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Biochimica et Biophysica Acta 1710 (2005) 24 – 33

BIOCHIMICA ET BIOPHYSICA ACTA

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Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UV-B protection

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Received 1 April 2005; received in revised form 26 August 2005; accepted 12 September 2005
Available online 3 October 2005

Abstract

Polyamines have been described to protect against numerous oxidative stresses in plants. Increasing UV-B radiation (280 – 315 nm) in the biosphere may also induce an increase in radical formation in tissues. This study employed the tobacco cultivars Bel B and Bel W3 to describe possible protective functions of polyamines against UV-B radiation in sun light simulators (GSF/Munich) with natural diurnal fluctuations of simulated UV-B. Polyamine measurements on a whole leaf basis in isolated chloroplasts and thylakoids were paralleled to photosynthetic and respiration rates, photosynthetic efficiency, leaf thickness and photosynthetic pigment compositions. The study revealed that an increase of polyamines, and especially of putrescine level in thylakoid membranes upon elevated UV-B exposure comprises one of the primary protective mechanisms in the photosynthetic apparatus of the tobacco variety Bel B against UV-B radiation. The tobacco cultivar Bel W3, sensitive to ozone, was also proved to be sensitive to UV-B. This sensitivity is attributed to its incapability to enhance putrescine level in thylakoid membranes. After prolongation of UV-B exposure, when endogenous plant balances are being gradually restored, due to secondary responses, (e.g., biosynthesis of carotenoids and of additional flavonoids) and the plant is adapting to the altered environmental conditions, then the polyamine level is being reduced. Thus, we can discriminate the UV-B induced stress period from a UV-B acclimation period.

Keywords: Putrescine; UV-B radiation; Spermidine; Spermine; Sun simulator chamber

1. Introduction

The depletion of stratospheric ozone, caused by a series of anthropogenic activities (mainly the release of halogenated hydrocarbons), has resulted in an increase in the UV-B radiation reaching the biosphere [1,2]. This increase is estimated to be about 1% per year [3]. Frederick [4] described that enhanced UV-B radiation affects the yield of a series of economically important agricultural crops [5–7], while others could not support this assumption [8]. Out of numerous inhibitory effects, there often appears inhibition of photosynthesis [9], but also enhancement of photosynthesis is reported [10]. Plants have evolved different avoidance mechanisms against UV-B. By transducing the light signal followed by altered gene expression, they result in synthesis of repair systems, such as UV-B absorbing pigments, like flavonoid and related phenolic compounds [11,12], thickening of the leaf to reduce UV-B penetration [13] and formation of specific peroxidases [14]. Further, the sensibility of plants exposed to UV-B radiation is dependent on the presence of UV-A plus photosynthetic active radiation to induce photorepair processes [2,13]. Major damage in plant tissues caused by UV-B radiation includes the destabilization of biomembranes [15,16], as well as induction of senescence-related gene products, which may explain the often-observed earlier

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Abbreviations: PUT, putrescine; SPD, spermidine; SPM, spermine; PAR, photosynthetically active radiation; LHC, light harvesting complex; PSII, photosystem II

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doi:10.1016/j.bbabio.2005.09.001

occurrence of senescence [17]. Kramer et al. [16] discussed that polyamines might play an important role in the protective mechanism of plants during exposure to UV-B radiation. Polyamines have been shown to be radical defense compounds, which can be enhanced if polyamines are added exogenously [18]. Direct application of polyamines to plant tissue has been shown to inhibit chilling injury [19].

Recently, it has been reported that polyamines play a main role in the regulation of structure and function of the photosynthetic apparatus [20,21]. Kotzabasis and coworkers [22] found that the main polyamines, putrescine (PUT), spermidine (SPD) and spermine (SPM) are associated with the light-harvesting (LHC) and the PSII complex of spinach. Highly purified PSII-core antenna and reaction centre particles of PSII contained in considerable concentration mainly the tetramine Spm. Del Duca et al. [23] showed the apoproteins of the chlorophyll-a/b antenna complex (LHCII, CP24, CP26, CP29) and the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to be substrates of a plastidic transglutaminase. This enzyme catalyses the incorporation of polyamines into their target proteins. Besford et al. [24] identified D1, D2, Cyt_f and the large subunit of Rubisco as proteins which can be stabilized by the addition of exogenous polyamines. Andreadakis and Kotzabasis [25] suggested that polyamines, as well as the corresponding plastidal enzyme activities of ornithine decarboxylase (ODC), arginine decarboxylase (ADC) and diamine oxidase (DAO), are photoregulated and undergo considerable changes during chloroplast photodevelopment. These data strongly support the hypothesis that polyamines could play an important role in the development of structure and function of the photosynthetic apparatus. This hypothesis is mainly based on the capability of polyamines to 'stabilize' chlorophyll protein complexes [24].

The purpose of the present study is to determine the effect of an increase in UV-B (about 30% compared to current measurements in Germany) on polyamines in whole leaves, in isolated intact chloroplasts and thylakoid membranes and to describe their protective role in the photosynthetic apparatus.

2. Materials and methods

2.1. Plant cultures and growth conditions

For all experiments plants of two tobacco cultivars (*Nicotiana tabacum* L. *cv* Bel W3 and Bel B) were used and cultivated similar to Langebartels et al. [26]: tobacco plants were sown in 2:1 (v/v) mixture of standard substrate (Fruhstorfer, type T, Archut, D-Lauterbach) and perlite. The emerged seedlings were transferred to 2-L pots after 3 weeks of further culture. The plants were grown in a controlled environment cabinet at 25/20 °C day/night temperatures, 16 h photoperiod and 100 μmol photon light intensity and 70% relative humidity, until used for exposure in the sun simulators.

