REGULAR ARTICLE

UV-B radiation does not limit carbohydrate level and carbohydrate metabolism in cucumber leaves

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ABSTRACT

Cucumber is a vegetable exhibiting relatively high sensitivity to environmental stress factors. When it is grown outdoors, from early stages of development there is a real risk of exposure to elevated UV-B radiation. In order to explain the effects of time-dependent UV-B doses on carbohydrate level and metabolism, the photosynthetic activity, accumulation of carbohydrates and activities of carbohydrate-related enzymes were determined in the cucumber leaves.

Elevated UV-B radiation led to an increase in the rate of photosynthesis, which was reflected by an increase in SPAD values. Higher photosynthetic activity resulted in an increase in levels of soluble sugars. In view of the above-mentioned results, radiation stress led to a UV-B time-dependent dose increase in the activity of two enzymes decomposing carbohydrate: invertase and glucosidase. Our results suggest that the exposure of cucumber plants to supplemental UV-B doses does not limit the availability of the photoassimilate. Carbohydrates are required to provide not only respiratory energy for protection, maintenance (and repair) of plant activity and structure, but also provide biosynthetic carbon skeletons for secondary metabolite synthesis

Key Words: *invertase; b-D-glucosidas; soluble sugars (fructose, glucose and sucrose); SPAD readings.*

INTRODUCTION

As photoautotrophic sessile organisms, plants have to use sunlight for photosynthesis. Light is not only the source of energy that drives photosynthesis, but also acts as an informational signal directing plant development during the whole plant life. Plants are able to sense the quality, intensity, duration and direction of light (Heijde and Ulm 2012). Ultraviolet-B radiation (UV-B) is an intrinsic part of sunlight. Solar radiation in the UV-B range (280–320 nm) generally accounts for less than 0.5% of the total solar energy reaching the Earth's surface (McKenzie et al. 2003, Paul and Gwynn-Jones 2003). The current level of

UV-B radiation during the cropping season falls anywhere between 2 and 12 kJ•m^{-2} per day on the Earth's surface (UNEP 2002). During the last three decades, UV-B dose-rates in the biosphere have increased as a consequence of ozone-layer depletion by the anthropogenic use of potent ozone destroying halogenated chemicals including chlorofluorocarbons. Increases in the UV-B radiation on the Earth's surface have been estimated to be in the range of 2-5% per decade for Central Europe (McKenzie et al. 2007, Jansen et al. 2008). Estimates from a three-dimensional chemistry-climate model indicate that ground level UV-B radiation is currently near its maximum level (Surabhi et al. 2009).

Solar radiation in the UV-B range accounts for a minor percentage of the total solar energy, but still could be potentially harmful because these short wave lengths are capable of causing deleterious effects in the cells (Salama et al. 2011). The depletion of the ozone layer has raised concerns over the ecological implication on agricultural production and natural plant ecosystems (McKenzie et al. 2007). Commonly observed UV-B effects include damage of the photosynthetic apparatus (Musil et al. 2003, Correia et al. 2005), damage to nucleic acids (Schmitz and Weissenbock 2003) and membranes (An et al. 2000), finally leading to decreased vegetative biomass and grain yield (Kakani et al. 2003, Yao et al. 2008, Kataria and Guruprasad 2012). Probably the most important effect of UV radiation on plants is related to genetic damages, because macromolecules such as DNA, RNA and protein have strong absorption at the level of 280-315 nm (Reboredo and Lidon 2012). Ultraviolet-B radiation has long been perceived a stress factor. However, UV-B damage is now rather rare. It was hypothesized that low UV doses cause "eustress" (good stress), that stimuli-specific signaling pathways pre-dispose plants to a state of low alert that includes activation of antioxidant defenses, and that ROS-mediated signaling contributes to UV-B acclimation, and that (Hideg at al. 2013). Additionally, negative effects of UV-B radiation can be greatly increased or decreased by a multiplicity of interacting stress factors in the natural habitat (Caldwell at al. 1998).

