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Isolation of *uvh1*, an Arabidopsis Mutant Hypersensitive to Ultraviolet Light and Ionizing Radiation

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A genetic screen for mutants of Arabidopsis that are hypersensitive to UV light was developed and used to isolate a new mutant designated *uvh1*. UV hypersensitivity in *uvh1* was due to a single recessive trait that is probably located on chromosome 3. Although isolated as hypersensitive to an acute exposure to UV-C light, *uvh1* was also hypersensitive to UV-B wavelengths, which are present in sunlight that reaches the earth's surface. UV-B damage to both wild-type and *uvh1* plants could be significantly reduced by subsequent exposure of UV-irradiated plants to photoreactivating light, showing that photoreactivation of UV-B damage is important for plant viability and that *uvh1* plants are not defective in photoreactivation. A new assay for DNA damage, the DraI assay, was developed and used to show that exposure of wild-type and *uvh1* plants to a given dose of UV light induces the same amount of damage in chloroplast and nuclear DNA. Thus, *uvh1* is not defective in a UV protective mechanism. *uvh1* plants were also found to be hypersensitive to ionizing radiation. These results suggest that *uvh1* is defective in a repair or tolerance mechanism that normally provides plants with resistance to several types of DNA damage.

INTRODUCTION

The UV spectrum is commonly divided into three ranges: UV-C (<280 nm), UV-B (280 to 320 nm), and UV-A (320 to 400 nm). No significant UV radiation of wavelength less than 295 nm reaches the earth's surface (Green, 1983). Stratospheric ozone shields the earth's surface from excess levels of harmful UV-B radiation (Harm, 1980). Plants are likely to be subjected in the future to increased UV radiation from sunlight due to depletion of stratospheric ozone levels by chemical pollution. Recent reports indicate that ozone levels are declining over the Antarctic continent and periodically over the middle and high latitudes of both the Northern and Southern Hemispheres (Madronich, 1992; Stolarski et al., 1992). The effects that such increased UV-B exposure might have on plant life are largely unknown. Field studies using levels of supplemental UV-B that correspond to various levels of ozone depletion have shown that some crop plants would be adversely affected by increased UV-B (Teramura et al., 1990, 1991).

UV-B light has been reported to have a variety of effects on plants, including induction of DNA damage (McLennan, 1988; Pang and Hayes, 1991; Quaite et al., 1992) and several physiological responses. The best documented of the physiological responses include photomorphogenesis, auxin inactivation, plasma membrane ATPase inactivation through oxygen damage, damage to photosystem II, and flavonoid induction (for a review, see Stapleton, 1992). However, there is still very little known about the mechanisms of UV resistance in higher plants.

The primary goal of this study was to analyze the resistance mechanisms of plants to UV damage using a combination of genetic and biochemical techniques. UV-C and UV-B wavelengths damage DNA primarily through the production of two photoproducts, cyclobutyl pyrimidine dimers (CPD) and pyrimidine(6,4)pyrimidone dimers (6-4 pyo), although at a lower efficiency at UV-B wavelengths (Quaite et al., 1992). Based on studies with model organisms, the damage is removed or bypassed by several mechanisms including excision repair, photoreactivation, recombinational repair, and replication bypass (for example, see Walker, 1984). Because UV repair mechanisms are highly conserved across species, we anticipated that these same mechanisms should occur in plants. Indeed, there is biochemical evidence for the existence of these types of repair mechanisms in plants (for a review, see McLennan, 1988). In addition, the response of plants to UV light should be unique in several ways: (1) they are exposed chronically to UV-B light, (2) they have UV-B receptors that influence metabolic responses, and (3) they have pigment molecules that filter UV-B light wavelengths from incident sunlight. Thus, plants are likely to possess unique genetic systems that regulate their response to UV-B light.

Characterization of the UV resistance mechanisms of bacteria, yeast, and mammalian cells has been facilitated greatly by genetic analysis (see Hanawalt et al., 1979; Walker, 1984; Friedberg, 1988; Sancar and Sancar, 1988). In this work, we have used Arabidopsis as a model organism for a genetic study of UV resistance mechanisms in higher plants. A screening procedure based upon the multicellular nature of whole plants

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was developed for isolating UV-hypersensitive mutants. The strategy was to expose test areas of leaf tissues to a low fluence of UV while shielding the meristem, which produces the inflorescence, with a UV-protective foam. We first determined the minimum UV-C fluence that caused detectable wilting and chlorosis of mature leaves of wild-type plants. We then screened plants grown from a mutagenized seed stock for mutants that showed similar types of damage after exposure to a severalfold smaller UV-C fluence. Using this screen, we were able to isolate mutants (*uvh* mutants) that are hypersensitive to acute fluences of UV-B and UV-C light. This paper describes a preliminary characterization of one of these mutants, *uvh1*.

