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Adequate phenylalanine synthesis mediated by G protein is critical for protection from UV radiation damage in young etiolated *Arabidopsis thaliana* seedlings

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ABSTRACT

Etiolated *Arabidopsis thaliana* seedlings, lacking a functional prephenate dehydratase1 gene (*PD1*), also lack the ability to synthesize phenylalanine (Phe) and, as a consequence, phenylpropanoid pigments. We find that low doses of ultraviolet (UV)-C (254 nm) are lethal and low doses of UV-B cause severe damage to etiolated *pd1* mutants, but not to wild-type (wt) seedlings. Furthermore, exposure to UV-C is lethal to etiolated *gcr1* (encoding a putative G protein-coupled receptor in *Arabidopsis*) mutants and *gpa1* (encoding the sole G protein α subunit in *Arabidopsis*) mutants. Addition of Phe to growth media restores wt levels of UV resistance to *pd1* mutants. The data indicate that the *Arabidopsis* G protein-signalling pathway is critical to providing protection from UV, and does so via the activation of PD1, resulting in the synthesis of Phe. Cotyledons of etiolated *pd1* mutants have proplastids (compared with etioplasts in wt), less cuticular wax and fewer long-chain fatty acids. Phe-derived pigments do not collect in the epidermal cells of *pd1* mutants when seedlings are treated with UV, particularly at the cotyledon tip. Addition of Phe to the growth media restores a wt phenotype to *pd1* mutants.

Key-words: phenylpropanoid; prephenate dehydratase; UV-A; UV-absorbing compounds; UV-B.

Abbreviations: PD, prephenate dehydratase; Phe, phenylalanine; UV, ultraviolet.

INTRODUCTION

It is widely agreed that chronic exposure to UV light – UV-B (290–320 nm) radiation, in particular – can cause damage to plants, including reduced photosynthetic capacity, biomass yield and nutritional quality of the seed, altered patterns of species competition, plant ultrastructure and pigment production, and increased susceptibility to disease (reviewed in Caldwell & Flint 1994; Stapleton, Thornber & Walbot 1997; Sullivan & Rozema 1999; Ries *et al.* 2000; Caldwell *et al.* 2003; reviewed in Frohnmeyer & Staiger 2003; Sullivan 2005; Jenkins & Brown 2007; reviewed in Caldwell *et al.* 2007). However, there is a wide variation in sensitivity to UV between species and even among varieties of the same species (Sato, Kang & Kumagai 1994; Torabinejad & Caldwell 2000; Cartwright *et al.* 2001; Li *et al.* 2003; Koti *et al.* 2005, 2007; Rozema, Boelen & Blokker 2005; Sullivan *et al.* 2007). The mechanisms of perception and signal transduction that determine the level of sensitivity to UV-B and invoke 'protective' responses remain largely unstudied and undetermined (Ulm & Nagy 2005; Sullivan *et al.* 2007).

Stratospheric oxygen and ozone molecules absorb >97% of the high-energy UV [composed of UV-C (150–280 nm) and shorter-wavelength UV-B (280–320 nm)] portions of the spectrum [NASA advanced supercomputing (NAS) division; http://www.nas.nasa.gov], but some wavelengths of UV-B can be detected in latitudes of temperate regions (Ajavon *et al.* 2003; reviewed in Caldwell *et al.* 2007). The United States Department of Agriculture (USDA) UV-B Monitoring and Research Network (UVBMRN) monitors the actual UV exposure at the earth's surface. Projects such as the UVBMRN allow for collaborative studies between physicists and plant biologists on understanding the effects of UV-B on plants (Sullivan *et al.* 2007). The question we hope to address with our research with young seedlings is, 'What are the mechanisms for sensing and responding to UV-B radiation?'

Plants have several strategies to deal with UV radiation, including both prevention of damage and repair after the occurrence of damage (reviewed in Jansen, Gaba & Greenberg 1998; Frohnmeyer & Staiger 2003). Among the most important means of preventing damage from exposure to UV-B is the prophylactic deployment of UV-screening pigments that derive from the phenylpropanoid pathway. In most plants, exposure to UV-B results in the increased or new synthesis of such screening pigments (Li *et al.* 1993; Stapleton & Walbot 1994; Caldwell *et al.* 1995; Landry, Chapple & Last 1995; Christie & Jenkins 1996; Gonzalez *et al.* 1996; Reuber, Bornman & Weissenböck 1996; Rozema *et al.* 1997; Burchard, Bilger & Weissenbock 2000; Mazza *et al.* 2000; Tattini *et al.* 2000;

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Bieza & Lois 2001; Rozema *et al.* 2002; Weinig *et al.* 2004; Casati & Walbot 2005).

The best-known example of how a cell repairs the damage caused by UV-B exposure is the repair of UV-induced thymidine dimers (reviewed in Jansen *et al.* 1998; Frohnmeyer & Staiger 2003). UV-B exposure can also regulate several gene families, including those associated with both the repair and production of screening pigments (Strid, Chow & Anderson 1994; Liu & McClure 1995; reviewed in Frohnmeyer & Staiger 2003; Oravecz *et al.* 2006; Blanding *et al.* 2007; Kaiserli & Jenkins 2007).

Pigments are not the only type of structures made by the phenylpropanoid pathway in response to UV. Other products of the phenylpropanoid pathway that may be important in signalling or protection include materials such as suberin and other structural products. For example, exposure to UV irradiation results in an increase in the amount of wax on the surface of leaves (Gonzalez *et al.* 1996; Long *et al.* 2003). In maize and *Arabidopsis*, wax and other cuticular materials can provide protection from UV radiation (Sieber *et al.* 2000; Long *et al.* 2003) and other stresses (Kim *et al.* 2007; reviewed in Nawrath 2006).

The aromatic amino acid Phe is required for protein synthesis and is therefore important for all plants. Much less studied is Phe's role as the precursor to the large number of secondary metabolites produced from the phenylpropanoid pathway (Hahlbrock & Scheel 1989; Chapple *et al.* 1994; Kliebenstein 2004). It is not clear what controls (i.e. the rate-limiting step) the synthesis of UV-screening molecules or stress responses associated with UV-B radiation in developing plants (Ulm & Nagy 2005; Sullivan *et al.* 2007). It is known that exposure to UV-B can result in the induction of genes encoding enzymes active in the phenylpropanoid pathway [e.g. phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS)], and that the expression of these genes and the activity of the encoded enzymes appear sensitive to both the intensity and the wavelengths of the incident UV (Margna 1977; Chappell & Hahlbrock 1984; Beerhues, Robeneck & Wiermann 1988; Ohl, Hahlbrock & Schafer 1989; Li *et al.* 1993; Christie, Alfenito & Walbot 1994; Leyva *et al.* 1995; Liu & McClure 1995; Fuglevand, Jackson & Jenkins 1996; Frohnmeyer, Bowler & Schafer 1997; Wade *et al.* 2001; Brown *et al.* 2005; Kaiserli & Jenkins 2007; Brown & Jenkins 2008).

Because of its two roles, as a precursor to both protein synthesis and the phenylpropanoid pathways, Phe can account for as much as ~30% of the dry mass of a plant (Lewis & Yamamoto 1989; Margna, VainJarv & Laanest 1989; van Heerden, Towers & Lewis 1996). Little is known about the pathways responsible for the synthesis of Phe in plants and almost nothing about the regulation of the biosynthetic pathways or any regulatory roles that Phe itself might have in the actual initiation of stress responses. Our laboratory recently defined the pathway responsible for Phe synthesis in the cytoplasm of etiolated seedlings of the model plant *Arabidopsis*. The process is similar to that identified in bacteria and fungi, wherein prephenate is converted to phenylpyruvate by PD, and phenylpyruvate is converted to Phe by phenylalanine aminotransferase (Gilchrist & Kosuge 1980; Warpeha *et al.* 2006). Given the very small pools of Phe in seeds and in developing seedlings, and the potential for large demands on this pool (Margna 1977; Margna *et al.* 1989), especially in times of stress or anticipation of stress, it remains possible that it is the ability to synthesize Phe that is limiting to the production of phenylpropanoid pathway products, and therefore is limiting to the ability to screen or protect in a variety of ways from harmful UV-B.

We recently demonstrated that exposure of etiolated *Arabidopsis* seedlings to blue light (BL) induces the synthesis of phenylpyruvate, Phe and, subsequently, a number of compounds derived from the phenylpropanoid pathway (Warpeha *et al.* 2006). This BL induction occurs within minutes through the activation of a signal transduction chain consisting of GCR1 (a putative G protein-coupled receptor), and GPA1 (the sole Gα subunit coded for in the *Arabidopsis* genome) and results in the activation of PD1 (one of six members of the *PD* gene family coded for in the *Arabidopsis* genome and the only member expressed in the etiolated seedling) within minutes. Enhanced Phe production occurs as a direct result of the activation of PD1 through its physical interaction with activated GPA1 (Warpeha *et al.* 2006). None of the other five PD proteins coded for in the *Arabidopsis* genome appear to be able to compensate for a lack of PD1 activity in etiolated seedlings (Warpeha *et al.* 2006). In Arabidopsis, six genes code for prephenate dehydratase/arogenate dehydratase (ADT), so named as it can work with either prephenate or arogenate although the latter as recently shown, may be the substrate of choice in some plants (Cho *et al.* 2007; Yamada *et al.* 2008). As we have published two papers using the PD1 name, we refer to the product encoded by At2g27808 as PD1.

The experiments described herein make use of T-DNA insertion mutants in the signal transduction components GCR1 and GPA1 and in the family members of PD, as well as supplementing Phe to the growth media, to determine if the defence responses to environmentally relevant levels of UV-B are dependent on Phe already present in the developing embryo, on UV-A- or UV-B-mediated induction of Phe synthesis, and/or on a more downstream mechanism-like enhanced levels of activity of specific enzymes within the phenylpropanoid pathway.

It is important that we attempt to identify the rate-limiting steps for the synthesis of compounds that protect against UV-B damage and that occur in response to UV-B and UV-B-caused damage. Studies that address early UV 'processing' in young seedlings are critical to understanding and gauging how plants will be able to cope with UV in the changing environment. It has been reported by several groups that adequate induction of the phenylpropanoid pathway within the first 3 weeks post-germination can influence the plant's ability to respond to or protect itself from stresses occurring much later in the life cycle (Liu, Gitz & McClure 1995; Bilger, Hohnsen & Schreiber 2001; Sullivan *et al.* 2007). Cosio & McClure (1984) reported that key enzymes of the flavonoid biosynthetic pathway were greatly reduced in activity by completion of leaf expansion.

