The influence of increased solar UV-B radiation on magnesium-deficient cowpea seedlings: Changes in the photosynthetic characteristics

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Abstract

The influence of increased solar UV-B radiation on the photosynthetic characteristics in cowpea seedlings (*Vigna unguiculata*) grown at optimal (Mg_s) and low (Mg_d) Mg levels were studied. Both higher UV-B and Mg_d treatments caused significant drops of photochemical activities and net CO_2 uptake rates (P_N). Yet the UV-B-induced decrease in the photosynthetic efficiency was lesser in Mg_d seedlings. The leaf Chl *a* fluorescence measurements proved that after receiving an enhanced UV-B radiation these seedlings showed a significant enhancement in their variable parts. The PSM oscillation of slow fluorescence kinetics was remarkably altered by both treatments. The P_N also followed a typical inhibitory pattern as seen in photochemical activities. Concentrations of several chloroplast proteins in trifoliate leaves were significantly reduced by Mg_d treatment and unaffected by the other two treatments. Whereas the contents of 43-47 kDa polypeptides in primary leaves were markedly reduced with a maximal effect in Mg_d seedlings, no major difference was noted for combined stress.

Additional key words: chlorophyll; chlorophyll fluorescence induction; chloroplast proteins; CO_2 uptake; net photosynthetic rate, photochemical activities; *Vigna unguiculata*.

Introduction

UV-B radiation inhibits photosynthesis (Bornman 1989, Kulandaivelu *et al.* 1997); several mechanisms have been suggested responsible for the loss of photosynthetic

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Abbreviations: Chl - chlorophyll; C_i - intercellular CO_2 concentration; DCPIP - 2,6-dichlorophenol indophenol; Mg_d - magnesium deficient; Mg_S - magnesium sufficient; MV - methyl viologen; O_3 - ozone; P_N - net photosynthetic rate; PFD - photon flux density; PPFD photosynthetic photon flux density; PS - photosystem; RC - reaction centre; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase; SDS-PAGE - sodium dodecyl sulphate polyacrylamide gel electrophoresis; UV-B - ultraviolet-B radiation between 280-320 nm.
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capacity (Bornman 1989, Vass *et al.* 1996). It was photosystem (PS) 2 that the UV-B radiation primarily affected while damage of PS1 was only slight (Premkumar and Kulandaivelu 1996, Kulandaivelu *et al.* 1997). Thus the UV-B radiation exerts a disproportionate impact on the two photosystems.

UV-B radiation directly influences the protein contents in thylakoid membranes (Nedunchezhian and Kulandaivelu 1991, Wilson and Greenberg 1993, Friso *et al.* 1995), and also the structure and functioning of RuBPCO (Nedunchezhian and Kulandaivelu 1991). Fiscus and Booker (1995) have proposed that these effects were unrealistic since most of the studies were carried out in greenhouse and/or growth cabinets where the spectral balance between UV-B and visible radiation was not optimal. Some field studies have indicated that the UV-B radiation had little or no effects on measurable photosynthetic characters (Gonzalez *et al.* 1997, Visser *et al.* 1997). Contrary to this, we found in two studies that field-grown cowpea seedlings exhibited lower photochemical efficiency under 16 and 20 % O_3 depletion simulation (Premkumar and Kulandaivelu 1996, Nedunchezhian and Kulandaivelu 1997).

The effectiveness of UV-B radiation is modifed by abiotic factors such as temperature (Caldwell 1994), irradiance (Deckmyn *et al.* 1994), nutrients (Musil and Wand 1994, Premkumar and Kulandaivelu 1996), CO_2 (Visser *et al.* 1997), O_3 (Zeuthen *et al.* 1997), and climatic factors (Drilias *et al.* 1997). Studies involving UV-B radiation and biotic factors like pathogens and insects showed that the UV-B radiation had altered the plant atraction to insects in *Datura ferox* and *Arabidopsis* (Ballare *et al.* 1996, Grant-Petersson and Renwick 1996). Similarly, P-deficient wheat plants suffered a severe infection by *Gaeumannomyces graminis*, a fungal pathogen (Brennan 1989).

