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REGULAR ARTICLE

Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd

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Abstract

Aims The objective of this research was to study the effects of ultraviolet-B (UV-B) radiation and cadmium (Cd), applied individually, in combination, and alternately in soybean (*Glycine max* L) seedlings.

Methods Pigment content, photosynthetic parameters, chlorophyll fluorescence and antioxidant enzymes (i.e., catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (POD)), and malondialdehyde (MDA) content were investigated. Observations began 6 days after the exposure to Cd (40 mgkg^{-1}), UV (7.1 kJ m^{-2}) and Cd+UV, as well as 3 days after the exposure to Cd/(or) UV followed by exposure to Cd+UV (i.e., Cd~Cd+UV, UV~Cd+UV).

Results After 6 days of treatment, all treatments showed lower shoot dry weight relative to controls, except Cd~Cd+UV. Cd and Cd+UV significantly ($P<0.05$) decreased root length and root dry weight. UV-B and Cd alone significantly ($P<0.05$) reduced pigment content, net photosynthetic rate, stomatal conduction, transpiration and SOD activity, and significantly ($P<0.05$) increased MDA content. Cd also significantly ($P<0.05$) reduced chlorophyll fluorescence but increased H_2O_2 contents. Compared to individual treatments, the combined effect was more detrimental to the above parameters. The sequential treatments (Cd~Cd+UV or UV~Cd+UV) were no or less detrimental compared to the combined treatments.

Conclusions This is the first report of the sequentially applying UV-B and Cd stress. Reduced detrimental effects by the sequential application compared to the combination suggested the cross-adaptation of Cd and UV-B mediated protection of Cd+UV in soybean.

Keywords UV-B radiation · Cadmium · *Glycine max* · Photosynthesis · Oxidative stress · Cross-adaptation

Introduction

Anthropogenic emission of chlorofluorocarbon compounds has resulted in a global reduction of the stratospheric ozone layer, leading to increased ultraviolet-B (UV-B) radiation (280–315 nm) on the earth's surface (McKenzie et al. 2007). Increases in UV-B have raised concerns regarding the damaging impact of this radiation on crop plants (Kakani et al. 2003; Caldwell et al. 2007). It may damage proteins, nucleic acids and lipids and thus inflict mutagenesis and depression of key physiological processes (Agrawal and Rathore 2007; Lizana et al. 2009).

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The photosynthetic apparatus and the free-radical scavenging system are major target sites of UV-B radiation (Prasad et al. 2005; Albert et al. 2010).

Under natural conditions, effects of UV-B radiation on plants are related to other environmental factors, including CO_2, O_3, drought and heavy metals (Larsson et al. 1998; Ambasht and Agrawal 2003; Poulson et al. 2006; Tegelberg et al. 2008). Thus, further studies of the relationships between UV-B radiation and other environmental factors would be more closely approximate natural conditions, and promote understanding of biochemical responses in plants. Among the other environmental factors cadmium (Cd) is a highly toxic element that has become widespread in the environment as a result of several sources of pollution. High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated into the food chain mainly through uptake by plants. Cd can directly or indirectly interfere with numerous biochemical and physiological processes (Wang et al. 2008; López-Millán et al. 2009; Zhang et al. 2009). Cd injury can likely be attributed to the alteration of oxidant levels in plants, as it causes oxidative stress by generating reactive oxygen species (ROS) (Romero-Puertas et al. 2004).

UV-B radiation and heavy metals are ‘novel’ man made abiotic stress factors. The potential interactions between UV-B radiation and Cd was studied because these two factors have an increasing negative impact in agriculture and combined environmental effects are often overlooked. Another reason for the choice of the two potential stressors is that UV-B irradiation and Cd have been demonstrated to stimulate the formation of ROS at various points in the photosynthetic and respiratory electron transport chain and induce lipid peroxidation, thereby causing extensive damage to higher plants (Arora et al. 2002; Ambasht and Agrawal 2003; Zhang et al. 2010).

Boussiba et al. (1975) proposed the phenomenon of “cross-adaptation” by which a given stress confers increased resistance to other, apparently unrelated stresses. Many studies have documented the existence of cross-adaptation in plants (Wang et al. 2004; Fujita et al. 2006). Chao et al. (2009) showed that heat shock and hydrogen peroxide induced Cd tolerance of rice seedlings. Hsu and Kao (2007) also found that heat shock pretreatment protected against subsequent Cd-induced oxidative damage of rice seedlings. Chmielowska et al. (2010) demonstrated the ability of copper stress to protect pepper plants against a plant disease, Verticillium wilt, and to trigger other plant defence mechanisms against pathogens. Research has demonstrated that elevated UV-B induces cross-adaptation to cold in *Rhododendron* leaf tissues (Chalker-Scott and Scott 2004) and to frost and heat in conifer seedling shoots (L’Hirondelle and Binder 2005). However, no work has been done on cross-adaptation between UV-B and heavy metals (particularly Cd).

