

Effect of Low Doses of UV-A and UV-B Radiation on Photosynthetic Activities in *Phaseolus mungo* L

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The effect of low doses of UV-A (320-400 nm) and UV-B (280-320 nm) radiation on photosynthetic activities in *Phaseolus mungo* L. was investigated under field condition. Supplementation of UV-A enhanced the synthesis of chlorophyll and carotenoids than the UV-B supplemented plants. Significant increase was seen in the concentration of UV-B absorbing compounds of UV-B treated plants. Increase of PS 2 activity in UV-A treated plants was seen. Changes in photosynthetic activity were measured in terms of PS 2 mediated O₂ evolution and Chl a fluorescence.

Keywords: chlorophyll, fluorescence induction, *Phaseolus mungo*, ozone depletion, UV-A radiation, UV-B radiation.

Anthropogenic chemical depletion of the stratospheric ozone layer has resulted in an increase in UV-B radiation reaching terrestrial organisms (Blumthaler and Ambach, 1990). Growth characteristics are altered in plants showing UV-B sensitivity. UV-B radiation supplied either artificially or naturally has resulted in decreased stem length, leaf area and plant height in cucumber, sunflower and soybean (Sullivan and Teramura, 1989; Tevini and Teramura, 1989). Supplementation of UV-A radiation has promoted the overall growth of the black gram plants than the control plants, while UV-B radiation has inhibited the growth of the plants (Jayakumar et al., 2003). Reductions in biomass accumulation with increased UV-B radiation have been observed in a range of agronomic species including wheat, barley, tomato, cucumber, and lettuce (Krupa and Kickert, 1989).

Most investigators studied UV-B effects from growth chamber or green house experiments, where the light spectrum is quite different from that of normal sunlight and the plants are generally more sensitive to UV-B (Runeckles and Krupa, 1994). Combination of visible radiation and UV-A at a particular ratio may be highly suitable for enhanced growth of seedlings (Middleton and Teramura, 1993). So far, conflicting reports arise about the regulatory effects of UV-A. Hashimoto and Tajima (1980) and Biswal et al. (1997) found the inhibition of total chlorophyll and carotenoid contents induced by UV-A. Promoting effects of UV-A on the synthesis of chlorophylls and carotenoids were reported by Senger and Schmidt (1986) and Rau and Schrott

(1987). Several researchers have discussed the potential effects of UV-B on vegetative growth and photosynthetic activities of higher plants (Bornman, 1989; Tevini and Teramura, 1989; Kulandaivelu et al., 1997; Choi and Roh, 2003). Both visible light and UV-A are important in modulating UV-B effects (Caldwell et al., 1994; Björn, 1996). To assess possible effects of UV-B on plants, it is important to conduct experiments in the field where a realistic balance between UV-B and UV-A as well as photosynthetically active radiation (PAR) is maintained.

In the past, research effort has mainly stressed the damaging effects of UV-B radiation on plant physiology and morphology, varying from DNA damage (Stapleton et al., 1997) and damage to the photosynthetic apparatus, proteins and membranes (Tevini and Teramura, 1989; Jansen et al., 1998) to destruction of photosynthetic pigments (Tevini et al., 1991; Strid and Porra, 1992). This paper reports the effect of UV-B and UV-A, supplemented solar radiation on photosynthetic and UV shielding pigments and photosynthetic activities in black gram.

MATERIALS AND METHODS

Plant and Growth Conditions

Pre-soaked seeds of *P. mungo* L. were germinated in the dark and grown in the outdoor conditions (UV-B of 300 mWm⁻²). The day/night temperature was 29/

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25 ± 2°C. The natural photoperiod varied between 11 and 13 h.

UV-A and UV-B Radiation Treatments

Seven days old seedlings were supplemented with UV-A/UV-B irradiation (0.5 Wm⁻²) for 30 min/day between 10.00 and 10.30 am (UV-B sun lamp, TL 20W/12, Philips, USA). Philips TL 20W/08 was used as an UV-A source. Radiation below 280 nm accounted to only 0.04% of total UV-B, which had negligible effect on seedlings, exposed for 30 min.