Cucumber is one of the most common vegetable species. It exhibits relatively high sensitivity to environmental stress factors. When it is grown outdoors from the early stages of plant development, there is a real risk of exposure to elevated UV-B radiation. Literature on UV-B radiation's influence on photosynthesis offers inconsistent results, most likely owing to different crop species analyzed, radiation dosage and other environmental conditions (Kakani et al. 2003). Some studies indicate that supplemental UV-B radiation significantly decreased leaf photosynthesis (Reboredo at al. 2012). Laboratory experiments have indicated that damage to photosystem II and electron transport and/or loss of Rubisco activity are the main causes of a decreased rate of photosynthesis under enhanced UV-B (Teramura and Sullivan 1994, Zhao et al. 2004). Other studies found little or no significant effect of increasing UV-B radiation on photosynthesis, as no effect was observed even under long-term exposure to reasonably elevated UV-B radiation when plants were grown under natural conditions (Feng et al. 2003, Reboredo at al. 2012). At early stages of cucumber growth, initially a decrease in phosynthesis was observed in cotyledons under UV-B stress, followed by no effect on photosynthesis rate (Rybus-Zając and Kubiś 2010).

The aim of the present study was to continue the previous research in order to investigate the effect of time-dependent UV-B doses on the photosynthesis rate and carbon skeleton availability, i.e., the accumulation of soluble carbohydrates and activities of some carbohydrate-related enzymes.

MATERIALS AND METHODS

PLANT MATERIAL

Seeds of cucumber plants (*Cucumis sativus* cv. Dar) were sown in perlite (5 per 1.0 l pot) and allowed to germinate and develop in a growth chamber under controlled growth conditions: fluorescent light supplied by Osram LUMILUX L18/840 lamps intensity 120

µmol m^{-2} s^{-1} photon flux density of 400-700 nm, at photoperiod of 14/10 h (day/night) and temperature of 25°C (day) and 20°C (night), and 60-70% relative humidity. Photosynthetically active radiation (PAR) intensity was measured with FF-01 fitophotometer (Sonopan). 21-day-old seedlings were subjected to UV-B irradiation, supplied by Philips TL 20 W/01 RS lamps, with max 315 nm at the intensity of 16 kJ m^{-2} day^{-1} for 8 h per day (3.25 µmol m^{-2} s^{-1} photon flux density), during the 14 h light period and the following 9 days. UV-B irradiation intensity was controlled using VLX 3W radiometer. Control plants were grown under PAR only. The second fully expanded leaf of each seedling was taken to the analysis. Each time-sample contained 25 plants (5 pots, 5 plants per pot).

PHOTOSYNTHESIS RATE

Photosynthesis rate was analyzed for the whole cucumber seedlings by measuring CO_2 loss in a closed system using an AirTECH 2500-P CO_2 analyzer. The results were expressed as mg CO_2 consumed per g fresh weight. Determinations were performed in five replicates, each using one second leaf of an individual plant

SPAD

The level of chlorophyll contents was defined using an optical apparatus known in Europe as Hydro N-Tester while in the USA as the SPAD-502 apparatus (Scharf et al. 2006, Dong et al. 2007). This apparatus operates by measuring light absorption by a leaf at the wave lengths of 650 and 940 nm. The quotient of these differences indicates the chlorophyll contents and is defined in SPAD units (Soil and Plant Analysis Development). Measurements were made on leaves of ten separate plants.

CARBOHYDRATE ANALYSIS

Frozen samples were ground in 80% ethanol and extracted in closed teflon tubes at 80°C for 1 h. After cooling, the extracts were centrifuged (10 min 12.000 g) and evaporated in a speed vacuum concentrator (Heto Lab Equipment A/S, Denmark). The residues were resuspended in 0.1 mM CaEDTA. The HPLC analysis was performed by Waters Alliance with a Sugar Pack I column. Parameters concerning temperature and the mobile phase flow were used according to Waters' protocol, whereas quantitative analysis was based on standard curves (Nygara et al. 1996). Determinations were performed in five replicates and expressed in mg per g fresh weight.