2.2. Conditions for simulation of solar and increased UV-B radiation

Plants of the same age (about 8 weeks), from both Bel B and Bel W3 cultivars, were placed in the sun simulator chambers. Half of the plants were exposed to the simulated sunlight excluding UV-B and thus were used as control plants. The other half of the plants received a high but still realistic UV-B irradiance. Fluctuations of the simulated solar irradiation followed natural diurnal fluxes referring to field records which were obtained from measurements on the site of the GSF (South Germany 48.22 °N, 11.6 °E, 495 m above).

After transfer of plants to the sun simulator [27,28], they were kept for an acclimation phase of 7 days at 25/20 °C (day 14 h/night), and at 70% relative humidity. Light conditions: during acclimation from 500 μmol PAR to 1500 μmol PAR over 3 days; during treatments in the control: (average maximum values): PAR: 1500 μmol photons; UV-A: 21 W/m^2 ; UV-B: <1 mW/m^2. The corresponding values during treatments were PAR: 1500 μmol photons; UV-A: 30 W/m^2; UV-B:1 W/m^2.

Bel B and Bel W3 control plants were harvested on the day before onset of the treatment, and also control and UVB-treated plants of both varieties were harvested 1, 3 and 7 days after onset of the UV-B treatment. Harvested plant leaves were further subjected to plastid and thylakoid isolations and together with intact leaf samples were analyzed as follows.

2.3. Isolation of chloroplasts and thylakoid membranes

The method is adapted from Lütz et al. [29]. After removal of major veins, leaf samples (approx. 7 g) were homogenized with 100 ml of Tricin buffer (0.05 M, pH 7.5, $MgCl_2$ 0.004 M, $MnCl_2$ 0.004 M, sorbitol 1 M) in a kitchen blender equipped with razor blades. The homogenate was filtered through gauze to remove debris and further centrifuged for 3–4 min in $200\times g$. The supernatant was placed on top of a two step gradient formed by 6 ml 50% Percoll and 8 ml 15% Percoll and the gradient was formed for 15 min at $5000\times g$, after which two bands developed. The lower band which contained intact chloroplasts was collected, and the plastids were gently pelleted by centrifugation to remove Percoll. After osmotic shock in 1:10 diluted grinding medium and homogenization, the thylakoids were pelleted by centrifugation ($10{,}000\times g$, 15 min). The thylakoid membranes were finally resuspended in 2 ml of buffer pH 8.8 (Tris 1.2 M, glycerol 3.2 M).

2.4. Polyamine analysis and estimation

Leaves, plastids or thylakoid membranes of both plant cultivars (Bel B and Bel W3) were homogenized in 1 N NaOH. 0.2 ml of this suspension were mixed with 36% (w/v) HCl in a proportion of 1:1 (v/v), in screw cap tubes and hydrolyzed at 110 °C for 18 h. The hydrolysis product was centrifuged for 10 min at $1400\times g$ in order to remove carbonized material. Samples were dried at 70–80 °C and redissolved in 0.2 ml of 5% (v/v) perchloric acid. The content in derivative polyamines was estimated and characterized after a specific benzoylation according to the modified method of Flores and Galston [30] and following HPLC separation. For the benzoylation 1 ml of 2 N NaOH and 10 μl of benzoylchloride were added to 0.2 ml of the sample aliquots and vortexed for 30 s. After 20 min incubation at room temperature, 2 ml of saturated NaCl stopped the reaction. The formed benzoylpolyamines were extracted with diethylether and evaporated to dryness. The remaining benzoylpolyamines were redissolved in 0.2 ml of 63% (v/v) methanol and 20 μl of this solution were injected into the HPLC and analyzed for polyamines according to Kotzabasis et al. [31]. The analysis was performed with a Hewlett-Packard 1090 HPLC system equipped with a DPU multi-channel integrator and a diode array detector. A narrow bore column (C_{18}, 2.1×200 mm, 5 μm particle size, Hypersil, Hewlett Packard) was used for the analysis of the polyamines.

2.5. Polarographic assay of photosynthetic oxygen evolution

Rates of oxygen evolution of the Bel B and Bel W3 leaves were determined polarographically at 25 °C in a Hansatech Instruments Ltd. (Kings Lynn, England) electrode system, under continuous temperature control, according to [32].

2.6. Analysis of photosynthetic pigments

The same leaf samples as were used in photosynthesis measurements were extracted with dimethylformamide (leaf area of each sample: 10 cm^2). The pigments were separated and quantified on a Waters 990+ HPLC with diode array detection as was described by Wildi and Lütz [33].

2.7. Measurement of fluorescence induction (photosystem II activity)

For the fluorescence induction measurements the "Plant Efficiency Analyser" (PEA) (Hansatech Instruments Ltd.; Kings Lynn, GB) was used. Maximum yield of photochemistry (F_v/F_m) was measured according to the JIP-method of Strasser and Strasser [34] after a pre-darkening period of 30 min.

2.8. Protein determination

Protein concentrations were determined following the method of Bradford [35], as modified by Jones et al. [36].

2.9. Data analysis

Each treatment was performed on three to four plants and the experiment was repeated three times. Bars on the diagrams represent the standard deviation of the average values. Samples were also analyzed by analysis of variance (ANOVA, $P < 0.05$), after performing Levene's test for equality of error variances. Individual differences were analyzed by Tukey HSD test.

3. Results

The analyses of polyamine contents in the leaves of control and high UV-B treated plants revealed a different pattern for endogenous polyamine regulation on exposure of the two plant cultivars (Fig. 1). Either on the first, third or seventh day of UV-B treatment, polyamine levels were kept more or less unchanged on a whole leaf protein basis in the case of the Bel B cultivar. Some minor, not significant, alterations (as indicated by the statistical analysis) appear in SPD and SPM, which may indicate their fast turnover into each other. Statistically significant differences appear among the controls of different days (not only for Bel B, but also for Bel W3 plants). However, according to unpublished data from our lab, polyamine levels always show such fluctuation depending on the age.

