Plant Physiology and Biochemistry 134 (2019) 73–80

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Acclimation of *Arabidopsis thaliana* to low temperature protects against damage of photosystem II caused by exposure to UV-B radiation at 9 °C☆

Matthias Schultze[a,b], Wolfgang Bilger[a,*]

[a] Botanical Institute, Christian-Albrechts Universität zu Kiel, Am Botanischen Garten 1-9, D-24118, Kiel, Germany
[b] BioConsult SH GmbH & Co KG, Schobüller Straße 36, D-25813, Husum, Germany

ARTICLE INFO

Additional Keywords:
Photoinhibition
Recovery
Chlorophyll fluorescence
Epidermal transmittance
Low temperature acclimation

ABSTRACT

Various environmental variables interact with UV-B radiation (280–315 nm), among them temperature. In many plants epidermal UV screening is induced by low temperature even in the absence of UV irradiation. On the other hand, low temperature can aggravate damage caused by UV-B radiation. We investigated the interaction of UV-B radiation and low temperature in *Arabidopsis thaliana* (L.) Heynh. Exposure of plants grown at moderate temperature (21 °C) to UV-B radiation at 9 °C resulted in significantly higher damage of photosystem II (PS II) as compared to exposure at 21 °C. The higher damage at low temperature was related to slower recovery of maximal PS II quantum efficiency at this temperature. Epidermal UV-B transmittance was measured using a method based on chlorophyll fluorescence measurements. Acclimation to low temperature enhanced epidermal UV-B screening and improved the UV-B resistance considerably. Differences in the apparent UV-B sensitivity of PS II between plants grown in moderate or acclimated to cool temperatures were strongly diminished when damage was related to the UV-B radiation reaching the mesophyll ($UV\text{-}B_{int}$) as calculated from incident UV-B irradiance and epidermal UV-B transmittance. Evidence is presented that the remaining differences in sensitivity are caused by an increased rate of repair in plants acclimated to 9 °C. The data suggest that enhanced epidermal UV-B screening at low temperature functions to compensate for slower repair of UV-B damage at these temperatures. It is proposed that the UV-B irradiance reaching the mesophyll should be considered as an important parameter in experiments on UV-B resistance of plants.

1. Introduction

UV-B radiation (280–315 nm) is one of the many environmental variables plants are confronted with. As UV-B radiation is an integral part of sunlight, photosynthesizing plants cannot get around being exposed to this potentially damaging radiation.

Resistance to UV-B radiation depends on the formation of epidermal screening pigments and repair processes such as the removal of cyclobutane pyrimidine dimers in the DNA by photolyase or the repair of photosystem II (PS II) (Britt, 2004; Barnes et al., 2015; Järvi et al., 2015).

Under natural conditions, ever-present environmental variables will not only affect plant performance in general, but also the action of the mechanisms of damage and resistance against UV-B radiation. Synergistic processes may aggravate or mitigate damage, or acclimatory processes may cause cross-resistance (Jansen et al., 2018). For example, low temperature has been reported to enhance the UV-B-induced damage of PS II (Strid et al., 1996). Especially in springtime, when many plants have their main growth phase, they can be exposed to a combination of low temperature and high sunlight (Poorter et al., 2016).

UV-B-induced damage to PS II is being assumed to be caused by the absorption of photons by the manganese cluster in the oxygen evolving complex (Hakala et al., 2005). Loss of electron donation to the reaction centre causes an extended lifetime of $P_{680}{}^{+}$, the central chlorophyll in the reaction centre of PS II, which is formed upon absorption of a photon (Ohnishi et al., 2005). $P_{680}{}^{+}$ is one of the strongest oxidants possible and will accordingly oxidize the D1 protein, leading to destruction of PS II (Hakala et al., 2005; Ohnishi et al., 2005). Such photophysical processes are temperature independent, i.e., they will proceed at low temperature with the same rate as at high temperature. For the UV-B-induced formation of cyclobutyl pyrimidine dimers (CPDs) at the DNA, the same reasoning applies. However, repair reactions, such as the de novo biosynthesis of the D1 protein or the action of the photolyase, are provided by enzymatic reactions which are strongly temperature dependent (Britt, 2004; Järvi et al., 2015). This leads to a

☆ This article is part of the Special Issue "Interactive effects of UV-B radiation in a complex environment" published at the journal Plant Physiology and Biochemistry 134, 2019.

* Corresponding author.
E-mail address: wbilger@bot.uni-kiel.de (W. Bilger).

https://doi.org/10.1016/j.plaphy.2018.10.017
Received 13 April 2018; Received in revised form 12 October 2018; Accepted 12 October 2018
Available online 13 October 2018
0981-9428/

shift in the balance between damage induction and its repair in favour of the damaging processes when the temperature decreases (Takeuchi et al., 1996; Nishiyama and Murata, 2014).

Higher plants protect themselves against UV-induced damage by the formation of epidermally located phenolic compounds, as, e.g., flavonoids, which are screening UV radiation (Emiliani et al., 2013). Flavonoid formation is not only induced by UV-B radiation, but also affected by many other environmental variables such as nutrient supply, visible irradiance and temperature (Agati et al., 2009; Albert et al., 2009; Olsen et al., 2009). Among the underlying signal transduction chains cross-talk presumably takes place, leading to cross-resistance (Jansen et al., 2018). Considering the increased risk of accumulation of damage at low temperature it seems beneficial to the plant if epidermal screening is enhanced at low temperature, even before the plant is exposed to UV-B radiation (Bilger et al., 2007). However, the functional significance of flavonoids at low temperature has so far not been interpreted in this context.

Because of the observation of a good correlation between freezing resistance and flavonoid contents among various flavonoid deficient mutants and the respective wild types, Schulz et al. (2016) proposed that flavonoids may enhance freezing resistance. However, to induce freezing resistance in plants a temperature of 4 °C is normally used (see e.g., Schulz et al., 2016), whereas temperatures already a few degree C below the optimum for growth result in enhanced flavonoid accumulation and epidermal screening (Bilger et al., 2007; Olsen et al., 2009; Petridis et al., 2016).