ACID INVERTASE ACTIVITY

Acid invertase activity was assayed using the method of Copeland and Lea (Copeland and Lea 1990) with some modifications. Leaves samples 400 mg were ground in 1.6 ml 50 mM cold Na phosphate buffer (pH 7.4) containing 10 µl mercaptoethanol. The homogenate was centrifuged for 30 min at 27.000 g at 4°C. The reaction mixture contained 0.18 ml extract, 0.57 ml 0.1 M acetate buffer (pH 5.0) with 0.1 M sucrose. The reaction was carried out in vials kept at 30°C for 45 min. After incubation, 0.05 M tricine buffer (pH 8.3) was added and boiled at 100°C for 3 min. Absorbance increase was followed 1 min at 560 nm. Determinations were performed in five replicates and the enzyme activity was expressed as the absorbance increase per 1min and 1g fresh weight.

THE ACTIVITY OF b-D-GLUCOSIDASE

The activity of b-D-glucosidase was determined on the basis of Nichols et al. (1980). The leaves were ground in 0.1 M phosphate buffer of pH 7.0 containing 0.5% of polyethylene glycol and 40 mg of Polyclar AT. Supernatant obtained after centrifugation at 10.000 g for 15 min were used to determine the enzyme activity. The mixture containing 0.2 ml extract and 0.2 ml 4-nitrophenyl-b-D-glucopyranoside as substrate was incubated for one hour at 35°C. After the time, 0.6 ml 0.2 M Na_2CO_3 was added. The formation of p-nitrophenol (p-NP) was followed at 400 nm. The activity was measured in five replications and expressed as µ moles p-NP per mg protein.

PROTEIN CONTENT

Protein content was determined by the Bradford method (Bradford 1976).

STATISTICAL ANALYSIS

Statistical analyses were performed based on five replications (ten in the case of SPAD measurements) and the data are presented as mean ± standard deviation (SD). A two-way analysis of variance (ANOVA) was carried out to verify the hypotheses about lack of effects of UV-B irradiation and days of stress as well as the hypothesis about lack of UV-B irradiation × days of stress interaction for photosynthesis rate, SPAD, glucose concentration, fructose concentration, sucrose concentration, acid invertase activity and glucosidase activity. Significant differences between means were determined by Tukey's multiple range test. Differences in the studied traits between UV-stressed plants and the corresponding control (for each day independently) were tested with linear contrasts based on the corresponding ANOVA model.

RESULTS

The ANOVA results indicated that all the tested effects – that is, the main effects of UV-B irradiation (with and without UV-B) and days of stress as well as UV-B irradiation × days of stress interaction – were significant ($P<0.01$) for all the observed traits. Generally, a higher photosynthetic activity was measured in UV-B stressed seedlings than in the control plants, especially at the beginning of the supplemental irradiation (Fig. 1). In UV-B treated seedlings the rate of photosynthesis increased to 154%, 142%, 115% and 113% of that of the respective control plants at 3, 5, 7, and 9 days, respectively (Table 1). Additionally, in control plants a nonsignificant increase in the rate of photosynthesis was also observed as compared to the value at the beginning of this experiment.

Higher photosynthetic activity measured in leaves of UV-B stressed seedlings was reflected in increased SPAD values (Figure 2) as compared to the respective control cucumber plants (Table 1). In UV-B stressed seedlings, SPAD readings increased significantly at 7 and 9 days of the experiment to 112% and 123% of that of the respective control. The increase observed in UV-B treated plants was not followed by the increase in insignificant time-dependent values in leaves not subjected to supplemental radiation versus the value at the beginning of the experiment.

Table 1. The contrast estimations for comparison between UV-stressed plants and control for the studied traits

Traits	Days				
	0	3	5	7	9
Acid invertase activity	-0.333**	0.294**	0.290**	0.314**	0.447**
Glucosidase activity	-16.66**	-1.63^{ns}	3.30*	3.85*	-0.09^{ns}
SPAD	-499**	8.40^{ns}	40.6*	62.47**	122.43**
Glucose concentration	-1.077**	0.797**	0.673*	1.727**	0.090**
Fructose concentration	-1.200**	0.573**	0.777**	0.910**	-0.190^{ns}
Sucrose concentration	-4.597**	4.523**	3.247**	1.993**	0.267^{ns}
Photosynthesis rate	-4.413**	2.450**	1.990**	0.797**	0.663**