Experimental treatments were designed to investigate mutual effects of UV-B radiation and Cd cross-adaptation. The soybean seedlings pre-exposed to UV-B radiation or Cd was then exposed to a combination of UV-B radiation and Cd. In the present study, growth parameters, photosynthesis, ultraviolet absorbing compounds (e.g. flavonoids), lipid peroxidation and anti-oxidative machinery were investigated. The aim of this study was to elucidate the possible mechanisms of interaction between enhanced UV-B radiation and Cd.

Materials and methods

Plant material and growth conditions

Soybean cultivar (*Glycine max* cv. Tiefeng 29) seedlings were grown from seed in pots. Each pot was filled with vermiculite and seedlings were watered to saturation on alternate days with Hoagland solution in a greenhouse (25°C day/20°C night, 16 h/8 h light/dark period, 800 μmol m^{-2} s^{-1} photosynthetically active radiation and 80% relative humidity).

UV-B and Cd treatment

Supplemental UV-B was provided artificially by 40 W UV-B fluorescent tubes (Beijing Electric light Source Research Institute, China). The tubes flanked both sides of a cool-white fluorescent lamp that provided photosynthetically active radiation of 800 μmol m^{-2} s^{-1}. Banks of four lamps (120 cm long) fitted 30 cm apart on a steel frame were suspended above and perpendicular to the rows of pots. The 50 cm distance between the top of the seedling canopy and UV-B lamps was kept constant by adjusting the steel frame. The radiation was filtered through 0.127 mm cellulose diacetate to remove all incident UV-C (<280 nm) and the radiation intensity was measured with a UV-B radiation detector produced at Beijing Normal University. The seedlings beneath cellulose diacetate film received UV-B_{BE} (7.1 kJ m^{-2}) that mimicked a 20% reduction in stratospheric ozone.

After 15 day from sowing the seedlings were subjected to six treatments: (a) control: without UV-B irradiation and without Cd^{2+}, (b) UV: increased UV-B irradiation, (c) Cd: increased Cd^{2+}, (d) UV+Cd: increased UV-B irradiation and increased Cd^{2+}, (e) Cd~UV+Cd: pre-exposed to increased Cd^{2+} for 3 day, then exposed to UV+Cd for 3 day, and (f) UV~UV+Cd: pre-exposed to increased UV-B irradiation for 3 day, then exposed to UV+Cd for 3 day.

In order to investigate the interactive effects between UV-B and Cd, these two factors were generally applied during the same period. Our design (e, Cd~UV+Cd and f, UV~UV+Cd) provided a chance to observe alternate effects from different initiation points. Seedlings were irradiated with UV-B radiation (280–320 nm), 6 hd^{-1}, from 10:00–16:00. Cadmium was added as $CdCl_2 \cdot 2.5H_2O$ (40 mg Cd kg^{-1} DW vermiculite). All parameters were measured at 6 day after the beginning of each treatment. Each treatment was carried out in triplicate.

Growth and biomass production

Biomass production and growth parameters were determined at 6 day after treatment. Seedling height and root length of ten seedlings were measured. Shoot and root dry weights were obtained by drying the samples at 80°C to a constant weight.

Photosynthetic pigment and photosynthetic parameter determination

Total chlorophyll (Chl) and carotenoid (Car) content were extracted from 0.1 g of leaf discs with 10 ml 80% acetone and quantified as described by Agrawal and Rathore (2007).

Between 10:00 and 12:00 on each sampling day, gas exchange by leaves (one leaf per plant, three plants per replicate) was measured with a portable photosynthesis system (Li-6400, Li-Cor Lincoln, NE, USA). Net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E) were measured under ambient CO_2 (370 μmol mol^{-1}). Photosynthetic photon flux density (PPFD) was set at 800 μmolm^{-2} s^{-1} in the cuvette containing the leaf for Pn measurement.

Chlorophyll fluorescence

Fluorescence parameters of intact leaves (one leaf per plant, three plants per replicate) were measured using Li-6400-40LCF (Li-Cor Lincoln, NE, USA). The minimal chlorophyll fluorescence (*F*o) level when photosystem II centres are open was measured after applying a far-red pulse for 6 s. The maximal fluorescence (*F*m) after 30 min of dark adaptation was measured after applying a saturating flash for 0.8 s. Maximal photochemical efficiency of PSII (*F*v/*F*m) was expressed as: $Fv/Fm=(Fm\text{-}Fo)/Fm$.