On the other hand, similarly to the induction of flavonoid accumulation, already mildly reduced temperature will slow down repair reactions. Therefore, we hypothesize that low temperature-induced formation of epidermal screening may serve to keep a stable balance between damage and repair. Following this hypothesis low temperature enhanced UV-B screening should be sufficient to compensate for the reduced repair activity caused by the same low temperature. Accordingly, one would expect: 1) plants not acclimated to low temperature would suffer more from short term treatment with UV-B radiation at low temperature than at their growth temperature; 2) acclimation to low temperature by extended growth at low temperature would protect against the enhancement of UV-B damage at low temperature; 3) low temperature-induced formation of epidermal screening should be sufficient to explain the difference in damage between acclimated and non-acclimated plants. While hypotheses 1 and 2 are relatively easy to test by growing plants at different temperatures and exposing them afterwards to short term treatments at different temperatures, expectation 3 needs a more involved procedure. Increased epidermal screening should cause a proportional decrease in the exposure of susceptible targets of UV-B radiation inside leaves. Accounting for epidermal screening in the UV-B dose dependency of the damage should then remove the difference between acclimated and non-acclimated plants.

We selected *Arabidopsis thaliana* as an experimental plant for practical reasons and since there exists already much experience with this species on the effects of UV-B radiation (e.g., Britt and Fiscus, 2003; Olsen et al., 2009). Recently, studies were published investigating the influence of natural environmental conditions on UV-B protection in this species (Coffey et al., 2017, Coffey and Jansen, 2018; Pescheck and Bilger, 2018). We first investigated the effect of low temperature on the damage to PS II caused by UV-B radiation in order to prove the interaction of temperature and UV-B radiation and to quantify the extent of additional damage caused by the applied low temperature. As low temperature, 9 °C was chosen as this temperature is clearly lower than the usual growth temperature of 21 °C but still allows appreciable plant growth. Second, we tested in a 2-factorial design with growth temperature and treatment temperature as the two factors, if acclimation of plants to this low temperature would result in cross-resistance and if this was sufficiently large to avoid the additional damage caused by exposure at the low temperature. In order to assess if potential cross-resistance was caused by enhanced epidermal screening, we used a chlorophyll fluorescence approach to determine epidermal UV-B transmittance (Bilger et al., 1997b) and developed a procedure to correct the UV-B exposure for the extent of epidermal screening. Finally, we explored if a reduced rate of repair contributed mechanistically to the enhanced damage at low temperature.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh. (ecotype Columbia) seeds were sown in pots of a diameter of 7 cm on TKS® 2 soil (Floragard, Oldenburg, Germany) and grown in a growth cabinet (Grobank BB-XL3 HID, CLF Plant Climatics, Wertingen, Germany) located in a climate chamber (BBC York, Mannheim, Germany) set at 21 °C air temperature day and night. A photosynthetic photon flux density (PPFD) of 50 μmol m^{-2} s^{-1} was supplied for 16 h a day by white fluorescent tubes (Philips TL741 Universal HiVision, CLF Plant Climatics) installed in the growth cabinet above the plants. Due to ventilation of the growth cabinets, environmental conditions in the cabinets were very close to those in the climate chambers, except the irradiance.

After 4 weeks, when the fourth leaf had appeared, the plants were subdivided in two groups. One group was further grown at the same conditions. The other one was moved to a growth cabinet of the same type located in a second climate chamber set at 9 °C air temperature day and night with otherwise identical conditions. These plants will henceforth be called “acclimated to 9 °C” although the 3 youngest but fully mature leaves had developed at 9 °C. Plants of an age of 6–7 weeks with 6–7 mature leaves without any visual symptoms of flowering were used for the experiments. During cultivation the plants were not exposed to UV-B radiation.

2.2. Chlorophyll fluorescence measurements

For determining the damage to PS II induced by UV-B radiation, the maximal quantum yield of PS II (F_V/F_M) was measured by an Imaging PAM fluorometer (M-Series Maxi Version, Walz GmbH, Effeltrich, Germany) after the UV-B treatments. For the measurements the duration of the saturating light pulse was set to 0.8 s, its intensity was adjusted to the maximal setting 10, corresponding to a PPFD of 2430 μmol m^{-2} s^{-1}. 30 min before the measurements, plants were transferred to darkness. For each plant, values of 3 mature leaves were averaged using a circular area of interest adjusted to the diameter of the leaf.

2.3. Determination of UV radiation

The spectrum of the applied UV-B lamp was measured by a DM-150 Bentham spectroradiometer (Bentham Instruments Ltd., Reading, UK). UV-B irradiance was routinely determined by a custom made broadband UV-B-sensor (Veit et al., 1996) calibrated against the spectroradiometer using the same lamps. Spectral UV irradiance was weighted according to the general plant damage action spectrum (Caldwell, 1971) using the equation published by Thimijan et al. (1978). This equation was preferred over the equation published by Green et al. (1974), because it includes also short-wave UV-A radiation, which was present in the lamp spectrum (see Fig. S1) and which is known to have a damaging effect on PS II.

2.4. Determination of epidermal transmittance and calculation of internal UV-B irradiance

A Xe-PAM fluorometer (Walz) with a UV-B excitation beam (λ_{max}, 314 nm, see Fig. S1) and a blue-green reference beam (λ_{max}, 475 nm, half band width 410–560 nm) was applied to quantify epidermal UV-B

transmittance, T(UV-B), (Bilger et al., 1997b). Fluorescence yields were normalized using the fluorescence yield of a blue plastic foil (Walz). Fluorescence excitation ratios F(UV)/F(BG) were calculated, with F (UV) and F(BG), fluorescence intensity induced by the UV-B or the blue excitation beam, respectively. Of each plant, values of three mature leaves were measured and averaged. Reference values for 100% transmittance were determined with chloroplasts isolated from *A. thaliana* applied to white filter paper (No. 1, Whatman International Ltd, Maidstone, UK) with varying chlorophyll concentrations (Bilger et al., 1997b). At a concentration range equivalent to that found in intact leaves constant fluorescence excitation ratios F(UV)/F(BG) were observed. This excitation ratio was used as 100% transmittance reference for calculating epidermal transmittance.

