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REGULAR PAPER

The UV-B photoreceptor UVR8 promotes photosynthetic efficiency in *Arabidopsis thaliana* exposed to elevated levels of UV-B

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Abstract The UV-B photoreceptor UVR8 regulates expression of genes in response to UV-B, some encoding chloroplast proteins, but the importance of UVR8 in maintaining photosynthetic competence is unknown. The maximum quantum yield of PSII (F_v/F_m) and the operating efficiency of PSII (Φ_{PSII}) were measured in wild-type and *uvr8* mutant *Arabidopsis thaliana*. The importance of specific UVR8-regulated genes in maintaining photosynthetic competence was examined using mutants. Both F_v/F_m and Φ_{PSII} decreased when plants were exposed to elevated UV-B, in general more so in *uvr8* mutant plants than wild-type. UV-B increased the level of *psbD*-BLRP (blue light responsive promoter) transcripts, encoding the PSII D2 protein. This increase was mediated by the UVR8-regulated chloroplast RNA polymerase sigma factor SIG5, but SIG5 was not required to maintain photosynthetic efficiency at elevated UV-B. Levels of the D1 protein of PSII decreased markedly when plants were exposed to elevated UV-B, but there was no significant difference between wild-type and *uvr8* under conditions where the mutant showed increased photoinhibition. The results show that UVR8 promotes photosynthetic efficiency at elevated levels of UV-B. Loss of the DI polypeptide is probably important in causing photoinhibition, but does not entirely explain the reduced photosynthetic efficiency of the *uvr8* mutant compared to wild-type.

Keywords Arabidopsis · ELIP · PSII · SIG5 · UV-B · UVR8

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Introduction

Ultraviolet B (UV-B) wavelengths (280–315 nm) are the most energetic that reach the earth and have the potential to damage macromolecules and impair cellular processes (Jordan 1996; Rozema et al. 1997; Frohnmeyer and Staiger 2003; Caldwell et al. 2007). The injurious effects of UV-B on humans and other organisms are well documented. Plants, however, are constantly exposed to UV-B in sunlight but rarely show signs of damage by UV-B. This is because plants have evolved mechanisms of protection

(Rozema et al. 1997). Plants reflect UV-B using surface waxes and hairs and synthesize phenolic compounds in the outer tissues that absorb UV-B and act as an effective sunscreen (Jordan 1996; Rozema et al. 1997; Frohnmeyer and Staiger 2003; Jenkins 2009; Lake et al. 2009). In addition, plants efficiently repair DNA damage and use antioxidants to ameliorate oxidative stress caused by UV-B (Jordan 1996; Brosché and Strid 2003; Frohnmeyer and Staiger 2003). A key feature of these protective mechanisms is that they are stimulated by UV-B exposure. This process of acclimation involves the differential regulation of many genes (Ulm and Nagy 2005; Casati and Walbot 2004; Jenkins 2009). For example, UV-B stimulates expression of genes encoding enzymes involved in the synthesis of UV-absorbing flavonoids (Jenkins et al. 2001; Brown et al. 2005; Stracke et al. 2010) and those encoding DNA photolyases that repair DNA damage (Brown and Jenkins 2008). Plants that are not acclimated to UV-B are much more likely to suffer cellular injury and necrosis when exposed to relatively high levels of UV-B. There are numerous examples in the scientific literature of physiological processes in plants being impaired by exposure to high ambient or above ambient levels of UV-B (Jordan 1996; Rozema et al. 1997; Frohnmeyer and Staiger 2003; Jansen et al. 1998).

The expression of genes involved in prevention and repair of UV-B damage is initiated by exposure of plants to relatively low doses of UV-B (Jenkins et al. 2001; Ulm et al. 2004; Brown and Jenkins 2008; Jenkins 2009). A key protein that regulates these gene expression responses is UV RESISTANCE LOCUS8 (UVR8). UVR8 mediates responses specifically to UV-B (Brown et al. 2005; Jenkins 2009). *Arabidopsis* mutants lacking UVR8 suffer necrosis when exposed to high ambient levels of UV-B because they lack UV-protection (Kliebenstein et al. 2002; Brown et al. 2005). Transcriptome analysis of wild-type and *uvr8* plants showed that UVR8 regulates over 100 UV-B-induced genes (Brown et al. 2005; Favory et al. 2009), including those involved in sunscreen biosynthesis, other metabolic pathways, DNA repair, protection against oxidative stress, and chloroplast function. UVR8 also controls morphological responses to UV-B (Favory et al. 2009; Wargent et al. 2009). The ELONGATED HYPOCOTYL5 (HY5) transcription factor regulates expression of most if not all UVR8-regulated genes. UVR8 controls the rapid induction of *HY5* expression specifically in response to UV-B (Brown et al. 2005, 2009). UVR8 interacts with chromatin via histones at the *HY5* gene and a number of other UVR8-regulated genes (Brown et al. 2005; Cloix and Jenkins 2008). A proposed model of UVR8 function is that its association with chromatin facilitates recruitment of transcription factor proteins that regulate target genes such as *HY5* (Brown et al. 2005; Jenkins 2009).

UVR8 is a 7-bladed β-propeller protein and its crystal structure has been determined (Christie et al. 2012; Wu et al. 2012). UVR8 is conserved among plant species, including in lower plants such as mosses and algae, and is constitutively expressed. Recent research shows that UVR8 is in fact a UV-B photoreceptor (Rizzini et al. 2011; Christie et al. 2012; Wu et al. 2012). In plants and in vitro, UVR8 molecules form dimers and UV-B acts directly on the protein to promote monomerization. This photoconversion is specific to UV-B wavelengths, requires only brief exposure to UV-B and occurs at low, physiological fluence rates that initiate changes in transcription. Monomerization of UVR8 occurs following illumination of plant extracts as well as intact plants (Rizzini et al. 2011). In addition, UV-B exposure stimulates rapid nuclear accumulation of UVR8 (Kaiserli and Jenkins 2007) and interaction with the CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) protein (Favory et al. 2009). COP1 acts as a positive regulator of photomorphogenic responses to UV-B (Oravecz et al. 2006). The *cop1-4* mutant lacks UV-B induction of essentially the same genes as *uvr8*, indicating that COP1 and UVR8 act in the same pathway (Favory et al. 2009).

