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REGULAR ARTICLE

Natural variation in UV-B protection amongst *Arabidopsis thaliana* accessions

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Abstract

Pronounced altitudinal and latitudinal UV-B gradients exist across the earth. Therefore, we hypothesised that plants from different geographic origins differ in the regulation and/or magnitude of UV-protection. Eight Arabidopsis accessions with different geographic origins (altitude between 32 and 3016 m) were raised under Photosynthetic Active Radiation (PAR), PAR + UV-A or PAR + UV-A + UV-B radiation for 10 days, after which UV-B protection of photosynthesis was assessed by measuring the consequences of exposure to a pulse of acute UV-B. We found significant variation in UV-B protection among accessions exposed to PAR or PAR + UV-A. Yet, all accessions raised under PAR + UV-A + UV-B were well protected. Thus, differences between accessions are not about UV-B protection per sé, but rather about regulation of UV-B protection which varies from constitutive to inducible by UV-A and/or UV-B. Particularly striking are differential UV-A responses, whereby some high altitude accessions lack UV-A regulated accumulation of UV-absorbing pigments, but show a strong UV-A induced morphogenic response. The adaptive relevance of the differential regulation of UV-protection is discussed.

Key words: *Arabidopsis*, Carotenoid, Phenolics, Photosynthesis, UV-radiation

Introduction

Plants can adapt to local environmental conditions resulting in the evolution of ecotypes. Where plants are exposed to gradients of particular environmental effectors, adaptation may give rise to a specific pattern of phenotypic diversity. *Arabidopsis thaliana* is a variable species that is native to Europe and central Asia where it is exposed to a wide range of altitudinal, climatic, and edaphic conditions (Koornneef et al., 2004). It is likely that at least some of the phenotypic variation in *Arabidopsis* reflects local adaptation and has ecological significance (Koornneef et al., 2004). Phenotypic variation in *Arabidopsis* has been identified in traits such as disease resistance, tolerance to oxidative stress, extreme temperatures, salt and drought, flowering time and morphology, biochemical make-up, growth rate and others (Koornneef et al., 2004). Analysis of such natural diversity can contribute to the identification of gene-function, but also inform about the ecological importance of particular traits. In recent years *Arabidopsis* accessions have been used to study, among others, latitudinal clines for flowering time (Stinchcombe et al., 2004; Balasubramanian et al., 2006) , and Red / Far-Red light responses (Stenøien et al., 2002) as well as a coastal cline for sodium accumulation (Baxter et al., 2010).

In this study, we have investigated the biological effects of ultraviolet (UV) radiation on eight different *Arabidopsis* accessions that have evolved under different UV-regimes. Ultraviolet radiation (UV) penetrating the earth's biosphere consists largely of UV-A (315-400nm) with a much smaller contribution of UV-B (280-315nm). The levels of UV-B in the biosphere vary spatially and temporally depending on the ozone layer and geographic position on earth, with near equator and mid-latitudes receiving the higher doses and higher latitudes substantially less UV-B (McKenzie et al., 2001). The levels of UV radiation also increase with altitude (Piazena, 1996; McKenzie et al., 2001) and annual total levels of UV-B and UV-A have been reported to increase by 19% and 11% per 1000 m altitude respectively, in the Austrian alps (Blumthaler et al., 1992). It can be hypothesised that this UV-gradient affects plants growing at higher altitudes. Indeed, several studies have reported a positive correlation between the altitude of the growing site and the content of UV-

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protecting flavonoids and phenolic acids (Zidorn et al., 2005; Jaakola and Hohtola, 2010). An interesting question is whether the increased accumulation of flavonoids in alpine plants is regulated by UV-radiation, and if so, whether this is an inducible response, or rather a constitutively expressed trait in high altitude plants. Van de Staaij et al. (1997) compared *Silene vulgaris* ecotypes from alpine and low land origins, and found that UV-B exposure resulted in decreased flower and seed production in a low land *Silene vulgaris*, but up to 2.5-fold more seeds per plant in an alpine ecotype (Van de Staaij et al., 1997). These data implicate a degree of genetic adaptation of the UV-response in alpine plants. Similarly, an *ex situ* study showed that *Rumex acetosella* and *Plantago lanceolata* ecotypes or *Lupinus* and *Taraxacum* species originating at equatorial alpine sites were relatively UV protected compared to low land and/or higher latitude plants when grown under standardised conditions (Barnes et al., 1987). In contrast, a large scale study demonstrated a non-significant weak association between the geographic origin of *Arabidopsis* accessions and their constitutive UV-B protection of photosystem-II (Jansen et al., 2010). These seemingly contradictory reports on plant adaptation to ambient UV-B radiation levels, may well reflect experimental conditions (ratio PAR to UV-A to UV-B radiation) and the use of different proxies for UV-impact (photosynthetic damage, reproduction, biomass). UV-B radiation can potentially induce a wide range of inhibitory, effects including slower plant growth, reduced biomass, damage to photosystem II (PSII) and decrease in chlorophyll content (Jansen et al., 1998; Xiong and Day, 2001; Germ et al., 2005; Kataria and Guruprasad, 2012). UV-B also triggers morphological, physiological and metabolic acclimation responses such as accumulation of UV-B absorbing compounds, increased quenching of Reactive Oxygen Species (ROS) and DNA-repair (Rozema et al., 1997; Jansen et al., 1998; Kakani et al., 2004), and as a result many studies show none, or minimal, UV-B stress in plants raised under ambient UV-B conditions (Ballaré et al., 2011). It has been reported that exposure to UV-A can also result in direct photosynthetic damage (Turcsanyi and Vass, 2000; White and Jahnke, 2002), resulting in further ROS formation. However, there is a degree of controversy regarding UV-A and its biological effects on plants as both damaging (Nayak et al., 2003), and protective (Helsper et al., 2003; Joshi et al., 2007) UV-A responses have been reported.

