The role of short-term high temperature pretreatment on the UV-B tolerance of barley cultivars

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Abstract: The impact of pretreatment with high temperature (45 °C for 45 min) on the UV-B tolerance of 4 barley cultivars (*Hordeum vulgare* L. 'Bülbül-89', 'Kalaycı-97', 'Tarm-92', and 'Tokak-157/37') was examined. The response of the plants to treatment was evaluated by measuring the pigment content, chlorophyll a fluorescence, oxygen evolution, fraction of oxygen-evolving complex, proline content, UV-B-absorbing compounds (A_{535} and A_{300}), and stress markers (malondialdehyde, H_2O_2, and UV-B marker). Regardless of high temperature pretreatment, UV-B irradiation decreased the photosynthetic pigment content, photosystem II activity, oxygen evolution, and the fraction of oxygen-evolving complex in almost all of the barley cultivars. UV-B treatment significantly increased the proline content, UV-B-absorbing compounds, and stress markers. According to the findings, it can be deduced that short-term high temperature pretreatment might not provide a cross-tolerance to UV-B irradiation in the 4 barley cultivars studied; in fact, such exposure was found to aggravate the responses. In addition, although plants substantially accumulated the UV-B-absorbing compounds, the photosynthetic process might not be adequately protected from UV-B radiation.

Key words: Chlorophyll fluorescence, high temperature, *Hordeum vulgare*, pigment, photosynthesis, UV-B

Arpa çeşitlerinin UV-B toleransında kısa süreli yüksek sıcaklık ön uygulamalarının rolü

Özet: Yüksek sıcaklık ön uygulamasının (45 °C, 45 dakika) 4 arpa (*Hordeum vulgare* L. 'Bülbül-89', 'Kalaycı-97', 'Tarm-92' ve 'Tokak-157/37') çeşidinin UV-B toleransı üzerine etkisi incelenmiştir. Bitkilerin uygulamalara cevabı, pigment içeriği, klorofil a fluoresansı, oksijen çıkışı, oksijen çıkış kompleksinin fraksiyonu, prolin içeriği, UV-B absorplayan pigmentler (A_{535} ve A_{300}) ve stres markörleri (malondialdehit, H_2O_2 ve UV-B markörü) ölçülerek değerlendirilmiştir. Yüksek sıcaklık ön uygulaması gözetilmeksizin, UV-B ışıması hemen hemen tüm arpa çeşitlerinin fotosentetik pigment içeriğini, fotosistem II aktivitesini, oksijen çıkışını ve oksijen çıkış kompleksinin fraksiyonunu azaltmıştır. UV-B uygulaması, prolin içeriğini, UV-B absorplayan bileşikleri ve stres markörlerini ise önemli düzeyde artırmıştır. Bulgulara göre; kısa süreli yüksek sıcaklık ön uygulamasının 4 arpa çeşidinde UV-B ışımasına bir çapraz uyum sağlamadığı, hatta uygulamanın tepkileri kötüleştirdiği sonucu çıkarılabilir. Ayrıca; bitkiler çok fazla UV-B absorplayan bileşikler biriktirmesine rağmen, fotosentetik süreç UV-B ışımasından yeterince korunamamıştır.

Anahtar sözcükler: Klorofil fluoresansı, yüksek sıcaklık, *Hordeum vulgare*, pigment, fotosentez, UV-B

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Introduction

Continuous climate change may concurrently induce several abiotic stress factors, such as drought, enhanced UV-B radiation, and high temperatures. Plants usually suffer from several abiotic or biotic stresses simultaneously. Responses to UV-B stress vary among the higher plant species (Hideg et al. 2006). The effect of UV-B irradiation on many metabolic processes can be very deleterious due to its high energy. It can affect DNA, proteins, and the photosynthetic machinery in the plants (Hollosy 2002). UV-B inhibits photosynthesis (Teramura and Sullivan 1994; Mackerness 2000; Surabhi et al. 2009; Albert et al. 2010) and plant growth and, at the same time, activates defense mechanisms such as the up-regulation of UV-B-absorbing compounds (flavonoid biosynthesis) (Mazza et al. 2000; Wilson et al. 2001; Kakani et al. 2003; Jansen et al. 2004) and antioxidant enzymes (Shi et al. 2005; Ren et al. 2007; Çakırlar et al. 2011). It has been suggested that flavonoids play a major role in UV-screening (Jansen et al. 2004), which is often proposed as an adaptive mechanism to prevent UV-B irradiation from reaching the mesophyll and affecting photosynthesis (Caldwell et al. 1983). Photosystem II (PS II) is widely recognized as the primary target of UV-B damage (Correia et al. 1999; Hollosy 2002; Wang et al. 2010).

In addition to inter- and intraspecific variations in UV-B sensitivity, other environmental stresses also alter and/or modify plant responses. The findings of interaction between UV-B irradiation and other environmental stresses in plants demonstrate that these factors may induce several responses that can be antagonistic, synergistic, and/or additive (Alexieva et al. 2001; Ren et al. 2007; Remorini et al. 2009). Hideg et al. (2003) found that the photosynthesis of drought pretreated plants was significantly higher than the photosynthesis of plants exposed to six days of UV-B stress. They proposed that reversible water withdrawal improved the tolerance of plants against subsequent UV-B irradiation. High levels of photosynthetically active radiation (PAR) during sunny days are inevitably accompanied by increased UV radiation (Stroch et al. 2008).

Although there are many studies related to the individual effect of UV-B and temperature on plants, only a limited number of papers have been devoted to the interactive effects of UV-B irradiation and high temperature pretreatment. Recently, in a study investigating the effect of pretreatment with salt stress on the responses of barley cultivars to UV-B stress, a cross-acclimation was demonstrated by Çakırlar et al. (2008).