As stated above, UVR8 regulates a range of genes in response to UV-B. Among these genes are several that encode chloroplast proteins, which raises the possibility that UVR8 may be important in maintaining the photosynthetic competence of plants (Brown et al. 2005). Numerous studies have shown that photosynthesis is susceptible to damage by UV-B (Teramura and Sullivan 1994; Jordan 1996; Jansen et al. 1998). In particular, UV-B is known to impair the activity of photosystem II (PSII) (Greenberg et al. 1989; Jansen et al. 1996; Booij-James et al. 2000; Takahashi et al. 2010). Hence, in this study we examined whether *uvr8* mutant *Arabidopsis* are altered in photosynthetic activity using measurements of chlorophyll fluorescence. Furthermore, we examined whether impaired expression of genes encoding chloroplast proteins could contribute to the hypersensitivity of the *uvr8* mutant to UV-B.

Materials and methods

Growth and treatment of plants

Seeds of wild-type *Arabidopsis thaliana*, both Landsberg *erecta* (L*er*) and Columbia (Col-0), were obtained from the European *Arabidopsis* Stock Centre (Nottingham, UK). The *uvr8-1* mutant (L*er* background) was obtained from Dr. Dan Kliebenstein (Kliebenstein et al. 2002). The *sig5-1* and *sig5-2* mutants, both in the Col background, were obtained from Professor Takashi Shina (Tsunoyama et al.

2004) and Dr. Kan Tanaka (Nagashima et al. 2004), respectively. The *elip1,elip2* double mutant in the Col background was obtained from Professor Carlo Soave (Rossini et al. 2006).

Seeds were sown on compost and stratified at 4 °C for several days before transferring to controlled environment cabinets, where they were grown at 20 °C in 120 μmol m^{-2} s^{-1} white light.

UV-B treatments were undertaken in controlled environment rooms at 20 °C. UV-B was obtained from UVB-313 UV fluorescent tubes (Q-Panel Co, USA) covered with cellulose acetate (West Design Products, London, UK), which was changed every 24 h (Brown and Jenkins 2008). This source provides broadband UV-B with maximal emission at 313 nm. Fluence rates of white light (photosynthetically active radiation: 400–700 nm) were measured using a Skye RS232 meter with a Quantum sensor (Skye Instruments, Powys, UK). Fluence rates of UV-B (280–315 nm) were measured either by a Skye RS232 meter equipped with a SKU 430 sensor or using a Macam spectroradiometer (model SR9910, Macam Photometrics, Livingston, UK).

Wild-type (L*er*) and *uvr8-1* plants were exposed to ambient solar conditions under three custom-made frames (height: 0.35 m width: 0.9 m length: 0.9 m) situated at an outdoor field site in the North West of the UK (latitude: 54.12 N, longitude: −3.25) at mid-summer. Frames were placed at a spacing of 0.5 m, with the top surface and 60 % of the upper sides covered in clear FEP film (Holscot Fluoroplastics Ltd, Grantham, UK), which has a transmission in the UV–visible regions of greater than 99 %. Spectral treatments were confirmed in situ on a cloudless, sunny day using a spectroradiometer as detailed above. Following initial growth from seed under controlled conditions, plants were transferred in equal numbers to a central location under each of the frame treatments prior to solar dawn (05.20 h), with sampling points including dawn and solar noon (13.20 h) for 2 days in total.

UV-B sensitivity assay

UV-B sensitivity of plants was assayed essentially as described by Brown and Jenkins (2008). Plants were grown in 120 μmol m^{-2} s^{-1} white light as described above for 12 days and then exposed to supplementary UV-B of 5 μmol m^2 s^{-1} for 60 h. Plants were returned to white light minus UV-B and photographed after 5 days. Control plants were not given the UV-B treatment. The sensitivity assay was repeated at least three times and the data shown are representative of the results obtained.

Assays of transcripts

Transcript levels of *SIG5*, *ELIP1*, and the *ACTIN2* control were measured by RT-PCR using cDNA derived from DNase-treated RNA samples isolated from leaf tissue as described by Brown and Jenkins (2008). For each transcript, the cycle number was optimized to ensure that relative transcript measurements were within the linear range of amplification.

Transcripts of the chloroplast *psbD* gene initiated specifically from the blue light responsive promoter (BLRP) and *psbA* gene were assayed in the above RNA samples. Synthesis of cDNA was undertaken as described previously (Brown and Jenkins 2008) except that random primers (Invitrogen) were used instead of oligo-dT. PCR was undertaken with the primers for *psbD*-BLRP transcripts reported by Mochizuki et al. (2004). Levels of *psbA* transcripts were assayed using the following primers (Wormuth et al. 2006): forward, 5′TTACCCAATCTGGGAAGCTG3′; reverse, 5′GAAAAT CAATCGGCCAAAAT3′. For both transcripts, the cycle number was optimized for measurement of relative transcript levels; 18 cycles was employed for *psbD*-BLRP and 16 cycles for *psbA*.

Protein analysis

The level of D1 protein was assayed by immunodetection on western blots. To isolate total protein, mature leaves were ground in liquid nitrogen and extraction buffer was added (250 mM Tris–HCl pH 8.5, 0.5 mM EDTA, 2 % SDS, 10 % glycerol, with protease inhibitors (Complete mini plus, Roche; 1 tablet/2 ml buffer)). The frozen samples were sonicated at 30 % power using a Soniprep 50 sonicator (Sanyo, UK) until just thawed. The samples were centrifuged at 10,000 rpm for 3 min at 4 °C and DTT added to 50 mM. Samples containing 10-μg protein were fractionated by SDS-PAGE and transferred to PVDF membrane (Amersham Bioscience) using standard methods. Western blots were stained with Ponceau S to reveal the prominent Rubisco rbcL band, which was used as a loading control (Kaiserli and Jenkins 2007).

The membrane was blocked in 8 % milk powder in TBST (10 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1 % (v/v) Triton X-100) overnight at 4 °C. The membrane was incubated with the D1 primary antibody (Agrisera) and subsequently a secondary antibody (anti-rabbit HRP, Promega), each for 1 h. Antibodies were diluted in 8 % milk-TBST according to the manufacturer's instructions. Bands were visualized with ECL+ solution. The analysis was repeated three times and D1 and rbcL bands were quantified using Quantity One software (BioRad).

Chlorophyll fluorescence

Measurements of chlorophyll fluorescence were obtained using a chlorophyll fluorescence imager using Fluorimager software (Technologica Ltd., Colchester, UK). Each block

of six plants was dark adapted for at least 30 min before the maximum efficiency of photosystem II (F_v/F_m) was measured to a blue light pulse at 3,000 μmol m^{-2} s^{-1} for 200 ms. Following this pulse, the plants were exposed to an actinic light of either 150 or 500 μmol m^{-2} s^{-1} for 6 min, followed by pulses of 3,000 μmol m^{-2} s^{-1} for 200 ms to obtain measurements of the operating efficiency of photosystem II (Φ_{PSII}) in light-adapted plant material. Mean values of F_v/F_m and Φ_{PSII} for each plant were taken from the image of each whole plant.

