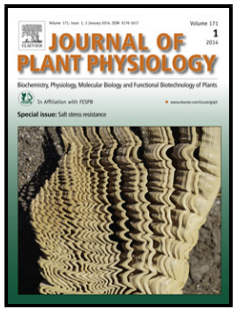
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Lutein-mediated photoprotection of photosynthetic machinery in *Arabidopsis thaliana* exposed to chronic low ultraviolet-B radiation

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Abstract

Ecologically relevant low UV-B is reported to alter reactive oxygen species metabolism and anti-oxidative systems through an up-regulation of enzymes of the phenylpropanoid pathway. However, little is known about low UV-B-induced changes in carotenoid profile and their impacts on light harvesting and photoprotection of photosystem II (PSII) in plants. We investigated carotenoids profile, chlorophyll pigments, phenolics, photosynthetic efficiency and growth in *Arabidopsis thaliana* (Col-0) plants grown under photosynthetically active radiation (PAR), PAR+ ultraviolet (UV)-A and PAR+UV-A+B regimes for 10 days in order to assess plant acclimation to low UV-B radiation. A chlorophyll fluorescence assay was used to examine UV-B tolerance in plants further exposed to acute high UV-B for 4 and 6 hours following a 10-day growth under different PAR and UV regimes. We found that both PAR+UV-A and PAR+ UV-A+ B regimes had no negative effect on quantum efficiency, electron transport rate, rosette diameter, relative growth rate and shoot dry weight of plants. Chronic PAR+UV-A regime considerably ($P < 0.05$) increased violaxanthin (26%) and neoxanthin (92%) content in plants. Plant exposure to chronic PAR+UV-A+B significantly ($P<0.05$) increased violaxanthin (48%), neoxanthin (63%), lutein (33%), 9-cis ß-carotene (28%), total ß-carotene (29%) and total phenolics (108%). The maximum photochemical efficiency (F_v/F_m) in leaves was found to be positively correlated with total phenolics (rho = 0.81 and rho = 0.91, $P<0.05$ for 4 and 6 hours, respectively) and non-photochemical quenching (q_N) (rho = 0.81 and rho = 0.84, $P<0.05$ for 4 and 6 hours, respectively) in plants exposed to acute high UV-B for 4 and 6 hours following a 10-day growth under chronic PAR+UV-A+B. There was also a significant positive correlation (rho = 0.93, $P<0.01$) between q_N and lutein content in the plants exposed to acute high UV-B stress for 4 hours following plant exposure to chronic PAR+UV-A+B. The findings from our study indicate that plants grown under chronic PAR+UV-A+B displayed higher photoprotection of PSII against acute high UV-B stress than those grown under PAR and PAR+UV-A regimes. An induction of phenolics and lutein-mediated development of q_N were involved in the photoprotection of PSII against UV-B-induced oxidative stress.

Keywords: *Arabidopsis thaliana*; carotenoids; lutein; phenolics; photoprotection; chl *a* fluorescence; UV-B acclimation; UV-B tolerance

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1. Introduction

Carotenoid pigments play important roles in light harvesting as well as protection of photosystem II (PSII) by quenching reactive oxygen species and dissipation of excess energy as heat via non-photochemical quenching (Asada, 1999). In the conditions of excess light, reversible de-epoxidation of violaxanthin to zeaxanthin occurs as a means of photoprotection through non-photochemical quenching. Conversely, zeaxanthin is reversibly converted to violaxanthin, which potentially functions as a light-harvesting antennae pigment under reduced light conditions (Demmig-Adams and Adams, 2006). Light-dependent conversion of epoxylutein to lutein has similar photoprotective functions by quenching reactive oxygen species and dissipation of excess energy, but this reaction has been less extensively studied (Bungard et al., 1999; Latowski et al., 2004). Furthermore, little is known about low UV-B-induced changes in the content and composition of carotenoids and their impacts on light harvesting and photoprotection of PSII in plants.

Plant response to UV radiations depends on the nature of UV-B treatments and the extent of plant adaptation and acclimation to UV-B (Jenkins, 2009; Biswas and Jansen, 2012). Studies have shown that the ratio of total carotenoids to chlorophyll may decrease (Jansen et al., 2008; Carletti et al., 2009) or increase in the UV-B-exposed plants (Xiong and Day, 2001; Jansen et al., 2008). The level of ß-carotene is generally found to be increased and decreased in the plants exposed to UV-A and high UV-B, respectively (White and Jahnke, 2002; Carletti et al., 2009). Similarly, plant exposure to UV-A alone increases the level of total carotenoids, while plant exposure to low UV-B decreases total carotenoid levels in some *Arabidopsis* ecotypes (Biswas and Jansen, 2012). Violaxanthin is known to be more sensitive to high UV-B radiation, compared to other xanthophyll pigments (Pfundel et al., 1992; Joshi et al., 2007; Lidon et al., 2012). Plant exposure to a moderate dose of UV-B (8.35 kJ m^{-2} day^{-1}) for three days results in a decreased level of zeaxanthin and ß-carotene (Jansen et. al., 2008). It has also been reported that high UV-B may cause a blockage in the functioning of violaxanthin de-epoxydase (i.e., light depended conversion of violaxanthin to zeaxanthin), indicating a possibility of limited role of xanthophyll zeaxanthin in protection of PSII under UV-B stress (Joshi et al., 2007). Available reports indicate that although advances have been made in understanding the impacts of high UV-B dose on carotenoids (Pfundel et al., 1992; Joshi et al., 2007; Jansen et. al., 2008), little attention has been paid to the ecologically relevant low UV-B-induced changes in carotenoid profile and their roles in photoprotection of PSII and UV-B tolerance in plants.

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Available research report on the effects of low UV-B on *Arabidopsis thaliana* demonstrates a reduction in rosette diameter and inflorescence length, but an increase in the numbers of flowering stems, indicating that chronic low UV-B treatment mostly causes redistribution of resources rather than cessation of growth (Hectors et al., 2007). Although low UV-B dose has no significant effect on growth, it can alter reactive oxygen species (ROS) metabolism through an up-regulation of enzymes of the phenylpropanoid pathway and anti-oxidative systems (Brown and Jenkins, 2008; Jenkins, 2009; Hideg et al., 2013). This may lead to changes in the content, composition and functions of carotenoids including xanthophyll cycle pigments, and hence plant UV-B tolerance (Biswas and Jansen, 2012). We therefore hypothesized that low UV-B might alter carotenoid profile, which can modulate light harvesting and photoprotection of PSII in plants. The low UV-B conditions are defined in this paper as conditions that do not cause a decrease in growth and photosynthetic efficiency, but do drive acclimation, i.e. phenolic accumulation and morphogenesis (Biswas and Jansen, 2012). In addition to low UV-B, a low PAR is also used in the present study as high-PAR intensities are reported to modify changes in gene expression and accumulation of phenylpropanoids induced by UV-B radiation (Rossel et al., 2002; Kaffarnik et al., 2006). The results from this study will be valuable in the understanding of plant acclimation to low UV-B and the role of specific carotenoid in light harvesting, photoprotection of PSII and plant UV-B tolerance.

