UV-B on D1 protein could be one of the possible reasons for the rapid inactivation of PSII. Based on various electron transport activities and fluorescence measurements Noorudeen and Kulandaivelu (30) have demonstrated UV-B induced changes in the reaction centre proteins which subsequently altered the electron transport reactions.However, electron transport mediated by PSII appears to be the most sensitive [20]. Within PSII, all parts from the Mn binding sites to the plastoquinone acceptor sites on thylakoid membrane are sensitive.

Several studies with artificial UV-B sources have shown that UV-B radiation strongly affects the PSII, whereas PSI appears to be rarely sensitive [31]. In radish, the PSI was not affected by enhanced UV-B radiation but in carrot, onion and beet root the PSI activity was slightly affected. Earlier reports indicate that UV-B had little or no effect on PSI as compared to PSII [32,33].In contrast to these reports ,Pang and Hays (34) reported that UV-B radiation inhibits the PSI-mediated cyclic photophosphorylation.Inhibition of the photosynthetic CO_2 assimilation and specifically on the activity, synthesis and degradation of Rubisco was reported in many studies [35,36,37].The Photochemical capacity of PSI remained largely unaffected under UV-B stress [38]. The decline in the efficiency of the P700 photoreaction is probably a universal response of PSI to high light stress and may be caused by enhanced charge recombination in the PSI reaction centre that confers photoprotection [39]. It is not known whether such alteration of PSI photochemistry is also induced by the UV-B component of solar radiation. Decreasing photosynthetic activity during/after UV exposure may be the result of direct damage to key components within the primary and secondary photosynthetic reactions (40,41].UV-B radiation also induces structural damage to the chloroplast and to the D1 and D2 proteins of the PSII core complex, which is linked to the decrease in photosynthetic capacity and growth rate [42]. The damage to the PSII activity may also be associated with the UV-B induced disruption of a structural integrity of the lamellar membrane systems of the chloroplast, probably due to the reduction in the saturated and unsaturated fatty acid ratio of polar lipids [43].

Chlorophyll fluorescence kinetics

In the absence of any significant loss in pigments, changes observed in Fv/Fm could be attributed to the changes in water oxidation and primary photochemical reactions. This is supported by the fact that these changes are almost in parallel with those of direct PSII activity measurements. Changes in the potential PSII efficiency were monitored by means of Fv to Fm ratio in dark adapted leaves. The reduction in fluorescence indicates the possible inhibition of energy transfer within the PSII reaction centre [44].

Decrease in variable fluorescence yield in enhanced UV-B irradiated plants observed in carrot and onion plants indicates the damage in PSII complex.[45]. The enhanced level of UV-B irradiation was shown to decrease the maximum photochemical efficiency (Fv/Fm) of the dark adapted leaves of beech [46]. The insensitivity of PSII under field conditions was not unexpected. Under field conditions Chl *fluorescence* kinetics has exhibited only minor short term effects (47,48). In addition, variable fluorescence can be used as an internal probe for measuring the redox state of PSII electron acceptor $Q_{A.}$ The ratio between Fv and Fm is lowered by the UV-B irradiation which cause damage on the thylakoid and produce structural changes in D1/D2 protein complex [49].

In radish, UV-B irradiation produced the significant increase in the variable fluorescence. It appears that in many cases, plants are capable of acclimatising and thereby, achieve efficient protection against increased UV-B levels (Allen *et al*., 1998). The UV-B absorbing compounds had little or no effect on morphological parameters and leaf photosynthesis as means of gas exchange and Chl fluorescence [50, 51, 52]. Changes in fluorescence parameters was observed in Mosses and Lichens [53]. Boucherd *et al*. [54] have also reported a high Fv/Fm due to the high proportion of light harvesting matrices in the reaction centre.

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