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UV-B RESPONSE OF GREENING BARLEY SEEDLINGS

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The relationship between the greening stage of barley seedlings and their response to UV-B irradiation was studied. Etiolated barley seedlings (*Hordeum vulgare* L., cv. Alfa) greened 12, 24 and 48 h were exposed to UV-B irradiation (312 nm) for 5 h. As a result of UV-B treatment the rate of CO_2 fixation and chlorophyll contents decreased but flavonoids, UV-B-induced compounds and carotenoids increased. The inhibition of photosynthesis in green plants was lower in comparison to greening ones. The 12 h greening plants were more sensitive to UV-B treatment than the plants greening 24 h and particularly 48 h, estimated by the quantum efficiency of PSII photochemistry and the oxygen production rate. The levels of flavonoids and UV-B induced compounds enhanced with increasing the greening time. Activity of antioxidant enzymes catalase, peroxidase and superoxide dismutase increased during the seedlings greening and as a result of UV-B irradiation, but the pattern of isoforms remained similar to those found in the controls. UV-B preferentially induced Cu,Zn-superoxide dismutase. Increase of UV-B induced synthesis of antioxidant enzymes is in line with their important role in the plant response to UV-B stress. Data presented show that the response of barley seedlings to UV-B irradiation is related to the development stage of photosynthetic apparatus.

Keywords: Antioxidant enzymes – chlorophyll fluorescence – flavonoids – oxygen evolution – UV-B radiation

INTRODUCTION

Ultraviolet-B radiation can damage plants, decreasing growth and productivity. Many studies have shown deleterious UV-B effects on plants including reduced photosynthesis, decreased protein synthesis, impaired chloroplast function, damaged nucleic acids and lipids [17, 19, 35]. UV-B-dependent inhibition of photosynthesis

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may involve the disruption of chloroplast membrane structure [45] changes of synthesis and degradation of Rubisco and light-harvesting chlorophyll a/b binding protein of PSII [43]. Plants are known to respond to UV-B radiation with morphological and biochemical alterations which may include expression of genes leading to the synthesis of protective pigments [41] the production of enzymes responsible for scavenging of reactive oxygen species [48], synthesis of DNA repair proteins [36]. The most common protective mechanism against potentially damaging irradiation is the biosynthesis of UV-absorbing compounds. Li et al. [23] showed that flavonoid-deficient mutants of *Arabidopsis* were hypersensitive to UV-B, and reported that UV-sensitive soybean cultivars were deficient in the production of UV-B-absorbing pigments. It was expected that plants with high levels of UV-B-absorbing compounds would be more tolerant than those with lower levels. However, such a trend could not be verified [7, 13].

UV-B radiation produces oxidative stress by increasing generation of active oxygen species (AOS) such as superoxide radicals ($^1O_2^-$*), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH*) [37]. Plants respond to UV-B oxidative stress in terms of activation of antioxidant enzymes as well as changes in the levels of antioxidants. The activities of antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, and glutatione reductase were enhanced by UV-B treatment in *Arabidopsis* [34], cucumber [44] and wheat [40]. The main source of AOS in plant tissues is photosynthetic electron transport chain [11]. Recently, the redox potential of the photosystems has been demonstrated to regulate chloroplast gene expression through the redox state of the plastoqionone pool [46]. Mackerness et al. [26] showed that amelioration of UV-B effects on gene expression by high light involved photosynthetic electron transport and photophosphorylation. Research on etiolated tissues is also indicative of a strong link between the photosynthetic apparatus and UV-B-induced gene expression [20]. This may, in part, account for the lack of UV-B effect on gene expression in etiolated tissue when the photosystems are not functional. In etiolated seedlings, the degree of increase in UV-induced cyclobutyl pyrimidine dimers (CPD) levels was the highest, the contents of UV-absorbing compounds was the lowest and no photorepair of CPD could be detected [21]. In addition, the levels of AOS and antioxidants have been related to UV-B response at different developmental stages of photosynthetic apparatus [27].

Radyuk and Homan [32] have obtained experimental evidence that the development of the photosynthetic apparatus in greening etiolated barley leaves is of an uneven rather than gradual nature. They suppose that each discrete step in development of the photosynthetic apparatus begins with the formation of photosystem cores, containing only Chl *a*, and ends with the synthesis of light-harvesting complexes, containing both Chl *a* and Chl *b* molecules. Despite of large number of papers on the effect of UV-B irradiation on plants, the reports on its effect on greening seedlings are limited.