For plants transferred to ambient sunlight, F_v/F_m was measured at each sampling point using a Plant Efficiency Analyser (Hansatech Instruments, Kings Lynn, UK). The youngest fully expanded leaf of each plant was dark-adapted for 30 min and F_v/F_m measured with a blue light pulse at 3,000 μmol m^{-2} s^{-1} for 3 s.

Results

UV-B has a more damaging effect on photosynthetic efficiency in *uvr8* mutant than in wild-type Arabidopsis

The chlorophyll fluorescence measurement, F_v/F_m, provides an estimate of the maximum efficiency (or maximum quantum yield) with which light absorbed by pigments of photosystem II (PSII) is used to drive photochemistry in dark-adapted material. Decreases in F_v/F_m can indicate photoinhibition, where there is a decrease in the optimal quantum yield of photosynthesis and the accumulation of photochemically inactive PSII reaction centres.

Maximum efficiency of PSII (F_v/F_m)

Spatial variation in the maximum efficiency of PSII (F_v/F_m) of wild-type and *uvr8* plants grown under fluorescent light (minus UV-B) and exposed to either no UV-B (control) or 20 h of 3 μmol m^{-2} s^{-1} UV-B is presented in Fig. 1. This fluence rate of UV-B is similar to that found in bright sunlight in the UK. These representative images illustrate how under control conditions both wild-type and *uvr8* plants have evenly distributed and healthy values of F_v/F_m ($\sim$0.77). Wild-type plants that were exposed to UV-B had decreased values of F_v/F_m (<0.6), particularly in older leaves. F_v/F_m values were less affected in the midrib region. Very low F_v/F_m values (<0.3), indicating damage to photosystem II, were measured in the *uvr8* plants.

The effect of UV-B on F_v/F_m is dependent on the fluence rate and duration of UV-B exposure. Figure 2a shows that F_v/F_m remained essentially constant in wild-type plants exposed to a relatively low fluence rate of UV-B (1 μmol m^{-2} s^{-1}) for up to 20 h. The *uvr8* plants maintained F_v/F_m values over 0.7 over the same period, although the values were significantly lower than for wild-type after 11 and 14 h.

Wild-type and *uvr8* plants exposed to 3 μmol m^{-2} s^{-1} UV-B maintained F_v/F_m values of 0.7–0.8 over the first 6 h of UV-B exposure (Fig. 2b). After 7 h exposure both wild-type and *uvr8* had decreased values of F_v/F_m, with the decrease in *uvr8* being more severe and F_v/F_m values being significantly lower than the wild-type after 11 and 14 h. After 20-h exposure, *uvr8* did not further decrease its F_v/F_m but the wild-type did.

The F_v/F_m values of wild-type and *uvr8* plants that were exposed to the highest level of UV-B (5 μmol m^{-2} s^{-1}) were significantly lower in the *uvr8* plants after 2–15-h UV-B exposure, where the *uvr8*F_v/F_m value was approximately 0.3 (Fig. 2c).

The operating efficiency of PSII (Φ_{PSII})

The operating efficiency of PSII is shown by the value Φ_{PSII} in light-adapted material. This measures the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry and so gives an indication of the rate of electron transport. Φ_{PSII} values of $\sim$0.5 are considered normal and healthy for *Arabidopsis*. A decrease in Φ_{PSII} indicates less efficient use of light and may reveal damage to the electron transport system.

Measurements were made using an actinic light level of either the growth fluence rate of 120 μmol m^{-2} s^{-1} or 500 μmol m^{-2} s^{-1}, which facilitated the statistical separation of the wild-type and *uvr8* measurements.

The operating efficiency of PSII (Φ_{PSII}) was affected by an increase in exposure time to UV-B at different fluence rates (Fig. 2d, e, f). At 120 μmol m^{-2} s^{-1} actinic light, wild-type and *uvr8* plants maintained relatively normal and very similar values for up to 6 h of exposure at 1 and 3 μmol m^{-2} s^{-1} UV-B. Thereafter, the mutant generally displayed lower values than the wild-type (Fig. 2d, e). At 5 μmol m^{-2} s^{-1}, UV-B, the difference between wild-type and mutant, was evident after 4-h exposure (Fig. 2f). At 500 μmol m^{-2} s^{-1}, actinic light the values of Φ_{PSII} were lower overall and, indicating less efficient use of the incident light, for all UV-B treatments the differences between wild-type and *uvr8* were clearer and statistically significant.

Impaired photosynthetic efficiency is seen in *uvr8* plants in sunlight

To determine whether UVR8 is likely to have a role in maintaining photosynthetic efficiency in the full balance of natural sunlight, we exposed wild-type and *uvr8* plants to typical summer solar conditions at a mid-Northern latitude location. At the point of transfer into solar conditions, plants exhibited strong F_v/F_m values of $\sim$0.76. After 32 h