Considering these aspects, in present study we investigated the changes in photosynthetic characteristics in cowpea seedlings grown under the combination of higher solar UV-B radiation and limited Mg supply. Mg, an important macroelement, plays an important role in plant growth, Chl synthesis, cellular pH control, protein synthesis, enzyme activation, and protein phosphorylation (Marschner 1986, Joslin and Wolfe 1994, Suichu *et al.* 1995, Rintamaki *et al.* 1996). That's why Mg was specifically selected for this study to investigate its combined effect with UV-B radiation.

Materials and methods

Plants: Seeds of *Vigna unguiculata* L. cv. Pusa-152 were germinated in vermiculite and grown during July 1995 in the natural field environments at Madurai (10°N), India. Seedlings were daily supplied with a standard nutrient solution. The composition of the basal nutrient solution was the same as described in Marschner and Cakmak (1989). To attain Mg deficiency, the seedlings were supplied with 20 μM $MgSO_4$, while control plants received 650 μM $MgSO_4$. Solar UV-B supplementation was created by adding 2.0 kJ m^{-2} d^{-1} irradiance equivalent to 20 % of ambient solar UV-B (10.0 kJ m^{-2} d^{-1}) radiation at Madurai. Seedlings were exposed to supplemental solar UV-B radiation for 6 h each day centered around solar noon. UV-B radiation was artificially provided through an output regulated field UV-B

irradiation system by *Philips TL 20W/12* sunlamps (*Philips Gloelampenfabrieken*, Holland).

Photochemical activities: Type II broken chloroplasts were extracted from both primary and first formed trifoliate leaves by the method of Reeves and Hall (1973). PS2 dependent DCPIP photoreduction was measured spectrophotometrically (*Hitachi 557*) by following the changes in absorbance at 590 nm caused by red actinic radiation (>640 nm) of 150 W m^{-2}. The photomultiplier was protected by a *Corning CS-4-90* blue filter. The reaction mixture (2 cm^3) was composed of 20 mm Tris-HCl (pH 7.5), 100 mM sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 5 mM NH_4Cl, and 50 μM DCPIP. Uncoupled electron transport mediated by PS1 was determined polarographically as oxygen consumption with $DCPIPH_2$, sodium ascorbate as electron donor system, and MV as terminal auto-oxidizable electron acceptor using an oxygen electrode (*Hansatech*, U.K.). The reaction medium in a final volume of 1 cm^3 contained 20 mM Tris-HCl (pH 7.5), 100 mM sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 2 mM sodium ascorbate, 50 μm DCPIP, 5 μM DCMU, 0.1 mM sodium oxide, 1 mM MV, and chloroplasts equivalent to 20 μg Chl. To uncouple the electron flow from phosphorylation, 5 mM NH_4Cl was added to the assay buffer. The PPFD amounted to 1500 μmol m^{-2} at the surface of the electrode cell. The Chl concentration was determined in 80 % acetone according to Arnon (1949).

CO_2 uptake rate by intact leaves was monitored using a *Li-Cor 6000* (*Li-Cor*, USA) portable infra-red gas analyser equipped with a 250 cm^3 leaf chamber.

Chl fluorescence measurements of detached intact primary and first trifoliate leaves at room temperature after a 10 min dark adaptation period were taken as described in Premkumar *et al.* (1996). The excitation was performed with a broad band blue radiation (400-460 nm, *Corning 5113*) at a PFD of 100 W m^{-2}. The signal was stored in a digital oscilloscope (*Iwatsu SS 5802*, Japan) and later copied on a recorder (*Hitachi 056*).

SDS-PAGE analysis of chloroplast proteins was made using the Laemmli (1970) buffer system with gels containing a linear gradient (8-18 %, m/v) of acrylamide. The extraction and processing of chloroplast proteins was the same as in Nedunchezhian *et al.* (1995), and protein contents were estimated according to Lowry *et al.* (1951).