In this study we have tested the hypothesis that *Arabidopsis* accessions from different geographic origins differ with respect to the regulation and/or magnitude of UV-protection of the photosynthetic machinery. To test this hypothesis we investigated the biological effects of low, chronic levels of UV-A and UV-B that facilitate acclimation, and of a high level UV-B that causes stress, on eight different *Arabidopsis* accessions. The data presented in this manuscript highlight a remarkable degree of specificity in UV-responses with UV-B protection of photosynthesis being controlled by genetic background, UV-A and UV-B radiation.

Materials and Methods

Plant material and growth conditions

Eight *Arabidopsis thaliana* accessions originating from different geographical locations were selected to study plant responses to UV radiation. These accessions (ecotypes) were selected to represent a range of latitudes and altitudes. Seeds were kindly donated by Prof. Koornneef (Wageningen University, The Netherlands and MPIZ, Cologne, Germany), and had been propagated for several generations under controlled conditions prior to use in the described experiments. Details of the selected *Arabidopsis* accessions are given in the Table 1. Following sterilization, seeds were germinated on MS plates. Seedlings that had reached the 3-4 leaf stage were transferred to individual 6 cm diameter plastic pots filled with a soil-based substrate (John Innes 2, Westland Horticulture, Winsford, UK) and perlite (John Innes 2: perlite = 4: 1 approx.). Following transplanting, plants were grown for 7 days in a growth chamber under 80 µmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) (Philips LLD 36W/840 reflex). Growth rooms were kept at 20 ºC, under a 14/10-h light/dark cycle and a relative humidity of 75%.

Treatments and exposure conditions

After 7 days of establishment, plants were raised for a further 10 days under different PAR and UV regimes. These were:

1) PAR (35 µmol m^{-2} s^{-1}),
2) PAR (35 µmol m^{-2} s^{-1}) + UV-A (0.159 mWcm^{-2})
3) PAR (35 µmol m^{-2} s^{-1}) + UV-A (0.159 mWcm^{-2}) + UV-B (0.026 mWcm^{-2})

Table 1. *Arabidopsis* accessions and their geographical distributions.

Full Name	Abbreviated name	Country of origin	Line code	Longitude (ºE)	Latitude (ºN)	Altitude (m)
Burren	Bur-0	Ireland	CS 6643	-9.0	53.1	32
Buskerud	Bus-1	Norway	JA 46	9.9	59.9	100
Vind-1olanda	Vind-1	UK	CS 22560	-2.3	55.0	122
Martuba	Mt-0	Libya	CS 6799	38	56	137
Argentat	Ang-0	France	JA.2[b]	1.9	45.1	196
Cape Verde Islands	Cvi-1	Portugal	N 8580	-24	16	1052
Hodja-Obi-Garm	Hog	Tajikistan	CS 6179	69.7	38.7	1414
Shadara	Sha	Tajikistan	CS 929	71.3	37.3	3063

PAR was generated by Philips LLD 36W/840 reflex tubes suspended approximately 55 cm above the plants. PAR levels were kept low to minimise photoprotection and induction of antioxidative defences, i.e. to unmask UV-induced differences between accessions. UV-A radiation was generated by UV-A lamps (Philips Black light Blue TLD 36W/08). UV-B radiation was generated using Philips 36W/TL12 tubes. The small ultraviolet-C (UV-C) component that is generated by these lamps was filtered out using a cellulose acetate filter (thickness 95 µm; Kunststoff-Folien-Vertrieb GmbH, Hamburg, Germany). Radiation levels used in the present study were quantified with a spectroradiometer (USB2000, RAD, Ocean Optics). The dose of Biologically Effective UV (UV_{be}) radiation was calculated using the formula derived by Flint and Caldwell (2003). UV_{be} during growth (PAR + UV-A + UV-B condition) was 0.84 $kJm^{-2}d^{-1}$, in comparison, a typical biologically effective daily dose during clear sky summer conditions in the UK (latitude 53°N) is in excess of 24 kJ m^{-2} when calculated using Flint and Caldwell (2003) (Wargent et al., 2009). Temperatures were approximately 20°C and relative humidity ranged between 65 and 75%. The plants were maintained in the UV-B box under a similar 14h day/ 10h night cycle as used in the growth chamber.