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The 6- to 7-day-old etiolated *Arabidopsis* cotyledon is an ideal setting in which to study the initial responses of a plant to UV radiation. It is a simple living laboratory – only a few-cells thick and semi-transparent – where discrete responses can be recorded in living plants by deconvolution microscopy and the cuticle and surface characteristics can be studied after fixation by scanning electron microscopy (SEM) or transmission electron microscopy (TEM). *Arabidopsis* genetics and development are well researched, which makes evaluating and understanding responses to UV a tractable problem. We have utilized a variety of mutants impaired in phenylpropanoid accumulation and have studied growth and developmental responses to UV radiation. We have focused much of our work on mutants deficient in PD1 protein (catalyses the penultimate step of Phe) as they do not produce Phe in the etiolated seedling in response to the known inducers [BL, abscisic acid (ABA)] of wild type (wt) (Warpeha *et al.* 2006). We have investigated the importance of Phe availability and the signal transduction components required for young etiolated *Arabidopsis* to respond to UV radiation.

MATERIALS AND METHODS

Plant materials and accessions

Matched seed lots of wt Columbia (col) *Arabidopsis* and siblings carrying T-DNA insertions within coding regions of *GCR1* (SALK_027808), *GPA1* (SALK_066823), *PD1* (SALK_013392, called *pd1-1* (*pd1* herein; At2g027820), *pd3* (SALK_030329), *pd4* (SALK_065483), *pd5* (SALK_124232) and *pd6* (SALK_28611). Mutants in the *PD2* gene (At3g07630) are unavailable and appear unobtainable, indicating that they may be embryonic lethals. All accessions were obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH, USA; Alonso *et al.* 2003), except that of the mutant *cry1cry2*, which was a generous gift of the Chentau Lin laboratory at University of California at Los Angeles (UCLA). Plants intended for production of seed stock were grown in Scott Metromix 200 (Scotts, Marysville, OH, USA) in continuous white light as described elsewhere (Lapik & Kaufman 2003). All lines are homozygous for the reported insertion. Gene sequence accessions were obtained from Genbank (http://www.ncbi.nlm.nih.gov) and SIGnAL (http://signal.salk.edu).

Plant growth and preparation of tissue

Six-day-old dark-grown seedlings of *A. thaliana* wt col or insertion mutants were grown on 0.8% agarose plates containing only 0.5× Murashige and Skoog media as described in Lapik & Kaufman (2003). The growth media contain no additional sugar, hormones, vitamins or other nutrients. Seedlings were irradiated with a single pulse (1260 s or less) of low-fluence UV (total fluence of 10^4 μmol m^{-2}; Warpeha & Kaufman 1990a,b; Warpeha *et al.* 1991) or no light (DK), placed back in the dark for 24 h or as indicated. Fluence levels used have been confirmed to be within reciprocity limits (Warpeha & Kaufman 1990a). Aerial portions were subsequently harvested to conduct experiments as described.

Chemicals

All chemicals including L-Phe, unless otherwise noted, were obtained from Sigma (St Louis, MO, USA).

UV radiation sources

The UV source (for wavelengths 300, 305, 311, 317, 325, 332 and 368 nm) was custom designed for specific UV-A and UV-B wavelengths (designated the Durham lamp) and assembled by the UVBMRN, Colorado State University, Fort Collins, CO, USA. Light is redirected by a mirror that reflects the UV radiation, and allows the near infrared (NIR) and infrared (IR) to pass through and out an exhaust port. Condensing lenses collect the reflected UV light and define the beam, which then passes through one of seven narrow band filters of type (UVMFRSR). The narrow band-pass filters (Barr Associates, Westford, MA, USA) have a 2 nm FWHM at the following centre wavelengths: 300, 305, 311, 317, 325, 332 and 368 nm. The lengths of irradiation were selected so as to avoid reciprocity failure.

All treatments consisted of a total fluence (dose) of 10^4 μmol m^{-2} (Warpeha & Kaufman 1990a,b; Warpeha *et al.* 1991). The 368 nm treatment was estimated to be the amount of 368 nm light in approximately 20 s of normal sunlight at midday, delivered over 240 s. The 317 and 300 nm treatments with a total fluence of 10^4 μmol m^{-2} approximating twice and five times the level of 317 and 300 nm radiation, respectively, in normal midday sunlight were delivered in 1260 s. 'Normal' sunlight at specific wavelengths was estimated from measurements made at noon in Illinois in June by the UVBMRN (see http://uvb.nrel.colostate.edu). The source for the UV-C 254 nm, the UV-A broad spectrum treatments and the broadband UV-A (320–380 nm, peak ~366 nm) treatments was a UV-P model UVGL-58 Mineralight lamp (UVP, Upland, CA, USA); total fluence for treatments was 10^4 μmol m^{-2}.

Gene expression

Total RNA was extracted from 7-day-old etiolated or white-light-grown seedlings as described previously (Warpeha *et al.* 2007). First- and second-strand synthesis for RT-PCR was conducted using PD-coding region gene-specific primers, according to directions using the Superscript First Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA). Results were visualized by ethidium bromide staining.

Microscope images

Fluorescent images of living seedlings were obtained 24 h post-UV treatment as described in the 'Plant Growth

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and Preparation of Tissue' section. Optical sectioning was achieved by using a Zeiss Axiovert 200 M microscope (Carl Zeiss, Oberkocken, Germany) equipped with a digital camera and the DAPI, FITC and Texas Red filter sets (Chroma, Rockingham, VT, USA) and, where indicated, the DAPI Long-Pass, which has excitation at 300–380 nm, peak at 350 nm with approximately 20% of excitation in the UV-B range, and emission at all wavelengths 400 nm and above to approximately 700 nm (Chroma). Photographs of whole-cotyledon fluorescence were taken on this microscope set-up, minus the ApoTome setting as previously described (Warpeha *et al.* 2006). Light images were taken on an Epson digital camera (Epson, Seiko-Epson Corp., Nagano, Japan).

Electron microscopy: TEM

The conditions for TEM are modifications of that described previously (Anderson *et al.* 1999). Etiolated cotyledons were harvested in darkness directly into 0.05 M sodium cacodylate buffer pH 6.9. Samples were rotated at room temperature for 2 h, then rotated gently overnight in darkness at 4 °C. Tissue was then washed three times for 10 min each in 0.05 M sodium cacodylate buffer pH 6.9. The cotyledons were then secondary fixed in 2% osmium tetroxide in 0.05 M sodium cacodylate pH 6.9 for 2 h at room temperature. The cotyledons were washed in buffer as described, aligned, then entrained at 4 °C in SeaPrep (Karlan, Cottonwood, AZ, USA) ultra-low gelling temperature agarose and then sequentially dehydrated in a graded series of ethanol consisting of 10, 25, 50, 65, 75 and 95% for 15 min each. Dehydration continued with 100% ethanol over molecular sieves with three changes lasting 15 min each. The dehydrated samples were infiltrated with Spurr's low-viscosity resin using the following ratios: two parts 100% ethanol to one part Spurr resin (for 24 h); one part 100% ethanol to one part Spurr resin (for 24 h); and one part ethanol to two parts Spurr resin (for 24 h). Infiltration was complete with three changes of pure Spurr resin over 24 h. All infiltration was carried out at room temperature on a rotator. The infiltrated samples were placed in flat embed moulds and polymerized in an oven at 70 °C for 3 d. Polymerized blocks were trimmed with razor blades, then thick sectioned (0.5 μm), stained and viewed with light microscopy. Once areas of interest were identified, samples were thin sectioned between 80 and 90 nm on a Reichert Ultracut E ultramicrotome (Leica, Wetzlar, Germany) using a DiATOME diamond knife (Diatome AG, Biel, Switzerland). Thin sections were collected on parlodion/carbon coated 200 hex mesh nickel grids then stained with 5% uranyl acetate in 50% ethanol. The grids were rinsed in distilled water, dried, then stained with Reynolds's lead citrate, then rinsed and dried as described. The dried stained grids were placed in a JEOL 1200EX (Jeol Ltd., Tokyo, Japan) TEM and cells of interest were photographed at 60 KV using a Kodak 4489 electron microscopy film (Kodak, Rochester, NY, USA). Images from film were then put into digital format.

Electron microscopy: SEM

All preparation steps were carried out at room temperature. The fixation, wash, dehydration and critical point drying steps were carried out with the seedlings in a microporous specimen holder (EM Sciences, Hatfield, PA, USA). The fixation, wash and dehydration steps were carried out in a vacuum chamber (Pelco Biowave; Ted Pella Inc., Redding, CA, USA), where the vacuum was cycled 30 s on and 60 s off. Seedlings were fixed for 1.0 h with 2.5% gluteraldehyde and 2% paraformaldehyde in 0.05 M sodium cacodylate buffer, pH 6.9. The material underwent two buffer washes for 10 min each in 0.05 M sodium cacodylate buffer, pH 6.9. The seedlings were post-fixed for 1.0 h with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer, pH 6.9, then washed with buffer twice for 10 min each with 0.05 M sodium cacodylate buffer, pH 6.9. Next, the seedlings were dehydrated in an ethanol concentration gradient of 10, 25, 50, 65, 75 and 95% for 10 min each. This was followed by three changes for 10 min each in 100% ethanol. After dehydration, the seedlings were dried in a Balzers CPD 030 critical point dryer (Balzers Union, Liechtenstein). The dried seedlings were removed from the microporous specimen holder, and a sample of each was used for light microscopy. The remaining seedlings were placed on 10 mm aluminum specimen stubs with carbon adhesive tabs and coated with 100 Å Au/Pd using an Anatech Hummer Jr sputter coater (Anatechelectronics, Garfield, NJ, USA). The seedlings were then viewed and their micrographs taken with a JEOL 5600LV scanning electron microscope (Jeol Ltd.).