Pigment Analysis

Fresh leaves were extracted with 80% acetone, and the chlorophyll and carotenoid contents were determined according to Lichtenthaler (1987). Anthocyanins and flavonoids were extracted from the fresh leaves using acidified methanol (79:20:1, v/v/v, methanol: water:HCl). Extract was measured at 657, 530 and 315 nm using a spectrophotometer (557, Hitachi, Japan) and contents of anthocyanin (Mancinelli et al., 1975) and flavonoid (Mirecki and Teramura, 1984) were determined.

Isolation of Chloroplasts

Type II broken chloroplasts were isolated following the method of Reeves and Hall (1973) using 20 mM Tris-HCl (pH 7.8), 0.4 M sucrose, 5 mM MgCl₂, and 10 mM NaCl at 4°C.

PS 2 Electron Transport Assay

Photosynthetic partial reaction mediated by PS 2 (H₂O → BQ) was measured using a Hansatech (UK) O₂ electrode as described by Noorudeen and Kulandaivelu (1982). Assay mixture was prepared similarly as in Lingakumar and Kulandaivelu (1996).

Variable Chla Fluorescence

Variable chl_a fluorescence was followed in vivo in intact leaves after excitation with broadband blue radiation (400-620 nm) filtered by the Corning CS 4-96 filter (Corning, USA). Prior to the excitation, the leaves were incubated in the dark at 28°C for 10 min, and care was taken to avoid wilting of leaves in the dark. The signal was stored in a digital oscilloscope (SR-1100, Iwatzu, Japan) and then transferred to a recorder (056, Hitachi).

RESULTS AND DISCUSSION

Changes in Pigment Content

Total chlorophyll content decreased marginally in UV-A (3%) and drastically in UV-B (40%) exposed plants on 30th day of growth. A similar trend showing 28% and 32% inhibition in the level of carotenoids was noticed in UV-A and UV-B exposed plants, respectively (Fig. 1 and 2). Reports indicate that UV-B enhanced radiation during growth showed a significant reduction in the chlorophyll concentration (Ziska et al., 1993; Musil and Wand, 1994; Nedunchezian and Kulandaivelu, 1996, 1997). Such reduction in chlorophyll and carotenoid content might be due to inhibition of biosynthesis or increased degradation of chlorophyll/carotenoid or breakdown of pigments or their precursors by the UV irradiation (El-Mansy and Salisbury, 1997).

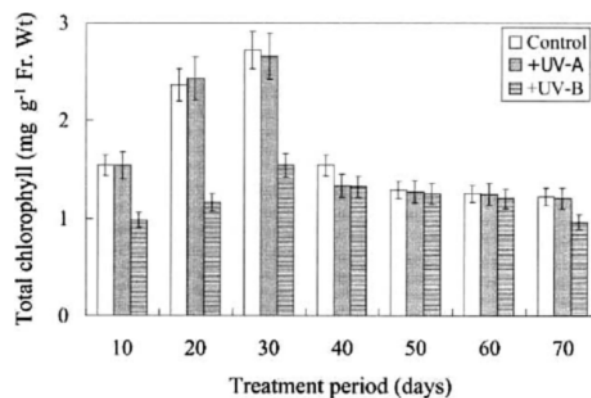


Figure 1. Effect of UV-A and UV-B enhanced radiation on total chlorophyll in *P. mungo* at different treatment period.

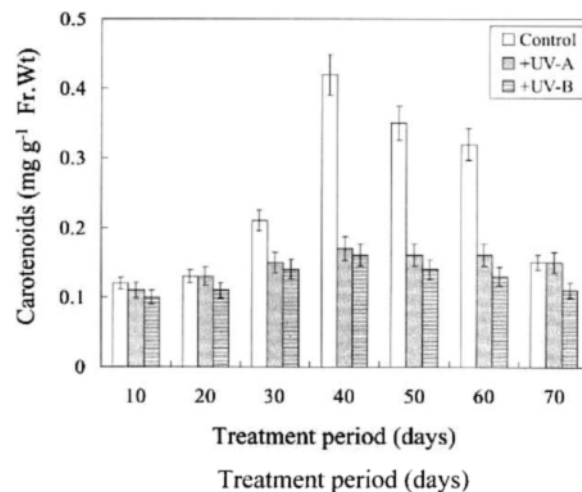


Figure 2. Effect of UV-A and UV-B enhanced radiation on carotenoids in *P. mungo* at different treatment period.