2. Materials and methods

2.1. Plant material, growth conditions and radiation treatments

The experiment was conducted at the plant growth facility at the School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland. *Arabidopsis thaliana* (Col-0) was selected to examine induction of carotenoids and their role in UV-protection under low UV-B. 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 using in the present experiment. Seeds were germinated on MS plates following sterilization. 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.). Plants were grown for 7 days in a growth chamber under 80 µmol m^{-2} s^{-1} PAR. The growth room was maintained at 20/17 ^{o}C under a 14/10-h light/dark cycle and a relative humidity of 75%.

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Plants were raised for further 10 days under different PAR and UV regimes (i.e., PAR (35 µmol m^{-2} s^{-1}), PAR (35 µmol m^{-2} s^{-1}) + UV-A (0.159 $mWcm^{-2}$) and PAR (35 µmol m^{-2} s^{-1}) + UV-A (0.159 $mWcm^{-2}$) + UV-B (0.026 $mWcm^{-2}$) after 7 days of establishment. PAR was generated by Philips LLD 36W/840 reflex tubes suspended approximately 55 cm above the plants. PAR levels were kept low to minimize photoprotection and induction of anti-oxidative defenses in order to unmask UV-induced protection in plants. UV-A radiation was generated by UV-A lamps (Philips Black light Blue TLD 36W/08) and UV-B radiation was generated using Philips 36W/TL12 tubes. The small amount of ultraviolet-C (UV-C) component that was 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 INC, FL, USA). The dose of biologically effective UV (UV_{be}) radiation was calculated using the formula derived by Flint and Caldwell (Flint and Caldwell, 2003). The level of UV_{be} during growth under PAR + UV-A+B treatment was 0.84 kJ $m^{-2}d^{-1}$ (Fig. 1), which was a typical biologically effective daily dose during clear sky summer conditions in the UK (latitude 53◦N) (Wargent et al., 2009). The plants were maintained in the UV-B box under a similar 14 h day/10 h night cycle and temperatures as used in the growth chamber. To determine plant tolerance to UV-B, plants were further exposed to an acute high UV-B dose following 10-day growth under different PAR and UV regimes. Detached leaves (young and fully expanded) were floated on water (adaxial site up) in open petri dishes and were exposed to UV-B (0.107 $mWcm^{-2}$; UV_{be} 3.46 kJ $m^{-2}d^{-1}$) radiation for 4 and 6 hours in absence of PAR and UV-A. The experiment consisted of two runs, which was carried out continuously by adjusting planting dates.

2.2. Analysis of photosynthetic efficiency

The photosynthetic efficiency was determined on young, but fully expanded detached leaves from the plants raised at three different chronic radiation regimes and the leaves exposed to acute high UV-B stress for 4 and 6 hours following a 10-day growth under chronic radiation treatments with a modulated Imaging PAM (M-Series, Walz, Effeltrich, Germany). Both dark and light adapted leaves were used for chlorophyll fluorescence assay. The maximum photochemical efficiency of PSII (F_v/F_m) was measured following at least 30 min dark-adaptation of leaves. The minimum fluorescence (F_0) was determined with modulated light, which was sufficiently low (<1 µmol m^{-2} s^{-1}), so as not to induce any significant variable fluorescence. The maximum fluorescence (F_m) was determined using a 0.8 s saturating pulse

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at 4950 µmol m^{-2} s^{-1}. After dark-adapted fluorescence measurements, the leaf was continuously illuminated with actinic light at the intensity of 150 µmol m^{-2} s^{-1}. The steady state fluorescence (F_s) was reached within 3 minutes then a saturating pulse was imposed to determine the maximum fluorescence in the light-adapted state (F_m'). The minimum fluorescence in the light-adapted state (F_0') was determined during a brief interruption of actinic illumination in the presence of far-red illumination. After recording the fluorescence key parameters in both dark and light-adapted state, we calculated: (1) variable fluorescence, $F_v = F_m - F_0$, (2) maximum photochemical efficiency in the dark-adapted state, F_v/F_m (Krause and Weis, 1991), (3) Actual yield = $(F_m' - F_0')/F_m'$, (4) photochemical quenching coefficient, $q_P = (F_m' - F_s)/(F_m' - F_0')$, (5) non-photochemical quenching coefficient, $q_N = 1-(F_m' - F_0')/(F_m - F_0)$ (Van Kooten and Snel, 1990) and electron transport rate, ETR = yield × PAR × 0.5 × 0.85 (Biswas and Jiang, 2011).

2.3. Determination of phenolics

The content of total phenolics in leaves was measured following 10 days of growth under three different radiation regimes. Fresh leaf sample (0.283 cm^2) was collected from young, but fully expanded leaves using a cork borer. The amount of total soluble phenolics was extracted with acidified methanol [MeOH: H_2O: HCl (v/v) = 80: 19: 1] by incubating samples for 4 days in the dark at 4ºC. Absorbance at 330 nm was taken as a proxy for total soluble phenolics (Mirecki and Teramura, 1984).

2.4. Carotenoids and chlorophyll extraction and analysis

Rosette leaves of each plant were harvested from three different chronic PAR and UV regimes, and immediately were frozen in liquid nitrogen and transferred to an ultra-freezer at -80 ºC until the time of assay. Frozen leaf samples (approx. 1.5 g) were ground in a mortar prechilled with liquid nitrogen placed on ice and were homogenized with hexane/ethanol/acetone (50:25:25, vol/ vol/vol) containing 0.1% BHT. The mixture was incubated for 10 min and centrifuged in a Sorvall TC6 (DuPont Instruments, Herts, UK) at 3000×*g* for 5 min at 4 ◦C. A recovery standard consisting of either β-Apo-8′- carotenol or lycopene was added to all samples, which were extracted three times with hexane/ethanol/acetone (50:25:25, vol/ vol/vol). The supernatant layers were removed, pooled, and dried under nitrogen gas. The residues were reconstituted in 100 µL of mobile phase. The contents of carotenoids and chlorophyll were determined using the methodology of Hart and Scott (1995) with a reverse-phase high pressure liquid chromatography (HPLC)

