Isolation of Deficiencies in the Arabidopsis Genome by γ-Irradiation of Pollen

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ABSTRACT

Chromosomal deficiencies are a useful genetic tool in fine-scale genetic mapping and the integration of physical and visible marker genetic maps. Viable overlapping deficiencies may permit gene cloning by subtractive procedures and provide a means of analyzing the functional importance of different chromosomal regions. A method is described for isolation of deficiencies in the Arabidopsis genome which encompass specific loci and other extended chromosomal regions. The technique employs pollen mutagenized by γ-irradiation to pollinate marker lines homozygous for recessive mutations. Deficiencies at specific loci were detected by screening for marker phenotypes in the F_1. Screening for lethal mutations in the F_1/F_2 confirmed specific deficiencies and revealed other deficiencies that did not overlap the marker loci. Further evidence for such mutations was provided by distorted F_2 segregation of the chromosomal markers linked to putative deficiencies. Maintainable (transmissible) and non-transmissible deficiencies were demonstrated by their pattern of inheritance in subsequent generations.

CYTOLOGICAL, genetic and, more recently, molecular evidence indicates that, in higher plants, mutations produced by ionizing radiation are most often the result of chromosomal rearrangements rather than point mutations (STADLER 1928; MCCLINTOCK 1944; SPARROW 1960; EVANS 1962; COE *et al.* 1988). If selected on the basis of viability and simple mutant phenotype, such mutations frequently include small deletions (WESSLER and VARAGONA 1985; OPPENHEIMER *et al.* 1991; WILKINSON and CRAWFORD 1991; SHIRLEY *et al.* 1992; SUN *et al.* 1992). Larger deletions (deficiencies) encompassing two or more genes (STADLER and ROMAN 1948; MOTTINGER 1970; RHOADES and DEMPSEY 1973) are characterized by lack of revertibility, pseudodominance, recessive lethality and cytologically, by the formation of deletion loops during meiosis (SUZUKI *et al.* 1989).

In maize, irradiation mutagenesis of pollen has been extensively employed to generate deletions, deficiencies, reciprocal and B-A translocations, duplications and inversions (COE *et al.* 1988; CARLSON 1988). A particular advantage of pollen irradiation is the ease with which a large number of haploid nuclei can be mutated. Seeds obtained after pollination using irradiated pollen will give rise to heterozygous plants, non-chimaeric for a given induced mutation, and these can be selfed or crossed with other lines to obtain large progenies for further genetic analysis. Furthermore, since the mature pollen grain contains a vegetative nucleus and two sperm nuclei, certain deleterious mutations induced in one sperm nucleus may be transmissible, if the functions of the other nuclei are unaffected (COE *et al.* 1988). X-irradiation of pollen has been used to generate new alleles of the *clavata* locus in Arabidopsis (CLARK *et al.* 1993).

It may not be possible to obtain transmissible mutations for certain regions of the genome, due to dominant lethality or haploid lethality. However, research with maize shows that, in practice, mutations in much of the genome, for example, a deficiency of 5% of the long arm of chromosome *3*, can be transmitted to a greater or lesser extent depending on genetic background, though usually through the egg rather than pollen (MCCLINTOCK 1944; STADLER and ROMAN 1948; RHOADES and DEMPSEY 1973).

Deficiencies have not been extensively studied in Arabidopsis, probably because such chromosomal rearrangements are difficult to distinguish by classical cytology, owing to the small size of the chromosomes (RÉDEI and KONCZ 1992). Furthermore, the efficiency of genetic detection of deficiencies is affected by the level of saturation of the genome by previously mapped point mutations, since detection methods are based on the pseudodominance of such markers when hemizygous with a deficiency. In Arabidopsis, despite much recent progress, the classical genetic map is still at a relatively low level of saturation with visible markers, having on average three mapped mutations per megabase (CHERRY *et al.* 1992; FRANZMANN *et al.* 1993). In contrast, 37 genes have been mapped per megabase of the Drosophila genome (MERRIAM *et al.* 1991)

Pollen irradiation as a means of generating deficiencies has not yet been widely exploited in Arabidopsis research. This report describes this approach in Arabidopsis and provides genetic evidence that the induced mutations include deficiencies both at specific loci and over extensive chromosomal regions. The possible applications of such mutations in Arabidopsis genome research are discussed.

