Preconditioning under high PAR or exposure to UV-A radiation both allow acclimation of the photosynthetic apparatus of barley transferred to UV-B radiation

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INTRODUCTION

Due to the depletion of the stratospheric ozone layer, which selectively absorbs UV-B radiation, the influence of UV-B on plants has been widely studied. The adverse effects of UV-B radiation on the structure and function of the photosynthetic apparatus are well-known and documented (reviewed in [1]). Enhanced UV-B radiation often leads to a decrease in photosynthetic pigment content and photosynthetic rates due to the impairment of photosystem II (PSII) function and reduced Rubisco content and activity [1,2]. On the other hand, less attention has been devoted to UV-A effects, although UV-A radiation represents the major component of the UV spectral region of solar radiation incident on Earth as it passes almost unaltered through the atmosphere. The effects of UV-A radiation on the photosynthetic apparatus may be damaging [3,4] or non-damaging, even mitigating the deleterious effect of UV-B [5,6].

This presentation describes the acclimation response of the barley photosynthetic apparatus after exposure of plants grown under low and high photosynthetically active radiation (PAR) to UV-A and/or UV-B radiation. The main aims were to evaluate (1) how the level of PAR during growth of barley affects the induction of regulatory and protective mechanisms during UV exposure and (2) whether UV-A radiation prevents UV-B induced damage to the photosynthetic apparatus in plants acclimated to low PAR.

CONCLUSIONS

Our data revealed that the PAR level itself during growth was a key factor determining the degree of epidermal UV-shielding. The short-term exposure (6 days) of barley to both UV-A and UV-B radiation had only a minor effect on the extent of UV-shielding.

PSII of barley acclimated to high PAR was resistant to excessive UV-B radiation. In spite of visual damage to the leaf tissue caused by UV-B radiation, PSII function of HL-acclimated barley was not significantly disrupted, while LL-acclimated plants showed a strong PSII photoinhibition after one day of high UV-B treatment. We suppose that the main contributions to the resistance of PSII to UV-B radiation are the ability to accumulate a considerable amount of UV-screening compounds under high PAR itself and the pool of carotenoids that may fulfil an antioxidative and screening function.

Contrary to UV-B radiation, UV-A exposure of barley plants acclimated to low PAR had no negative impact on the photosynthetic apparatus function. UV-A treatment even resulted in the synthesis of Chls and Cars and twofold higher photosynthetic capacity. This implies that the long-wave UV-A spectral region (350-400 nm), used in our experiments, can compensate a low level of PAR. In addition, the presence of UV-A radiation during acclimation to a simultaneous UVA and UVB treatment not only mitigated but even completely eliminated the negative effect of UV-B radiation on the function of photosynthetic apparatus.

RESULTS AND DISCUSSION

High PAR (without UV) induced considerable accumulation of UV-screening substances in barley leaves. We found 4-6 times higher epidermal UV-shielding degree in HL-acclimated barley as compared to LL plants (**Fig. 1**). UV-A radiation had no effect on UV-shielding, whereas UVB and UVAB treatments resulted in increased UV-shielding in both LL and HL plants (up to 66%).

UV-B treatment resulted in a decrease in both total Chls and Cars (**Fig. 2**). Only under low UV-B the content of Cars remained the same. On the contrary, UV-A exposure induced synthesis of Chls and Cars in plants acclimated to low PAR. Both Chl *a+b* and Car *x+c* contents increased by 26% after 6 days under UV-A radiation. In HL plants, Chls were more sensitive than Cars to both UV-B and UV-A, as Cars decreased less. Similarly as in UV-A-treated plants, Chls and Cars contents tended to increase after exposure to combined UV-A and low UV-B radiation. Analysis of photosynthetic pigments indicated damage of core complexes of photosystems in LL-acclimated plants exposed to high UV-B radiation, as we observed a large decrease of the Chl *a/b* ratio (from 3.1 to 2.0) and β-carotene content (by 62%, per Chl *a+b*) (*not shown*).

We observed a pronounced decrease of F_V/F_M (to 0.645) in LL plants already after the 1st day of high UV-B treatment. After the 3rd day F_V/F_M was close to zero. Surprisingly, high UV-B radiation together with high PAR resulted only in a slight reduction of F_V/F_M (to 0.772) (**Fig. 3**). Similarly, as in the case of both high and low UV-B treatment, F_V/F_M was reduced in UV-A-treated HL plants. However, neither UV-A nor UVAB treatment affected F_V/F_M in LL plants. Analysis of slow fluorescence induction kinetics revealed that utilization of absorbed light energy within PSII was optimized in HL plants exposed to high UV-B radiation, as indicated by an elevated actual photochemical efficiency of PSII (*not shown*). We suppose that one of the factors contributing to efficient photoprotection of PSII in HL plants exposed to high UV-B radiation is accumulation of the fraction of xanthophylls not bound to pigment-protein complexes which might act as a filter for excitation energy. Excitation energy captured by these unbound xanthophylls is not transferred to Chls. This is supported by the excitation spectra of Chl fluorescence that indicate a pronounced decrease of efficiency of excitation energy transfer from carotenoids to emission forms of Chl *a* (**Fig. 4**).