Contrary to Bel B, Bel W3 plants show remarkable changes in polyamine levels on a leaf basis, already after one day of exposure to UV-B irradiation. All polyamines in Bel W3 plants decreased (Fig. 1B). As UV-B treatment continued (i.e., on the third day) plants have already started to increase polyamine levels, with PUT showing already higher values than the 1 day control, and SPD and SPM were not further reduced. This UV-B mediated induction of polyamines was more obvious on the seventh day, when each of the three polyamines reached values nearly twice of the corresponding control values.

Direct measurements of polyamine contents were also done both in isolated chloroplasts and in isolated thylakoid membranes from all plant treatments. Fig. 2 presents the changes observed in chloroplast polyamine amounts after 1, 3 and 7 days of UV-B treatment. One-day UV-B treatment caused a decrease by 34% and 30% in SPD and SPM respectively, but an increase of PUT in the chloroplasts of Bel B plants (about 55% higher than the control), and this increase became highest during the third day of treatment (285% higher than the control), while it declined after prolonged UV-B stress (7th day, 175%). Although with a significant delay, SPD started to increase by the third day (265%) and became highly elevated (about 9-fold higher than the control, 892%) by the seventh day of UV-B exposure. Changes occurred also in SPM, although much smaller and they also demonstrate a positive response under high UV-B. When comparing the data on a basis of control (=100%), it has to be considered, that the control is exposed in the absence of UV-B, versus the treatment of approx. 1 W/m^2 UV-B radiation.

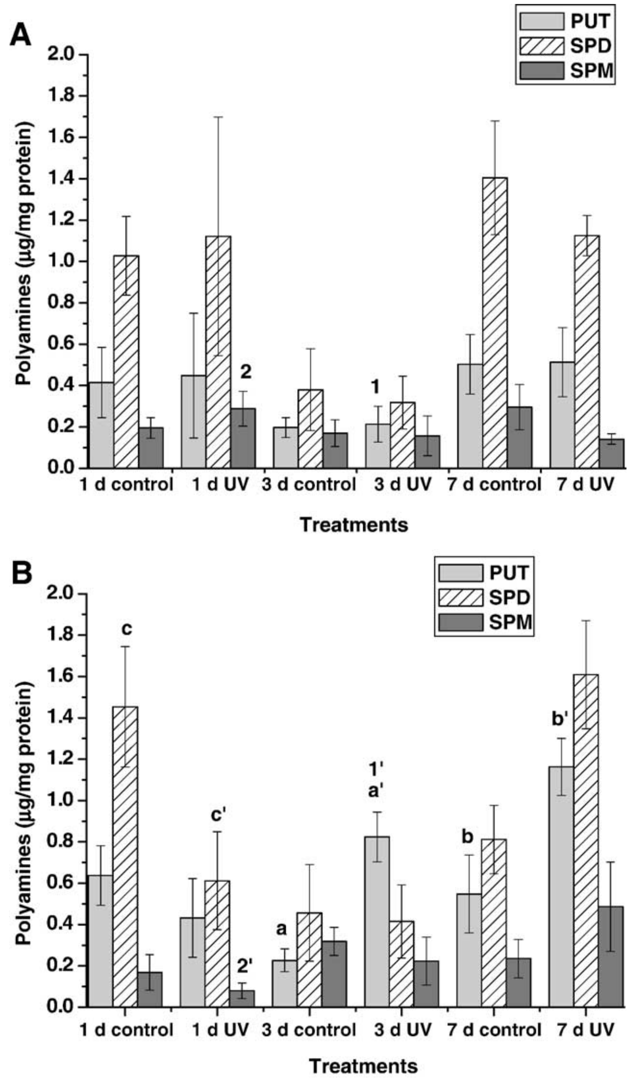

Fig. 1. Alterations in the levels of polyamine contents induced in the leaves of Bel B (A) and Bel W3 (B) plants, not treated (controls) or treated with UV-B irradiation, for 1, 3 or 7 days. Letters above the bars are used to designate significant differences (ANOVA statistics were performed with the log10 transformed data $P < 0.05$). Same letter (e.g., a and a′) is used for the bars which differ (comparisons always between control and treated samples of the same cultivar). Same numbers (e.g., 1 and 1′) are used to designate the statistically significant differences on the bars that represent corresponding polyamine values between the two cultivars Bel B (A) and Bel W3 (B). Polyamine levels were also measured before treatment (day 0) and the corresponding values in µg/mg protein were as follows: (a) Bel B: Put = 0.29 ± 0.10, Spd = 0.36 ± 0.21, Spm = 0.17 ± 0.04, (b) Bel W3: Put = 0.52 ± 0.18, Spd = 0.65 ± 0.03, Spm = 0.16 ± 0.08.

This pattern, seen in Bel B chloroplasts, is almost totally different from the corresponding pattern in Bel W3 plants. Indeed, there seemed to be a great delay in polyamine

