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Mutants of *Arabidopsis thaliana* Hypersensitive to DNA-Damaging Treatments

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Manuscript received October 28, 1996
Accepted for publication January 27, 1997

ABSTRACT

A simple screening method was developed for the isolation of *Arabidopsis thaliana* mutants hypersensitive to X-ray irradiation. The root meristem was used as the target for irradiation with sublethal doses of X rays, while protection of the shoot meristem by a lead cover allowed the rescue of hypersensitive individuals. We isolated nine independent X-ray-hypersensitive mutants from 7000 M2 seedlings. Analysis of three chosen mutants (*xrs4*, *xrs9* and *xrs11*) showed that alterations in single recessive alleles are responsible for their phenotypes. The mutations are not allelic but linked and map to chromosome *4*, suggesting mutations in novel genes as compared to previously mapped mutant alleles. Importantly, hypersensitivity to X rays was found to correlate with hypersensitivity to the DNA-alkylating agent mitomycin C, which provokes interstrand crosslinks, and/or to methyl methanesulfonate, which is known as a radiomimetic chemical. These novel phenotypes suggest that the mutants described here are altered in the repair of DNA damage, most probably by recombinational repair.

THE genetic information of all organisms is constantly subjected to damage and replication errors but efficient repair systems maintain functional genomes. Plant germ cells, for example, differentiate from somatic tissues relatively late in development and the genetic material must be preserved during somatic growth. Although the enzymatic and regulatory processes responsible for the maintenance of genomic integrity in plants are largely unknown, several DNA repair pathways activated by specific types of DNA damage have been characterized in prokaryotes and simple eukaryotes (Friedberg *et al.* 1991; Petes *et al.* 1991; Wood 1996). DNA strand breaks, which for their efficient repair require rather complex interactions, may be the result of repair reactions or the direct hydrolysis of phosphodiester bonds by the action of free radicals and/or high-energy radiation. Damage caused by DNA-damaging chemicals may be repaired by a base excision repair pathway or by a nucleotide excision repair process. Endonucleolytic cleavages are part of both pathways. Recently, the Arabidopsis homologue of apurinic endonuclease has been cloned (Babiychuk *et al.* 1994); however, its physiological role in the living plants needs further elucidation.

Single-strand breaks and gaps can be repaired, after nucleolytic processing of ends, by DNA polymerase and ligase using the second DNA strand as a template. The repair of double-strand breaks (DBS) via homologous recombination pathway requires intact homologous templates (Szostak *et al.* 1983). Most present knowledge about recombinational repair in eukaryotes is derived from studies in yeast, where the genes controlling these processes are referred to as the *RAD*52 epistasis group (Friedberg *et al.* 1991; Petes *et al.* 1991). Mutation of these genes causes hypersensitivity to X-ray irradiation and to particular DNA-damaging chemicals and most often results in a deficiency of strand-break repair and in altered mitotic or meiotic recombination properties. An increased level of DNA damage also induces homologous recombination in plants (Lebel *et al.* 1993; Puchta *et al.* 1995), suggesting an active role for recombination in DBS repair. However, similar to other complex eucaryotes (Wood 1996), illegitimate recombination seems to be the main activity for DBS repair in plants (Puchta and Hohn 1996). Therefore plant mutants hypersensitive to X-ray radiation may include deficiencies in homologous or illegitimate recombination pathways.

The recovery and characterization of mutants defective in DNA repair could help to define these molecular interactions. Mutants hypersensitive to UV and γ-radiation have been recently recovered from mutagenized M2 populations of *Arabidopsis thaliana* (Britt *et al.* 1993; Davies *et al.* 1994; Harlow *et al.* 1994; Jenkins *et al.* 1995). The genetic lesion causing one such UV-hypersensitive mutant leads to a reduction in the repair of UV-induced pyrimidine-pyrimidone (6–4) photoproducts (Britt *et al.* 1993).

We designed a screening procedure for the isolation of *A. thaliana* mutants specifically hypersensitive to X-ray-induced DNA damage and isolated nine independent X-ray-hypersensitive (*xrs*) mutants. Phenotypic and genetic characterization of three representative *xrs* mutants demonstrated that their X-ray-hypersensitive phenotypes are linked to sensitivity to specific DNA-damaging agents, suggesting that these mutants are indeed altered in DNA-damage repair.