RESULTS

Presence of Phe in the cotyledon is key to UV response signalling

Distribution of UV-absorbing compounds in the cotyledons of etiolated wt and pd1 *mutants following brief exposure to UV-A (368 nm; 320–380 nm) or UV-B (300 nm) radiation*

Seven-day-old etiolated wt *Arabidopsis* seedlings, but not *pd1* mutants, synthesize a range of UV-absorbing fluorescent compounds in response to irradiation with a single short pulse of low-fluence BL (Warpeha *et al.* 2006). These compounds are located in specific regions of the etiolated cotyledon, with the cotyledon tip epidermal cells being the most prevalent location (Warpeha *et al.* 2006). The lack of such compounds in the BL-treated *pd1* mutant suggested that the compounds are dependent on PD1 activity, are derived from Phe and are products of the phenylpropanoid pathway (Warpeha *et al.* 2006).

To determine if exposure to UV-A or UV-B radiation can similarly stimulate PD1 activity and the production of Phe and UV-absorbing phenylpropanoids, we examined the ability of select narrow band (10 nm) wavelengths of UV-A (368 nm) and UV-B (300 nm), and of broadband UV-A to elicit the synthesis of fluorescent compounds that ordinarily locate to the tip of the cotyledon in wt or *pd1* seedlings as

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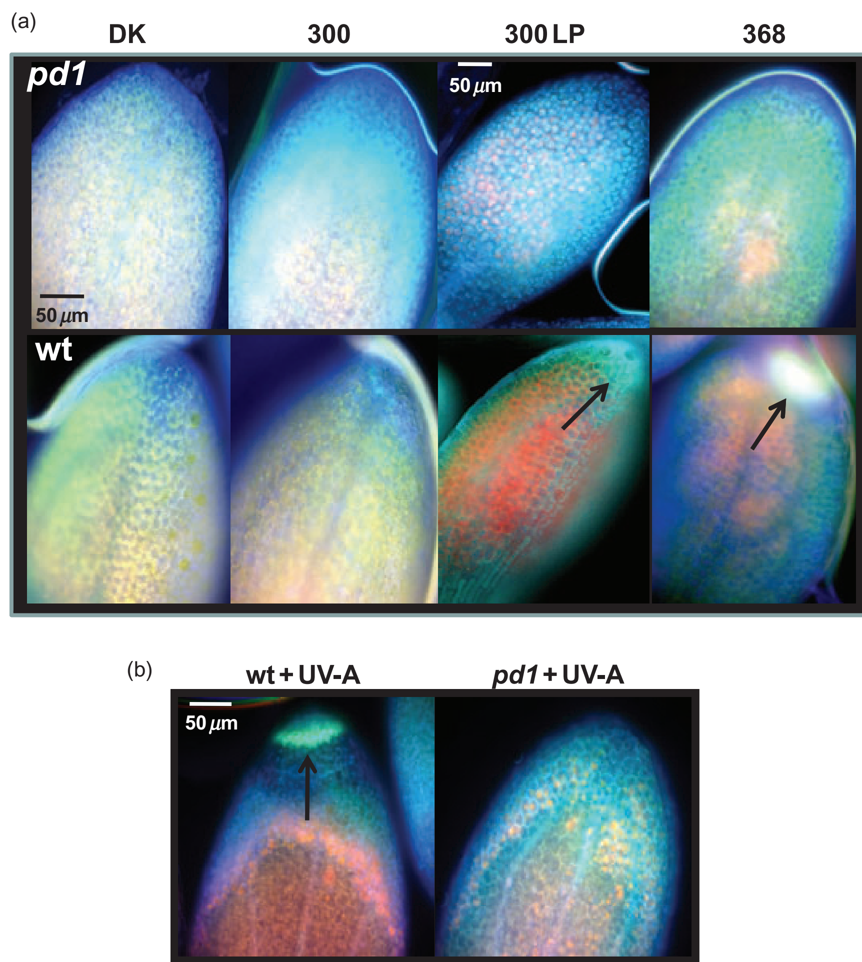

Figure 1. Natural fluorescence in the cotyledons of 7-day-old wild-type (wt) and *pd1* mutant seedlings treated with individual wavelengths of UV (a) and broad UV-A (b). Seedlings of wt and *pd1* mutant seedlings were grown for 6 d in complete darkness. Seedlings were mock-irradiated (DK) or irradiated with 10^4 μmol m^{-2} of UV (300 = 300 nm; 368 = 368 nm) or broad UV-A (320–380 nm), indicated by 'wt + UV-A' or '*pd1* + UV-A', then returned to the dark for 24 h, after which, the cotyledons were photographed on a deconvoluting microscope. The images shown are natural fluorescence and represent optical sections of the Z-stack focused at the adaxial epidermal surface of the cotyledon (fluorescent materials are also visible from the two cell layers 'in between' the epidermal layers of adaxial and abaxial surfaces) using DAPI, FITC and Texas Red cut-off filters with excitations and emissions of 365, 460; 480, 535; and 560, 645 nm, respectively (Chroma, Rockingham, VT, USA), except for '300 LP', which shows in wt and *pd1* fluorescence captured using a DAPI Long-Pass filter, where excitation is in the UV-B and UV-A ranges (300–380, peak 350 nm) and emission cut-off is 400 nm and above, which will show all visible light wavelengths present. The 300 LP illustrates, in particular, that the *pd1* mutant cannot make fluorescing compounds compared with wt (arrow and general increase in fluorescence intensity and colours). Shown in '368', after treatment with 368 nm, *pd1* mutants fail to develop the pigments that localize to the tip of the cotyledon in wt seedlings, the aqua-coloured, fluorescent focus is designated by arrow on the wt seedlings photograph.

shown in Fig. 1. Six-day-old etiolated wt and *pd1* mutants were exposed to a brief pulse of the specified UV treatment or no UV (indicated by 'DK'), then returned to darkness for 24 h. The presence of fluorescing materials in living unfixed cotyledons was observed via fluorescent deconvolution microscopy utilizing filter sets that have excitation in UV and BL wavelengths (Fig. 1).

Fluorescent materials accumulate in the tips of living wt cotyledons treated with either UV-A or UV-B radiation compared with untreated (DK) seedlings. *pd1* mutant seedlings do not appear to respond to UV of any wavelength, where the fluorescence appears unchanged regardless of UV treatment. In wt cotyledons, fluorescing compounds are localized in the cotyledon tip, similar to that elicited in response to BL (compare with Fig. 7 in Warpeha *et al.* 2006) but with considerable qualitative differences. To demonstrate the enormity of the difference in the ability of wt versus that of *pd1* mutants to respond to UV-B and UV-A, the '300 LP' photograph depicts seedlings treated identically to '300' but visualized by using a DAPI Long-Pass filter, where the excitation is both in the UV-B and UV-A range (see 'Materials and Methods') and the emission captured is all wavelengths at 400 nm and above, which is likely to display the majority of the pigments present (Fig. 1). Specific foci (glowing cotyledon tip) are indicated by arrows and are only seen in wt, as *pd1* is unable to make these pigments.

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The broadband UV-A treatment elicits a more intense and broader spectral response than either the narrowband UV-A or UV-B treatments in wt seedlings (Fig. 1b). The UV- and BL-absorbing fluorescent material that accumulates in the tip of the etiolated cotyledons of wt seedlings in response to UV-A is absent in the *pd1* mutants, as could be expected from the data shown in Fig. 1a. Furthermore, overall accumulation of fluorescent material in the cotyledons of the *pd1* mutant is substantially reduced (photographic exposure had to be almost twice as long in order to visualize fluorescence adequately) compared with that in the cotyledons of wt seedlings in general.

The quality of the fluorescent emissions from materials that accumulate in the cotyledons of UV-A-treated wt seedlings (368 nm or UV-A) is different from that elicited by UV-B wavelengths (300 nm), suggesting that the population of compounds produced in response to UV-A and UV-B exposure differ. Absorbance spectra of cotyledon extracts indicate that this is the case (data not shown).

Supplementing the growth media with Phe restores pd1 *mutants to a wt response to UV radiation*

Phe is the starting point for the phenylpropanoid pathway and represents a potentially concentration-limiting substrate for the synthetic sub-pathways within the phenylpropanoid pathway (Gilchrist & Kosuge 1980). Etiolated *pd1* mutants lack the ability to synthesize Phe (Warpeha *et al.* 2006).

To confirm that the loss of *PD1* activity and the inability to synthesize Phe is responsible for the lack of ability to synthesize UV-B-absorbing materials in etiolated cotyledons in response to UV-A or UV-B, we examined the possibility that the provision of exogenous Phe to etiolated *pd1* seedlings could restore normal wt responses to UV-treated (e.g. accumulate fluorescent materials in the cotyledon tip) *pd1* mutants. Phe was added to the growth medium at the time of planting. Plants were grown for 6 d in darkness, irradiated with no radiation (DK = control), UV-B (300 nm) or broadband UV-A, then returned to darkness for 24 h. The live cotyledons were then photographed on the deconvoluting microscope as shown in Fig. 2a. Data from Fig. 1a showing non-Phe supplemented plants are included for comparison (300 – Phe). Addition of Phe to the growth media restores cotyledon tip fluorescence to etiolated *pd1* seedlings treated with UV-B (300) or UV-A (compare Fig. 2 with Fig. 1). Both the wt and *pd1* seedlings have significant fluorescence due to the exogenously applied Phe, indicating that we are probably supplying a saturating level of Phe. The overall fluorescence of the *pd1* mutants supplemented with Phe has some minor qualitative and quantitative spectral differences compared with wt, but the patterning and localization of fluorescence is highly similar (compare Fig. 2a with Fig. 1b).

Phe allows pd1 *to survive exposure to high-energy radiation*

If etiolated *pd1* mutants are not able to synthesize Phe, or products of the phenylpropanoid pathway, and subsequently deploy phenylpropanoid materials to the cotyledon, then they would be expected to be hypersensitive to high-energy UV. High-energy UV in the UV-C range is characteristic for causing DNA damage, induced cell death and can cause oxidative damage, which can spread to neighbouring cells and elicit antioxidant responses (Zacchini & de Agazio 2004). Etiolated wt and *pd1* mutant seedlings were grown in the dark for 6 d, irradiated with a brief pulse of high-energy radiation (UV-C, 254 nm) returned to the dark for 24 h and subsequently photographed. The results shown in Fig. 2b indicate that the pulse is lethal to *pd1* mutants but not to wt seedlings. A similar experiment where seedlings were treated with 300 nm confirms that *pd1* is also hypersensitive to high-energy UV-B albeit less with less severe outcomes (damaged but not dead; data not shown) than observed for 254 nm, where the nature of UV-B damage is explored in experiments later in the paper.