RESULTS

Genetic Screening Strategy

We have developed a method to screen for Arabidopsis mutants that are hypersensitive to the damaging effects of UV light (see Methods). To avoid killing mutants during a test UV exposure, we used a protective foam to cover the meristem and a portion of the leaves, as we have illustrated in Figure 1A. The foam dried a short time after irradiation, and damage could be assessed later by the response of the exposed regions of the leaves. Plants grown from an ethyl methanesulfonate (EMS)–mutagenized seed stock (M_2) were screened to increase the yield of potential mutants. Initially, we wished to screen for mutants with defects in repair processes other than photoreactivation (Pang and Hayes, 1991). Photoreactivation was avoided by growing irradiated plants under gold fluorescent lights, which lack the wavelengths necessary for photoreactivation. In this screen for UV-hypersensitive mutants, meristematic cells, which are undergoing cell division, were protected by the foam treatment. Mature plant leaves, which exhibit very little cell division, were irradiated. Mutants that are hypersensitive due to the effects of UV light on cell division might therefore be excluded from the screen. However, as shown below, the first mutant obtained also appeared to exhibit hypersensitivity of the meristematic region to UV radiation. It is therefore likely that a DNA damage repair or tolerance mechanism that is utilized in both meristematic and mature leaf cells is defective in this mutant.

The test UV exposure for mutant screening was determined by first finding a dose that produced visible damage on wild-type plants and then by a using a fourfold lower dose in the actual screening process. For wild-type plants, a dose of 200 J/m^2 UV-C and subsequent growth under nonphotoreactivation conditions produced yellowing and shriveling of exposed leaf regions 3 days after irradiation. To screen the M_2 seed stock, a dose of 50 J/m^2 was used therefore. The irradiated population was then grown under gold fluorescent lights and visually scanned 3 days later for mutants that had yellowed or shriveled outer leaf segments. Approximately 49,000 M_2 plants were screened in this fashion, and 31 putative UV-hypersensitive mutants were isolated. One of the most hypersensitive mutants was chosen for further study.

Figure 1. Genetic Screen for Identifying UV-Hypersensitive Mutants of Arabidopsis.

(A) Appearance of PABA foam–protected EMS-mutagenized M_2 plants immediately prior to UV-C irradiation.

(B) Appearance of *uvh1* in the mutant screen. At the top left is the *uvh1* plant. Two leaves on *uvh1* are partially green due to PABA foam protection. The inflorescence (slightly out of focus) of the *uvh1* plant was not protected by the PABA foam and subsequently died. However, secondary inflorescences developed from the foam-protected meristem and produced seed.

The plant in the upper left of Figure 1B is one of the mutants as it appeared in the screening procedure. This mutant plant (denoted *uvh1* for UV hypersensitive) showed extensive damage, i.e., yellowing and shriveling of leaves, except on two leaves that had been partially covered with UV-protective foam.

The other M_2 plants in Figure 1B were unaffected by this low fluence of UV but showed the size variation and occasional lack of germination expected in EMS-treated seed stocks. To confirm the UV-hypersensitive behavior of the *uvh1* mutant, seed was collected from the *uvh1* mutant and used to produce progeny plants that were also UV-C hypersensitive.

Genetic analysis was used to show that the UV-hypersensitive phenotype of the *uvh1* mutant is due to a single, recessive Mendelian trait. The *uvh1* mutant was crossed to the ecotype Columbia (*UVH1/UVH1*) parent line. The resulting F_1 plants (*uvh1/UVH1*) all had the normal UV-resistant phenotype of the *UVH1* parent. Of 101 F_2 plants examined, 22 were UV hypersensitive. These data are a close fit to the 3:1 ratio expected if UV hypersensitivity is due to a single recessive mutation ($\chi^2 = 0.56$, $P = 0.25$ to 0.5). The *uvh1* mutant is homozygous for the *glabrous1* (*gl1*) mutation on chromosome 3 (Koornneef et al., 1983). In a cross between the *uvh1* mutant and a strain that is *UVH1/UVH1*, *GL1/GL1*, the F_1 generation (*uvh1/UVH1*, *gl1/GL1*) was UV resistant. Among 88 F_2 progeny plants that were UV hypersensitive (*uvh1/uvh1*), 35 were also glabrous (*gl1/gl1*). If *uvh1* and *gl1* were unlinked, one-quarter of the F_2 plants (22/88 plants) were expected to be homozygous for both *uvh1* and *gl1*. Therefore, these data suggest that *uvh1* and *gl1* are probably linked ($\chi^2 = 10.24$, $P < 0.005$) and that *uvh1* may be located on chromosome 3.

uvh1 Plants Are Hypersensitive to UV-B Light

Although UV-C was used to screen for the *uvh1* mutant, plants are usually exposed to UV-B wavelengths in sunlight. To determine if *uvh1* plants are hypersensitive to UV-B light, 3-week-old plants were exposed to increasing fluences of UV-B light and subsequently incubated in the presence (white fluorescent lights) or absence (gold fluorescent lights) of photoreactivating light. The sensitivity of the plants to UV-B light was revealed by the degree of yellowing and shriveling of leaves, as seen in Figure 2. When photoreactivating light was supplied after UV-B irradiation, wild-type plants were resistant to even the highest test UV-B fluence (2.7 minimum erythemal dose units [MED]; one MED is ~210 J/m^2). In contrast, *uvh1* plants showed visible effects after UV-B fluences of 1.8 MED or more. In the absence of photoreactivating light, wild-type plants showed slight damage at the highest test UV-B fluence (2.7 MED), but *uvh1* plants showed visible effects after the lowest fluence of UV-B (0.2 MED) and extreme effects at fluences of 0.4 MED or more.