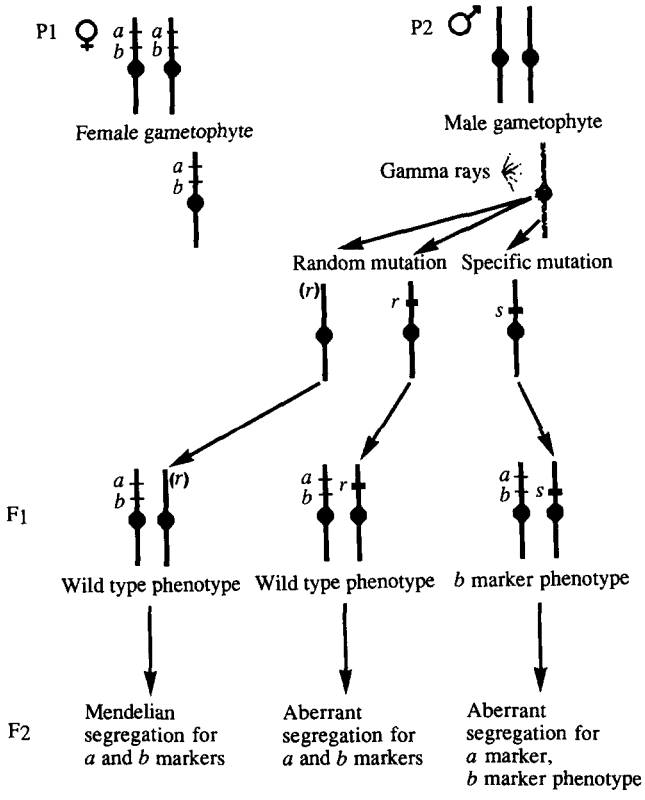

FIGURE 1.—Experimental scheme for induction and detection of deficiencies by pollen irradiation. *(r)* denotes a deficiency unlinked to markers *a* and *b*; *r* denotes a deficiency linked to markers *a* and *b*; *s* represents a deficiency covering a specific locus (*e.g.*, locus *b* as shown in the figure).

MATERIALS AND METHODS

Experimental scheme: The experimental approach adopted in the present work is outlined in Figure 1. The method depends on the availability of multimarker lines of Arabidopsis. These are used as the female parent in crosses with irradiated wild-type (Ler) pollen. If a mutation is induced in the pollen in a chromosomal region corresponding to a recessive marker locus, then the F_1 plant, derived from fertilization of the female recessive line by the mutant pollen grain, will show the recessive phenotype. If induced mutations do not encompass the marker locus, phenotypically wild-type F_1 plants will result. The F_2 progeny obtained by selfing individual F_1 plants will show different patterns of segregation of the recessive marker phenotypes, indicating chromosomes derived from the female parent, depending on the location of the mutations induced in the chromosomes carried by the pollen. In the case where mutations are induced at sites unlinked to the marker loci, the F_2 will show normal Mendelian segregation of the appropriate recessive marker phenotypes. With linked mutations, the recessive phenotypes may be in excess of the expected 25% in the F_2, owing to elimination (reduced transmission), to a varying extent, of the mutated chromosomes. Similarly, when the induced mutation encompasses a specific marker locus, all the F_2 will show the recessive marker phenotype. However, any other linked recessive markers will show aberrant segregation. Thus, mutations induced in chromosomes carried by the pollen can be detected from the aberrant segregation of markers carried in the same region of chromosomes carried in the egg.

Plant materials and growth conditions: The Nottingham Arabidopsis Stock Centre provided *Arabidopsis thaliana* Ler (wild type, NW20), the multimarker line W100, which carries mutations distributed over all five chromosomes (NW100; *an' ap1' er' py' hy2' gl1' bp' cer2' ms1' tt3*), the chromosome *5* marker line *lu' co' ms1' ttg* (N240), the chromosome *3* marker line *hy2' gl1' tt5* (NW7) and the chromosome *4* marker line carrying the semidominant mutation *alb4* (N215; 31.5 cM; I. Y. VIZIR, unpublished). M. KOORNNEEF (Wageningen) provided seeds of the chromosome *5* marker line *ms1' ttg* (Ler background). Another chromosome *3* line, *ap3' tt5* (Ler background), was constructed during the present work.