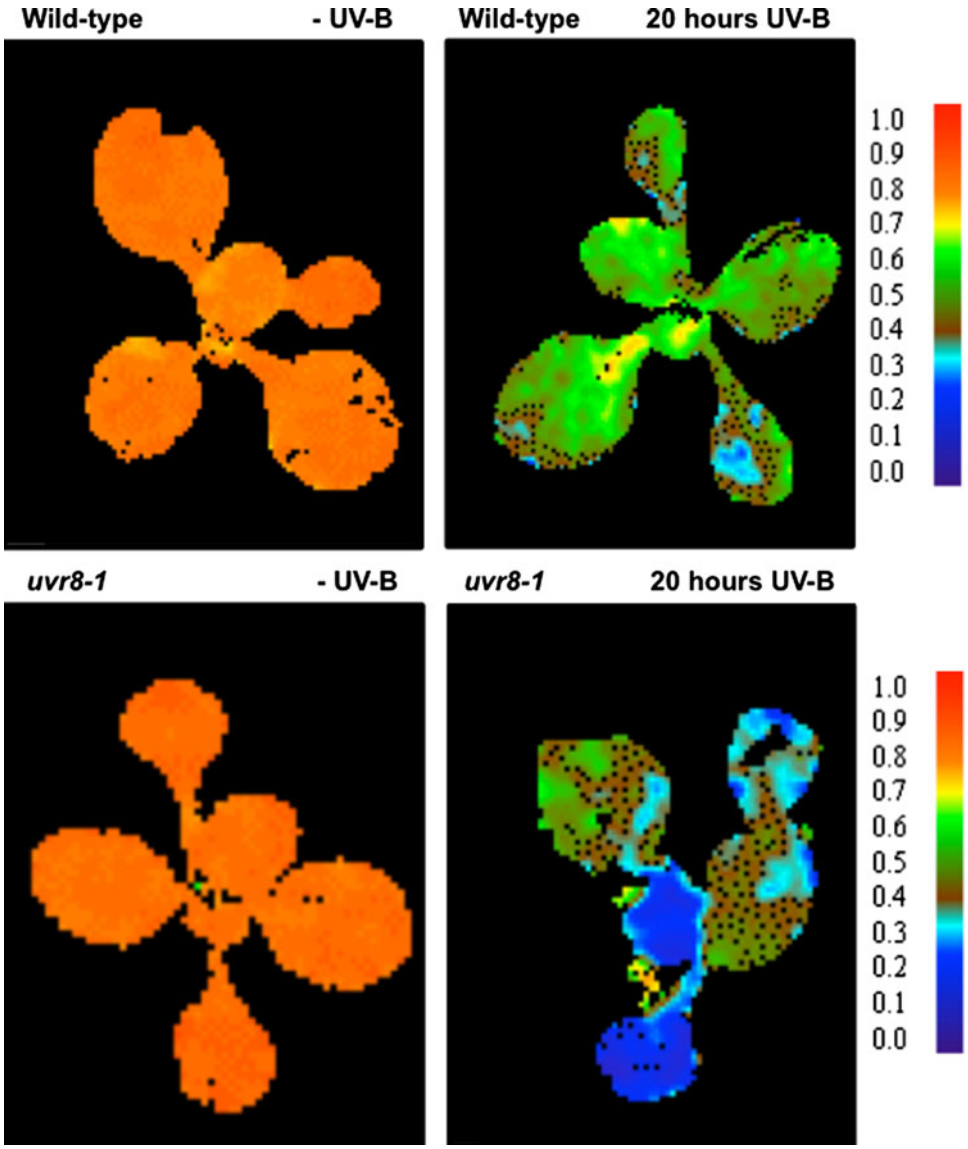

Fig. 1 Spatial changes in the maximum efficiency of PSII (F_v/F_m) of wild-type L*er* and *uvr8* mutant *Arabidopsis* plants grown in 120 μmol m^{-2} s^{-1} white light and exposed to either no or 3 μmol m^{-2} s^{-1} UV-B for 20 h. Images represent F_v/F_m in single representative plants (false colour imaging)

of sunlight exposure, while F_v/F_m decreased in all plants regardless of genotype, F_v/F_m was significantly decreased in *uvr8* plants compared to wild-type (Fig. 3).

SIG5 mediates a UV-B-induced increase in the level of *psbD-BLRP* transcripts

UVR8 mediates UV-B induction of several transcripts encoding chloroplast proteins (Brown et al. 2005; Brown and Jenkins 2008). For instance, the *ELIP1* and *ELIP2* transcripts, which show large fold-inductions by UV-B are regulated by UVR8, although the function of the ELIP proteins in UV-B responses is unknown. In addition, UVR8 regulates an increase in *SIG5* transcript levels in response to UV-B. SIG5 is one of six sigma factors for the plastid-encoded RNA polymerase and regulates expression of the *psbD* transcript, encoding the D2 protein of the PSII reaction centre, specifically from the blue light responsive promoter (BLRP) (Tsunoyama et al. 2004; Nagashima et al. 2004; Lerbs-Mache 2011). In addition to regulation by blue light, SIG5 mediates an increase in *psbD*-BLRP transcripts in response to several abiotic stresses (Nagashima et al. 2004). However, no information was reported on regulation of *psbD*-BLRP transcripts by UV-B.

We wished to test the hypothesis that the greatly reduced level of *SIG5* transcripts in *uvr8* plants exposed to UV-B would impair *psbD* expression and hence account for the reduced photosynthetic competence of the mutant. We, therefore, examined whether UV-B increased the level of *psbD* transcripts and whether SIG5 mediated the response.

UV-B stimulated the expression of *psbD* transcripts from the BLRP in wild-type plants (Fig. 4a). The *psbD*-BLRP transcript level increased with the duration of UV-B

