Radiation-Sensitive Mutants of *Arabidopsis thaliana*

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ABSTRACT

Five Arabidopsis mutants have been isolated on the basis of hypersensitivity of leaf tissue to UV light. For each mutant, the UV-hypersensitive phenotype *(uvh)* was inherited as a single recessive Mendelian trait. In addition, each *uvh* mutant represented a separate complementation group. Three of the mutations producing the UV hypersensitive phenotype have been mapped relative to either genetic markers or physical microsatellite polymorphisms. Locus *UVH1* is linked to nga76 on chromosome *5*, *UVH3* to *GL1* on chromosome three, and *UVH6* to nga59 on chromosome *1*. Each *uvh* mutant has a characteristic pattern of sensitivity based on UV sensitivity of leaf tissue, UV sensitivity of root tissue, and ionizing radiation sensitivity of seeds. On the basis of these patterns, possible molecular defects in these mutants are discussed.

PLANTS are exposed daily to UV-B radiation (wavelengths 280–320 nm) present in sunlight at the earth's surface. This radiation damages DNA as well as other cellular targets. Photosystem II, the hormone auxin and a plasma membrane ATPase are examples of such targets (reviewed in STAPLETON 1992). In addition to producing damage, UV-B light also causes a distinct physiological response in plants. This response includes the induction of flavonoid biosynthesis, changes in cuticular wax composition, and epidermal deformities (reviewed in STAPLETON 1992).

UV-B radiation causes the same types of DNA damage as UV-C light (wavelengths 230–280 nm), by producing primarily cyclobutane pyrimidine dimers and pyrimidine(6,4)pyrimidone dimers, although at a lower efficiency (QUAITE *et al.* 1992). Evidence exists that three of the major DNA repair mechanisms found in other species, photoreactivation, excision repair, and postreplication repair (SANCAR and SANCAR 1988) occur in plants. Photoreactivation utilizes one or more photolyase enzymes and visible light wavelengths to directly reverse pyrimidine cyclobutane dimers (SANCAR 1994) and also pyrimidine(6,4)pyrimidone dimers (KIM *et al.* 1994). A plant photolyase gene was recently identified by ability to complement an *Escherichia coli* mutant defective in photoreactivation (BATSCHAUER 1993).

An endonuclease with a specificity similar to the enzyme complex which carries out excision repair in *E. coli*, the *UvrABC* enzyme complex, has been detected in plants (MCLENNAN and EASTWOOD 1986). In *E. coli*, excision repair removes pyrimidine dimers and other types of DNA damage. The process requires several steps including incision on either side of the damaged site, followed by removal and repair synthesis of the damaged area (SANCAR and SANCAR 1988).

In postreplication repair, recombination is used to repair gaps left opposite UV lesions after replication of damaged chromosomes. In *E. coli*, this mechanism requires RecA protein. A homologous recombination system which potentially may be involved in DNA repair, has been characterized in the chloroplasts of higher plants (FEJES *et al.* 1990). An *Arabidopsis thaliana* cDNA that has a sequence similar to that of the *E. coli recA* gene and that encodes a chloroplast localization signal has been isolated (CERUTTI *et al.* 1992). Although the function of the encoded protein has not been proven, a DNA strand transfer activity like that of RecA protein has been found associated with chloroplasts (CERUTTI and JAGENDORF 1993).

In addition to UV-damage repair mechanisms, plants also synthesize UV-B absorbing molecules that prevent UV damage. These molecules can prevent ≤95% of incident UV-B radiation from penetrating beyond the epidermal layer (ROBBERECHT and CALDWELL 1978). Mutants that are defective in biosynthesis of UV-B absorbing flavonols, sinapic acid esters and related compounds have been shown to be hypersensitive to long-term exposure to UV-B light (LI *et al.* 1993). Flavonoids have been shown to protect maize DNA from the induction of ultraviolet radiation damage (STAPLETON and WALBOT 1994). Protection mutants can be identified by assays that measure levels of DNA damage immediately after UV exposure (HARLOW *et al.* 1994; STAPLETON and WALBOT 1994).

Radiation hypersensitive mutants have been useful for elucidating important resistance mechanisms in several species, but few plant mutants of this type have

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been isolated (BRITT et al. 1993; DAVIES et al. 1994; HARLOW et al. 1994). In this study, *A. thaliana* plants derived from mutagenized seeds were screened for hypersensitivity to UV-C light. These mutants, designated *uvh* for UV hypersensitive, were classified on the basis of hypersensitivity of leaf and root tissues to UV radiation and sensitivity of seeds to ionizing radiation. On the basis of these sensitivities, possible molecular defects are proposed.

MATERIALS AND METHODS

Growth conditions: Seedlings used for determining UV and ionizing radiation sensitivity were grown as previously described (HARLOW et al. 1994) except F40 agro fluorescent lights were substituted for one half of the cool white fluorescent lights.

