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UV-B response of green and etiolated barley seedlings

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Abstract

7-d-old etiolated and green barley seedlings (*Hordeum vulgare* L. cv. Alfa) were irradiated with UV-B for 30 min and then kept for 24 h in light or darkness. Chlorophyll (Chl) synthesis was inhibited by about 30 % as a result of UV-B irradiation, but there were no significant changes in photochemical activity measured by variable to maximum fluorescence ratio (F_v/F_m), quantum yield (Φ_{PS2}) and oxygen evolution rate. Electron transport of etiolated seedlings was similar to that of green ones, nevertheless, the Chl content was more then 2-fold lower. Ribulose-1,5-bisphosphate carboxylase/oxygenase large and small subunits were diminished as a result of UV-B irradiation in etiolated and green plants, especially in those kept in the darkness. Catalase activity decreased and total superoxide dismutase activity increased in green and etiolated plants following UV-B treatment. When benzidine was used as a substrate, an isoform located between guaiacol peroxidases 2 and 3 (guaiacol peroxidase X) appeared, which was specific for UV-B treatment. As a result of irradiation, the contents of UV-B absorbing and UV-B induced compounds increased in green seedlings but not in etiolated seedlings.

Additional key words: chlorophyll fluorescence, flavonoids, *Hordeum vulgare*, oxygen evolution, ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

UV-B radiation (280 - 320 nm) induces multiple morphological and physiological responses in plants. Many of the effects of UV-B in plants are likely to be a more or less a direct result of cellular damage caused by aberrant photoproducts in macromolecules such as DNA (Britt 1996) and proteins (Gerhardt *et al*. 1999). UV-B also induces the production of potentially harmful active oxygen species, AOS (Foyer *et al.* 1994, Malanga *et al*. 1999). The most common protective mechanism against potential damage is the biosynthesis of UV-absorbing compounds (Hahlbrock and Scheel 1989). These secondary metabolites, mainly phenolic compounds, flavonoids, and hydroxycinnamate esters, accumulate in the vacuoles of epidermal cells and attenuate the penetration of the UV-B into deeper cell layers with little effect on the penetration of visible or the photosynthetically active radiation (Cen and Bornman 1993), allowing photosynthesis to continue while UV-B wavelengths are attenuated in the epidermis. Species with higher contents of these compounds prior to the onset of UV-B treatment (Gonzales *et al*. 1996) or species that can rapidly accumulate these compounds (Murali and Teramura 1986) are protected against UV-B damage and would be UV-B tolerant. However, such a trend was not observed in many studies. Smith *et al.* (2000) established that mean contents of UV-B absorbing compounds did not differ significantly between the tolerant and sensitive groups, not did an ability to increase the content of UV-B screening pigments in response to UV-B necessarily reduce sensitivity. Dunlap and Yamamoto (1995) reported that mycosporine-glycine might function as a biological antioxidant or only absorb UV in marine organisms. In barley seedlings an accumulation of UV-B induced compounds, with maximum absorbance at

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Abbreviations: AOS - active oxygen species; Car - carotenoids; CAT - catalase; Chl - chlorophyll; GXP - guaiacol peroxidase; F_0 - chlorophyll fluorescence of dark-adapted state; F_m - maximal fluorescence of PS 2 in dark-adapted state; F_v - variable chlorophyll fluorescence; q_N - non-photochemical fluorescence quenching; q_P - photochemical quenching; PMSF - phenylmethylsulfonyl fluoride; PPFD - photosynthetic photon flux density; PS - photosystem; RLS and RSS - ribulose-1,5-bisphosphate carboxylase/oxygenase large and small subunits; SOD - superoxide dismutase; TCA - trichloracetic acid; Φ_{PS2} - the quantum yield of PS 2 photochemistry in the light-adapted state; UV-B - ultraviolet B radiation.

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438 nm was established (Fedina *et al*. 2003). These compounds could play an important role in overall UV-B protection or probably could be a consequence of stress-induced damage to the cells and serve as stress markers.

Plants respond to UV-B oxidative stress by activation of antioxidant enzymes or changes in the contents of antioxidants. The activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase, and glutatione raductase are enhanced by UV-B treatment in *Arabidopsis* (Rao *et al.* 1996), cucumber (Tekchandani and Guruprasad 1998), wheat (Sharma *et al.* 1998) and cyanobacterium (Prasad and Zeeshan 2005). The main source of AOS in plant tissues is photosynthetic electron transport system (Foyer *et al*. 1994).

