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Evidence That Heat and Ultraviolet Radiation Activate a Common Stress-Response Program in Plants That Is Altered in the *uvh6* Mutant of *Arabidopsis thaliana*[1]

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The *uvh6* mutant of Arabidopsis was previously isolated in a screen for increased sensitivity to ultraviolet (UV) radiation. *uvh6* mutant plants were killed by incubation at 37°C for 4 d, a treatment not lethal to wild-type plants. Furthermore, under permissive conditions, *uvh6* plants were yellow-green with an approximately one-third lower chlorophyll content. Genetic analysis of the *uvh6* mutant strongly suggested that all three mutant phenotypes were due to mutation at the same genetic locus. To understand *UVH6* function more fully, the response of wild-type plants to growth at elevated temperatures and exposure to UV radiation was analyzed. Wild-type plants grown at 30°C were as UV-hypersensitive and yellow-green as *uvh6* mutant plants grown at 24°C. Mutant *uvh6* plants induced heat-shock protein HSP21 at a lower threshold temperature than wild-type plants, indicating that the *uvh6* mutant was exhibiting signs of heat stress at a 4 to 5°C lower temperature than wild-type plants. We propose that UV damage and heat induce a common stress response in plants that leads to tissue death and reduced chloroplast function, and that the UVH6 product is a negative regulator of this response.

Plants are subjected to a variety of stress conditions associated with their natural environment and have developed protective mechanisms against the types of damage produced. Sunlight, which is required for photosynthesis, exposes plants to damaging levels of UV-B radiation and to heat stress (Green, 1983). Adsorption of UV-B radiation by DNA generates two major photoproducts, cyclobutyl pyrimidine dimers and pyrimidine (6–4) pyrimidinone dimers, which block DNA replication and transcription (Britt, 1995). Protein unfolding is probably an important type of damage resulting from heat stress (Boston et al., 1996). PSII, a component of the light reactions of photosynthesis, is damaged by both UV radiation and heat stress (Renger et al., 1989; Bredenkamp and Baker, 1994).

Plants usually protect themselves from heat shock and UV radiation damage by mechanisms very similar to those used by other organisms. When plants are exposed to temperatures five or more degrees above optimal growth temperature, the synthesis of most normal proteins is repressed and a set of proteins called HSPs is induced. HSP expression also occurs when plants experience a gradual increase in temperature, and HSP synthesis under such conditions is thought to provide thermotolerance to normally lethal temperatures. These HSPs have a variety of molecular weights and are very similar to HSPs from other eukaryotes, but a group of low-molecular-weight species is particularly abundant in plants (for review, see Vierling 1991).

HSPs are thought to act as molecular chaperones by binding to proteins that are in an unfolded configuration, such as during heat stress. Their activities include preventing aggregation of unfolded proteins and promoting renaturation of aggregated proteins (for review, see Boston et al., 1996). To regulate the heat-shock response, organisms produce heat-shock factors that regulate transcription of HSP mRNAs. These factors become activated in response to temperature increases (Sorger, 1991). The promoters of plant HSP genes include specific sequences that are necessary for heat-induced transcription in other organisms (Vierling, 1991). Thus, HSP induction mechanisms are likely to be highly conserved in plants. However, treatments such as arsenite also increase the synthesis of many HSPs, whereas other treatments such as salt stress do not (Vierling, 1991). UV radiation has also been reported to induce HSP-like proteins in *Vigna sinensis* seedlings (Nedunchezian et al., 1992). Therefore, plants may have additional mechanisms for HSP induction.

DNA-repair and damage-tolerance mechanisms, which provide resistance to UV damage in other organisms, also occur in plants (Britt, 1995). These mechanisms include photoreactivation (Pang and Hays, 1991; Chen et al., 1994; Ahmad et al., 1997; Landry et al., 1997), postreplication repair (Cerrutti et al., 1992), and nucleotide excision repair (McClennan and Eastwood, 1986). These mechanisms remove cyclobutyl pyrimidine dimers and pyrimidine (6–4) pyrimidinone dimers from UV-damaged plants or provide ways to avoid their lethal effects (Britt, 1995). Plants also respond to UV radiation exposure by increased flavonoid biosynthesis (Beggs and Wellman, 1985). Intermediates of this pathway protect plants from the UV-B component of sunlight (Li et al., 1993; Stapleton and Walbot, 1994; Landry et al., 1995). Exposure of plants to UV radiation also activates certain signal transduction pathways, leading to in-

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Abbreviations: HSP, heat-shock protein; PCD, programmed cell death.

creased expression of the pathogen-responsive gene *PR1* in tobacco (Green and Fluhr, 1995) and wound-defense genes in tomatoes (Conconi et al., 1996). In these cases, the UV radiation may induce these pathways through the production of oxygen radicals that damage the cell membrane and activate a membrane-associated signal transduction protein or through a DNA damage signal sent from the nucleus (Mount, 1996).

Another response of organisms to DNA damage, heat stress, and other types of biological damage is the PCD response or apoptosis. PCD plays an important role in the development and maintenance of cell populations in the tissues of multicellular organisms. Cells undergoing PCR are characterized by plasma membrane blebbing, cytoplasmic and nuclear volume loss, nuclear condensation, and endonucleolytic cleavage of DNA between nucleosomes. PCD is also associated with the induction of specific gene transcripts for degradative enzymes, and this induction is mediated by signal transduction pathways. Another form of cell death called necrosis has also been described in animal systems. However, PCD and necrosis are biochemically and morphologically distinct. In necrosis the cytoplasm and organelles swell, the DNA is randomly fragmented, and the cell lyses (Umansky, 1996).