Genetic screen for isolating UV-hypersensitive mutants: The screen described previously was used (HARLOW et al. 1994) to obtain *uvh 3–6,* except that the plants recovered from UV exposure under conditions that allowed photoreactivation.

Strains: *A. thaliana* seeds of the Columbia ecotype used for mutagenesis were a gift from Dr. FRED LEHLE (Lehle Seeds, Tucson). The ethyl methane sulfonate mutagenesis has been described previously (HARLOW et al. 1994). For the UV and ionizing radiation sensitivity studies, *uvh1* (backcrossed twice), *uvh6* (backcrossed once) and the original mutant isolates of *uvh 3, uvh4, and uvh5* were used.

UV sources: UV-C radiation (lambda max. = 254) was supplied by a UV Stratalinker (Stratagene model 2400) by selecting the desired UV dose. UV-B radiation was supplied by substituting UV-B bulbs (lambda max. = 302 nm) for the UV-C bulbs in the Stratalinker. The dose rate (12 $J/m^2/s$) was determined using a recently calibrated UVX Digital Radiometer equipped with a UV-B sensor (UV-Products) and the time required for the proper dose was programmed into the Stratalinker.

Mapping of *uvh* loci: *uvh1, uvh3 and uvh6,* which were isolated in a Columbia ecotype background, were outcrossed to the Landsberg ecotype. F_2 plants from this cross were analyzed visually for segregation of the glabrous and UV hypersensitive phenotypes. UV hypersensitivity was scored by protecting all but one leaf of 22-day-old plants with aluminum foil and irradiating plants with 400 J/m^2 UV-C light. Plants recovered from UV irradiation under conditions that did not support photoreactivation (see below). UV-hypersensitive plants were identified by complete shriveling of the unprotected leaf.

To map *uvh1* and *uvh6* relative to SSLP markers, DNA from the above UV-hypersensitive F_2 plants was prepared as previously described (HARLOW et al. 1994). PCR reactions utilizing primers specific for simple sequence length polymorphisms (SSLPs) (BELL and ECKER 1994) were then scored for Columbia and Landsberg polymorphisms. PCR reactions and gel electrophoresis were performed as reported previously (BELL and ECKER 1994) with the exception that metaphor agarose (FMC BioProducts, Rockland, ME) was used instead of polyacrylamide to visualize polymorphisms of 10 bp. Map distances were calculated utilizing the computer program Mapmaker version 3.0 (LANDER et al. 1987). All LOD scores for reported linkages were better than −20, indicating a very high likelihood of linkage.

Measurement of UV survival of seedlings: For each UV dose, ~30 14-day-old plants were irradiated with UV-B or UV-C light and then grown under one of two lighting conditions: the lighting used in normal growth conditions, which produced photoreactivating wavelengths or F40GO gold fluorescent lights, which produced light not containing photoreactivating wavelengths (PANG and HAYS 1991) for 3 days followed by transfer to the lighting used for normal growth conditions. A preliminary scoring of survival was performed 10 days after irradiation, as shown in Figure 1. Final scoring of survival was performed 6 weeks after irradiation at which time the plants had either produced seed or withered and died.

Measurement of sensitivity of roots to UV light: The assay was performed as previously described (BRITT et al. 1993) with modifications. UV-B light was not filtered through cellulose acetate, and the plants did not contain the *tt5* mutation which inhibits flavonoid biosynthesis. For the data shown in Table 3, 100 plants were scored at each UV dose.

Measurement of ionizing radiation sensitivity of plants: Irradiation and scoring of sensitivity were performed as previously described (HARLOW et al. 1994) except true leaf formation was scored 12 days after exposure to ionizing radiation.

RESULTS

Isolation of *uvh* mutants: Five *A. thaliana* mutants were isolated by screening individual plants derived from a mutagenized seed stock for damage to leaf tissue after UV irradiation. The screening procedure and preliminary characterization of one of these mutants, *uvh1,* have been described elsewhere (HARLOW et al. 1994). To identify mutants, the meristematic region of mutagenized seedlings was covered with a UV opaque foam and the remaining leaf tissue was exposed to a small UV-C dose. Three days after UV irradiation, plants were scored for damage detectable as bronzing and shriveling of leaf tissue. Of ~49,000 mutagenized seedlings screened, 31 exhibited UV damage normally seen in wild-type plants only after exposure to higher UV-C doses. Of these 31 plants, five were successfully propagated by seed and produced lines that were reproducibly UV hypersensitive.