In this study we used greening and green barley seedlings in order to investigate the relationship between activity of photosynthetic apparatus and accumulation of UV-B-induced and UV-B-absorbing compounds and the susceptibility of plants to

UV-B. The activities and isoforms of catalase, peroxidase and superoxide dismutase were analysed. The physiological response to UV-B irradiation was evaluated by measuring the oxygen evolution rate and chlorophyll fluorescence in intact leaves.

MATERIALS AND METHODS

Plant material and experimental design

Barley (*Hordeum vulgare* L. cv. Alfa) seedlings were grown in a growth chamber under white fluorescent lamps (160 μmol m^{-2} s^{-1} PPFD) with a 12 h light/dark cycle, 25/22 °C and 60% humidity. Etiolated seedlings were grown for 7 d in the dark. After 12 h, 24 h and 48 h greening of etiolated seedlings under continuous white light (160 μmol m^{-2} s^{-1} PPFD) they were subjected to UV-B irradiation.

The seedlings were irradiated with UV-B (312 nm) fluorescent tubes (TL 20W/12 R; Philips, Germany) for 5 h (biological effectiveness of UV-B radiation (UVB_{BE}) 14.4 kJ m^{-2} d^{-1}). The 30 cm distance between the top of the plants and UV-B lamp was kept constant. The biological effectiveness of UV-B radiation (UVB_{BE}) was calculated using the plant action spectrum of Caldwell et al. [5], normalized to unity at 300 nm.

Chlorophyll fluorescence

Chlorophyll fluorescence induction of leaf disks was measured with a pulse amplitude modulation fluorometer (PAM 101–103, H. Walz, Germany) as described by Schreiber et al. [38]. The initial fluorescence yield in weak modulated light (0.075 μmol m^{-2} s^{-1} PPFD), F_o, 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 illuminated with continuous red light (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 a red light were used to obtain the fluorescence intensity F_m' with all PSII reaction centres closed in any light adapted state. Induction kinetics were registered and analysed with a program FIP 4.3, written by Tyystjarvi and Karunen [46].

Oxygen production

Oxygen production rate was determined using a leaf disk electrode (Type LD2/2, Hansatech, Norfolk, UK). It was measured 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 and UV-B induced compounds

Barley leaves (150 mg) were homogenized in 6 ml 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 [28] to determine UV-B absorbing compounds.

UV-B induced compounds (A438) were determined as follow: 150 mg barley leaves were homogenized in 3 ml of 0.1% trichloracetic acid (TCA) (4 °C) and centrifuged at 10,000 *g* for 15 min. Absorbance was read at 438 nm.

Determination of proline, malondialdehyde and hydrogen peroxide

Proline content was determined by the method of Bates et al. [3]. A 500 mg leaves were homogenized in 10 ml of 3% aqueous sulphosalicylic acid and the homogenate was centrifuged at 2,000 *g* for 5 min. Two ml of the extract reacted with 2 ml of acid-ninhydrine and 2 ml of glacial acetic acid for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluen. The chromophore containing toluene was separated and the absorbency read at 520 nm (Specol 11 Jena, Germany).

Malondialdehyde content was determined by the method of Heath and Packer [15]. A 150 mg barley leaves were homogenized in 3 ml of 0.1% TCA (4 °C) and centrifuged at 10,000 *g* for 15 min and the supernatant was used in the subsequent determination. To 0.5 ml of the supernatant were added 0.5 ml of 0.1 M tris/HCl, pH 7.6, and 1 ml of TCA-TBA-HCl reagent (15% w/v trichloracetic acid, 0.375% w/v thiobarbituric acid, 0.25 N hydrochloric acid). This solution was boiled 15 min in water bath centrifuged at 2,000 *g* for 5 min and the absorbency read at 352 nm and 600 nm (Specol 11 Jena, Germany) for determination of malondialdehyde.

Hydrogen peroxide was determined by the method of Esterbauer and Cheeseman [8]. To 0.5 ml of the supernatant were added 0.5 ml 0.1M Tris/HCl pH 7.6 and 1 ml 1M KJ. After 90 min the hydrogen peroxide content was measured spectrophotometrically ($\lambda = 390$ nm).