It has been shown that blue light may not be a good reference wavelength for the determination of epidermal transmittance since this spectral region is absorbed by carotenoids (Nichelmann et al., 2016). High light acclimation affects carotenoid contents and reduces the fluorescence excited by a blue beam. However, in contrast to Nichelmann et al. (2016) we used here a blue-green reference beam, which is spectrally too broad to detect carotenoid contents (data not shown). Furthermore, the irradiances used to manipulate epidermal transmittance proved in other experiments to be too low to induce significant increases in carotenoid contents in *A. thaliana*. Therefore, the epidermal transmittances determined here should be reliable.

By multiplying the incident UV-B irradiance ($UV\text{-}B_{inc}$) by the epidermal UV-B transmittance, the internal UV-B irradiance ($UV\text{-}B_{int}$) was calculated, which reaches the chloroplasts in the mesophyll of the leaves:

$$UV\text{-}B_{int} = T(UV\text{-}B) \times UV\text{-}B_{inc} \quad (1)$$

2.5. UV-B treatment experiments

A general outline of the UV-B treatment experiments is shown in Fig. 1. Here, growth, treatment and recovery conditions are indicated for the two groups of plants used: those grown at 21 °C (solid lines) and those transferred after 4 weeks and acclimated for further three weeks to 9 °C (dashed lines). The single experiments described below did not always use all possible commutations. In some experiments plants were grown only at one temperature or, in another one, treated at only one temperature. This is explained in detail below.

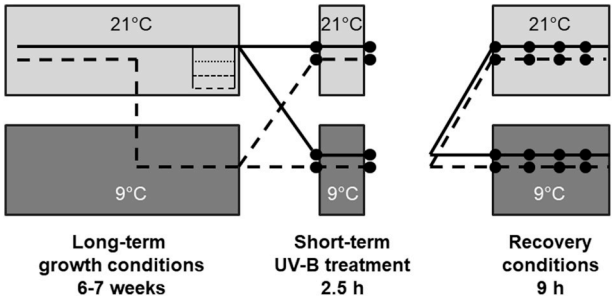

Fig. 1. Scheme of the general experimental set-up. Lines denote the history of the experimental plants. Plants were initially grown at 21 °C. After 4 weeks half of the plants were transferred to 9 °C for further 3 weeks of growth (dashed lines), while the other half remained at 21 °C (solid lines). For the experiment, in which plants with varying epidermal transmittance were treated, plants growing at 21 °C were subdivided and grown for 1 week at 4 different PPFDs. This is indicated by the three additional thin dotted and dashed lines at the right hand side of the left light grey box. When the plants had reached a stage with 6–7 leaves, they were exposed at growth temperature or at the other temperature for 2.5 h to UV-B radiation. F_V/F_M was determined after the treatment (black circles) and in one experiment also before the treatment. For the recovery experiment (right part of the scheme), plants grown at 21 °C and 9 °C were exposed at 9 °C and F_V/F_M determined directly after exposure and in 3-hourly intervals while they were placed at either 9 °C or 21 °C. Plants were exposed to UV-B radiation only during the treatment period. For further details, see section 2.

2.5.1. Experiment 1: UV-B treatment at 21 °C and at 9 °C of plants grown at 21 °C

This experiment was conducted to test the hypothesis that there is an interaction between treatment temperature and UV-B irradiance. In the middle of the 16 h light period, UV-B irradiation in a range from 0 to 0.45 W m^{-2} (all UV-B irradiances refer to biologically effective UV-B weighted according to Thimijan et al. (1978)) was given for 2.5 h together with a PPFD of 150 μmol m^{-2} s^{-1} of white light. The increase in PPFD should at least partially improve the unnatural relation between UV-B irradiance and PPFD. The treatments took place in the same growth cabinets used for growth. For UV-B irradiation UV-B fluorescent tubes (Philips TL12, Eindhoven, The Netherlands) covered with cellulose acetate filters (0.1 mm, Rachow GmbH, Hamburg, Germany) were used. White and UV-B lamps were located side by side in the ceiling of the growth cabinets and were connected to two different electric circuits, which could be regulated independently to achieve different irradiances of white light and UV-B radiation. After the UV-B treatment plants were predarkened for 30 min prior to F_V/F_M determination. This experiment was conducted four times, each with independent batches of plants.

2.5.2. Experiment 2: UV-B treatment of plants grown at 21 °C and of plants acclimated to 9 °C

In a two factorial design plants were either grown at 21 °C or acclimated to 9 °C and both groups were treated at both temperatures. This should test the hypothesis that acclimation to low temperature would compensate for the increased damage occurring at low temperature. Plants from both temperatures were treated in groups of 6 plants at different UV-B irradiances in the growth cabinets as described above (2.5.1). After treatment F_V/F_M was determined using the Imaging-PAM fluorometer as described in 2.2. For calculating the decrease of F_V/F_M all values were compared with the mean of 6 control plants, of which 3 mature leaves per plant were averaged. The controls were plants that had been exposed to the same temperature and PPFD during the 2.5 h of treatment but without irradiating them with UV-B. The F_V/F_M values of the controls are shown in the legend of Fig. 3. This experiment was repeated once, albeit without treating plants acclimated to 9 °C at 21 °C.