To determine plant tolerance to UV-B, plants were exposed to a further, acute, 4 hour UV-B dose following 10 day growth under chronic UV. Detached leaves (young, fully expanded) were floated on water (adaxial site up) in open petri dishes and were exposed for 4 hour to UV-B radiation in the absence of PAR or UV-A (0.107 $mWcm^{-2}$; UV_{be} 3.46 $kJm^{-2}d^{-1}$).

Analysis photosynthetic efficiency

Young but fully expanded leaves were detached from plants raised for 10 days under one of the three different radiation regimes and the maximum photochemical efficiency of PSII (F_v/F_m) was measured following 20-25 min dark-adaptation of leaves. The maximum photochemical efficiency of leaves treated for a further four hours with acute UV-B was also determined using same procedure. The maximum photochemical efficiency of PSII (F_v/F_m) of plants was assessed using a modulated PAM (Imaging PAM, M-Series, Walz, Effeltrich, Germany) and calculated as $F_v/F_m = (F_m-F_0)/Fm$ (Krause and Jahns, 2003), where, F_m and F_0 are the maximum and minimum fluorescence, respectively. F_v represents variable fluorescence.

Analysis rosettes and extractable pigments

Levels of Chlorophyll-a (Chl-a), Chl-b, total chlorophyll (Chls), phenolics (Phe) and total carotenoids (Car) were measured following 10 days of growth under three different radiation regimes. For biochemical assays, 0.283 cm^2 of fresh leaf was used for extraction purposes. Both chlorophyll and carotenoids were extracted with methanol (MeOH: H_2O = 96: 4), while phenolics were extracted with acidified methanol [MeOH: H_2O: HCl (v/v) = 80: 19: 1] by incubating samples for 4 days in the dark at 4°C. Absorbance was determined spectrophotometrically (Genesis 10 series, Thermo Electron Scientific Instruments LLC, Madison, WI, USA) and pigments peaks were used to calculate the content of chlorophyll a, chlorophyll b and total carotenoid using the formulas of Lichtenthaler and Wellburn (1983). Absorbance at 330nm was taken as a proxy for total soluble phenolics (Mirecki and Teramura, 1984). Contents of total chlorophyll and carotenoid, and absorbance for total phenolics (i.e., 330 nm) were normalized on the basis of leaf area. Expressing pigment data on the basis of leaf weight does not substantially change results.

Following 10 days growth under PAR and UV, the rosette diameter (cm) of each plant was measured using a ruler. Two readings was taken per rosette and from opposite directions, after which the mean rosette diameter of each plant was calculated.

Statistical analysis

The experimental design consisted of three blocks each containing PAR, PAR + UV-A and PAR + UV-A + UV-B exposure treatments.

Statistical analyses of data were performed using analysis of variance (ANOVA) in the General Linear Model procedure of the SPSS package (version 18, SPSS, Chicago, IL, USA). The overall treatment effects (i.e., PAR, PAR + UV-A, PAR + UV-A + UV-B) on grouped accessions were tested using a nested ANOVA, while responses of individual accessions to different treatments as well as the responses of different accessions to each treatment were analysed separately using one-way ANOVA on the measured variables. Linear regression and Pearson's correlation of different variables with altitude were performed within the SPSS. Differences between treatments were considered significant if $P < 0.05$.

Results

Photosynthetic performance

Arabidopsis accessions were raised for 10 days under one of three distinct radiation regimes (PAR, PAR + UV-A or PAR + UV-A + UV-B) to study UV-acclimation. Following 10 days of growth, no macroscopic effects of UV-A or UV-B exposure were discernible, and the maximal quantum efficiency of photosystem II was found to vary between 0.77 and 0.80 (Figure 1a), values typically associated with healthy plants. Subsequent exposure of leaves to 4 hours acute high UV-B resulted in decreases of F_v/F_m values (Figure 1b). However, F_v/F_m values varied significant between accessions depending on the radiation regime under which plants were raised. Overall, plants raised under the PAR + UV-A + UV-B regime were least affected by acute UV-B (i.e. highest UV-B tolerance), while plants raised under a PAR-only regime displayed the largest decreases in F_v/F_m. Variations on this pattern can be observed for individual accessions. For example, Hog, Vind-1, Ang-0, Cvi-1, Bus-1, Mt-0 and Bur-0 raised under PAR + UV-A + UV-B all displayed statistically ($P < 0.001$) higher F_v/F_m values following exposure to acute UV-B than plants raised under PAR only. In contrast, Sha plants raised under PAR + UV-A + UV-B displayed a similar level of protection as plants raised under PAR-only, i.e. neither UV-A nor UV-B induced additional protection. UV-A increased protection in all tested accessions except Sha. Addition of UV-B to the PAR + UV-A mixture did not induce in additional UV-B protection in Hog and Ang-0, but increased the level of protection in Cvi-1 and Bur-0 significantly ($P < 0.001$ for both accessions).

