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Effects of ultraviolet-B enhanced radiation and temperature on growth and photochemical activities in *Vigna unguiculata*

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Abstract

Changes in growth characteristics and photochemical activities in *Vigna unguiculata* L. Walp seedlings maintained at constant temperature of 10, 20, 30 and 40 °C under control and ultraviolet-B enhanced radiation (UV-B) were investigated. UV-B retarded the shoot elongation and also leaf expansion to a great extent at 30 °C but produced only marginal changes at 20 and 40 °C. Similar response was also observed with respect to changes in leaf fresh and dry masses and total chlorophyll (Chl) content under these temperatures. At 10 °C the total Chl content was 3-fold higher under the treatment than under control conditions. In seedlings growing at 20 and 30 °C the overall photosynthetic electron transport ($H_2O \rightarrow$ methyl viologen) showed a significant enhancement during the 36-h UV-B treatment and thereafter a gradual reduction. Although a similar trend was found in photosystem 1 (PSl), the inhibition even after 60 h of UV-B treatment was not statistically significant. Photosystem 2 (PS2) activity was inhibited in seedlings treated for 60 h by UV-B at 20 and 30 °C. However, no inhibition was observed at 40 °C. No detectable photochemical activity was found in seedlings grown at 10 °C under either control or UV-B enhanced irradiation although the chloroplasts contained Chl.

Additional key words: chlorophyll, dry mass, fresh mass, fluorescence induction, leaf expansion, mung bean, photosystem 1, photosystem 2, shoot elongation.

Introduction

Plants growing under natural conditions often experience some degree of environmental stress, due to the shortage of water, temperature and nutrients.

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Abbreviations: DCPIP - 2,6-dichlorophenol indophenol; Chl - chlorophyll; MV - methyl viologen; PS - photosystem; UV-B - ultraviolet-B (280 - 320 nm) radiation.

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Considering world vegetation types, plant productivity is more closely related to availability of water and temperature than to any other environmental factors. Temperature stress affects plant growth primarily through the alteration of most physiological processes (Downes and Slatyer 1972, Pearcy *et al.* 1977, Bauer 1979). A combination of stresses can be either antagonistic, synergistic or independent in its effects on physiological or morphological growth characteristics.

The stratospheric ozone layer, which attenuates solar UV-B radiation is being depleted by pollutants such as chlorofluorocarbons (Rowland 1989, Frederick 1990). If, as a result of ozone loss, UV-B fluence at the surface of the earth increases negative impacts on biological organisms are inevitable (Coohill 1991). Exposure to high UV-B irradiances inhibits plant growth (Vu *et al.* 1978), alters photosynthetic enzyme activities (Nedunchezhian and Kulandaivelu 199la), disrupts PS2 reaction centres (Iwanzik *et al.* 1983, Noorudeen and Kulandaivelu 1982), and modifies stomatal closure (Negash 1987). Growth characteristics are also 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, sunllower, soybean and loblolly pine (Sullivan and Teramura 1988, Tevini and Teramura 1989, Teramura and Murali 1986). Reduction in biomass accumulation with increased UV-B radiation was observed in wheat, barley, soybean, tomato, cucumber and lettuce (Krupa and Kickert 1989).

Many studies related to effect of combined stresses like water stress, metal stress, nutrient stress, drought, CO_2 enhancement and UV-B have also been made (Teramura *et al.* 1983, 1984, Murali and Teramura 1985, Sullivan and Teramura 1990, 1994, Dube and Bornman 1992, Musil and Wand 1994). No report is available regarding the combined effect of UV-B and temperature on growth and physiological activities of crop plants. The aim of the present study was to investigate the effect of UV-B enhanced radiation on the morphological and photochemical activities in *Vigna* seedlings growing at various temperatures. The purpose of this specific study was to ascertain whether temperature would modify the response of *Vigna* seedlings to UV-B radiation.

Materials and methods

Plants: Pre-soaked seeds of *Vigna unguiculata* L. Walp were germinated in the dark at 30 °C for 2 d. When the primary leaves were fully expanded, 2 d-old seedlings were given radiation (control and UV-B enhanced) treatments in environmental growth chambers maintained at 10, 20, 30 and 40 °C (*Hotpack Corporation*, Philadelphia, USA) as described earlier (Nedunchezhian *et al.* 1992).