2.5.3. Experiment 3: UV-B treatment at 21 °C and at 9 °C of plants with varying epidermal UV-B transmittance

While in exp. 1 plants were treated at varying UV-B irradiances, in this experiment the same UV-B irradiance was chosen, but plants used which had varying epidermal UV-B transmittances. This was done to prove the hypothesis that epidermal transmittance has a direct UV-B protective effect. In order to achieve the variation in epidermal transmittance, plants grown at 21 °C were 7 days before the experimental treatment subdivided in 4 groups with 10 plants each and positioned in growth cabinets such that they were exposed to different irradiances. This was achieved by placing the plants in two different tiers in the same cabinet set at different PPFD. To further vary irradiance the distance to the lamps was adjusted. One group stayed at a PPFD of 50 μmol m^{-2} s^{-1}, while the other groups were transferred to 80, 180 and 220 μmol m^{-2} s^{-1}, respectively. The different light conditions had only slight effects on the leaf temperatures (less than 1 °C increase). During the UV-B treatment, all plants were exposed to one single UV-B irradiance of 0.52 W m^{-2} UV-B (weighted according to Thimijan et al., 1978) in otherwise the same way as for experiment 1. UV-B treatment was given in the middle of the light period in growth cabinets of the same type, located in climate chambers set at 9 and 21 °C. Since exp. 1 had shown that plants exposed to no UV-B had no reduction of F_V/F_M, in this experiment controls without UV-B exposure were renounced. $F_V/$

F_M was determined as described in 2.2.

2.5.4. Experiment 4: inhibition of repair of PS II by lincomycin

It was hypothesized that the differences between the damage occurring during treatments at low or moderate temperature were due to the temperature dependence of repair processes. Therefore, repair by de novo synthesis of the D1 protein of PS II was inhibited by application of lincomycin, an inhibitor of protein synthesis in chloroplasts. Leaf discs (0.62 cm^{-2}) from plants grown at 21 °C were floated for 12 h on a 6 mM lincomycin (AppliChem, Darmstadt, Germany) solution in darkness. Lincomycin treatment had no effect on F_V/F_M as compared to control leaf discs floating on distilled water. After the preincubation, leaf discs were irradiated with 0.38 W m^{-2} UV-B and 150 µmol m^{-2} s^{-1} PPFD in the same growth cabinets as used before at 9 or 21 °C for 2.5 h while lying on wet filter paper. F_V/F_M was determined as in 2.2.

2.5.5. Experiment 5: recovery kinetics at 9 and 21 °C after UV-B treatment

The influence of temperature on the recovery kinetics was tested again using plants inhibited to an extent as similar as possible. 8 plants grown at 21 °C or acclimated to 9 °C, respectively, were treated at 9 °C with UV-B irradiances of 1.13 and 0.24 W m^{-2}, respectively, leading to an equal internal UV-B irradiance of 0.21 W m^{-2}. The treatment lasted 3 h, with a background PPFD of 150 µmol m^{-2} s^{-1} as described in 2.5.1. To quantify the temperature dependence of recovery of PS II after the treatment, 4 plants of each group were placed in the climate chambers at temperatures of 9 °C or 21 °C in the absence of UV-B radiation and with 50 µmol m^{-2} s^{-1} PPFD as background irradiance. F_V/F_M was measured on 3 leaves per plant every 3 h after 30 min predarkening. This was experiment was conducted three times.

2.5.6. Statistical analysis

Standard methods of statistical analysis such as two way ANOVA were performed with the software GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

3. Results

As a first step, the effect of decreased temperature on UV-B-induced damage in *A. thaliana* was investigated by treating plants grown at 21 °C at different UV-B irradiances at their growth temperature and at 9 °C. Whereas after treatment of the plants in the absence of UV-B F_V/F_M remained at normal values of around 0.8, maximal PS II quantum yield decreased with increasing UV-B irradiance in a nonlinear fashion (Fig. 2). Treatment at low temperature doubled the decrease of F_V/F_M.

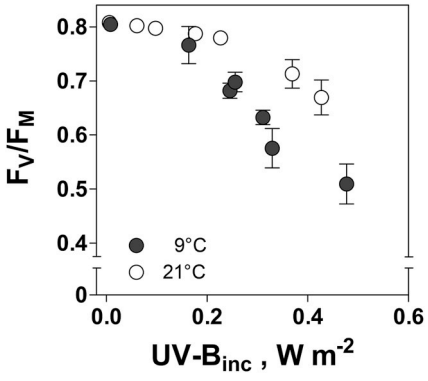

Fig. 2. Maximal quantum yield of PS II (F_V/F_M) after treatment at 9 °C (filled circles) and 21 °C (open circles) as a function of the incident UV-B irradiance. All plants were grown at 21 °C. Plants with equal values for epidermal UV-B transmittance (T(UV-B) = 0.470, SD = 0.047) were treated with different UV-B irradiances for 2.5 h. UV-B irradiance was biologically weighted according to Caldwell (1971) as parameterized by Thimijan et al. (1978). n = 6 plants. Error bars denote 95% confidence interval.

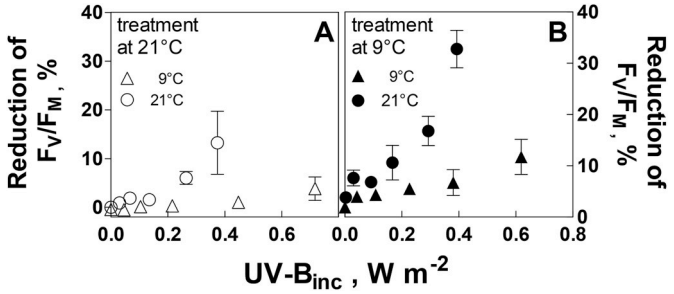

Fig. 3. UV-B induced decrease of F_V/F_M in plants acclimated to 9 °C (triangles) or grown at 21 °C (circles) as a function of incident UV-B irradiance. Plants were treated for 2.5 h with a background PPFD of 150 µmol m^{-2} s^{-1} at either 21 °C (A) or 9 °C (B). Decrease of F_V/F_M after treatment was calculated as difference to the mean of 6 control plants treated in the absence of UV-B. Control values of F_V/F_M were: A: 0.799 (21 °C grown), 0.779 (9 °C acclimated); B: 0.783 (21 °C grown), 0.772 (9 °C acclimated). n = 6. Error bars denote 95% confidence interval.