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(Shimadzu, Kyoto, Japan) at the Department of Food and Nutritional Sciences, University College Cork, Cork, Ireland. The HPLC system consisted of a LC10-AD pump connected to a SIL-10A auto-injector and SPD-10AV UV-visible detector. The column system consisted of a Spherisorb ODS-2 C18 5µm PEEK guard column (Alltech Associate Applied Science Ltd., Lancs, UK) connected to a Vydac 201TP54 (250 × 4.6 mm) reversed phase C18 column (supplied by AGB Scientific Ltd, Dublin, Ireland). Column temperature was maintained at 28°C by an internal column oven. The injection volume was 50 µL and the samples were eluted using isocratic mobile phase composed of acetonitrile/methanol/dichloromethane (75:20:5, vol/vol/vol) containing 10 mmol/L ammonium acetate, 4.5 mmol/L butylated hydroxytoluene, and 3.6 mmol/L triethylamine at a flow rate of 1.5 mL/min. Carotenoids were detected at 450 nm. The mobile phase was filtered through a 0.5-µm organic filter and degassed using ultrasonic agitation. Results were collected and analyzed using ChromQuest software (version 4.2, Thermo Fisher Scientific). The concentration of specific carotenoids was determined from the standard curves and expressed as µg mg^{-1} total chlorophylls. Lutein, lycopene, and β-Apo-8′-carotenol recovered from all analyzed samples were extrapolated from the pure carotenoid standard curves after correction for extraction efficiency based on recovery of lycopene or β-Apo-8′- carotenol then they were quantified after correction for initial sample weight and dilution factors.

2.5. Growth analysis

Plants were sampled for initial dry weight of shoot on the day of start of chronic radiation treatments. Following 10 days growth under different PAR and UV regimes, the rosette diameter (cm) of each plant was measured using a ruler. Two readings was taken per rosette and from opposite directions then the mean rosette diameter of each plant was calculated. After measurement of rosette diameter, plants were harvested for above-ground shoot biomass, which was dried to constant weight in an oven at 72 ^{o}C for 7 days. Relative growth rate of shoots was calculated as described by Hunt (1990).

2.6. Statistical analysis

The experiment consisted of two runs (i.e. two blocks) and each run included chronic PAR, PAR + UV-A and PAR + UV-A+B treatments. Data from two runs were checked for homogeneity of variance then they were combined for statistical analysis. Statistical analysis of data was performed using analysis of variance (ANOVA) in the General Linear Model procedure of the SPSS package (version 18, SPSS, Chicago, IL, USA). A Tukey comparison

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of means was performed when the F-test showed significant at $P \leq 0.05$. Associations between different parameters were examined using non-parametric method of correlation by determining Spearman's Rank correlation coefficient (rho).

3. Results

3.1. Growth and photosynthetic performance

Chronic radiation treatments had no significant effect on relative growth rate (RGR), rosette diameter and shoot dry mass (Table 1). A 10-day growth under different PAR and UV regimes also showed no visible effect on dark-adapted fluorescence parameters such as F_0, F_m, F_v and F_v/F_m (Table 1). However, the plant exposed to chronic radiation treatments exhibited a significant ($P < 0.05$) effect on light-adapted fluorescence parameters. For example, plants exposed to both PAR +UV-A and PAR+UV-A+B had significantly higher F_v'/F_m' than those exposed to chronic PAR (Fig. 3). Plants raised at both chronic PAR and PAR+ UV-A+B regimes displayed higher q_N than those raised at PAR+UV-A. Plants grown under both PAR+UV-A and PAR+UV-A+B showed a statistically higher ETR than those grown under PAR-alone.

The acute high UV-B stress for 4 hours following plant exposure to chronic radiation treatments resulted in the highest F_0 in plants raised at PAR and the lowest F_0 in the plants grown at PAR+UV-A+B. Exposure to acute UV-B for 4 hours also showed higher F_m, F_v and F_m/F_v in plants exposed to PAR+UV-A+B than in those exposed to both PAR+UV-A and PAR regimes (Fig. 2). The acute high UV-B for 6 hours displayed higher F_m, F_v and F_v/F_m in the plants raised at PAR+UV-A+B than in those raised at PAR+UV-A and PAR. Acute high UV-B treatment for both 4 and 6 hours showed significant effect on F_v'/F_m', q_N and ETR, but had no effect on q_P (Fig. 3). Acute high UV-B for both 4 and 6 hours following chronic radiation treatments resulted in higher levels of F_v'/F_m', q_N and ETR in the plants grown under PAR+UV-A+B than in those grown under both PAR+UV-A and PAR, which displayed a statistically similar effect on those parameters.

Analysis of data showed that chronic radiation treatments contributed to the development of UV-B tolerance of photosynthetic machinery in plants in terms of F_v/F_m, F_v'/F_m' and ETR as assessed by acute high UV-B stress (Fig. 4). However, the magnitude of relative UV-B tolerance was found to be varied in plants exposed to different chronic radiation treatments.

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For instance, plants raised under PAR+UV-A+B showed higher levels of F_m, F_v, F_v/F_m, F_v'/F_m', q_N and ETR than those raised under both PAR and PAR+UV-A treatments.

3.2. Accumulation of UV-screening compound

Chronic radiation treatments showed significant effects on the level of total phenolics with remarkably ($P < 0.001$) higher levels of total phenolics in plants under PAR+UV-A+B than both under PAR+UV-A and PAR regimes (Table 1). Data analysis also indicated a slight increase in the level of total phenolics in plants raised at PAR+UV-A (+9%), but a dramatic increase in total phenolics content in the plants grown at PAR + UV-A+B (+118%), relative to the PAR-alone (Fig. 4).

3.3. Photosynthetic pigments and carotenoids

Chronic radiation treatments displayed a significant effect only on Chl *a*, but not on Chl *b*, Chl *a*/Chl *b* and total chlorophyll (Table 1). Plants exposed to both chronic PAR and PAR+UV-A resulted in an higher level of Chl *a* than those exposed to PAR+UV-A+B. Chronic radiation treatments had significant effects on the levels of violaxanthin, xanthophyll cycle pool size (V+A+Z), neoxanthin, lutein, 9-cis ß-carotene, total ß-carotene, but had no effect on the levels of antheraxanthin and epoxidation state (EPS) (Table 1). The level of zeaxanthin was undetectable in plants raised under different chronic radiation treatments. Plants grown at both PAR+UV-A and PAR+UV-A+B showed significantly higher levels of violaxanthin and neoxanthin than those grown at PAR-alone. The level of xanthophyll cycle pool size in the plants was significantly greater for PAR+UV-A+B than for either PAR +UV-A or PAR treatments. Plants exposed to both PAR and PAR+UV-A regimes had a similar level of xanthophyll cycle pool size. The levels of lutein, 9-cis ß-carotene and total ß-carotene in plants were significantly higher in PAR+UV-A+B than in both PAR and PAR+UV-A treatments. The plants exposed to both PAR and PAR+UV-A regimes showed statistically similar levels of lutein, 9-cis ß-carotene and total ß-carotene. Data analysis displayed a lower relative decrease in the level of Chl *a* in the plants exposed to PAR+UV-A (-11%) than in those exposed to PAR+UV-A+B (-23%), compared to PAR-alone (Fig. 4). While both PAR +UV-A and PAR +UV-A+B treatments showed an increase in the levels of violaxanthin, xanthophyll cycle pool size, neoxanthin, lutein and total ß-carotene increased in plants, the greater increases were observed in PAR +UV-A+B than in the PAR+UV-A regime. Both antheraxanthin and 9-cis ß-carotene were found to be decreased and increased in the plants grown under PAR+UV-A and PAR+UV-A+B, respectively, relative to PAR alone.