Analysis of U V-absorbing compounds (flavonoids)

Fresh leaf sample was immersed in an 80% ethanol 20% water solution (*v*/*v*) at a concentration of 10 mg of tissue/ml of solution and incubated at 55°C for 30 min. Flavonoid in the crude extract was determined by measuring absorbance at 334 nm (Lois 1994).

Extraction and assay of antioxidant enzymes

Fresh leaf sample (0.5 g) was homogenized in 5 ml extraction buffer (0.1 M phosphate buffer pH 6.8) with mortar and pestle on ice. The homogenate was then centrifuged at 12,000× *g* for 15 min at 4°C and the supernatant was used as the crude extract for superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POD). SOD activity was assayed by measuring the ability of the enzyme in the crude extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated photochemically (Beyer and Fridovich 1987). One unit of SOD was defined as the amount of enzyme required to inhibit the rate of reduction NBT by 50% at 25°C. POD and CAT activities were assayed following the method of Chance and Maehly (1955) with some modification. The POD reaction solution contained 50 mM phosphate buffer (pH 7.8), 25 mM guaiacol, 200 mM H_2O_2, and the enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined. CAT activity was assayed in a reaction mixture containing 50 mM potassium phosphate buffer (pH

7.0, containing 0.1 mM EDTA), 200 mM H_2O_2, and the enzyme extract. The reaction was started with the addition of the supernatant, and the decomposition rate of H_2O_2 was followed at 240 nm.

MDA and H_2O_2 content

The estimation of MDA content was made according to the method by Islam et al. (2008). Fresh leaf sample (1.0 g) was homogenized with 4 ml of 0.1% (*w*/*v*) trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 12,000× *g* for 20 min and the supernatant was used for lipid peroxidation analysis. To 1 ml aliquot of the supernatant, 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was incubated in boiling water for 30 min. MDA content was then determined spectrophotometrically at 532 nm and corrected for nonspecific turbidity at 600 nm.

Hydrogen peroxide (H_2O_2) content was determined according to the method by Mukherjee and Choudhuri (1983). Fresh leaf sample (0.3 g) was ground with 4 ml ice-cold acetone. The homogenate was centrifuged at 12,000× *g* for 20 min at 4°C. Subsequently, the supernatant was added to 5% (*w*/*v*) titayl sunlphate and conc. NH_4OH solution. The post-reacted compound was centrifuged at 3,000× *g* for 10 min; the pellet was dissolved in 2 M H_2SO_4 and the absorbance value at 415 nm was determined. H_2O_2 content was calculated from a standard curve prepared in a similar way.

Statistical analysis

All experiments were conducted with three replicates, and results were expressed as mean ± standard deviation (SD). All data were subjected to one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p<0.05$) using the SPSS statistical package.

Results

Plant growth parameters and biomass production

No significant differences in seedling height were found between treatments (Table 1 and Fig. 1). All treatments showed lower shoot dry weight than the controls, except Cd~Cd+UV. Cd and Cd+UV significantly decreased root length and root dry weight, while other treatments had no effects compare to controls.

Changes in pigment content of leaves

Compared with the controls, the Chl a/b ratios showed no significant differences for all exposed treatments (Fig. 2a). Total chlorophyll content significantly decreased with Cd and Cd+UV treatments compared to the controls, and were considerably lower relative to UV~Cd+UV treatment (Fig. 2b). There was a significant ($P<0.05$) reduction of Car content with Cd and Cd+UV, but there were no differences among UV, Cd~Cd+UV and UV~Cd+UV treatments compared to controls (Fig. 2c). UV and Cd~Cd+UV had no significant effects on total chlorophyll content. UV radiation and Cd+UV significantly increased flavonoid content, but Cd, Cd~Cd+UV and UV~Cd+UV did not exert any influence on flavonoid (Fig. 2d).

Table 1 Effects of UV-B radiation and/or Cd^{2+} on growth characteristics of *Glycine max* seedlings. Values are the means ± SD ($n=3$). The different letters indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

Treatment	Seedling height (cm)	Root length (cm)	Shoot dry weight (g/10 plant)	Root dry weight (g/10 plant)
control	23.19±2.59a	15.75±2.04ab	1.33±0.21a	0.32±0.04ab
Cd	22.10±2.47a	12.88±1.61c	0.89±0.13c	0.24±0.04c
UV	21.17±3.08a	14.20±2.91bc	0.97±0.17bc	0.31±0.03ab
Cd+UV	20.06±1.59a	11.98±1.83c	1.10±0.11b	0.23±0.04c
Cd~Cd+UV	19.45±3.90a	14.00±1.29bc	1.17±0.25ab	0.36±0.03a
UV~Cd+UV	21.44±3.33a	16.56±0.94a	0.97±0.09bc	0.27±0.05bc