Genetic analysis of *uvh* mutants: To determine the genetic basis of UV hypersensitivity, each mutant was backcrossed to the parent Columbia ecotype (see MATERIALS AND METHODS). In every case, the F_1 progeny from these crosses had wild-type levels of UV resistance. When these F_1 plants self-pollinated, ~25 percent of the F_2 progeny were UV hypersensitive in each case (Table 1). Therefore, the UV hypersensitivity of each mutant appeared to be due to a single, recessive Mendelian trait. To determine the number of complementation groups represented, the five mutants were crossed in all combinations and the resulting F_1 progeny were tested for UV hypersensitivity. In each cross, the F_1 progeny had wild-type UV-resistance levels (data not shown). Therefore, each *uvh* mutant appeared to represent a separate complementation group.

The map locations of the mutations leading to UV hypersensitivity in the *uvh1, uvh3* and *uvh6* mutants were determined. First, the *uvh* mutants were outcrossed to ecotype Landsberg to take advantage of genetic and physical polymorphisms between the Columbia and Landsberg ecotypes. During this analysis, it became apparent that the *UVH3* locus and the *GL1* locus on chromosome three were very tightly linked.

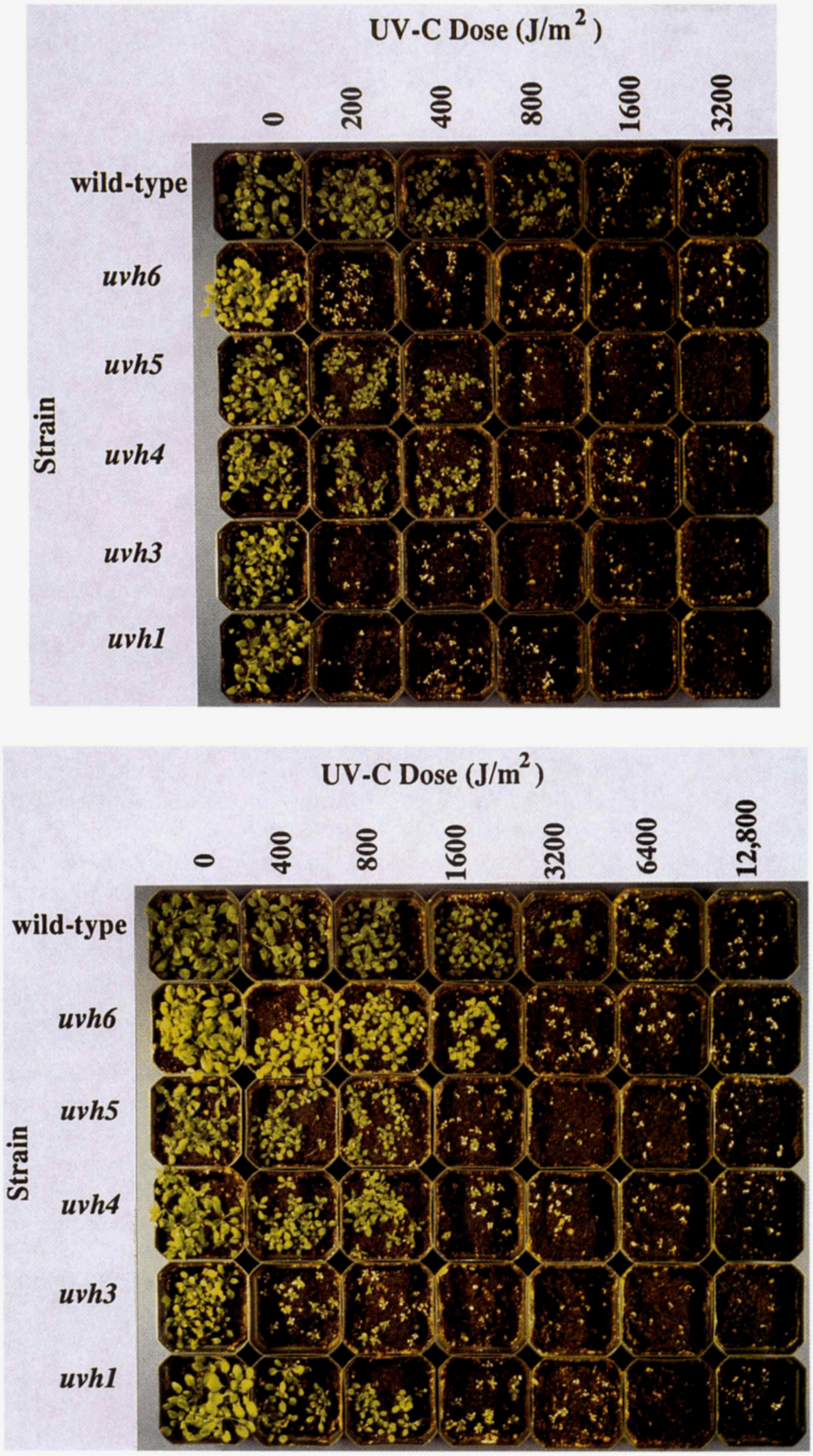

FIGURE 1.—UV-C sensitivity of *uvh* mutants. Fourteen-day-old seedlings were irradiated with UV-C light at the indicated doses. The plants were then grown under conditions that either (A, top) did not allow photoreactivation or (B, bottom) did allow photoreactivation, as described in MATERIALS AND METHODS. Plants were photographed 10 days after UV irradiation.