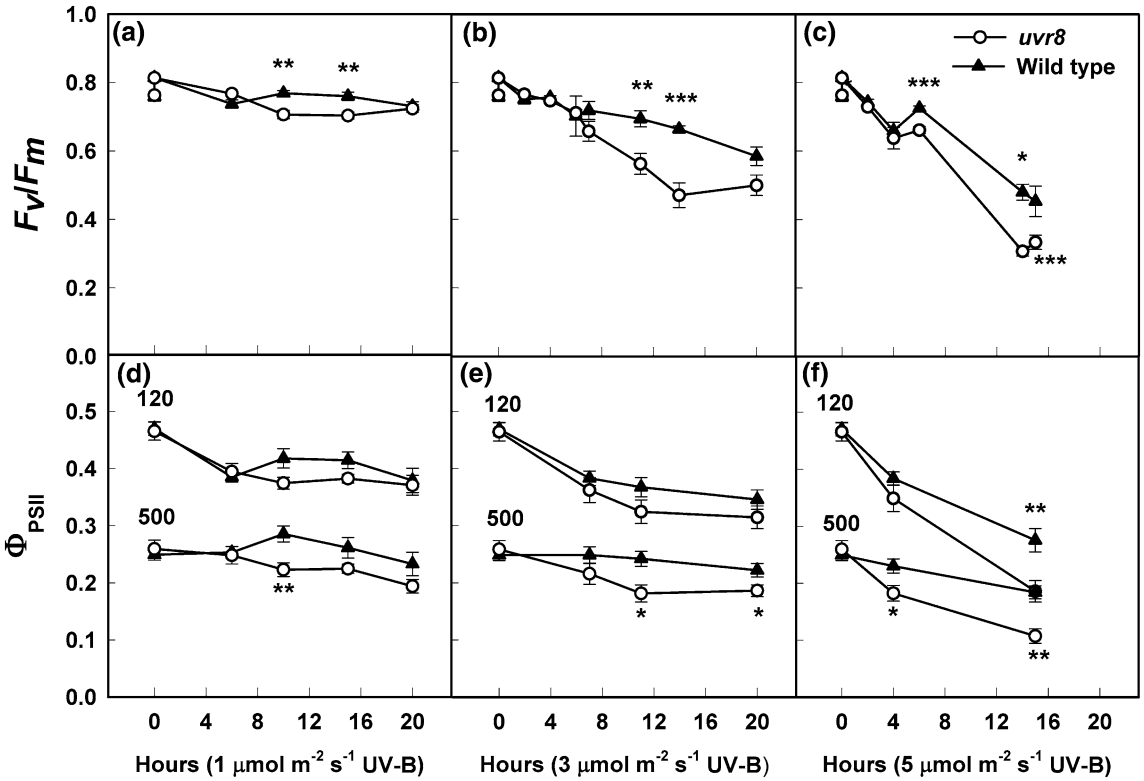

Fig. 2 Maximum efficiency of PSII (F_v/F_m) (**a**–**c**) and the operating efficiency of PSII (Φ_{PSII}) (**d**–**f**) of wild-type L*er* (*closed triangle*) and *uvr8* mutant (*open circle*) plants grown in 120 μmol m^{-2} s^{-1} white light and exposed to 1 (**a, d**), 3 (**b, e**), or 5 (**c, f**) μmol m^{-2} s^{-1} UV-B for 0, 2, 4, 6, 7, 11, 14, or 20 h. Φ_{PSII} values were obtained at an actinic light level of either 120 or 500 μmol m^{-2} s^{-1}. The data points are average values obtained for whole plant images, $n = 6 \pm$ SE. Statistically significant differences between wild-type and *uvr8* are indicated by $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

exposure and fluence rate, whereas *ELIP1* and *SIG5* transcripts were strongly induced even at low fluence rates of UV-B (Fig. 4a). The *sig5-1* and *sig5-2* mutant alleles, which lack *SIG5* transcripts (Fig. 4b), did not show an increase in *psbD*-BLRP transcripts in response to UV-B, demonstrating that SIG5 mediates the response (Fig. 4c).

Loss of SIG5 and ELIP proteins does not reduce photosynthetic efficiency or viability in UV-B

To test the possibility that impaired expression of either SIG5 or the ELIP1 and ELIP2 proteins might be responsible for reduced photosynthetic efficiency and viability of the *uvr8* mutant under UV-B stress, we assayed photosynthetic parameters and viability of *sig5* and *elip* mutants exposed to UV-B.

The response of F_v/F_m to UV-B in the *sig5-1* and *sig5-2* mutant alleles was similar to that measured in the *elip1, elip2* double mutant, when compared to the wild-type (Fig. 5a, b). Similar results were obtained for Φ_{PSII} (Fig. 5c, d). In fact, the mutants had significantly higher values of F_v/F_m and Φ_{PSII} than wild-type at some time points. To test sensitivity to UV-B, wild-type, *uvr8*, the sig*5-1* and *sig5-2* mutants and the *elip1, elip2* double mutant were exposed to a relatively high level of UV-B for 60 h. Viability was then assessed 5 days later. Control plants not exposed to UV-B continued to grow and the different genotypes were largely indistinguishable. Growth of wild-type plants was reduced when exposed to UV-B, but they survived, whereas the *uvr8* plants essentially all died (Fig. 6a). In contrast, the *sig5-1* and *sig5-2* mutants (Fig. 6b) and the *elip1, elip2* double mutant (Fig. 6c) showed very similar survival to wild-type.

UV-B causes a loss of PSII D1 protein in wild-type and *uvr8*

The D1 protein of the PSII reaction centre is regarded as the principal target of photoinhibition in vivo (Edelman and Mattoo 2008; Takahashi and Murata 2008). D1 is subject to rapid turnover and photoinhibition occurs when the rate of degradation exceeds that of synthesis. The damaged D1 protein is replaced in a PSII repair cycle (Nixon et al. 2010). We examined expression of D1 in wild-type and *uvr8* mutant plants exposed to UV-B. The level of *psbA* transcripts, encoding D1, was essentially

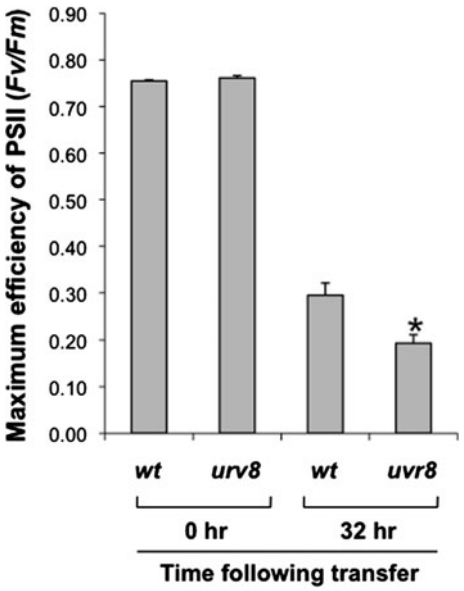

Fig. 3 Maximum efficiency of PSII (F_v/F_m) in wild-type L*er* and *uvr8* mutant plants following exposure to ambient sunlight. Plants were grown in 120 μmol m^{-2} s^{-1} white light for 14 days then transferred to outdoor frames covered with clear FEP film (>99 % transmission in the UV–visible range). 10 plants per genotype were placed under each frame, and there were three replicate frames per treatment ($n = 3 \pm 1$ SE). Measurements were made at the time of transfer (05.20 h) and 32 h later (13.20 h the following day). Statistically significant differences between wild-type and mutants are indicated by $*P \leq 0.05$