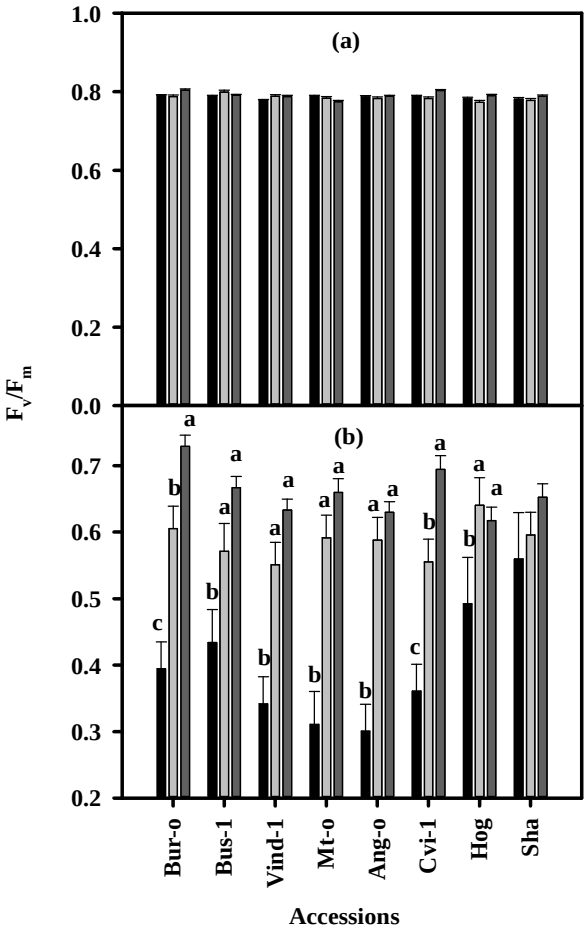

Figure 1. Photochemical efficiency (F_v/F_m) of *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days (a) and subsequently exposed to acute UV-B (0.35 Wm^{-2}) for 4 hours (b).

Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B growth conditions, respectively. The intensities of PAR, UV-A and UV-B during growth were 35 µmol m-2 s-1, 0.159 mWcm-2 and 0.026 mWcm-2 respectively. Following exposure to acute UV-B (b), Fv/Fm was higher in plants raised under PAR + UV-A + UV-B compared to either PAR or PAR + UV-A ($P<0.01$). Variations among accessions were significant under PAR ($P<0.05$) and PAR + UV-A + UV-B ($P<0.01$) but not under PAR + UV-A. For each individual accession, different letters denote significant differences ($P<0.01$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

Accumulation of UV-screening pigments

Levels of UV-screening pigments were determined in leaf extracts of accessions raised under different radiation conditions. Overall, plants raised under the PAR + UV-A + UV-B regime contained the highest levels of phenolics, while plants raised under a PAR-only regime displayed the lowest levels ($P < 0.001$). Variations on this pattern can be observed for individual accessions. The levels of phenolics varied significantly ($P < 0.05$) among *Arabidopsis* accessions raised under either PAR or PAR + UV-A. In contrast, all accessions were found to accumulate statistically similar levels of phenolics

under PAR + UV-A + UV-B (Figure 2). The induction of phenolics was found to be significantly ($P < 0.05$) altered by growth conditions (i.e. PAR, PAR + UV-A, PAR + UV-A + UV-B) in all accessions except Sha ($P = 0.510$) and Hog ($P = 0.216$) (Figure 2) which had similar levels of phenolics irrespective of growth conditions. In Vind-1 and Cvi-1 accumulation of phenolics was induced ($P < 0.001$) by growth under PAR + UV-A + UV-B. UV-A did not cause accumulation of phenolics in these two accessions, with statistically similar levels of phenolics in plants raised under PAR or PAR + UV-A. In contrast, substantial accumulation of phenolics was triggered by UV-A in Ang-0, and Bur-0 (both $P < 0.001$). The UV-A mediated increase in inducible total phenolics negatively correlated with altitude, i.e. the increase in phenolics content in plants raised under PAR + UV-A compared to those raised under PAR-only was negatively associated with the altitudes of origin in this small subset of *Arabidopsis* accessions (rho = 0.81, $P < 0.025$) (Figure 5b).