In plants PCD removes cells with a temporary function in development, promotes cell and tissue specialization, and participates in the senescence of tissues (for review, see Pennell and Lamb, 1997, and Bleeker and Patterson, 1997). For example, nuclear shrinking and cleavage of DNA have been observed in Arabidopsis cells undergoing senescence in tissue culture. Transcription of specific gene products is also associated with PCD in plants. These products include Cys proteases and a glycosyl hydrase, an activity associated with senescing plant tissues (Callard et al., 1996; see Pennell and Lamb, 1997). Other studies have suggested that PCD and senescence in plants are coordinated by a signal transduction pathway (Pennell and Lamb, 1997).

Several types of stress have been shown to elicit a PCD response in plants. In soybean cells oxidative damage was shown to produce localized cell death with features of PCD, including plasma membrane breakdown and nuclear condensation. Similar effects were also produced during the onset of the hypersensitive response to microbial pathogens in soybeans and Arabidopsis (Levine et al., 1996). Such observations raise the possibility that other forms of stress such as heat and UV damage could also induce PCD in plants.

To analyze the mechanisms that control the response of plants to UV radiation, mutants of *Arabidopsis thaliana* with increased sensitivity to UV radiation (*uvh* and *uvr* mutants) have been isolated (Britt et al., 1993; Harlow et al., 1994; Jenkins et al., 1995; Landry et al., 1997). Several of these mutants appear to be defective in a specific DNA repair mechanism (Britt et al., 1993; Ahmad et al., 1997; Landry et al., 1997). The UV-sensitive mutant *uvh6* does not appear to be a DNA-repair mutant. Instead, it exhibits additional phenotypes, including failure to grow at elevated temperatures and a yellow-green color. The properties of this mutant, which are phenocopied in wild-type plants in response to moderate heat stress, suggest that UV damage and heat induce a common stress response that leads to tissue death in plants, and that the *UVH6* gene regulates this response.

MATERIALS AND METHODS

Strains and Growth Conditions

Arabidopsis refers to the species *Arabidopsis thaliana*, and Columbia and Landsberg refer to two ecotypes of this species. The wild-type ecotype Columbia parent and the back-crossed strain of the *uvh6* mutant have been described previously (Jenkins et al., 1995). Both strains lack trichomes due to a mutation at the glabrous1 (*GL1*) locus (Oppenheimer et al., 1991).

Seeds were planted in soil comprised of Metromix 350 (Grace-Sierra Horticultural Products, Milpatis, CA) overlaid with a 1-cm layer of potting mix (Sunshine All-Purpose Pottery Mix, Fisons, Vancouver, BC, Canada). Normal laboratory conditions for growth included lighting with a 1:1 mixture of cool-white fluorescent lights (model F40C/W; Sylvania, Danvers, MA) and F40 agro-fluorescent lights (model F40AGRO; Philips, Somerset, NJ) at a distance of 35 cm above the plants (PAR = 30 μm m^{-2} s^{-1}) and at an approximate room temperature of 23°C. In several experiments, plants previously germinated under the above conditions were subsequently transferred to a growth chamber (Conviron, Controlled Environments Ltd., Winnipeg, MB, Canada) under ambient humidity.

Mutagenesis and Selection of Temperature-Resistant Derivatives of the *uvh6* Mutant

Approximately 3000 *uvh6* seeds were mutagenized with ethyl methane sulfonate, as previously described (Estelle and Somerville, 1987). The *uvh6* strain used for mutagenesis was a *GL1* derivative that resulted from an outcross between the *uvh6* mutant and the ecotype Landsberg strain. This latter strain was used so that the presence of trichomes and a characteristic pattern of physical map polymorphisms could be used to confirm the genetic origin of the derivatives. To expedite obtaining independent derivatives, seeds from approximately 75 M_1 plants were pooled to produce a total of 32 M_2 pools. Approximately 900 25-d-old plants from each of 20 pools were grown and tested for survival after transfer to 37°C for 5 d, and 5 survived this selection. Seed was collected from four surviving derivatives and was used to produce progeny plants that were also tested for temperature resistance. One true-breeding line was subsequently established from each derivative. These lines were independent isolates, since each was derived from a different M_2 pool.

Measurement of UV Sensitivity

The UV-C source and UV light measurements were as described previously (Jenkins et al., 1995). UV-C radiation was supplied by a germicidal lamp (model G8T5, Sylvania; predominant wavelength = 254 nm) at a rate of 5 J m^{-2} s^{-1}. UV-C radiation was measured using a digital radiometer

(UVX, UVP Inc., Upland, CA) equipped with a UV-C sensor (UVX-25, UVP Inc.).

Two different conditions for measuring UV-C sensitivity were used to emphasize strain similarities and differences. For the data in Figure 1, plants to be irradiated were grown under standard laboratory conditions for 14 d, and, following irradiation, were placed under gold fluorescent lights (F40GO, Sylvania), which lack photoreactivating wavelengths (Jenkins et al., 1995). Irradiated plants were photographed 7 d later. UV-irradiated plants are more UV-hypersensitive when placed under gold light because photoreactivation, a major DNA-repair mechanism in plants (Pang and Hays, 1991; Chen et al., 1994), is not supported by the wavelengths produced by these lamps. These conditions were used because they favor the identification of small differences in UV hypersensitivity, as observed between wild-type plants and the resistant revertants of the *uvh6* mutant.