Seeds were sown on moistened compost (J. Arthur Bowers New Horizon, WILLIAM SINCLAIR, Lincoln, United Kingdom), imbibed for 24 hr at 22° and transferred to 4° for 4 days. Germination was carried out in a growth room (22 ± 2°; 2000 lux cool white fluorescent illumination with a 20-hr photoperiod). After 2 weeks, seedlings were transferred to a glasshouse (24°; supplemented with 2000 lux fluorescent illumination with a 16-hr photoperiod).

Irradiation conditions: Inflorescences were harvested and placed in closed plastic tubes to avoid desiccation during the irradiation period. Inflorescences were irradiated at room temperature shortly after harvest with up to 150 krad γ-rays from a ^{137}Cs source (Gammacell-1000 Elite, Nordion International Inc., Kanata, Ontario, Canada) which provided 0.923 krad min^{-1}. Mature flowers (stage 13–14; SMYTH *et al.* 1990) were selected from inflorescences and used immediately to pollinate multimarker lines. Where necessary (plants not carrying the *ms1* mutation), multimarker lines were emasculated and pollinated under a stereomicroscope.

Estimation of frequency of mutations: The proportion of mutated chromosomes carrying a specific mutation and taking part in formation of the zygote was estimated as the percentage of F_1 plants showing the recessive mutation characteristic of the parental marker line. The frequency of mutations causing lethality in the haploid phase or lethality after fertilization was estimated from the percentage of F_1 plants showing abnormalities in the silique as described below.

RESULTS

Effects of γ-irradiation of pollen on seed set: To determine an irradiation dose which was suitable for the induction of mutations and yet which allowed sufficient viable seed set, pollination of Arabidopsis multimarker lines was carried out with γ-irradiated Ler pollen. Pollen treated with between 30 and 60 krad resulted in siliques containing 50% aborted seeds, while complete seed abortion was observed only at doses above 100 krad. For a dose of 60 krad, 20% of F_1 seed was viable.

Seed abortion might be explained by either dominant lethality due to loss of chromosomal DNA and consequent chromosomal imbalance (CAVE and BROWN 1954; PFAHLER 1967) or abnormal development of the endosperm (LIN and COE 1986).

Dose dependence and mutagenic effects of γ-irradiation: Siliques of F_1 plants were analyzed for lethal mutations in the F_2 generation. Five general phenotypic classes were detected in the F_1 population. In the first, normal and abnormal or shrivelled seeds (abs) were observed. In the second, siliques contained normal seeds but these were interspersed with gaps representing

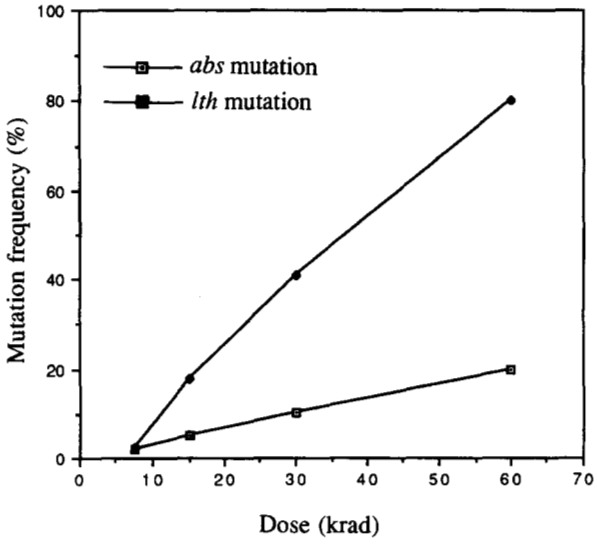

FIGURE 2.—Dose dependence of the mutagenic effects of γ-irradiation analyzed in siliques of F_1 plants.

lethality in the haploid phase or lethality after fertilization (lethal; lth). The third combined both abs and lth. The fourth class was totally sterile or showed very low fertility. The fifth class represented phenotypically normal plants. The frequency of lth and abs mutations increased at different rates with irradiation dose applied (Figure 2). For a dose of 60 krad, 95% of F_1 plants showed in their siliques a phenotypic result of radiation (85% had siliques showing abs and/or lth and 10% showed very low fertility or complete sterility). The appearance of F_1 plants showing lth combined with abs suggested the occurrence of multiple mutation events. Similarly, low fertility or sterility may result from a combination of different lth mutations. Other visible mutations (*e.g.*, albino, dwarf and variegated) were observed at a total frequency of 1% in the F_2.