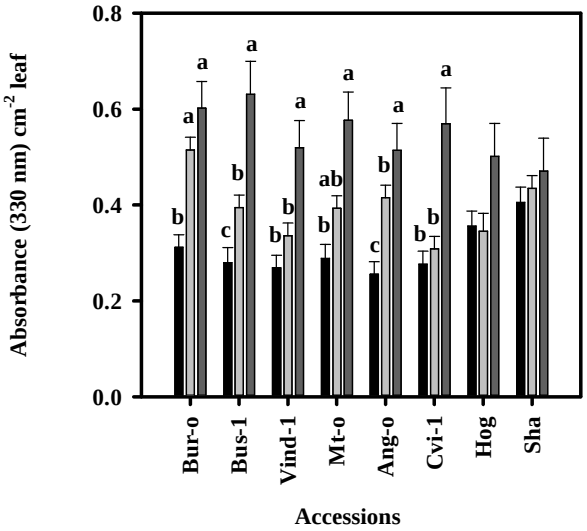

Figure 2. Induction of total phenolics (i.e., absorbance at 330 nm) in *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days.
Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B treatments, respectively. Plants raised under PAR + UV-A + UV-B contained more phenolics than those raised under PAR or PAR + UV-A ($P<0.01$). Variations in phenolic content between accessions were significant under PAR ($P<0.05$) and PAR + UV-A ($P<0.01$) but not under PAR + UV-A + UV-B. For each individual accession, different letters denote significant differences ($P<0.01$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

Photosynthetic pigments and carotenoids

Radiation conditions during growth had a significant ($P < 0.05$) effect on the levels of total chlorophyll and carotenoids in *Arabidopsis*. Overall, growth under PAR + UV-A increased the levels of total chlorophyll and carotenoids compared to levels in plants raised under PAR + UV-A + UV-B or just PAR, across all accessions ($P < 0.002$ and $P , < 0.001$, respectively) (Figure 3a,b). Significant ($P < 0.01$) UV-A induced increases in chlorophyll levels were noted for Sha and Hog. Levels of total carotenoids were significantly ($P < 0.05$) increased in Hog, Mt-0 and Bur-0 grown under PAR + UV-A compared to plants grown under PAR-only. Both Mt-0 and Bur-0 exhibited significantly ($P < 0.05$ and $P < 0.001$; respectively) lower levels of total carotenoids in plants exposed to PAR + UV-A + UV-B compared to plants raised under PAR + UV-A only.

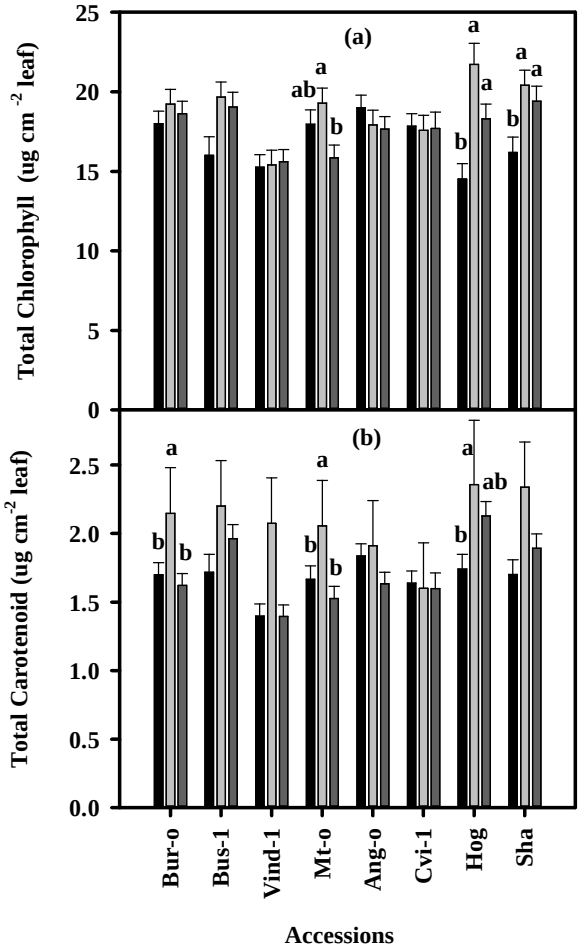

Figure 3. Levels of total chlorophyll and total carotenoids in *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days.
Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B treatments, respectively. Plants raised under PAR + UV-A contained more chlorophyll and carotenoids than those raised under PAR or PAR + UV-A + UV-B ($P<0.05$). Variations in chlorophyll among accessions were significant under PAR ($P<0.01$) under PAR + UV-A ($P<0.01$) and under PAR + UV-A + UV-B ($P<0.05$), but for carotenoids only under PAR + UV-A + UV-B ($P < 0.01$). For each individual accession, different letters denote significant differences ($P<0.05$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

Rosette Diameter

Rosette diameters were determined for accessions raised under different radiation conditions (Figure 4). Overall, plants raised under the PAR + UV-A regime had the greatest rosette diameter. Plants raised under PAR-only or under a PAR + UV-A + UV-B regime were considerably smaller ($P < 0.001$). Slight variations on this pattern can be observed for individual accessions. *Arabidopsis* accessions showed significant ($P < 0.001$) variation in rosette diameter when raised under PAR or PAR + UV-A + UV-B, although less under PAR + UV-A ($P = 0.726$). A significant negative association (rho = -0.67; $P < 0.025$) was noted between altitude of origin and rosette diameter of PAR-raised plants (Data not shown). Some inducible changes in rosette diameter were also associated with altitude. Thus, the change in rosette diameter of plants raised under PAR + UV-A, compared to these raised under PAR was positively associated with altitude (rho = 0.74, $P < 0.025$) (Figure 5c). There was also considerable (rho = -0.55, $P = 0.058$) negative association between the effect of UV-B and altitude (Figure 5i).