$^{14}CO_2$ *fixation*

The rate of photosynthesis was determined radiometrically with leaf segments (200 mg) in a closed chamber under following conditions: irradiance 1000 μmol m^{-2} s^{-1}, 25 °C, plus 10 mg $NaHCO_3$ and 7.4 MBq ^{14}C ($NaH^{14}CO_3$, 72 μCi/μmol^{-1} specific activity) in 2500 cm^3 volume. After exposure for 20 min the leaf segments were boiled in 10 ml 80% ethanol. The leaf material was homogenised in 10 ml distillate water and 0.1 ml of this sample was placed into scintillation vials. Fifty microlitres of acetic acid were added to liberate any remaining CO_2, then 0.1 ml of ethanol and 1 ml of scintillant (Ultima gold XR; Packard Bioscience BV, Groningen, The Netherlands) were added and the counts per minute (CPM) were measured for each vial in a liquid scintillation counter.

Antioxidant enzymes activities assay

For enzyme analyses leaves were ground in 0.1 M tris-HCl buffer, pH 7.1 in the ratio 1 : 5. The homogenate was centrifuged at 12,000×*g* for 30 min at 4 °C. The supernatant was used as a crude enzyme extract. Native PAGE in 7.5% gel was carried out by the method of Davis [6].

Peroxidase (POX) (EC 1.11.1.7) isoenzymes were detected by incubating the gels for 60 min in a reaction mixture containing 0.5 mM benzidine hydrochloride and 0.04 mM H_2O_2 in 0.05 M acetat buffer, pH 4.9 according to procedure of Ornstein [31].

Superoxide dismutase (SOD) (EC 1.15.1.1) isoenzymes were detected by the method of Greneche et al. [12]. The gels were incubated for 30 min in the dark in a solution containing 10 mg NBT, 75 mg Na_2-EDTA and 3 mg riboflavin dissolved in 100 ml tris-HCl buffer, pH 8.2 and then illuminated for 15 min.

Catalase (CAT) (EC 1.11.1.6) isoenzymes were stained as described by Woodbury et al. [49]. The gels were incubated in the dark for 20 min in 10 mM H_2O_2 in K/Na phosphate buffer, pH 7.0, followed by incubation in the 1% $K_3Fe(CN)_6$ and 1% $FeCl_3$ for 15 min.

SDS-PAGE

SDS-PAGE was conducted on 12.5% acrylamide gels according to the description of Laemmli [22]. An electrophoresis calibration kit of MBI Fermentas was used to determine the molecular weight of proteins. Protein bands were detected by the silver staining outlined by Nesterenko et al. [30].

Protein and pigments assay

Protein content was determined by the method of Lowry et al. [25].

Leaf pigments were extracted in an aliquot of 80% acetone and estimated as described by Lichtenthaler [24]. For determination of pigments content the middle part (3 cm long) of the leaf was used.

Statistical analysis

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

RESULTS

Exposure to UV-B irradiation reduced chlorophyll content and increased carotenoids content both in green and greening seedlings (Table 1). As a result of UV-B treatment the primary photochemical activity of PSII, estimated by the ratio Fv/Fm and the actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII) were reduced by 24–27% in plants greening 12 h but only 10% in the plants greening 48 h (Fig. 1a). After 12 h of light exposure of etiolated plants the quantum efficiency of PSII photochemistry and the oxygen production rate were very close to those measured in control green plants (Fig. 1b). Nevertheless, plants greening 12 h were more sensitive to UV-B treatment than the plants greening 24 h and particularly 48 h. The rate of O_2 production was more sensitive to UV-B radiation (Fig. 1c) than the quantum efficiency of PSII. O_2 production was reduced by 60% and 20% as a result of UV-B treatment of 12 h and 48 h greening plants, respectively. Exposure of green barley plants to UV-B for 5 h decreased the functional efficiency of PSII by 10% (Fig. 1b). However, when green barley plants were treated twice (2 consecutive days for 5 h every day) with UV-B the quantum yield of PSII electron transport decreased by 25% and the rate of O_2 production about 40%. Such treatment inhibited PSII activity and O_2 production in 24 h greening plants by 60% and 94%, respectively (data not shown). The rate of $^{14}CO_2$ fixation decreased after UV-B treatment (Fig. 2). The rate of photosynthesis in the seedlings illuminated 48 h was a little higher in comparison to the green seedlings but was more influenced by UV-B-inhibition was 38% versus 28%. As a result of UV-B treatment $^{14}CO_2$ fixation decreased by 57% and 44% in the greening seedlings illuminated 12 h and 24 h, respectively.