* $P<0.05$; ** $P<0.01$; ns - not significant

The increase in photosynthesis activity (Fig. 1) was mirrored in an increase in carbohydrate contents in cucumber leaves (Fig. 3). Monosaccharides, i.e. glucose and fructose, and a disaccharide, i.e. sucrose, were analysed under UV-B-stress conditions. A glucose level under UV-B radiation increased to 200%, 128%, 230% at 3, 5, 7 days of stress versus the respective control value (Table 1). The level of another monosaccharide, fructose, rose at the 3, 5, and 7 days of exposure to UV-B to 166%, 142%, 164% in relation to the respective control leaves (Table 1). Sucrose content under supplemental UV-B radiation increased to 207%, 147%, 121% at 3, 5, 7 days of stress in comparison to the respective control value. Additionally, in the control plants, some increase in carbohydrate contents was found in relation to the value at the beginning of the experiment.

The higher availability of primary metabolites, monosaccharides and a disaccharide (Fig. 3), was probably in part affected by UV-B time-dose dependent increase of two enzymes, particularly (140%) invertase and less significantly (110%) glucosidase activities, connected with carbohydrate metabolism. Invertase activity (Fig. 4) rose in cucumber leaves to 184%, 155%, 168% and 190% at 3, 5, 7 and 9 days of stress duration versus the respective control. Glucosidase activity (Fig. 5) amounted to 120% and 125% at 5 and 7days of UV-B stress in comparison to the respective control.
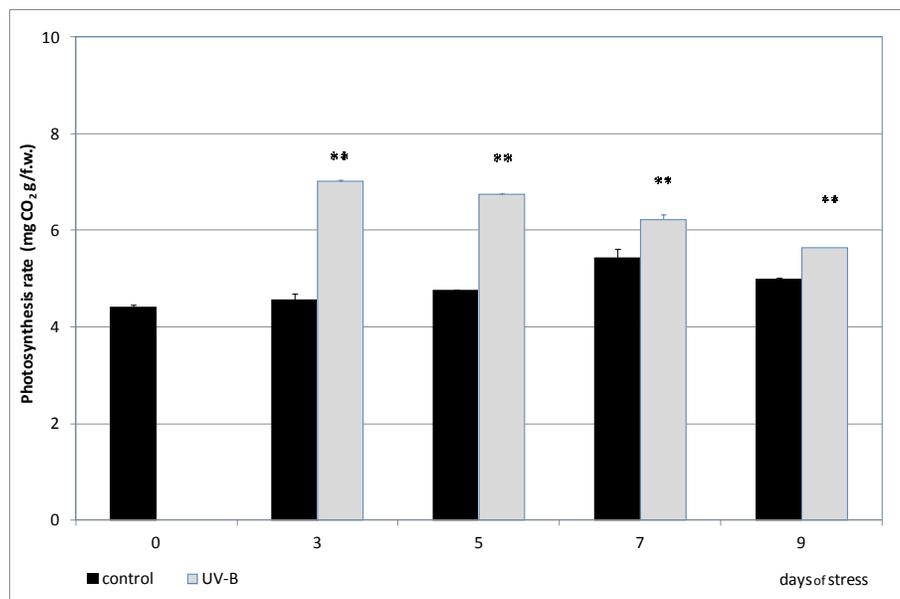