As a next step we investigated if acclimation to low temperature would lead to cross-resistance against UV-B radiation. When plants grown at 21 °C or acclimated to 9 °C were treated with UV-B at 21 °C, the plants acclimated to 9 °C showed only at the highest UV-B irradiances a reduction of maximal PS II quantum yield by 3%, whereas it was similarly reduced as observed before in plants grown at 21 °C (Fig. 3A). When treated at 9 °C, the differences in F_V/F_M between both growth conditions remained, but the UV-B-induced decrease of F_V/F_M was much stronger, reaching up to 10% and 33% in plants grown at 9 °C and 21 °C, respectively (Fig. 3B). It is instructive to note that the reduction of F_V/F_M experienced during treatment at the respective growth temperature was in the same order of magnitude in both groups of plants.

Since it is well known that growth at low temperature induces flavonoid biosynthesis and accordingly epidermal screening of UV-B radiation (Leyva et al., 1995; Bilger et al., 2007; Olsen et al., 2009), we tested if cultivation at low temperature also here increased epidermal screening. Plants acclimated to 9 °C displayed significantly lower epidermal transmittance as compared to those grown at 21 °C (Fig. 4). Therefore, the question arises if the lower UV-B-induced reduction of F_V/F_M experienced by plants acclimated to 9 °C was caused by the reduced epidermal transmittance and if this was the only cause.

In order to evaluate this question it was helpful to plot the reduction of F_V/F_M as a function of the UV-B irradiance reaching the chloroplasts. To validate the calculation of this internal UV-B irradiance (UV-B_{int}) an experiment was conducted (exp. 3), in which exposure of the

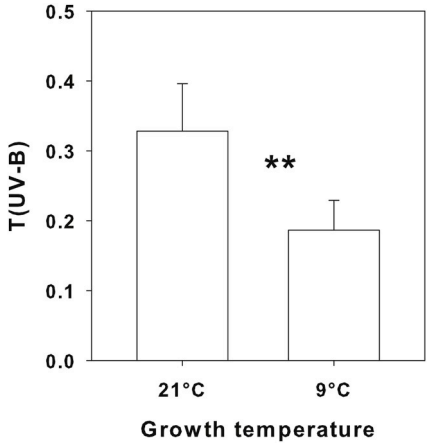

Fig. 4. Transmittance of *A. thaliana* leaves after growth at 21 °C (white bar) and at 9 °C (black bar). The error bars refer to the SEM, n = 6. A Mann-Whitney *U* test showed a significant difference between the two columns (p = 0.002).

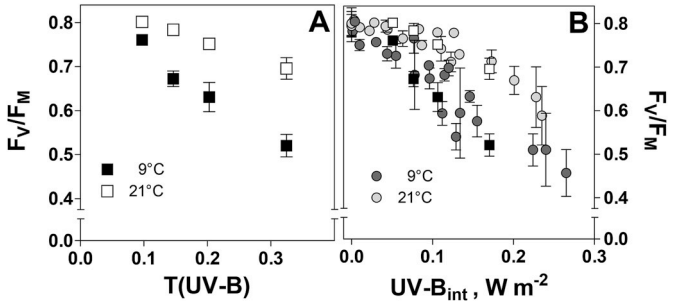

Fig. 5. A: F_V/F_M after UV-B treatment at 9 °C (filled squares) and 21 °C (open squares) as a function of epidermal UV-B transmittance of the leaves. All plants were grown at 21 °C at four different irradiances of photosynthetically active radiation. Plants were treated with 0.52 W m^{-2} UV-B (biologically weighted according to Caldwell (1971) as parameterized by Thimijan et al. (1978)) for 2.5 h. n = 15 leaves per exposure. Error bars denote 95% confidence intervals. **B:** F_V/F_M after UV-B treatment at 9 °C (black or dark grey symbols) and at 21 °C (open or light grey symbols) as a function of the calculated internal UV-B irradiance ($UV\text{-}B_{int}$). $UV\text{-}B_{int}$ was calculated by multiplying T(UV-B) and $UV\text{-}B_{inc}$ (see 2.4). Data points are taken from 4 different experiments in which either epidermal transmittance (squares; same experiment as shown in Fig. 5A; n = 15) or incident UV-B radiation (circles; 3 different independent experiments with n = 3 or n = 6 per exposure) were varied. All plants were grown at 21 °C. Error bars denote 95% confidence intervals. When no error bar is visible it is smaller than the symbol size.

photosynthetic apparatus in the plants was varied by treating plants with different epidermal UV-B transmittance at the identical UV-B irradiance of 0.52 W m^{-2}. The variation of epidermal transmittance was induced by growth at four different PPFD. Acclimation to high PPFD has been reported before to result in increased accumulation of screening pigments (Agati et al., 2009; Kolb et al., 2001). In our experiments, growth of *A. thaliana* at 220 µmol m^{-2} s^{-1} reduced epidermal UV-B transmittance to only 0.1 (Fig. 5A). As expected, PS II function was negatively correlated to the epidermal transmittance. Again, treatment at low temperature doubled the damage.

If the reduction of F_V/F_M by UV-B radiation is dependent on the irradiance reaching PS II, the data from Figs. 2 and 5A should fall on the same line. This is indeed shown in Fig. 5B. Here, results from three experiments in which plants grown at 21 °C were exposed to varying $UV\text{-}B_{inc}$ are drawn together with the results from the experiment shown in Fig. 5A. . The coincidence of the data from two different types of experiments indicates that the concept for the calculation of internal UV-B radiation is applicable.