Both high and low UV-B treatments led to a reduction in light-saturated CO_2 assimilation rate (A_{Nmax}; **Fig. 5**). In addition to increased contents of Chls and Cars, a positive effect of UV-A radiation in LL plants was also documented by a twofold higher A_{Nmax} as compared to control plants. A_{Nmax} was even elevated under UVAB exposure.

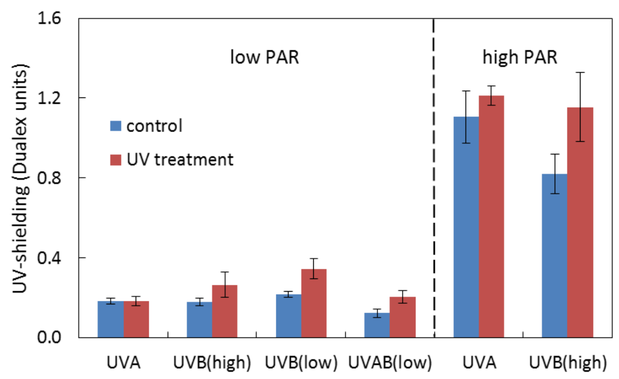

Fig. 1. Epidermal UV-shielding for barley leaves grown under low or high PAR in the absence of UV radiation after the acute UV treatments. n=4-6±SD

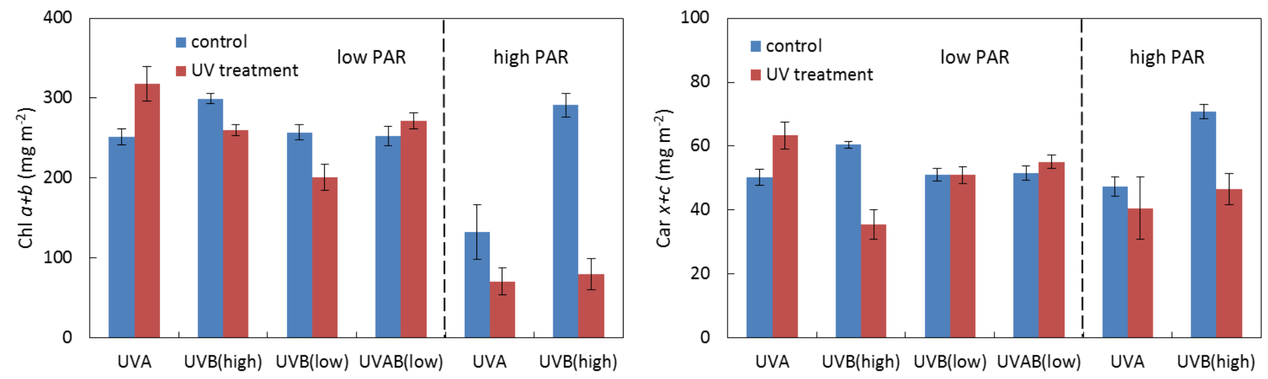

Fig. 2. The contents of total chlorophylls (Chl *a+b*) and total carotenoids (Car *x+c*) expressed per leaf area for barley leaves grown under low or high PAR in the absence of UV radiation after the acute UV treatments. n=5-6±SD

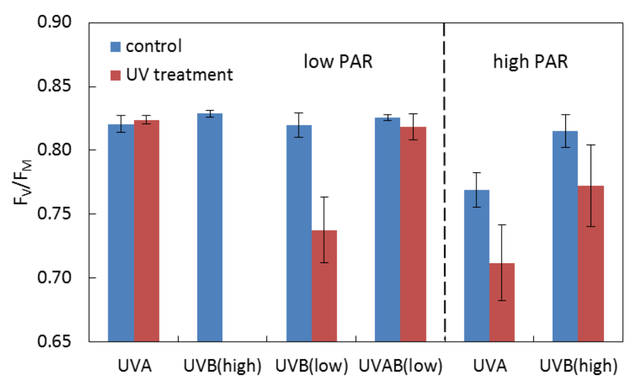

Fig. 3. The potential quantum yield of PSII photochemistry (F_V/F_M) for barley leaves grown under low or high PAR in the absence of UV radiation after the acute UV treatments. n=4-6±SD