Seven-day-old etiolated T-DNA insertion mutants of *pd3*, *pd4*, *pd5* and *pd6* (there are no insertion mutants of *pd2*) were tested for their ability to survive a UV-C (254 nm) treatment. In all cases (*pd3*, *pd4*, *pd5* and *pd6*), the mutant *pd* seedlings, like wt seedlings, survived 254 nm radiation confirming that *pd3*, *pd4*, *pd5* and *pd6* (unlike *pd1*) are not responsible for a critical level of production of UV-screening pigments in the cotyledons of etiolated *Arabidopsis* seedlings (Fig. 3).

Expression of the PD gene family in etiolated seedlings

The PD gene family in *Arabidopsis* has six members (*PD1* through *PD6*). The cotyledons of young etiolated *pd1* mutants do not exhibit UV-absorbing fluorescent compounds (Figs 1 & 2; Warpeha *et al.* 2006), and etiolated *pd1* mutants do not survive the 254 nm radiation treatment (Figs 2 & 3). Both of these observations indicate that the remaining members of the family are not capable of complementing the *pd1* insertion mutants, at least in the etiolated seedlings, suggesting that the PD members have either a different enzymatic activity, a different sub-cellular location or are not expressed in the cotyledons of 7-day-old etiolated seedlings.

Member-specific primers were used in RT-PCR to measure the expression of *PD1–PD6* in the cotyledons of 7-day-old etiolated and 7-day-old white-light-grown wt seedlings. The data shown in Fig. 4 confirm that *PD1* is the only family member expressed in the 7-day-old etiolated seedlings. The data also indicate that *PD2* is the only family member expressed in the 7-day-old white-light-grown seedlings. Thus, PD3–PD6 are not expressed in 7-day-old etiolated tissue and are not expressed in 7-day-old plants grown in continuous white light.

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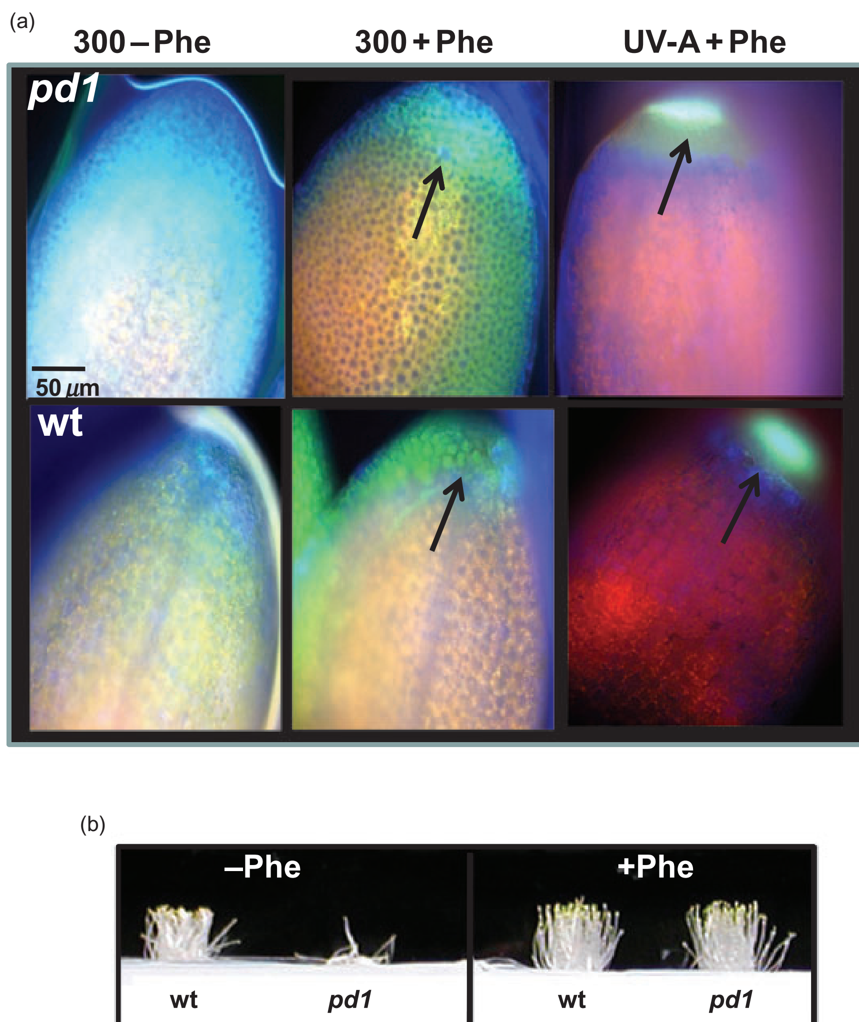

Figure 2. Inclusion of Phe in growth media suppresses the lethal UV-C (254 nm)-hypersensitive phenotype of *pd1*. (a) Inclusion of Phe in the growth media enables *pd1* cotyledons to respond to UV. Seedlings were grown identically to that described in Fig. 1, except where indicated, and 1 mM Phe final concentration was included in the media (+Phe) at planting. Seedlings were irradiated (300 = 300 nm; 368 = 368 nm) then returned to darkness. Seedlings were then processed for deconvoluting microscopy as described in Fig. 1. The fluorescence characteristics of *pd1* are similar to wild type (wt) as a direct result of Phe supplementation (compare data with that of Fig. 1), where arrows indicate the pooling of the green-aqua-coloured fluorescent materials in the cotyledon tip, visualized by using DAPI, FITC and Texas Red filter sets. (b) Growth response of wt and *pd1* mutants to brief treatment of high-energy UV-C (254 nm). Seedlings were grown identically to that described in Fig. 1, except that 100 seedlings were grown on media from seed with (+) and without (–) 1.0 mM Phe. All seedlings were treated with a brief pulse of UV-C (254 nm) then returned to darkness for 24 h. Seedlings were then photographed in white light from the side as shown.

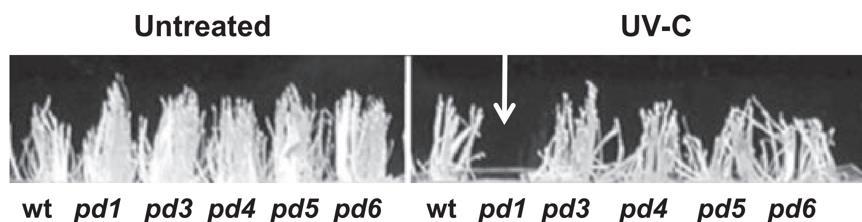

Figure 3. High-energy UV (254 nm) is lethal to etiolated *pd1* mutants, not wild type (wt) or other members of the PD family. Fifty-six-day-old dark-grown wt and members of the PD family (*pd1*, *pd3*, *pd4*, *pd5* and *pd6* mutant seedlings) were irradiated with no light (untreated) or a single pulse of low-fluence UV at 254 nm (UV-C; 10^4 μmol m^{-2}), returned to darkness and photographed 24 h later in white light, side view. Note the apparent 'absence' of the *pd1* mutants where the UV treatment is lethal only to *pd1* seedlings (lying flat on the agar surface).

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Figure 4. Expression of the members of the PD family in etiolated *Arabidopsis* and light-grown seedlings. Seedlings were grown for 7 d in either continuous darkness (DK) or continuous white light (LT). Total RNA was extracted (Warpeha *et al.* 2007), and expression of individual members was determined using gene-specific primers by RT-PCR. Numbers across the lanes pertain to the gene member family, that is, '1' refers to *PD1*, '2' to *PD2* and so on.

Signal transduction

In addition to PD1, the signal transduction components GCR1 and GPA1 are also required to survive high-energy (UV-C, 254 nm) UV radiation

We have previously demonstrated that BL irradiation of etiolated *Arabidopsis* activates a signal transduction chain composed of GCR1, GPA1 and PD1, with the result being an immediate increase in phenylpyruvate and subsequently Phe, and that it is the direct physical interaction between BL-activated GPA1 and PD1 that leads to the specific activation of PD1 activity (Warpeha *et al.* 2006). The data presented in Figs 2 and 3 indicate that PD1 activity and the Phe are critical in order for etiolated *Arabidopsis* to survive exposure to UV-C. We sought to determine if GCR1 and GPA1 have a critical role in the means by which wt seedlings protect themselves from damage or death by UV-C exposure. To do so, we tested multiple independent T-DNA insertion lines for each component of the pathway (GCR1, GPA1 and PD1), as well as a *cry1cry2* double mutant as a control, for their ability to survive exposure to high-energy UV-C radiation.

Six-day-old etiolated wt and T-DNA insertion mutants of GCR1, GPA1 and PD1 seedlings were exposed to UV-C radiation, returned to the dark for 24 h and subsequently observed for effects of the UV-C treatment. The results, shown in Fig. 5, indicate that mutants in all three genes, GCR1, GPA1 and PD1, are UV sensitive to the point of lodging and lethality. The wt seedlings survive the treatment, as do *cry1cry2* double mutants. The fact that *cry1cry2* mutants survive the treatment indicates that the CRY1 and CRY2 photoreceptors are likely not part of the BL- or UV-A-elicited GCR1, GPA1 and PD1 signal transduction chain, and in fact are not critical in terms of mechanisms of protection from UV damage.

Characterization of etiolated *pd1*

The cotyledons of dark-grown pd1 *mutants have no waxy exudate or osmium tetroxide staining materials localized at the very tip of the cotyledon*

The fluorescing pigments at the tip of the cotyledon become visually apparent within 5 h of irradiation (unpublished data) and, subsequently, within 24 h, spread/appear throughout to base of the cotyledon with an appearance similar to layers of lava leaving the top of a volcano. We have speculated what these UV- and BL-absorbing materials might be based on certain phenomena observed by others during early *Arabidopsis* development in etiolated or light-grown seedlings. Many wax- and lipid-based materials, including those that coat the surface of leaves or cotyledons, are derived from the phenylpropanoid pathway (reviewed in Jenks, Eigenbrode & Lemieux 2002; Nawrath 2006). The growing tip of the cotyledon is thought to be the source and location for much of the wax that eventually coats the cotyledons as they mature from pro-cuticle to mature cuticle (Gonzalez *et al.* 1996; Kunst & Samuels 2003; Szczuka & Szczuka 2003). We speculate that the UV- and BL-absorbing materials observed at the tip of the cotyledon might be secreted as part of the waxy exudates secreted from the epidermal cells at the tip of the cotyledon.