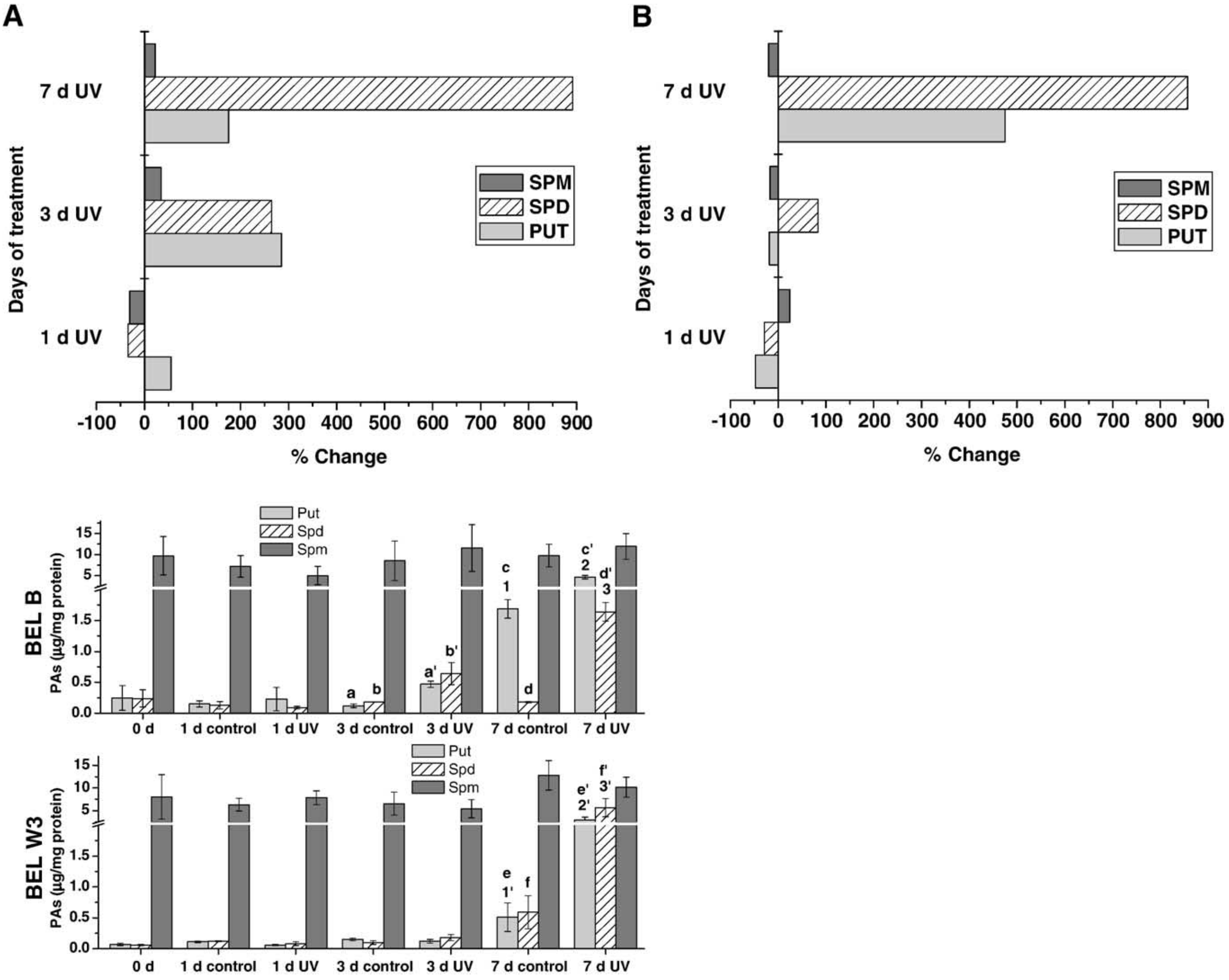

Fig. 2. Changes in polyamine levels (expressed as percent changes of corresponding control values) from chloroplast preparations of Bel B (A) and Bel W3 (B) plants, after UV-B irradiation treatment, for 1, 3 or 7 days, as well as the absolute values measured for both control and UV-B treatments in Bel B and Bel W3 chloroplasts, for 0, 1, 3 and 7 days of experiment. Letters above the bars are used to designate significant differences (ANOVA statistics were performed with the original data $P < 0.05$). Same letter (e.g., a and a′) is used for the bars which differ (comparisons always between control and treated samples of the same cultivar). Same numbers (e.g., 1 and 1′) are used to designate the statistically significant differences on the bars that represent corresponding polyamine values between the two cultivars Bel B (A) and Bel W3 (B).

enhancement in the chloroplasts of Bel W3 plants (Fig. 2B). Only a minor increase is measured in SPM after 1 day high UV-B radiation, which disappeared during continued treatment. The strongest effect developed in SPD, from 83% after 3 days incubation to 857% after 7 days incubation—similar to the corresponding value in Bel B, but delayed in comparison to the wild type samples. In contrast to Bel B, PUT synthesis needed more time to produce considerable amounts of this polyamine, which finally reached about twice the levels in Bel W3 compared to Bel B.

Polyamine amounts bound to thylakoid membranes are shown in Fig. 3. Thylakoid membranes, isolated from Bel B plants, have more than doubled their amounts of PUT and SPD after one day high UV-B, while SPM remained reduced. After 3 days of exposure, all three polyamines accumulated drastically, especially PUT (815%) and SPD (479%). Surprisingly, by the 7th day all values were found strongly reduced to levels near the control or similar to values of the 1-day exposure (PUT, 116%). Thylakoids prepared from Bel W3 plants showed a completely different pattern: biosynthesis of all polyamines was reduced by 1 day high UV-B exposure compared to the normal UV-B controls, while also PUT and SPD were still reduced after 3 days exposure. In comparison to the large changes observed in Bel B thylakoids, the most prominent changes in polyamine induction are those of SPD (+79%) after 3 days and of PUT (+79%) after 7 days, which clearly shows that polyamine induction is much less enhanced by UV in the Bel W3 plants, than in the Bel B strain.

The physiological state of all the plants used in the experiment seemed unchanged, namely neither control nor UV-treated plants did develop any visible injury, like chlorosis or necrotic lesions. However, when comparing the specific leaf weight (fresh weight per leaf area) of the plants, an effect appeared (Fig. 4). Bel B plants showed no treatment effect, but Bel W3 plants (from day 1 to day 7) exhibited a small increase in specific leaf weight of the leaves. This effect was more