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3.4. Correlations between different biochemical and physiological parameters

The values of F_v/F_m in leaves exposed to acute high UV-B stress for 4 hours were positively correlated (rho = 0.71, P<0.05) with the total phenolics in plants raised at chronic PAR+UV-A. The F_v/F_m values in leaves exposed to acute high UV-B for both 4 and 6 hours were also found to be significantly correlated with total phenolics in plants grown under chronic PAR+ UV-A+B (Fig. 5). A significant positive association (rho = 0.93, P<0.01) was detected between q_N and lutein content in the plants exposed to acute high UV-B for 4 hours following a 10-day growth under PAR+UV-A+B (Fig. 6). There were also positive correlations between F_v/F_m values and q_N in the leaves exposed to acute high UV-B for both 4 and 6 hours following plants grown under chronic PAR+UV-A+B regime.

4. Discussion

Plant exposure to chronic radiation treatments (i.e., PAR, PAR+UV-A and PAR+UV-A+B) for 10 days showed that plants raised under PAR and UV regimes had a F_v/F_m value close to 0.80, which is in the range found in healthy, non-stressed plants. High levels of UV-B and/or low levels of accompanying PAR are usually required to impede PSII activity (Lud et al., 2003; Jansen et al., 2010). In this study, despite the use of low level of PAR, no damage to the photosynthetic machinery was observed in plants grown under chronic low UV-B. This indicates that damaging reactions might have been balanced with the defense responses (i.e. the plants appeared to have been acclimated to the exposure conditions) (Jansen et al., 2008). This was further evidenced by a higher level of actual yield (F_v'/F_m') in the plants raised under PAR+UV-A and PAR+UV-A+B regimes than in those grown under PAR-only.

To assess plant UV-B acclimation response, we measured phenolics inductions, carotenoid profile and photosynthetic performance in terms of chlorophyll fluorescence in plants after the end of chronic radiation treatments. Moreover, we measured maximum photochemical efficiency along with quenching parameters and electron transport rate in the leaves exposed to acute high UV-B for 4 and 6 hours following chronic radiation treatments to examine plant UV-B tolerance. We found that despite no visible UV-B stress in terms of maximum photochemical efficiency, plants raised under PAR + UV-A+B had higher level of total phenolics than those raised under both PAR-only and PAR+UV-A regimes. The observed high level of total phenolics induction by low UV-B suggests a clear UV-B acclimation in plants (Biswas and Jensen, 2008). The UV-B absorbing phenolics accumulate in plants and protect cellular components from UV-B radiation through their anti-oxidative capacity

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(Jansen et al., 2008; Agati and Tattini, 2010). However, there was no induction of phenolics in plants raised under PAR+UV-A regime in our study as found in some Arabidopsis ecotypes and soybean plants exposed to UV-A radiation (Mazza et al., 2000; Biswas and Jensen, 2012).

Carotenoids protect light harvesting core complex, photosystem II against oxidative stress by multiple mechanisms including quenching of chlorophyll triplet states, scavenging of both superoxide and hydroxyl radicals and quenching of singlet oxygen, and therefore prevent protein oxidation and lipid peroxidation (Niyogi et al., 1997; Telfer, 2005). We found that plants raised under both PAR+UV-A and PAR+UV-A+B regimes showed higher level of violaxanthin and neoxanthin than those raised under PAR-only. An increased level of violaxanthin in plants grown at the two chronic radiation treatments indicates either a lower requirement of the enzymatic conversion of violaxanthin to zeaxanthin as excitation pressure might be minimum due to low UV-B or an efficient UV-B screening with phenolics. The high violaxanthin content in the plants exposed to low UV-B might result from a blockage in the functioning of violaxanthin de-epoxidase (Joshi et al., 2007). Plant exposure to acute high UV-B stress for 4 hours (4H) following chronic radiation treatments indicated an occurrence of photoinhibition of PSII in plants raised under both PAR and PAR+UV-A due to an increase in non-radiative thermal deactivation as documented by an increase in F_0 (Biswas et al., 2013). On the other hand, plant exposure to acute high UV-B for 6 hours (6H) resulted in an occurrence of damage to the PSII reaction centers as evidenced by a significant decrease in F_m (Biswas et al., 2013) in the plants exposed to both chronic PAR and PAR+UV-A. This implies that high accumulation of violaxanthin and neoxanthin along with no induction of phenolics failed to show tolerance in the plants exposed to acute high UV-B stress following an exposure to chronic PAR+UV-A in terms of both F_v/F_m and F_v'/F_m'. The results also indicated a potential role of phenolics in plant UV-tolerance, but a little or no involvement of the two xanthophyll cycle pigments in plant UV-B protection. Our results are consistent with the findings of Niyogi et al. (1997), which demonstrate that violaxanthin and neoxanthin are less efficient in quenching of triplet chl and scavenging of singlet oxygen than zeaxanthin, antheraxanthin and lutein. It should be noted that the zeaxanthin was undetectable in the leaf samples from all three radiation regimes, although plants raised under PAR+UV-A+B showed higher level of xanthophyll cycle pool size (violaxanthin and antheraxanthin) than in those raised under PAR-alone and PAR+UV-A regimes. However, an increase in xanthophyll cycle intermediate, antheraxanthin in the plants grown under PAR+UV-A+B relative to PAR+UV-A along with a lack of zeaxanthin, confirms a blockage inhibiting conversion of zeaxanthin