The aim of the present work was to investigate the effect of pretreatment with high temperature (45 °C for 45 min) on the UV-B tolerance of 4 barley cultivars in order to determine whether there was a cross-tolerance. The photosynthetic pigment content, photosynthetic efficiency, proline content, and UV-absorbing and UV-induced compounds were measured.

Materials and methods

Plant material

The experiment was carried out in 2007 and 2008. In 2007, the seeds of 4 barley cultivars (*Hordeum vulgare* L. 'Bülbül-89', 'Kalaycı-97', 'Tarm-92', and 'Tokak-157/37') were provided by the Republic of Turkey's Ministry of Food, Agriculture, and Livestock's Variety Registration and Seed Certificate Center. These cultivars were chosen because they were grown in the southern and southwestern regions of Turkey, where the climate is warmer.

Growth conditions and treatments

After imbibition in distilled water for 3 h, 10 seeds were sown in plastic pots (10 cm in diameter and 8.5 cm in height) containing perlite. Pots were placed in a controlled growth chamber with a day temperature of 25 ± 0.2 °C and a night temperature of 20 ± 0.2 °C. The chamber featured a 16-h photoperiod under a white fluorescent light (200 µmol m^{-2} s^{-1} PPFD) and a relative humidity of 60 ± 5%; a completely randomized design was employed. Pots were irrigated every second day with tap water and no fertilizer was added during the experiment. Following germination, seedlings were grown for 6 days, and the pots were then divided into 4 groups per cultivar: a control group (in controlled growth conditions), a high temperature treatment group, a UV-B treatment group, and a high temperature pretreatment + UV-B treatment group. Six-day-old plants were subjected to exposure to 45 °C for 45 min. Twenty-four hours after the high temperature

treatment, the UV-B treatment group and the high temperature pretreatment + UV-B treatment group were irradiated for 5 h with UV-B (312 ± 25 nm) fluorescent tubes (G15T8E, USHIO, Cypress, CA, USA) (UV-B radiation (UVB$_{BE}$): 2.88 kJ m^{-2} day^{-1}). The distance between the top of the plants and the UV-B lamp was about 30 cm. During the UV-B treatment, no white light was applied. The biological effectiveness of UVB$_{BE}$ was calculated using the plant action spectrum of Caldwell (1971) normalized to unity at 300 nm.

Measurements

All measurements were taken 24 h after the administration of the UV-B treatment and the first leaves were used from each plant.

Photosynthetic pigment content

For the determination of the pigment content, the middle leaf region was used. Leaf samples (50 mg) were extracted in 10 mL of 100% acetone and centrifuged at 3500 rpm for 5 min. The absorbance of the extracts was measured at 470, 644.8, and 661.6 nm. The contents of chlorophyll (chl) *a*, chl *b*, and carotenoids (car) xanthophyll and carotene were calculated using formulae set by Lichtenthaler (1987).

Polyphasic chlorophyll a fluorescence measurements

Approximately 24 h following the UV-B treatment, fluorescence measurements were taken at room temperature using a Handy PEA fluorimeter (Hansatech Instruments, Norfolk, UK). Dark-adapted leaves (at least 1 h) were illuminated homogeneously with continuous light (650 nm peak wavelength, 3000 µmol m^{-2} s^{-1} maximum light intensity for 500 ms) over an area of 4 mm in diameter with an array of 3 red LEDs. The chl *a* fluorescence signals were recorded within a time scan from 10 µs to 500 ms according to the method of Strasser and Strasser (1995). Polyphasic chl *a* fluorescence (OJIP) transient was analyzed using the JIP test. This testing model, based on the energy flux theory for biomembranes in a photosynthetic sample, leads to equations and calculations for specific energy fluxes (per reaction center, RC) and phenomenological energy fluxes (per excited cross-section, CS), as well as for flux ratios or yields (Strasser and Strasser 1995; Strasser et al. 2000, 2010; Tsimilli-Michael and Strasser 2008) (see Table 1 for explanation).

Oxygen evolution and the fraction of oxygen-evolving complex

The oxygen evolution rate was determined using a leaf disk electrode (Type LD2/2, Hansatech). Measurements were carried out at an illumination of 800 µmol m^{-2} s^{-1} PPFD and a saturating CO$_2$ concentration (CO$_2$ provided by a carbonate/bicarbonate buffer) at room temperature. The fraction of oxygen-evolving complex (OEC) was calculated according to the formulae (Han et al. 2009) presented in Table 1.

UV-B-absorbing compounds

Barley leaves (150 mg) were homogenized in 6 mL of medium containing methanol, HCl, and dH$_2$O (79:1:20) and centrifuged at 10,000 rpm for 15 min. Supernatant was used for the photometric measurements of absorbance (Mirecki and Teramura 1984). UV-B-absorbing compounds were estimated by absorbance at 300 nm of diluted extract. The same acidified methanol extract was used for the determination of anthocyanins while reading the absorbance at 535 nm, using a Shimadzu Mini-1240 UV-Vis spectrophotometer (Fedina et al. 2006).

Proline determination

Proline content was determined by the method of Bates et al. (1973). First, 500 mg of leaves were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 3500 rpm for 10 min. Next, 2 mL of the extract was reacted with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid for 1 h at 100 °C. The reaction was stopped in an ice-bath and the reaction mixture was extracted with 4 mL of toluene. The chromophore containing toluene was separated and the absorbance was read at 520 nm.

Determination of malondialdehyde, hydrogen peroxide, and UV-B marker

For this test, 150 mg of barley leaves were homogenized in 3 mL of 0.1% trichloroacetic acid (TCA) at 4 °C and centrifuged at 10,000 rpm for 15 min, and the supernatant was used in the subsequent determination.

Malondialdehyde (MDA) was determined by the method of Heath and Packer (1968). To 0.5 mL of the supernatant, 0.5 mL of 0.1 M Tris-HCl (pH 7.6) and

Table 1. Summary of the JIP test formulae using data extracted from the chlorophyll a fluorescence (OJIP) transient in this study (Tsimilli-Michael and Strasser 2008; Han et al. 2009; Strasser et al. 2010).