Figure 1. Photosynthesis rate (expressed as assimilated CO_2 mg/g fresh weight) measured at 0, 3, 5, 7 and 9 day in cucumber leaves, as a function of UV-B time-dose irradiation. Data of the stressed plants significantly different (at $P<0.01$) from the respective control are indicated with ** ($HSD_{0.01}$ for UV-B irradiation: 0.0599, days of stress: 0.0947, UV-B irradiation × days of stress interaction: 0.1339).
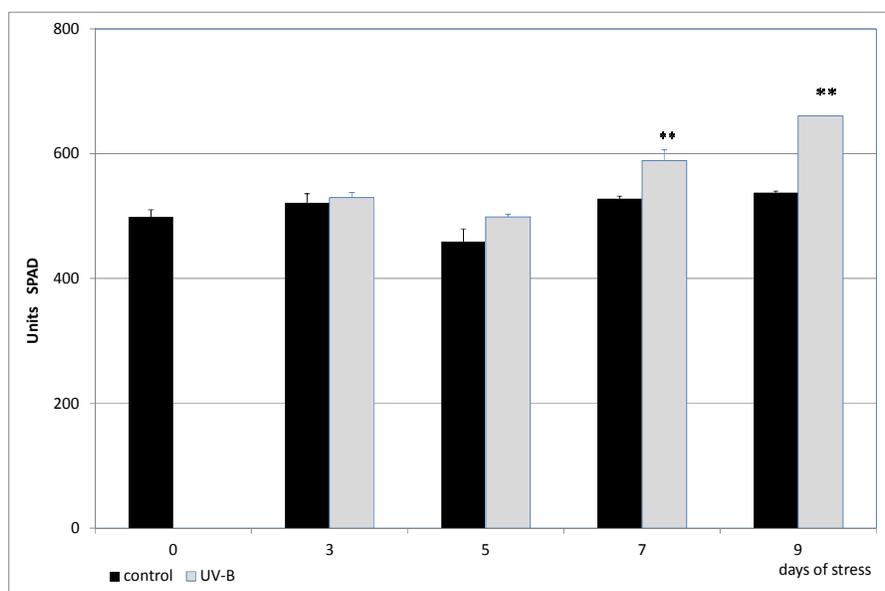
Figure 2. Chlorophyll meter readings indicates the chlorophyll contents and it is defined in SPAD units – SPAD (Soil and Plant Analysis Development), measured at 0, 3, 5, 7 and 9 day in cucumber leaves, as a function of UV-B time-dose irradiation. Data of the stressed plants significantly different (at $P<0.01$) from the respective control are indicated as ** ($HSD_{0.01}$ for UV-B irradiation: 11.14, days of stress: 17.61, UV-B irradiation × days of stress interaction: 24.90).
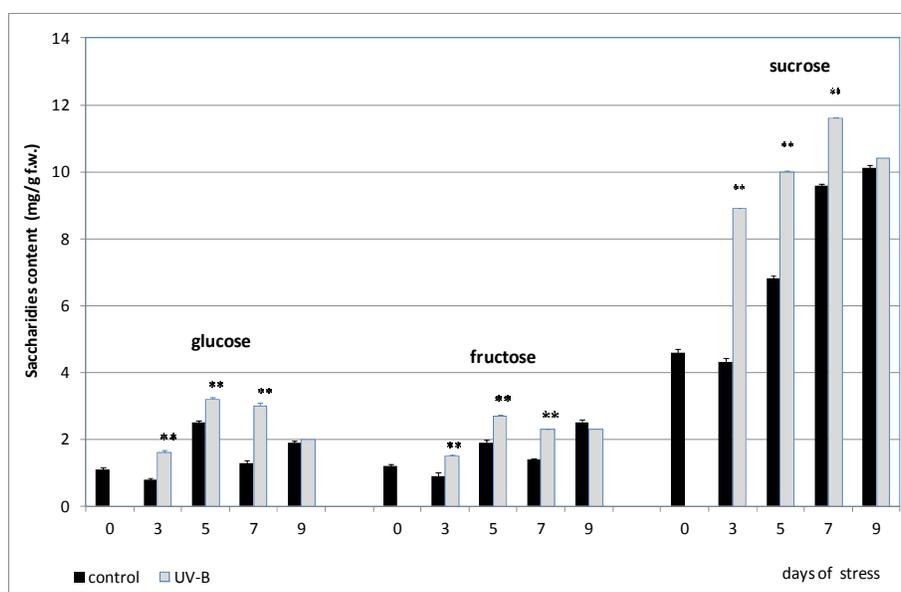