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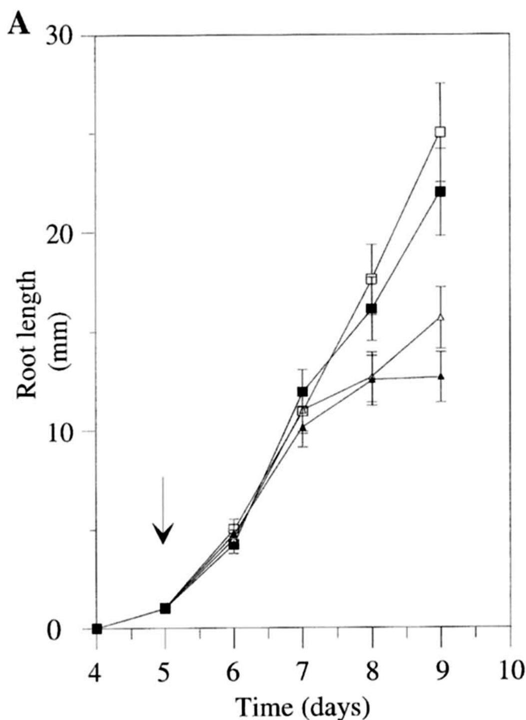

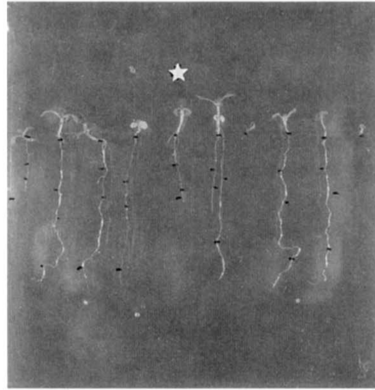

FIGURE 1.—Isolation of X-ray hypersensitive mutants of *Arabidopsis thaliana*. (A) X-ray inhibition of root growth of wild-type seedlings. The length of the main root is shown for control wild-type seedlings (□) and seedlings irradiated with X rays at day 5 (arrow) with 20 Gy (■), 40 Gy (△) and 80 Gy (▲). The vertical bars show the standard deviations from the means for 20 seedlings tested. (B) Screening of M2 seedlings for X-ray-hypersensitive mutants. The arrow indicates the distal root position of the M2 seedlings at the time of irradiation. The star shows an X-ray-hypersensitive candidate stopped in its root development after irradiation with 20 Gy.

MATERIALS AND METHODS

Plant material: EMS-mutagenized seeds of *A. thaliana*, ecotype *Landsberg erecta* (M2 generation) were provided by Dr. ERWIN GRILL, ETH Zürich. Albino mutants were found at a frequency of 5%. *L. erecta* and C24 ecotypes were used for backcrosses. The *L. erecta* NW tester lines were supplied by the Nottingham Arabidopsis Stock Center. Surface-sterilized seeds were used for *in vitro* experiments (MASSON and PASZKOWSKI 1992).

Plant growth conditions: Plants were grown under aseptic conditions with 16 hr light of 25 $\mu E/m^2/s$ (Osram Natura de Luxe) at 22.5° and 8 hr dark at 22.5° (condition A). The composition of the germination medium has been described previously (MASSON and PASZKOWSKI 1992). Culture vessels were placed on temperature-regulated shelves at 1° below air temperature. This prevented water condensation on the lids of the vessels and thus minimized variation in medium composition throughout the experiment.

Plants grown in soil were illuminated for 16 hr at 120 $\mu E/m^2/s$ using Phillips Cool White lamps. The temperature during the day period was 21° and during the night was 16°. The relative humidity was adjusted to 80% for all growth conditions.

X-ray and UVC irradiation of seedlings: Sterilized M2 seeds were plated in a row of 10 seeds/plate (Sterilin, square, 10 cm) containing 60 ml germination medium. Plates were kept for 48 hr at 4° in the dark and then transferred into growth condition A in a vertical position. This allowed light penetration exclusively from above and resulted in uniform growth of the roots on the surface of the agar. The elongation of the main root of each seedling was marked each day. The root tips were irradiated 5 days after sowing by placing the plates in a horizontal position and covering the shoot meristems with a 4-cm-thick lead sheet. The root apices were irradiated with 20 Gy using a Phillips 300 kV/9 mA generator at the rate of 5 Gy/min. Root growth was continuously monitored for a further 10 days. From our monitoring of root growth after application of an increasing dose of X rays (see RESULTS), we expected that the root growth of radiation-hypersensitive individuals would be inhibited by 20 Gy irradiation. Such hypersensitive candidates were recovered and transferred in soil for further plant development and seed production. Similar to X rays, a UVC radiation (254 nm, lamp OSRAM HNS 55W ORF) was applied to the roots while the upper part of the plantlet was protected by a Plexiglas cover. Seedlings were irradiated with increasing doses up to 5 kJ/m^2. After irradiation they were transferred for 2 days into the dark or directly grown under standard conditions.