We investigated the role of photosynthetic apparatus in accumulation of UV-B-induced and UV-B absorbing compounds in greening etiolated barley seedlings. Additionally we compared the changes of antioxidant system and soluble proteins in green and etiolated seedlings as a result of UV-B treatment. A physiological response to UV-B irradiation was evaluated by measuring the oxygen evolution rate and chlorophyll (Chl) fluorescence.

Materials and methods

Plant growth and treatment: Barley (*Hordeum vulgare* L. cv. Alfa) seedlings were grown in a controlled environment with a 12-h photoperiod, irradiance of 130 μmol m^{-2} s^{-1}, day/night temperature of 25/22 °C and relative humidity 60 %. Etiolated seedlings were grown for 6 d in the dark. Etiolated and green barley seedlings were irradiated with UV-B for 30 min and then kept for 24 h in the light or in darkness. We used as a control the green seedlings kept in the light since there were no significant differences between responses of green plant kept in the light or in darkness. As a source of UV-B irradiation a mercury lamp was used with a characteristic emission in the range 280 - 320 nm (type *HPQ 125W; N.V. Philips Gloeilampenfabriken*, Einhoven, the Netherlands), 64.4 μmol m^{-2} s^{-1}, where the UV-B radiation was about 80 % of the total emission. The distance between the lamp and plants was 25 cm. To cut off the radiation below 292 nm a cellulose acetate filter (0.13 mm) was used and plants received 49 kJ m^{-2} d^{-1} of biologically effective UV-B radiation.

Chlorophyll fluorescence induction of leaf disks was measured with a pulse amplitude modulation fluorometer (*PAM 101-103, H. Walz,* Effeltrich, Germany) as described by Schreiber *et al*. (1986). The initial fluorescence yield in weak modulated radiation (75 nmol m^{-2} s^{-1} PPFD), F_0, and maximum total fluorescence yield emitted during a saturating white light pulse (1 s, over 3 500 μmol m^{-2} s^{-1} PPFD, by *Schott KL 1500* light source), F_m, were determined. The leaf disc (1 cm diameter) was then irradiated with continuous red radiation (125 μmol m^{-2} s^{-1} PPFD). When the measuring light was applied alone, a modulation frequency of 1.6 kHz was used, otherwise the modulation frequency was set to 100 kHz. The short pulses (at 20-s intervals) on the background of red radiation were used to obtain the fluorescence maximum F_m' with all photosystem (PS) 2 reaction centres closed in light-adapted state. Induction kinetics were registered and analysed with a program *FIP 4.3*, written by Tyystjärvi and Karunen (1990). The q_P and q_N were calculated according to Van Kooten and Snel (1990): $q_P = (F_m' - F)/(F_m' - F_0')$ and
$q_N = 1 - (F_m' - F_0')/(F_m - F_0)$.

Oxygen evolution rate was determined using a leaf disk electrode (*type LD2/2, Hansatech*, Norfolk, UK) at 800 μmol m^{-2} s^{-1} PPFD at saturating CO_2 concentration (provided by a carbonate/bicarbonate buffer) at room temperature.

UV-B absorbing compounds: Leaves (150 mg) were homogenized in 6 cm^3 of medium containing methanol:HCl:H_2O (79:1:20), centrifuged at 10 000 *g* for 15 min, and the absorption at 300 nm was read according to standard procedure (Mirecki and Teramura 1984). Additionally, the absorption spectra in the region 310 - 460 nm were registered (*Shimadzu*, Tokyo, Japan) and analysed to evaluate the changes of the absorption of different bands.

UV-B induced compounds: Leaves (150 mg) was homogenized in 3 cm^3 of 0.1 % trichloracetic acid, TCA (4 °C) and centrifuged at 10 000 *g* for 15 min. The absorbance spectra of these compounds were recorded between 300 and 650 nm (*Shimadzu*). The maximum absorbance was at 438 nm.