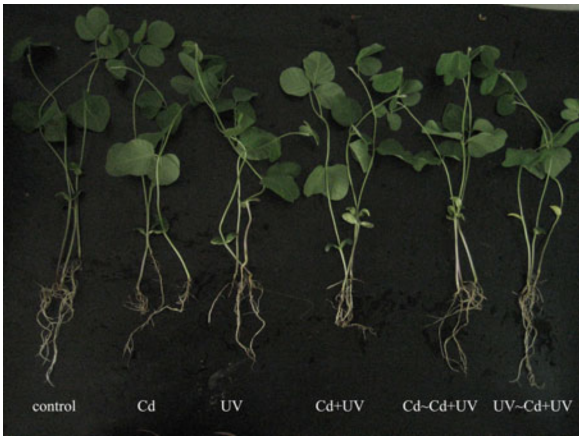

Fig. 1 Effects of UV-B and/or Cd on growth of *Glycine max* seedlings at the time of harvest

Photosynthetic characteristics

Net photosynthetic rate (Pn) showed a significant ($P<0.05$) decrease following all treatments except Cd~Cd+UV, moreover, inhibitory effects of UV, Cd and Cd+UV on Pn were more severe than those of UV~Cd+UV (Fig. 3a). Stomatal conduction (gs) and transpiration (E) showed significant ($P<0.05$) decreases following treatments with UV and Cd, either alone or in combination, with no significant changes following subsequent treatment with Cd and UV (Fig. 3b, d). There were no significant effects on internal CO_2 concentration (Ci) following all treatments compared to controls (Fig. 3c).

Chlorophyll fluorescence

Maximal photochemical efficiency *F*v/*F*m was used as an indicator of the potential quantum yield (photochemical efficiency) of PSII (Genty et al. 1989), while the ratio between variable and minimum fluorescence, *F*v/*F*o indicated the potential activity of PSII. After 6 day, Cd treatment showed lower values of *F*v/*F*m and *F*v/*F*o, while other treatments showed no significant changes compared with controls (Fig. 4). The ratio *F*v/*F*m is often used as a stress indicator and the decreases in *F*v/*F*m implied that the plants were under stress (Domínguez et al. 2011).

Changes in CAT, SOD and POD activity

Activities of antioxidative enzymes such as SOD, POD and CAT were measured in the leaves of *Glycine max*

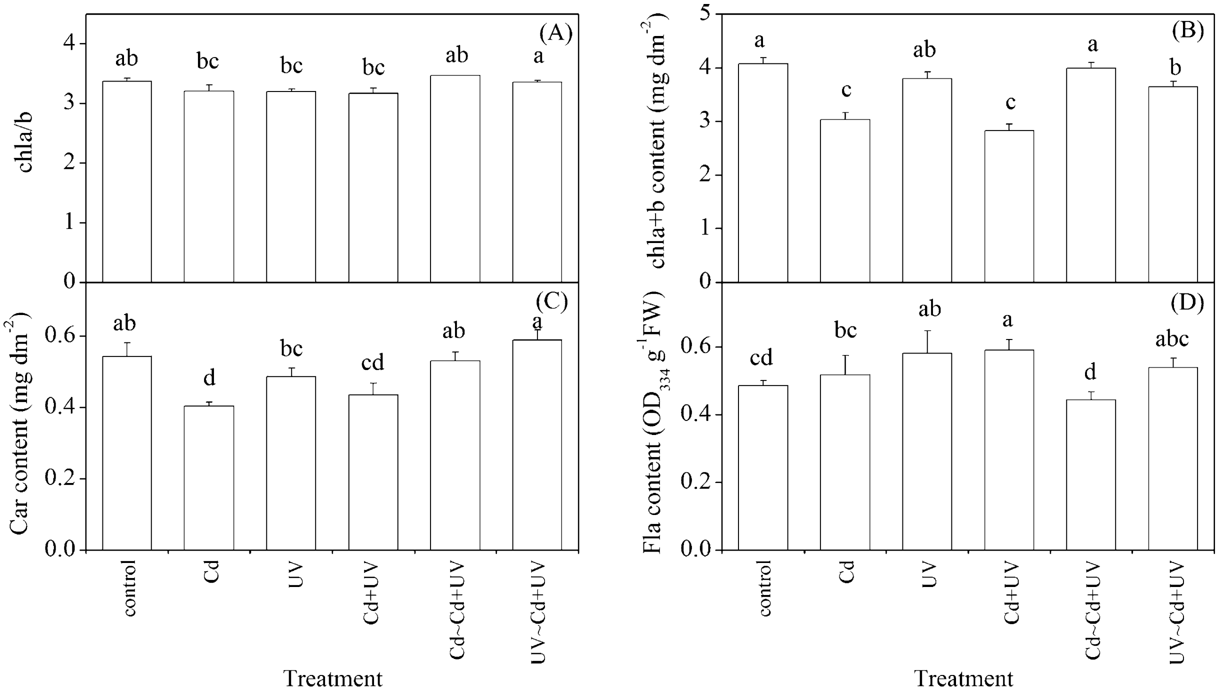

Fig. 2 Effects of UV-B and/or Cd on photosynthetic pigments and flavonoid content of *Glycine max* seedlings. *Vertical bars* represent standard deviations ($n=3$). The *different letters* indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