To test the X-ray sensitivity of segregating F_2 progeny, seeds were plated on germination medium (25 seeds/plate), kept for 48 hr at 4° in the dark and then transferred into growth condition A. Five-day-old seedlings (at the early open cotyledon stage) were irradiated with 20 Gy three times at 24-hr intervals. This treatment was the minimum producing lethal effects on whole seedlings. The plates were kept in condition A for a further 4 weeks and the number of sensitive and resistant seedlings were scored.

Assays of mitomycin C and methyl methanesulfonate sensitivity: Four days after germination, seedlings were transferred into 24-multiwell plastic plates (Falcon). Each well contained one seedling in 0.5 ml of liquid germination medium supplemented with mitomycin C (MMC) (Sigma) at concentrations of 2.5, 5, 10, 15 or 20 mg/liter or with methyl methanesulfonate (MMS) (Fluka) at concentrations of 25, 50, 75, 100 or 150 ppm. Most assays were performed with 10 mg/liter MMC or 100 ppm MMS, which had been previously established to be sublethal doses for the wild type. The plates were incubated in condition A for 4 weeks.

Genetic analysis: Plants grown in soil were used for crosses at the beginning of the flowering period. After mature flowers were removed, immature flower buds were emasculated and cross-pollinated under the binocular microscope using mature anthers as pollen donors. The segregation of mutant traits and complementation analysis of the mutants were performed on F_2 seedlings, using the multiple X-ray irradiation procedure on whole seedlings or sensitivity tests on MMC

or MMS. The significance of differences in the results were analyzed by the χ^2 test at $P \leq 0.05$.

RESULTS

Isolation of X-ray hypersensitive mutants: To establish conditions for the uniform growth of the main root and delayed formation of secondary roots, an array of parameters was tested: medium composition, spectrum and intensity of light, and temperature (data not shown). The optimal conditions established (condition A) resulted in uniform main root growth for 10 days without the formation of secondary roots.

The effect of X-ray dose on root growth inhibition was investigated. The growth of wild-type roots was completely inhibited by 80 Gy; after an initial elongation phase (for 2 days after irradiation), the meristems did not recover from the treatment (Figure 1A). A dose of 20 Gy did not significantly reduce growth compared with nonirradiated controls (Figure 1A), and this dose was chosen for screening for hypersensitive mutants. We screened four independent batches of M2 seeds to ensure the recovery of independent mutations. From 7000 M2 seedlings examined, 11 primary candidates, which in all respects exhibited the desired phenotype (Figure 1B), were recovered and grown to maturity. Seeds (M3) were obtained from nine of them. M3 seedlings were reexamined for sensitivity to X-ray irradiation (20 Gy) by comparing the effects on root growth to that of the wild-type and nonirradiated controls, as shown for mutant *xrs4* in Figure 2. This test confirmed the hypersensitivity to X-rays of all nine candidates (data not shown). Mutants (M3) were backcrossed to the wild-type *L. erecta*. Segregated homozygous lines for the *xrs* allele (BC 1) were used as a starting material for the examination of sensitivity to DNA-damaging chemicals and for genetic crosses described below.

Sensitivity of the *xrs* mutants to UVC and DNA-damaging chemicals: Roots of pregerminated seedlings of *xrs*, wild-type and UVC hypersensitive strains (D. Albinsky, J. E. Masson and J. Paszkowski, unpublished results) were subjected to increasing doses of UVC radiation. The minimal lethal dose of UVC for *xrs* strains and the wild type was the same (Table 1).