The Columbia parent carried a recessive mutation at the *GL1* locus and as a result did not produce trichomes (glabrous phenotype) (OPPENHEIMER *et al.* 1991), and the Landsberg strain used was wild type at this locus. Of 455 F_2 progeny from the above outcross, all 366 plants that produced trichomes had wild-type UV-resistance levels and all 89 plants that lacked trichomes were UV hypersensitive. Seed was collected from each of the 366 individual F_2 plants that had trichomes and were UV resistant. The F_3 progeny of these plants were subsequently tested to determine if the parent F_2 plant was homozygous or heterozygous for the *uvh3* and *gl1* markers. One of the 366 lines analyzed produced progeny plants that were UV hypersensitive and possessed trichomes, indicating a recombination event between the *uvh3* and *gl1* markers. Analysis of these data by the maximum likelihood method placed the *UVH3* locus within a centimorgan (cM) of the *GL1* locus, which is

TABLE 1

Segregation of the UV-hypersensitive phenotype in F_2 progeny from crosses of *uvh* mutants to wild-type plants

Cross	UV^r	UV^h	χ^2	P^a
uvh1 × WT	79[b]	22[b]	0.31	0.5–0.7
uvh3 × WT	71	30	0.95	0.3–0.5
uvh4 × WT	79	30	0.25	0.5–0.7
uvh5 × WT	99	39	0.62	0.3–0.5
uvh6 × WT	71	25	0.013	0.7–0.9

UV^r, wild-type UV resistance; UV^h, UV hypersensitive.

[a] Calculated using the Yates correction factor (STRICKBERGER 1976).

[b] Values from HARLOW *et al.* (1994).

located at position 46.2 on chromosome three (KOORNNEEF 1994).

The *UVH1* and *UVH6* loci were mapped by analyzing F_2 progeny from the above outcross for linkage of UV hypersensitivity to SSLPs (BELL and ECKER 1994). SSLP polymorphisms are due to microsatellite length differences between different ecotypes of Arabidopsis and are visualized by gel electrophoresis after PCR amplification of specific microsatellite sequences. DNA was prepared from F_2 plants of the above outcross that were homozygous for UV hypersensitivity (MATERIALS AND METHODS).

When 34 *uvh1/uvh1* plants were tested for linkage to the SSLP nga76 on chromosome *5*, only Columbia polymorphisms were observed, suggesting the *UVH1* locus is tightly linked to nga76. When linkage to SSLP nga139 was examined in these same plants, 14 plants were homozygous for the Columbia polymorphism, seven plants were heterozygous and two plants were homozygous for the Landsberg polymorphism. The calculated linkage between the *UVH1* and nga139 loci was 24.1 cM. This distance is consistent with the published distance of 26.9 cM between nga76 and nga139 (BELL and ECKER 1994).

Thirty-one *uvh6/uvh6* plants were similarly analyzed for linkage of the *UVH6* locus to various SSLP markers. When linkage between the *UVH6* and SSLP nga59 on chromosome *1* was analyzed, 30 plants were homozygous for the Columbia polymorphism, one plant was heterozygous, and none was homozygous for the Landsberg polymorphism. The calculated distance between the *UVH6* and nga59 loci is 1.6 cM. Linkage between the *UVH6* and nga63 loci was also demonstrated. Of the 31 plants tested, 27 were homozygous for the Columbia polymorphism, four were heterozygous and none was homozygous for the Landsberg polymorphism. These data indicate a linkage of 6.9 cM between the *UVH6* and nga63 loci. Because the published linkage between the nga59 and nga63 loci is 9.6 cM (BELL and ECKER 1994), these data are consistent with the location of the *UVH6* locus between these two markers.

Growth characteristics of *uvh* mutants: Two of the mutants, *uvh3* and *uvh6*, showed growth abnormalities without UV exposure. *uvh6* plants appeared yellow-green compared with wild-type plants but otherwise grew normally. This phenotype can be seen in the unirradiated *uvh6* plants shown in Figure 1, A and B. The yellow green phenotype cosegregated with the UV-hypersensitive phenotype in 237 backcrossed lines. The two phenotypes are therefore probably due to the same mutation.

At early stages of development, unirradiated *uvh3* plants could not be distinguished from wild-type plants, as seen in the unirradiated plants in Figure 1, A and B. However, after the onset of reproductive growth (production of the inflorescence), the rosette leaves died and seed set was severely reduced (data not shown). Increasing either light intensity or humidity resulted in loss of this phenotype. This phenotype cosegregated with UV hypersensitivity in 556 backcrossed lines suggesting that the two phenotypes are due to the same mutation.