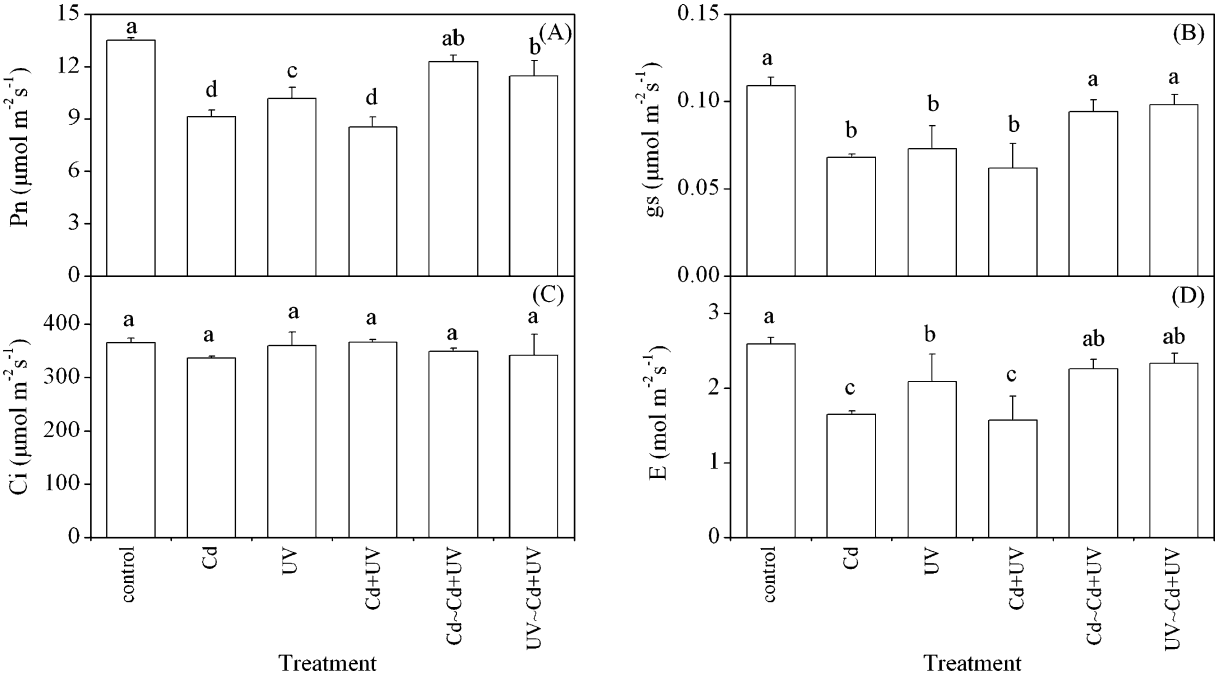

Fig. 3 Effects of UV-B and/or Cd on net photosynthetic rate (Pn), stomatal conduction (gs), internal CO2 concentration (Ci) and transpiration (E) of Glycine max seedlings. Vertical bars represent standard deviations (n=3). The different letters indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

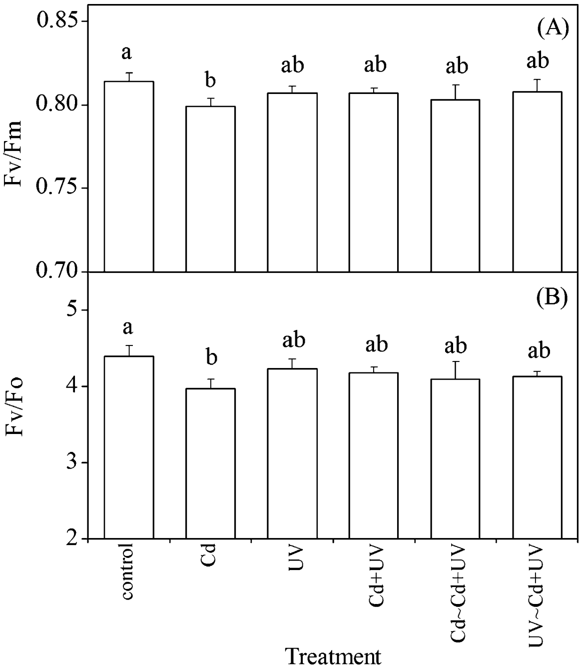

Fig. 4 Effects of UV-B and/or Cd on Fv/Fm and Fv/Fo of *Glycine max* seedlings. *Vertical bars* represent standard deviations (n=3). The *different letters* indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

seedlings exposed to Cd, UV, Cd+UV, Cd~Cd+UV and UV~Cd+UV (Fig. 5).