To establish a reliable assay system for sensitivity to MMC and MMS, seedlings of uniform size were transferred to multiwell plates containing medium supplemented with these chemicals at different concentrations. After 2–3 wk, the effects of MMC and MMS were manifested by arrest of development and bleaching (Figure 3). The minimal lethal dose of MMC for wild-type seedlings was 20 mg/liter and for MMS was 150 ppm. Using this test, all *xrs* mutants appeared to be

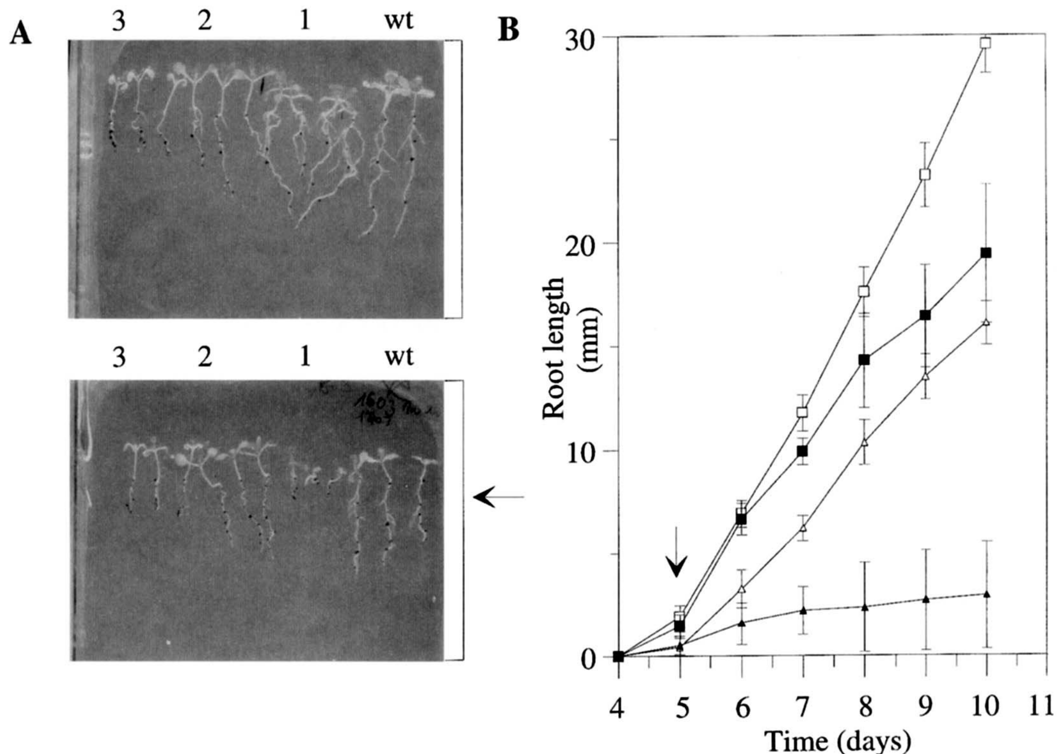

Figure 2.—Rescreening of X-ray-hypersensitive candidates. (A) The progeny from selfing of three mutant lines and the wild type (three seedlings each) were grown under control conditions (top) or irradiated with 20 Gy X rays (bottom). The arrow indicates the distal root position at the time of irradiation. Lane 1 shows mutant with X-ray-specific inhibition of root growth (*xrs4*), lanes 2 and 3 show mutants with constitutively slower root growth, which were eliminated from further studies. (B) The length of the main root is shown for wild type (□) and *xrs4* (△) unirradiated seedlings and for wild-type (■) and *xrs4* (▲) seedlings irradiated with 20 Gy at day 5 (arrow). The vertical bars show the standard deviation from the means for 10 seedlings.

TABLE 1

Lethal doses of DNA-damaging treatments for the mutants *xrs4*, *xrs9*, *xrs11* and the wild-type *L. erecta*

Line tested	Minimal lethal dose of DNA-damaging treatments			
	X rays (Gy)	MMC (mg/l)	MMS (ppm)	UVC (kJ/m^2)
wt	80	20	150	3
xrs4	20	10	75	3
xrs9	20	20	100	3
xrs11	20	10	150	3

wt, wild type.