The extent of lipid peroxidation was determined by measuring changes in malondialdehyde (MDA) in greening and green seedlings after UV-B treatment (Fig. 3a). The MDA content in all seedlings increased after UV-B irradiation. The level of

Table 1
Effect of UV-B radiation on chlorophyll and carotenoid contents in green and greening etiolated barley seedling

Variants	mg g^{-1} f. m.		
	Chl *a*	Chl *b*	carotenoids
Greening 12 h	0.359 ± 0.047	0.167 ± 0.022	0.121 ± 0.017
Greening 12 h + UV-B	0.170 ± 0.030*	0.078 ± 0.019*	0.132 ± 0.015
Greening 24 h	0.501 ± 0.042	0.276 ± 0.034	0.140 ± 0.011
Greening 24 h + UV-B	0.418 ± 0.039	0.223 ± 0.040	0.165 ± 0.022
Greening 48 h	0.770 ± 0.064	0.359 ± 0.042	0.188 ± 0.018
Greening 48 h + UV-B	0.616 ± 0.051	0.310 ± 0.028	0.211 ± 0.019
Green plants	1.266 ± 0.101	0.636 ± 0.030	0.321 ± 0.026
Green + UV-B	0.955 ± 0.022*	0.553 ± 0.030	0.368 ± 0.022

The mean values ±SE were calculated from 3 independent experiments.
*$P < 0.05$.

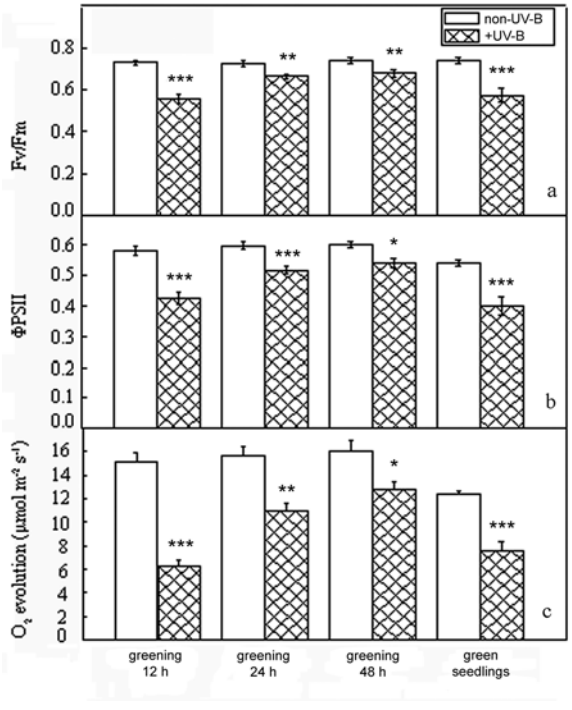

Fig. 1. Changes in the ratio Fv/Fm and ΦPSII and in oxygen evolution rate of green and greening etiolated barley seedlings after UV-B irradiation. The mean values ±SE were calculated from 3 independent experiments. *P < 0.05; **P < 0.01 and ***P < 0.001

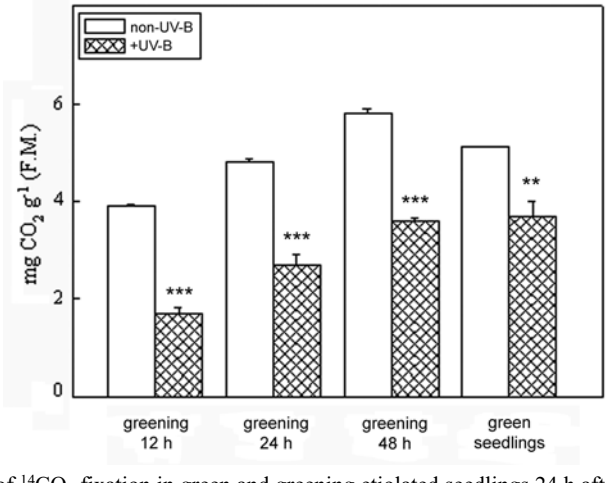

Fig. 2. The rate of $^{14}CO_2$ fixation in green and greening etiolated seedlings 24 h after exposure to UV-B irradiance. The mean values ±SE were calculated from 3 independent experiments. **P < 0.01 and ***P < 0.001