Because of the location of Phe-derived fluorescing compounds (Fig. 1), the fact that epicuticular wax can serve as a UV-protection barrier, and that epicuticular wax is reported to both increase and change in chemical composition in response to UV exposure (Cen & Bornman 1993; Gonzalez *et al.* 1996; Grammatikopoulos *et al.* 1998; Pilon *et al.* 1999; Long *et al.* 2003), we examined the effects of UV radiation on wax deposition on the surfaces of cotyledons of etiolated wt and *pd1* seedlings. SEM was used to examine the surface characteristics of cotyledons from etiolated wt and *pd1* mutants grown in complete darkness irradiated with either UV-C (254 nm), UV-A or no radiation (Fig. 6). The SEM data indicate that etiolated wt seedlings accumulate a wax deposit at the dome of the cotyledon tip in untreated (DK)

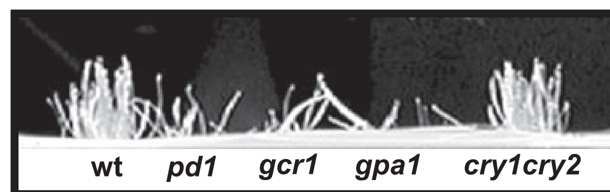

Figure 5. Brief irradiation with 254 nm is lethal to the mutants of the signalling components of the *Arabidopsis* GCR1–GPA1–PD1 signalling chain. Fifty seedlings of wild type (wt) and mutants of the GCR1–GPA1–PD1 signalling chain (i.e. *gcr1*, *gpa1*, *pd1*) and *cry1cry2* seedlings were grown for 6 d in complete darkness. Seedlings were irradiated with a single pulse of 254 nm (10^4 μmol m^{-2}), returned to the darkness and photographed 24 h later in white light, side view. wt, *pd1*, *gcr1*, *gpa1* and *cry1cry2* are shown in the figure.

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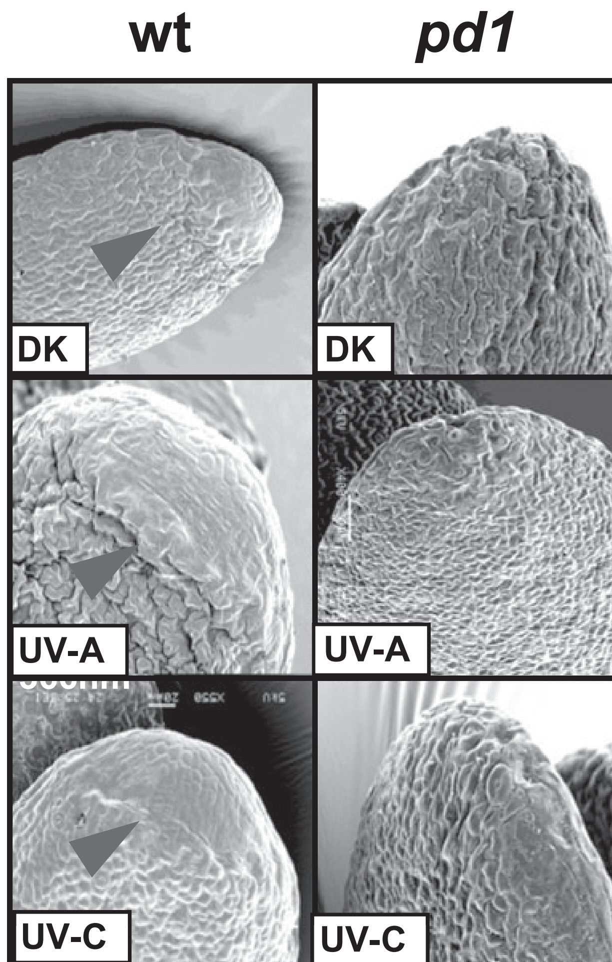

Figure 6. The cotyledon tip of the *pd1* mutant does not accumulate wax. Seedlings of wild-type (wt) and *pd1* mutant seedlings were grown for 6 d in complete darkness. Seedlings were mock-irradiated with either no radiation (DK) or irradiated with a brief pulse of UV-A or UV-C (254 nm), then returned to the dark for 24 h. Cotyledons were harvested directly into fix for scanning electron microscopy (SEM) procedures. Representative photographs of the SEM results are shown. Waxy substances appear to originate at the tip of wt cotyledons indicated by arrows. These waxes are not present at the tips of the *pd1* mutant cotyledons.

and UV-treated seedlings within 24 h of exposure. In contrast, etiolated *pd1* mutants do not accumulate waxy material in this location, regardless of the irradiation treatment.

Osmium tetroxide is known to adhere to and therefore act as a visible stain for long-chain lipids (>C_{16}; Dykstra 1992). Cotyledons from wt and *pd1* mutant seedlings, treated exactly as for SEM, were fixed and stained with osmium tetroxide. The osmium staining pattern occurs in the same locations as the waxy material observed in the SEM data (Fig. 7). These data indicate that the materials that accumulate at the tip of the cotyledon of wt seedlings were absent from the tip of the cotyledon of *pd1* mutant seedlings, and that this material is composed, in part, of long-chain lipids (Figs 6 & 7).

The cotyledons of UV-C-treated pd1 *mutants accumulate a fibrous material on the cotyledon surface*

The surface characteristics of the cotyledons of etiolated *pd1* and wt seedlings were examined in detail by SEM. In contrast to the surfaces of the wt-derived cotyledons, both the adaxial and the abaxial surfaces of the *pd1* mutant have visible cuticular/epicuticular materials. The cotyledon experiences radiation from above (where the very tip bears the impact of most of the radiation) after experiencing very brief 254 nm radiation (Fig. 8). On the other hand, the actual cotyledon tip of *pd1* seedlings displays almost no deposition of materials (Figs 6 & 7). The diffuse speckles of osmium stain across the surface of the *pd1* mutant cotyledons in Fig. 7 may be indicative of these same materials.

The cotyledons of etiolated pd1 *mutants have proplastids in contrast to wt seedlings, which have etioplasts*

Because etiolated *pd1* mutants appear to have less than wt levels of proto-chlorophyllide (Warpeha *et al.* 2006) and are slow to accumulate chlorophyll upon transfer to continuous light, we used TEM to examine the state of plastid development in the cotyledons of etiolated wt and *pd1* seedlings. The micrographs shown in Fig. 9 indicate that the cotyledons of etiolated *pd1* seedlings have proplastids. This is in contrast to the etioplasts normally present in the cotyledons of etiolated wt seedlings. These data are very interesting

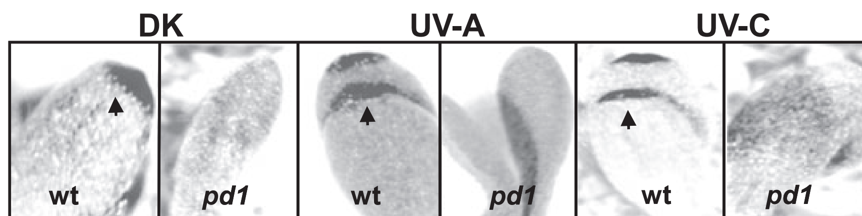

Figure 7. The cotyledon tip of the *pd1* mutant does not accumulate waxes/lipids that stain with osmium tetroxide. Seedlings of wild-type (wt) and *pd1* mutant seedlings were grown and irradiated exactly the same way as described in Fig. 6. Cotyledons were harvested directly into fixative buffer for scanning electron microscopy and were then treated with osmium tetroxide. Osmium-fixed cotyledons were photographed on a light dissection microscope. Representative cotyledons are shown. Arrows indicate visible concentration of osmium stain.

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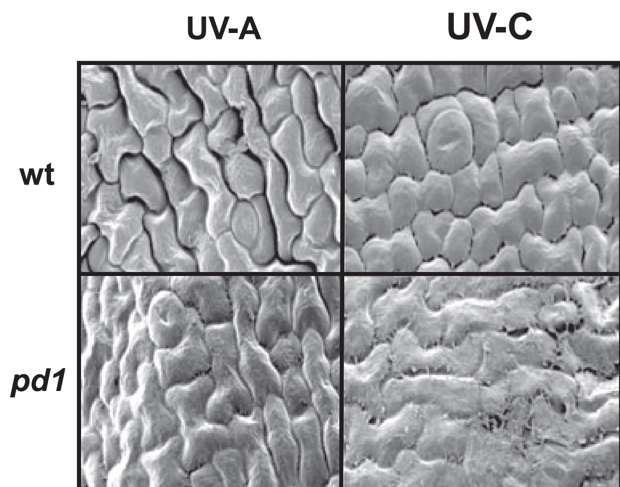

Figure 8. The cotyledon surface of the *pd1* cotyledon accumulates wax in response to UV treatment. Seedlings were grown and treated identically to that described in Fig. 6, except that the scanning electron microscopy photographs display a closer view of the cotyledon surface. Photographs are representative.

when considered with the data from Fig. 1, where much of the reddish fluorescence appears to be coming from the developing plastid, as determined during our microscopy.

DISCUSSION

UV-B radiation has resulted in measurable DNA damage in native plant species, changes in secondary metabolites (reviewed in Caldwell *et al.* 2007) and altered patterns of herbivory (reviewed in Caldwell *et al.* 2007). These effects of UV-B exposure are most pronounced and long lived when it occurs during early seedling development (Liu *et al.* 1995; Bilger *et al.* 2001; Sullivan *et al.* 2003; Sullivan *et al.* 2007). Hence, examining the effects of UV-B and other UV radiations on young etiolated seedlings would advance scientific understanding of the metabolic and developmental responses in plants.

Phe is the immediate precursor to the phenylpropanoid pathway and either the concentration of Phe or the rate of synthesis Phe appears to be the limiting step in the ability to produce UV-B-absorbing pigments and other stress-related compounds from the phenylpropanoid pathway. Depending on the quality and quantity of the materials stored in the endosperm, newly emergent seedlings may or may not have sufficient Phe to serve both the immediate need for protein, as well as for UV protection and/or other stresses or developmental processes that rely on the phenylpropanoid pathway. Etiolated *pd1* mutants would appear to be so deficient in Phe so as not to be able to produce the pigments that would shield against UV-B or UV-C or have adequate plastid development. Other researchers have also found that the phenylpropanoid pathway is the most active and responsive in the first 3 weeks of growth (Liu & McClure 1995; Bilger *et al.* 2001; Sullivan *et al.* 2003). We would conclude that Phe is more critical to early development than was previously supposed.