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from antheraxanthin in the plants grown under chronic low UV-B. The results are in agreement with earlier reports (Pfundel et al., 1992; Joshi et al., 2007) that demonstrate a negative impact of high UV-B on the functioning of violaxanthin de-epoxidase cycle, which results in an accumulation of antheraxanthin and reduction in the level of zeaxanthin under high irradiance levels. This indicates that both low and high UV-B had detrimental impacts on the conversion of zeaxanthin from antheraxanthin. The results also imply that an increased UV-B protection of PSII in plants raised under chronic low UV-B treatment was not essentially related to xanthophyll zeaxanthin in our study and even in the study with high UV-B treatment (Finazzi et al., 2004). On the other hand, we found that plants raised under PAR+UV-A+B accumulated higher level of lutein than those raised under PAR-only and PAR+UV-A regimes. It has been documented that lutein can replace zeaxanthin in photoprotection in shade canopies and has similar potential as like zeaxanthin in development of non-photochemical quenching and protection of PSII against photodamage (Esteban et al., 2010). A significant higher level of lutein and non-photochemical quenching (q_N) in the plants grown under PAR+UV-A+B than those grown under PAR and PAR+UV-A, suggests an enhanced UV-B photoprotection of PSII in plants grown under chronic low UV-B radiation. The lutein-mediated enhanced UV-B protection of PSII via development of non-photochemical quenching can be further confirmed by a significant positive association between q_N and lutein content in the plants exposed to acute high UV-B for 4 hours following a 10-day growth under chronic PAR+UV-A+B (Fig. 6). However, no such correlation was observed in the plants exposed to acute high UV-B for 6 hours following chronic PAR+UV-A+B treatment. This suggests that lutein-mediated UV-B protection was limited by higher acute UV-B dose.

The effect of UV-B on the level of ß-carotene has been reported as conflicting conclusions including an increase, decrease and no change in ß-carotene in plants, depending on UV-B dose, exposure duration and plant species (Carletti et al., 2003; Joshi et al., 2007). The levels of 9-cis ß-carotene and total ß-carotene in plants exposed to chronic PAR+UV-A+B were significantly higher compared to those raised under PAR and PAR+UV-A regimes. High accumulation of ß-carotene in plants exposed to chronic low UV-B as found in our study might be contributed to additional UV-B protection of PSII through an enhanced level of quenching of triplet chl and scavenging of singlet oxygen (Telfer, 2005). This can be further explained by a lack of accumulation of ß-carotene in plants exposed to both chronic PAR and PAR+UV-A regimes, which resulted in an oxidative stress to PSII due to acute high UV-B

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stress. The results are consistent with a report of Götz et al. (1999), which showed that the photosynthetic efficiency of genetically modified *Synechococcus* with higher levels of β-carotene was UV-B protected. The effects of UV-A on plant have been reported as both damaging (White and Jahnke, 2002) and non-damaging (Joshi et al., 2007). We found that plants grown at PAR+UV-A slightly had higher accumulation of neoxanthin and a reduction in Chl *a* than those grown at PAR-only, suggesting that neoxanthin might be involved in photoprotection against UV-A-induced oxidative stress (Niyogi et al., 1997). High UV-B treatment generally results in a loss of photosynthetic pigments due to UV-B-induced oxidative stress (Deckmyn and Impens, 1996; Joshi et al., 2007). A significant reduction in Chl *a* and increase in the level of lutein and ß-carotene in plants raised under PAR+UV-A+B than in those raised under PAR+UV-A and PAR-only suggested an increased UV-B-induced oxidative stress and photoprotection mediated by lutein and ß-carotene. Plants raised under PAR+UV-A+B showed higher F_v/F_m, F_v'/F_m' and ETR values than those raised under both PAR-only and PAR+UV-A in response to acute high UV-B stress. The results also indicate that plants raised under PAR+UV-A+B had higher photoprotection of PSII as documented by higher dissipation of excess energy as heat via non-photochemical quenching (q_N) compared to those raised under both PAR and PAR+UV-A regimes. This can be further explained by a significant positive association between F_v/F_m and q_N in the leaves exposed to acute high UV-B for both 4 and 6 hours following plant exposure to chronic PAR+UV-A+B regime (Fig. 6). The results indicate that an induction of phenolics and lutein mediated development of q_N contributed to higher UV-B protection of PSII in plants raised under PAR+UV-A+B than in those raised under PAR and PAR+UV-A regimes.

5. Conclusions

The exposure of plants to chronic low UV-B for 10 days had no adverse effect on PSII function and plant growth. Despite a lack of visible UV-B stress, plants showed differential acclimation responses to chronic radiation treatments in terms of induction of phenolics and carotenoid profile. Plants raised at PAR+UV-A+B showed higher induction of total phenolics than those raised at both PAR+UV-A and PAR-only regimes. A higher accumulation of violaxanthin and neoxanthin was observed in plants grown under PAR+UV-A and PAR+UV-A+B than in those grown under PAR-only. The levels of lutein, 9-cis ß-carotene and total ß-carotene were found to be increased in plants raised under PAR+UV-A+B than in those raised under PAR and PAR+UV-A. Plant UV-B tolerance as determined by plant exposure to acute high UV-B stress for 4 hours following chronic radiation treatments indicated an occurrence

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of photoinhibition of PSII due to an increase in a non-radiative thermal deactivation as documented by an increase in F_0 for the plants raised under both PAR and PAR+UV-A regimes. On the other hand, plant exposure to acute high UV-B for 6 hours following plant exposure to chronic PAR and PAR+UV-A regimes resulted in an occurrence of damage to the PSII reaction centers as evidenced by significant decrease in F_m in those plants. The results also indicated that UV-A-induced higher accumulations of violaxanthin and neoxanthin along with low induction of phenolics were not associated with plant UV-B protection. Besides, plants exposed to acute high UV-B stress following a 10-day growth under PAR+UV-A+B displayed an improved UV-B tolerance of PSII in terms of F_v/F_m, F_v'/F_m', q_N and ETR mediated by total phenolics and lutein, but not by the xanthophyll cycle pigments. Taken together, the findings of this study indicate that xanthophyll cycle pigments were not involved in plant UV-B protection, but induction of phenolics and lutein-mediated development of q_N were involved in photoprotection of PSII in the plants against UV-B-induced oxidative stress.

Authors Statement

DKB conceived and conducted research, analyzed data and wrote the manuscript; BLM, XH, YGL and GMJ commented and revised the manuscript.

Declaration of Competing Interest

None. We state that all the authors agree for the submission of this paper and that we have no undeclared competing financial interests.

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Figure Legends

Fig. 1. Biologically Effective UV-B (BE UV-B, $Wm^{-2}nm^{-1}$) of three chronic (PAR, PAR+UV-A and PAR+UV-A+B) and acute UV-B radiation treatments used in the present study.