The minimal lethal irradiation dose (X rays or UV) is defined as the lowest dose that stopped the growth of roots of pregerminated seedlings. The minimal lethal dose for chemical DNA-damaging agents is defined as the lowest concentration of MMC or MMS in the germination medium causing inhibition of growth and necrosis of pregerminated seedlings. Bleaching and necrosis is clearly visible after 3–4 weeks of incubation (for details see MATERIALS AND METHODS).

hypersensitive to one or both DNA-damaging agents. Three phenotypes were found: hypersensitivity to both MMC (MMC^s) and MMS (MMS^s), only MMC^s and only MMS^s. One line of each type was chosen for further analysis: line *xrs4*, line *xrs11* and line *xrs9*, respectively (Table 1). For both MMC^s lines, the minimal lethal dose was reduced to 10 mg/liter as shown for *xrs4* in Figure 3A. Furthermore, *xrs4* (Figure 3B) and *xrs9* were sensitive to MMS at concentrations of 75 and 100 ppm, respectively (Table 1). It was tentatively concluded that these concomitant sensitivities of the mutants are linked to the *xrs* alleles and reflect an inability to repair a particular type of DNA damage. This assumption was tested by genetic analysis.

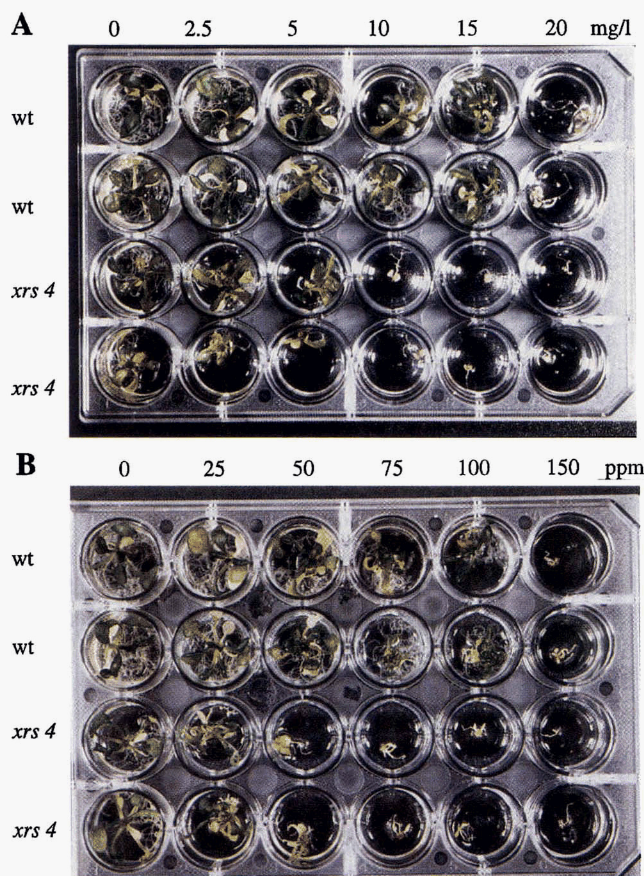

FIGURE 3.—The response of the wild type and mutant *xrs4* to DNA-damaging chemicals. Pregerminated seedlings were transferred to germination medium containing increasing concentrations of MMC (A) or MMS (B). The picture was taken after 4 weeks treatment.

Genetic analysis of the *xrs* mutants: A set of test crosses was performed to determine whether the X-ray hypersensitivity of the mutants is linked to hypersensitivity to MMC and MMS and to assess the nature and the number of genetic loci involved. The F_1 hybrids between wild-type *L. erecta* and all three *xrs* mutants had phenotypes indistinguishable from the wild type, indicating recessive mutations. Segregating F_2 seedling populations were then subjected to X-ray sensitivity tests, the number of hypersensitive seedlings being scored after 4 weeks as shown for F_2 (wt × *xrs4*) in Figure 4. For all three mutants tested (*xrs4*, *xrs9* and *xrs11*), the segregation data indicate single, recessive alleles (Table 2). This was confirmed in F_2-segregating populations after outcrosses to another Arabidopsis ecotype, C-24, and to mapping lines NW4 and NW5 (data not shown).

To test whether the hypersensitivity to X rays and to DNA-damaging agents is the result of mutations in different genes, several randomly chosen individuals of segregating F_2 populations were grown to maturity and their F_3 progeny were examined for hypersensitivity to X rays, MMC and MMS. For example, F_2 seedlings of a cross between *xrs4* (X ray^s, MMC^s and MMS^s) and the wild type segregated 3:1 for sensitivity to all three DNA-damage inducers. Seventy-six randomly chosen F_2 individuals were grown to maturity and were allowed to self-pollinate. Eighteen plants produced F_3 progeny all sensitive to MMC, MMS and X rays. Progeny of 45 plants segregated 3:1 for sensitivity to MMC, MMS and X rays. In 13 F_3 families, seedlings showed the same phenotype as the wild type. These data suggest that identical or closely linked mutant alleles confer hypersensitivity to X rays, MMC and MMS. Further genetic analysis of backcrosses (data not shown) indicated that the hyper-