Chlorophyll analysis: Chl (*a*+*b*) content was estimated spectrophotometrically in 80 % acetone (Lichtenthaler 1987).

Activities of electron transport: *Vigna* chloroplasts, prepared as described by Kulandaivelu and Daniell (1980) were suspended in a medium containg 400 mM saccharose, 10 mM NaCl, and 20 mM Tris-HCl buffer, pH 7.8. Photosynthetic

reactions mediated by PS1 and PS2 were measured as described by Noorudeen and Kulandaivelu (1982).

Fluorescence measurement: *In vivo* Chl *a* fluorescence induction was followed at 25 °C in intact leaves as described by Noorudeen and Kulandaivelu (1982). The signal was stored in a digital oscilloscope (*Iwatsu SS -5802*) and then transferred to a *Hitachi* recorder.

Resuts and discussion

Vigna seedlings, when grown under control and UV-B enhanced radiation at 10, 20, 30 and 40 °C, showed much variation in their growth. At 30 °C, UV-B retarded the shoot elongation and also leaf expansion to a great extent. The leaves were small, thick and leathery in texture with heavy cuticular waxes on their upper surface (Fig. 1). The changes observed at 20 °C were comparatively less than those seen at

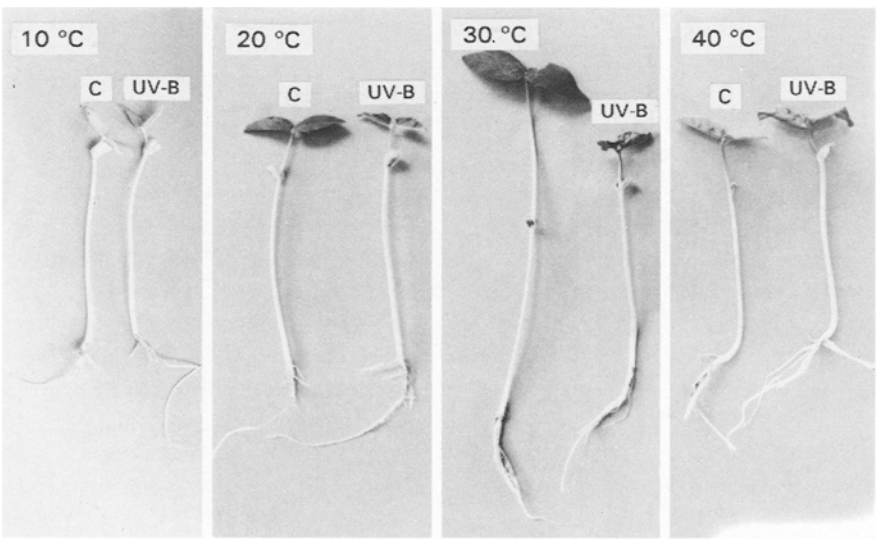

Fig. 1. Typical morphological responses in *Vigna* seedlings grown for 48 h under control (C) and UV-B enhanced (UV-B) radiations at various temperatures.

30 °C. At 10 and 40 °C, UV-B produced only a marginal effect on seedling growth. UV-B progressively decreased the seedlings height with increase in the duration of treatment (Fig. 2). At the end of 60 h of treatment an 11, 31 and 3 % inhibition was observed at 20, 30 and 40 °C, respectively. At 10 °C, UV-B enhanced the seedling height by approximately 6 %. The retardation of shoot growth under UV-B at 20, 30 and 40 °C was likely due to its direct action on auxins. UV-B lowers the auxin concentration in fronds of *Spirodella oligorhiza* (Witztum *et al.* 1978). Indol-3-yl-

acetic acid (IAA) absorbs UV-B and induces structural changes and inhibition of vegetative growth (Kulandaivelu *et al.* 1989).