Extracted and technical fluorescence parameters	Description
Fo	Initial fluorescence intensity when all PS II RCs are open
F_{300}	Fluorescence intensity at 300 µs
F_J	Fluorescence intensity at the J-step (at 2 ms)
F_I	Fluorescence intensity at the I-step (at 30 ms)
F_M	Maximal fluorescence intensity when all PS II RCs are closed
t_{fmax}	Time to reach F_M, in ms
V_J	$(F_{2ms} - Fo) / (F_M - Fo)$, relative variable fluorescence at the J-step (2 ms)
V_I	$(F_{30ms} - Fo) / (F_M - Fo)$, relative variable fluorescence at the I-step (30 ms)
V_K	$(F_{300µs} - Fo) / (F_M - Fo)$, relative variable fluorescence at the K-step (300 µs)
Mo or **(dV/dt)o**	$4(F_{300µs} - Fo) / (F_M - Fo)$, approximated initial slope (in ms^{-1}) of the fluorescence transient $V = f(t)$
Area	Total complementary area between fluorescence induction curve and F_M
Sm	Area / $(F_M - Fo)$, normalized total complementary area above the OJIP (reflecting multiple-turnover QA reduction events) or total electron carriers per RC
OEC	$[1 - (V_K / V_J)]_{treated} / [1 - (V_K / V_J)]_{control}$, fraction of OECs
Quantum efficiencies or flux ratios	
φ_{Po} or **TRo/ABS**	$(1 - Fo) / F_M$ or Fv / F_M, maximum quantum yield of primary photochemistry at $t = 0$
φ_{Eo} or **ETo/ABS**	$(1 - Fo / F_M) \times Yo$, quantum yield for electron transport at $t = 0$
Yo or **ETo/TRo**	$1 - V_J$, probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond QA^-
δRo or **RE/ETo**	$(1 - V_I) / (1 - V_J)$, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PS I end electron acceptors
φ_{Ro} or **REo/ABS**	$\varphi_{Po} \times \Psi o \times \delta Ro$, the quantum yield of electron transport from Q_A^- to the PS I end electron acceptors
Specific fluxes or specific activities	
ABS/RC	$Mo \times (1 / V_J) \times (1 / \varphi_{Po})$, absorption flux per RC at $t = 0$ or a measure for an average antenna size
TRo/RC	$Mo \times (1 / V_J)$, trapped energy flux per RC at $t = 0$
ETo/RC	$Mo \times (1 / V_J) \times \Psi o$, electron transport flux per RC at $t = 0$
Phenomenological fluxes or phenomenological activities	
ABS/CSo	Fo or other useful expression, absorption flux per CS at $t = 0$
TRo/CSo	$\varphi_{Po} \times (ABS / CSo)$, trapped energy flux per CS at $t = 0$
ETo/CSo	$\varphi_{Po} \times \Psi o \times (ABS / CSo)$, electron transport flux per CS at $t = 0$
RC/CSo	$\varphi_{Po} \times (V_J / Mo) \times Fo$, amount of active PS II RCs per CS at $t = tF_M$
PI_{total}	$[(RC / ABS) \times (\varphi_{Po} / (1 - \varphi_{Po})) \times (\Psi o / (1 - \Psi o)) \times (\delta Ro / (1 - \delta Ro))]$, performance index (potential) for energy conservation from photons absorbed by PS II to the reduction of PS I end acceptors

1 mL of TCA-TBA-HCl reagent [15% TCA (m/v), 0.375% TBA (m/v), 0.25 M HCl] were added. The mixture was heated at 95 °C for 30 min and then was rapidly cooled in an ice bath. After centrifugation at 10,000 rpm for 5 min to remove suspended turbidity, the absorbance of the supernatant at 532 nm was recorded. Nonspecific absorbance was measured at 600 nm and subtracted from the readings recorded at 532 nm. The concentration of MDA was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}.

For the determination of hydrogen peroxide, 0.5 mL of 0.1 M Tris-HCl (pH 7.6) and 1 mL of 1 M KI were added to 0.5 mL of supernatant. After 90 min, the absorbance was read at 390 nm (Esterbauer and Cheeseman 1990).

To determine the UV-B marker, supernatant was used directly. Absorbance at 440 nm was recorded (Fedina et al. 2003).

Statistical analysis

Experimental data were analyzed with the SPSS statistical program. Statistical evaluation of the data with 5 replicates was performed using ANOVA and was compared with the least significant differences (LSDs) at the 5% level.

Results

UV-B decreased the chl a content by approximately 13% in Kalaycı-97 and 36% in Tarm-92, whereas UV-B following exposure to 45 °C for 45 min decreased the content by about 22% in Tokak-157/37 and 41% in Tarm-92 compared to the untreated controls. High temperature alone was also found to decrease the chl a contents (25%-31%) (Table 2). UV-B irradiation adversely affected the chl b content, especially in Tarm-92 and Tokak-157/37

Table 2. The effects of UV-B irradiation on the photosynthetic pigment contents in the leaves of 4 barley cultivars pretreated with high temperature (Chl: chlorophyll, Chl $a + b$: total chlorophyll, Car: total carotenoids). Means ± SE, n = 5.