When the data of Fig. 3A and B were plotted against the internal UV-B irradiance ($UV\text{-}B_{int}$) (Fig. 6A and B), the data points were much

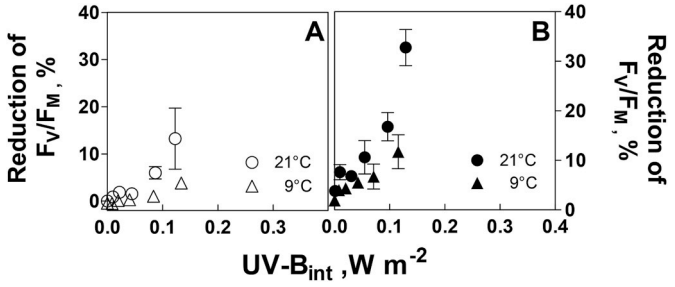

Fig. 6. UV-B induced decrease of F_V/F_M in plants acclimated to 9 °C (triangles) or grown at 21 °C (circles) as a function of internal UV-B irradiance. The F_V/F_M data are the same as in Fig. 3, but now plotted against internal UV-B irradiance instead of incident UV-B irradiance. Plants were treated for 2.5 h with a background PPFD of 150 µmol m^{-2} s^{-1} at either 21 °C (A) or 9 °C (B). Decrease of F_V/F_M after treatment was calculated as difference to the mean of 6 control plants treated in the absence of UV-B. For control values of F_V/F_M, see Fig. 3 n = 6. Error bars denote 95% confidence interval.

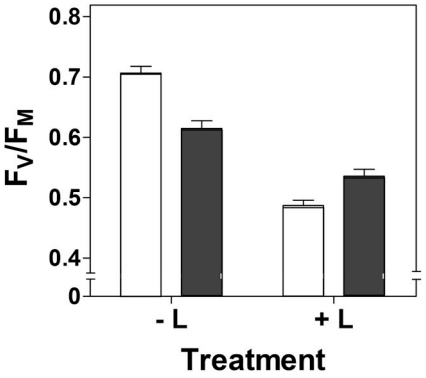

Fig. 7. F_V/F_M of leaf discs after irradiation with 0.38 W m^{-2} $UV\text{-}B_{inc}$ for 2.5 h. Discs were taken from 10 different plants grown at 21 °C. UV-B treatment was at 21 °C (white bars) and at 9 °C (black bars) in the absence (-L) and in the presence (+L) of 6 mM lincomycin. Bars represent the mean value of 10 single measurements. Error bars denote 95% confidence intervals. Two way ANOVA revealed significant differences ($p < 0.0001$) with respect to treatment temperature and lincomycin treatment and a significant interaction ($p < 0.0001$).

closer, but plants acclimated to 9 °C still showed a 50% lower damage as compared to those grown at 21 °C. Presumably, an additional acclimation reaction besides enhanced UV-B screening contributed in this experiment to the improved UV-B resistance.

Inhibition of repair is considered as a cause for enhanced photoinhibition at low temperature (Nishiyama and Murata, 2014). If this was also true during the UV-B treatments, the difference in F_V/F_M between plants exposed at low and moderate temperature should disappear in the absence of repair. Therefore, leaf discs of plants grown at 21 °C were treated with lincomycin to inhibit D1 synthesis. As expected, lincomycin treatment enhanced the decrease of F_V/F_M (Fig. 7). Also, the difference between F_V/F_M after treatment at 9 and 21 °C was not only reduced, as hypothesized, but even inverted in the presence of lincomycin. The difference between F_V/F_M in absence and presence of lincomycin, which reflects the part of repairable damage, was 3 times higher at 21 °C than at 9 °C.

To further explore the role of repair at different temperatures, plants were irradiated at low temperature to obtain a given damage and afterwards transferred for repair to low light at either 21 or 9 °C. For the treatment, $UV\text{-}B_{inc}$ was adjusted to yield about the same $UV\text{-}B_{int}$ for all plants. Two-way ANOVA for the F_V/F_M values directly after treatment with the factors growth temperature and (the following) recovery temperature showed still a significant difference between the plants with respect to growth temperature ($p = 0.0115$; comparison of circles vs. triangles in Fig. 8), indicating that the attempted equal damage was not fully reached. On the other hand, the analysis showed no difference with respect to the following recovery temperature ($p = 0.435$; comparison of filled vs. open symbols). F_V/F_M did not fully recover over 9 h (Fig. 8) at all temperatures. Recovery at 21 °C was largely independent of the growth temperature of the plants. At 9 °C, plants grown at 21 °C showed strongly retarded recovery as compared to plants kept at 21 °C, but also plants acclimated to low temperature showed incomplete recovery. After 9 h recovery there were no significant differences between the plants recovered at the same temperature, whereas comparisons at all other times were significant.

4. Discussion

4.1. At low temperature PS II is more vulnerable to UV-B-induced damage

To test for the interaction of UV-B radiation and low temperature, we have chosen the functioning of PS II as a parameter. UV-B treatment was given with a background of rather low PPFD, at which photoinhibition or induction of longer lasting non-photochemical quenching

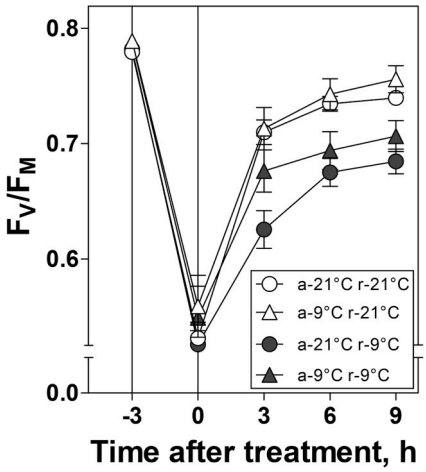

Fig. 8. Recovery of maximal PS II quantum yield at 9 °C (filled symbols) and 21 °C (open symbols) after irradiation with UV-B_{int} = 0.21 W m^{-2} at 9 °C for 3 h. UV-B irradiance was biologically weighted according to Caldwell (1971). Plants were acclimated to 9 °C (triangles) or grown at 21 °C (circles). n = 12 leaves from 4 different plants. Error bars denote 95% confidence interval. Differences between F_V/F_M of plants acclimated to 9 and 21 °C were tested using a Kruskal-Wallis test followed by a Dunn's multiple comparison test.