The other *uvh* mutants chosen for study grew with a rate and vigor similar to wild-type plants under laboratory conditions. During the genetic screen, several UV hypersensitive plants were observed that grew slowly and with poor vigor. These plants were excluded from this study because of the concern that their UV hypersensitivity could be related to poor growth. These excluded plants may represent another class of mutants sensitive to a wide variety of stress conditions.

Sensitivity of mutant seedlings to UV light: To compare UV-sensitivities of the five *uvh* mutants with that of wild-type plants, 14-day-old seedlings were irradiated with increasing doses of UV-C light. After irradiation, seedlings were grown under conditions that either did not permit (Figure 1A) or did permit (Figure 1B) photoreactivation (see MATERIALS AND METHODS). Each of the *uvh* mutants was more extensively damaged than wild-type plants at the UV doses tested. Furthermore, the degree of sensitivity among the mutants varied. For example, in Figure 1, A and B, *uvh3* appeared to be one of the most hypersensitive mutants, while *uvh4* was one of the least hypersensitive mutants. All of the plants also showed increased UV resistance when photoreactivation was allowed (Figure 1B), suggesting that all of the mutants are capable of photoreactivation.

To compare the UV sensitivities of the mutants more quantitatively, the approximate doses of UV-C light required to kill one half of the seedlings (lethal dose 50%) were determined for each strain and are shown in Table 2. The mutants could be divided into three categories based on their UV-C survivals compared with wild-type plants: extreme hypersensitivity under both photoreactivating and nonphotoreactivating conditions [*uvh3*], intermediate hypersensitivity under both photoreactivating and nonphotoreactivating conditions [*uvh4* and *uvh5*], and extreme hypersensitivity under nonphotoreactivating conditions but intermediate hypersensitivity under photoreactivating conditions [*uvh1* and *uvh6*]. The *uvh* mutants were also hypersensitive to UV-B wave-

TABLE 2

UV hypersensitivity of *uvh* seedlings

Strain	UV dose in kJ/m^2								Lethal dose 50%, kJ/m^2
	0	0.2	0.4	0.8	1.6	3.2	6.4	12.8	
	A. Recovery without photoreactivating wavelengths								
WT	100	100	100	86	38	14	0	0	0.8–1.6
uvh1	100	0	0	0	0	0	0	0	<0.2
uvh3	100	0	0	0	0	0	0	0	<0.2
uvh4	100	100	94	14	20	0	0	0	0.4–0.8
uvh5	100	100	71	0	0	0	0	0	0.4–0.8
uvh6	100	16	0	0	0	0	0	0	<0.2
	B. Recovery with photoreactivating wavelengths								
WT	100	100	100	94	100	54	13	0	3.2
uvh1	100	100	100	83	71	9	0	0	1.6–3.2
uvh3	100	100	38	10	0	0	0	0	0.2–0.4
uvh4	100	100	100	83	50	0	0	0	1.6
uvh5	100	100	100	88	40	0	0	0	1.6
uvh6	100	100	100	93	77	0	0	0	1.6–3.2

Table values are percentage of seedlings surviving UV-C irradiation (see MATERIALS and METHODS). This experiment was repeated three times with similar results.

lengths and showed variations in UV sensitivity similar to those shown in Figure 1 (data not shown).

UV sensitivity of mutant root tissue: The above assay of UV hypersensitivity was based primarily upon damage to already formed leaf tissue by UV light. To determine if additional mutant tissues were also hypersensitive to UV light, and if mutants could be classified on this basis, the sensitivity of root tissue was measured. The assay used to examine root sensitivity to UV light has been previously described (BRITT *et al.* 1993). In this assay, plants were grown on agar plates held vertically, which caused the roots to grow along the agar surface. Three days after planting, the plates were UV irradiated, turned 90 deg and placed in the dark for 3 days. UV-B radiation was used to facilitate the comparison with a previously described mutant, *uvr1* (BRITT *et al.* 1993). Different UV doses were administered to plants by moving a UV opaque card across the plate during subsequent exposures. Continued downward root growth after turning the plate 90 deg resulted in bending of the root and this growth was inhibited by exposure to UV-light (Figure 2). The fraction of irradiated roots that continued to grow after irradiation was scored as a measure of sensitivity and is reported in Table 3. Presumably, the root bending assay measures the ability of root tip cells to divide and elongate after UV irradiation.