Cultivars	Treatments	Chl a mg g^{-1} FW	Chl b mg g^{-1} FW	Chl $a + b$ mg g^{-1} FW	Car mg g^{-1} FW	Chl a/b	Chl/Car
Bülbül-89	Control	1.55 ± 0.10 a*	0.49 ± 0.04 b	2.04 ± 0.14 a	0.39 ± 0.02 bc	3.15 ± 0.05 a	5.19 ± 0.12 a
	UV-B	1.20 ± 0.04 b	0.47 ± 0.00 b	1.67 ± 0.04 b	0.38 ± 0.04 c	2.56 ± 0.08 f	4.47 ± 0.30 ef
	45 °C	1.10 ± 0.09 c	0.37 ± 0.00 d	1.47 ± 0.08 cd	0.28 ± 0.03 f	2.97 ± 0.27 c	5.23 ± 0.28 a
	45 °C + UV-B	1.00 ± 0.01 de	0.36 ± 0.00 d	1.35 ± 0.02 ef	0.31 ± 0.01 e	2.80 ± 001 d	4.34 ± 0.20 f
Kalaycı-97	Control	1.27 ± 0.15 b	0.43 ± 0.03 c	1.70 ± 0.18 b	0.34 ± 0.03 d	2.98 ± 0.11 bc	5.02 ± 0.02 b
	UV-B	1.10 ± 0.06 c	0.42 ± 0.02 c	1.52 ± 0.08 c	0.40 ± 0.02 bc	2.63 ± 0.00 f	3.75 ± 0.02 j
	45 °C	0.96 ± 0.04 ef	0.32 ± 0.02 f	1.27 ± 0.06 fg	0.27 ± 0.01 f	3.00 ± 0.08 ab	4.66 ± 0.15 cd
	45 °C + UV-B	0.91 ± 0.06 f	0.33 ± 0.03 ef	1.24 ± 0.09 g	0.31 ± 0.02 e	2.74 ± 0.03 e	3.96 ± 0.08 gh
Tarm-92	Control	1.55 ± 0.02 a	0.49 ± 0.00 b	2.04 ± 0.03 a	0.41 ± 0.01 ab	3.15 ± 0.03 a	4.92 ± 0.00 b
	UV-B	0.99 ± 0.02 de	0.37 ± 0.01 d	1.36 ± 0.04 e	0.35 ± 0.00 d	2.67 ± 0.04 ef	3.85 ± 0.08 hj
	45 °C	1.11 ± 0.00 c	0.36 ± 0.00 d	1.47 ± 0.00 cd	0.31 ± 0.00 e	3.13 ± 0.02 ab	4.76 ± 0.05 c
	45 °C + UV-B	0.91 ± 0.04 f	0.34 ± 0.01 ef	1.24 ± 0.05 g	0.33 ± 0.02 de	2.69 ± 0.02 ef	3.82 ± 0.10 hj
Tokak-157/37	Control	1.53 ± 0.11 a	0.57 ± 0.04 a	2.10 ± 0.07 a	0.43±0.01 a	2.73 ± 0.39 e	4.93 ± 0.04 b
	UV-B	1.20 ± 0.02 b	0.43 ± 0.01 c	1.63 ± 0.03 b	0.40±0.00 bc	2.79 ± 0.05 de	4.05 ± 0.05 g
	45 °C	1.05 ± 0.03 d	0.35 ± 0.01 de	1.41 ± 0.04 de	0.31±0.01 e	2.98 ± 0.04 bc	4.57 ± 0.24 de
	45 °C + UV-B	1.13 ± 0.04 bc	0.41 ± 0.01 c	1.53 ± 0.05 c	0.38±0.01 c	2.78 ± 0.06 de	4.00 ± 0.02 g
LSD 5%		**0.07**	**0.02**	**0.09**	**0.02**	**0.15**	**0.17**

* Values followed by different letters in a column are significantly different.

(with decreases of approximately 24%). The combination of UV-B together with high temperature pretreatment enhanced the adverse effect on chl *b* content (22%-31%). UV-B irradiation following the high temperature pretreatment led to an additional decrease in the total chl content (27%-39% compared to control). In addition, the chl *a*-to-chl *b* ratio was also affected by UV-B irradiation. The chl *a*-to-chl *b* ratio decreased by 11%-19% in all of the cultivars except Tokak-157/37. The decrease in the ratio of chl *a* to chl *b* is mainly due to the decrease of chl *a*. The

ratio of chl *a* to chl *b* in Tokak-157/37, however, was increased by the high temperature treatment (Table 2). After UV-B irradiation, the carotenoid content increased in Kalaycı-97, whereas it significantly decreased in Tarm-92 and Tokak-157/37. However, UV-B irradiation following the high temperature pretreatment significantly decreased the carotenoid content in all 4 cultivars. All of these changes affected the chl-to-car ratio, with decreases of 14%-25%.