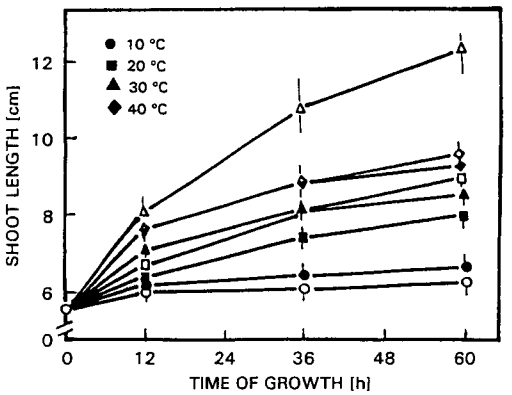

Fig. 2. Changes in the height of *Vigna* seedlings grown under control (*open symbols*) and UV-B enhanced (*closed symbols*) irradiations at various temperatures. *Vertical bars* represent the SD of the mean of 50 samples.

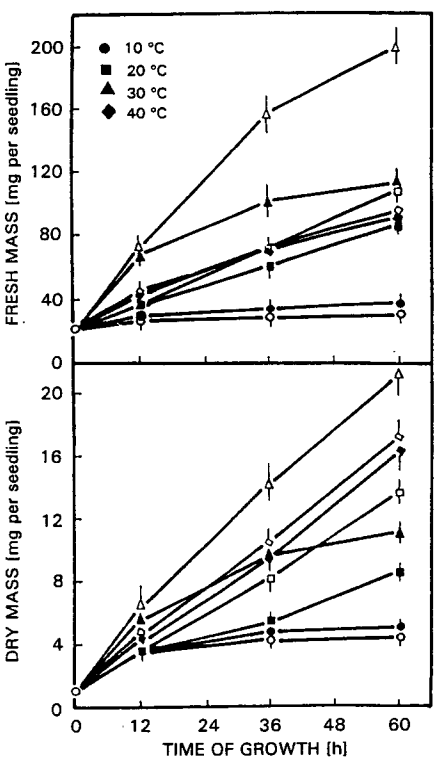

Fig. 3. Changes in the leaf fresh and dry masses in *Vigna* seedlings grown under control (*open symbols*) and UV-B enhanced (*closed symbols*) irradiations at various temperatures. *Vertical bars* indicate the SD of the mean of 50 samples.

UV-B treated seedlings had lower fresh and dry masses than the respective controls (Fig. 3). Significant reduction in leaf fresh and dry masses was observed at 30 °C, but the reduction was only marginal in seedlings grown at 20 and 40 °C. At 10 °C, a marginal increase in fresh and dry masses occurred. Investigations carried out in growth chambers, greenhouses and field have indicated that UV-B significantly depressed growth and biomass accumulation of many sensitive plant species (Sisson and Caldwell 1976, Vu *et al.* 1978, Kulandaivelu *et al.* 1989, Cen and Bornman 1990, Wilson and Greenberg 1993, Musil and Wand 1994). Such reduction in leaf area could be an adaptive mechanism to mininimize the area of exposure to UV-B. No such reduction was observed at 10 °C (Fig. 1). Among the several growth parameters studied, marginal increase in seedling height, leaf area, and leaf biomass was observed under UV-B enhanced radiation at 10 °C. This indicates that low temperature alters the UV-B effects.

When determined per dry mass (Fig. 4), the Chl (*a*+*b*) content showed a gradual reduction from the beginning of UV-B treatment at 30 and 40 °C. In seedlings grown

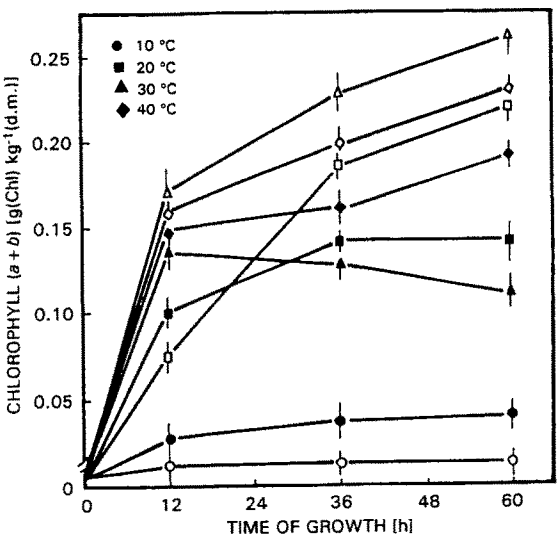

Fig. 4. Changes in the total Chl content in *Vigna* seedlings under control (*open symbols*) and UV-B enhanced (*closed symbols*) irradiations at various temperatures. Each point represents the mean of 4 measurements.