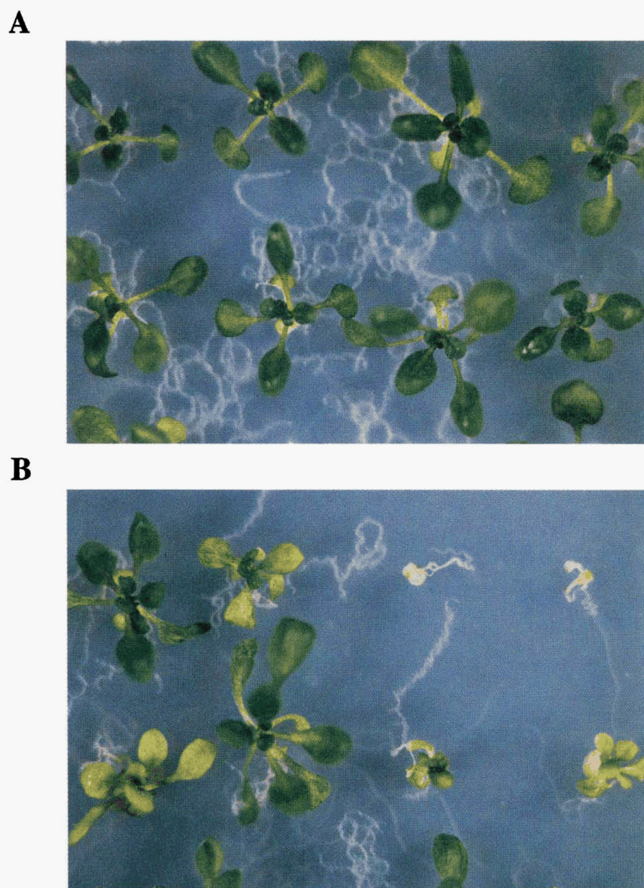

FIGURE 4.—The response of mutant *xrs4* to X-ray irradiation. The F_2 (wt × *xrs4*) population was grown under standard conditions (A) or irradiated with 20 Gy (B) at the early cotyledon stage. The picture was taken 4 wk after irradiation.

sensitivity traits are either encoded by the same gene or by genes < 1 cM apart. Similar data for the cosegregation of concomitant sensitivities were obtained for the other two mutants, *xrs9* and *xrs11*.

To identify the number of independent loci responsible for the *xrs* phenotypes, the segregation patterns of alleles in F_1 and F_2 seedling populations of the crosses *xrs4* x *xrs9*, *xrs4* x *xrs11* and *xrs9* x *xrs11* were determined. The sensitivity of F_1 seedlings of each cross was indistinguishable from that of the wild-type *L. erecta*, suggesting complementation of mutations at different loci in the F_1 hybrids. This was confirmed by analysis of F_2 populations for sensitivity to X rays and DNA-damaging agents. In the F_2, two subpopulations expected for the segregation of nonallelic mutations were observed. The data for *xrs4* × *xrs11* and *xrs9* × *xrs11* suggest linkage between these two alleles (Table 2); thus, *xrs4* and *xrs9* are also probably are linked, but the data obtained actually are ambiguous (Table 2). In summary, the phenotypes of *xrs4*, *xrs9* and *xrs11* appear to be the result of the mutation of three distinct but probably linked genes.

Genetic mapping of the *xrs4*, *xrs9* and *xrs11* mutations: To assort *xrs* mutations to particular chromosome(s), *xrs4*, *xrs9* and *xrs11* alleles were crossed to five marker strains carrying a set of visible mutations on one chromosome each (NW lines) (MEYEROWITZ and MA 1994) (Table 3). Individual plants of segregating F_2 populations with wild-type phenotypes for marker mutations were examined for their status of *xrs* alleles by subjecting their progeny to treatments with DNA-damaging chemicals (MMS for *xrs4* and *xrs9*; MMC for *xrs11*). Genotypes heterozygous for marker alleles and homozygous for *xrs* allele should be recovered in one of six F_3 families assuming independent segregation of *xrs* allele and marker loci in F_2. This was the case in all but one NW line with markers residing on chromosome *4* (Table 3). This result confirmed suggested linkage between the three *xrs* mutations and placed them on chromosome *4*.