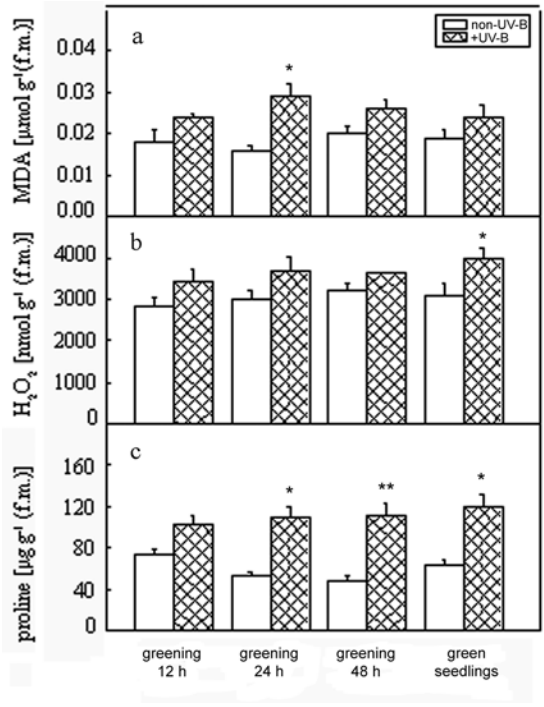

Fig. 3. The content of proline, MDA and H_2O_2 in green and greening etiolated barley seedlings after UV-B irradiation. The mean values ±SE were calculated from 3 independent experiments. *P < 0.05 and **P < 0.01

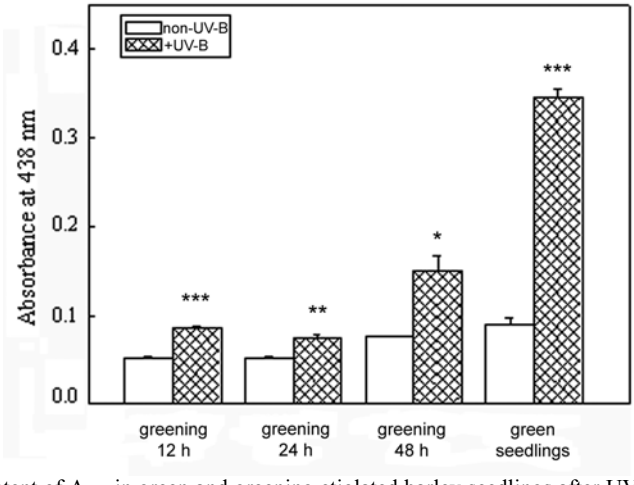

Fig. 4. The content of A_{438} in green and greening etiolated barley seedlings after UV-B irradiation. The mean values ±SE were calculated from 3 independent experiments. *P < 0.05; **P < 0.01 and ***P < 0.001

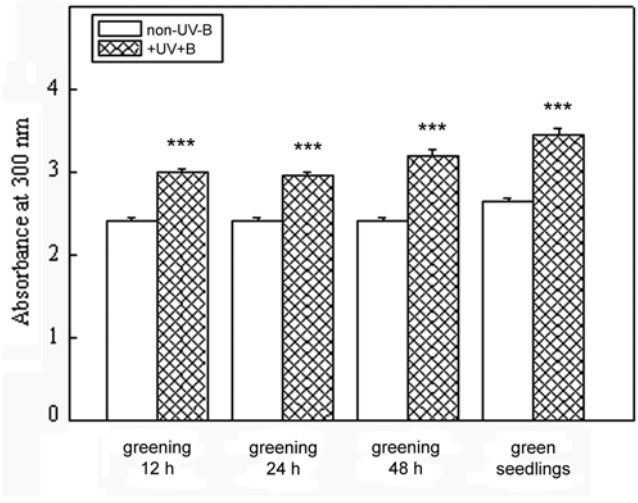

Fig. 5. The absorbance at 300 nm of leaf extracts of green and greening etiolated barley seedlings 24 h after exposure to UV-B irradiance. The means values ±SE were calculated from 4 independent experiments. ***$P < 0.001$

H_2O_2 in non-irradiated seedlings was very similar in green and greening plants (Fig. 3b). An increase of H_2O_2 content in all seedlings is observed as a result of UV-B irradiation. In the green and the 24 and 48 h greening seedlings the proline content increased almost in the same extent (about 2-fold) after UV-B irradiation (Fig. 3c). In non-irradiated seedlings we established very low level (traces) of UV-B-induced compounds with maximum absorbance at 438 nm (A_{438}). As a result of UV-B irradiation the level of A_{438} increased significantly in green plants (Fig. 4). An increase was established in the greening seedlings and the content of A_{438} depended on the illumination time of etiolated seedlings.