Proline content was quantified using the ninhydrin method (Bates *et al.* 1973). Approximately 0.5 g of leaves was homogenized in 10 cm^3 of 3 % aqueous sulphosalicylic acid and the homogenate was centrifuged at 2 000 *g* for 5 min. Two cm^3 of the extract reacted with 2 cm^3 of acid-ninhydrine (50 cm^3 of 2.5 % ninhidrine; 30 cm^3 glacial acetic acid; 20 cm^3 6 M phosphoric acid) and 2 cm^3 of glacial acetic acid for 1 h at 100 °C. The reaction mixture was extracted with 4 cm^3 of toluene. The chromophore containing toluene was separated and the absorbance was read at 520 nm (*Specol 10*, *Zeiss*, Jena, Germany).

Malondialdehyde (MDA) and hydrogen peroxide contents were determined by the method of Esterbauer and Cheeseman (1990). Leaves (150 mg) were homogenized in 3 cm^3 of 0.1 % TCA (4 °C) and centrifuged at 10 000 *g* for 15 min. To 0.5 cm^3 of the supernatant 0.5 cm^3 of 0.1 M Tris/HCl pH 7.6 and 1 cm^3 of

TCA-TBA-HCl reagent [15 % TCA (m/v) 0.375 % (m/v) thiobarbituric acid, 0.25 M HCl] were added. This solution was boiled for 15 min in water bath, centrifuged at 2 000 *g* for 5 min, and the absorbance was read at 532 and 600 nm (*Specol 10*). To 0.5 cm^3 of the supernatant 0.5 cm^3 0.1 M Tris/HCl (pH 7.6) and 1 cm^3 1 M KI were added. After 90 min, the absorbance was read at 390 nm for determination of the hydrogen peroxide.

SDS PAGE of soluble leaf proteins: The leaves were homogenized (1:5 m/v) at 4 °C with ice cold 100 mM Tris-HCl buffer (pH 8) containing 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride (PMSF), 12.5 % glycerol (v/v), 20 mM β-mercaptoethanol, 3 % Polyclar (m/v). After incubation at 4 °C for 30 min, the homogenate was centrifuged at 13 000 *g* for 20 min. The leaf total soluble proteins were separated by 12 % SDS-PAGE (Laemmli 1970). Equal amounts of 30 μg protein per lane were loaded.

Enzyme assays: 0.5 g frozen leaf samples were ground and extracted (1:10, m/v as previously described (Demirevska-Kepova *et al*. 2004). Extracts were desalted on *Sephadex-G 25* mini-columns. The activities were determined using a *Shimadzu* spectrophotometer. SOD (EC 1.15.1.1) activity was measured at 560 nm based on the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich 1971). One unit of SOD was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50 %. Catalase (CAT) (EC 1.11.1.6) activity was assayed following H_2O_2 decomposition at 240 nm (ε = 0.0394 mM^{-1} cm^{-1}) according to Aebi (1984). Guaiacol peroxidase (GPX) (EC 1.11.1.7) activity was assayed according to McRae and Thompson (1983). The product tetraguaiacolchinone (ε = 26 mM^{-1} cm^{-1}) was registered at 420 nm.

Isoenzyme staining: In gel staining methods were used after native 7.5 % PAGE for CAT and GPX and 10 % native PAGE for SOD at 4 °C, loading 30 μg protein per lane. SOD activites were visualized and SOD types were differentiated according to Gonzáles *et al*. (1998). CAT isoenzymes were stained according to Woodbury *et al*. (1971). GPX isoenzymes were separated and revealed according to Hart *et al*. (1971).

Protein and pigment content: Protein was determined by the method of Bradford (1976). Chlorophyll and carotenoid (Car) contents were determined according to Lichtenthaler (1987).

Statistics: The data are means of three different experiments, each including at least three replications. They were analysed with the Student's *t*-test**.**

Results

Chl was synthesized at a reduced rate in etiolated UV-B irradiated plants compared to non-irradiated ones (Table 1). Chl *a/b* ratio was increased, mainly due to decreased Chl *b* accumulation (Chl *b* 59 %, Chl *a* 70 % of the control). In green seedlings the contents of Chl *a* and *b* slightly decreased while Car synthesis was increased as a result of UV-B treatment.