The UV-B sensitivity of *uvh1* plants was next measured by a quantitative survival assay. Percent survival has been used to gauge the sensitivity of bacterial (Hill, 1958) and yeast (Cox and Parry, 1968) UV-hypersensitive mutants. To measure plant survival, large numbers of 9-day-old seedlings were grown so

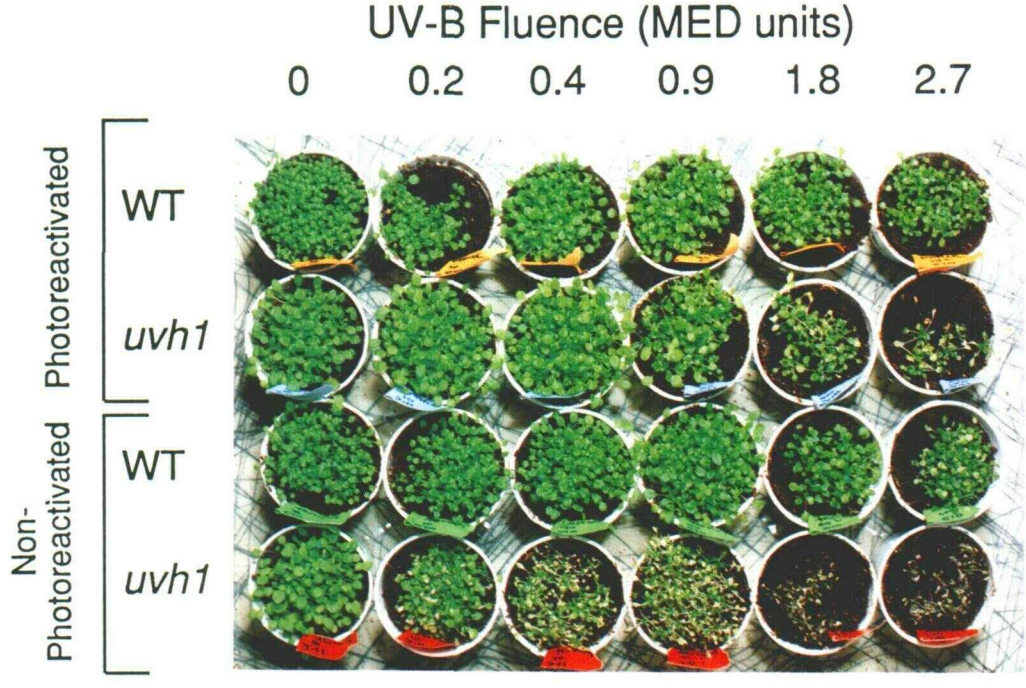

Figure 2. UV-B Sensitivities of Wild-Type and *uvh1* Mutant Plant Strains.

Three-week-old plants growing in soil were irradiated with increasing fluences of acetate-filtered UV-B light. Following UV irradiation, plants were either photoreactivated (grown under cool-white fluorescent lights) or not photoreactivated (grown under gold fluorescent lights). Plants are shown 2 days after the initial UV exposure. One MED unit is ~210 J/m^2 UV-B. WT, wild type.

that their leaves did not overlap and were irradiated from above with various fluences of UV-B light. Because cells in the meristem, immature leaves, and mature leaves were UV irradiated in these seedlings, this assay provided a measure of sensitivity of a variety of cell types to UV exposure. Within ~11 days, the irradiated plants showed one of two responses: (1) survival, indicated by the continued presence of viable green tissue, or (2) death, indicated by browning and drying of the plants. Using this assay, the fraction of irradiated plants that survived each UV exposure was determined.

Survival rates for *uvh1* and wild-type plants using this assay are shown in Figure 3. Without photoreactivation, the *uvh1* strain was ~8.5-fold more sensitive than the wild-type strain (50% survival occurred at 2 MED for *uvh1* plants and at 17 MED for wild-type plants). Photoreactivating light increased survival for both mutant and wild-type plants. When subsequently grown in the presence of photoreactivating light, wild-type plants were virtually unaffected by even the highest UV-B fluence given. However, *uvh1* plants showed a higher degree of sensitivity under these conditions. Photoreactivation increased survival of *uvh1* plants by a factor of ~9 (50% survival at 2 MED without photoreactivation compared to 18 MED with photoreactivation). We conclude that the *uvh1* mutant is not likely to be defective in photoreactivation. Furthermore, because photoreactivation removes CPD (Sancar and Sancar, 1988; Pang and Hayes, 1991) and possibly 6-4 pyo products (Todo et al., 1993), these results suggest that *uvh1* is defective in tolerance or repair of these types of damage.

Measurement of UV Damage Induction in Nuclear and Chloroplast DNA of *uvh1* Plants

We have developed an assay (the DraI assay) for comparing induction of UV damage in *uvh1* and wild-type plants. This assay was used to determine whether DNA was less protected from UV damage in *uvh1* plants than in wild-type plants. The basis for this assay is the finding (Whittaker and Southern, 1986) that restriction enzyme activity can be inhibited by the presence of DNA damage at the recognition sequence. Partial DNA digests result when UV-irradiated DNA is digested with enzymes whose recognition sequences contain adjacent thymidines (Hall and Larcom, 1982). Because the DraI recognition sequence, TTTAAA, contains a minimum of four potential sites for induction of CPD or 6-4 pyo, two on each strand, we reasoned that inhibition of DraI activity by UV damage should provide an assay for CPD or 6-4 pyo induction.