The concentration of UV-absorbing compounds was estimated by the absorption at 300 nm (A_{300}) of leaf extracts in acidified methanol. The values for absorbance at 300 nm in green and etiolated barley seedlings after different periods of illumination are shown in Fig. 5. A high amount of UV-B induced compounds was found in UV-B treated green seedlings. The value of A_{300} in 48 h illuminated seedlings is close to that in green plants.

The activities and isoforms of POX, SOD and CAT were investigated in barley seedlings subjected to UV-B irradiation. During greening of etiolated seedlings the activities of investigated enzymes increased (Fig. 6). POX, SOD and CAT activities increased as a result of UV-B treatment. POX isoenzymes were in tree main areas – slowly, medium and fast migrating (Fig. 6a). The fast moving isoenzymes were the most active. No new isoenzymes were detected, but an increase of the activity of available isoenzymes was established. UV-B decreased the activity of the slow migrating peroxidase isoenzyme 3, increased activity of medium migrating isoenzyme 5 and the fastest migrating isoenzymes 6 and 7.

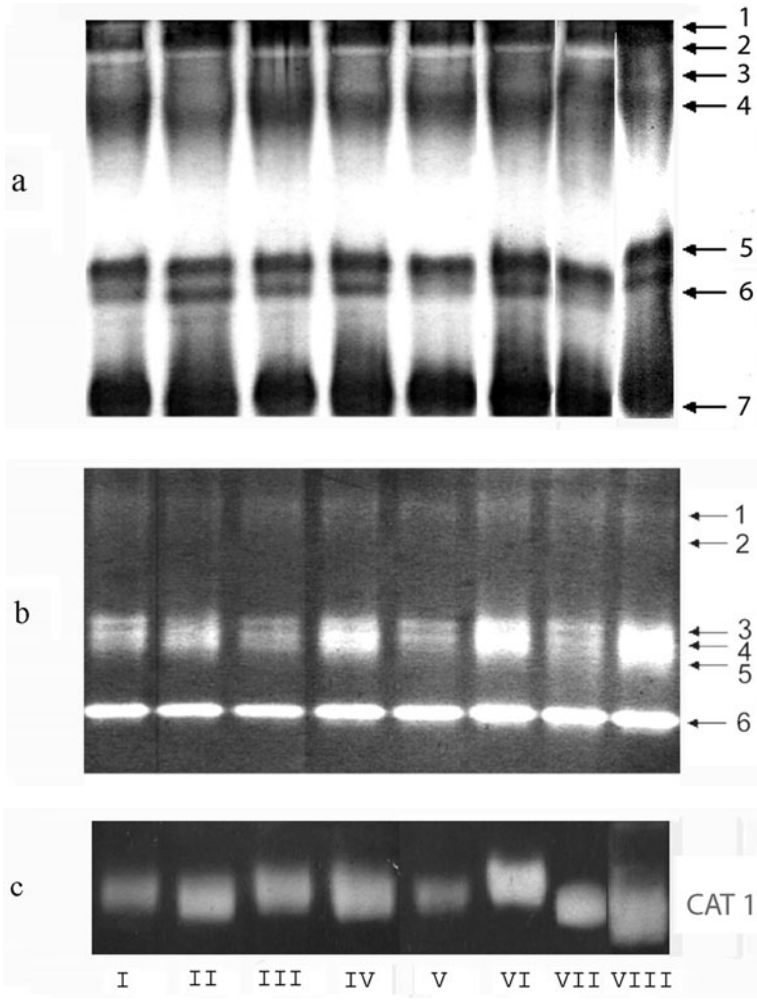

Fig. 6. Isoenzyme profiles. a – Peroxidase – 50 μg protein was loaded in each lane. b – Superoxide dismutase – 25 μg protein was loaded in each lane. c – Catalase – 50 μg protein was loaded in each lane. Lanes: I – greening 12 h; II – greening 12 h + UV-B; III – greening 24 h; IV – greening 24 h +UV-B; V – greening 48 h; VI – greening 48 h +UV-B; VII – green seedlings; VIII – green seedlings + UV-B 5 h

There is no difference in SOD activity of non-irradiated greening and green seedlings (Fig. 6b). After UV-B treatment activity of SOD isoenzymes 3, 4 and 5 (Cu,Zn-SOD) increased (lanes II, IV, VI, VIII) and the effect of UV-B was well expressed in 48 h illuminated seedlings and in green ones (lanes VI, VIII) whereas in 12 h greening seedlings it was comparatively slight (lane II).