The UV-B irradiation of green plants decreased the photochemical efficiency of PS 2, estimated by the ratio F_v/F_m and Φ_{PS2} (Fig. 1) and oxygen evolution rate (Fig. 1), but the effect of UV-B radiation was similar in plants exposed for 24 h in the light or for 24 h in darkness. The variable Chl fluorescence, F_v, an indicator for the electron flow capacity through PS 2, and oxygen evolution were more sensitive to UV-B than other parameters. The decrease in PS 2 activity as a result of UV-B exposure was accompanied by a corresponding increase in 1 - (F_v'/F_m'), which indicated increased proportion of thermal energy dissipation in the antenna (Fig. 2). The F_v/F_m measured after 24-h irradiation of etiolated seedlings was only slightly lower than that in green plants indicating that 24 h were enough for development of fully active photosynthetic apparatus even after UV-B irradiation. The UV-B treatment followed by 24-h irradiation influenced significantly only F_v (Fig. 1). The activity of PS 2 in UV-B irradiated etiolated plants kept 24 h in the dark was very low and O_2 uptake was registered.

Table 1. Chlorophyll (Chl) and carotenoid (Car) content [mg g^{-1} (f.m.)] in etiolated and green barley seedlings kept 24 h in the light or in darkness after 30 min UV-B irradiation. Means ± S.E. of 3 independent experiments.

Treatments		Chl *a*	Chl *b*	Car	Chl *a*/*b*	Chl/Car
Etiolated plants	C - non UV-B	0.667 ± 0.150	0.211 ± 0.040	0.174 ± 0.020	3.15	5.12
	UV-B 24 h light	0.468 ± 0.090	0.125 ± 0.040	0.120 ± 0.010	3.74	4.92
	UV-B 24 h dark	n.d.	n.d.	n.d.		
Green plants	C - non UV-B	1.464 ± 0.020	0.637 ± 0.018	0.322 ± 0.016	2.29	6.52
	UV-B 24 h light	1.402 ± 0.020	0.580 ± 0.056	0.347 ± 0.019	2.41	5.72
	UV-B 24 h dark	1.380 ± 0.130	0.531 ± 0.131	0.330 ± 0.006	2.47	5.11

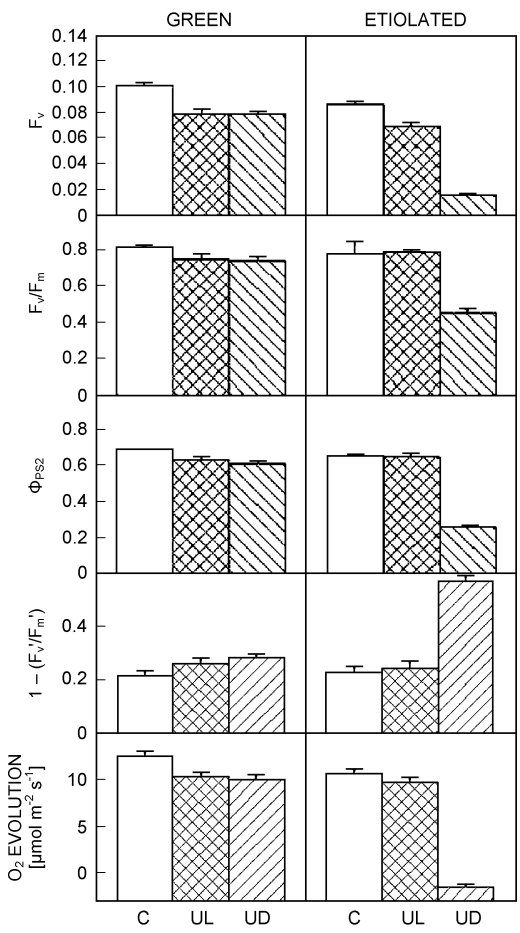

Fig. 1. Changes in variable chlorophyll fluorescence, variable to maximum fluorescence ratio, quantum yield, proportion of thermal energy dissipation in the antenna, and oxygen evolution rate in green and etiolated barley seedlings kept in continuous light or in the dark for 24 h after UV-B irradiation. C - control (non UV-B irradiated), UL - UV-B treated plants kept in light for 24 h, UD - UV-B treated plants kept in dark for 24 h. The mean values ± SE were calculated from 3 independent experiments.

The content of H_2O_2 in non UV-B treated green seedlings was about 2-fold higher in comparison to non-irradiated etiolated seedlings (Fig. 2). As a result of UV-B irradiation, H_2O_2 content increased both in green and etiolated seedlings. In the etiolated UV-B irradiated seedlings kept in the light the content of H_2O_2 increased more then 2-fold in comparison to etiolated control, while this increase in green seedlings was about 30 %. There was no difference between MDA content in green and etiolated controls (Fig. 3). UV-B irradiation lead to increase of MDA, the most pronounced in the green plants kept in light.