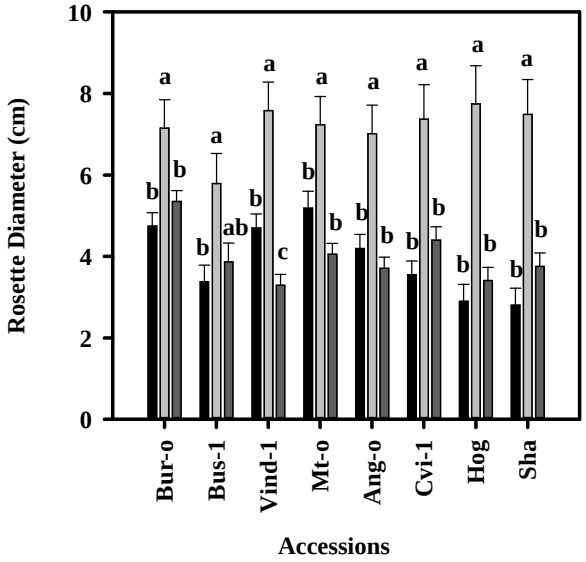

Figure 4. Rosette diameter of *Arabidopsis* accessions raised under PAR, PAR + UV-A or PAR + UV-A + UV-B for 10 days.

Black, light grey and dark grey bars denote PAR, PAR + UV-A, PAR + UV-A + UV-B treatments, respectively. Plants raised under PAR, or PAR + UV-A + UV-B were smaller than those raised under PAR + UV-A ($P<0.001$). Significant variations in rosette diameter across accessions were noted under PAR and under PAR + UV-A + UV-B ($P<0.001$) but not under PAR + UV-A. For each individual accession, different letters denote significant differences ($P<0.05$) between plants raised under the different growth conditions. Mean ± 1 SEM. n = 6-9.

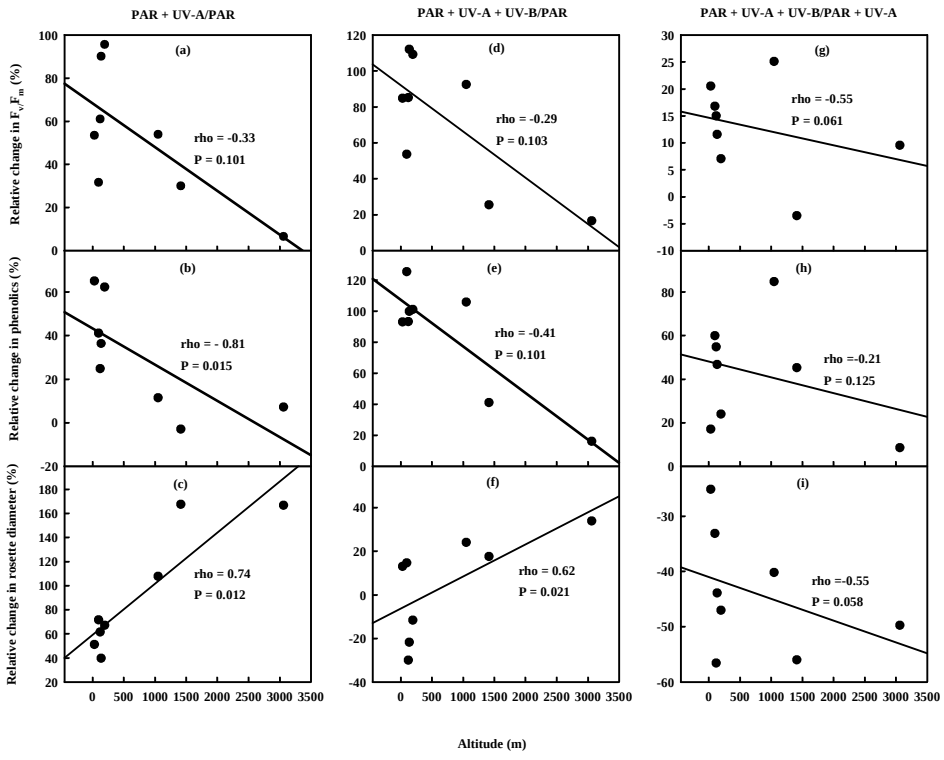

Figure 5. Relationship between altitude and changes in F_v/F_m (a, d, g), total soluble phenolics (b, e, h) and rosette diameter (c, f, i) among *Arabidopsis* accessions.