was improbable. This is evident from the observation of F_V/F_M values above or around 0.8 after treatment in the absence of UV-B radiation (Figs. 2 and 5). The actinic effect of UV-B itself must have been very low due to its low PPFD such that one should also not expect any additional down regulation in the UV-B treatments. Therefore, F_V/F_M was taken as an indicator of PS II function.

In our experiments, treatment at low temperature of plants grown at 21 °C showed the instantaneous influence low temperature had on PS II function (Fig. 2), whereas the treatment of plants acclimated to 9 °C at both temperatures showed the protection attained during acclimation to low temperature (Fig. 3). In all experiments, F_V/F_M was more strongly reduced by treatment at 9 °C as compared to 21 °C (Figs. 2–5). These data prove that UV-B exposure at 9 °C would predispose plants to stronger damage and confirm results of Strid et al. (1996). Although plants acclimated to 9 °C showed much less decrease of F_V/F_M, they still were more sensitive to treatment at 9 °C as compared to treatment at 21 °C.

4.2. Acclimation to low temperature mitigates UV-B-induced damage of PS II

Plants acclimated at 9 °C displayed much less PS II damage upon exposure to UV-B as compared to those grown at 21 °C (Fig. 3). Although this experiment was only independently repeated once with the same principal result, its conclusion is also supported by experiments as that used for Fig. 8, which were conducted three times independently. In these experiments, much higher UV-B doses were necessary for treating plants acclimated to 9 °C in order to reach the same degree of reduction of F_V/F_M as observed in plants grown at 21 °C. Comparison of Fig. 3B with Fig. 3A shows that plants acclimated to and treated at 9 °C have become about as resistant toward UV-B radiation as plants grown and treated at 21 °C. In other words, *A. thaliana* was able to compensate for the stress induced by low temperature under the given radiation conditions.

The presented data allow some conclusions about the mechanisms behind the increased resistance to UV-B radiation after acclimation at low temperature. A prominent mechanism was apparently the enhanced epidermal screening in low temperature acclimated leaves (Fig. 4). Relating the UV-B-induced decrease in F_V/F_M directly to internal UV-B irradiance minimized the differences between plants grown at 21 °C and those acclimated to 9 °C again. This is on one hand in line with our third hypothesis stating that low-temperature-induced formation of screening compounds was sufficient to compensate for the enhanced damage by UV-B exposure at low temperature. In a second experiment, the difference between the two types of plants was even smaller. On the other hand, the existence of such a remaining difference suggests the potential existence of one or more additional protective mechanisms induced by acclimation to 9 °C. These will be further addressed in section 4.3.

The improved UV-B screening induced at low temperature has presumably an important protective function under natural conditions. UV-B irradiance and temperature do not follow the same time course through the year. Although both peak during summer and have a minimum in winter, temperature lags always about two months behind, leading to a situation with still low temperatures and already high irradiance during spring (Poorter et al., 2016). On the other hand, in Central Europe spring is the time when *A. thaliana* flowers (Wilczek et al., 2009), making coinciding low temperature and at least moderately high irradiance to a commonly experienced event in springtime. Also with increasing altitude high irradiation and low temperatures coincide quite often. For the mountain plant *Arnica montana* low temperature has been suggested as the decisive factor for the formation of high amounts of flavonoids in its natural habitat (Albert et al., 2009).

Enhanced screening may not only reduce the disequilibrium between damage and repair of PS II at low temperature. Also the repair of another type of major UV-B-induced damage, CPD formation in the DNA, could be reduced at low temperature. It has been reported that activity of the photolyase, which removes UV-B-induced CPDs in the DNA, was strongly reduced at low temperature (Takeuchi et al., 1996; Hall et al., 2003). If enhanced screening indeed reduces CPD accumulation at low temperature remains to be shown.

When seen in the context of decreased repair rates at low temperature it makes sense that formation of UV screening compounds is induced at low temperature despite low UV-B irradiation or even in its absence. In this context the high contents of screening pigments could be explained, which were observed in *A. thaliana* or *Nostoc commune* during winter time (Bilger et al., 1997a; Coffey et al., 2017; Coffey and Jansen, 2018; Pescheck and Bilger, 2018). Low temperature-induced enhancement of epidermal screening represents an example of cross-resistance, presumably caused by signalling cross-talk (Petridis et al., 2016). It has been emphasized that UV-B induced damage is rare under natural conditions (Hideg et al., 2013). One of the reasons may be that during evolution plants not only adapted to UV-B radiation itself but also to environmental factors which are enhancing sensitivity.

4.3. Low temperature reduces repair of PS II, but acclimation to 9 °C positively affects the repair rate

The results shown in Figs. 7 and 8 suggest that the enhanced decrease of F_V/F_M when plants were treated with UV-B radiation at low temperature was caused by an insufficient repair. The experiment shown in Fig. 8 was repeated two times with the same principal result. However, the degrees of damage reached and the following final recovery varied to some extent. Therefore, before the causes of this variability are not understood, one should consider these data with some caution.