The data in Table I were generated by transferring plants previously grown under standard laboratory conditions for 15 d to a growth chamber for 7 d at the temperature indicated, and then treating them with UV-C radiation. Following irradiation, the plants were returned to the growth chamber at the same temperature and then scored for survival at 10 d and at later times, as described previously (Jenkins et al., 1995). The dose giving 50% survival (UV-C lethal dose 50%) was then determined. In this experiment, all unirradiated plants survived the treatment. The fluorescent lights in the growth chamber provided photoreactivating wavelengths of light that photoreverse UV damage. Relative survivals were therefore higher than those shown in Figure 1.

Table I. *Effects of temperature on chlorophyll content and UV-C hypersensitivity of wild-type and mutant uvh6 plants*

UV-C survival was determined as described in "Materials and Methods." Chlorophyll levels were determined on plants first grown under normal laboratory conditions for 7 d to ensure uniform germination and growth rates, and were then transferred to a growth chamber at the temperature indicated for 8 d. Chlorophyll content was determined as described in Figure 2 and each value shown is the average ± SD of three separate determinations.

Treatment	Strain	
	uvh6	Wild type
	J/m^2	
UV lethal dose		
24°C	800–600	3200–6400
30°C	100–200	800–1600
	$mg\ g^{-1}$ *fresh wt*	
Chlorophyll content		
24°C	1.01 ± 0.03	1.37 ± 0.01
30°C	0.74 ± 0.05	0.98 ± 0.04

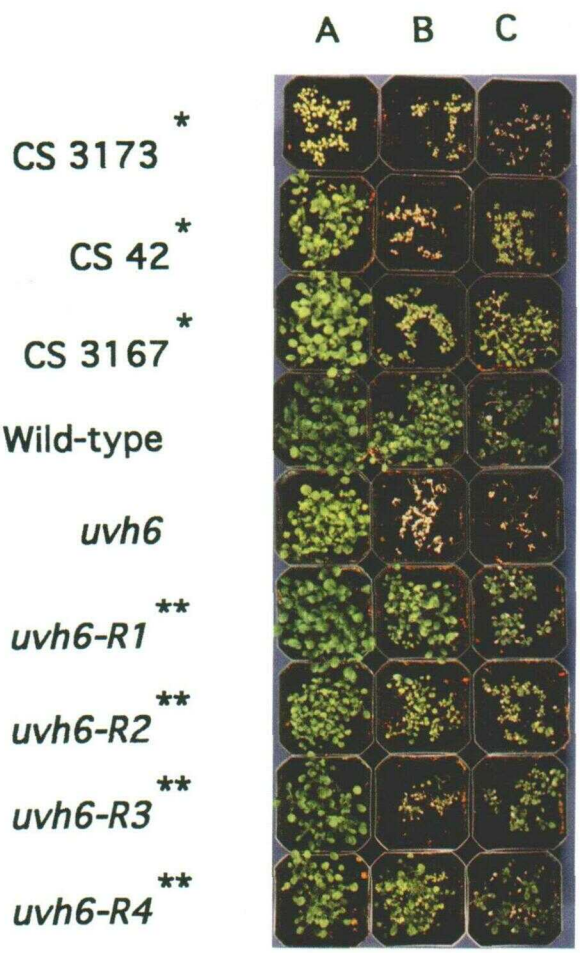

Figure 1. Sensitivity of the *uvh6* mutant, additional yellow-green mutants (*) of Arabidopsis, and resistant revertants of the *uvh6* mutant (**) to increased temperature and UV-C radiation exposure. Plants were grown under normal laboratory conditions for 14 d with no further treatment (A), transferred to 37°C for 4 d (B), or exposed to 500 J/m^2 UV-C radiation (C), as described in "Materials and Methods."

Measurement of Temperature Sensitivity

The ability of plants to survive a 37°C heat stress was determined by transferring 14-d-old plants previously grown under standard laboratory conditions to a growth chamber at 37°C. At later times, the plants were transferred back to normal laboratory growth conditions and survival was scored 1 week later. A shriveled plant lacking chlorophyll was scored as a nonsurvivor.

Polyacrylamide Gels and Immunoblotting

Protein isolation, polyacrylamide electrophoresis, and immunoblotting were performed as described previously (Osteryoung and Vierling, 1994). To assess protein concentrations in each extract, an initial polyacrylamide gel loaded with the extracts was run and then stained with Coomassie blue. A second gel was then loaded with equal amounts of extract in each lane. After transfer of the electrophoresed proteins to a membrane, protein levels were again assessed by staining the membrane with a Ponceau stain.

Measurement of Leaf Temperatures

Leaf temperatures were measured with a thermocouple (model 872, Omega Engineering, Stamford, CT) using a

standard iron-constant electrode. Plants for this experiment were grown for 28 d under a day/night cycle (18 h/6 h) under standard laboratory conditions to maximize leaf area and thus facilitate attachment of the electrode. The plants were then transferred to a growth chamber at ambient humidity at the temperature specified. Readings were taken every minute from 10 to 30 min after transfer. Values reported are the average of five such readings, each made on a different plant.

RESULTS

Pleiotropic Behavior of the *uvh6* Mutant

The procedures used to isolate the *uvh6* mutant (Harlow et al., 1994) and a preliminary characterization of its response to UV and ionizing radiations have been described previously (Jenkins et al., 1995). To summarize, leaves of 14-d-old *uvh6* seedlings were shown to be extremely sensitive to acute doses of UV-B and UV-C radiation wavelengths. In contrast, roots of *uvh6* plants showed near normal sensitivity to UV radiation using a root-bending assay. Additionally, development of the first true leaves by *uvh6* seedlings was not abnormally inhibited by previous exposure of seeds to ionizing radiation, as were other mutants such as *uvh1* and *uvh3*. These studies suggest that the *uvh6* mutant is altered in a resistance or response mechanism for UV damage (Jenkins et al., 1995). The *uvh6* mutant is now shown to have several additional phenotypes that suggest the increased sensitivity to UV radiation is due to an altered stress-response program.