Figure 3. Soluble sugar (glucose, fructose and sucrose) concentration (expressed as mg/g fresh weight) measured at 0, 3, 5, 7 and 9 day in cucumber leaves, as a function of UV-B time-dose irradiation. Data of the stressed plants significantly different (at $P<0.01$) from the respective control are indicated as ** (glucose $HSD_{0.01}$ for UV-B irradiation: 0.1118, days of stress: 0.1768, UV-B irradiation × days of stress interaction: 0.25; fructose $HSD_{0.01}$ for UV-B irradiation: 0.0609, days of stress: 0.0964, UV-B irradiation × days of stress interaction: 0.1363; sucrose $HSD_{0.01}$ for UV-B irradiation: 0.1418, days of stress: 0.2243, UV-B irradiation × days of stress interaction: 0.3172).
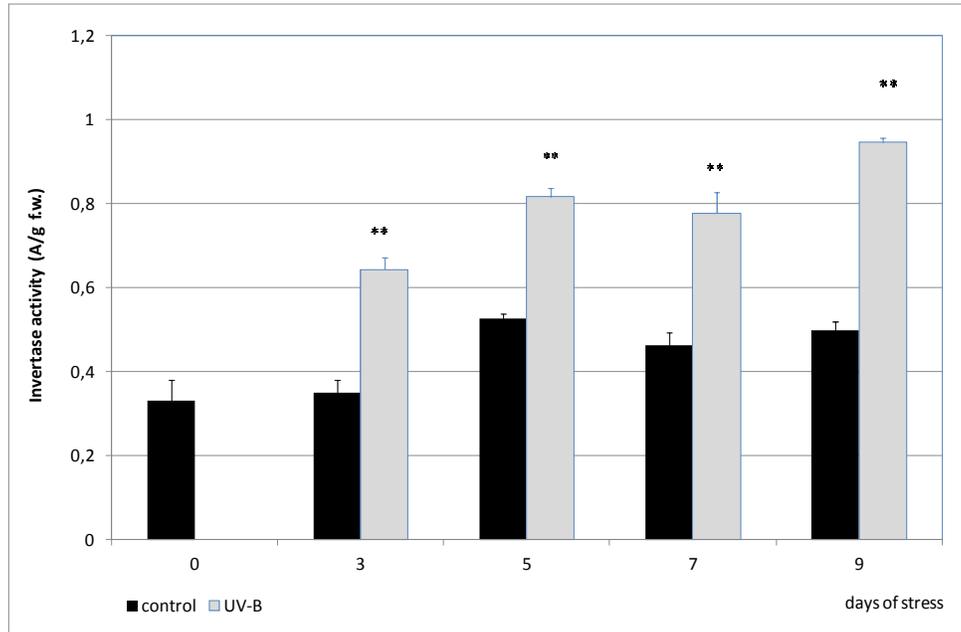
Figure 4. Acid invertase activity (expressed as A/g fresh weight) measured at 0, 3, 5, 7 and 9 day in cucumber leave, as a function of UV-B time-dose irradiation. Data of the stressed plants significantly different (at P<0.01) from the respective control are indicated as ** (HSD$_{0.01}$ for UV-B irradiation: 0.0160, days of stress: 0.0253, UV-B irradiation × days of stress interaction: 0.0358).