Induction of mutations at specific loci: The frequency of recessive marker traits appearing in the F_1 was between 0.1 and 1.6%, depending on the particular marker. These F_1 plants showed a range of other traits including full sterility, lth, and/or abs (Table 1). For example, the F_1 frequency of the *ap3* phenotype was 0.11%, while at the upper level of the range, *bp* plants were observed at 1.6%. This indicates differences in frequency of radiation-induced events in different regions of the genome. The occurrence of plants carrying both specific visible marker phenotypes and lth or abs suggests that mutations were deficiencies, that is large deletions, covering more than one gene. Alternatively, two separate events may have occurred. These possibilities were distinguished by F_2/F_3 segregation analysis (see below).

Assessment of apomixis as a possible cause of marker phenotypes in the F_1: To confirm the participation of irradiated pollen in fertilization, the multimarker W100 was pollinated with irradiated pollen from a plant heterozygous for the semidominant, seedling lethal mutation *alb4*. The same procedure permitted detection of deletions and deficiencies pseudodominant to specific W100 marker loci. The *alb4//alb4* genotype gives a yellowish albina seedling; *alb4//+* plants appear yellow-green. In a small scale experiment, normal green plants and 200 F_1 yellow-green plants (*alb4//+*) were obtained. Normal green plants were not analyzed further. Since all of the progeny were *alb4//+* or *+//+* and no plants were homozygous for all W100 markers, apomixis did not occur at a detectable frequency. The *alb4//+* plants were screened for single markers from W100. Two *gl1*, two *an* and one *ap1* plants were obtained, indicating possible locus-specific mutations. In addition, in an analysis of 1128 F_1 plants obtained from W100 crossed with irradiated Ler pollen, no mutants homozygous for all W100 markers were detected. These observations indicate that fertilization was due to irradiated pollen and that apomixis did not occur at a detectable frequency.

Assessment of somatic recombination in early embryogenesis causing appearance of marker phenotypes in the F_1: An alternative possibility to account for the appearance of a recessive marker phenotype in F_1 plants could be somatic recombination, very early in embryogenesis, between the centromere and the marker locus concerned. The genetic distance between centromere and marker locus will be reflected in the frequency of somatic recombination.

In principle, the total frequency of mutations observed in the F_1 is the sum of the frequency of mutations at a specific locus and the frequency of somatic recombination between the centromere and the locus. To distinguish between somatic recombination and deletion, one or more markers distally located to the locus can be used.

Using the *ap3' tt5* (chromosome *3*) line, the frequency of occurrence of *ap3* together with *tt5* in the F_1 was 0.16%, which should represent the frequency of somatic recombination between the centromere and *ap3* plus the frequency of deficiencies covering both loci. The frequency of *ap3* alone was 0.11%; this figure may represent the frequency of double recombination events between the *centromere-ap3* and between *ap3-tt5* plus the frequency of deficiencies encompassing the *ap3* locus. However, based on the small map distance between *ap3* and *tt5* (0.6 cM; I. Y. VIZIR, unpublished) and marker order *gl1* (39.6 cM)-*centromere*(?)-*ap3* (77.2 cM)-*tt5* (77.8 cM) (KOORNNEEF *et al.* 1983; I. Y. VIZIR, unpublished), the probability of double somatic recombination in this region is so small that it can be ignored. Gene conversion in mitosis at the *ap3* locus can also be disregarded since such events are very rare even in meiosis. The only remaining explanation for the observed frequency of *ap3* events is induced deficiencies.