PD1 is critical to the synthesis of phenylpropanoids and the resistance to the harmful effects of UV radiation, and serves as a GPA1 effector

PD1 serves unique roles in the newly emergent etiolated seedling. Genetic, phenotypic and biochemical analyses presented both previously (Warpeha *et al.* 2006) and herein, indicate that etiolated young seedlings synthesize Phe via PD1. PD1, as the only member of the PD family expressed or active in etiolated seedlings, is immediately responsible for Phe synthesis, phenylpropanoid production, protection from high-energy UV-radiation and timely plastid (chloroplast) development in young etiolated seedlings. Thus, the presence of sufficient Phe is much more important than just a primary amino acid or initiation of secondary metabolic pathways. Data presented herein indicate that UV can also elicit the GCR1–GPA1–PD1 signalling mechanism.

Most striking is that *pd1* mutants are considerably more sensitive to UV irradiation than wt seedlings, so much so that exposure to even low doses of high-energy UV (254 nm) is lethal to etiolated *pd1* mutants. Homozygous null T-DNA insertion mutations in *pd1* are not lethal under laboratory white light growth conditions, although *pd1* mutants do exhibit a 1–2 d delay in germination, overall growth and greening (data not shown). *PD1* is the only member of the six-member *PD* gene family to be expressed in etiolated *Arabidopsis* seedlings. It is possible that *pd2* mutants represent a dominant lethal given the lack of availability of an insertion mutant. The inability to synthesize phenylpropanoid products presents a combination of

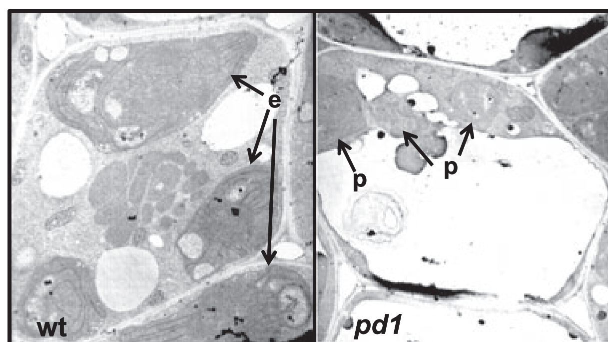

Figure 9. Etiolated *pd1* seedlings have less developed plastids than the etiolated wild-type (wt) seedlings. Seedlings of wt and *pd1* mutant seedlings were grown in complete darkness. On day 6, cotyledons were harvested directly into transmission electron microscopy (TEM) fixative in the dark, then immediately processed for TEM. Sections were cut perpendicular to the adaxial surface, and a representative section is shown for wt and *pd1* seedlings, where plastids are labelled and indicated by arrows. e, etioplast; p, proplastid.

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developmental issues for etiolated *pd1* plants when exposed to UV radiation.

Phe is the rate-limiting substrate for the production of phenylpropanoids and UV-protective materials

Phe is the first committed precursor of the phenylpropanoid pathway, including that branch leading to photoprotective compounds such as cinnamoyl esters, flavonols, flavonoids and anthocyanins identified accumulating in the epidermal cells of a variety of plants (Robberecht & Caldwell 1978; Flint, Jordan & Caldwell 1985; Tevini, Braun & Fieser 1991; Rozema *et al.* 2002). Phe is also thought to be a concentration-limiting substrate (Noh & Spalding 1998; Rozema *et al.* 2002; Warpeha *et al.* 2006). Therefore, protective pigment production observed in response to UV-B alone, and the enhanced pigment production observed in response to UV-A, requires that the plant either synthesize more Phe and/or that the plant divert Phe normally used by other branches of the phenylpropanoid pathway for the production of pigments. Almost no published data are available about the synthesis of, or the regulation of the synthetic pathway responsible for, Phe.

In response to UV, etiolated *pd1* mutants are unable to produce Phe and, as a result, all subsequent phenylpropanoids, as shown in our data herein. Phe cross-feeding is able to restore wt phenotypes to *pd1* seedlings, including surviving exposure to a high-energy UV treatment and the synthesis of UV-absorbing compounds localized to the cotyledon tip.

The ability to restore resistance to UV-C and UV-B to *pd1* mutants by the feeding of Phe, in the absence of any pre-irradiation, would strongly suggest that all of the enzymes necessary to produce the pigments that absorb and prevent UV-C and UV-B damage, such as PAL and CHS, are present and active at sufficient levels to produce sufficient pigments so as to protect the young etiolated seedlings. These data would appear to make the emergent seedling and etiolated seedling different from a more mature seedling, or adult plant, where the adult plant would seem to require the activity of genes or enzymatic activities specific to the phenylpropanoid pathways, and not a particular rate or level of Phe synthesis (Margna 1977; Chappell & Hahlbrock 1984; Beerhues *et al.* 1988; Ohl *et al.* 1989; Li *et al.* 1993; Christie *et al.* 1994; Leyva *et al.* 1995; Liu & McClure 1995; Fuglevand *et al.* 1996; Frohnmeyer *et al.* 1997; Wade *et al.* 2001; Brown *et al.* 2005; Kaiserli & Jenkins 2007; Brown & Jenkins 2008). Studies are underway in etiolated seedlings to determine the identity of the existing and UV-elicited compound pool.

GCR1 and GPA1, like PD1, have a critical role in protecting against the deleterious effects of UV irradiation

Data presented herein and in Warpeha *et al.* (2006) indicate that GCR1 and GPA1 are critical to the BL-induced activity of PD1, Phe synthesis and the synthesis of UV-screening pigments. As is the case for *pd1* mutants, exposure of etiolated *gcr1* or *gpa1* mutants to high-energy UV radiation (UV-C, 254 nm) is lethal at fluences that do not kill wt seedlings.

Heterotrimeric G protein-mediated cell signalling is one of the most highly conserved signalling mechanisms in eukaryotes (Hamm 1998). Analysis of *Arabidopsis* plants mutant in *GPA1* gene reveal that G proteins are likely to be involved in a number of processes critical for proper plant development (reviewed in Perfus-Barbeoch, Jones & Assmann 2004; reviewed in Temple & Jones 2007).

A number of biotic and abiotic stimuli are known to activate the GCR1–GPA1–PD1 pathway within minutes and result in an increase in the production of Phe (Warpeha *et al.* 2006). The fact that Phe is the ultimate precursor to all of the compounds produced by the phenylpropanoid pathway, and the fact that many of these compounds are associated with either preventing the deleterious effects of biotic and abiotic stress or in the response to those stresses, suggests that the GCR1–GPA1–PD1 pathway may have a role in the response to many different stresses, in addition to UV radiation. Reports that the phenylpropanoid pathway is similarly stimulated by cold stress make this a likely possibility (McClure 1986; Christie *et al.* 1994; Leyva *et al.* 1995; Wade, Sohal & Jenkins 2003).

PD1 is critical to *Arabidopsis* plastid development in etiolated young leaves

Etiolated *pd1* mutants do not appear different in gross form and structure when compared with wt. However, TEM reveals that plastid development of *pd1* mutants is retarded as the cotyledons of etiolated seedlings have proplastids or an embryonic plastid rather than the expected etioplast. These proplastids lack a prolamellar body, the lipid–protein crystalline signature structure found in all etioplasts.

These observations would suggest the need for specific products synthesized from, or with, Phe – be the specific proteins rich in Phe or the products that derive from the phenylpropanoid pathway, or both – in order to progress from the proplastid to the etioplast. Given that the etioplast is not an intermediary between the proplastid and the chloroplast but rather an alternative pathway, it is possible that the proplastids present in the etiolated *pd1* mutants develop directly into chloroplasts upon light exposure. If so, this would suggest a role for PD2 (expressed strongly in white light) in chloroplast development and the other PD proteins, whereby most online protein signature tools indicate that they are expressed in the chloroplast.

Moreover, this is not the first time we have observed proplastids in TEM examinations of young plants. In 1999, Anderson *et al.* reported that pea seedlings grown in continuous dim red light failed to develop etioplasts and instead developed proplastids and, as such, were unable to respond to high fluences of BL. It is possible that the lack of plastid development includes the inability to synthesize

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certain lipids and pigments made in more mature plastids that would help defend the young growing plant from high light or UV radiation insult.

PD1 and its role in the synthesis and deposition of cuticular constituents

The *Arabidopsis* tissue used herein is unexpanded etiolated cotyledons of 6–7 d in age; therefore, it is likely that there is a pro-cuticle with little epicuticular complexity in the wt seedlings (reviewed in Bird & Gray 2003; Kunst & Samuels 2003). Our TEM results indicate that wt seedlings contain etioplasts and are still immature, which may slow the building of the cuticle. Many of the early primary wax biosynthetic reactions during epidermal cell development are reported to occur in the plastid (reviewed in Bird & Gray 2003; Kunst & Samuels 2003). Given that under certain growth conditions young pea seedlings with proplastids failed to respond to high fluencies of BL (Anderson *et al.* 1999), it is likely that this developmental issue is a result of the presence of Phe rather than an overall difference in protein synthesis.

The SEM and osmium staining studies herein indicate that as a result of UV treatment, the tips of the cotyledons of wt *Arabidopsis* accumulate or extrude a wax-like substance, and that this does not occur in the *pd1* mutants. The wax-like substance may itself contain or be translocated at the same time with UV-absorbing pigments. Suberin and other molecules reportedly involved with the cuticle do have constituent molecules derived from the phenylpropanoid pathway (i.e. phenolics and hydroxycinnamates; reviewed in Bernards 2002; Jenks *et al.* 2002; Franke *et al.* 2005). Perhaps the cuticular waxes and associating pigments are unable to be formed properly or transported properly in *pd1* mutants.

The diffuse patches of materials deposited on the epidermal cells of the *pd1* mutants may be wax crystals with appearance similar to those observed for certain mutants described by Aharoni *et al.* (2004). The epicuticular wax correlates with the diffuse pattern of osmium staining observed.