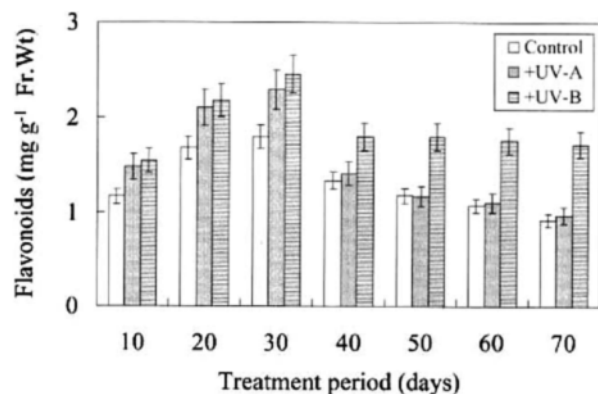


Figure 3. Effect of UV-A and UV-B enhanced radiation on flavonoids in *P. mungo* at different treatment period.

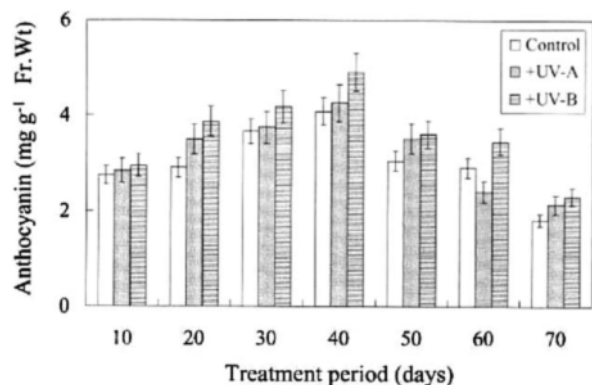


Figure 4. Effect of UV-A and UV-B enhanced radiation on anthocyanin in *P. mungo* at different treatment period.

Less inhibition in the content of photosynthetic pigments was observed due to UV-A than due to UV-B. The relative roles of UV-B and UV-A have not been described comprehensively, because even though the weighed damage due to UV-B is greater than that due to UV-A, the ratio between UV-B and UV-A photons in natural radiation is only about 3:100 (Cullen and Neale, 1994).

The UV protective pigments, flavonoids, showed about 28% and 36% increase in UV-A and UV-B treated plants, respectively, when compared to control plants. The same decreasing trend was followed in the level of anthocyanin. The increase was only 4% and 20% in UV-A and UV-B treated plants, respectively (Fig. 3 and 4).

Flavonoids and/or anthocyanins are induced by UV-B exposure. These pigments absorb UV-B radiation and they generally accumulate in the epidermis, where they could keep UV radiation from reaching photosynthetic tissues (Hahlbrock and Scheel, 1989). Possi-

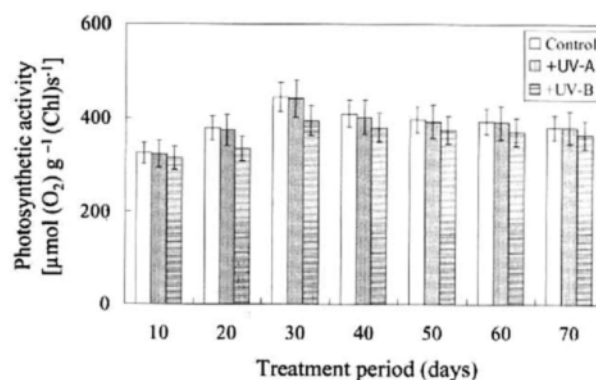


Figure 5. Changes in photosynthetic activity of *P. mungo* leaves exposed to UV-A and UV-B enhanced radiation.