Results and discussion

Photochemical activities significantly decreased under both UV-B and Mg deficiency (Table 1). Mg_d seedlings exhibited a higher level of such reduction than those that received UV-B supplementation only. Increased solar UV-B radiation tended to alleviate the loss in Mg_d seedlings to a minor extent. Such alleviating ability was absent in PS2 activity of chloroplasts isolated from trifoliate leaves of seedlings under the combined stress. In agreement with the present study, previous studies have revealed that both Mg deficiency (McSwain *et al.* 1976) and increased solar UV-B radiation (Nedunchezhian and Kulandaivelu 1997) individually reduced the photochemical activities in several crop species. A more significant reduction of

photosynthetic electron flow in Mg_d seedlings as compared to increased solar UV-B radiation could be due to ultrastructural changes in chloroplasts (McSwain *et al.* 1976, Marschner 1986).

Table 1. Photochemical activities of chloroplasts isolated from cowpea seedlings grown under four different treatments. Seedlings were grown under control, Mg_s (sunlight, standard nutrient solution with 650 μM $MgSO_4$), Mg-deficient (Mg_d, sunlight, nutrient medium with 20 μM $MgSO_4$), increased solar UV-B radiation (ambient + 2 kJ m^{-2} d^{-1} UV-B), and Mg_d + increased solar UV-B radiation. After 20 d of growth under these conditions, chloroplasts were isolated from the primary leaves and photosystem (PS) 1 [nmol(O_2) kg^{-1}(Chl) s^{-1}] and 2 [nmol(DCPIP red.) kg^{-1}(Chl) s^{-1}] activities, net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}], intercellular CO_2 concentration (C_i) [cm^3 m^{-3}], and stomatal resistance, r_s [s cm^{-1}] were measured. Values are means of 5 replicates. Figures in parentheses are percentage activity with reference to control (Mg_s).

	Leaves	Mg_s	Mg_d	UV-B	Mg_d + UV-B
PS2	primary	168.1±2.9	102.9±1.6 (61.2)	115.1±1.4 (68.5)	122.1±4.9 (72.6)
	trifoliate	151.3±3.9	88.8±2.6 (58.6)	109.1±1.9 (72.1)	93.2±1.3 (61.6)
PS1	primary	432.6±2.6	307.3±1.8 (71.0)	318.7±1.0 (73.6)	346.8±1.0 (80.2)
	trifoliate	416.7±7.5	284.9±2.4 (68.4)	374.7±3.3 (89.9)	384.9±1.2 (92.4)
P_N	primary	38.5±1.7	29.1±1.3 (75.7)	34.1±1.9 (88.5)	36.1±1.2 (93.8)
	trifoliate	26.3±1.0	16.7±1.2 (63.5)	21.4±1.1 (81.0)	19.4±1.4 (73.8)
C_i	primary	148.1±16.7	122.4±12.9 (82.6)	169.9±16.2 (114.1)	142.7±20.7 (96.3)
	trifoliate	136.2±18.3	129.9±16.5 (95.4)	152.5±11.9 (112.0)	134.3±12.4 (98.6)
r_s	primary	0.81±0.26	1.16±0.17 (143.2)	1.67±0.19 (207.2)	1.29±0.12 (159.2)
	trifoliate	0.64±0.12	0.72±0.28 (112.5)	0.96±0.21 (150.0)	1.52±0.13 (237.5)

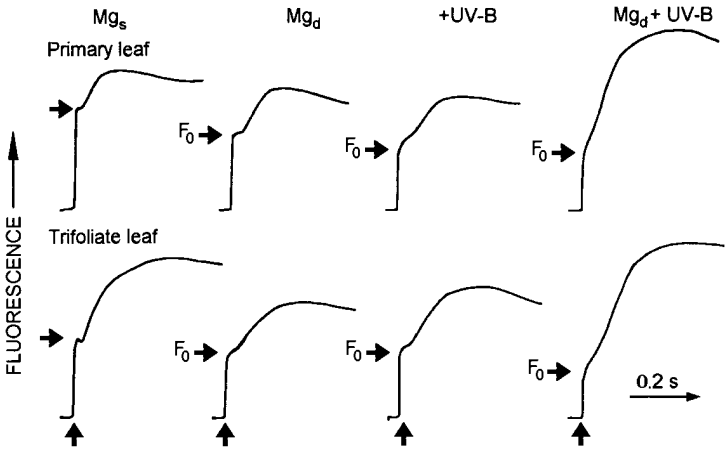

Fig. 1. Typical fast fluorescence transients in primary and trifoliate leaves of 20-d-old cowpea seedlings grown under various treatments as described in Materials and methods.