SOD activity showed significant ($P<0.05$) decreases following treatments with UV and Cd~Cd+UV. The inhibitory effect became more severe under Cd and Cd+UV, while no significant changes under UV~Cd+UV were observed (Fig. 5a). POD activity increased under UV and Cd treatments, either alone or in combination, with no significant changes under Cd~Cd+UV and UV~Cd+UV (Fig. 5b). Compared with the controls, CAT activity showed no significant differences for all exposed treatments (Fig. 5c).

Changes in MDA and H_2O_2 content

The level of lipid peroxidation was determined by means of the MDA content in the leaves of treated seedlings. All treatments (except Cd~Cd+UV) showed significantly ($P<0.05$) higher MDA content than the controls (Fig. 6).

Compared with the controls, H_2O_2 content showed significant ($P<0.05$) increases following treatments with Cd and Cd+UV (Fig. 7). Other treatments

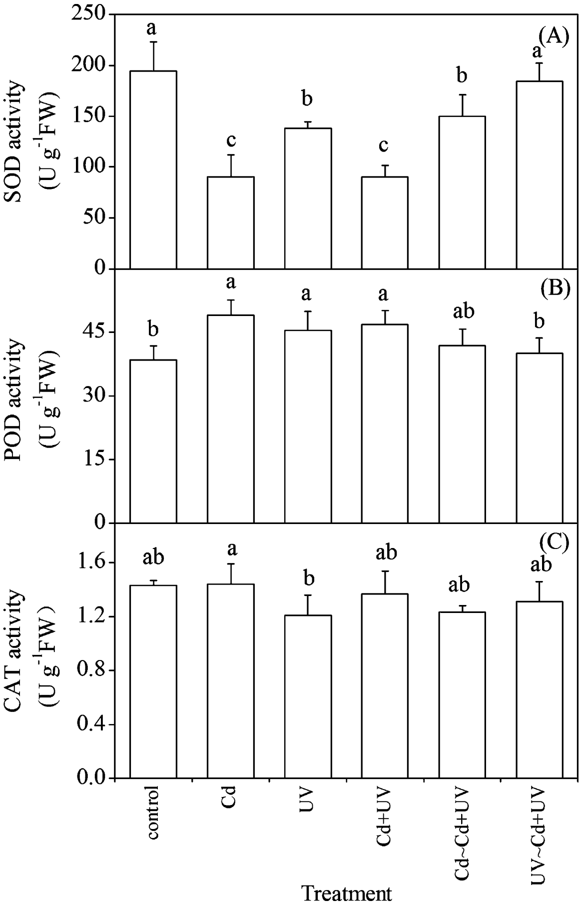

Fig. 5 Effects of UV-B and/or Cd on SOD, POD and CAT activity in leaves of *Glycine max* seedlings. *Vertical bars* represent standard deviations (n=3). The *different letters* indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

showed slightly higher but not significant ($P>0.05$) H_2O_2 content relative to controls.

Discussion

In the present investigation Cd and/or UV-B (except Cd~Cd+UV) reduced shoot dry weight, and Cd and Cd+UV significantly decreased root length and root dry weight (Table 1). Growth reductions due to heavy metals have been described for a large number of plant species at different developmental stages (Sharma and Agrawal 2005; Mishra and Agrawal 2006; Agrawal and Rathore 2007). Growth reductions due to Cd and/or UV-B can be explained as a consequence of adverse changes in metabolic or developmental processes. The results of this inhibitory role of Cd and/or UV-B on growth are consistent with other reported studies (Larbi et al. 2002; López-Millán et al. 2009).