ble receptors for anthocyanin and flavonoid induction after UV-B exposure have been analysed by Hasimoto et al. (1992) in sorghum. According to them the action spectrum for anthocyanin induction in sorghum has several maxima, indicating that both phytochrome and separate UV-B photoreceptor are involved in anthocyanin induction. Likewise phytochrome and UV-B photoreceptor have all been shown to be involved in flavonoid induction in parsley (Bruns et al., 1986). Feeding riboflavin to parsley cells increases the UV-B induction of chalcone synthase, a key enzyme in the flavonoid biosynthesis pathway. It was suggested that the UV-B photoreceptor might be a chromophore (Ensminger and Schafer, 1992). In our experiments the effects of UV-A on the plants are not remarkable, so the contents of the anthocyanins and flavonoid in the UV-A exposed plants are less than in the UV-B exposed plants.

Changes in Photosynthetic Characteristics

Overall photosynthetic electron transport activity measured as O_2 evolution showed a marginal loss in both UV-A (6%) and in UV-B (12%) exposed plants after 30 days of growth (Fig. 5). The UV-A treatment had no observable effect on photosynthesis or productivity. Many field studies have predicted that increasing exposure to UV-B will reduce plant productivity and photosynthesis (Teramura et al., 1990; Naidu et al., 1993; Sullivan, 1994; Nikolopoulos et al., 1995; Drilias et al., 1997). Many reports exist on the target site of UV-B radiation in PS 2 activity of the photosynthetic system of higher plants. The decrease in PS 2 activity might be due to the damage in the PS 2 complex. This is in agreement with the reports of Nedunchezian and Kulandaivelu (1997) and Jayakumar et al. (1999,

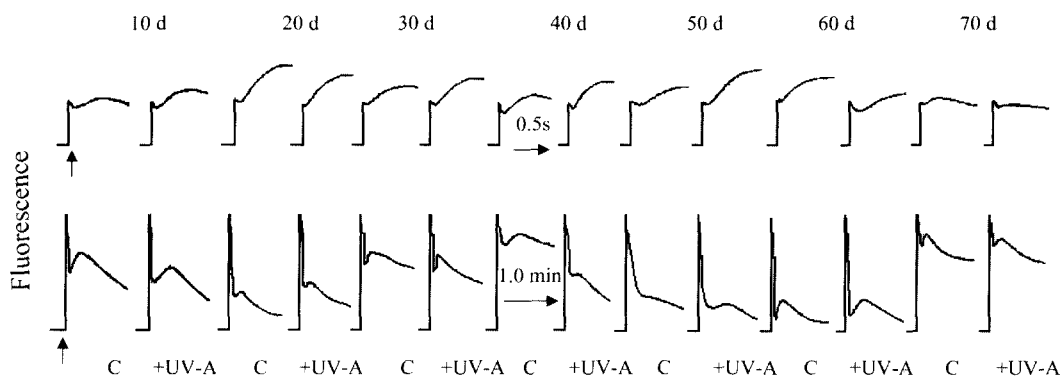


Figure 6. Typical fast and slow fluorescence transients obtained with intact leaves of *P. mungo* plants exposed to enhanced UV-A radiation for different period. The leaves were incubated in darkness for at least 15 min before measurement. Short vertical arrows indicate the switching on the excitation light.