The proline content in non-irradiated green and etiolated seedlings was similar (Fig. 3). After UV-B irradiation the seedlings accumulated proline, more in the green plants in comparison to the etiolated ones.

Recently we established that the exposure to UV-B radiation induced accumulation of coloured compounds with maximum absorbance at 438 nm (A_{438}). These compounds appeared 4 h after UV-B treatment, their contents reached maximum after 24 h and then declined, while UV-B absorbing compounds (mainly flavonoids) extracted in methanol + HCl existed in non irradiated seedlings and their synthesis continued for a long period (more then 96 h) after UV-B exposure. In non-irradiated seedlings we established very low signal of A_{438}. Accumulation of these compounds after UV-B irradiation depended on irradiation (Fedina *et al*. 2003, 2005). Considering this fact, we compared the accumulation of A_{438} in green and etiolated barley seedlings 24 h after UV-B irradiation. As a result of UV-B irradiation, the A_{438} signal increased significantly in green plants (Fig. 3). However, in etiolated seedlings the content of A_{438} compounds was very low both in the control and in UV-B irradiated seedlings. Some increase was established in the seedlings kept in light in comparison to the seedlings kept in darkness.

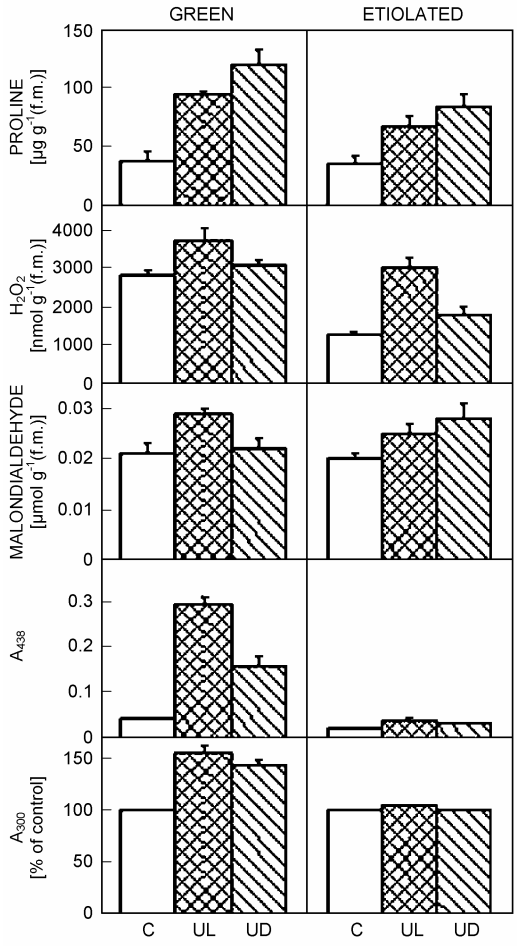

Fig. 2. The contents of proline, H_2O_2, malondialdehyde (MDA), A_{438} and A_{300} in green and etiolated barley seedlings kept in continuous light or in the dark for 24 h after UV-B irradiation. C - control (non UV-B irradiated), UL - UV-B treated plants kept in light for 24 h, UD - UV-B treated plants kept in dark for 24 h. The mean values ± SE were calculated from 3 independent experiments (A_{300} form 4).

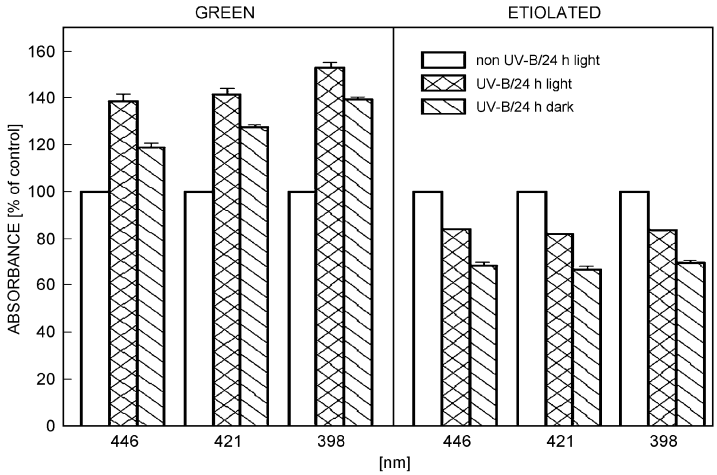

Fig. 3. Absorbances at 446, 423 and 398 nm, measured 24 h after UV-B treatment of etiolated and green barley seedlings. 100 % corresponds to absorbances of extracts from control non-UV-irradiated green and etiolated plants. The mean values ± SE were calculated from 4 independent experiments.