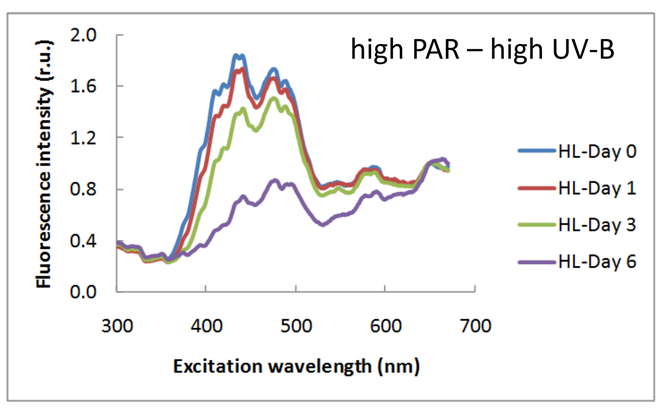

Fig. 4. Chlorophyll *a* fluorescence excitation spectra at room temperature for barley leaves grown under high PAR in the absence of UV radiation during acclimation to high UV-B treatment. The excitation spectra were normalized in the red spectral region. The mean spectra from 8-12 samples are presented.

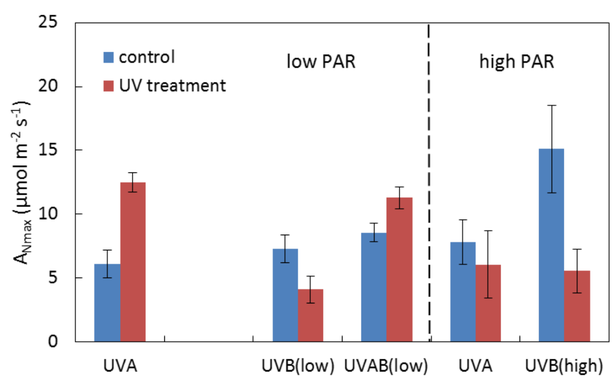

Fig. 5. The light-saturated CO_2 assimilation rate (A_{Nmax}) at steady-state for barley leaves grown under low or high PAR in the absence of UV radiation after the acute UV treatments. n=3-6±SD

MATERIALS AND METHODS

Plant material and acclimation conditions

Barley plants (*Hordeum vulgare* L. cv. Bonus) were grown from seeds inside growth chambers (HB1014, VB1014, Vötsch, Germany) under low and high PAR (LL and HL; 50 and 1000 µmol m^{-2} s^{-1}, 16/8 h day/night cycle), 20 °C and 65% relative air humidity in the absence of UV radiation. After 8 days of development, plants were exposed to UV-A (8 W m^{-2} for 16 h per day, together with PAR illumination) and/or UV-B radiation (0.05 and 2 W m^{-2} for 16 h per day; **low and high UVB treatment**) for the next 6 days. LL-acclimated plants did not survive 6 day-high UV-B treatment. Therefore, measurements on LL plants exposed to high UV-B radiation (2 W m^{-2}) could only be made for 3 days of UV-B exposure. The combined effect of UV-A and low UV-B radiation is denoted as **UVAB treatment**. UV irradiation was achieved using UV-A lamps emitting in the 350-400 nm range (Actinic BL TL-D 18W, Philips) and UV-B broadband lamps with a peak at 302 nm (TL 20W/12 RS SLV, Philips). The middle segments of primary leaves were used for the measurements. Sampling of leaves was done after at least 2 h-dark adaptation in order to assure a reproducible conditions for estimation of Chl fluorescence parameters.

Methods

Epidermal UV-shielding was determined using a portable leaf-clip sensor (Dualex 4 Scientific, Force-A, Orsay, France) by comparing Chl fluorescence excited by red (λ_{max} = 655 nm, reference) and UV-A radiation (λ_{max} = 375 nm) on dark-adapted leaves [7]. The measurements were carried out on a spot 3.5 cm from the leaf tip. Total chlorophylls (Chl *a+b*) and carotenoid contents (Car *x+c*) were estimated spectrophotometrically (UV/VIS 550, Unicam, UK) from pigment extracts in 80% acetone [8]. The potential quantum yield of PSII photochemistry, $F_V/F_M = (F_M - F_0)/F_M$ was determined using a PAM 101/103 fluorometer equipped with the emitter-detector unit ED-101BL employing a blue LED as a source of excitation light (H. Walz, Effeltrich, Germany). Chl fluorescence was detected above 660 nm. The maximal CO_2 assimilation rate (A_{Nmax}) was measured at steady-state using an open-path gas-exchange system Li-6400 (Li-Cor, Lincoln, NE, USA) under saturating PAR (1200-1600 µmol m^{-2} s^{-1}), with controlled CO_2 concentration (380ppm) and leaf temperature of 20°C. Chl *a* fluorescence excitation spectra at room temperature were measured using spectrofluorometer LS50B (Perkin Elmer, UK). The excitation spectra were measured at the emission wavelength of 685 nm. The slit widths of excitation and emission monochromators were 5 nm.

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