In the DraI assay, DNA was extracted from UV-irradiated plants, digested with DraI, electrophoresed, and gel blotted. The dimer content of DraI sites in specific DNA fragments was then determined by using a suitable DNA hybridization probe. To enhance the detection of partial digest products, hybridization probes that gave strong signals on gel blots were used. The nuclear gene probe was a fragment of the 18S rRNA gene, which is present in many copies arranged as tandem repeats.

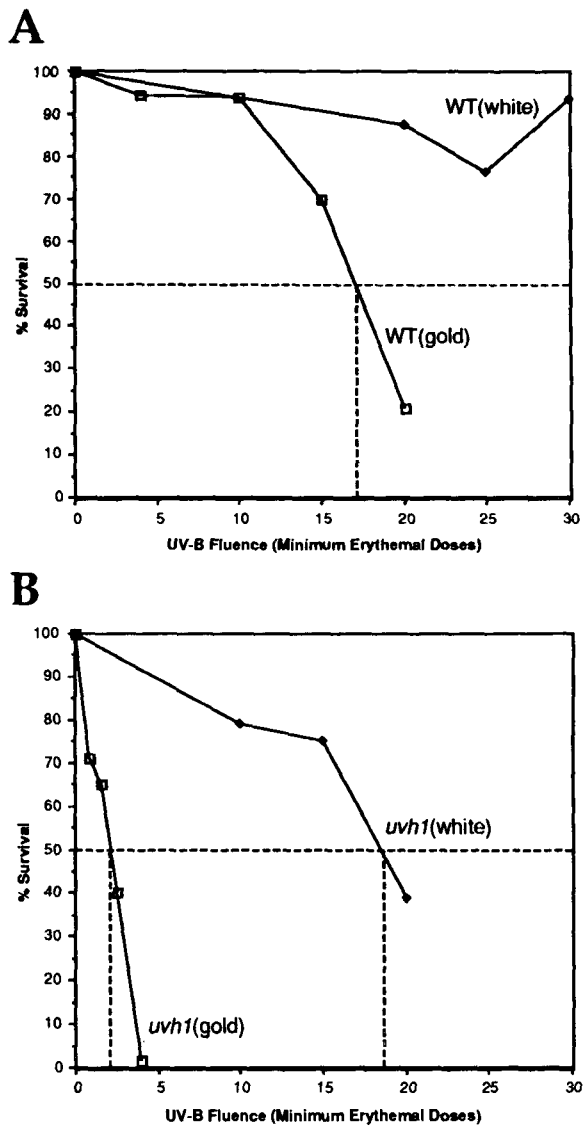

Figure 3. Survivals of Wild-Type and *uvh1* Seedlings Following Exposure to UV-B Radiation.

(A) Wild-type (WT) plants.
(B) *uvh1* plants.
Nine-day-old seedlings were irradiated with an acute fluence of UV-B. Plants of this age have cotyledons and the first true leaves. Dividing cells found in the meristematic tissue and in immature leaves should be damaged in this assay. Following irradiation, plants were grown under either photoreactivating (white light) or nonphotoreactivating (gold light) conditions for an additional 11 days before determining survivals (see text). The survivals shown are averages from two independent experiments that showed similar values and the same differences between strains.

The chloroplast gene probe was a fragment of the single-copy ribulose bisphosphate carboxylase large subunit (*rbcL*) gene, which gave an intense signal because of the multicopy nature of the chloroplast genome. The extent of DNA damage in each DNA sample was estimated by the ratio of radioactivity in a suitable partial DraI digest band to that in the complete DraI digest band. A phosphorimager device was used to measure radioactivity in the partial and complete digest bands.

As predicted, partial digest bands were obtained when DNA extracted from irradiated plants was digested with DraI and probed with either nuclear 18S rRNA, as seen in Figure 4, or chloroplast *rbcL* DNA, as seen in Figure 5. Because the probe sequences themselves did not contain DraI sites, only one band was expected following complete digestion of plant DNA with DraI. At lower doses, one (18S rRNA probe) or two (*rbcL* probe) partial digest bands were observed, presumably due to damage to one of the two DraI sites that flank the probe. At higher doses, higher molecular weight bands arose due to damage to two or more of these flanking sites. Because the location of the DraI sites flanking these regions was not known, the sizes of the DraI partial bands could not be predicted.

