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BRIEF COMMUNICATION

Effect of Cd and UV-B radiation on polypeptide composition and photosystem activities of *Vigna unguiculata* chloroplasts

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Abstract

Rates of whole chain and photosystem 2 activities in chloroplasts isolated from *Vigna unguiculata* L. seedlings grown under ultraviolet-B (UV-B) enhanced radiation were less affected by 3, 6 and 9 mM $CdCl_2$ for 60 min at 0 °C in the dark than the rates in chloroplasts from control plants grown under normal irradiation. The results are in agreement with changes in contents of chloroplast 55, 47, 43, 33, 29, 27-25, 23 and 17 kDa polypeptides that were significantly lowered at 3, 6 and 9 mM $CdCl_2$ only in chloroplasts from control plants. On the other hand, in the simultaneous treatment of chloroplast isolated from control plants the UV-B supported the inhibitory effect of all applied concentrations of $CdCl_2$. The photosystem 1 activity was only marginally affected in the all experimental variants.

Key words: chloroplast proteins, environmental stresses, interaction, photosynthesis

Both cadmium (Baszynski *et al.* 1980, Roynet *et al.* 1981, Bazzaz and Govindjee 1974, Hampp *et al.* 1976, Clijsters and Van Assche 1985, Barua and Jana 1986, Becerril *et al.* 1988) and UV-B radiation (Brandle *et al.* 1977) affect the activity of photosystem 2 (PS 2) particularly affecting its reaction centre (Li and Miles 1975, Noorudeen and Kulandaivelu 1982) and the water splitting reaction (Renger *et al.* 1989, Kulandaivelu *et al.* 1991, Nedunchezhian and Kulandaivelu 1991). Since both UV-B radiation and Cd^{2+} act at a similar site, we were interested to find out how these two environmental stresses interact and alter the photosynthetic function of the *Vigna unguiculata* chloroplasts.

Three-day old seedlings of *Vigna unguiculata* L. were exposed to radiation from four *Philips* 40 W fluorescent tubes (type *TL/33*) plus one *Philips 20 W/12* sunlamp (*N.V. Philips, Gloelampenfabrieken*, The Netherlands), (UV-B treated plants), or

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from four 40 W white fluorescent tubes (control plants). After continuous irradiation at 28 °C for 48 h, type II broken chloroplasts were isolated for photosynthetic measurements according to Kulandaivelu *et al.* (1989). Chlorophyll (Chl) content was estimated according to method of Lichtenthaler and Wellburn (1983).

In the first experiment, chloroplasts isolated from both the control and UV-B treated plants were suspended in 10 cm^3 of isolation medium (Tris-HCl, pH 7.8, 5 mM NaCl, 10 mM $MgCl_2$, 100 mM saccharose) at a final Chl concentration of 500 g m^{-3} in a conical flask and $CdCl_2$, where mentioned, was added at a final concentration of 3, 6 or 9 mM. The conical flasks were kept on ice and gently shaken using a shaker (*Labline*, model *3575-1*). In the second experiment, suspension of chloroplast [500 g(Chl) m^{-3}] isolated from control plants was uniformly spread on a Petri dish and irradiated with a *Philips Sunlamp* type *20 W/12* filtered with cellulose acetate *5 mil* filter as described by Noorudeen and Kulandaivelu (1982). The control

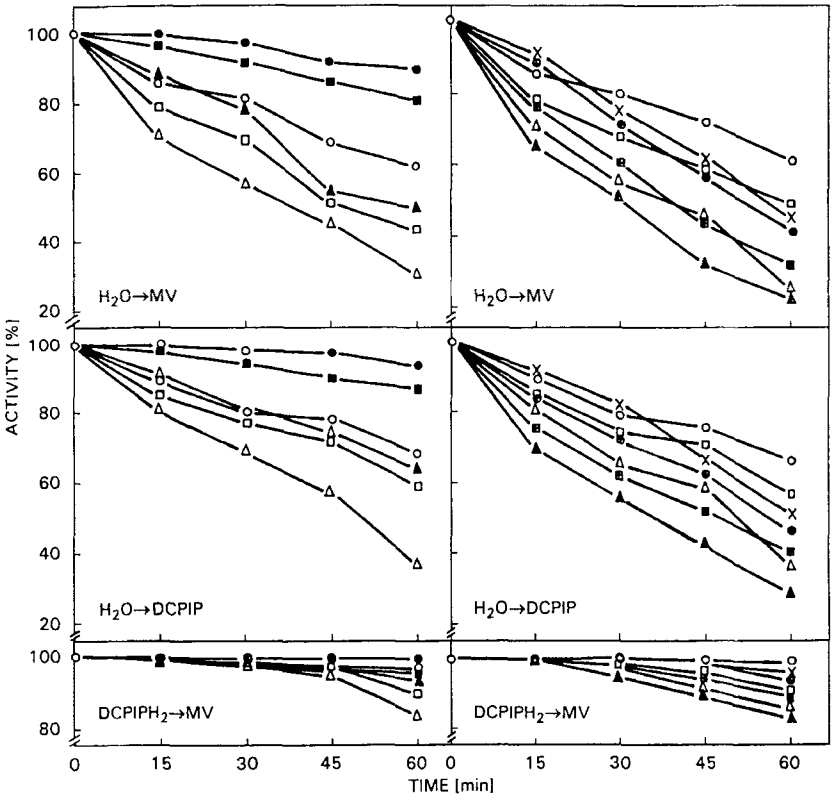

Fig. 1. Changes in the rate of whole chain, PS2 and PS1 electron transport activities in chloroplasts of control (*open symbols*) and UV-B treated (*closed symbols*) *Vigna* plants (*left*) and in chloroplasts of control plants treated with $CdCl_2$ (*open symbols*), UV-B (*crosses*) and combination of UV-B and $CdCl_2$ (*closed symbols*) (*right*) as a function of incubation time. Chloroplasts were incubated with 3 (*circles*), 6 (*squares*) and 9 (*triangles*) mM $CdCl_2$ at 0 °C. The 100 % values are $H_2O \rightarrow MV$: 24, 22; $H_2O \rightarrow DCPIP$: 39, 36; $DCPIPH_2 \rightarrow MV$: 72, 71 [$\mu mol(O_2)\ kg^{-1}(Chl)\ s^{-1}$] for chloroplasts of control and UV-B treated plants, respectively. Values represent means of 3 experiments and differences are significant at 5 % level.

samples were covered with *Mylar A 5 mil* filter. $CdCl_2$ was added at final concentrations of 3, 6 or 9 mM.