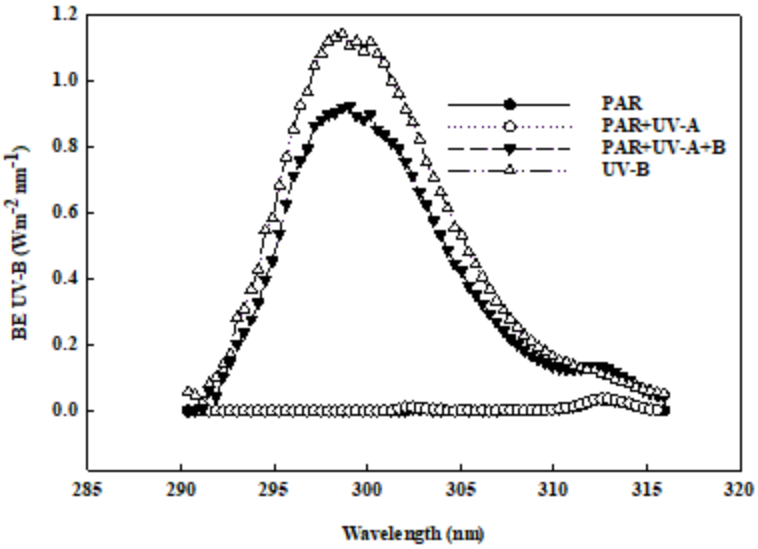


Fig. 2. Minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum photochemical efficiency of PSII (F_v/F_m) in the recently developed leaves of *Arabidopsis thaliana* (Col-0) exposed to acute high UV-B (0.35 Wm^{-2}) in absence of PAR and UV-A for 4 and 6 hours following 10 days growth under chronic PAR, PAR+UV-A and PAR+UV-A+B. Mean ± 1 SEM. n = 6. Similar letters indicate a non-significant difference at $P < 0.05$.

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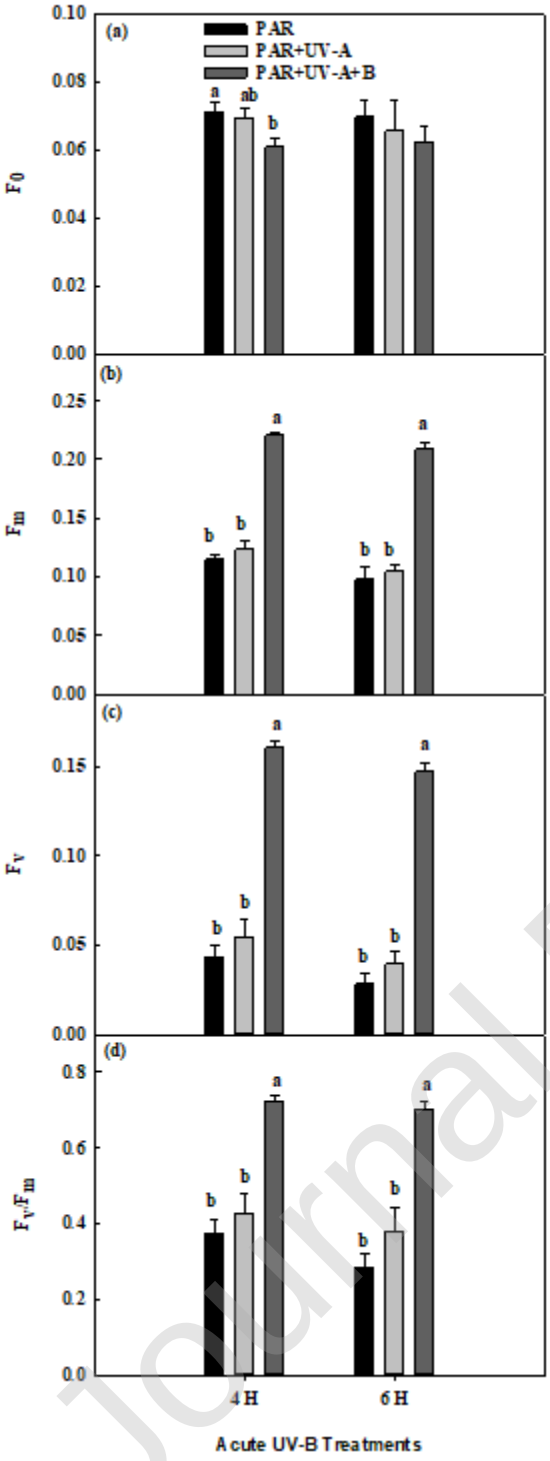


Fig. 3. Actual photochemical efficiency of PSII (F_v'/F_m'), photochemical quenching (q_P), non-photochemical quenching (q_N) and electron transport rate (ETR) in the recently developed leaves of *Arabidopsis thaliana* (Col-0) exposed to acute high UV-B (0.35 Wm^{-2}) in the absence of PAR and UV-A for 4 and 6 hours following 10 days growth under chronic

PAR, PAR+UV-A and PAR+UV-A+B. Mean ± 1 SEM. n = 6. Similar letters indicate a non-significant difference at $P < 0.05$.

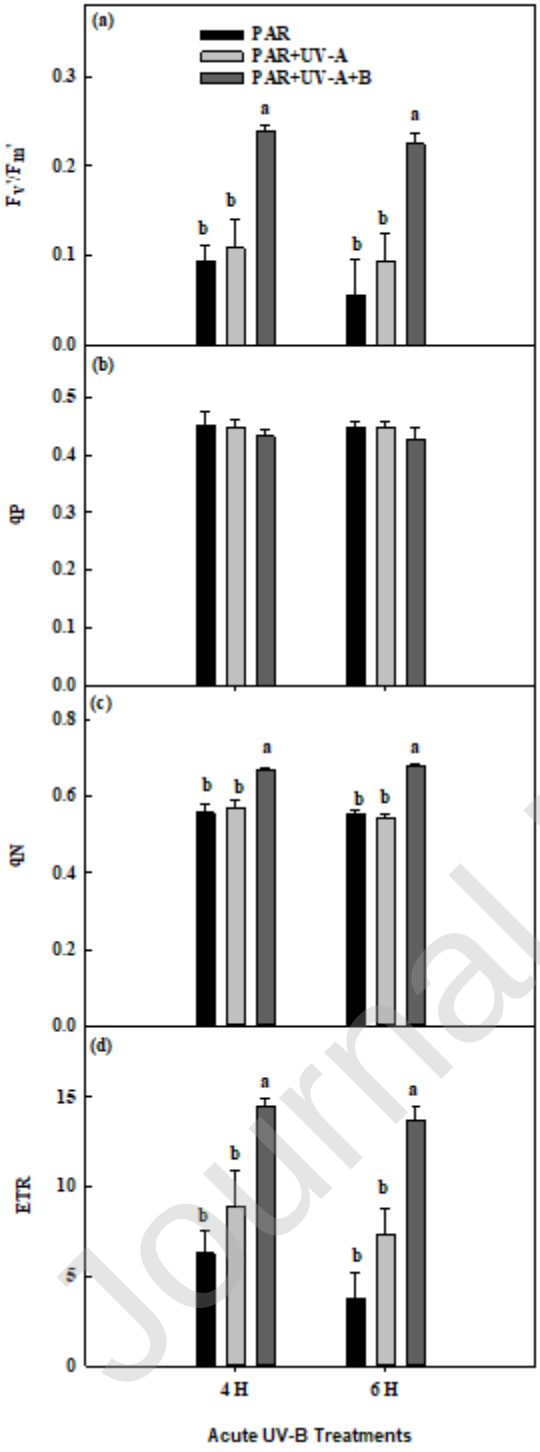


Fig. 4. Percent relative changes in physiological, biochemical and growth parameters in *Arabidopsis thaliana* (Col-0) in PAR+UV-A and in PAR+UV-A+B relative to PAR, and in the PAR+UV-A+B compared to PAR+UV-A. n = 6.