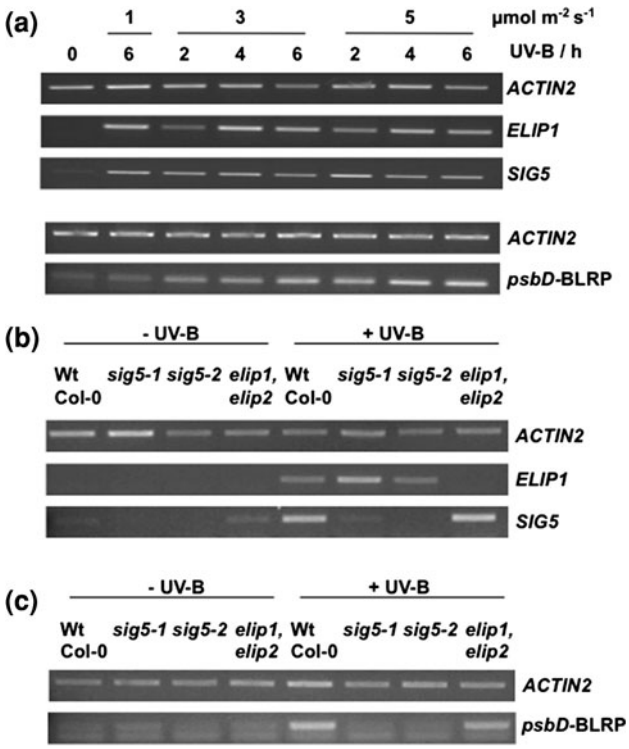

Fig. 4 Levels of *ELIP1,SIG5*, and *psbD*-BLRP transcripts relative to control *ACTIN2* transcripts in **a** wild-type L*er* plants grown in 120 μmol m^{-2} s^{-1} white light and exposed to 1, 3, or 5 μmol m^{-2} s^{-1} UV-B for 2, 4, or 6 h; **b**, **c** wild-type Col-0 plants, *sig5-1*, *sig5-2* and *elip1, elip2* plants grown in 120 μmol m^{-2} s^{-1} white light and exposed to 3 μmol m^{-2} s^{-1} UV-B for 14 h. Transcripts were assayed in leaf RNA samples by RT-PCR optimized to ensure that relative transcript measurements were within the linear range of amplification

unaltered following prolonged UV-B exposure of wild-type and *uvr8* plants to 3 μmol m^{-2} s^{-1} UV-B (Fig. 7). However, the level of D1 protein, detected using a specific antibody on western blots showed a significant reduction both in wild-type and in *uvr8* mutant plants 14 h after UV-B exposure, a time when F_v/F_m is reduced (Fig. 8). Although *uvr8* showed lower values of F_v/F_m than wild-type at this time point (Fig. 2b), there was no evidence from replicated experiments that the reduction in D1 was significantly greater in *uvr8* than wild-type.

Discussion

In this study, we show that *Arabidopsis* plants lacking the UV-B photoreceptor UVR8 are more susceptible than wild-type to photoinhibition of photosynthesis when exposed to high ambient levels of UV-B. Exposure of plants to low doses of UV-B promotes acclimation, which enhances survival at elevated levels of UV-B. UVR8 is key to acclimation because it mediates the production of sun-screen compounds, antioxidants, DNA repair enzymes, and other components in response to UV-B (Kliebenstein et al. 2002; Brown et al. 2005; Favory et al. 2009). As, in the present experiments, plants were exposed to UV-B without any prior acclimation, they had limited initial capacity for screening UV-B wavelengths and for amelioration and repair of damage by UV-B.

The effect of UV-B on the maximum quantum yield of PSII, as measured by F_v/F_m, was clearly dependent on the fuence rate and duration of exposure. When exposed to 1 μmol m^{-2} s^{-1} UV-B, which is well below the maximum level of UV-B in bright sunlight but sufficient to induce expression of acclimation-related genes and some stress-related genes (Brown and Jenkins 2008), plants were able to carry out efficient photosynthesis, as indicated by F_v/F_m values of approximately 0.7. At this fluence rate, there was relatively little difference in F_v/F_m values for wild-type and *uvr8* even after 20-h exposure to UV-B. When the fluence rate was raised to 3 μmol m^{-2} s^{-1}, roughly equivalent to the level of UV-B in UK sunlight, the plants did not show any obvious photoinhibition for the first 6 h. After this time there was a decrease in F_v/F_m for wild-type plants and a larger decrease for *uvr8*, indicating a degree of photoinhibition or other forms of photochemical damage. Older leaves appeared more susceptible to photoinhibition.

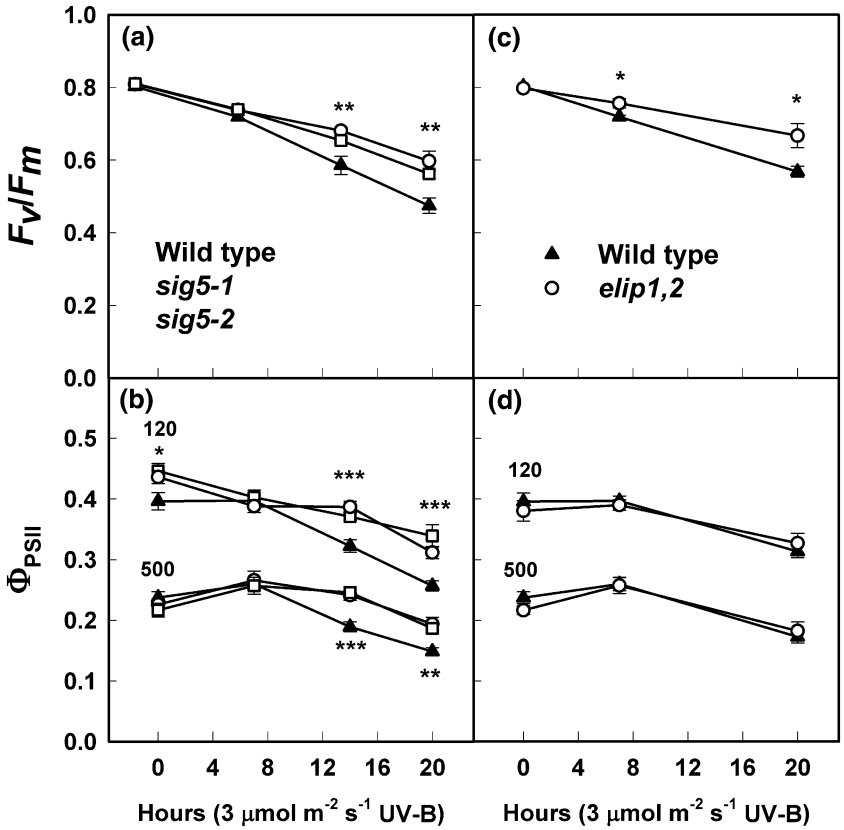

Fig. 5 Maximum efficiency of PSII (F_v/F_m) (**a, b**) and the operating efficiency of PSII (Φ_{PSII}) (**c, d**) of (**a, c**) wild type Col-0 (*closed triangle*), *sig5-1* (*open circle*) and *sig5-2* (*open square*) and (**b, d**) wild-type Col-0 (*closed triangle*) and *elip1,elip2* double mutant (*open circle*) plants grown in 120 μmol m^{-2} s^{-1} white light and exposed to 3 μmol m^{-2} s^{-1} UV-B for 0, 7, or 20 h. Values of Φ_{PSII} were obtained at an actinic light level of either 120 or 500 μmol m^{-2} s^{-1}. The data points are average values obtained for whole plant images, $n = 6 \pm$ SE. Statistically significant differences between wild-type and mutants are indicated by $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

Following exposure to 5 μmol m^{-2} s^{-1} UV-B, which is a little higher than the UK ambient level, a rapid decrease in F_v/F_m was observed for both the genotypes, with *uvr8* showing the larger reduction.