Photosynthetic reactions mediated by photosystem 2 (PS2) and photosystem 1 (PS1) were measured as described by Noorudeen and Kulandaivelu (1982). Whole chain electron transport ($H_2O \rightarrow MV$) was measured according to Armond *et al.* (1978).

Chloroplast proteins were separated by SDS-PAGE according to Laemmli (1970). For this purpose, chloroplast suspension was mixed with an equal volume of cold 10 % trichloroacetic acid. The mixture was shaken and incubated in ice bath for 1 h to precipitate proteins. The protein pellet was collected by centrifugation at 2000 *g* for 15 min and redissolved in 10 % sodium dodecyl sulphate. Protein content was estimated according to Lowry *et al.* (1951).

The changes in various photosynthetic electron transport reactions were followed after 15 to 60 min incubation at 0 °C in dark. The decline in whole chain and PS2 electron transport activities showed a more or less linear decay during the 60 min incubation and the extent of decay increased proportionately with the concentration of added $CdCl_2$ (Fig. 1, *left*). The decay was always much less in chloroplasts of UV-B treated plants than in the control ones. Changes in the PS1 activity due to the addition of $CdCl_2$ were only marginal, and even at 9 mM $CdCl_2$ a decline of less than 20 % after 60 min incubation was observed in chloroplasts isolated from control plants. In contrast to the above results in the simultaneous treatment with UV-B and

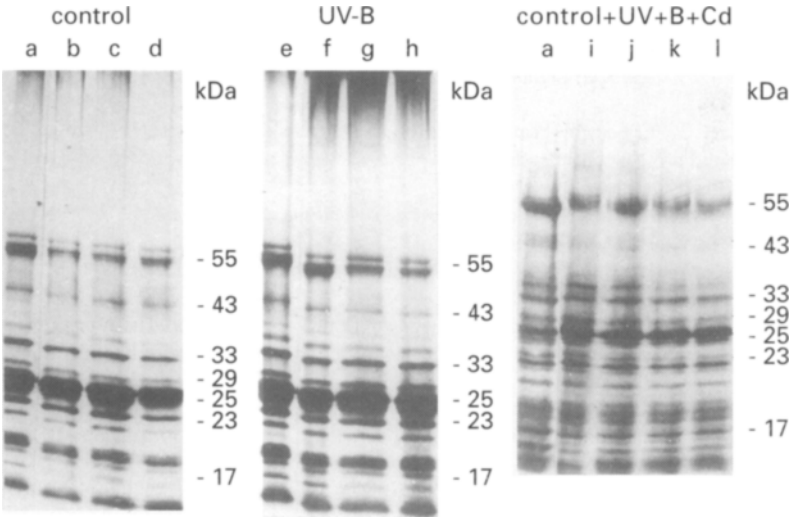

Fig. 2. SDS-PAGE profiles of chloroplast proteins of *Vigna* seedlings. Chloroplasts from control (*a*) and UV-B treated (*e*) plants were incubated for 60 min at 3 (*b*), 6 (*c*) and 9 (*d*) mM $CdCl_2$; *i* - *a* + UV-B; *j* - UV-B + 3 mM $CdCl_2$; *k* - UV-B + 6 mM $CdCl_2$; *l* - UV-B + 9 mM $CdCl_2$. For details see text.

$CdCl_2$ on chloroplasts from control plants the UV-B supported the inhibitory effect of all applied concentrations of $CdCl_2$ (Fig. 1, *right*).

In order to check if Cd^{2+} treatment induced any structural changes, the chloroplast proteins were analyzed by SDS-PAGE. The analyses (Fig. 2) supported the results of photosystem activities determination: control chloroplasts showed large decrease in the content of 55, 43, 33, 29, 27, 23 and 17 kDa polypeptides that was proportional to the concentration of Cd^{2+}, while chloroplasts from UV-B treated plants showed only marginal loss in the contents of 55, 43, 33, 23 and 17 kDa polypeptides even at the highest $CdCl_2$ concentration (9 mM) used. The simultaneous treatment of chloroplasts from control plants with UV-B + Cd^{2+} induced a severe loss in 43, 33, 23 and 17 kDa polypeptides. Extrinsic polypeptides like 33, 23 and 17 kDa are essential for long term stability and function of the O_2 evolving reactions. Release of these polypeptides from the chloroplasts causes inactivation of O_2 evolution (Kuwabara and Murta 1983, Murata and Miyao 1987, Shen *et al.* 1988). Therefore, the observed loss of PS2 activity in chloroplasts from control plants could be correlated with the release of the three extrinsic polypeptides. From the above results we conclude that chloroplasts isolated from *Vigna* seedlings grown under UV-B enhanced radiation showed a relatively high stability to Cd^{2+} damage which was mainly due to stabilization of a few key polypeptides, responsible for water oxidation and PS2 electron transport, while the combination of UV-B and Cd^{2+} stresses resulted in cumulative inhibition of the PS2 activity.

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