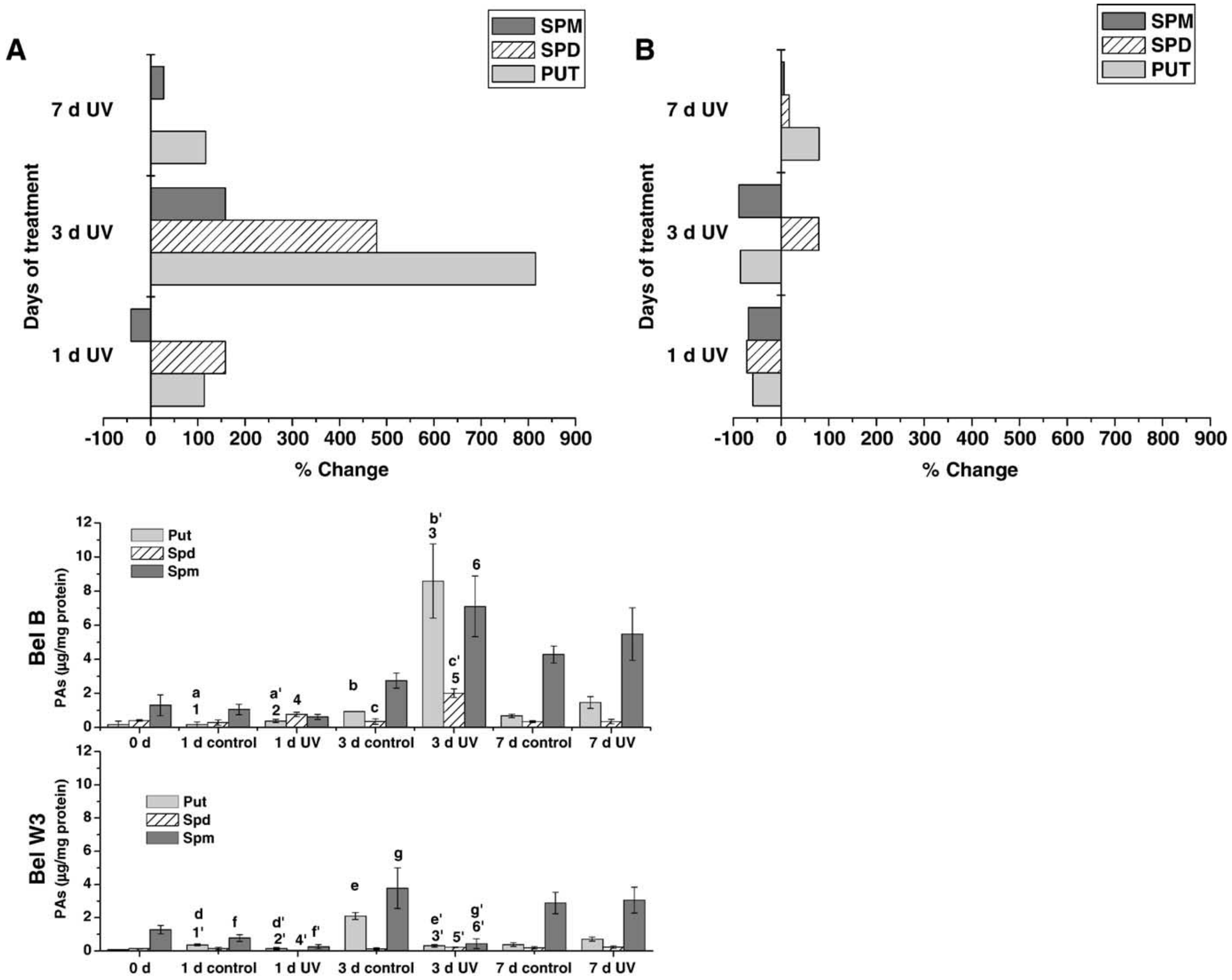

Fig. 3. Changes in polyamine levels (expressed as percent changes of corresponding control values) from isolated thylakoids of Bel B (A) and Bel W3 (B) plants, after UV-B irradiation treatment, for 1, 3 or 7 days, as well as the absolute values measured for both control and UV-B treatments in Bel B and Bel W3 thylakoids, for 0, 1, 3 and 7 days of experiment. Letters above the bars are used to designate significant differences (ANOVA statistics were performed with the log10 transformed data $P < 0.05$). Same letter (e.g., a and a′) is used for the bars which differ (comparisons always between control and treated samples of the same cultivar). Same numbers (e.g., 1 and 1′) are used to designate the statistically significant differences on the bars that represent corresponding polyamine values between the two cultivars Bel B (A) and Bel W3 (B).

obvious after the first day of the treatment, and then slowly reduced, to become almost similar to control plants after the 7th day.

The F_v/F_m ratio, as a measure for the photochemical state of photosystem II and of the photosynthetic efficiency of the cultivars, was also determined (Fig. 5). The results show that both plant cultivars exhibit a gradual decrease in the F_v/F_m values during growth under control light conditions. Although photosynthetic efficiency seems to be reduced in the high-UV treatment, the lowest value of 0.77 indicates that no cultivar shows a photodestructive effect in photosystem II.

The influence of increased UV-B impact on oxygen turnover of leaves (net photosynthesis, respiration) is shown in Fig. 6. It is obvious that Bel B did not decrease photosynthesis by UV irradiation. After 7 days of simulation, the high-UV treatment developed somewhat reduced oxygen, but not significantly. The general trend of photosynthesis reduction with time might be attributed to the high-light conditions in both light regimes. The variety Bel W3 developed no different reaction under the sunlight simulation conditions. This holds also for respiration activities in both sets of plants, which did not change over time or depending on the treatment. In general, photosynthesis measured as leaf oxygen evolution, is not affected by the treatment (as indicated by ANOVA).