The mutants could be divided into three groups based on the UV hypersensitivity of root tissue (Table 3): extreme hypersensitivity [*uvh1* and *uvh3*], intermediate hypersensitivity [*uvh6*], and wild-type levels of resistance [*uvh4* and *uvh5*]. The most hypersensitive group was approximately as sensitive to UV radiation as the Arabidopsis mutant *uvr1*, recently isolated by screening for hypersensitivity of root tissue (BRITT *et al.* 1993). Similar variations in root UV sensitivities were observed after exposure to UV-C wavelengths (data not shown).

Sensitivity of *uvh* mutants to ionizing radiation: Sensitivity to another DNA damaging treatment, ionizing radiation, has been useful in the classification of UV sensitive mutants of other species. In contrast to UV light, ionizing radiation is extremely penetrating and damages DNA through single and double strand DNA breaks and other types of damage (AGER *et al.* 1990). Accordingly, a mutant with a defect in protection against UV light or in repair of a UV specific photoproduct should not be hypersensitive to ionizing radiation. On the other hand, sensitivity of a mutant to both types of radiation would suggest a defect in a mechanism that confers resistance to both types of radiation.

Sensitivity to ionizing radiation was measured by irradiating seeds with ^{60}Co γ-rays and scoring the ability of the resulting seedlings to produce the first set of true leaves. Examples of the appearance of wild-type and γ-ray sensitive *uvh* mutant plants grown from ^{60}Co irradiated seeds are shown in Figure 3. As previously observed (HARLOW *et al.* 1994), exposure of seeds to ionizing radiation did not effect germination or expansion of the preformed cotyledons, processes not requiring cell division. However, the ability to produce the first true leaves, a developmental process that requires cell division, was affected, as previously observed (VAN'T HOFF and SPARROW 1963; LAPINS and HOUGH 1970; HARLOW *et al.* 1994).

Absence of the first set of leaves 12 days after planting was used as a measure of ionizing radiation sensitivity. The average numbers of leaves per plant grown from

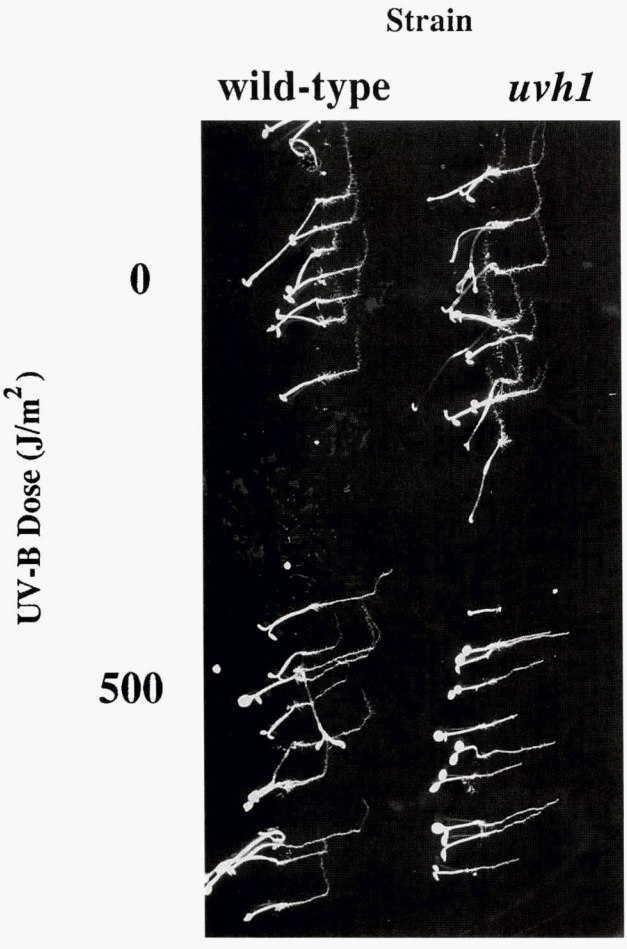

FIGURE 2.—UV-B sensitivity of *uvh1* roots. Plants were grown on agar plates held vertically, which resulted in the roots growing on top of the agar surface. The exposed roots were irradiated and the plates were rotated 90 deg so that continued root growth appeared as bending of the root. Without UV-B irradiation, all roots showed bending but after irradiation with 500 J/m^2 UV-B light, roots of wild-type plants were bent but roots of *uvh1* roots were not bent, indicating inhibition of growth and hypersensitivity to UV-B light of the *uvh1* roots. To improve visualization of roots, 0.6% charcoal was included in the growth media to provide greater contrast.