Figure 1 presents the effects of UV-B alone and following pretreatment with high temperatures using
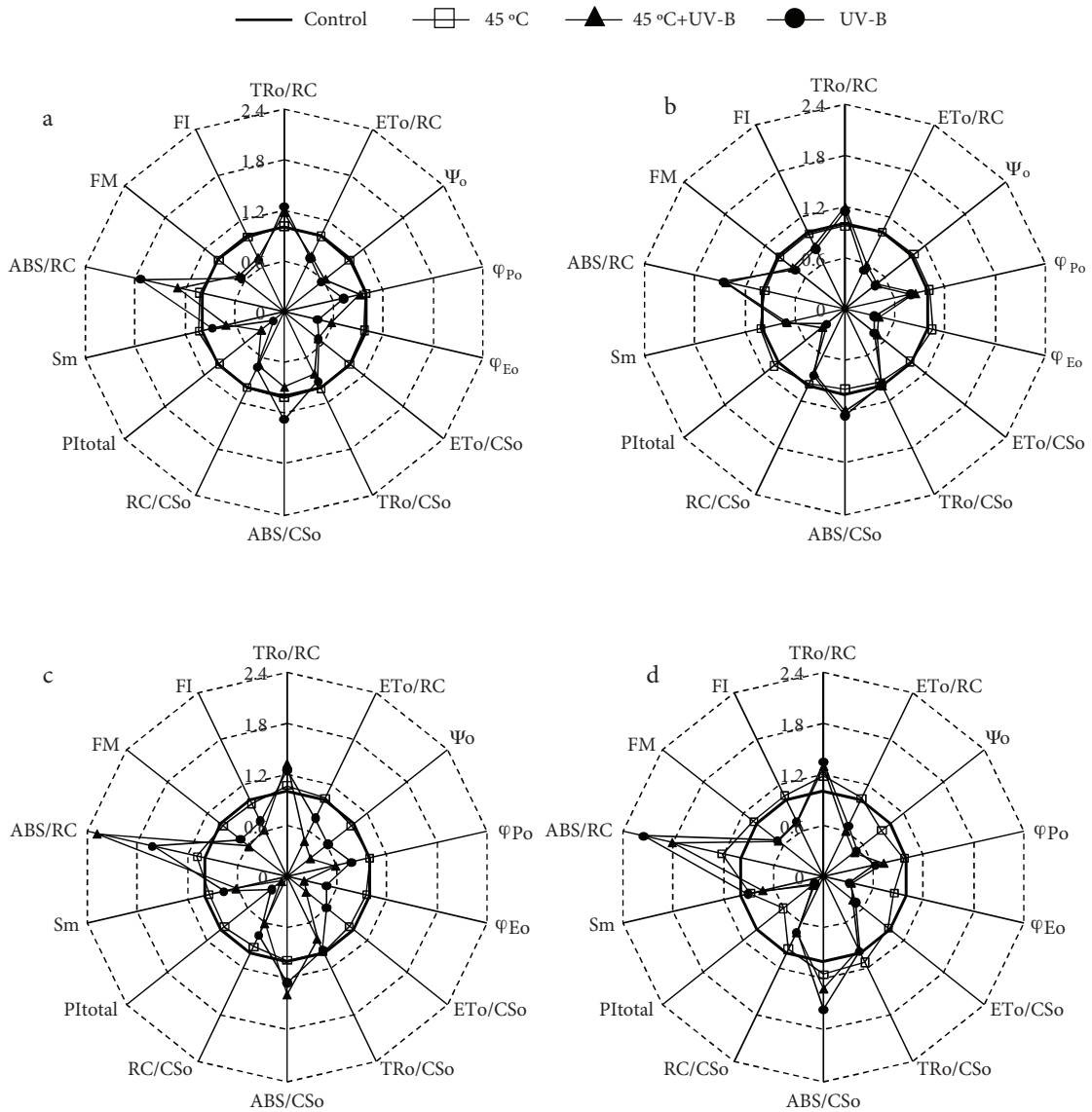

Figure 1. Effects of UV-B irradiation alone or after high temperature pretreatment on some polyphasic chlorophyll *a* fluorescence parameters plotted relative to their respective controls in the barley plants: a) Bülbül-89, b) Kalaycı-97, c) Tarm-92, d) Tokak-157/37 (n = 15).

radar plots of some OJIP parameters from the 4 barley cultivars. The parameters of seedlings derived from UV-B alone and from exposure following high temperature pretreatment are plotted relative to the parameters of the control plants. Both UV-B treatments resulted in a significant decrease in the I (F_I) and P (F_M) phases of the fluorescence transients (Figure 1), whereas they slightly increased the O phase (data not shown). The high temperature alone led to a response nearly similar to that of the control (Figure 1). With UV-B irradiation, the maximum rate of electron transport per reaction center (ETo/RC) and the maximum yield of electron transport (φ_{Eo}) decreased pronouncedly, and the value of the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (Ψ_o) was reduced significantly in all cultivars. In addition, the decrease in the maximum quantum yield of primary photochemistry ($\varphi_{Po} = F_V/F_M$) was less than these 2 flux ratios. UV-B irradiation increased the average antenna size of an active RC (ABS/RC) and the maximum trapping rate of PS II (TRo/RC) of specific fluxes or specific activities, whereas it decreased the electron transport in an active RC (ETo/RC) in all of the barley cultivars studied. Additionally, UV-B treatments decreased the density of the active RC in a cross-section (RC/CSo, slightly), the maximum trapping rate in a PS II cross-section (TRo/CSo, slightly in Kalaycı-97 and Tokak-157/37), and the electron transport in a PS II cross-section (ETo/CSo, significantly) (Figure 1). The total electron carriers per RC (Sm) and PI_{total}, measuring the performance up to the photosystem I (PS I) end electron acceptors, were also decreased by UV-B irradiation. The decrease in PI_{total} was more prominent than those of the other OJIP parameters (Figure 1).

The effect of UV-B treatment on O_2 evolution indicated variation between treatments and cultivars (Figure 2a). UV-B treatments (alone or after exposure to 45 °C for 45 min) decreased O_2 evolution in Bülbül-89 and Tarm-92. All treatments increased evolution in Kalaycı-97, while UV-B and heat alone increased it in Tokak-157/37. Furthermore, UV-B irradiation alone or after high temperature pretreatment significantly decreased the fraction of OEC in all of the barley cultivars when compared to the untreated plants (Figure 2b).
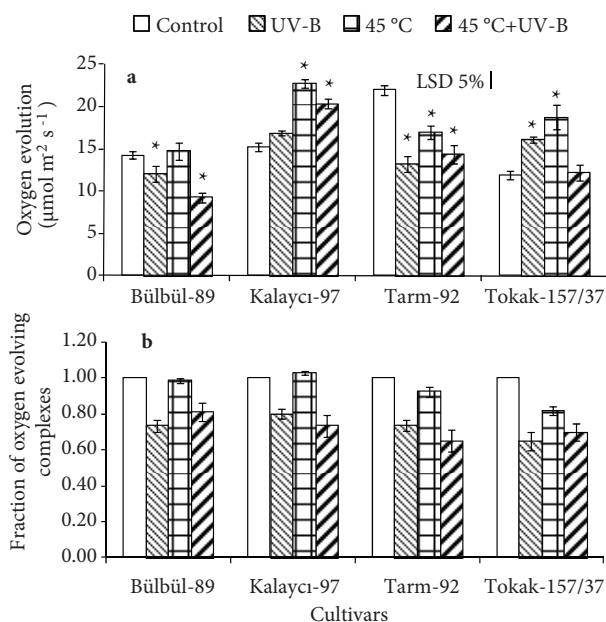

Figure 2. Changes in a) oxygen evolution (n = 5) and b) fraction of OEC (n = 15) of barley cultivars treated with high temperature prior to UV-B exposure. The values for OEC are normalized by the value of control plants for every genotype. *Means are significantly different from the control at P < 0.05.