The changes observed for chlorophylls and carotenoids, respectively, during treatment appear in parallel to each other (Fig. 7), which means that none of both pigment fractions is affected differently. However, some cultivar-specific differences occur: 1 day of high-UV treatment does not change pigments in Bel B; after 3 days under high-UV both pigment pools increase. This “overshooting” is down-regulated in the 7-day sample to values slightly, but not significantly (as indicated by ANOVA), higher compared to the one day-samples. Leaf pigment extracts prepared from Bel W3 show

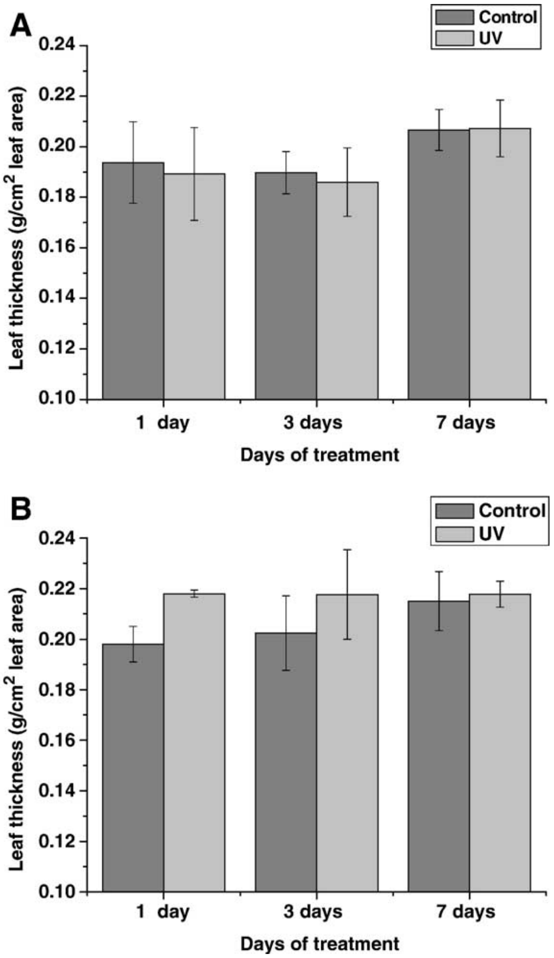

Fig. 4. Changes in specific leaf weight (g fresh weight per unit of leaf area) of Bel B (A) and Bel W3 (B) plants, after UV-B irradiation treatment, for 1, 3 or 7 days, compared to the corresponding control plants.

that already 1 day treatment with high-UV decreases the photosynthetic pigments by nearly 50%. During the following period, pigment contents increase strongly. Some increase (not significant) is also measured in the controls after 7 days. In general, the pigment levels, expressed on a FW basis, are about 20% higher in the Bel W3 variety.

4. Discussion

The aim of the present study was to follow the response of the two plant cultivars (Bel B and Bel W3) in their ability to change polyamine contents upon enhanced UV-B radiation over a treatment time of up to 7 days. The experiments should show whether polyamines might be induced as additional protective compounds.

A first indication that the two plant cultivars respond differently to enhanced UV-B irradiation came from their leaf polyamine pattern. On a whole leaf basis, the Bel B cultivar, which is known as tolerant to ozone, did not change polyamine contents during UV treatment. At the same time, Bel W3 was unable to retain its polyamine amounts close to control values after 1 day of the UV treatment. It needed 3 days of UV-B treatment, until polyamines were finally upregulated to avoid leaf damages. These results support what has formerly been reported about polyamines being elevated in Bel B plants during ozone stress to provide efficient protection of the plant, while being unchanged or even reduced in the sensitive Bel W3 variety [18]. However, in case of applied ozone stress, it could be shown that the thylakoid and/or plastid polyamines play the major role in protecting photosynthesis.

The pattern of polyamine changes in isolated intact chloroplasts and in thylakoids, proved that UV-B treatment resulted in different alterations in the two plant cultivars. Similarly to the above discussion about whole-leaf polyamine alterations, Bel W3 plants delayed to increase their polyamine amounts in the chloroplasts, especially for Put. However, polyamines, measured in whole leaf preparations, as well as in isolated chloroplast preparations, include total polyamines, i.e., soluble, conjugated and bound forms. Instead, polyamine amounts measured in isolated thylakoid membranes represent bound (and to a lesser extend also conjugated) forms. According to the results of Bors et al. [37], it is the conjugated and bound forms of polyamines that are mostly involved in protective mechanisms like scavenging of active oxygen species.

Indeed, in our study, the pattern of thylakoid bound polyamine changes under UV-B treatment in the two plant

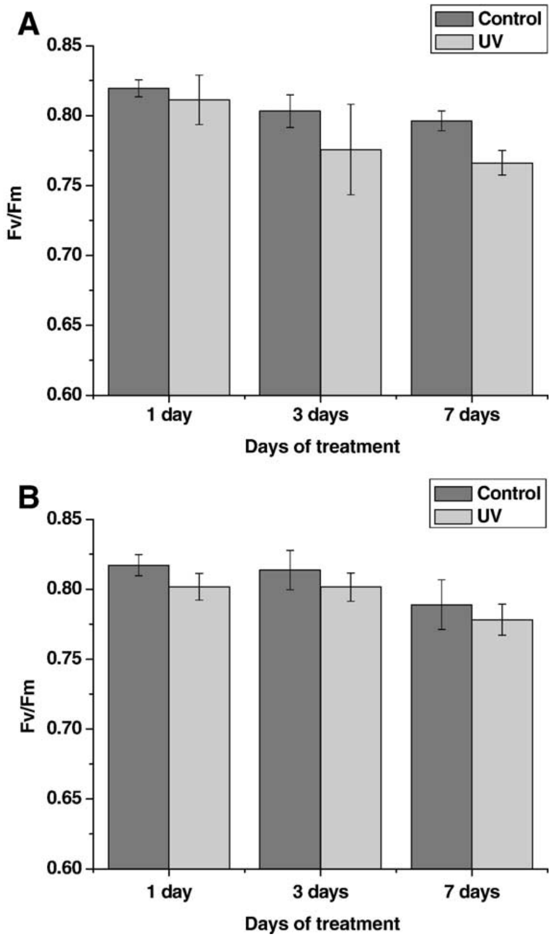

Fig. 5. Photosynthetic efficiency (F_v/F_m ratio) of Bel B (A) and Bel W3 (B) plants, after UV-B irradiation treatment, for 1, 3 or 7 days, compared to the corresponding control plants.