Exposure to UV irradiation results in an increase in the amount of wax on the surface of leaves (Gonzalez *et al.* 1996; Long *et al.* 2003), and in both maize and *Arabidopsis*, surface wax can provide protection from UV radiation (Sieber *et al.* 2000; Long *et al.* 2003) and other stresses (Kim *et al.* 2007; reviewed in Nawrath 2006). It is uncertain if the epicuticular materials on the abaxial and adaxial surfaces are a normal secondary response to the UV exposure that occurs in the *pd1* mutant because (1) it lacks the primary tip-based response of phenylpropanoid accumulation, or (2) this represents a developmental change in the cotyledons that results in the misdirection of wax deposition and/or other compounds as a direct result of the mutation in the *PD1* gene, or (3) the fibrous-appearing material is damaged pro-cuticle or waxes protruding from the epicuticular surface. While the data indicate that the cuticle structure and formation is affected by PD1 function, it is unclear if PD1 itself has a role in synthesis or deposition of cuticular and epicuticular materials, or both.

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REFERENCES

Aharoni A., Dixit S., Jetter R., Thoenes E., van Arkel G. & Pereira A. (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. *The Plant Cell* **16,** 2463–2480.

Ajavon A.N., Albritton D.L., Megie G. & Watson R.T. (2003) *Scientific Assessment of Ozone Depletion: 2002. Global Ozone and Monitoring Project – Report # 47*. World Meteorological Organization, Geneva, Switzerland.

Alonso J.M., Stepanova A.N., Leisse T.J., *et al.* (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301,** 653–657.

Anderson M.B., Folta K.M., Warpeha K.M., Gibbons J., Gao J. & Kaufman L.S. (1999) Blue light-directed destabilization of the pea *Lhcb1*4* transcript depends upon sequences within the 5′-UTR. *The Plant Cell* **11,** 1579–1589.

Beerhues L., Robeneck H. & Wiermann R. (1988) Chalcone synthesis from spinach (*Spinacia aleracea* L.). II. Immunofluorescence and immunogold localization. *Planta* **173,** 544–553.

Bernards M.A. (2002) Demystifying suberin. *Canadian Journal of Botany* **80,** 227–240.

Bieza K. & Lois R. (2001) An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. *Plant Physiology* **126,** 1105–1115.

Bilger W., Hohnsen T. & Schreiber U. (2001) UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. *Journal of Experimental Botany* **52,** 2007–2014.

Bird S.M. & Gray J.E. (2003) Signals from the cuticle affect epidermal cell differentiation. *New Phytologist* **157,** 9–23.

Blanding C., Simmons S.J., Casati P., Walbot V. & Stapleton A.E. (2007) Coordinated regulation of maize genes during increasing exposure to ultraviolet radiation: identification of ultraviolet-responsive genes, functional processes and associated potential promoter motifs. *Plant Biotechnology Journal* **5,** 677–695.

Brown B.A. & Jenkins G.I. (2008) UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature *Arabidopsis* leaf tissue by requirement for UVR8, HY5, and HYH. *Plant Physiology* **146,** 576–588.

Brown B.A., Cloix C., Jiang G.H., Kaiserli E., Herzyk P., Kliebenstein D.J. & Jenkins G.I. (2005) A UV-B-specific signaling component orchestrates plant UV protection. *Proceedings of the National Academy of Sciences of the United States of America* **102,** 18225–18230.

Burchard P., Bilger W. & Weissenbock G. (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. *Plant, Cell & Environment* **23,** 1373–1380.

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Caldwell M.M. & Flint S.D. (1994) Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Climatic Change* **28,** 375–394.

Caldwell M., Teramura A.H., Tevini M., Bornman J.F., Bjorn L.O. & Kulandevalu G. (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio* **24,** 166–173.

Caldwell M.M., Ballare C.L., Bornman J.F., Flint S.D., Bjorn L.O., Teramura A.H., Kulandaivelu G. & Tevini M. (2003) Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical & Photobiological Sciences* **2,** 29–38.

Caldwell M.M., Bornman J.F., Ballare C.L., Flint S.D. & Kulandaivelu G. (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. *Photochemical & Photobiological Sciences* **6,** 252–266.

Cartwright H.N., Baucom C., Singh P., Smith K.L. & Stapleton A.E. (2001) Intraspecific comparisons reveal differences in the pattern of ultraviolet radiation responses in four maize (*Zea mays* L.) varieties. *Journal of Photochemistry and Photobiology B: Biology* **62,** 88–96.

Casati P. & Walbot V. (2005) Differential accumulation of maysin and rhamnosylisoorientin in leaves of high-altitude landraces of maize after UV-B exposure. *Plant, Cell & Environment* **28,** 788–799.

Cen Y.P. & Bornman J.F. (1993) The effect of exposure to enhanced UV-B radiation on the penetration of monochromatic and polychromatic UV-B radiation in leaves of *Brassica napus*. *Physiologia Plantarum* **87,** 249–255.

Chappell J. & Hahlbrock K. (1984) Transcription of plant defense genes in response to UV light or fungal elicitor. *Nature* **311,** 76–78.

Chapple C.C.S., Shirley B.W., Zook M., Hammerschmidt R. & Somerville S.C. (1994) Secondary metabolism in Arabidopsis. In *Arabidopsis* (eds E.M. Meyerowitz & C.R. Somerville), pp. 989–1030. CSH Laboratory Press, Plainview, NY, USA.

Cho M.H., Corea O.R.A., Yang H., Bedgar D.L., Laskar D.D., Anterola A.M., Moog-Anterola F.A., Hood R.L., Kohalmi S.E., Bernards M.A., Kang C., Davin L.B. & Lewis N.G. (2007) Phenylalanine biosynthesis in Arabidopsis thaliana. Identification and characterization of arogenate dehydratases . *Journal of Biological Chemistry* **282,** 30827–30835.

Christie J.M. & Jenkins G.I. (1996) Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in *Arabidopsis* cells. *The Plant Cell* **8,** 1555–1567.

Christie P.J., Alfenito M.R. & Walbot V. (1994) Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* **194,** 541–549.

Cosio E.G. & McClure J.W. (1984) Kaempferol glycosides and enzymes of the flavonol biosynthesis in leaves of a soybean strain with low photosynthetic rates. *Plant Physiology* **74,** 877–881.

Dykstra M.J. (1992) *Biological Electron Microscopy: Theory, Techniques and Trouble Shooting*. Plenum Press, New York, USA.

Flint S.D., Jordan W.P. & Caldwell M.M. (1985) Plant protective response to enhanced UV-B radiation under field conditions: leaf optical properties and photosynthesis. *Photochemistry and Photobiology* **41,** 95–99.

Franke R., Briesen I., Wojciechowski T., Faust A., Yephremov A., Nawrath C. & Schreiber L. (2005) Apoplastic polyesters in *Arabidopsis* surface tissues – a typical suberin and a particular cutin. *Phytochemistry* **66,** 2643–2658.

Frohnmeyer H. & Staiger D. (2003) Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiology* **133,** 1420–1428.

Frohnmeyer H., Bowler C. & Schafer E. (1997) Evidence for some signal transduction elements involved in UV-light-dependent responses in parsley protoplasts. *Journal of Experimental Botany* **48,** 739–750.

Fuglevand G., Jackson J.A. & Jenkins G.I. (1996) UV-B, UV-A, and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene expression in *Arabidopsis*. *The Plant Cell* **8,** 2347–2357.

Gilchrist D.G. & Kosuge T. (1980) Aromatic amino acid biosynthesis and its regulation. In *The Biochemistry of Plants* (eds P.K. Stumpf, E.E. Conn & B.J. Miflin) Vol. 5, pp. 507–531. Academic Press, New York, USA.

Gonzalez R., Paul N.D., Percy K., Ambrose M., McLaughlin C.K., Barnes J.D., Areses M. & Welburn A.R. (1996) Responses to ultraviolet-B radiation (280–315 nm) of pea (*Pisum sativum*) lines differing in leaf surface wax. *Physiologia Plantarum* **98,** 852–860.

Grammatikopoulos G., Kyparissis A., Drilias P., Petropoulou Y. & Manetas Y. (1998) Effects of UV-B radiation on cuticle thickness and nutritional value of leaves in two Mediterranean evergreen sclerophylls. *Journal of Plant Physiology* **153,** 506–512.

Hahlbrock K. & Scheel D. (1989) Physiology and molecular biology of phenylpropanoir metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 347–369.

Hamm H.E. (1998) The many faces of G protein signaling. *Journal of Biological Chemistry* **273,** 669–672.

van Heerden P.S., Towers G.H. & Lewis N.G. (1996) Nitrogen metabolism in lignifying *Pinus taeda* cell cultures. *Journal of Biological Chemistry* **271,** 12350–12355.

Jansen M.A.K., Gaba V. & Greenberg B.M. (1998) Higher plants and UV radiation: balancing damage repair and acclimation. *Trends in Plant Sciences* **3,** 131–135.

Jenkins G.I. & Brown B.A. (2007) UV-B perception and signal transduction. In *Light and Plant Development* (eds G.C. Whitelam & K.J. Halliday), pp. 155–182. Blackwell Publishing, Oxford, UK.

Jenks M.A., Eigenbrode S.D. & Lemieux B. (2002) Cuticular waxes of *Arabidopsis*. In *The Arabidopsis Book* (eds C.R. Somerville & M.E. Meyerowitz), American Society of Plant Biologists, Rockville, MD, USA. doi: 10.1199/tab.0016, http://www.aspb.org/publications/arabidopsis/

Kaiserli E. & Jenkins G.I. (2007) UV-B promotes rapid nuclear translocation of the *Arabidopsis* UV-B-specific signaling component UVR8 and activates its function in the nucleus. *The Plant Cell* **19,** 2662–2673.

Kim K.S., Park S.H., Kim D.K. & Jenks M.A. (2007) Influence of water deficit on leaf cuticular waxes of soybean (*Glycine max* [L.] Merr.). *International Journal of Plant Science* **168,** 307–316.

Kliebenstein D.J. (2004) Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. *Plant, Cell & Environment* **27,** 675–684.

Koti S., Reddy K.R., Reddy V.R., Kakani V.G. & Zhao D.L. (2005) Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. *Journal of Experimental Botany* **56,** 725–736.

Koti S., Reddy K.R., Kakani V.G., Zhao V.G. & Gao W. (2007) Effects of carbon dioxide, temperature and ultraviolet-B radiation and their interactions on soybean (*Glycine max* L.) growth and development. *Environmental and Experimental Botany* **60,** 1–10.

Kunst L. & Samuels A.L. (2003) Biosynthesis and secretion of plant cuticular wax. *Progress in Lipid Research* **42,** 51–80.

Landry L.G., Chapple C.C.S. & Last R.L. (1995) *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. *Plant Physiology* **109,** 1159–1166.