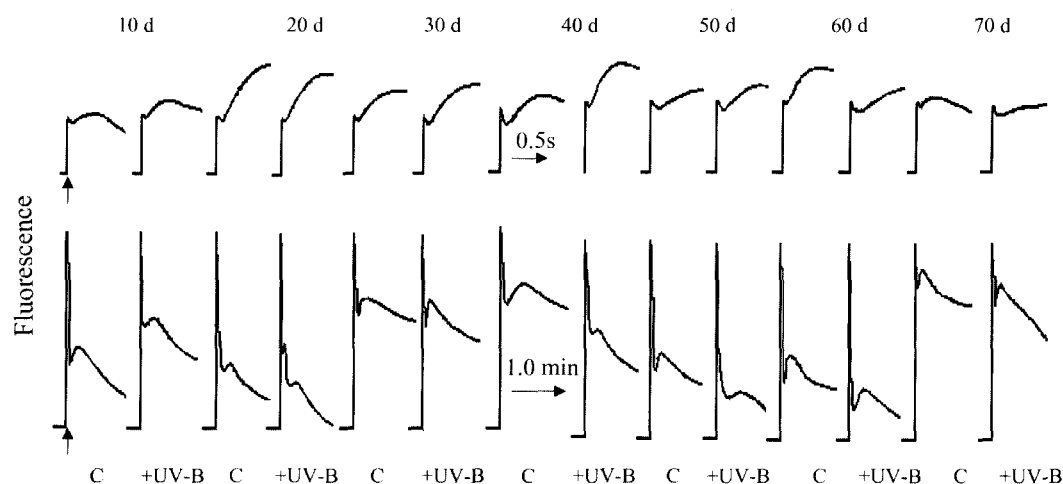


Figure 7. Typical fast and slow fluorescence transients obtained with intact leaves of *P. mungo* plants exposed to enhanced UV-B radiation for different period. The leaves were incubated in darkness for at least 15 min before measurement. Short vertical arrows indicate the switching on the excitation light.

2002).

The effect of UV-B radiation is probably not evenly distributed between the two photosystems. PS 1 mediated cyclic photophosphorylation was inhibited by UV-B radiation (Brandle et al., 1977). The damaging effects are mostly caused by direct damage to macromolecules involved (DNA, PS 2 elements, membranes, Rubisco, chlorophyll, and etc.) and by the generation of reactive oxygen species. Reduction in photosynthesis due to PS 2 damage is often by the reduced growth under enhanced UV-B (Deckmyn and Ceulemans, 2000). Amelioration of PS 2 activity by the addition of UV-A to UV-B irradiated chloroplast was shown by Panagopoulos et al. (1990).

The overall changes in photosynthetic activities in intact leaves of *Phaseolus* seedlings grown under UV-

A and UV-B were followed using Chl a fluorescence transients. Typical fast and slow Chl a fluorescence transients recorded are shown in Fig. 6 and 7. Fluorescence induction curves reveal the efficiency of PS 2 electron transport. The fluorescence transients show a typical Kautsky curve. The Chl a fluorescence emission exhibits a fast rise to a maximum followed by a slow decline to reach a steady level after a few minutes (Kautsky and Hirsch, 1931). The fluorescence induction experiments showed that F_0 values were unaffected. It is affected by UV-B irradiation, which causes alterations at the pigment level of PS 2. The ratio between F_v and F_m is lowered by the irradiation of UV-B, which causes damage on the thylakoids. UV-B treatment causes an increase of F_v and a decrease of F_m so that the PS 2 activity will be inhibited. No such

remarkable change has occurred by UV-A irradiation. Increase of F_o observed in UV-B treated *Helianthus annuus* cotyledons was due to reduced excitation transfer in the antennae or to an increase in the antenna cross section (Mark and Tevini, 1997). A steady increase in the ratio between F_v and F_m was observed in the *Vigna unguiculata* seedlings grown under ambient condition. These seedlings under ambient condition showed decline in PS state and a slow attainment of the T state (Lingakumar et al., 1999). Renger et al. (1982) observed structural changes in D1 and D2 protein complex in the PS 2 membrane fragments of UV-B irradiated spinach. This indicates that the reduced photosynthetic O_2 evolution could be correlated with the poor supply of ATP and NADPH₂, reduced stability of water oxidation system, and energy redistribution between the photosystems. UV-B radiation directly influences the protein contents in thylakoid membranes and also the structure and function of RuBPCase.

The present investigation shows that UV-A enhanced radiation affects the photosynthetic activities of *P. mungo* plants moderately when compared to UV-B exposed plants.

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