UV-B irradiation significantly increased the UV-B-absorbing pigment [anthocyanin (A_{535}) and A_{300}] contents in all of the cultivars (Figures 3a and 3b, respectively). UV-B alone was more effective than UV-B following high temperature pretreatment. Both UV-B treatments (UV-B administered alone and following pretreatment with high temperature) increased the proline contents of all of the barley cultivars (Figure 3c), but UV-B alone was more effective in increasing the proline content.

Exposure to UV-B irradiation alone or after pretreatment with high temperature significantly increased the product of lipid peroxidation (MDA), H_2O_2 content, and the level of UV-B marker in comparison to the controls in all 4 of the barley cultivars (Figures 4a-4c). The stress marker responses of barley cultivars to high temperature showed differences. High temperature exposure significantly increased the MDA content in all of the cultivars except Kalaycı-97, but the increase was lower than that in cultivars exposed only to UV-B treatments (Figure 4a). While high temperature
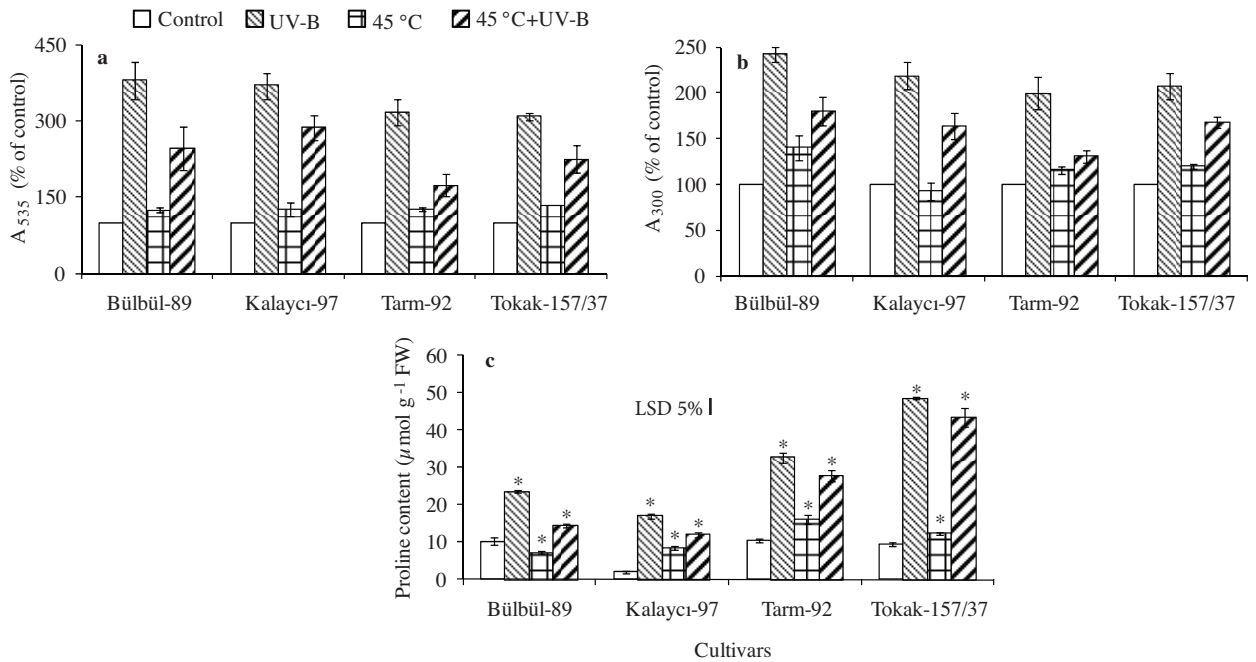
Figure 3. Changes in the contents of a) ABS$_{535}$, b) ABS$_{300}$, and c) proline of the leaves of barley cultivars subjected to UV-B stress applied alone or after high temperature pretreatment (n = 5). *Means are significantly different from the control at P < 0.05.
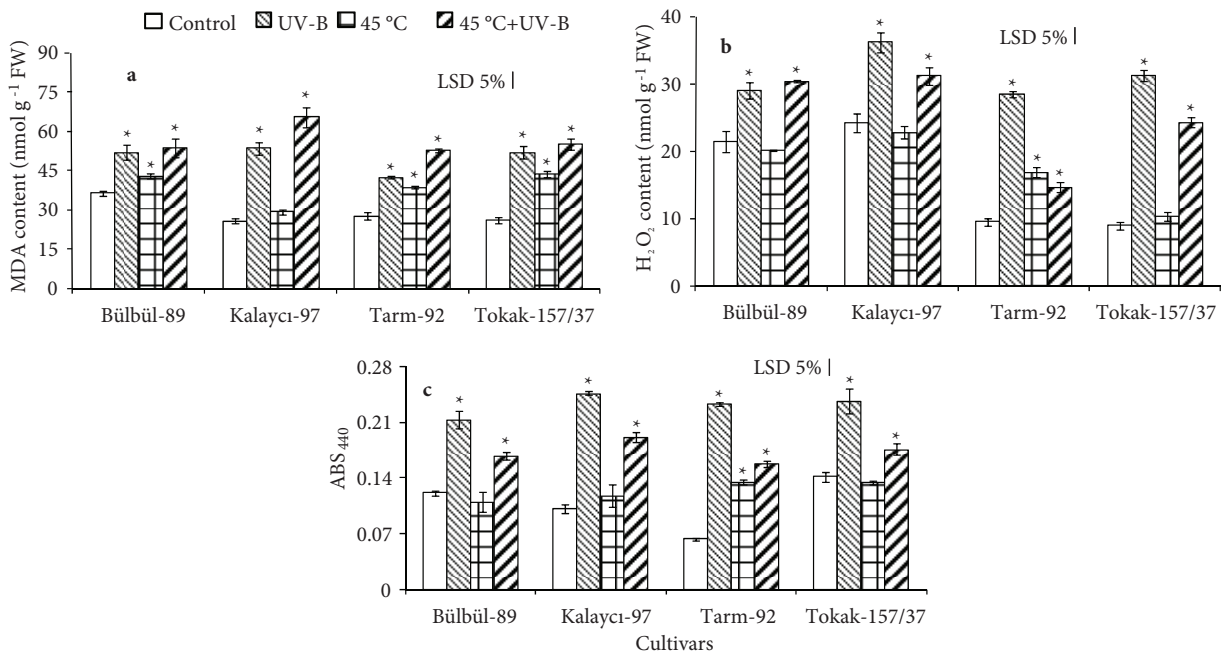