As it can be seen in Fig. 6c, control as well as treated seedlings showed one CAT isoform (CAT 1). The CAT activity in non-UV-B irradiated seedlings increased dur-

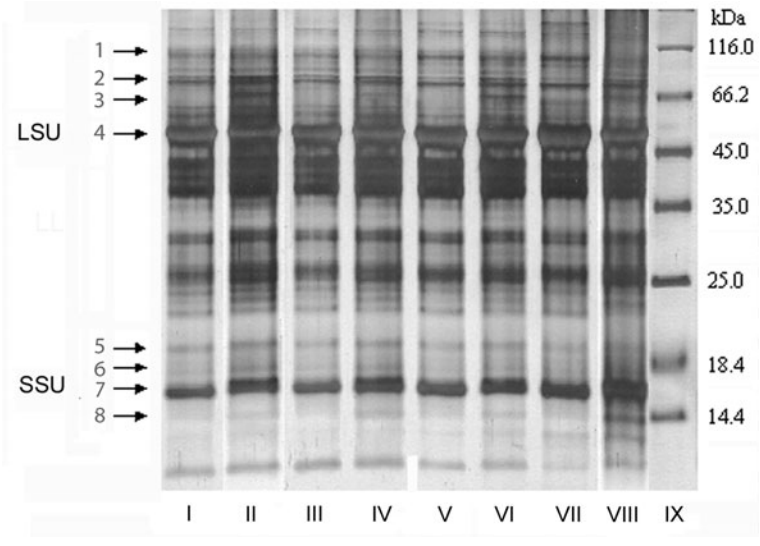

Fig. 7. Effect of UV-B irradiation on polypeptide patterns after SDS-PAGE of soluble proteins in barley leaves. The experimental details are the same as in Fig. 6. Lane IX – molecular mass markers β-galactosidase; bovine serum albumin; ovalbumin; lactate dehydrogenase; restriction endonuclease *Bsp*981; β-lactoglobulin; lysozyme. In each lane 20 μg protein was loaded

ing the greening (lane I, III, V). As a result of UV-B treatment CAT activity definitely enhanced, especially in green and greening 48 h seedlings (lanes VI, VIII).

Figure 7 shows the effect of UV-B irradiation on polypeptide patterns after SDS-PAGE of soluble proteins in barley leaves. Rubisco LSU diminished as a result of UV-B irradiation. Rubisco SSU is barely influenced. A new polypeptid band with Mr 66.2 kDa was established after UV-B treatment. In greening and green seedlings the band with Mr 21 kDa was slightly enhanced by UV-B treatment.

DISCUSSION

The data presented here showed, that UV-B radiation caused reduction in the amount of chlorophyll, oxygen production, activity of PSII, the rate of CO_2 fixation and chloroplast proteins, especially ribulose-5-bisphosphate carboxylase/oxygenase in barley seedlings. Several mechanisms have been suggested to be responsible for UV-B-induced photosynthetic damage. Indoor supplementation studies have identified changes in stomatal conductance [29], Rubisco content, reduction in capacity for photosynthetic electron transport [2, 14] as possible limitations on photosynthesis. Some studies suggested that PSII is the primary target of UV-B damage reducing PSII activity [41] and the amount D_1 protein [18]. Results of our study indicated that the damaging UV-B effect on photosynthesis depended also on the development

stage of photosynthetic apparatus. The plants greening 12 h were more sensitive to UV-B treatment than the plants greening 24 h and particularly 48 h and this was evident from our data about the quantum efficiency of PSII, the rate of O_2 production and $^{14}CO_2$ fixation. ΦPSII was the most affected by UV-B treatment in the seedlings greening 12 h. Large Rubisco subunits were diminished and this was closely related on the development stage of photosynthetic apparatus. It has been demonstrated that white light ameliorated UV-B-induced responses and was suggested that these effects might be the consequence of photosynthetic electron transport and photophosphorylation [33]. A typical repair mechanism is the light-dependent photoreactivation by photolyases resulting in the restoration of UV-damaged DNA to its native form [16]. High PPFDs may confer protection from UV-B damage by increasing photosynthesis and the available biochemical energy for defence and/or repair processes.