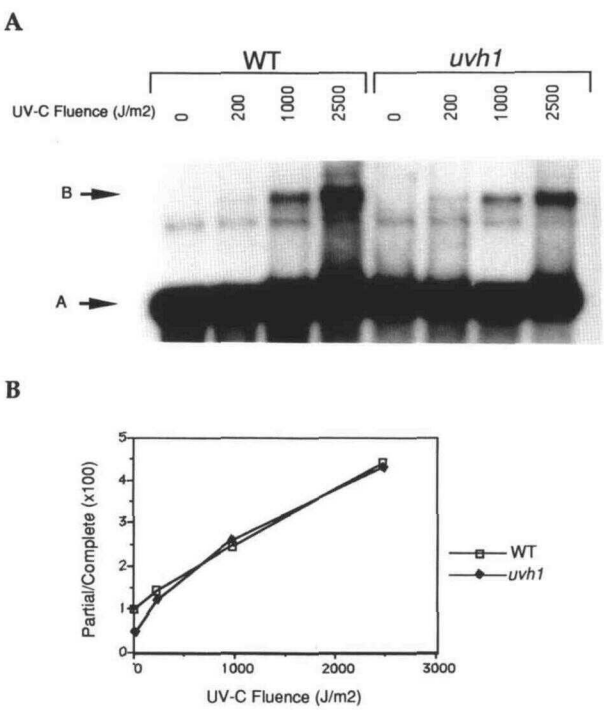

Figure 4. Induction of UV Damage in Nuclear DNA.

(A) Autoradiograph from the DraI assay. Wild-type and *uvh1* plants were irradiated with increasing fluences of UV-C. DNA was extracted immediately after irradiation, digested to completion overnight with the restriction enzyme DraI, electrophoresed, and gel blotted using an 18S rRNA probe. Complete digestion of the DNA sample resulted in the band labeled A; inhibition of DraI activity at either of the two enzyme recognition sites that flank the probe region resulted in a partially digested fragment, labeled B.
(B) Amount of UV damage expressed as percent radioactivity in the partial digest band to radioactivity in the complete digest band.
WT, wild type. The gel blot shown in **(A)** is representative of two experiments, and the data in **(B)** are the average of two experiments.

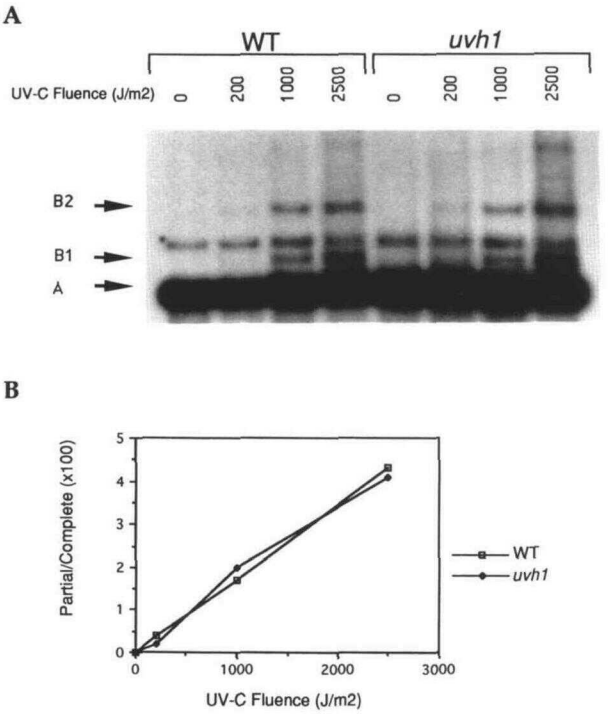

Figure 5. Induction of UV Damage in Chloroplast DNA.

(A) Autoradiograph from the DraI assay, as shown in Figure 4A. Shown are the complete band (A) and two partial digest bands (B1 and B2) that result when UV-C–induced DNA damage inhibits DraI activity at either of the two DraI sites that flank the *rbcL* gene.
(B) Amount of UV damage, calculated as in Figure 3B.
WT, wild type. The gel blot shown in **(A)** is representative of at least two experiments, and the data in **(B)** are the average of two experiments.

The primary 18S rRNA partial digest band that was induced by UV irradiation (band B in Figure 4A) was compared to the complete digest band (band A). The ratio of partial to complete band radioactivities (B/A) is plotted as a function of UV-C fluence in Figure 4B. This ratio was linear with respect to UV fluence over the range of doses tested. These single-hit kinetics suggest that production of the primary partial digest band was due to inactivation of a single DraI cleavage site. The proportion of damaged molecules thus represents the average frequency of dimer production in one of the two DraI sites that flank the probed region. These data indicate that the extent of damage in wild-type and *uvh1* plants was the same for each of the fluences used in this experiment.

The DraI assay was used to measure damage induction in chloroplast DNA from the irradiated plants used above. The

same DraI-restricted DNA extracts were alternatively probed with *rbcL*, and the resulting gel blot is shown in Figure 5A. One partial band (B2) was clearly visible and was easily quantified. A second band (B1) was partially obscured by the strong signal created by the complete digest band. The chloroplast DNA changes were similar to those obtained for nuclear DNA. First, induction of UV damage in chloroplast DNA was linear with respect to UV fluence (Figure 5B). Second, the amount of chloroplast DNA damage per incident UV fluence was the same in wild-type and *uvh1* plants. We conclude that the *uvh1* mutant is not defective in shielding either nuclear or chloroplast DNA from UV-C light damage. Therefore, the *uvh1* mutant is probably not defective in a UV-protective mechanism.

uvh1 Plants Are Hypersensitive to Ionizing Radiation

Patterns of sensitivity to agents that damage DNA, including ionizing radiation, have been useful for classifying DNA repair mutants in other species. Testing for sensitivity to ionizing radiation can also help to identify mutants that are defective in mechanisms that shield DNA from damage. Because ionizing radiation is so highly penetrating, such defective shielding mutants should not be hypersenstitive to ionizing radiation.