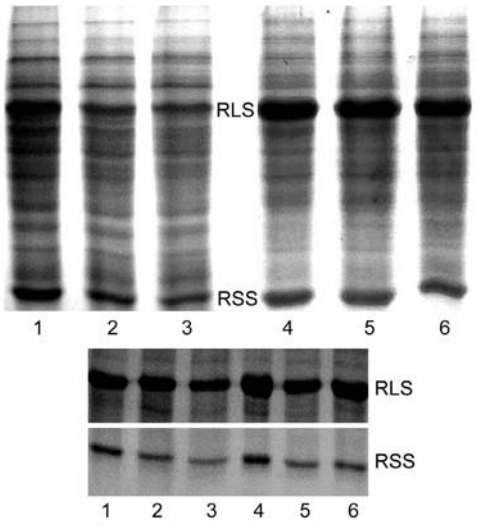

Fig. 4. Effect of UV-B irradiation on polypeptide patterns after SDS electrophoresis of extracts of barley leaves stained with Coomassie Brilliant Blue R 250. Gels were loaded with equal amounts of protein (30 µg per lane). RLS - RuBPCO LS; RSS - RuBPCO SS. *Lanes*: *1* - control etiolated plants; *2* - UV-B treated etiolated plants kept in the light 24 h; *3* - UV-B treated etiolated plants kept in the dark 24 h; *4* - control green plants; *5* - UV-B treated green plants kept in the light 24 h; *6* - UV-B treated green plants kept in the dark 24 h.

The content of UV-absorbing compounds was estimated by the absorption at 300 nm (A_{300}) of leaf extracts in acidified methanol (Fig. 2). A high amount of UV-B induced compounds was found in UV-B treated green seedlings. No increase of A_{300} was registered for etiolated plants. The post-treatment irradiation of plants did not affect considerably the content of UV-absorbing compounds in green plants; the values for plants kept in darkness and in the light were similar. Data of A_{300} in Fig. 2 are % from the respective controls, *i.e.* non-irradiated green and etiolated plants. Absolute values of A_{300} for etiolated and green control did not differ significantly. The absorption spectra of acidified methanol extracts in the region 310 - 460 nm were also recorded. Apart from high absorption near 360 nm, three different maxima at 446, 423, and 398 nm were observed. 24 h after UV-B exposure, the differences of intensities of these maxima between controls and UV-B treated green seedlings were significant (Fig. 3). For etiolated samples the values for absorbance at 446, 423, and 398 nm were lower by about 17 % for UV-B irradiated seedlings in comparison with etiolated control. This decrease was more pronounced for etiolated seedlings, kept in the dark (28 %).

The effect of UV-B irradiation on the polypeptide profile of leaf extracts from barley plants is presented in Fig. 4. We did not find qualitative changes, but some quantitative changes were available. The content of total leaf soluble protein in green seedlings was higher than etiolated ones (data not shown). It decreased both in green and etiolated plants following UV-B exposure mainly due to the change in the content of the key photosynthetic enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). SDS electrophoresis analysis detected a diminishment of RuBPCO LS (RLS) and SS (RSS) as a result of UV-B irradiation of etiolated or green barley plants, especially in those kept in the dark (Fig. 4). Hence RLS and RSS subunits were highly sensitive to UV-B irradiation.

The total SOD activity in leaf extracts increased following UV-B treatment of green and etiolated plants (Fig. 5*A*). More detailed analysis revealed three isoforms of SOD: a weak band of mitochondrial Mn SOD, cytosolic Cu/Zn SOD 1 and a predominant plastidial Cu/Zn SOD 2 (Fig. 6*A*). Cu/Zn SOD 1 and Cu/Zn SOD 2 contents increased when etiolated barley plants were exposed 24 h to light or to dark after 30 min UV-B

irradiation (Fig. 6*A*). Similar tendency was obtained in green barley plants (Fig. 6*A*). Thus, the observed Cu/Zn SOD 2 activity and Cu/Zn SOD 1 increase was connected with UV-B irradiation.