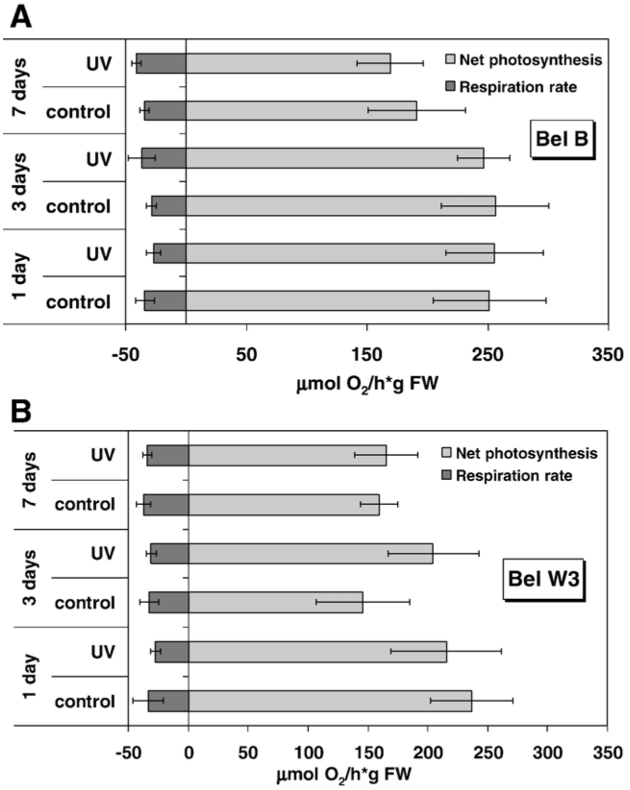

Fig. 6. Net photosynthesis and respiration rate of Bel B (A) and Bel W3 (B) plants, non-treated (controls) and UV-B-treated, for 1, 3 or 7 days.

cultivars responded differently to the stress condition. Even from the onset of the UV-B treatment, increased rapidly the thylakoid-associated polyamines in Bel B plants to stabilize the photosynthetic membranes, but in the case of Bel W3 plants, the onset of this response was greatly delayed. This reaction is most prominent when comparing leaves versus plastids and plastids versus thylakoids.

In the case of the resistant Bel B plants, they (and especially Put) are being upregulated primarily in thylakoids from the first day of UV-B treatment, whereas later they are also being upregulated in chloroplasts (third day) and finally on a whole cell basis (seventh day). Contrary, in the sensitive Bel W3 plants polyamine levels (and especially Put) fail to increase in thylakoids throughout the treatment period (although on the seventh day they seem more or less stabilized), and also in chloroplasts, they are being induced not earlier than the seventh day, whereas on a whole cell basis they are upregulated quite early, ever since the third day of UV-B treatment. Thus, it is concluded that an increase in polyamines bound to thylakoid membranes confers some level of resistance against UV-B. After some time, when endogenous plant balances have been restored due to secondary responses, (e.g. biosynthesis of carotenoids, in the case of UV-B treatment) and the plant is adapting to the altered environmental conditions, then polyamine level is reduced.

Similar results have also been reported by Kramer et al. [16,38]. These authors compared the effects of UV-B treatment between cultivars, a sensitive one and a tolerant cucumber or soybean species, respectively. They concluded that intracellular polyamines, especially Put and Spd, increased during this stress treatment. The upregulation of these polyamines was more pronounced in the tolerant cultivar than in the sensitive. But these authors discussed that low polyamine levels are not primarily responsible for UV-B sensitivity. Our results seem to disagree to their conclusion: they used a prolonged UV-B treatment over 2 weeks, which may have led to an adaptation of plants, while we followed the kinetics of UV-B effects from the first until the 7th day of treatment. In addition, the polyamine analysis of the present work was performed not only on a cellular level (as was done in the case of polyamine analysis of the mentioned study), but also on isolated intact chloroplasts and thylakoid membranes.

The similarity between the effects of ozone [18] and UV-B on polyamine changes in thylakoid membranes shows that ozone and UV-B, by forming free radicals, evoke oxidative stress in plants, which concomitantly form polyamines as radical scavengers. Thus, ozone and UV-B induce the same response mechanisms in plants. This is also supported by the results of Besford et al. [24], who identified D1, D2, Cyt *f* and the large subunit of Rubisco as proteins which can be stabilized by the addition of exogenous polyamines. Moreover, Kotzabasis et al. [22] reported that the putrescine, spermidine and spermine are associated with the Light Harvesting Complex (LHC) and the photosystem II (PS II).

Another evidence comes from a previous study [39] where it was shown that light intensity is a factor affecting the range of UV-B effects to the plants. Plants grown under high light conditions were most resistant to UV-B radiation (similar to our study), whereas low light conditions enhanced inhibitory responses of UV-B radiation. Strid et al. [40] also reported that supplementary UV-B light under high PAR-irradiation show deleterious effects with field and glasshouse plants being less sensitive to enhanced UV-B radiation, when compared to plants raised under lower irradiance. For this reason, the visible-light conditions is of major importance for the study of UV-B effects on the plants. The use of sun light simulator chambers in GSF/Munich, where fluctuations of the simulated solar irradiation followed diurnal fluxes referring to field records (South Germany) was the best solution of this problem.