Chlorophyll and carotenoid are central to energy manifestation for virtually every green plant system and significant alterations in their levels are likely to cause marked effects on the entire metabolism of a plant (Agrawal and Mishra 2009). Cadmium induced significant decreases in chlorophylls and carotenoids (Fig. 2). Stobart et al. (1985) suggested that Cd^{2+} inhibited the production of chlorophyll by affecting the synthesis of 5-aminolaevulinic acid and the protochlorophyllide reductase ternary complex with its substrates. According to Larbi et al. (2002), the decrease was attributed to a Cd-induced Fe deficiency. Previous studies reported negative, neutral and, rarely, positive effects of enhanced UV-B radiation on chlorophyll content in plants, the response of chlorophyll to UV-B radiation depended on developmental phase and environmental conditions (Larsson et al. 1998; Yu et al. 2005; Lizana et al. 2009). In this study no effects of UV-B and Cd~Cd+UV on chlorophyll content were found, and chlorophyll content under UV~Cd+UV was higher than under Cd+UV treatment. Car stabilizes and protects the lipid phase of the thylakoid membrane, and is quenchers of the excited triplet state of chlorophyll and singlet oxygen (Siefermann-Harms 1987). Car has been shown to increase (Middleton and Teramura 1993) under UV-B or decrease (Lizana et al. 2009) depending on species,

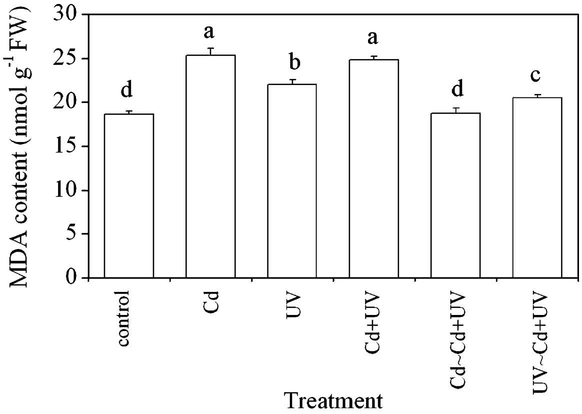

Fig. 6 Effects of UV-B and/or Cd on MDA content in leaves of *Glycine max* seedlings. *Vertical bars* represent standard deviations (n=3). The *different letters* indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

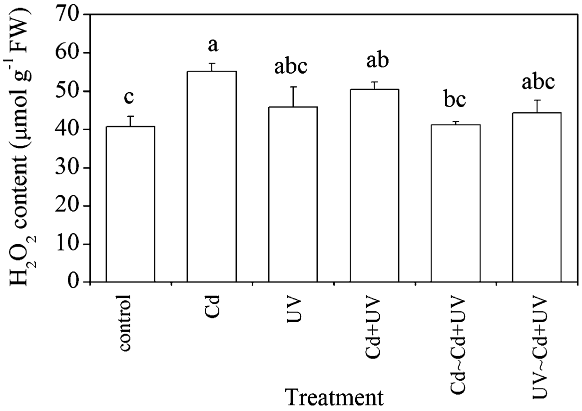

Fig. 7 Effects of UV-B and/or Cd on H_2O_2 content in leaves of *Glycine max* seedlings. *Vertical bars* represent standard deviations (n=3). The *different letters* indicate significant difference at $p<0.05$ (Tukey's multiple comparison test)

growth conditions, and other factors. In this study UV-B reduced the amount of Car, but not significantly. Otero et al. (2006) reported that in an aquatic liverwort *Jungermannia exsertifolia subsp. cordifolia*, the stronger influence of Cd masked the effects of UV-B on many variables, but an additive effect of Cd and UV-B may have promoted the decrease in chlorophyll and chl a/b. Since decreases in chlorophyll and Car in *Glycine max* leaves were induced by Cd and Cd+UV (Fig. 2), pre-exposure of seedlings to Cd or UV-B was expected to reduce subsequent Cd+UV-induced decreases in chlorophyll and Car. Sequential treatments (Cd~Cd+UV or UV~Cd+UV) confirmed this phenomenon. The results indicated cross-adaptation of Cd or UV-B with the combination of Cd+UV in plants.

The chlorophyll a/b ratios of UV-B and Cd, either alone or in combinations, were lower than those of UV~Cd+UV. This may indicate that pre-exposure of seedlings to UV-B reduced subsequent Cd+UV-induced decreases in chlorophyll a/b ratios.

There is substantial evidence that after UV-B exposure, certain flavonoids are selectively produced (Warren et al. 2003; Poulson et al. 2006). They have effective radical scavenging capabilities, and can contribute directly to enhance photoprotection against UV-B radiation (Smith and Markham 1998; Karioti et al. 2008). Therefore, flavonoids can help to maintain levels of photosynthetic pigments and normal photosynthetic activity. In the present study, flavonoid concent significantly increased under UV and Cd+UV treatments (Fig. 2d). Although there were no increases in flavonoid accumulation in sequential treatments, chlorophyll and Car content were higher than under Cd+UV treatment. These results showed that sequential treatments exhibited less influence on *Glycine max* seedlings. Chalker-Scott and Scott (2004) demonstrated that elevated UV-B irradiation induced cross-protection to cold temperatures in leaves of *Rhododendron* under field conditions. UV-B-induced production of phenolic compounds may be involved in increasing cold hardiness of *Rhododendron* leaf tissues. Fedina et al. (2006) found that salt pretreatment resulted in considerable decreases in levels of UV-B absorbing compounds after UV-B irradiation. Our data indeed showed that there was no change in flavonoid content after pretreatments.