Additional phenotypes of the *xrs* mutants: All three *xrs* lines differ in appearance from the wild type. Mutant *xrs4* shows the most striking alterations: slow growth, small rosette and fertility reduced to 5–10% of the wild type. Mutant *xrs9* is also weaker than the wild type, with

TABLE 2

Analysis of X ray, MMC, MMS hypersensitivity of F_2 seedlings derived from crosses between the mutants *xrs4*, *xrs9* and *xrs11* and the wild type

F_2 population tested	Germination (%)	No. of resistant seedlings	No. of sensitive seedlings	χ^2		
				3:1	1:1	9:7
(wt × *xrs11*)	93	164	69	2.65*	—	—
(wt × *xrs9*)	90	77	36	2.52*	—	—
(wt × *xrs4*)	100	75	25	0*	—	—
(*xrs4* × *xrs11*)	98	85	111	—	3.45*	65.27
(*xrs4* × *xrs9*)	90	31	59	—	8.72	17.39
(*xrs9* × *xrs11*)	95	135	151	—	0.90*	9.61
(*xrs4* × *xrs11*)[a]	—	98	105	—	0.24*	5.25
(*xrs4* × *xrs9*)[b]	—	179	147	—	3.14*	0.23*

* Not significantly different from the segregation ratio tested, $P \leq 0.05$.
[a] The F_2 seedlings were irradiated with 20 Gy or grown in germination medium containing 10 mg/liter MMC.
[b] The F_2 seedlings were irradiated with 20 Gy or grown in germination medium containing 100 ppm MMS.

TABLE 3

Genetic mapping of the *xrs4*, *xrs9* and *xrs11* mutations

Lines crossed		Chromosome with marker mutations	No. of F_3 families tested	No. of families heterozygous for tester mutations and homozygous for *xrs* allele	Expected no.	χ^2
X-ray-hypersensitive line	Marker line					
xrs4	NW 4	1	8	2	1.33	0.40
	NW 5	2	11	2	1.83	0.04
	NW 7	3	20	2	3.33	0.63
	NW 9	5	19	4	3.16	0.26
	NW 148	4	24	0	4	4.80*
xrs9	NW 4	1	13	1	2.16	0.74
	NW 5	2	34	2	5.66	2.83
	NW 7	3	20	6	3.33	2.56
	NW 9	5	25	1	4.16	2.88
	NW 148	4	30	0	5	5.10*
xrs11	NW 4	1	20	3	3.33	0.03
	NW 5	2	34	4	5.66	0.58
	NW 148	4	26	0	4.33	5.19*

NW tester strains were crossed to *xrs* mutants. F_1 plants were grown and F_2 seeds were harvested. F_3 seeds were harvested from individual F_2 plants showing a wild-type phenotype for tester mutations. Expected ratio for a family homozygous for *xrs* allele and heterozygous for tester alleles from NW strain is 1:6. * Significantly different from the expected ratio at $P \leq 0.05$.

approximately 50% seed yield. In contrast, mutant *xrs11* is vigorous but with delayed flowering (~5 days) and about 30% more seeds. To address the question whether possible reduction of overall plant fitness, in the case of *xrs4* and *xrs9*, may be the reason for unspecific increase of sensitivity to DNA damaging treatments, we have introduced *ER* wild-type allele, which reverts slow growth of *er/er* ecotype of *L. erecta* (MEYEROWITZ and MA 1994). *ER*-containing *xrs4*, *xrs9* and *xrs11* had increased vigor; however, characteristic *xrs* phenotypes for each mutant (Table 1) remained unchanged. Also, in backcrosses to *L. erecta*, C24 and NW tester lines, these specific phenotypes cosegregated together.

DISCUSSION

We describe here the isolation and characterization of Arabidopsis mutants (*xrs*) hypersensitive to X-ray radiation, which was found to coincide with an elevated sensitivity to chemical DNA damage. Mutants were recovered with a frequency of ~1 among 1000 screened M2 individuals. This high frequency implies that complex pathways consisting of multiple genes are involved in maintaining the wild-type levels of resistance to ionizing radiation. In this respect, our results agree with the recovery of γ-ray hypersensitive (*rad*) mutants (DAVIES *et al.* 1994), although the frequency of *rad* mutant recovery was higher, at one mutant strain per 300 M2 families tested.