In addition to these previously reported results, Sfichi et al. [21] have well established that under UV-B conditions, the exogenously added Put induces an increase of Put concentration which is bound to thylakoid membranes as well and this subsequently causes a decrease in the oligomeric forms of LHCII, similar to that induced by high light conditions. Thus, resistance is conferred to the photosynthetic apparatus against UV-B. This is consistent with the data of the present work. Since high light confers some kind of resistance against UV-B treatment, and we could describe that resistance of Bel B cultivar versus Bel W3 cultivar is exerted by PUT upregulation in thylakoid membranes, it is concluded that LHC II antenna size regulation is one of the primary plant responses against UV-B. The antenna size could be directly regulated by PUT being bound to or loosened from thylakoids [21], thus also directly regulating the physiological state of the photosynthetic

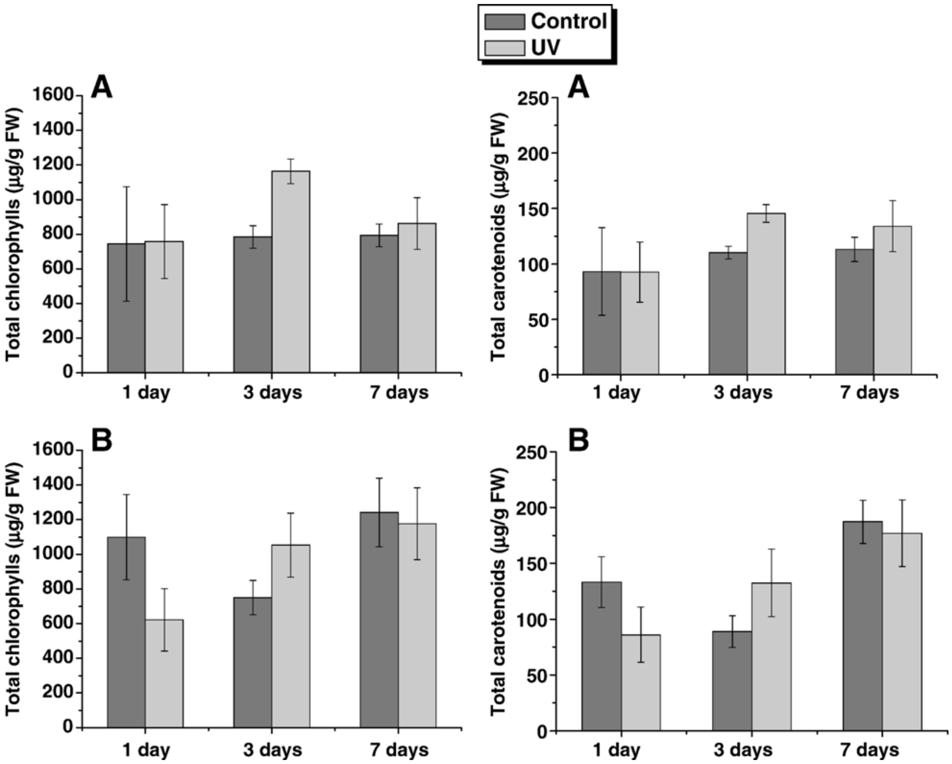

Fig. 7. Total chlorophyll and carotenoid contents in the leaves of Bel B (A) and Bel W3 (B) plants, after UV-B irradiation treatment, for 1, 3 or 7 days, compared to the corresponding control plants.

apparatus itself and consequently the mechanism of photosynthesis. This conclusion is supported by other works [41–43] implying that one of the primary effects of UV-B in the photosynthetic apparatus lies between photosystem II (PS II) subcomplexes.

The changes in the most sensitive part of photosynthesis, photosystem II, were followed via chlorophyll fluorescence measurements. The data clearly show that there is no treatment influence in both cultivars; only a slight reduction in activity as an adaptation to the high PAR light regime with time was found. The literature describes varying results with regard to sensitivity of photosystem II and several different target sites have been proposed [44–47]. All those different results can be explained by different UV-B irradiation regimes, which were sometimes unnatural.

Whole-chain-photosynthesis of both plant cultivars supports the fluorescence studies. The general oxygen development seems to be slightly lower in Bel W3 compared to Bel B, but again both plant varieties appear stable in all exposures. The reduction of oxygen development after 7 days of incubation is similar to the F_v/F_m readings and supports these results. Similarly, Shi et al. [48] found that in acclimated plants high UV-B radiation did not reduce photosynthesis, and Johnson and Day [10] described a small increase in photosynthesis of *Sorghum* plants by UV radiation.

Our findings are in contrast to studies reporting that photosynthetic rates are reduced upon UV-B treatment [41,49]. Again, the experimental setup determines whether a true UV effect will be measured, as is outlined by Fiscus and Booker [8].

Kirchgessner et al. [50] studied the effects of light, temperature and UV radiation on photosynthetic pigments in spruce needles. UV-B radiation alone did not influence the pigments. A similar stability was seen in our experiments with Bel B pigment pools.

The phenotype of the treated plants was unaffected, at least to the extent that no visible lesions appeared. There was only a small, not significant increase of the specific leaf weight in the leaves of Bel W3, under enhanced UV radiation, because leaf development was nearly finished at the onset of the experiment. If plants grow up under high UV, inhibition of internode elongation and leaf thickening can be observed, and often reduced leaf area [51,52]. Bornman and Vogelmann [46], could also show that sensitivity to UV-B is displayed by responses as the leaf thickening. The UV-B induced thickening of the Bel W3 leaves compared to the Bel B leaves, could be an alternative protection (or adaptation) mechanism against enhanced UV-B radiation, because of the absence of polyamine protecting mechanism.

Conclusively, polyamines and especially the thylakoid-associated polyamines play a decisive role in protecting the photosynthetic apparatus, and consequently the plant organism, against various anthropogenic impacts (ozone, UV-B). After some time, when endogenous plant balances have been restored, due to secondary responses, (e.g. biosynthesis of carotenoids, in the case of UV-B treatment) and the plant is adapting to the altered environmental conditions, then the thylakoid associated polyamine level is reduced. Thus, it was possible to discriminate the early stress period from a later adaptation period.

Acknowledgements

The authors would like to thank Dr. Christian Langebartels (GSF) for providing the tobacco plants cultivars Bel B and Bel W3. We thank I. Darchinger, R. Kuhnke-Thoss, and S. Thiel for expert technical assistance. This study was supported by a binational grant to K. Kotzabasis and to C. Lütz (No. 6.KOA.1B).

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