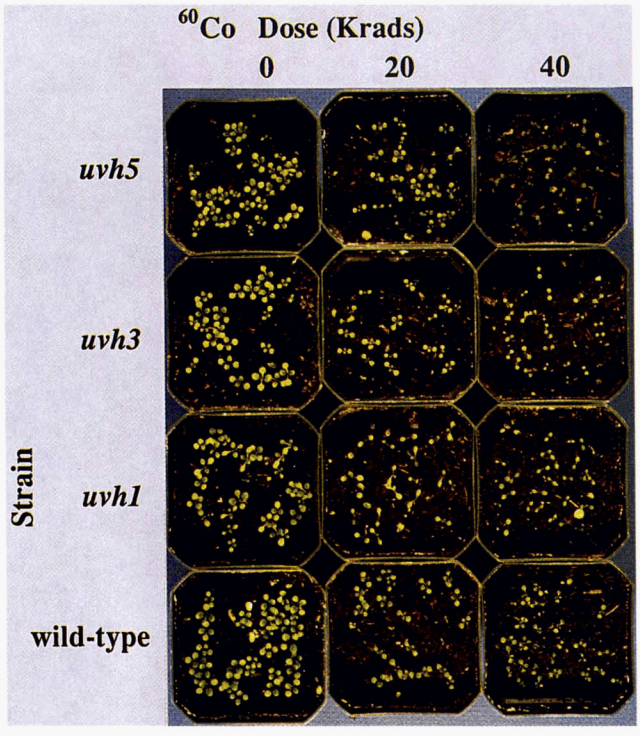

FIGURE 3.—Ionizing radiation sensitivity of *uvh* mutants. Shown are examples of 14-day-old seedlings grown from seeds previously γ-irradiated with the indicated doses. Seed germination and production of the two cotyledons, which are preformed in the seeds, were not affected. Damage was revealed by inability to produce one or both of the first two true leaves, which normally develop at approximately right angles to the cotyledons. *uvh1* produced fewer true leaves than wild-type plants at the 20-krad dose and did not produce any leaves at the 40-krad dose, thus indicating extreme hypersensitivity. *uvh3 and uvh5* plants produced fewer leaves than wild-type plants at the 40-krad dose, indicating intermediate hypersensitivity.

irradiated seeds were determined in ~200 mutant and wild-type plants at each dose tested (Table 4). The *uvh* mutants could be divided into three classes based on the comparison of the response of seeds to ionizing radiation to wild-type plants (Table 4): extreme hypersensitivity, [*uvh1*], in agreement with previously published results (HARLOW *et al.* 1994), intermediate hypersensitivity, based on the 40-krad dose results, [*uvh3* and *uvh5*], and wild-type levels of resistance, [*uvh4 and uvh6*].

DISCUSSION

Five UV-hypersensitive mutants of *A. thaliana* (*uvh*) have been isolated and characterized. Each mutant also had a characteristic pattern of UV and ionizing radiation sensitivity in specific plant tissues, suggesting that these mutants have different molecular defects, as summarized in Table 5. The UV-hypersensitive phenotype of each mutant was due to a single recessive mutation and each mutant represented a separate complementation group. It is therefore likely that other mutants of this phenotype representing additional genes may be readily isolated.

The *UVH1* and *UVH6* loci were mapped to chromo-

TABLE 3

Sensitivity of *uvh* mutant roots to UV-B radiation

	UV-B dose in J/m^2			
Strain	0	500	1000	2000
WT	99	97	93	38
uvh1	100	1	0	0
uvh3	100	1	0	0
uvh4	100	100	100	23
uvh5	100	98	100	12
uvh6	99	87	28	5

Root growth was measured using the root bending assay illustrated in Figure 2 and described in the text. Values are the percentage of irradiated roots that continued growth as indicated by bending.

TABLE 4

Sensitivity of *uvh* mutants to ionizing radiation

Strain	^{60}Co dose		
	0	20 krad	40 krad
WT	2.0	1.9	1.0
uvh1	2.0	0.6	0.0
uvh3	2.0	1.5	0.6
uvh4	1.9	1.9	1.0
uvh5	2.0	1.7	0.3
uvh6	2.0	1.9	1.0

Two sets of ~100 seeds were irradiated for each dose of ^{60}Co γ-rays. Total numbers of leaves produced by these plants were determined and the average numbers of first two true leaves per plant were calculated.

somes *5* and *1*, respectively, using SSLP markers (BELL and ECKER). The *UVH3* locus was found to be tightly linked to the *GL1* locus on chromosome *3* (KOORNNEEF 1994). This location is within 5 cM of the only other UV-resistance locus which has been mapped, *UVR1* (BRITT *et al.* 1993). However, the *UVH3* and *UVR1* loci have significantly different linkages to *GL1*. In addition, the *uvr1* mutant is defective in the repair of a UV-induced photoproduct, the 6,4 pyrimidine pyrimidone dimer. Such a defect is unlikely to result in hypersensitivity to ionizing radiation, a property of the *uvh3* mutant. For these reasons, the *uvh3* and *uvr1* mutations are probably not allelic. The *uvh4* and *uvh5* mutants have not yet been mapped. Complementation analysis suggests that they are not allelic with any of the remaining *uvh* mutants. Because the roots of *uvh4* and *uvh5* plants are not sensitive to UV light, as are roots of *uvr1* plants, it is unlikely that they are *uvr1* alleles.