At growth temperatures of 22 to 24°C, *uvh6* plants grew with the same rate and vigor (Fig. 1, column A) and were more sensitive to UV radiation than wild-type plants (Fig. 1, column C). When 14-d-old *uvh6* and wild-type plants were grown at 37°C, the *uvh6* plants died after 4 d (Fig. 1, column B). When this treatment was given to a total of approximately 10,000 *uvh6* mutant plants in several experiments, none survived. In contrast, wild-type plants grew more slowly at 37°C than at 22 to 24°C, but all survived the 37°C treatment. Growth of wild-type plants at 45°C for 4 d produced a degree of killing comparable to that observed in *uvh6* plants at 37°C (data not shown). These observations suggest that *UVH6* function is essential for growth at 37°C.

An additional phenotype of *uvh6* plants was a chlorotic, yellow-green appearance (Fig. 1, column A), and *uvh6* leaves contained approximately one-third less chlorophyll than wild-type leaves (Fig. 2). To determine if increased UV and heat sensitivity is commonly associated with a reduction in chlorophyll, 10 other yellow-green mutants (Koornneef et al., 1983) were obtained from the Arabidopsis Stock Center (Columbus, OH). These mutants were analyzed for UV and temperature sensitivity and chlorophyll levels, as described above, and the results are shown in Figures 1 and 2. Eight of these additional yellow-green mutants showed reduced levels of chlorophyll but were not significantly hypersensitive to either UV-C exposure (Fig. 1, column C) or growth at 37°C (Fig. 1, column B). A typical example of these was strain Cs3167, shown in Figure 1, row 3, and Figure 2, column 8.

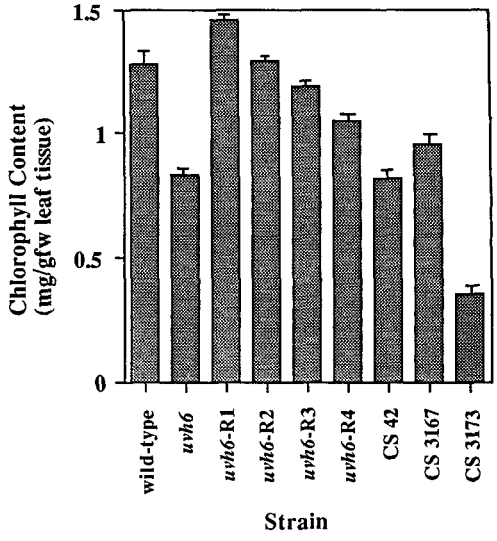

Figure 2. Chlorophyll levels of the *uvh6* mutant, resistant revertants of the *uvh6* mutant, and additional yellow-green mutants of Arabidopsis. Total chlorophyll A plus B levels were determined as described previously (Arnon, 1949) in 15-d-old plants previously grown under standard laboratory conditions. Each value shown is the average and SD of measurements on three independent plant preparations. fw, Fresh weight.

The remaining two strains showed increased sensitivity to either heat stress or UV radiation but not to both treatments. Strain Cs3173, which had one of the lowest chlorophyll levels (Fig. 2, last column), grew extremely poorly at all temperatures but was not particularly temperature sensitive, and also exhibited slight hypersensitivity to UV-C radiation at 22°C (Fig. 1, row 1). Strain Cs42 (Fig. 2, column 7), which has chlorophyll levels comparable to those of the *uvh6* mutant (Fig. 2, row 7), was sensitive to growth at 37°C but was not hypersensitive to UV-C radiation at 22°C (Fig. 1, row 2). Thus, a combined increase in UV and heat sensitivity is not a common property of other yellow-green mutants, and the *uvh6* mutant is unusual in this respect.

Phenotypes of *uvh6* Mutant Are Due to a Single Mutation

Previous genetic analysis of the *uvh6* mutant revealed that the UV-hypersensitivity phenotype resulted from a single, recessive, nuclear-encoded Mendelian trait located approximately 6 cM from the top of Arabidopsis chromosome *1* (Jenkins et al., 1995). To analyze whether all three mutant phenotypes were due to the same mutation, *uvh6* plants were crossed to wild-type plants and the F_1 progeny of this cross were allowed to self-fertilize. Thirty-two pools of seed representing progeny of individual F_2 plants were then collected. F_3 plants grown from these pools were tested for segregation of the mutant phenotypes. Cosegregation of all three phenotypes was observed, suggesting that they are due to a mutation at a single locus. However, the possibility that the phenotypes were due to two or more closely linked mutations or to a deletion cannot be excluded by this analysis.

If a mutation at a single locus caused the mutant phenotypes of the *uvh6* mutant, it should be possible to obtain secondary mutant derivatives with a single additional mutation that reverts all mutant phenotypes to the wild-type state. Four independent *uvh6* derivatives, designated *uvh6-R1*, *uvh6-R2*, *uvh6-R3*, and *uvh6-R4*, were obtained by selecting for mutagenized *uvh6* plants able to survive for 4 d at 37°C (see "Materials and Methods"). Although selected for increased heat resistance, these derivatives all showed increased UV resistance and chlorophyll levels compared with their *uvh6* parent, as illustrated in Figures 1 and 2. One derivative, *uvh6-R2*, was no longer yellow-green (Fig. 1, column A) with wild-type chlorophyll content (Fig. 2, column 4), temperature resistance (Fig. 1, column B), and UV resistance (Fig. 1, column C).