Comparisons were made between plants raised under PAR + UV-A relative to PAR (a, b, c), PAR + UV-A + UV-B relative to PAR (d, e, f) and PAR + UV-A + UV-B relative to PAR + UV-A (g, h, i). % relative changes were calculated as {(treatment – control)/control}*100. Fv/Fm was measured following exposure to acute UV-B. Mean ± 1 SEM. n = 6-9.

Table 2. Pearson's correlation between leaf physiological, biochemical and growth parameters and among different accessions raised under PAR + UV-A for 10 days.

Parameter	Phe	Rd	Chl-a	Chl-b	Chls	Car
F_v/F_m	0.36	0.28	0.81**	0.88***	0.84***	0.64*
Phe		-0.23	0.30	0.26	0.29	0.39
Rd			-0.14	0.20	-0.06	0.02
Chl-a				0.92***	0.99***	0.60
Chl-b					0.95***	0.71**
Chls						0.63*

Asterisks denote significance difference at *, P <0.1; **, P < 0.05 and ***, P < 0.01. Chlorophyll-a (Chl-a); Chlorophyll-b (Chl-b); total chlorophyll (Chls); phenolics (Phe); total carotenoids (Car); and rosette diameter (Rd).

Table 3. Pearson's correlation between leaf physiological, biochemical and growth parameters and among different accessions raised under PAR + UV-A + UV-B for 10 days.

Parameter	Phe	Rd	Chl-a	Chl-b	Chls	Car
F_v/F_m	0.66*	0.95***	0.19	0.43	0.25	-0.26
Phe		0.59	-0.04	0.07	0.01	-0.14
Rd			0.19	0.48	0.27	-0.22
Chl-a				0.93***	0.99***	0.78**
Chl-b					0.96***	0.59
Chls						0.75**

Asterisks denote significance difference at *, P <0.1; **, P < 0.05 and ***, P < 0.01.

Chlorophyll-a (Chl-a); Chlorophyll-b (Chl-b); total chlorophyll (Chls); phenolics (Phe); total carotenoids (Car); and rosette diameter (Rd).

Correlations among different parameters

Pearson's correlation coefficients were determined between UV-B protection (F_v/F_m) following acute UV-B exposure and physiological, biochemical and growth parameters across 8 accessions that had been grown for 10 days under PAR + UV-A or PAR + UV-A + UV-B (Table 2 and Table 3), respectively. The results indicate that UV-B protection of PSII (i.e. higher F_v/F_m) in plants raised under PAR + UV-A was significantly ($P < 0.1$) associated with the levels of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids, but not UV-absorbing phenolics. On the other hand, UV-B protection in plants raised under PAR + UV-A + UV-B significantly ($P < 0.1$) correlated with induction of total phenolics, but not with carotenoid levels.

Discussion

Induction of UV-B tolerance

Arabidopsis accessions raised for 10 days under various mixtures of PAR and UV radiation showed F_v/F_m values close to 0.80, which is in the range found in healthy, non-stressed plants. Many outdoor studies, using natural sunlight, also fail to show a UV effect on photosynthetic performance and growth (Ballaré et al., 2011). Generally, high levels of UV-B and/or low levels of accompanying PAR are required to impede PSII activity (Lud et al., 2003; Jansen et al., 2010). Here, despite the use of low levels of PAR, no damage to the photosynthetic machinery was measured in *Arabidopsis* grown under chronic, low UV-B (Figure 1a) indicating that damaging reactions were balanced by defence responses, i.e. the plants acclimated to the exposure conditions.

To assess the protective capacity of repair and acclimation responses we also measured F_v/F_m values in plants exposed for an additional 4 hours to acute high intensity UV-B. Under these extreme stress conditions, higher F_v/F_m values were interpreted as a greater protective capability, i.e. increased UV-B tolerance. The relative high UV-B tolerance in plants raised under PAR + UV-A + UV-B is consistent with previous reports that a key consequence of UV-exposure is the induction of UV-protection (Jansen et al., 2010; Ballaré et al., 2011). Interestingly, substantial UV-B protection is induced by UV-A radiation, emphasising the importance of solar UV-A for environmentally relevant assessments of the impacts of UV-B (Middleton and Teramura, 2003; Kotilainen et al., 2008).

The UV-B induced accumulation of phenolics is a key UV protection response that has been extensively demonstrated (de la Rosa et al., 2001). UV absorbing phenolics accumulate in vacuoles, cell walls, chloroplasts and even nuclei, and protect internal tissue of leaves and stem from UV-B radiation through their anti-oxidative capacity (Agati and Tattini, 2010). Accessions raised under PAR + UV-A + UV-B contained the highest

phenolic concentrations while the lowest levels of phenolics were noted in PAR-raised plants (Figure 2). UV-B tolerance in accessions raised under PAR + UV-A + UV-B is statistically associated with accumulation of total phenolics (Table 3). Substantial induction of total phenolics was also observed in accessions raised under PAR + UV-A (Figure 2). There seems to be considerable variation between species with respect to UV-A responses. Kotilainen et al. (2008) reported that in alder and birch specific phenolic metabolites are induced by either UV-A or UV-B, while in some cases there is even evidence for opposing effects of the two types of radiation. In contrast, in soybean (*Glycine max* L.), UV absorbing pigments are almost exclusively induced by UV-B, and not UV-A, wavelengths (Mazza et al., 2000). Levels of UV-absorbing metabolites in Scots pine (*Pinus sylvestris*) are mainly responsive to UV-A (Martz et al., 2007). It appears that some of this interspecific variation in regulation of phenolic accumulation, is present at the intraspecific level in *Arabidopsis*, thus offering scope for genetic analysis.