Seeds of mutant and wild-type plants were exposed to ionizing radiation, as described in Methods, germinated, and scored for the appearance of the first two true leaves. As observed previously, germination, growth, and expansion of the cotyledons were not affected as the dose of γ-rays was increased. However, the ability of plants to produce the first two rosette leaves was inhibited (Ivanov and Sanina, 1967; Fershtat and Stepanenko, 1973). In our experiments, three responses of plants to γ-rays were observed: (1) both of the first true leaves were produced, as observed in normal growth; (2) only one of the first two leaves was produced and a small, abnormal, pigmented structure that never grew into a leaf was observed at the position normally occupied by the missing leaf; and (3) neither of the first two leaves was produced, but two abnormal structures were observed at the normally occupied leaf positions. Previous studies have shown that exposure of root and flower bud meristems to increasing doses of ionizing radiation gradually destroys a subset of cells. At lower doses, surviving cells can replace the destroyed ones and make plant structures. However, a threshold in the number of destroyed cells is eventually reached when replacement is no longer possible and the structure is not made (Van't Hoff and Sparrow, 1963; Lapins and Hough, 1970).

Based on the above considerations, sensitivity to γ-rays was scored by measuring the number of plants with zero, one, or two leaves 6 days after germination. The average number of leaves per plant on *uvh1* plants was then calculated and compared to the average number on unirradiated control plants. By this assay, *uvh1* plants were approximately four times more sensitive to γ-rays than wild-type plants, as illustrated in Figure 6. Because a backcrossed line of *uvh1* was used in these experiments, UV and γ-ray hypersensitivity are probably due to the same mutation. The observed hypersensitivity of *uvh1* to γ-rays provides evidence that *uvh1* is likely to be defective in a repair or tolerance mechanism for both UV and γ-ray damage.

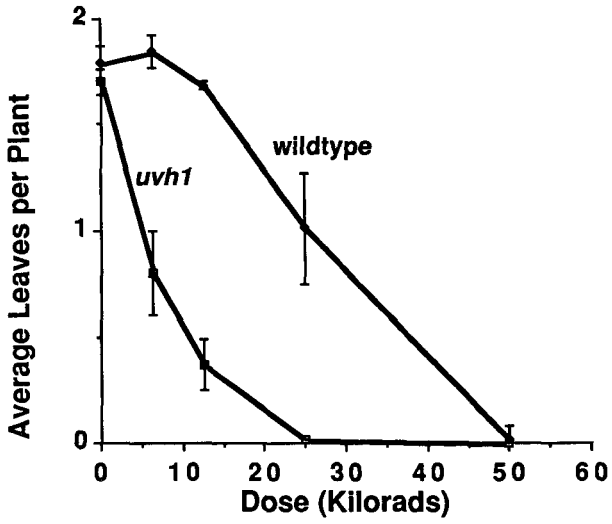

Figure 6. Sensitivity of Wild-Type and *uvh1* Strains to Ionizing Radiation.

Imbibed seeds were irradiated with increasing doses of ^{60}Co γ-rays and immediately planted in soil. The number of plants with zero, one, or two of the first set of true leaves was measured, and these data were used to calculate the average number of leaves per plant, as described in the text. Shown are the average and standard deviation of data from two experiments.

DISCUSSION

We have isolated and partially characterized a mutant of Arabidopsis, designated *uvh1*, which is hypersensitive to both UV-B and UV-C light wavelengths and to ionizing radiation. *uvh1* plants showed chlorosis, wilting, and extensive cell death following exposure of leaves to small, acute fluences of UV-B or UV-C light that did not affect wild-type plants. In addition, irradiation of *uvh1* seeds with γ-rays inhibited the production of the first true leaves at much lower doses than those needed to similarly affect wild-type plants. These hypersensitive mutant phenotypes are due to a single, recessive mutation probably located on chromosome 3.

For a more complete genetic analysis of radiation resistance in plants, additional mutants and alleles will be required. We have isolated additional *uvh* mutants, and five of these mutants are currently being characterized in detail. Other radiation-sensitive mutants of Arabidopsis have recently been described. A UV-B–hypersensitive mutant was isolated using a root bending assay and was shown to have a defect in repair of 6-4 pyo (Britt et al., 1993). Another set of γ-ray

hypersensitive mutants of Arabidopsis has also been obtained (C. Davies, personal communication).

Mutants of Arabidopsis that lack anthocyanins and sinapic acid esters have been shown to be hypersensitive to a chronic exposure of UV-B (Li et al., 1993). Two experiments indicate that the *uvh1* mutant is not defective in such a protective mechanism or in a repair mechanism specific for UV damage. First, the DraI assays (Figures 4 and 5) demonstrated that chloroplast and nuclear DNAs were not damaged more in *uvh1* plants than in wild-type plants by a given UV exposure. Second, *uvh1* plants were also hypersensitive to γ-rays. γ-Rays have been shown to cause double-strand breaks in plant nuclear and mitochondrial DNA (Hall et al., 1992). Ionizing radiation can also cause base damage and single-strand breaks in DNA (Dizdaroglu and Bergtold, 1986; McLennan, 1988). It seems highly unlikely that the same protective mechanism could be used by plants for both UV light and γ-rays.