Of the three remaining derivatives, *uvh6-R1* had elevated chlorophyll levels but wild-type UV and temperature resistance, *uvh6-R3* had slight temperature sensitivity but otherwise wild-type properties, and *uvh6-R4* had chlorophyll levels between those of wild-type and *uvh6* plants, but otherwise wild-type properties. These results strongly suggest that chlorophyll reduction, UV hypersensitivity, and temperature sensitivity in the *uvh6* mutant were all due to a mutation at the same genetic locus. These highly pleiotropic properties of the *uvh6* mutant suggest that there may be a genetic program in plants that responds to both UV radiation and heat stress, and that the *uvh6* mutant may be altered in such a program.

Wild-Type Arabidopsis Plants Grown at 30°C Are UV-Hypersensitive and Yellow-Green

To search for further evidence of a relationship between plant responses to heat and UV stress, UV sensitivities and chlorophyll levels of *uvh6* and wild-type plants grown at 24 and 30°C to increase heat stress were compared. As shown in Table I, growth of wild-type plants at 30°C increased their sensitivity to UV radiation and reduced their chlorophyll levels to extents similar to those observed in *uvh6* plants grown at 24°C, thus producing a phenocopy of the *uvh6* mutant. These results suggest that wild-type Arabidopsis plants exhibit a stress response to UV radiation and heat such that the response is observed in wild-type plants under more extreme conditions of heat stress than in mutant *uvh6* plants.

HSP21 Is Hyperinducible in the *uvh6* Mutant

One of the best-characterized responses to heat stress is the induction of HSPs. Since the *uvh6* mutant was killed by growth at 37°C, a temperature that did not affect wild-type plants, the heat-shock response could be altered in this mutant. To examine this possibility, induction of a well-characterized small HSP (HSP21), a major chloroplast HSP, was examined. Synthesis of HSP21 following heat stress is a reliable indicator of the heat-shock response in plants. Accumulation of HSP21 and kinetics of induction of HSP21 mRNA closely parallel those of another well-characterized HSP, HSP70 (Chen et al., 1990).

To test for HSP21 induction, plants were grown for 14 d at 22°C under normal laboratory conditions and then transferred to a growth chamber at 37, 33, or 28°C. At intervals after transfer, total protein was isolated and analyzed for HSP21 by immunoblotting following electrophoresis. When plants were transferred to 37°C, HSP21 was detected in both *uvh6* and wild-type extracts (Fig. 3A). Initially, the HSP21 levels were similar, but 6 h and later, *uvh6* extracts contained significantly more HSP21 than wild-type extracts. After transfer to a lower temperature of 33°C, HSP21 was detected only in *uvh6* extracts. HSP21 was initially present at 34 h, but the amount continued to increase until 70 h (Fig. 3B). HSP21 was not detectable at 33°C in either *uvh6* or wild-type extracts prior to 34 h (data not shown). After transfer to an even lower temperature of 28°C, HSP21 could not be detected in extracts of either wild-type or *uvh6* plants (data not shown). This analysis revealed that the *uvh6* mutation influences HSP21 induction by lowering the threshold temperature for induction and by inducing increased HSP21 levels at temperatures above this threshold. These results suggest that a heat-stress response was present in *uvh6* plants at a lower temperature than in wild-type plants.

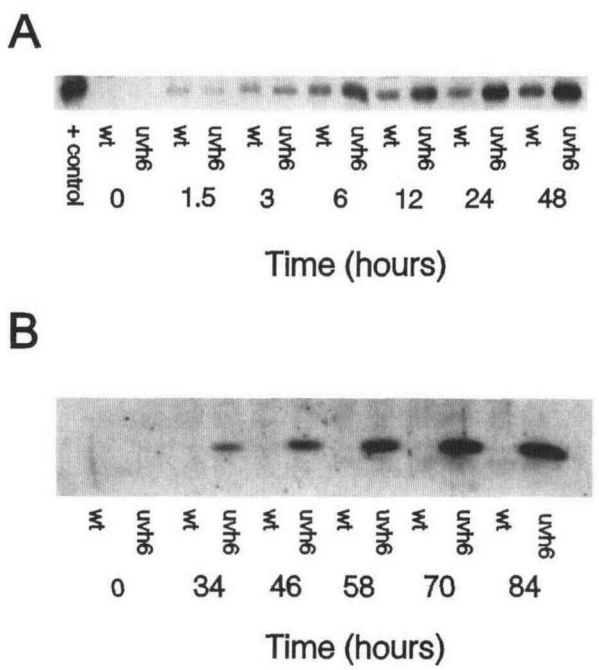

Figure 3. Induction of HSP21 in *uvh6* mutant and wild-type plants after transfer to elevated temperatures. Mutant (*uvh6*) and wild-type (wt) plants were grown under normal laboratory conditions. Total protein was isolated from whole plants, subjected to electrophoresis, and immunoblotted using HSP21 antibody, with loading controls as described in "Materials and Methods." A, Plants were transferred to 37°C for the times indicated. B, Plants were transferred to 33°C for the times indicated, and also for 1, 1.5, 3, 6, 12, and 24 h. The first lane in A is a control assay using purified HSP21.