To our knowledge, *uvh1*, *uvh3*, and *uvh5* are the first plant mutants to be isolated that are hypersensitive to both ionizing radiation and UV light (Table 5). Sensitivity to both types of radiation is characteristic of yeast mutants in the *RAD6* epistasis group, which represents a wide variety of molecular defects, possibly related to error-prone repair (FRIEDBERG 1988). One member of the *RAD6* epistasis group, *rad9*, decreases UV induced intergenic and intragenic recombination (KOWALSKI and LASKOWSKI 1975). *uvh1* plants have been shown to be partially defective in Agrobacterium-mediated transformation by both T-DNA and ri-DNA plasmids (R. SONTI, unpublished data), a process that requires illegitimate recombination. In contrast, *uvh1* plants showed normal linkage between genetic markers, suggesting that meiotic recombination occurs at normal frequencies (G. R. HARLOW, unpublished data). Thus, *uvh1* plants may be defective in a recombination function required for transformation and radiation resistance, but not for meiotic recombination.

In addition to UV hypersensitivity, the *uvh3* mutation also caused the death of rosette leaves late in development and reduced seed set. This phenotype may be a result of increased cell lethality or a defective developmental program and is reminiscent of the low plating efficiency of *rad6* mutants (KUPIEC and SIMCHEN 1985). In addition, *rad6/rad6* diploid cells do not produce spores (KUPIEC and SIMCHEN 1985). Although it is known that the RAD6 protein is an E2 ubiquitin-conjugating enzyme (JENTSCH *et al.* 1987), how the *rad6* defect leads to radiation sensitivity remains unclear.

uvh5 plants, like *uvh1* and *uvh3* plants, were hypersensitive to both ionizing radiation and UV light, suggesting a DNA repair defect. However, the degree of hypersensitivity to UV light was lower, suggesting a partially defective gene product. Alternatively, intermediate radiation sensitivities are also observed in some members of the yeast RAD6 epistasis group, especially the *rev* loci (FRIEDBERG 1988) and *uvh5* could be a similar plant mutant. Roots of *uvh5* plants were not particularly hypersensitive to UV radiation, suggesting the mechanism affected may not be present in root tissue,

TABLE 5

Summary of properties and possible defects of *uvh* mutants

Strain	Seedling sensitivity		Root sensitivity	Seed sensitivity	Possible defect
	UV-C (−phr)	UV-C (+phr)	UB-V (D)	Ionizing radiation	
WT	−	−	−	−	
uvh1	++	+	++	++	Recombination defect
uvh3	++	++	++	+	Recombination defect; cell viability and developmental abnormality
uvh4	+	+	−	−	Leaf specific UV repair
uvh5	+	+	−	+	Leaf specific DNA repair
uvh6	++	+	+	−	Chloroplast defect, stress response

The scoring of radiation sensitivities shown is based upon the data given in Tables 2–4. (+phr) and (−phr) indicate irradiated plants recovered under photoreactivating or nonphotoreactivating light wavelengths, respectively, and (D) denotes recovery in the dark. −, wild-type resistance levels; +, intermediate hypersensitivity; ++, extreme hypersensitivity.

Alternatively, the root bending assay may not be sensitive enough to detect an intermediate UV hypersensitivity of *uvh5* roots.

Leaf tissue of *uvh6* and *uvh4* plants was UV hypersensitive but root tissue of these plants was not particularly hypersensitive. In addition, these two mutants had wild-type levels of ionizing radiation resistance. We conclude that these two mutants may be defective in a mechanism involving repair of UV damage or protection from UV damage in leaf tissue. It would not be surprising to find that mechanisms exist that provide resistance to UV light in leaf tissue but not root tissue, since roots are not normally exposed to UV light. It has been previously observed that the mRNA for photolyase is induced by light in leaf, but not root tissue, in *Sinapis alba* (BATSCHAUER 1993). *uvh4* and *uvh6* plants may fail to produce a similar tissue-specific response.

uvh6 plants have two additional phenotypes linked to UV hypersensitivity, a yellow-green appearance and sensitivity to elevated temperature (M. E. JENKINS, unpublished results). The yellow-green phenotype suggests an alteration in chloroplast development. One possibility is that *uvh6* plants are UV hypersensitive due to a defect in repair of chloroplast UV damage or in protection of chloroplasts from UV damage. Alternatively, *uvh6* chloroplasts may be altered structurally in a manner that makes them more UV hypersensitive. The fact that *uvh6* root tissue, which does not contain mature chloroplasts, is not particularly UV hypersensitive is consistent with a chloroplast defect. The observation of several stress-related phenotypes in *uvh6* plants also raises the possibility that a stress response mechanism is defective in this mutant.

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