Photosynthesis by *Glycine max* seedlings was adversely affected by UV-B irradiation and/or Cd treatments. Similar effects were noted in other studies (An et al. 2003; Prasad et al. 2005; López-Millán et al. 2009). The decrease in net CO_2 assimilation may be correlated with the decrease in content of photosynthetic pigments, gs and E (Figs. 2, 3) as observed in the present study. The results obtained from chlorophyll fluorescence measurements showed that Fv/Fm and Fv/Fo significantly decreased under Cd treatment (Fig. 4). Similar results have been obtained in other studies of plants under Cd (Ekmekçi et al. 2008; Shi and Cai 2008; Shi et al. 2010). The Fv/Fm ratio estimates the potential photochemical efficiency of PSII and a decrease in this parameter may be explained in part by the negative effects of Cd on the photochemical reactions (Liu et al. 2011).

Cd and UV-B either alone or in combination influenced antioxidative enzyme activities depending on species, exposure time, and dose (Prasad et al. 2004; Agrawal and Mishra 2009; Zu et al. 2010). Prasad et al. (2005) observed that Cd+UV-B further enhanced the activity of SOD and CAT, and they suggested that both the stresses together exhibited synergistic effects. Contrary to this, the result of Bhargava et al. (2007) suggested that Cd mediated protection of UV-B in the cyanobacterium. The reduced SOD activity obtained in the present study is consistent with the observations of Prasad et al. (2004) for Cd+UV-B treated *Riccia* sp. The activity of SOD under sequential treatments was higher than the value under Cd+UV alone, and the activity of POD showed no changes compared to the controls. A common response to UV-B and Cd stress in higher plants is changes in the antioxidative defence system. Chmielowska et al. (2010) sugested that cross-protection of copper stressed pepper plants against a vascular pathogen was accompanied by the induction of a defence response. We observed an increase in POD activity in Cd/UV-B stressed seedlings, which may be related to Cd/UV-B tolerance, but may also influence the response against UV-B/Cd or Cd+UV. This observation leads us to conclude that Cd or UV-B alone induced the antioxidative defence system, and then promoted resistance to the combination of Cd+UV.

In higher plants, increase in lipid peroxidation and H_2O_2 was demonstrated following Cd and UV-B treatments (Smeets et al. 2009; Mishra et al. 2011). Enhanced lipid peroxidation in soybean seedlings may be a result of higher amounts of reactive oxygen species such as H_2O_2, ·OH and $^1O_2$. Many studies demonstrated that Cd and UV-B induced oxidative stress via indirect mechanisms such as inhibition of antioxidative defense systems, or via the activation of ROS-producing enzymes such as NADPH oxidases (Romero-Puertas et al. 2004; Smeets et al. 2009; Zu et al. 2010). Many reports have shown the early accumulation of H_2O_2 in heat shock or exogenous H_2O_2 pretreatment protects against subsequent changes caused by Cd (Hsu and Kao 2007; Lin et al. 2010). In the present study, H_2O_2 was increased in Cd and UV, which implies that H_2O_2 is involved in Cd or UV-induced protection against subsequent changes in MDA content caused by Cd+UV. Mishra and Agrawal (2006) have shown that simultaneous exposure of UV-B+Cd and UV-B+Ni caused increased accumulation of MDA content in spinach. In the present study, all treatments (except Cd~Cd+UV) showed significantly higher MDA content than the controls, indicating a loss of membrane function and induction of oxidative damage. Increases in MDA and H_2O_2 content, as a consequence of imposed stress was least pronounced in Cd~Cd+UV, which could be attributed to its superior cross-adaptation in comparison with other treatments.

Conclusions

This study concluded that UV-B and Cd stress treatments inhibited soybean seedling growth, pigment content, net photosynthetic rate, stomatal conduction, transpiration and SOD activity, while they increased MDA content. Cd also significantly reduced chlorophyll fluorescence but increased H_2O_2 contents. The interactive effects of the combined stresses were, however, synergistic for all of the estimated parameters and showed an additive effect. This suggests that the environmental hazards of UV-B radiation would be intensified far greater than expected in soils already contaminated with Cd. However, the sequential effects were no or less detrimental compared to the combination. This is the first report of the sequential effects involving UV-B and Cd stress. Comparatively lower detrimental effects by the sequential treatment relative to the combination suggested the cross-adaptation of Cd and UV-B mediated protection of Cd+UV in soybean.

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