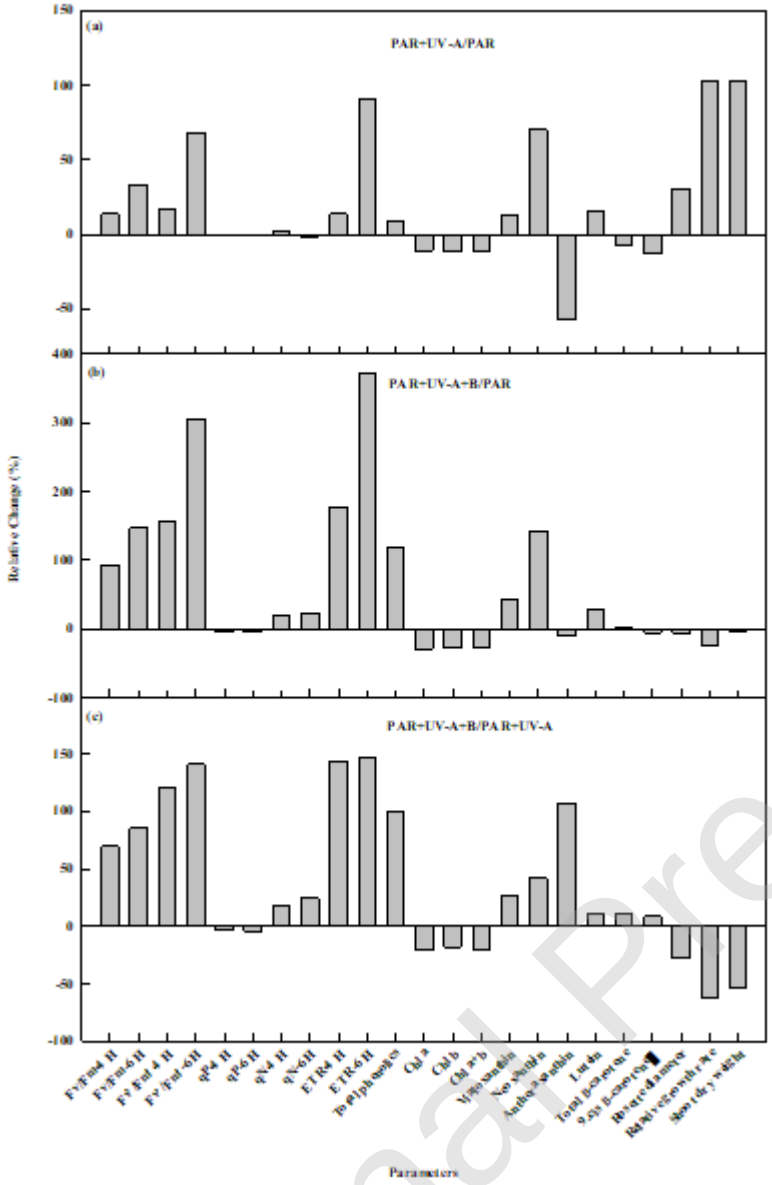


Fig. 5. Functional relationships between total soluble phenolics and maximum photochemical efficiency of PSII in the recently developed leaves of *Arabidopsis thaliana* (Col-0) exposed to acute UV-B for 4 and 6 hours after the plants were grown in PAR+UV-A (a) and PAR+UV-A+B (b) for 10 days. The relationships were assessed as determined by Spearman's correlation coefficient (rho). ns = non-significant, * P<0.05, *** P<0.001.

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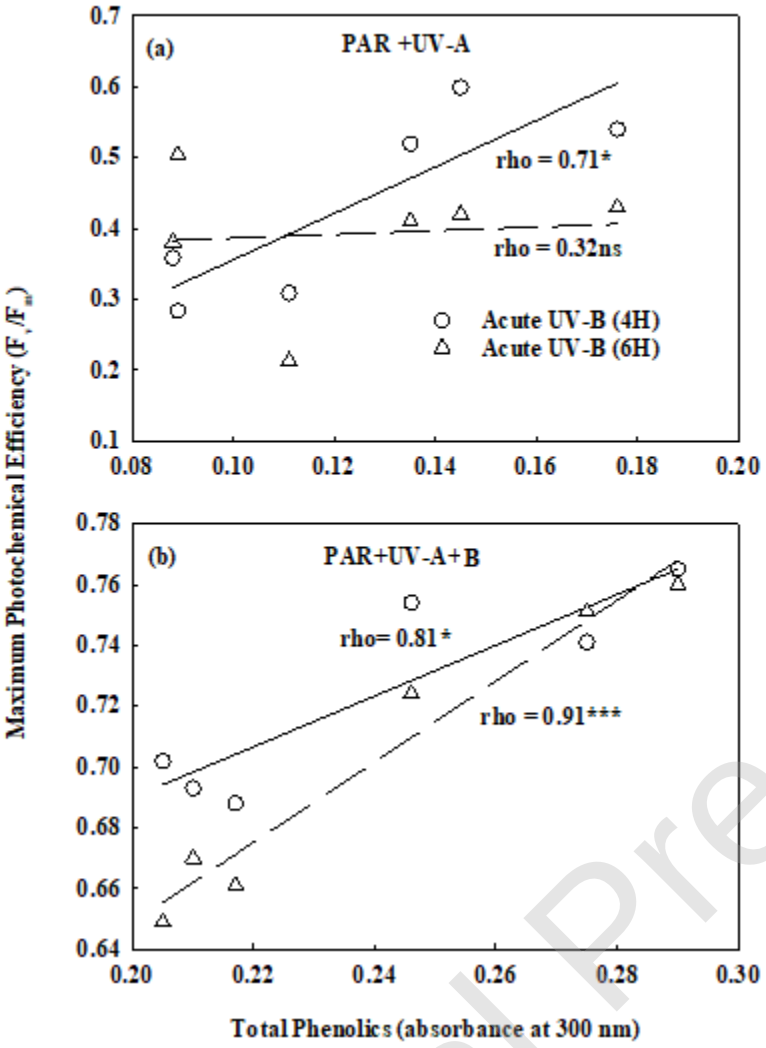
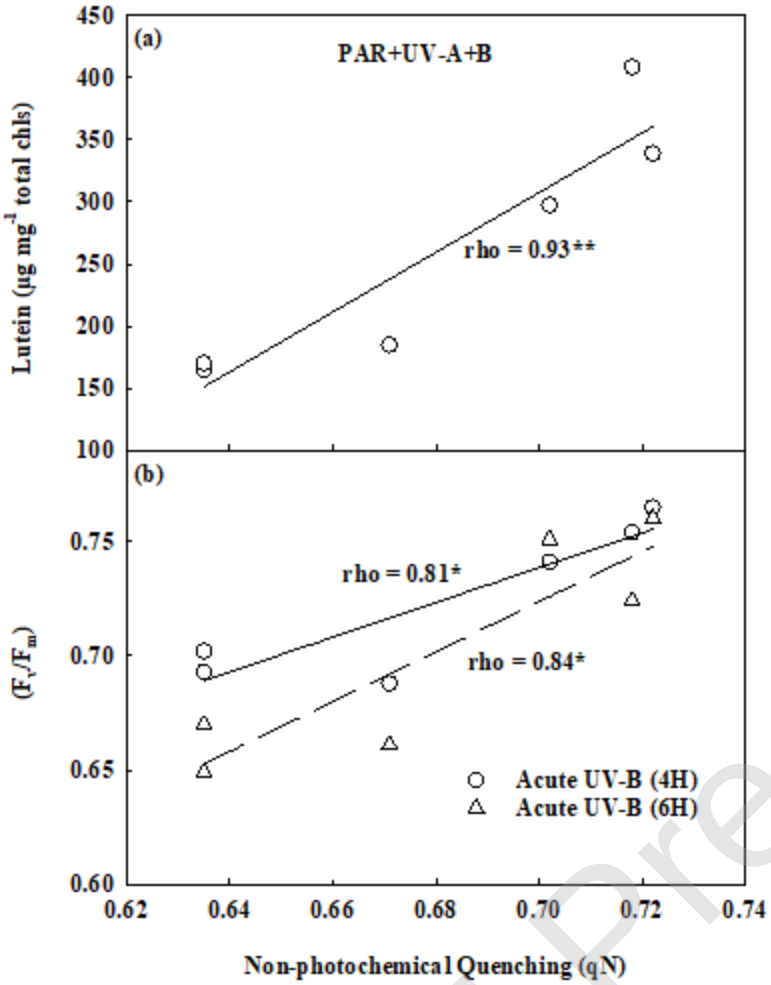