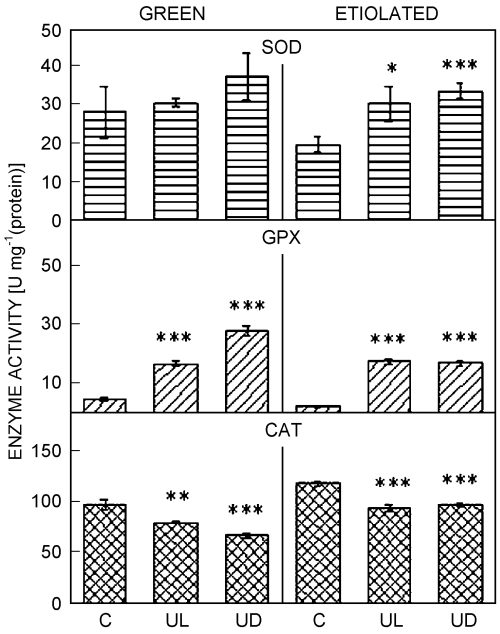

Fig. 6. Influence of UV-B irradiation on the activities of SOD (A), GPX (B) and CAT (C) in extracts from barley leaves. C - control (non UV-B irradiated), UL - UV-B treated plants kept in light for 24 h, UD - UV-B treated plants kept in dark for 24 h. The means values ± SE were calculated from 3 independent experiments.

Total GPX activity, determined in barley extracts, increased significently in UV-B irradiated green and etiolated seedlings (Fig. 5*B*). We found three isoforms - GPX 1, 2, and 3. GPX 3 increased in response to UV-B especially in light. When we used benzidine as a substrate of iso-peroxidases, we revealed a different isoform located between GPX 2 and GPX 3 (GPX X), which was specific for UV-B treatment, but was very unstable and disappeared during the development of the staining. Thus, peroxidases are highly responsive to UV-B stress in barley seedlings. The total CAT activity was higher in green compared to etiolated plants (Fig. 5*C*). After native PAGE, CAT was revealed as only one isoform (Fig. 6*C*). Its activity decreased slightly under UV-B irradiation of green barley leaves.

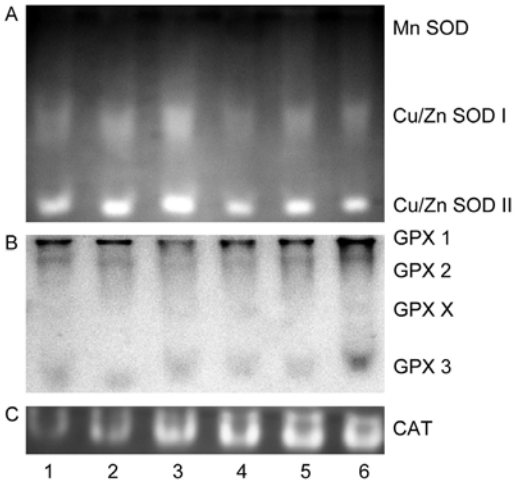

Fig. 7. Effect of UV-B irradiation on isoenzyme patterns of SOD (*A*), GPX (*B*) and CAT (*C*) of extracts from barley leaves. Native gels were loaded with equal amounts of protein (30 µg per lane). Lanes: *1* – control etiolated plants; *2* - UV-B treated etiolated plants kept in light 24 h; *3* - UV-B treated etiolated plants kept in dark 24 h; *4* - control green plants; *5* - UV-B treated green plants kept in light 24 h; *6* - UV-B treated green plants kept in dark 24 h. The different isoforms are numbered from cathode to anode.