During photoinhibition the integrity of PS II is the result of the simultaneously ongoing processes of damage and repair (Nishiyama and Murata, 2014). It has been shown, that after photoinhibition low temperature inhibits the recovery of F_V/F_M in *A. thaliana* (Vijayan and Browse, 2002) and in *Synechocystis* sp. PCC6803 (Nishiyama et al., 2006; see also Gombos et al., 1994). In the latter organism low temperature inhibits the synthesis of the D1 protein (Allakhverdiev and Murata, 2004) which seems to be the cause for the slow repair. Since the basic repair mechanisms of PS II are assumed to proceed in a similar way after damage induced by high light and UV-B (Sass et al., 1997; Cheregi et al., 2007) it seems evident that low temperature also slows

the repair of PS II damage by UV-B. This interpretation is also supported by the results obtained in lincomycin treated leaf discs (Fig. 7).

In the presence of lincomycin, damage was even lower at 9 °C than at 21 °C. This may be explained by results of Aro et al. (1990), who observed that in spinach thylakoid membranes D1 degradation is strongly retarded at 7 °C. Richter et al. (1990) found the same at 0 °C in conjunction with a distinct retardation of the loss of F_V/F_M. This was explained by a reduced proteolytic activity at low temperature.

After correction for epidermal transmittance, the plants acclimated to 9 °C still had a 50% better performance than those grown at 21 °C (Fig. 6). This difference could be attributed to the higher recovery rate of these plants at low temperature (Fig. 8), which was also observed in two other independent experiments. At low temperature, repair may be limited by the fluidity of the thylakoid membrane. Damaged reaction centres must move from the grana stacks to the stromal regions which will be affected by the fluidity of the thylakoid membrane (Barbato et al., 1992; Moon et al., 1995). It is well known that plants adjust the fluidity of their membranes to the growth temperature by changing the degree of saturation of the fatty acids of the membrane lipids (Cossins, 1994; Nishida and Murata, 1996). At 21 °C membrane fluidity may not be limiting for repair processes explaining similar repair rates in plants grown at 21 °C and acclimated to 9 °C (Fig. 8).

4.4. The product of epidermal transmittance and UV-B irradiance is a useful measure for the UV-B dose

In order to evaluate the contribution of epidermal screening to UV-B protection, we developed the concept of internal UV-B irradiance as the product of incident UV-B irradiance and epidermal transmittance (equation (1)). Treatments where either incident UV-B irradiance or T(UV-B) were varied showed very similar dependence of F_V/F_M when plotted against $UV\text{-}B_{int}$ (Fig. 5B) supporting the feasibility of our calculation.

This conclusion is further underpinned by the experiment shown in Fig. 8. Here, incident UV-B irradiance was adjusted to yield the same internal UV-B irradiance for all plants. The treatment resulted in F_V/F_M values not more than 0.03 relative units apart, although strongly different UV-B irradiances were applied. The small remaining significant difference in the decrease of F_V/F_M between the groups may have been caused by a higher repair activity in the plants acclimated to 9 °C. Pescheck et al. (2014) used the same concept when comparing UV-B induced reduction of F_V/F_M in a screening and a non-screening species of green algae. Correction for screening led to identical sensitivities of PS II towards UV-B radiation. Barnes et al. (2015) used similar calculations to estimate internal UV-B radiation during diel time courses from epidermal UV-A transmittance and absorption spectra of leaf extracts.

We feel that it is important and in our eyes indispensable to use internal UV-B irradiance to quantify exposure in experiments in which plants are artificially exposed to UV-B radiation. UV-B screening is quite variable in plants and strongly depending on environmental factors (Day et al., 1992; Bilger et al., 1997b; Løvdal et al., 2010). In most cases experimental plants are grown in greenhouses or climate chambers at optimal conditions (20 °C, high nutrient supply, comparatively low irradiance, low or no UV-B irradiance; see Poorter et al., 2016). Under these conditions epidermal screening is generally low, but extremely variable causing low reproducibility of experiments. Fig. 5A demonstrates that treatment with the same incident UV-B irradiance may lead to vastly different reduction of F_V/F_M in plants grown at irradiances which reflect the range commonly used for the culture of *A. thaliana*. Accordingly, studies would gain considerably if internal UV-B radiation would be determined in the experimental plants. The proposed approach is especially suitable when PS II damage is investigated, as in this case the sensor below the screening epidermis, i.e. chlorophyll, and the target are identically located in the chloroplasts. UV-B irradiance at other sensible targets, as e.g. DNA in the nucleus, may vary to some extent from that reaching the chloroplasts.

For the use of internal UV-B irradiance it is important to determine epidermal transmittance at about the wavelength at which the plants are treated. In our experiment, the wavelength maximum of the radiation emitted by the UV-B source and that where T(UV-B) was determined, coincided and both spectra were quite narrow (Fig. S1). This was a prerequisite that indeed $UV\text{-}B_{int}$ could be calculated. If $UV\text{-}B_{int}$ needs to be determined over a broader waveband, measurements of fluorescence excitation spectra will be necessary (Cerovic et al., 2002). This would be especially suitable for the determination of action spectra for photoinhibition based on the irradiance reaching the chloroplasts. Published action spectra for photoinhibition are only based on incident irradiance (Takahashi et al., 2010).

5. Concluding remarks

In this study we show that acclimation of *A. thaliana* plants to low temperature led to cross-resistance to UV-B radiation by at least two mechanisms. The first one was enhanced epidermal screening. Correction for this factor by plotting reduction of F_V/F_M as a function of internal UV-B irradiance revealed in some experiments the presence of a further mechanism. This may constitute of a faster rate of PS II repair. We speculate that this faster repair was due to a higher fluidity of the membrane. The observed enhanced UV-B resistance after acclimation at 9 °C compensated for the enhanced damage observed in unacclimated plants when treated at 9 °C.

Acknowledgement

This work was funded by the Graduiertenkolleg 820 of the Deutsche Forschungsgemeinschaft, Germany. We also want to thank Prof. Karin Schwarz for additional funding to Matthias Schultze. Comments of three anonymous reviewers greatly improved the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.10.017.

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