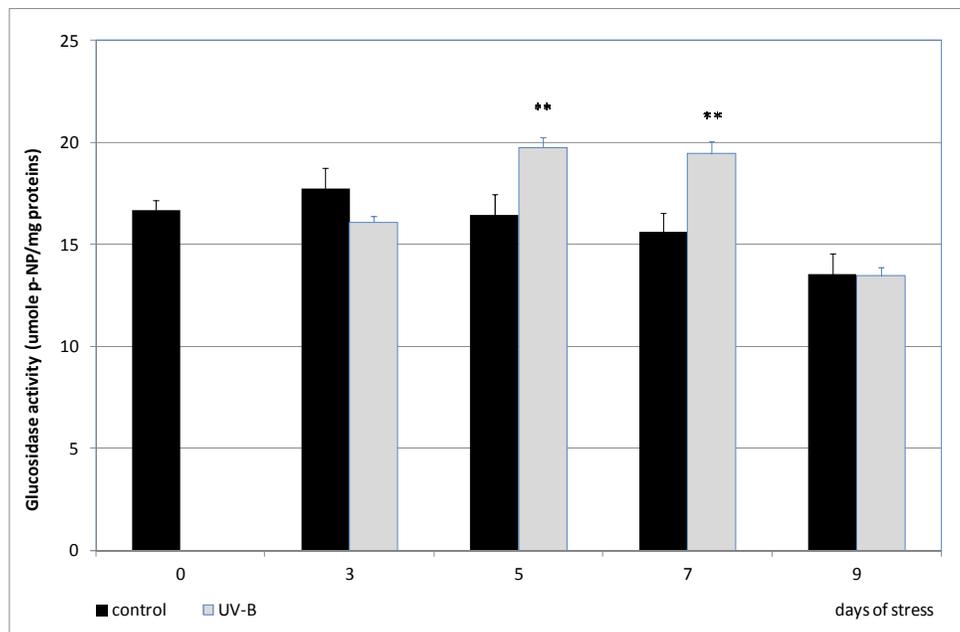Figure 5. Glucosidase activity (expressed as μ moles p-NP/mg protein) measured at 0, 3, 5, 7 and 9 day in cucumber leaves, as a function of UV-B time-dose irradiation. Data of the stressed plants significantly different (at P<0.01) from the respective control are indicated as ** (HSD$_{0.01}$ for UV-B irradiation: 1.752, days of stress: 2.770, UV-B irradiation × days of stress interaction: 3.917).

DISCUSSION

Although UV-B accounts for only less than 0.5% of total light energy reaching the Earth's surface, UV-B radiation has major biological effects. Plants are photoautotrophic organisms and thus light in particular is an environmental factor of utmost importance for plants (Heijde and Ulm 2012). It was earlier observed that UV-B dosage on the near ambient level (16 kJ m^{-2} day^{-1}) alter reactive oxygen species (ROS) metabolism in cucumber cotyledon and leaves (Kubiś and Rybus-Zając 2008, Rybus-Zając and Kubiś 2010). Such ROS-mediated signaling can lead to acclimation to UV-B stress condition (Noctor 2006).

In cucumber leaves elevated UV-B radiation leads to an increase in photosynthesis (Fig. 1). Higher photosynthetic activity was measured in UV-B stressed seedlings as compared with the respective control plants, especially at the early days of supplemental irradiation. In our earlier study on cucumber cotyledons under UV-B dosage on the ambient level (Rybus-Zając and Kubiś 2010), first an opposite effect was observed, i.e. a decrease in the rate of photosynthesis to 92-69% versus the control value, but only at a very early developmental stages, up to the 7th day of UV-B stress. Later, however, at day 10, the intensity of photosynthesis reached the level found in the control plants. The conclusion based on these results was that the cucumber plants displayed an organ-variety-specific photosynthesis rates under exposure to the enhanced UV-B radiation. Inhibition of photosynthesis is often observed either in sensitive species or under high UV-B doses (often in combination with unnaturally low PAR doses) (Caldwell et al. 1989, 1995, 1998, 2003, Johanson et al. 1995). In a comprehensive review on field crop responses to UV-B radiation, Kakani et al. (2003) suggested that a decrease in photosynthesis, particularly at higher UV-B doses, was due to both direct (on the photosystem) and indirect (on pigments and leaf area) effects of UV-B radiation. This decrease results in lower biomass and yields of most crop plants. In contrast, there was a tendency for photosynthesis to increase in well-adapted plant species (Shi et al. 2004, Haapala et al. 2010, Reboredo at al. 2012) or cultivars displaying differences in the photosynthetic performance and biomass accumulation (Feng et al. 2003).

Higher photosynthetic activity measured in leaves of UV-B stressed seedlings was reflected in an increase in SPAD values (Fig. 2). Chlorophyll meter readings exhibited a high positive correlation with leaf total chlorophyll concentration (TCHL), such correlation having been shown in several crops (Kapotis et al. 2003, Dong et al. 2007). In cotyledons of cucumber plants an increase in the photosynthetic pigment chlorophyll and carotenoids was previously observed (Rybus-Zając and Kubiś 2012). A similar result, i.e. accumulation of chlorophylls following enhanced UV-B, was evidenced in wheat (Zheng et al. 2003, Zu et al. 2004) and similar patterns were reported for other species as well (Brzezińska et al. 2006). Shi et al. (2004) measured also an increase in the photosynthetic pigments when expressed on the leaf area basis. They hypothesized that the increase of leaf thickness in alpine species after a long-term exposure of enhanced UV-B radiation could compensate for the photodestruction of photosynthetic pigments. Haapala et al. (2010) suggested that in the natural ecosystem, even a long-term exposure to reasonably elevated UV-B radiation does not affect the chlorophyll fluorescence or photosynthesis.