Table II. *Leaf temperatures of wild-type and uvh6 plants grown at two ambient temperatures*

Ambient Temperature[a]	Leaf Temperature	
	uvh6	Wild type
	°C	
22.6	22.1 ± 0.1	22.1 ± 0.1
37.2	34.3 ± 0.5	33.9 ± 0.4

[a] Ambient temperature is the internal temperature of the growth chamber.

uvh6 Mutant Defect Is Not Due to Increased Leaf Temperature

One simple explanation for the complex phenotype of *uvh6* plants is that the genetic defect results in increased leaf temperature. For example, a defect in transpiration, a process that cools leaves by evaporation, could conceivably lead to a 4 to 5°C temperature increase in *uvh6* leaf tissue. To examine this possibility, leaf temperatures of *uvh6* and wild-type plants were compared using a thermocouple, as described in "Materials and Methods." As shown in Table II, when plants were grown at 22.6°C in a growth chamber at ambient humidity (less than 20% RH), no difference was observed between the two strains. When plants were grown at 37°C under similar conditions, leaf tissue was approximately 3°C cooler than the ambient temperature in both *uvh6* and wild-type plants. A slight temperature difference was observed between the two strains but the difference was within the margin of error of the experiment. Although these data do not rule out a small difference in leaf temperature between mutant and normal plants, the 4 to 5°C difference that would be required to account for the *uvh6* phenotype was not observed.

DISCUSSION

An important observation in these studies was that wild-type plants grown under moderate heat-stress conditions at 30°C exhibited increased UV sensitivity and decreased chlorophyll levels. These changes were similar to those observed in mutant *uvh6* plants grown at a significantly lower temperature of 22 to 24°C. In addition, *uvh6* plants were killed by exposure to 37°C, a much lower temperature than necessary to kill wild-type plants. Genetic analysis strongly suggested that this increased sensitivity to heat was due to the same mutation as increased UV sensitivity and decreased chlorophyll levels. These observations suggest that there may be a regulatory relationship between the responses of plants to heat stress and UV damage, and that this relationship is altered in the *uvh6* mutant. Consistent with such a relationship, the induction of HSP-like proteins has been observed in UV-irradiated *Vigna sinensis* seedlings (Nedunchezhian et al., 1992).

The simplest interpretation of these results is that the increased UV sensitivity of wild-type plants under moderate heat stress and the increased sensitivity of *uvh6* mutant plants to both UV radiation and heat are due to the activation of a stress-response program, rather than to increased levels of lethal damage from the treatments themselves. One intriguing possibility is that plants respond to heat and UV damage through activation of a common signal transduction pathway, which triggers a PCD response. There is a strong precedent for such a response, since DNA damage and heat stress are among stress treatments that induce a PCD response in a number of other organisms (Umansky, 1996).

Although we have not demonstrated other features characteristic of PCD under these conditions, such as nuclear condensation and DNA fragmentation, features of PCD have been observed in plant tissues and tissue culture cells undergoing senescence (Callard et al., 1996; see Pennell and Lamb, 1997), in plants cells following oxidative damage, and in the hypersensitive response of plants to microbial pathogens (Levine et al., 1996). Further experiments are needed to determine whether plants respond to UV radiation and heat stress by showing the biochemical and morphological changes characteristic of the PCD response.

We hypothesize that plants first sense heat and UV radiation by receptors or by damage-detection mechanisms, and that these sensing mechanisms then each transmit signals to a common receiver in a signaling pathway. The signals are integrated, and if the resulting integrated signal is strong enough, the pathway then activates the PCD program. For wild-type plants, the increased UV sensitivity observed under conditions of moderate heat stress might be accounted for by the proposed combined signaling effects of heat and UV radiation. Therefore, the program may be partially triggered by heat stress so that a small UV dose is sufficient to induce the program fully, detectable as an increase in sensitivity to UV radiation.

The effect of the *uvh6* mutation could be to lower the temperature at which the program can be activated. Accordingly, *uvh6* plants at normal laboratory growth temperatures may have a partially triggered program similar to that of wild-type plants under conditions of moderate heat stress. Small doses of UV radiation or slight heat stress may then be sufficient to induce the program fully. We propose that the UVH6 gene product negatively regulates the proposed PCD pathway by preventing the activation of the program until a certain signaling threshold is reached, such as in those plant tissues that have undergone extensive heat and/or UV damage. According to this model, the recessive *uvh6* mutation inactivates UVH6 function, thus reducing the response threshold and, as a result, the mutant plants respond to a weaker signal.

The properties of the *uvh6* mutant can be compared with those of mutants defective in the programmed response to pathogen invasion. Arabidopsis accelerated cell death (*acd*) (Greenberg and Ausubel, 1993) and lesions simulating disease resistance (*lsd*) response mutants (Dietrich et al., 1994) form spontaneous lesions normally associated with the hypersensitive response that occurs after pathogen exposure. It has been suggested that the *ACD2* gene negatively regulates a genetically programmed hypersensitive response (Greenberg et al., 1994) and that the *lsd1* mutant has an extremely sensitive signal perception mechanism, leading to an inability to control a cell death program (Deitrich et al., 1994). Several features of PCD, including plasma membrane breakdown and nuclear condensation during

the onset of the hypersensitive response to bacterial pathogens, have been documented (Levine et al., 1996).

Two additional classes of Arabidopsis mutants that may have an altered response program have also been described. First, Arabidopsis de-etiolated (*det*) mutants exhibit changes characteristic of light-grown plants when they are grown in the dark (Chory et al., 1989), possibly due to alterations in a signal transduction pathway responding to light stimulation (Chory, 1993). Second, the *ctr1* mutant exhibits characteristics of plants grown in ethylene, even in the presence of inhibitors of ethylene biosynthesis. The CTR1 gene product appears to be a member of the raf family of protein kinases and a negative regulator of the ethylene signal transduction pathway (Kieber et al., 1993).