Fig. 6. Functional relationship between lutein and non-photochemical quenching (q_N) in the recently developed leaves of *Arabidopsis thaliana* (Col-0) exposed to acute UV-B for 4 hours after the plants were grown in chronic PAR+UV-A+B for 10 days (a) and the relationship between non-photochemical quenching (q_N) and maximum photochemical efficiency (F_v/F_m) in the recently developed leaves of *Arabidopsis thaliana* (Col-0) exposed to acute UV-B for 4 and 6 hours after the plants were raised in PAR+UV-A+B for 10 days (b). The relationships were assessed as determined by Spearman's correlation coefficient (rho). * P<0.05; ** P<0.01.

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Table 1. Levels of chlorophyll pigments, carotenoids, phenolics, chlorophyll fluorescence and growth in *Arabidopsis thaliana* (Col-0) grown under chronic PAR, PAR+UV-A and PAR+UV-A+B for 10 days. ND: Non-detected. Mean ± 1 SEM. n = 6. Similar letters indicate a non-significant difference at $P < 0.05$.

Parameters	PAR	PAR+UV-A	PAR+UV-A+B
(a) Chlorophyll pigments (mg g^{-1} FW)			
Chl *a*	0.71 ± 0.06^{a}	0.63 ± 0.05^{ab}	0.52 ± 0.07^{b}
Chl *b*	0.34 ± 0.03	0.30 ± 0.03	0.29 ± 0.03
Chl *a* /Chl *b*	2.14 ± 0.12	2.12 ± 0.11	1.81 ± 0.13
Chl *a+b*	1.05 ± 0.09	0.93 ± 0.08	0.81 ± 0.10
(b) Carotenoids (µg mg^{-1} Total chl.)			
Violaxanthin (V)	13.66 ± 2.41^{b}	17.24 ± 2.20^{ab}	25.44 ± 2.69^{a}
Antheraxanthin (A)	2.88 ± 0.55	1.46 ± 0.50	3.70 ± 0.61
Zeaxanthin (Z)	ND	ND	ND
V+A+Z	16.54 ± 2.73^{b}	18.70 ± 2.49^{b}	29.13 ± 3.05^{a}
Neoxanthin	5.18 ± 2.41^{b}	9.94 ± 2.20^{ab}	16.23 ± 2.70^{a}
Lutein	194.55 ± 17.33^{b}	251.10 ± 15.82^{b}	334.97 ± 19.37^{a}
9-cis ß-carotene	10.76 ± 0.73^{b}	10.40 ± 0.67^{b}	13.30 ± 0.82^{a}
Total ß-carotene	50.64 ± 4.75^{b}	51.89 ± 4.34^{b}	67.12 ± 5.31^{a}
EPS	0.92 ± 0.01	0.96 ± 0.01	0.93 ± 0.01
(c) Phenolics (330 nm) cm^{-2} leaf			
Total phenolics	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.25 ± 0.02^{a}
(d) Chlorophyll fluorescence			
F_0	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
F_m	0.30 ± 0.01	0.29 ± 0.01	0.27 ± 0.02
F_v	0.24 ± 0.01	0.23 ± 0.01	0.21 ± 0.01
F_v/F_m	0.79 ± 0.00	0.79 ± 0.00	0.79 ± 0.00
F_v'/F_m'	0.04 ± 0.01^{b}	0.14 ± 0.05^{a}	0.19 ± 0.01^{a}
q_P	0.29 ± 0.03	0.34 ± 0.00	0.30 ± 0.02
q_N	0.68 ± 0.01^{a}	0.60 ± 0.03^{b}	0.68 ± 0.02^{a}
ETR	3.67 ± 1.02^{b}	14.20 ± 0.15^{a}	11.30 ± 0.92^{a}
(e) Growth			
RD (cm)	6.41 ± 0.54	7.38 ± 0.54	6.04 ± 0.54
RGR (mg $mg^{-1}d^{-1}$)	0.63 ± 0.19	1.19 ± 0.19	0.59 ± 0.22
SDW (mg $plant^{-1}$)	15.33 ± 4.60	26.85 ± 4.60	16.64 ± 5.71

Rosette diameter, RD; Relative growth rate, RGR; Shoot dry weight, SDW