Similarly, the contribution of somatic recombination events to the frequency of phenotypically *ttg* plants in

TABLE 1

Frequencies of specific mutations induced by 60-krad γ-irradiation of pollen

Locus (chr.)	*N*	Total % (*n*)	Sterile % (*n*)	lth % (*n*)	abs % (*n*)	lth and abs % (*n*)
an (*1*)	1128[a]	0.71 (8)	0.53 (6)	0.09 (1)	— (0)	0.09 (1)
ap1(*1*)	1128[a]	1.51 (17)	0.98 (11)	0.36 (4)	— (0)	0.18 (2)
py (*2*)	1128[a]	0.89 (10)	0.36 (4)	0.36 (4)	— (0)	0.18 (2)
hy2 (*3*)	1237[a,b]	0.57 (7)	0.32 (4)	0.08 (1)	— (0)	0.16 (2)
gl1 (*3*)	1237[a,b]	0.89 (10)	0.32 (4)	0.24 (3)	— (0)	0.24 (3)
ap3 (*3*)	1849[c]	0.11 (2)	0.11 (2)[f]	— (0)	— (0)	— (0)
tt5 (*3*)	1958[b,c]	0.72 (14)	0.20 (4)	0.51 (10)	— (0)	— (0)
bp (*4*)	1128[a]	1.60 (18)	0.27 (3)	0.53 (6)	0.62 (7)	0.18 (2)
cer2 (*4*)	1128[a]	1.06 (12)	0.27 (3)	0.27 (3)	— (0)	0.53 (6)
lu (*5*)	1071[d]	0.84 (9)	0.56 (6)	0.19 (2)	— (0)	0.09 (1)
ttg (*5*)	2960[d,e]	0.74 (22)	0.37 (11)	0.27 (8)	0.03 (1)	0.07 (2)
tt3 (*5*)	1128[a]	0.80 (9)	0.36 (4)	0.27 (3)	— (0)	0.18 (2)

Female parent in crosses with irradiated Ler pollen:
[a] W100.
[b] *hy2' gl1' tt5.*
[c] *ap3' tt5.*
[d] *lu' co' msl' ttg.*
[e] *msl' ttg.*
[f] Since *ap3* homozygotes are normally male sterile, the sterility of the two *ap3* plants observed in the F_1 was assessed as female sterility (*i.e.*, lack of seed set after crossing with unirradiated Ler pollen).

the F_1 was assessed as the simultaneous appearance of *ttg* (35.5 cM; chromosome 5) with the distal markers *ms1* (29.8 cM)-*co* (20.3 cM)-*lu* (17.6 cM) (HAUGE *et al.* 1993). A frequency of somatic recombination of 0.12% was observed. The same considerations can be applied to the *gl1* (44.5 cM; chromosome *3*) locus which was analyzed in the same manner using the *hy2* (8.6 cM) distal markers. In this case somatic recombination was not observed.

For the other loci analyzed, for which distal markers were not used (Table 1), further analysis is required to distinguish between somatic recombination or deficiency. In this regard, however, the low frequency of somatic recombination measured for the *centromere-ap3* (0.11%), *centromere-ttg* (0.12%) and *centromere-gl1* (not detected) regions suggests that most of the induced events are deficiencies.

Co-segregation analysis and inheritance of mutations: For detailed analysis of random deficiencies induced within extended regions of the genome, the top arm of chromosome *5* was chosen as a model. Pollen from Ler, irradiated with 60 krad of γ-rays, was used to fertilize the chromosome *5* multimarker line *lu' co' ms1' ttg*. F_2 families were scored for segregation of the *lu* marker (Figure 3). The proportion of *lu* seedlings in F_2 families from F_1 plants that were fully fertile ranged with normal distribution around 25% (Figure 3B). This distribution is as expected for segregation of recessive markers in F_2. Analysis of F_2 families from F_1 plants showing abs and/or lth, revealed some families with a distorted, higher than expected proportion of *lu* seedings (Figure 3C). These data indicate elimination of the irradiated paternal chromosomes resulting in an increased frequency of *lu* carried by the non-irradiated chromosome. Of the F_1 plants showing abs, lth or abs with lth, 19.7% showed distorted *lu* segregation in F_2. Since *lu* is a marker for chromosome *5* only and this chromosome represents about 19.8% of the Arabidopsis genome (HAUGE *et al.* 1993), this implies that most induced mutations cause distorted segregation and are deficiencies with or without other associated chromosomal mutations.

In experiments using pollen irradiated with different doses, 35 F_1 plants with lth and/or abs together with the *ttg* phenotype were obtained. Further analysis of these lines showed strong distortion of F_2 segregation of *lu* (Figure 3D). This suggests that, in addition to the general cases described above, specific deficiencies covering *ttg* result in lth or abs also, due to reduced transmission of chromosomes carrying these deficiencies.