To get an overall picture on the changes in primary photochemistry, fast and slow fluorescence kinetics (Figs. 1 and 2) were followed in dark-adapted intact leaves. Low Mg supply lowered the slow rise of variable fluorescence in trifoliate leaves as compared to primary leaves. Consistent with this, Godde and Hefer (1994) found that

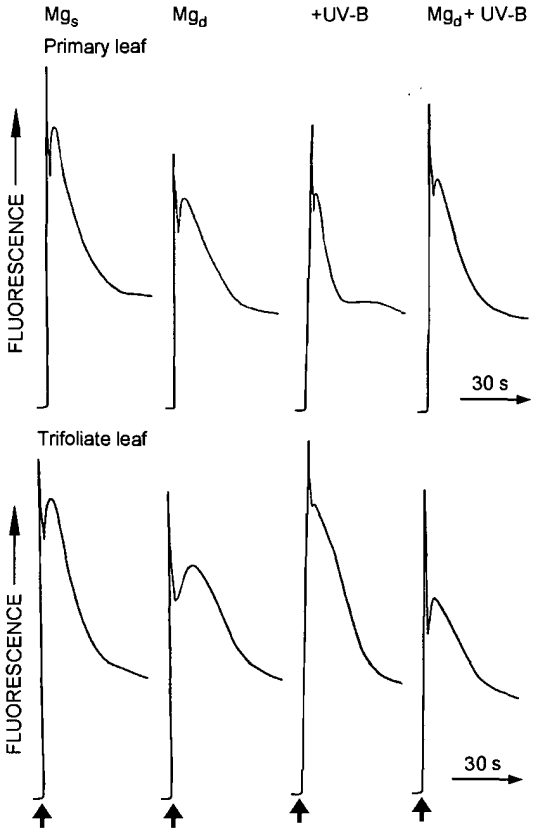

Fig. 2. Changes of slow fluorescence kinetics in primary and trifoliate leaves of cowpea seedlings grown for 20 d under various treatments. For details on fluorescence measurements see Materials and methods.

the plants grown in Mg-deficient medium had decreased their F_v/F_m ratio and this clearly indicates a proportional reduction in the relative concentrations of both reducing and non-reducing Q_B centres (Zeuthen *et al.* 1990, Vass *et al.* 1996, Mulo *et al.* 1997) by a low Mg supply and enhanced solar UV-B radiation. UV-B-induced damages in PS2-RC components were earlier confirmed by Melis *et al.* (1992). Such damages were minimized in UV-B irradiated Mg-deficient cowpea seedlings. Fluorescence at P level was slightly decreased by increased solar UV-B radiation when compared to the Mg_s seedlings. In contrast, Mg_d seedlings showed a significant enhancement in the variable part when they received a supplemental solar UV-B radiation. This enhancement was evident in both primary and trifoliate leaves. PSM oscillations of slow fluorescence induction kinetics were greatly altered by both low

Mg supply and enhanced solar UV-B radiation. The P level, an indicator of maximum transient yield of fluorescence, declined greatly by a low Mg availability. The rate of P-S quenching was rapid in trifoliate leaves of Mg_s and UV-B irradiated Mg_d seedlings. The slow P-S quenching in Mg_d and UV-B exposed leaves indicates an impairment of photosynthetic CO_2 uptake. UV-B irradiated Mg_d seedlings exhibited a significant recovery at P in primary leaves. Although P level was reduced by combined treatment to only 15 % in trifoliate leaves, the reduction at M was two times higher. This could be the main cause for the reduced alleviating ability of enhanced solar UV-B radiation on Mg deficiency induced PS2 loss.