Figure 4. The effects of UV-B irradiation alone or following high temperature pretreatment on the content of a) MDA, b) H$_2$O$_2$, and c) UV-B marker in the leaves of barley cultivars (n = 5). *Means are significantly different from the control at P < 0.05.

significantly increased the H$_2$O$_2$ content in Tarm-92 and Tokak-157/37, it slightly decreased the content in Bülbül-89 and Kalaycı-97 as compared to the controls (Figure 4b). High temperature significantly increased the UV-B marker in Kalaycı-97 and Tarm-92, whereas it slightly decreased the content of this marker in Bülbül-89 and Tokak-157/37 compared to the controls (Figure 4c).

Discussion

UV-B exposure decreased the photosynthetic pigment contents of the barley cultivars (except for chl b in Bülbül-89 and Kalaycı-97 subjected to UV-B alone) in the present study (Table 2). It has been reported that photosynthetic pigments seem to be altered after UV-B irradiation (Teramura and Sullivan 1994; Agrawal and Rathore 2007). Reduction in chlorophyll content has been ascribed to the inhibition of its biosynthesis or to the degradation of the pigments and their precursors (Teramura and Sullivan 1994). Strid and Porra (1992) suggested another possibility for this observed reduction, however, proposing that the decreased photostability of chlorophyll is a direct result of UV-B and the down-regulation of the gene responsible for chl a/b binding proteins, thereby inhibiting chlorophyll biosynthesis. The decrease in chlorophyll content was also found in other recent studies (Joshi et al. 2007; Ibanez et al. 2008; Singh et al. 2008). In addition, a significant reduction of carotenoids was also determined in this study, with the exception of Kalaycı-97 and Bülbül-89 exposed to UV-B alone (Table 2). Carotenoids protect chlorophyll from photooxidative destruction, so a reduction in carotenoids could have serious consequences for the effect of UV-B radiation on chlorophyll pigments (Agrawal and Rathore 2007; Mishra et al. 2008). Conversely, it was found that UV-B induced an increase in the carotenoid content of *Pisum sativum* (Strid and Porra 1992), as in the Kalaycı-97 cultivar. Carletti et al. (2003) theorized that an imbalance in the photosynthetic pigment composition may be due to the effects of UV-B radiation on photosynthetic membranes and that changes in the composition of these photosynthetic pigments may be indicative of perturbations in the photosynthetic apparatus. Changes in the pigment contents reflect on the chl a-to-chl b and chl-to-car ratios, as well (Table 2). The chl a-to-chl b ratio was detrimentally affected by UV-B irradiation. Similarly, the chl-to-car ratio decreased under UV-B irradiation (Table 2). The results obtained in the present study indicate that UV-B radiation alone or after pretreatment with high temperature damages membrane structure and the integrity of the photosynthetic apparatus. Membrane damage can also be detected from data on MDA. UV-B irradiation increased the content of MDA, a product of lipid peroxidation and an indicator of oxidative damage, in all 4 barley cultivars examined in this study (Figure 4a). UV-B-induced accumulation of MDA has previously been observed in *Nicotiana tabacum* (Hideg et al. 2003), *Arabidopsis thaliana* (Gao and Zhang 2008), and *Olea europaea* (Remorini et al. 2009).

It can be assumed that the reduction in chlorophyll contents due to the breakdown of the structural integrity of chloroplasts (Caasi-Lit et al. 1997) may result in the reduction of photosynthesis under UV-B radiation. UV-B treatment or the administration of UV-B following high temperature pretreatment significantly decreased the I (F_I) and P (F_M) phases of fluorescence transient in all barley seedlings (Figure 1). It is hypothesized that the IP phase is related to the electron transfer through PS I and the induction of a traffic jam of electrons caused by a transient block on the acceptor side of PS I (Schansker et al. 2006). The significant decrease in PI_{total} is consistent with this hypothesis. From the fluorescence transient, it is also possible to appraise the maximum yield of electron transport (φ_{Eo}), which is the product of the maximum quantum yield of the primary photochemistry (φ_{Po}), and the yield of electron transport per trapped exciton (Ψo). UV-B irradiation significantly decreased the maximum yield of electron transport in all barley cultivars. This reduction was due more to a decrease in the efficiency with which a trapped exciton can move an electron into the electron transport chain further than QA^- (Ψo) than to a decrease of the maximum quantum yield of primary photochemistry (φ_{Po}) (Figure 1). However, it was observed that the antenna size of PS II (ABS/RC) increased with UV-B irradiation (Figure 1). Strasser et al. (1999) suggested that the increase in antenna size can result from an increase in the number of chl molecules per RC and/or from the inactivation of some RCs. In the present study, because of the decreasing chl content, the increase in the ABS/RC parameters may be due to the inactivation of RCs. In addition, owing to the pronounced decreases in φ_{Po}, φ_{Eo}, and Ψo, it might be deduced that UV-B treatment possibly caused an increase in the fraction of QB-nonreducing PS II centers. This seems reasonable when taking into consideration data about the unstacking process that may occur after heat and UV-B treatments (Bukhov and Mohanty 1999). Many studies have indicated that PS II is the component of the thylakoid