F_1 lines D28, D36, D38 and D52 carried specific deficiencies at the *ttg* locus and lth. With D52, lth was not inherited due to complete elimination of the deficient chromosome. Thus all F_2 seedlings showed the *ttg* phenotype and were *lu//lu*. Strong distortion of *lu* segregation was found in the F_2 of D28, suggesting a high degree of deficient chromosome elimination. F_3 seeds were collected from all F_2 D28 plants which had the *ttg* and normal green phenotype and showed lth. F_3 analysis revealed inheritance of the same degree of distortion of *lu* segregation as observed in the F_2 (Table 2). Similarly, inheritance in the F_3 of the distortion of F_2 *lu* segregation ratio was observed for lines D38 and D36 (Table 2). Seed from some phenotypically normal (non-lth) F_2 plants was also harvested; F_3 progenies showed the same range of *lu* segregation (*i.e.*, undistorted) as shown in Figure 3B (data not shown).

Similarly, F_2 and F_3 progenies were obtained from F_1 lines A6.7, A8.7 and A12.7 showing lth and lines A4.7 and B255 which showed abs. Different extents of chromosome elimination were observed which were re-

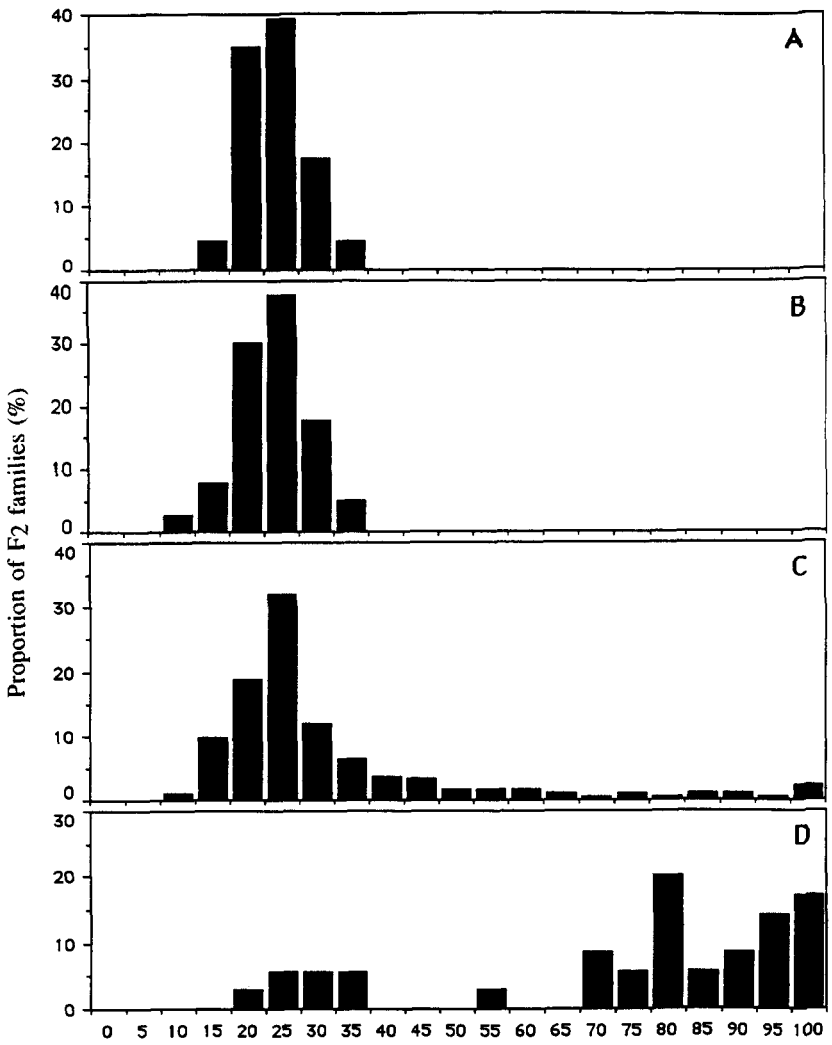

FIGURE 3.—Segregation ratios of the *lu* marker observed in F_2 families harvested from (*A*) F_1 plants obtained from crosses with unirradiated Ler pollen ($n = 30$), (*B*) from phenotypically non-mutant ($n = 52$) and (*C*) mutant (abs and/or lth) ($n = 187$) F_1 plants obtained after crosses using 60 krad irradiated Ler pollen. (*D*) Distortion of F_2 segregation of the *lu* marker in F_2 families obtained from F_1 plants ($n = 35$) with *ttg* phenotype together with abs and/or lth, produced by crosses with pollen irradiated with various doses up to 60 krad.