Discussion

Plants respond differently to UV-B irradiation, either by stimulating protection mechanisms or by activating repair mechanisms to cope with stress impact. The most common protective mechanism against potentially damaging irradiation is the biosynthesis of UV-absorbing compounds (Hahlbrock and Scheel 1989). In green seedlings 24 h after UV-B irradiation, content of methanol:HCl extracted flavonoids increased (Fig. 4). In etiolated seedlings the content of these compounds was not influenced by UV-B and was as high as in the control (non UV-B irradiated). UV-B induced compounds, extracted with 0.1 % TCA responded in the same manner (Fig. 3). Their content sharply increased after UV-B irradiation in the leaves of green seedlings kept 24 h in the light. In the irradiated etiolated seedlings, A_{438} slightly increased in the light. Obviously Chl (or photosynthetic apparatus) is essential for synthesis of these compounds. However, there was no correlation between accumulated Chl in etiolated UV-B-treated seedlings and the content of UV-B induced compounds. As we established recently (Fedina *et al*. 2005), accumulation of these compounds in green plants begins 4 h after UV-B irradiation. In our case lag period for accumulation of A_{438} coincided with lack of enough Chl in the first hours of irradiation. Therefore we suggest that the time course for UV-B induced compounds accumulation in etiolated seedlings was delayed relative to green seedlings. Jordan *et al*. (1994) studying etiolated tissue also indicated a strong link between the photosynthetic apparatus and UV-B-induced gene expression. The redox potential of photosystems regulates chloroplast gene expression through the redox state of the plastoquinone pool (Tullberg *et al*. 2000). This may be connected with its interaction with UV-B signal transduction and gene expression. Mackerness *et al*. (1996) showed that amelioration of UV-B effects on gene

expression by strong irradiation involved photosynthetic electron transport and photophosphorylation. This may, in part, account for the lack of UV-B effect on gene expression in etiolated tissue when photosystems are not functional.

UV-absorbing compounds, extracted by acidified methanol, include different classes of flavonoids. Despite the changes of absorption at 300 nm, we observed changes at longer wavelengths. Flavonoids differ by the position of maximum of absorption band I – *e.g.*, for flavonones and flavones is 310 - 330 and 330 - 350 nm, while for flavonols and anthocyanins is 350 - 390 and 475 - 545 nm, respectively. Therefore, the changes of intensities of different absorption bands could reflect the domination of synthesis of different UV-B absorbing compounds. The absorbance of three characteristic maximums at 446, 423, and 398 nm increased as a result of UV-B irradiation in green seedlings what coincides with the increase of absorbance at 300 nm. However, this is not the case in etiolated seedlings. Firstly, no increase of the content of all UV-absorbing compounds, evaluated by the absorbance at 300 nm, was observed. It seems that UV-B irradiation caused an increase of UV absorbing compounds only when the photosynthetic apparatus was well developed and completed. Moreover, in etiolated seedlings the absorbance at absorption maxima at 446, 423, and 398 nm was lower after UV-B irradiation and more pronounced in seedlings kept in dark after UV-B treatment. At the moment it is difficult to explain this observation, however, it can be speculated that some destruction of synthesized UV-B absorbing compound proceeds in etiolated plants. According to Beggs and Wellmann (1994) the synthesis of isoflavonoids in legumes, may be induced by DNA damage because the wavelength dependency of the response is similar to that for DNA absorption and acceleration of DNA repair by photoreactivation. Shinkle *et al*. (personal communication) hypothesized that DNA damage is the sensory mechanism for the response to short UV wavelength. After UV-B exposure, some flavonoids are selectively produced (Markham *et al.* 1998). This accumulation does not relate to any enhanced capability to absorb UV-B, but rather reflects a greater potential to dissipate energy or produce greater antioxidant capacity. In addition to enhanced antioxidant capacity provided by specific flavonoids, plant cell produces a range of alternative antioxidant systems to protect against free radicals generated by UV-B (Strid 1993). Thus, increased UV-B induces the rapid synthesis of antioxidant enzymes (SOD, CAT, GPX) to cope with the free superoxide radicals. We suppose that peroxidases under UV-B stress can use flavonoids as substrates to detoxify hydrogen peroxide.

The first step of defence system against AOS is to convert them to hydrogen peroxide and O_2. CAT dismutates hydrogen peroxide into H_2O and O_2, whereas GPX detoxifies hydrogen peroxide by oxidation of co-substrates. Our observation of higher contents of two Cu-Zn forms of SOD in leaves of etiolated and green barley plants after exposure to UV-B radiation is probably due to enhanced formation of superoxide free radicals. The increased content of hydrogen peroxide induced by UV-B irradiation is scavenged by CAT and peroxidases. We obtained increased peroxidase activity in plants under UV-B irradiation kept in dark and light.

Our results showed that the response of etiolated and green seedlings to UV-B irradiation was similar concerning protein synthesis, induction of antioxidant enzymes, accumulation of stress metabolites, and activity of electron transport and was different with regard to synthesis of UV-B-induced and UV-B absorbing compounds.

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