Similar results were obtained when plants were exposed to natural sunlight; although, in this case photoinhibition was likely exacerbated by high irradiance in addition to the level of UV-B, it should also be noted that plants were exposed for longer to UV-B in sunlight than in the growth room experiments. Our observations add to a growing body of evidence which supports the concept that adaptation to UV-B is necessary for plant survival, adaptation which may also form a vital component of plant tolerance to other stresses routinely encountered in the growing environment, particularly in cultivated crops, e.g., herbivory (Foggo et al. 2007), and high visible light irradiance (Wargent et al. 2011).

Values obtained for the operating efficiency of PSII (Φ_{PSII}) followed a similar trend to those for F_v/F_m. There was no difference between the wild-type and the *uvr8* within 6 h of UV-B exposure at 1 μmol m^{-2} s^{-1} but there was a significant decrease within 4 h at 5 μmol m^{-2} s^{-1} UV-B, at least with the higher actinic light level.

A likely cause of the photoinhibition observed in wild-type plants is that PSII reaction centres were damaged by UV-B and could not be replaced at a sufficient rate through the processes of the PSII repair cycle (Nixon et al. 2010; Takahashi and Badger 2010). The observed impairment of PSII function by UV-B is consistent with numerous previous studies (e.g., Greenberg et al. 1989; Jansen et al. 1996; Booij-James et al. 2000; Takahashi et al. 2010). The more substantial photoinhibition observed for *uvr8* indicates that PSII is more sensitive to damage, and there are several possible explanations for this. First, the *uvr8* plants were unable to respond to UV-B to induce protective gene expression and hence would acquire less capacity to prevent or repair UV-B damage than wild-type following transfer to UV-B. In particular, *uvr8* would have accumulated less UV-absorbing flavonoids and it is known that flavonoid biosynthesis mutants are more susceptible to damage to PSII caused by UV-B (Booij-James et al. 2000). However, since it takes several hours for transcript accumulation to peak following UV-B exposure (Casati and Walbot 2004; Brown et al. 2009) and longer for screening pigments to accumulate it is not clear whether differences between the genotypes in flavonoid levels would account for the observed effects on photosynthetic efficiency. Alternatively, the *uvr8* mutant may be less able than wild-type to replace damaged PSII reaction centres. UVR8 mediates the accumulation of several transcripts encoding chloroplast proteins in response to UV-B, including *SIG5*, which encodes the plastid RNA polymerase sigma factor that regulates *psbD* expression and hence synthesis of the D2 polypeptide (Kanamaru and Tanaka 2004; Nagashima et al. 2004; Tsunoyama

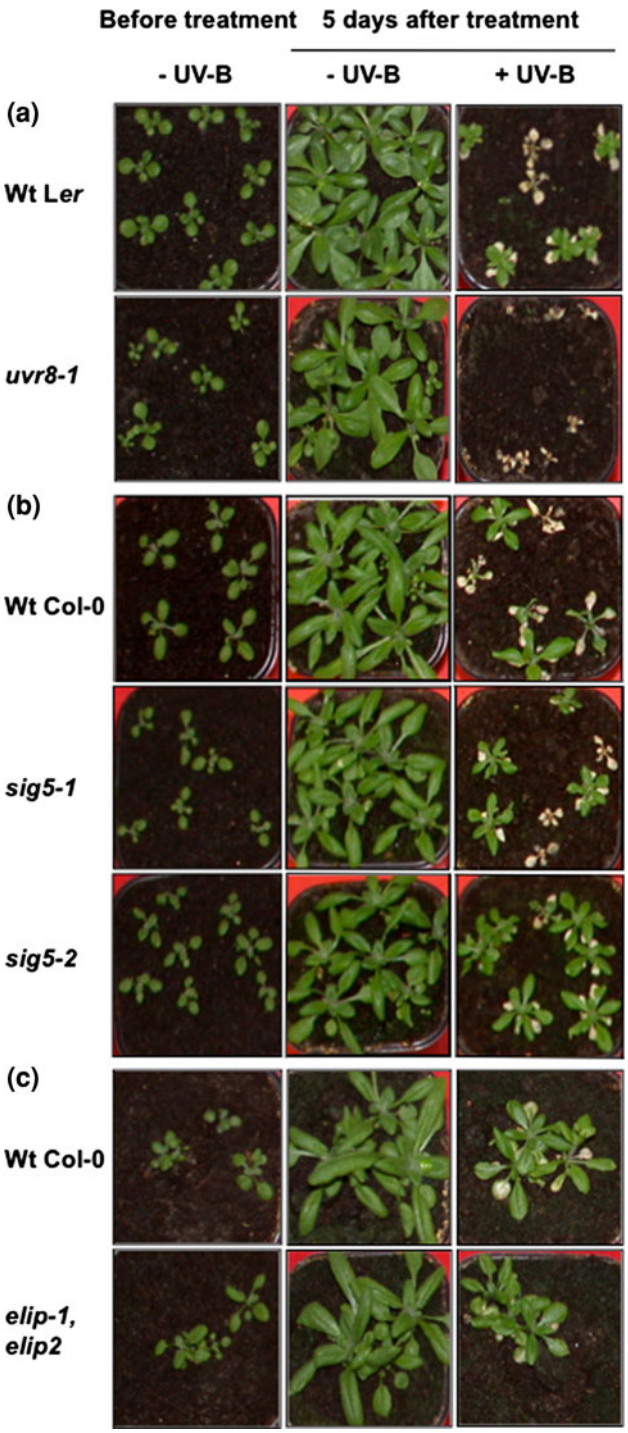

Fig. 6 UV-B sensitivity assay for **a** wild-type L*er* and *uvr8-1*,**b** wild-type Col-0, *sig5-1* and *sig5-2*, and **c** wild-type Col-0 and *elip1/2*. Plants were grown under continuous white light (120 μmol m^{-2} s^{-1}) for 12 days and transferred (or not in controls) to UV-B (5 μmol m^{-2} s^{-1}) for 60 h. After treatment plants were returned to white light to recover. Photographs were taken before transfer and after 5 days of recovery

et al. 2004). We, therefore, examined whether the reduced level of *SIG5* transcripts in *uvr8* plants exposed to UV-B would impair the replacement of D2 polypeptides and hence

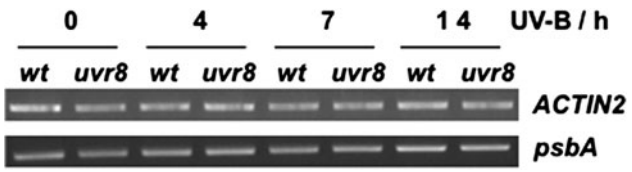

Fig. 7 Levels of *psbA* transcripts relative to control *ACTIN2* transcripts in wild-type L*er* and *uvr8-1* mutant plants exposed to either no or 3 μmol $m^{-2}s^{-1}$ UV-B for 4, 7, or 14 h. Transcripts were assayed in leaf RNA samples by RT-PCR optimized to ensure that relative transcript measurements were within the linear range of amplification

account for the reduced photosynthetic efficiency of the mutant.