Exposure of plants to ultraviolet radiation results in multiple responses including increased synthesis of UV-B absorbing and UV-B induced compounds which are an important defence against UV-B radiation and in addition can enhance antioxidant capacity [5, 10, 39]. The endogenous level of these compounds was similar in greening and green seedlings. As a result of UV-B irradiation, an increase of the content of flavonoids (A_{300}) was established. The value for A_{300} for 12, 24 and 48 h greening seedlings was similar and close to that for green plants. This increase was not related to development stage of photosynthetic apparatus, and we suggest that in this case higher resistance of the plants to UV-B might not be due to higher flavonoids content. Other authors also did not find the relationship between UV-B plant's resistance and endogenous flavonoids level. Adamse and Britz [1] showed that UV-B-induced plant damages were alleviated by elevated CO_2 in the absence of any change in flavonoid content. We suggest that the increase in carotenoids content may represent a biochemical response to alleviate UV-B stress. Carotenoid content was lower in the seedlings greening 12 h, which were more sensitive to UV-B, and higher after 48 h greening. Green seedlings were more resistant and with the highest carotenoid level. We suggest that carotenoids play an important role in the photoprotection of the photosynthetic system by dissipating excess excitation energy through the xanthophylls cycle. The changes in the content of UV-B induced compounds (A_{438}) [9, 10] were similar to those of carotenoids. As a result of UV-B treatment an increase in the content of A_{438} was established in the greening seedlings most pronounced in 48 h illuminated seedlings. The highest amount of UV-B induced compounds was found in green seedlings, in which the damaging UV-B effect was lower. These compounds appeared 4 h after UV-B treatment, reached their maximum after 24 h and then declined. Under salt-stress conditions A_{438} accumulation as a result of UV-B irradiation was the lowest and proline content was the highest [10]. The nature and the role of A_{438} are not understood, but it is possible to be related to UV-B protection.

The inhibition of photosynthesis or electron transport under UV irradiation may elevate the photosensitization process as well as the formation of AOS in this way. The formation of singlet oxygen via photosensitization was suggested to play an

important role in damaging the D_1 protein. The probable electron transfer from electron transport chain, especially in photosystem I (PSI), to molecular oxygen, the way to quench extensive energy, is an alternative source of AOS. Photoreduction of molecular oxygen by primary electron acceptor in PSI complex is thought to be the main source of superoxide in illuminated chloroplasts. Oxidative stress induces degradation of a variety of biological molecules, with the consequent release of malondialdehyde. Under UV-B irradiation MDA content increased. Green plants were less affected by UV-B in respect to MDA content. The increased lipid peroxidation could reflect more UV-B damages and/or reduced ability to scavenge free radicals produced by the UV-B. It should be noted that these lipid peroxidation products were present in plants not treated with UV-B. It is not surprising taking in mind that the electron transport chain and photorespiration are the main source of AOS even when no UV-B is supplied. The content of UV-B induced proline accumulation increased with increase the greening time and was the highest in green seedlings. It could be supposed that in this case proline is not related to osmoregulation. Endogenous free proline could mediate plant's defense responses to UV-B enhancing their antioxidant system due to its free radical scavenging capability. The antioxidant defence system include also enzymatic antioxidants such as superoxide dismutase catalase, peroxidase. SOD converts $^1O_2^-$* radicals into H_2O_2 and O_2. In plants CAT is one of the main H_2O_2-scavenging enzymes that dismutates H_2O_2 into water and O_2, and it is a constituve component of peroxisomes. Peroxidases catalyse the H_2O_2-dependent oxidation of a wide variety of substrates, mainly phenolics and they are often found in multiple molecular forms. The levels of antioxidants were related to UV-B response at different developmental stages of photosynthetic apparatus. Enzymes activities increased depending on greening time and as a result of UV-B treatment but the pattern of isoforms did not change. We established that UV-B preferentially induced Cu,Zn-SOD and had no significant effect on Mn-SOD. The function of these isozymes and its regulation remain largely unknown. Increase in POX activity suggested a higher potential for H_2O_2 destruction and greater protection to the plants. CAT increased its activity depending on the greening time and UV-B treatment but the pattern of isoforms remained similar to those found in the control. POX and SOD did not alter the number of isoforms, but increased activity of some of them under UV-B radiation. The induction of antioxidant enzymes with peroxidase activity indicated that hydrogen peroxide participates actively on UV-B plant response and that those antioxidant enzymes might play an important role in UV-B tolerance.

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