at 20 °C, UV-B induced a rapid accumulation of Chl during the first 12 h. However, prolonged treatment decreased this level. In contrast to this, at 10 °C, the seedlings under UV-B had always higher contents of total Chl than the respective control. The reason for such increase might be the activation of 5-aminolevullinic acid synthetase by UV-B, the activity of which is suppressed at low temperatures. Blue radiation induces changes in algae and higher plants (Senger 1982) including the increase in the number of chloroplasts and in pigment concentration. Seedlings grown at 30 and 40 °C showed low total Chl concentration under UV-B. Similar reduction in total Chl concentration level under UV-B has already been reported (Vu *et al.* 1982,

Kulandaivelu *et al.* 1989, Strid and Porra 1992, Wilson and Greenberg 1993, Deckmyn *et al.* 1994). However, in experiments under low irradiance, Chl concentrations are often increased by UV-B (Tevini and Iwanzik 1983, Takeuchi *et al.* 1989). The increase in Chl content was slightly greater during de-etiolation under supplementary UV-B than under control irradiance (Jordan *et al.* 1994). The reason for the loss of Chl may be inhibition of Chl precursors (El-Mansy and Salisbury 1971). According to Jordan *et al.* (1991) and Strid and Porra (1992) there are no specific effects of enhanced UV-B irradiation on the enzymes of the Chl biosynthetic pathway, but a down-regulation of the expression of genes crucial for Chl binding proteins which parallels the increased degradation of chlorophylls. UV-B radiation also induces non-enzymatic photo-oxygenation of the Chl and carotenoids resulting in the accumulation of their oxygenated forms (Monties 1975, Kulandaivelu *et al.* 1989). The loss of Chl may also be due to general photochemical degradation of the photosynthetic apparatus.

Fast fluorescence transients in detached leaves give an overall picture of various steps of early electron transport reactions. Seedlings grown at 10 °C under control irradiation showed no variable fluorescence (F_v) while in those under UV-B a significant F_v was noticed (Fig. 5). F_v of control seedlings grown at 20, 30 and 40 °C was large, maximum in 30 °C grown seedlings, and its extent was reduced in seedlings grown under UV- B. Reduction in F_v yield indicates an impairment of PS2 activity, particularly at donor side (Klimov *et al.* 1977, Schreiber *et al.* 1978). The changes in F_v indicate the relative efficiency of PS2 activity. In seedlings grown at 10 °C no large changes in F_v were observed due to UV-B. Large decrease in F_v in seedlings grown at 30 °C under UV-B indicated the presence of more UV-B damaging sites than in plants grown at 40 and 20 °C.

The whole electron transport chain ($H_2O \rightarrow MV$) showed a significant enhancement during the first 12 h of UV-B treatment at 20 and 30 °C, while at 40 °C

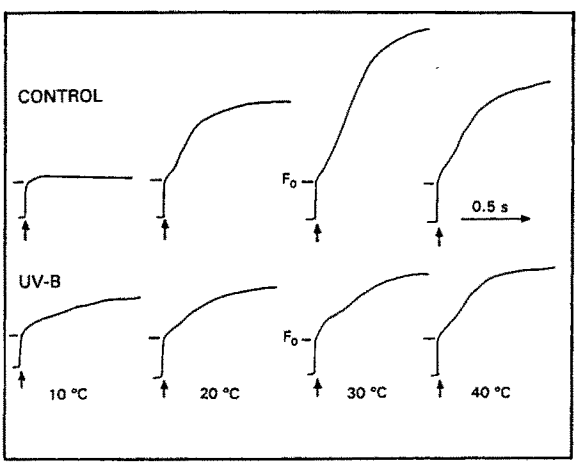

Fig. 5. Typical fluorescence transients of detached leaves from *Vigna* seedlings grown for 48 h under control and UV-B enhanced irradiations at various temperatures. *Open arrows* indicate actinic irradiation.