membrane of photosynthetic structures that is most sensitive to UV-B irradiation (Correia et al. 1999; Bolink et al. 2001; Shi et al. 2005; Mishra et al. 2008; Wang et al. 2010). It has been suggested that UV-B might damage the D1 and D2 proteins of PS II (Friso et al. 1994; Babu et al. 1999; Olsson et al. 2000) and, as a result, decrease the quantum efficiency or lower the photosynthetic capacity due to chlorophyll degradation (Sullivan 1997). In the present study, UV-B exposure also caused a decline in oxygen evolution (except in Kalaycı-97 and Tokak-157/37) and the fraction of OEC (Figure 2). It has been widely accepted that UV-B mainly damages the donor side of PS II by inactivating the Mn cluster of water oxidation (Vass et al. 1996; Larkum et al. 2001); in addition, it affects the Tyr-Z and Tyr-D electron donors (Vass et al. 1996) as well as the quinone electron acceptors (Melis et al. 1992; Vass et al. 1999). Szilard et al. (2007) also suggested that the damaging effect of UV-B could be located within the catalytic site and may cause a structural and/or functional change that renders the whole complex inactive. At the same time, Joshi et al. (2007) and Mishra et al. (2008) determined that the rate of photosynthetic oxygen evolution decreased considerably when plants were exposed to UV-B irradiation. According to all of the results above, UV-B damage to the photosynthetic apparatus may consist of many aspects, including the PS II reaction center, the OEC, and electron transfer. In the literature, reports of UV-B radiation's effect on photosynthesis are contradictory, presumably due to the differences between species or cultivars of the same species, variations in growth conditions, and disparities in the UV-B levels and the duration of UV-B exposure (Rozema et al. 1997; Kakani et al. 2003).

A well-known acclimation response of plants to UV-B is the induction of flavonoids and other UV-absorbing compounds. UV-B irradiation increased the UV-B-absorbing compounds and proline content in the 4 barley cultivars examined in the present study (Figure 3). Many researchers agree about which UV-absorbing pigments provide plants with protection from UV-B irradiation (Wilson et al. 2001; Jansen et al. 2004; Singh et al. 2008; Zu et al. 2010; Mohammed and Tarpley 2011). Smillie and Hetherington (1999) further indicated that anthocyanins may protect photosynthetic tissues against photoinhibition.

In addition, a strong correlation between the accumulation of UV-B-absorbing compounds (flavonoids) and UV-B tolerance has been shown for several plant species (Tevini et al. 1991; Gonzalez et al. 1998; Wilson et al. 2001). It has been suggested that UV-B radiation stimulates gene transcription and the expression of key enzymes in the flavonoid biosynthetic pathway (Tevini et al. 1991). Conversely, Haselgrove et al. (2000) reported that high temperatures reduced synthesis and were associated with net pigment loss. Moreover, Steyn et al. (2002) claimed that there is a negative relationship between temperature and anthocyanin.

In addition to its role as an osmolyte for osmotic adjustment, proline contributes to the stabilization of membranes and proteins, the scavenging of free radicals, and the buffering of cellular redox potential under stress conditions (Ashraf and Foolad 2007). The accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentrations have generally been shown to be higher in stress-tolerant plants than in their stress-sensitive counterparts. Alexieva et al. (2001) claimed that the removal of excess H^+ occurring as a result of proline synthesis may have a positive effect on the reduction of UV-B-induced damage. It has also been demonstrated that plants exposed to UV radiation accumulate proline, which may protect plant cells against peroxidative processes (Saradhi et al. 1995).

Hydrogen peroxide is increased in response to various stresses, and it is known to diffuse across biological membranes and cause cellular damage. UV-B increased the H_2O_2 content and UV-B marker of barley cultivars in the present study (Figures 4b and 4c). As anticipated, UV-B radiation may provoke oxidative damage, which is increased in UV-B-induced compounds.

In the present study, even though different responses to UV-B irradiation were observed among the barley cultivars, the responses occurred in almost the same manner. For example, changes in MDA content or other parameters of the cultivars were different under UV-B conditions, whereas UV-B irradiation significantly down-regulated the photosynthesis of all of the barley cultivars examined. UV-B irradiation alone or following high temperature pretreatment unfavorably affected

photosynthetic processes because of the breakdown of the membrane structure and integrity. At the same time, the amounts of A_{300} and anthocyanins (A_{535}) were significantly increased. This may mean that the increases were inadequate to serve as a UV-B protectant. Therefore, it may be concluded that pretreatment with high temperature did not mitigate the damaging effect of UV-B radiation in these 4 barley cultivars. Although all of the barley cultivars were adversely affected by UV-B treatments, Bülbül-89 demonstrated better responses than the others in terms of certain parameters, including PI_{total}, φ_{Po}, φ_{Eo}, Ψo, and the content of UV-B-absorbing and UV-B-induced compounds. Finally, as our study was

conducted in controlled conditions different from the field, further research is needed to elaborate on the effects of temperature pretreatments (different degrees, duration, etc.) on the responses of plants to UV-B radiation and its mechanisms.

Acknowledgments

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