In agreement with photosynthetic electron transport activities, P_N also exhibited a similar inhibitory pattern (Table 1). However, the magnitude of reduction was comparatively lesser than with photochemical activities. Reductions in P_N by both Mg deficiency (Terry and Ulrich 1974, Marschner 1986, Marschner and Cakmak 1989) and UV-B radiation (Lingakumar and Kulandaivelu 1993, Premkumar and Kulandaivelu 1996) have been reported for several plant species. Enhanced solar UV-B radiation increased the intercellular CO_2 (C_i) in both Mg_d and Mg_s seedlings and this was due to decreased P_N (Naidu *et al.* 1993). A linear relationship between C_i and P_N was not obtained in this study. Therefore, the reductions in P_N could not be attributed to stomatal limitations only.

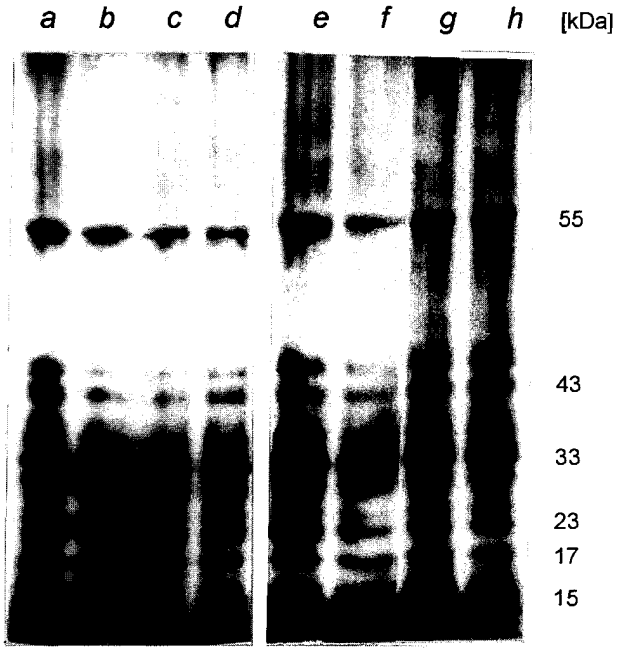

Fig. 3. Coomassie blue stained protein profiles of chloroplast proteins isolated from primary (lanes *a-d*) and trifoliate (lanes *e-h*) leaves of 20-d-old grown under Mg sufficiency (lanes *a,e*), Mg deficiency (lanes *b,f*), enhanced solar UV-B radiation (lanes *c,g*), and combination of both Mg deficiency and enhanced solar UV-B radiation (lanes *d,h*). For details on sample preparation and electrophoresis see Materials and methods.

Although UV-B radiation and Mg-deficiency reduced photosynthetic activities, Mg deficiency alone elicited adverse impacts on several chloroplast proteins of trifoliate leaves (Fig. 3). In trifoliate leaves, other two treatments did not reveal any marked differences except a slight decrease in 25 and 19 kDa polypeptides in seedlings exposed to combined stress. In primary leaves, polypeptides in the range of 43-47 kDa showed significant decrease in all the treatments and the extent of such decrease was higher when Mg deficiency and increased solar UV-B radiation were given independently. Surprisingly, there was no apparent damage to important thylakoid proteins as PS2 activity was affected by all treatments.

UV-B-induced changes in chloroplast proteins seen in this study are in a sharp contrast with previous growth cabinet studies where cowpea seedlings were used for impact assessment (Lingakumar and Kulandaivelu 1993, Nedunchezhian *et al.* 1995). This discrepancy could be ascribed to the growth environment and UV-B treatment. Our results suggest that Mg deficiency alone had a pronounced impact on protein machinery in the photosynthetic apparatus. The role of Mg in protein synthetic process has been well documented by Marschner (1986). Contrary to the effects of Mg deficiency, increased solar UV-B radiation alone altered the functional aspects of photosynthetic apparatus in field-grown cowpea seedlings.

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