Notwithstanding the UV-A induced induction of phenolics in several *Arabidopsis* accessions, it appeared that there is no simple statistical association between levels of phenolics and UV-B protection of photosynthesis among the accessions raised under PAR + UV-A (Table 2). Rather, UV-B tolerance in PAR + UV-A-raised plants was significantly correlated with the levels of carotenoids, suggesting a role for these antioxidants in UV-B protection. This is consistent with work by Götz et al. (1999) who showed that the photosynthetic activities of genetically modified *Synechococcus* with higher levels of β-carotene and zeaxanthin are UV-B protected (Götz et al., 1999). It has also been shown that accumulated β-carotene in *Dunaliella bardawil* prevents UV-related photosynthetic damage through absorption of blue-light/ultraviolet-A radiation (White and Jahnke, 2002). The observed accumulation of carotenoids in plants exposed to UV-A, but not UV-B (Figure 3b), is consistent with literature reports (Jahnke, 1999; Mogedas et al., 2009). The decrease in carotenoid level in plants raised under PAR + UV-A + UV-B is less easily explained. However, Jansen et al. (2008) previously reported that UV-B mediated changes in carotenoid levels depend in a complex manner on plant species, developmental stage and UV-B dose.

UV-B mediated morphogenesis has been suggested to be a protective response with more dwarfed plants being less impacted upon by UV-B (Bogenrieder and Klein, 1982). Our data do not reveal an association between smaller rosettes and UV-B protection. In contrast, we found that a substantial increase in rosette diameter in plants raised under PAR + UV-A (Figure 4) was matched by an increase in UV-protection (Figure 1b). Furthermore, for accessions raised under PAR + UV-A + UV-B bigger rosettes were positively associated with higher F_v/F_m values after exposure to acute UV-B (Table 3) contradicting that dwarfing can simply be linked to UV-B protection.

Accession specific UV responses

We have shown substantial variation among *Arabidopsis* accessions in terms of responses to UV-A and UV-B, consistent with previous studies by Cooley et al. (2001) and Jansen et al. (2010). Protection from acute UV-B was constitutively expressed in Sha, but strongly induced in Vind-1, Ang-0, Bus-1, Mt-0, Bur-0 and Cvi-1. Analysis of individual accessions shows that the relationship between accumulation of UV-absorbing pigments and UV-B protection is complex. For example, Cvi-1 and Vind-1 raised under PAR + UV-A are considerably more UV-protected than the same accessions raised under PAR-only (Figure 1b). Yet, growth under PAR + UV-A does not result in significantly increased accumulation of UV-absorbing pigments in these two accessions (Figure 2). Vind-1 does, however, accumulate substantial amounts of carotenoids when raised under PAR + UV-A, and this may have contributed to UV-protection, although Cvi-1 displays minimal carotenoid induction under UV-A. Levels of UV-screening pigments are not significantly increased in Sha in response to growth conditions (Figure 2), matching the relatively constant expression of UV-B protection (Figure 1b). Nevertheless, Sha is capable of responding to both UV-A and UV-B as demonstrated by the accumulation of carotenoids (Figure 3) and the decrease in rosette diameter (Figure 4), respectively.

The observed phenotypic differences between accessions trigger the question of adaptive relevance. Herbivory studies have shown that the balance between constitutive and inducible protection is related to risk of attack, the extent of damage and/or cost of defence (Zangerl and Rutledge, 1996). Thus, the constitutive UV-B protection of Sha might reflect its high altitude origin and exposure to a relatively harsh climate including high levels of ambient UV. We also found that induction of total phenolics by UV-A is negatively related to altitude (Figure 5b). Thus, accessions from higher altitudes (Hog, Cvi-1 and Sha) display no, or limited, responses to UV-A.

This is surprising as these accessions would be the most UV-A exposed accessions in their high altitude habitat (Blumthaler et al., 1992).

Conclusion

Our data show that all tested *Arabidopsis* accessions achieve UV-protection when raised under PAR + UV-A + UV-B. The key finding is that the differences between accessions are not so much about protection per sé, but rather about the regulation of UV-protection. Given the complex regulatory mechanisms involved in flavonoid biochemistry (Koes et al., 2005) and anti-oxidant defences (Lidon et al., 2012), the presence of phenotypic differences in flavonoid accumulation and UV-protection should not come as a surprise.

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