© 2008 The Authors

Lapik Y. & Kaufman L.S. (2003) The *Arabidopsis* cupin domain protein AtPirin1 and AtGPA1, the Arabidopsis Gα subunit interact with each other and regulate seed germination and early seedling development. *The Plant Cell* **15,** 1578–1590.

Lewis N.G. & Yamamoto E. (1989) *Chemistry and Significance of Condensed Tannins* (eds R.W. Hemingway & J.J. Karchesy), pp. 23–47. Plenum Press, New York, USA.

Leyva A., Jarillo J.A., Salinas J. & Matrinez-Zapater J.M. (1995) Low temperature induces the accumulation of phenylalanine ammonia lyase and chalcone synthase mRNAs of *Arabidopsis thaliana* in a light dependent manner. *Plant Physiology* **108,** 39–46.

Li J., Ou-Lee I.-M., Raba R., Amundson R.G. & Last R.L. (1993) *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. *The Plant Cell* **5,** 171–179.

Li Y., Zu Y.Q., Chen H.Y. & Chen J.J. (2003) Intraspecific differences in physiological response of 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. *Environmental and Experimental Botany* **50,** 87–97.

Liu L. & McClure J.W. (1995) Effects of UV-B on activities of enzymes of secondary phenolic metabolism in barley primary leaves. *Physiologia Plantarum* **93,** 734–739.

Liu L., Gitz D.C. & McClure J.W. (1995) Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Physiologia Plantarum* **93,** 725–733.

Long L.M., Patel H.P., Cory W.C. & Stapleton A.E. (2003) The maize epicuticular wax layer provides UV protection. *Functional Plant Biology* **30,** 75–81.

McClure J.M. (1986) Physiology of flavonoids in plants. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships* (eds V. Cody, E. Middleton & J.B. Harborne), pp. 77–85. Alan Riss, Inc., New York, USA.

Margna U. (1977) Control at the level of substrate supply – an alternative in the regulation of phenylpropanoid accumulation in plant cells. *Phytochemistry* **16,** 419–426.

Margna U., VainJarv T. & Laanest L. (1989) Different L-phenylalanine pools available for the biosynthesis of phenolics in buckwheat seedling tissues. *Phytochemistry* **28,** 469–475.

Mazza C.A., Boccalandro H.E., Giordano C.V., Battista D., Scopel A.L. & Ballare C.L. (2000) Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. *Plant Physiology* **122,** 117–125.

Nawrath C. (2006) Unraveling the complex network of cuticular structure and function. *Current Opinion in Plant Biology* **9,** 281–287.

Noh B. & Spalding E.P. (1998) Anion channels and the stimulation of anthocyanin accumulation by blue light in *Arabidopsis* seedlings. *Plant Physiology* **116,** 503–509.

Ohl S., Hahlbrock K. & Schafer E. (1989) A stable blue-light-derived signal modulates ultraviolet-light-induced activation of the chalcone-synthase gene in cultured parsley cells. *Planta* **177,** 228–236.

Oravecz A., Baumann A., Mate Z., Brzezinska A., Molinier J., Oakeley E.J., Adam E., Schafer E., Nagy F. & Ulm R. (2006) CONSTITUTIVELY PHOTOMORPHOGENIC1 is required for the UV-B response in *Arabidopsis*. *The Plant Cell* **18,** 1975–1990.

Perfus-Barbeoch L., Jones A.M. & Assmann S.M. (2004) Plant heterotrimeric G protein function: insights from *Arabidopsis* and rice mutants. *Current Opinion in Plant Biology* **7,** 719–731.

Pilon J.J., Lambers H., Baas W., Tosserams M., Rozema J. & Atkin O.K. (1999) Leaf waxes of slow-growing alpine and fast-growing lowland Poa species: inherent differences and responses to UV-B radiation. *Phytochemistry* **50,** 571–580.

Reuber S., Bornman J.F. & Weissenböck G. (1996) Phenylpropanoid compounds in primary leaf tissues of rye (*Secale cereale*): light response of their metabolism and the possible role in UV-B protection. *Physiologia Plantarum* **97,** 160–168.

Ries G., Heller W., Puchta H., Sandermann H., Seidlitz H.K. & Hohn B. (2000) Elevated UV-B radiation reduces genome stability in plants. *Nature* **406,** 98–1010.

Robberecht R. & Caldwell M.M. (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet radiation. *Photochemistry and Photobiology* **41,** 95–99.

Rozema J., van de Staaij J., Bjorn L.O. & Caldwell M. (1997) UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology & Evolution* **12,** 22–28.

Rozema J., Bjorn L.O., Bornman J.F., *et al.* (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. *Journal of Photochemistry and Photobiology B: Biology* **66,** 2–12.

Rozema J., Boelen P. & Blokker P. (2005) Depletion of stratospheric ozone over the Antarctic and Arctic: responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview. *Environmental Pollution* **137,** 428–442.

Sato T., Kang H.S. & Kumagai T. (1994) Genetic study of resistance to inhibitory effects of UV-radiation in rice (*Oryza sativa*). *Physiologia Plantarum* **91,** 234–238.

Sieber P., Schorderet M., Ryser U., Buchala A., Kolattukudy P., Metraux J.P. & Nawrath C. (2000) Transgenic *Arabidopsis* plants expressing a fungal cutinase show alterations in the structure and properties of the cuticle and postgenital organ fusions. *The Plant Cell* **12,** 721–737.

Stapleton A.E. & Walbot V. (1994) Flavonoids can protect maize DNA from induction of ultraviolet radiation damage. *Plant Physiology* **105,** 881–889.

Stapleton A.E., Thornber C.S. & Walbot V. (1997) UV-B component of sunlight causes measurable damage in field-grown maize (*Zea mays* L): developmental and cellular heterogeneity of damage and repair. *Plant, Cell & Environment* **20,** 279–290.

Strid A., Chow W.S. & Anderson J.M. (1994) UV-B damage and protection at the molecular level in plants. *Photosynthesis Research* **39,** 475–489.

Sullivan J.H. (2005) Possible impacts of changes in UV-B radiation on North American trees and forests. *Environmental Pollution* **137,** 380–389.

Sullivan J.H. & Rozema J. (1999) UV-B effects on terrestrial plant growth and photosynthesis. In *Stratospheric Ozone Depletion: The Effects of Enhanced UV-B Radiation on Terrestrial Ecosystems* (ed. J. Rozema), pp. 39–57. Backhays Publishers, Leiden, the Netherlands.

Sullivan J.H., Gitz D.C. III, Liu-Gitz L., Xu C., Gao W. & Slusser J. (2007) Coupling short-term changes in ambient UV-B levels with induction of UV-screening compounds. *Photochemistry and Photobiology* **83,** 863–870.

Szczuka E. & Szczuka A. (2003) Cuticle fluorescence during embryogenesis of *Arabidopsis thaliana* (L.) Heynh. *Acta Biologica Cracoviensa Series Botanica* **45,** 63–67.

Tattini M., Gravano E., Pinelli P., Mulinacci N. & Romani A. (2000) Flavonoids accumulate in leaves and glandular trichomes of *Phillyrea latifolia* exposed to excess solar radiation. *New Phytologist* **148,** 69–77.

Temple B.R.S. & Jones A.M. (2007) The plant heterotrimeric G-protein. *Annual Review of Plant Biology* **58,** 249–266.

Tevini M., Braun J. & Fieser F. (1991) The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. *Photochemistry and Photobiology* **53,** 329–333.

Torabinejad J. & Caldwell M.M. (2000) Inheritance of UV-B

© 2008 The Authors

tolerance in seven ecotypes of *Arabidopsis thaliana* L. Heynh. and their F1 hybrids. *Heredity* **91,** 228–233.

Ulm R. & Nagy F. (2005) Signaling and gene regulation in response to ultraviolet light. *Current Opinion in Plant Biology* **8,** 477–482.

Wade H.K., Bibikova T.N., Valentine W.J. & Jenkins G.I. (2001) Interactions within a network of phytochrome, cryptochrome, and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. *The Plant Journal* **25,** 675–685.

Wade H.K., Sohal A.K. & Jenkins G.I. (2003) *Arabidopsis* ICXI is a negative regulator of several pathways regulating flavonoid biosynthesis genes. *Plant Physiology* **131,** 707–715.

Warpeha K.M.F. & Kaufman L.S. (1990a) Two distinct blue-light responses regulate epicotyl elongation in pea. *Plant Physiology* **92,** 495–499.

Warpeha K.M.F. & Kaufman L.S. (1990b) Two distinct blue-light responses regulate the levels of transcripts of specific nuclear-coded genes in pea. *Planta* **182,** 553–558.

Warpeha K.M.F., Hamm H.E., Rasenick M.M. & Kaufman L.S. (1991) A blue-light activated GTP binding protein in the plasma membrane of etiolated pea. *Proceedings of the National Academy of Sciences of the United States of America* **88,** 8925–8929.

Warpeha K.M., Lateef S.S., Lapik Y., Anderson M.B., Lee B.S. & Kaufman L.S. (2006) G-protein-coupled receptor1, G-protein Gα-subunit1, and prephenate dehydratase1 are required for blue light-induced production of phenylalanine in etiolated *Arabidopsis*. *Plant Physiology* **140,** 844–855.

Warpeha K.M., Upadhyay S., Yeh J., Adamiak J., Hawkins S.I., Lapik Y.R., Anderson M.B. & Kaufman L.S. (2007) The GCR1, GPA1, PRN1, NF-Y signal chain mediates both blue light and ABA responses in *Arabidopsis*. *Plant Physiology* **143,** 1590–1600.

Weinig C., Gravuer K.A., Kane N.C. & Schmitt J. (2004) Testing adaptive plasticity to UV: costs and benefits of stem elongation and light-induced phenolics. *Evolution* **58,** 2645–2656.

Yamada T., Matsuda F., Kasai K., Fukuoka S., Kitamura K., Tozawa Y., Miyagawa H. & Wakasa K. (2008) Mutation of a rice gene encoding a phenylalanine biosynthetic enzyme results in accumulation of phenylalanine and tryptophan. *The Plant Cell* **20,** 1316–1329.

Zacchini M. & de Agazio M. (2004) Spread of oxidative damage and antioxidative responses through cell layers of tobacco callus after UV-C treatment. *Plant Physiology and Biochemistry* **42,** 445–450.

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