Compared with wild-type plants, synthesis of HSP21 was detected in *uvh6* mutant plants at a lower threshold temperature and was induced more strongly at temperatures above this threshold. These results suggest that the UVH6 product normally prevents HSP induction until a certain level of heat stress is reached. Since the UVH6 product may also negatively regulate a UV-heat PCD response, these results further imply regulatory interactions between the PCD response and the well-characterized HSP induction pathway in plants (Vierling, 1991). There is evidence for such an interaction between HSP induction and stress-induced PCD in mammalian cells (Mehlen et al., 1996; Gabai et al., 1997). This potential interaction between HSP induction and the PCD response suggests a promising new area for investigating the responses of plants to UV damage and heat stress.

Another effect of the *uvh6* mutation was a yellow-green phenotype due to a reduced chlorophyll content. This effect was also observed in wild-type plants grown at an elevated temperature of 30°C. These results confirm and extend previous findings that chlorophyll levels in Arabidopsis decrease as growth temperatures increase (Hugly and Somerville, 1992). The yellow-green phenotype observed in wild-type and *uvh6* mutant plants might be accounted for by a partial loss of chloroplast membrane function due to expression of the proposed stress-response program. Degradation of the cell membrane of eukaryotic cells is one of the major events accompanying apoptosis (Umansky, 1996). This proposed loss of chloroplast function could be tested by analyzing the photosynthetic processes under conditions of heat stress.

The *UVH6* gene appears to act specifically in influencing the response of Arabidopsis plants to UV radiation and heat, since the *uvh6* mutant did not have increased sensitivity to several other known stress-inducing treatments. The *uvh6* mutant does not have increased sensitivity to ionizing radiation (Jenkins et al., 1995), which acts primarily through the production of reactive oxygen species (Ward, 1988; Reilly, 1994), to an acute dose of ozone (Patricia L. Conklin, personal communication), to salt, or to bacterial pathogens (M.E. Jenkins, unpublished data). Thus, the *UVH6* gene does not appear to influence the responses of plants to oxidative stress, salt stress, or microbial pathogens. These observations suggest that the proposed *UVH6*-response pathway to UV radiation and heat stress is not influenced by these other agents. However, exposure of plants to UV radiation can induce pathogen-responsive and wound-defense genes through activation of signal transduction pathways (Green and Fluhr, 1995; Conconi et al., 1996). Thus, UV radiation damage may activate at least one additional pathway that is not influenced by the UVH6 product.

Two questions come to mind concerning the proposed PCD response of plants to UV damage and heat stress. First, since the known types of biological damage produced by heat and UV radiation and the mechanisms used to protect plants from these treatments are quite different, why should responses to these particular types of stress be coordinately regulated? One possible explanation is that heat and UV radiation stress in plants are likely to occur concurrently during exposure to sunlight. Thus, a common response rather than two separate responses may provide a more efficient survival strategy. Second, since plants have DNA-repair mechanisms for removing UV damage and HSPs to increase thermotolerance, why should they also have a PCD response to such treatments? Heat stress and UV radiation damage are probably highly variable within the individual plant tissues depending upon the degree of exposure to sunlight. Tissues may be protected from exposure by repair and damage-avoidance mechanisms, but only up to a certain level. Above this level, these mechanisms may no longer be sufficient and a PCD response may serve to localize the damage, to remove cells that are unable to function correctly, and to minimize infection by delaying leakage of cell constituents (Pennell and Lamb, 1997).

Alternative explanations for the *uvh6* mutant phenotype have been eliminated. The possibility that decreased chlorophyll levels can cause the phenotypic changes observed in the *uvh6* mutant was eliminated by showing that other yellow-green mutants do not show the increased stress-response patterns of the *uvh6* mutant. A possible defect in leaf cooling of the *uvh6* mutant seems unlikely based on leaf-temperature measurements.

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LITERATURE CITED

Ahmad M, Jarillo JA, Klimczak LJ, Landry LG, Peng T, Last RL, Cashmore AR (1997) An enzyme similar to animal type II photolyases mediates photoreactivation in Arabidopsis. Plant Cell **9:** 199–207

Arnon DL (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15

Beggs C, Wellman E (1985) Analysis of light-controlled anthocyanin formation in coleoptiles of *Zea mays* L.: the role of UV-B, blue, red and far-red light. Photochem Photobiol **41:** 481–486

Bleeker AB, Patterson SE (1997) Last exit: senescence, abcission, and meristem arrest in Arabidopsis. Plant Cell **9:** 1169–1179

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol **32:** 191–222

Bredenkamp GJ, Baker NR (1994) Temperature-sensitivity of D1 protein metabolism in isolated *Zea mays* chloroplasts. Plant Cell Environ **17:** 205–210

Britt AB (1995) Repair of DNA damage induced by ultraviolet radiation. Plant Physiol **108:** 891–896

Britt AB, Chen JJ, Wykoff D, Mitchell D (1993) A UV-sensitive mutant of Arabidopsis defective in the repair of pyrimidine-pyrimidinone (6–4) dimers. Science **261:** 571–1574

Callard D, Axelos M, Mazzolini L (1996) Novel molecular markers for late phases of the growth cycle of *Arabidopsis thaliana* cell-suspension cultures are expressed during organ senescence. Plant Physiol **112:** 705–715