it continued to increase upto 36 h. Further increase in the treatment period resulted in a gradual reduction in its activity. Even after 60 h of treatment the whole electron transport chain activity decreased only by about 10 % of the respective control. A similar trend was also noticed for the PS1 and PS2 mediated electron transport. The changes in photochemical activities under the *in vivo* UV-B treatment were different from those under the *in vitro* conditions, where drastic loss of the whole chain and PS2 electron transport activities has been reported (Van *et al.* 1977, Noorudeen and Kulandaivelu 1982, Bornman 1989, Renger *et al.* 1989, Nedunchezhian and Kulandaivelu 1991b, Melis *et al.* 1992).

Under the *in vivo* treatment all electron transport activities showed an increase during the initial period of treatment followed by a small decrease. Increase in activity was observed in spite of the fact that these seedlings had slow growth and low total Chl content. This could be explained as follows: Since the electron transport activities are determined on unit Chl basis, the number of Chl molecules per reaction centre may be reduced in seedlings grown under UV-B. In addition, UV-B induces structural changes, such as swelling of grana (Bornman *et al.* 1983) and

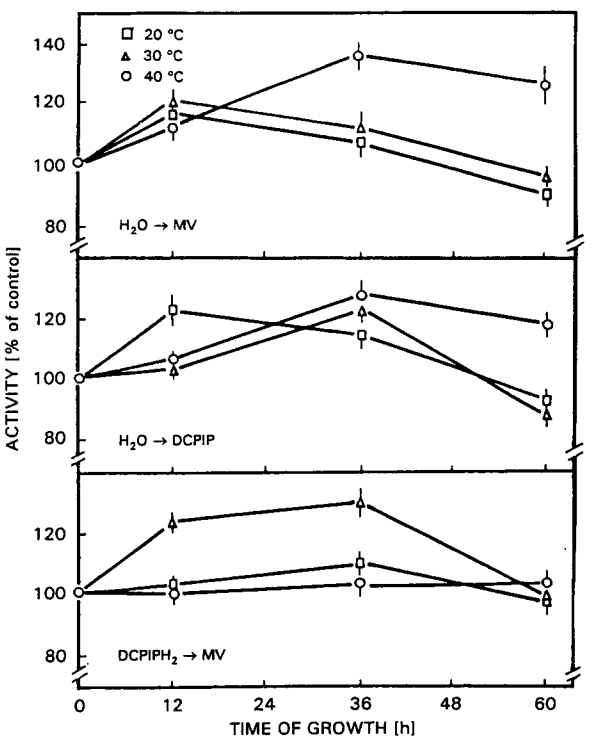

Fig. 6. Changes in the whole chain ($H_2O \rightarrow MV$), PSI ($DCPIPH_2 \rightarrow MV$) and PS2 ($H_2O \rightarrow DCPIP$) electron transport in *Vigna* seedlings grown under control and UV-B enhanced irradiation at 20, 30 and 40 °C. Values are expressed as per cent change to the respective control. The 100 % values at 0 h were: $H_2O \rightarrow MV$ - 49 (20 °C), 51 (30 °C), 69 (40 °C); $DCPIPH_2 \rightarrow MV$, 88 (20 °C), 94 (30 °C), 103 (40 °C) [mmol kg^{-1}(Chl) s^{-1}]; $H_2O \rightarrow DCPIP$ - 51 (20 °C), 44 (30 °C), 38 (40 °C) [mmol(DCPIP) kg^{-1}(Chl) s^{-1}]. Values represent means of 5 measurements, *vertical bars* represent SE.

reorganization of thylakoid components (Nedunchezhian and Kulandaivelu 1991b). These changes may increase the accessibility of added electron acceptors and donors.

In conclusion, *Vigna* seedlings grown under different temperatures but constant UV-B showed large differences in growth rate, morphology, photosynthesis and pigmentation. Nonetheless, these plants showed similar reductions of photosynthesis and growth when exposed to UV-B at 20 and 30 °C. The ambient temperature only increased the sensitivity of plants. In contrast to this, in plants grown under high (40 °C) and low (10 °C) temperatures the extent of such UV-B induced changes was decreased. Hence low and high temperatures altered the UV-B effects.

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