The increase of photosynthesis activity (Fig. 1) was generally followed by an increase in soluble carbohydrate contents in cucumber leaves (Fig. 3). Monosaccharides, i.e. glucose and fructose, and a disaccharide, i.e. sucrose, were analyzed under UV-B-stress. In cucumber cotyledons (Rybus-Zając and Kubiś 2012) no such relation was observed, in spite of the negative effect of supplemental UV-B radiation on photosynthetic activity, whereas soluble carbohydrate content was not reduced. This maintained high sugar level suggests that in cucumber cotyledons and leaves supplementary UV-B radiation did not decrease the availability of primary metabolites, that is, glucose, fructose and sucrose. Similarly, an increase in soluble carbohydrate levels was recorded in grass and oak leaves (Gwynn-Jones 2001, Newsham et al. 2001). It was suggested that carbohydrates are also required to provide

respiratory energy for protection and maintenance (repair included) of plant activity and structure (Farrar 1989). In contrast, supplemental UV-B level caused a decrease of the soluble carbohydrate level (Yue and Wang 1998), the rate of photosynthesis, biomass accumulation and yields of wheat plants (Ambasht and Agrawal 2003, Agrawal et al. 2004, Zheng et al. 2003, Li et al. 2010). In cotton, exposure to higher UV-B radiation decreased leaf soluble sugar and leaf starch concentrations due to a lower rate of net photosynthesis, loss of Rubisco activity and electron transport (Zhao et al. 2004). Similarly, in an outdoor study, elimination of UV-B radiation from the solar spectrum caused an increase in dry matter accumulation and yield parameters in wheat varieties (Kataria and Guruprasad 2012).

The UV-B treatment of cucumber leaves had no negative effect on the photosynthetic CO_2 assimilation rate and the soluble carbohydrate level. The above-mentioned results concerning the higher availability of primary metabolites were probably in part affected by the UV-B time-dose dependent increase of two enzymes, connected with carbohydrate metabolism, particularly (140%) invertase (Fig. 4) and less significantly (110%) glucosidase (Fig. 5) activities. Invertase is engaged in decomposing of sucrose, while glucosidase of glucosides; they both elevate sugars availability. In cucumber cotyledons a similar UV-B time-dose dependent increase (157%) of invertase activity was observed (Rybus-Zając and Kubiś 2012) versus the time-dependent decrease (83%) in control cotyledons. The data are scant; UV-B irradiated fruits of Asian pear (Muriguchi et al. 1992) and lemons (Interdonato et al. 2011) also exhibited an increase in invertase activity. In the case of glucosidase activity in cucumber cotyledons, a similar UV-B time-dose dependent increase (128%) was recorded (Rybus-Zając and Kubiś 2012) versus the generally stable activity in the control cotyledons. In leaves of UV-B irradiated buckwheat (*Fagopyrum esculentum*) also a high – as high as 360% of the increase in the control plants – increase in glucosidase activity was reported (Suzuki et al. 2005).

CONCLUSIONS

From the results obtained in this paper it can be concluded that photosynthesis is not significantly affected by changes in UV-B radiation when cucumber plants grow under realistic UV-B conditions. Moreover, sufficient availability of soluble carbohydrates was found. UV-B radiation on the ambient level did not cause metabolic disruption. Exposure to mild "eustress" induced active acclimation responses, while sufficient carbohydrates' level provide biosynthetic carbon skeletons for secondary metabolite synthesis. One of the main protection mechanisms against UV radiation is the accumulation of secondary metabolites, phenolic compounds and flavonoids, to filter out UV-B photons before they reach sensitive molecules (Harborne and Williams 2000, Kolb et al. 2001, Reboredo and Lindon 2012)

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