Ionizing radiation applied to living cells provokes a broad range of cellular responses due to the generation of active radicals, among them oxidative stress, in addition to direct DNA damage (LEVINE *et al.* 1994). This resembles UV hypersensitive mammalian cells treated with inhibitors of the AP-1 activation cascade (DEVARY *et al.* 1992) or UV hypersensitivity of rodent cells mutated for *c-fos*, which is a component of this cascade (SCHREIBER *et al.* 1995). Since *c-fos*-mutated cells are fully capable of DNA repair, it was postulated that this signaling pathway mediates protection against UV by neutralizing the hostile effects of free radicals (ANGEL 1995). Interestingly, cell death in plants, which is normally triggered by pathogen attack, is indeed mediated through rapid oxidative bursts (JONES and DANGL 1996). Therefore, a test of sensitivity to specific chemical DNA-damaging agents was an important step in the characterization of the *xrs* mutants. The mutants *xrs4*, *xrs9* and *xrs11* described here combine hypersensitivities to X rays and to the DNA-damaging chemicals MMC and MMS, but not to UVC (Table 1). MMC and MMS are alkylating agents (ITO *et al.* 1994; SZYBALSKI and IYER 1964). MMC provokes interstrand crosslinks, and treatment with MMS results in DNA strand breaks (DHILLON and HOEKSTRA 1994), which may be a secondary effect of the repair of MMS-induced damage. The damage caused by X rays, MMC or MMS can be repaired by homologous or illegitimate recombination pathways (PETES *et al.* 1991, WOOD 1996). X ray and γ-ray hypersensitivity of *Saccharomyces cerevisiae* or *S. pombe* mutants is often combined with hypersensitivity to DNA-damaging agents (FRIEDBERG *et al.* 1991; PETES *et al.* 1991; HAYLES and NURSE 1992) and these mutants are found to be defective in homologous recombination processes. Similar combined phenotypes were found in ionizing-radiation hypersensitive rodent mutant lines (COLLINS 1993). In plants, it has been shown that the damage caused by X rays, MMC or MMS induces intrachromosomal homologous recombination (LEBEL *et al.* 1993; PUCHTA *et al.* 1995). It is therefore

possible that the proteins encoded by the XRS4, XRS9 and XRS11 alleles are involved in recombinational DNA repair. Hypersensitivity to radiation and to DNA-damaging agents in these Arabidopsis mutants is not due to overall reduction of fitness; *xrs4* and *xrs9* mutant alleles combined with the *ER* wild-type allele resulted in restoration of the erecta phenotype with increased plant vigor (MEYEROWITZ and MA 1994). The phenotypes characteristic of *xrs* mutations were unchanged in this different genetic background. Furthermore, outcrosses of *xrs* alleles to another Arabidopsis ecotype (C24) did not suppress hypersensitivity. This is in contrast to the *rad* mutants, whose phenotypes were affected by the genetic background of particular Arabidopsis ecotypes (DAVIES *et al.* 1994). Genetic analysis suggested that *xrs4*, *xrs9* and *xrs11* are linked and map to chromosome *4*. To our knowledge, no mutation conferring hypersensitivity to radiation and DNA damaging chemicals has been mapped to chromosome *4* so far. Therefore, mutants described in this study represent, most likely, genes that have not been described previously in Arabidopsis.

Recently, it was shown that two γ-ray-hypersensitive mutants of Arabidopsis (*uvh1* and *rad5*) are affected in the *Agrobacterium tumefaciens*-mediated transformation process (SONTI *et al.* 1995). It still is not clear which plant factors are responsible for this effect in the mutant background, but this exciting observation may provide new insights into the molecular mechanisms of T-DNA integration and the repair of chromosomal damage. Thus, studies of the molecular defects of DNA-damage hypersensitive mutants such as those reported here should increase our understanding of the ways in which plants maintain genome integrity and also will benefit plant genome engineering.

We thank B. HOHN, F. NAGY and L. ROSSI for helpful suggestions in editing. A. BOGUCKI is thanked for technical help. We acknowledge the Seed Stock Center of Nottingham for providing *Arabidopsis thaliana* lines, E. GRILL for providing M2 seeds and F. CORDT and H. WALDNER for X-ray irradiation equipment. J.E.M. is a member of INRA (Institut National Recherche Agronomique), France.

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Communicating editor: M. E. ZOLAN