The observed increase in *psbD*-BLRP transcripts in response to UV-B has not been reported previously. Moreover, the lack of induction in mutants lacking SIG5 indicates that the UV-B response is mediated by SIG5 and that there is no functional redundancy with other sigma factors. Experiments in other laboratories have shown that SIG5 mediates transcription of *psbD*-BLRP to blue light and other environmental stimuli (Nagashima et al. 2004; Tsunoyama et al. 2004). Given the apparent importance of SIG5 in stimulating *psbD*-BLRP transcript levels in response to UV-B it is perhaps surprising that *sig5* null mutants do not show reduced photosynthetic efficiency or lower viability when exposed to UV-B compared to wild-type. It is possible that other mechanisms, not requiring SIG5 and the BLRP, stimulate an increase in *psbD* transcripts in response to UV-B. Alternatively, the stimulation of *psbD* transcript levels by UV-B may be unimportant with regard to maintaining photosynthetic efficiency.

Similar to *sig5*, the *elip1, elip2* double mutant did not show impaired photosynthetic efficiency or viability following exposure to UV-B, indicating that the ELIP proteins are not required to protect the photosynthetic apparatus under these conditions. The role of the ELIP proteins in chloroplast function remains unclear (Rossini et al. 2006). It is a mystery why *ELIP1* and *ELIP2* transcripts are so strongly induced by UV-B.

In contrast to the UV-B-stimulated increase in *psbD*-BLRP transcripts, there was no effect of UV-B on the levels of *psbA* transcripts, which encode the D1 polypeptide, either in wild-type or in *uvr8*. However, there was a substantial decrease in immuno-detectable D1 polypeptide in both wild-type and *uvr8* following exposure to 3 μmol m^{-2} s^{-1} UV-B for 14 h. Although there was no significant difference in the amount of D1 polypeptide between the genotypes at this time point, the *uvr8* mutant had a significantly lower value of F_v/F_m compared to wild-type. Therefore, while the loss of the D1 polypeptide is likely to be a major contributing factor to the photoinhibition observed (Edelman and Mattoo 2008; Takahashi and Murata 2008; Nixon et al. 2010), it may not

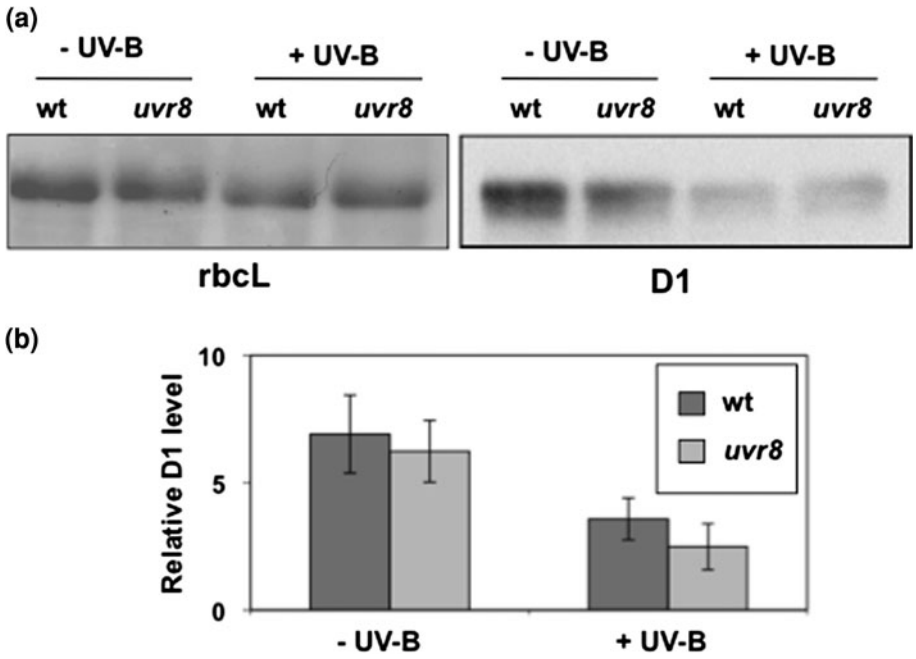

Fig. 8 Levels of the D1 protein of PSII in wild-type L*er* (*wt*) and *uvr8-1* mutant plants exposed to 3 μmol $m^{-2}s^{-1}$ UV-B for 14 h. **a** Leaf protein samples (10 μg per lane) were fractionated by SDS-PAGE and a western blot stained with Ponceau (*left panel*) to reveal Rubisco large subunit (rbcL, 47.5 kDa), as a loading control, and probed with an antibody against D1 protein (*right panel*). A representative image is shown. **b** Quantification of D1 protein levels normalized to rbcL abundance, in arbitrary units. Data are mean ± SE ($n = 3$ independent experiments)

account fully for the more severe photoinhibition observed in the *uvr8* mutant.

In conclusion, we have found that the UVR8 photoreceptor is required to maintain photosynthetic efficiency when *Arabidopsis* plants are exposed to elevated levels of UV-B. UVR8 mediates the regulation of gene expression and it is, therefore, likely that an inability to express specific genes is the cause of the reduced photosynthetic competence of the *uvr8* mutant. Although UVR8 regulates expression of genes encoding the chloroplast proteins ELIP1, ELIP2, and SIG5, along with others (Brown et al. 2005; Favory et al. 2009), there is no evidence that these proteins are required to maintain photosynthetic efficiency and viability at elevated levels of UV-B. It is likely that a loss of the D1 polypeptide is a major determining factor in photoinhibition, but is perhaps not the only factor responsible for the reduced photosynthetic efficiency of the *uvr8* mutant.

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