In our experiments, both dividing cells in immature leaves and nondividing cells in mature leaves appeared to be hypersensitive to UV light in *uvh1* plants. DNA damage caused by either UV-C or UV-B light was the probable cause of leaf damage scored in these experiments. The damaging effects of UV could be lessened by exposing the irradiated plants to photoreactivating light, which is known to remove UV damage. UV damage to DNA causes blockage of DNA replication (Moore et al., 1982; Shwartz and Livneh, 1987; Griffiths and Ling, 1989) and transcription (Sauerbier and Hercules, 1978). Studies on plants have shown that UV irradiation can block nuclear DNA and RNA synthesis (Ohyama et al., 1974) and can decrease the steady state levels of RNA transcripts in both the nucleus and chloroplast (Jordan et al., 1991). The UV hypersensitivity of *uvh1* plants could be due to an inability to tolerate or remove damage that is affecting these metabolic processes.

At present, the nature of the biochemical defect in *uvh1* is unknown. The *uvh1* mutant is similar in phenotype to *Saccharomyces cerevisiae* mutants in the *RAD6* epistasis group, which are also hypersensitive to both UV light and ionizing radiation. *rad6* mutants are variably pleiotropic, including sometimes showing defects in sporulation and UV mutagenesis and sometimes showing increased mitotic recombination (Friedberg, 1988). Mutants in the *RAD6* group are considered to be defective in post-replication repair of DNA damage, a recombination mechanism that promotes DNA strand exchanges in damaged DNA following abortive replication past DNA lesions. If similar to *rad6* mutants, *uvh1* plants might be expected to show additional pleiotropic effects such as alterations in meiotic or mitotic recombination and in mutagenesis (Friedberg, 1988).

METHODS

Common Names

Arabidopsis refers to the plant species *Arabidopsis thaliana*.

Growth Conditions

Arabidopsis (ecotype Columbia) seed was obtained from Lehle Seeds (Tucson, AZ). This strain is homozygous for glabrous (*gl1*) on chromosome 3, which confers a glabrous (lack of trichomes) phenotype on the leaves and stems of the plant. Seed from this stock showed a great deal of variation in germination and growth rate, which could interfere with screening of UV-sensitive mutants, because detection depended upon our ability to determine the influence of UV light on plant growth. To control this variability, we first generated a nonvarying derivative of ecotype Columbia, called C10. C10 was made by first generating a purified stock by single-seed descent, i.e., by successively amplifying seed from a single-parent plant, one that germinated and grew reliably under our conditions over several generations. Ethyl methanesulfonate (EMS)–mutagenized C10 seed was prepared using standard protocols (Estelle and Somerville, 1987).

Plants were grown at ambient room temperature and humidity in soil (Sunshine All Purpose Potting Mix Plus; Fisons, Vancouver, B.C.) with 24-hr illumination from two 40-W cool white fluorescent bulbs set 35 cm above the plants (2000-Lux intensity) and watered as needed with tap water.

Ultraviolet Light Sources

UV-C radiation (predominant wavelength = 254 nm) was supplied by a germicidal UV lamp (model G8T5; Sylvania, Danvers, MA) at a rate of 5 J/m^2 sec. UV-C levels were measured using a UVX Digital Radiometer equipped with a recently calibrated UVX-25 UV-C sensor (UVP, Inc., Upland, CA). For the higher UV-C fluences needed for the DraI assay, UV-C irradiation was performed in a recently calibrated UV crosslinker (Stratalinker 2400; Stratagene) by selecting the particular energy setting desired. UV-B was supplied by two UV-B lamps (model UBL FS40T12-UVB; National Biological Supply, Twinsburg, OH) at a rate of 2.7 minimum erythemal dose units (MED)/hr. UV-B light was filtered through two layers of 0.127-mm cellulose acetate (ProArt Supply, Beaverton, OR). UV-B levels were measured using a UV intensity meter (model 2D Erythema; Solar Light Co., Inc., Philadelphia, PA) and are expressed in MED units. Minimum erythemal dose is defined as the dose causing fair Caucasian skin to barely redden. UV-B was also measured with the UVX Digital Radiometer equipped with a UVX-31 sensor (UVP, Inc., Upland, CA). One MED unit corresponds approximately to 210 J/m^2 UV-B.

Following UV irradiation, plants were either incubated under normal growth conditions (see above), allowing photoreactivation, or were incubated under gold lights (model F40GO; Sylvania), which are deficient in wavelengths necessary for photoreactivation (Pang and Hayes, 1991), under the same growth conditions.