Cerutti H, Osman, M, Grandoni P, Jagendorf, AT (1992) A homolog of *Escherichia coli* RecA protein in plastids of higher plants. Proc Natl Acad Sci USA **89:** 8068–8072

Chen J, Mitchell DL, Britt AB (1994) A light-dependent pathway for the elimination of UV-induced pyrimidine (6,4) pyrimidinone photoproducts in Arabidopsis. Plant Cell **6:** 1311–1317

Chen Q, Lauzon LM, DeRocher A, Vierling E (1990) Accumulation, stability and localization of a major chloroplast heat-shock protein. J Cell Biol **110:** 1873–1883

Chory J (1993) Out of the darkness: mutants reveal pathways controlling light-regulated development in plants. Trends Genet **9:** 167–172

Chory J, Peto C, Feinbaum R, Pratt L, Ausubel F (1989) *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell **58:** 991–999

Conconi A, Smerdon MJ, Howe GA, Ryan CA (1996) The octadecanoid signalling pathway in plants mediates a response to ultraviolet radiation. Nature **383:** 826–829

Dietrich RA, Delaney TP, Uknes SJ, Ward ER, Ryals JA, Dangl JL (1994) Arabidopsis mutants simulating disease resistance response. Cell **77:** 565–577

Estelle M, Somerville C (1987) Auxin-resistant mutants of *Arabidopsis thaliana* with an altered morphology. Mol Gen Genet **206:** 200–206

Gabai VL, Meriin AB, Mosser DD, Caron AW, Rits S, Shifrin VI, Sherman MY (1997) Hsp70 prevents activation of stress kinases: a novel pathway of cellular thermotolerance. J Biol Chem **272:** 18033–18037

Green AES (1983) The penetration of ultraviolet radiation to the ground. Physiol Plant **58:** 351–359

Green R, Fluhr R (1995) UV-B-induced PR-1 accumulation is mediated by active oxygen species. Plant Cell **7:** 203–212

Greenberg JT, Ausubel FM (1993) Arabidopsis mutants compromised for the control of cellular damage during pathogenesis and aging. Plant J **4:** 327–341

Greenberg JT, Guo A, Klessig DF, Ausubel FM (1994) Programmed cell death in plants: a pathogen-triggered response activated coordinately with multiple defense functions. Cell **77:** 551–563

Harlow GR, Jenkins ME, Pittalwala TS, Mount DW (1994) Isolation of *uvh1*, an Arabidopsis mutant hypersensitive to ultraviolet light and ionizing radiation. Plant Cell **6:** 227–235

Hugly S, Somerville, C (1992) A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature. Plant Physiol **99:** 197–202

Jenkins ME, Harlow GR, Liu Z, Shotwell MA, Ma J, Mount, DW (1995) Radiation sensitive mutants of *Arabidopsis thaliana*. Genetics **140:** 724–732

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell **72:** 427–441

Koornneef M, van Eden J, Hanhart CJ, Stam P, Braaksma FJ, Feenstra WJ (1983) Linkage map of *Arabidopsis thaliana*. J Hered **74:** 265–272

Landry LG, Chapple CC, Last RL (1995) Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol **109:** 1159–1166

Landry LG, Stapleton AE, Lim J, Hoffman P, Hays JB, Walbot V, Last RL (1997) An Arabidopsis photolyase mutant is hypersensitive to ultraviolet-B radiation. Proc Natl Acad Sci USA **94:** 328–332

Levine A, Pennell RI, Alvarez ME, Palmer R, Lamb C (1996) Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Curr Biol **6:** 427–437

Li J, Ou-Lee T-M, Raba R, Amundson RG, Last RL (1993) Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell **5:** 171–179

McClennan AG, Eastwood AC (1986) An endonuclease activity from suspension cultures of *Daucus carota* which acts upon pyrimidine dimers. Plant Sci **46:** 151–157

Mehlen P, Schulze-Osthoff K, Arrigo A-P (1996) Small stress proteins as novel regulators of apoptosis. J Biol Chem **271:** 16510–16514

Mount D (1996) DNA repair: reprogramming transcription. Nature **383:** 763–764

Nedunchezhian N, Annamalainathan K, Kulandaivelu G (1992) Induction of heat shock-like proteins in *Vigna sinensis* seedlings growing under ultraviolet-B (280–320 nm) enhanced radiation. Physiol Plant **85:** 503–506

Oppenheimer DG, Herman PL, Sivakumaran S, Esch J, Marks MD (1991) A *myb* gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. Cell **67:** 483–493

Osteryoung K, Vierling E (1994) Dynamics of small heat shock protein distribution within the chloroplasts of higher plants. J Biol Chem **269:** 28676–28682

Pang Q, Hays JB (1991) UV-B inducible and temperature sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. Plant Physiol **95:** 536–543

Pennell RI, Lamb C (1997) Programmed cell death in plants. Plant Cell **9:** 1157–1168

Reilly PA (1994) Free radicals in biology: oxidative stress and effects of ionizing radiation. Int J Radiat Biol **65:** 27–33

Renger G, Volker M, Eckert HJ, Fromme R, Hohm-Veit S, Graber P (1989) On the mechanism of photosystem II deterioration by UV-B irradiation. Photochem Photobiol **49:** 97–105

Sorger PK (1991) Heat shock factor and the heat shock response. Cell **65:** 363–366

Stapleton AE, Walbot V (1994) Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiol **105:** 881–889

Umansky SR (1996) Apoptosis: molecular and cellular mechanisms (a review). Mol Biol **30:** 285–295

Vierling E (1991) The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol **42:** 579–620

Ward JF (1988) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acids Res Mol Biol **35:** 95–125