Genetic Screen for UV-Hypersensitive Mutants

Three-week-old EMS–mutagenized M_2 plants growing in soil were screened for UV hypersensitivity by applying a UV-absorbing foam solution over the center of the rosette. This foam covered the inner half of the mature leaves and also protected the primary meristem of the plant. The foam solution consisted of 0.5% para-amino-benzoic acid (PABA, sodium salt, grade I-S; Sigma) and 10% BSA (fraction V powder; Sigma) in water. PABA was selected as the UV-filtering compound because it absorbs UV strongly (absorption maximum in H_2O = 266 nm, $E^{1\%}_{1cm}$ = 1070) and is not toxic to Arabidopsis. After experimenting with several different carriers for the UV-absorbing PABA

compound, we settled on a viscous BSA solution that when whipped with a wire whisk would produce a foam that was light enough not to bend the plant parts and that would dry and flake off well enough not to affect growth and development of the plant following UV irradiation. When whipped, this solution allowed only 0.2% UV (measured at 260 nm) transmittance in a 1-cm quartz spectrophotometer cuvette.

γ-Irradiation of Seeds

uvh1 and wild-type strains used in this experiment were derived from two successive backcrosses of the original *uvh1* mutant to ecotype Landsberg. Prior to γ-ray exposure, seeds in lots of 105 to 115 were imbibed by presoaking them in 200 μL of water for 24 hr in the wells of a microtiter dish. Imbibition of Arabidopsis seeds has been shown to increase the mutagenic effectiveness of ionizing radiation 12-fold (Robbelen, 1964). The seeds and remaining water in the microtiter dish were irradiated from above by ^{60}Co γ-rays from a Theratron 80 machine (Theratronics, Ottawa, Ontario, Canada) at a dose rate of 284 rad/min and then immediately planted in soil. Irradiated seeds were then placed at 6°C for 4 days to synchronize germination and then transferred to 22°C. Germination rates, scored 3 days after this transfer, varied from 83 to 91% for *UVH1* plants and from 83 to 97% for *uvh1* plants. Plants were scored 9 days after transfer to 22°C for formation of the first pair of true leaves, and the average number of leaves per plant was determined for each γ-ray dose, as described in the text.

DNA Damage Induction Assays

Mature leaves from 3- to 4-week-old plants were excised, laid flat on aluminum foil with the petiole sandwiched between layers of paper towels moistened with tap water, and irradiated with UV-C. Immediately following irradiation, plant nuclear and organelle DNA was prepared by a modified hexadecyltrimethylammonium bromide procedure (J.J. Doyle and J.L. Doyle, Isolation of Plant DNA from Fresh Tissue, Focus, volume 12, 1990, published by Bethesda Research Laboratories). Plant material was frozen in liquid nitrogen, ground to a fine powder in a cold mortar and pestle, and then transferred to a 30-mL tube containing 5 mL isolation buffer/0.5 g powder and incubated at 55°C for 30 min. Isolation buffer contained 2% hexadecyltrimethylammonium bromide (Sigma), 1.4 M NaCl, 0.2% 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0. Samples were extracted with chloroform/isoamyl alcohol (24:1) and then precipitated with two-thirds volume of isopropanol. The nucleic acid pellet was resuspended in 200 μL of isolation buffer and incubated for 5 to 30 min at 55°C, reextracted with chloroform/isoamyl alcohol (24:1), and precipitated with two-thirds volume isopropanol. DNA pellets were then rinsed with 70% ethanol, dried, and resuspended in water. Digestions with the restriction enzyme DraI (Boehringer Mannheim) were incubated overnight in the buffer supplied. The possibility that contaminants in the DNA preparation were the cause of incomplete DNA digestion was eliminated by demonstrating that exogenous λ DNA, when added to duplicate plant DNA samples, was cut to completion.

The nuclear gene probe was a 1526-bp 18S rRNA gene fragment created by the polymerase chain reaction using primers 5′-GTGTAAGTATGAACGAATTC-3′ and 5′-GGAATTCCTCGTTGAAGACC-3′, which correspond to positions 144 to 163 and 1651 to 1670, respectively (Unfried et al., 1989). Amplification reactions were performed in 100 μL using 5 ng of Arabidopsis genomic DNA (ecotype Columbia) in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM each dATP, dCTP, dGTP, and dTTP, and 0.5 units of Taq polymerase (Promega) final concentrations. Polymerase chain reaction was performed using a thermal cycler (model 480; Perkin-Elmer-Cetus). After a 3-min hold at 94°C, samples were cycled 30 times through 1 min at 94°C, 1 min at 42°C, and 2 min at 72°C. After a 4-min extension at 72°C, the amplified product was held at 4°C until purified by centrifugation in a microconcentrator (Centricon-30; Amicon, Beverly, MA). The chloroplast DNA probe was a 1458-bp BamHI-PstI fragment of the *rbcL* gene from *Amaranthus hypochondriacus* (Michalowski et al., 1990). Probes were radiolabeled by random priming (Prime-It Kit; Stratagene) using $\alpha^{32}P$-dCTP.

Prehybridization and hybridization were performed in 0.25 M Na_2HPO_4, 7% SDS at 68°C. Washes were performed twice in 20 mM Na_2HPO_4, 5% SDS, pH 7.2, for 15 min at 68°C and twice in 20 mM Na_2HPO_4, 1% SDS, pH 7.2, for 15 min at 68°C. Individual bands on radioactive gel blots were quantified using a PhosphorImager (model 425; Molecular Dynamics, Sunnyvale, CA).

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