flected in the inheritance of aberrant *lu* segregation ratios (Table 2). For cases in which induced mutations lie in chromosomal regions unlinked to *lu*, normal Mendelian segregation of *lu* was observed. As typical examples of such cases, data for lines P12.1 showing lth, P14.4 showing abs and P14.9 is presented, all of which had normal (*i.e.*, undistorted) segregation of *lu*.

DISCUSSION

Deficiencies induced by ionizing radiation have proved valuable in fine genetic mapping in maize and Drosophila. The availability of similar mutations in Arabidopsis would provide a genetic tool to complement molecular studies of genome organization and function. While, in other organisms, cytological approaches have been the prinicipal means of demonstrating deficiencies, in Arabidopsis, owing to the small size of the chromosomes, cytological methods cannot be used alone for routine characterization of deficiencies. Genetic approaches, for which Arabidopsis is very suitable, potentially provide an efficient alternative.

In the present work, of the possible mutations induced by irradiation, point mutations (including nucleotide replacement and transpositions) can be discounted due to the relatively very low frequency of visible viable mutations observed. With point mutations, a much higher ratio of viable visible to embryo lethal mutations would be expected than that induced by irradiation (MÜLLER 1965; KOORNNEEF *et al.* 1982). The remaining possibilities are: reciprocal translocations, inversions and deficiencies. Lethality of the gametophyte is characteristic of deficiencies (Figure 4). If a deficiency is induced in a region of the genome unlinked to a particular recessive marker mutation, then in the F_2, normal 3:1 segregation of the corresponding wild-type:recessive marker phenotype will be observed. The phenotypic consequences of such deficiencies can only be detected by examining the siliques of phenotypically wild-type plants for abs or lth. If induced in a region completely linked to a marker locus, a variety of effects on idealized F_2 segregation of linked marker phenotypes may be observed (Figure 4). If the

TABLE 2

Inheritance in deficient lines of segregation ratio of the lu marker

Name of line	Generation	Segregation in F_n		*lu lu* (%)	P^a (n:1)	P^b (F_n/F_{n+1})
		Lu	*lu lu*			
D52	F_2	0	104	100.0	>0.9 (0:1)	—
D28	F_2	3	65	95.6	>0.9 (0.05:1)	—
	F_3[c]	8	195	96.1	>0.5 (0.05:1)	>0.9
D38	F_2	9	39	81.3	>0.5 (0.2:1)	—
	F_3[c]	33	125	79.1	>0.1 (0.2:1)	>0.5
D36	F_2	12	42	77.8	>0.5 (0.25:1)	—
	F_3[c]	98	315	76.3	>0.5 (0.25:1)	>0.1
A4.7	F_2	59	27	31.4	>0.5 (2:1)	—
	F_3[d]	64	38	37.3	>0.1 (2:1)	>0.1
B255	F_2	73	36	33.0	>0.9 (2:1)	—
	F_3[d]	36	25	41.0	>0.1 (2:1)	>0.05
	F_4[d]	39	22	36.1	>0.1 (2:1)	>0.1
A6.7	F_2	17	24	58.5	>0.1 (1:1)	—
	F_3[c]	117	162	58.1	<0.01 (1:1)	>0.5
A8.7	F_2	56	44	44.0	>0.1 (1:1)	—
	F_3[c]	99	70	41.4	<0.05 (1:1)	>0.1
A12.7	F_2	6	97	94.2	>0.5 (0.05:1)	—
	F_3[c]	19	372	95.1	>0.9 (0.05:1)	>0.1
	F_4[c]	8	208	96.3	>0.5 (0.05:1)	>0.1
P12.1	F_2	70	24	25.5	>0.9 (3:1)	—
P14.9	F_2	47	16	25.4	>0.9 (3:1)	—
P14.4	F_2	47	14	23.0	>0.5 (3:1)	—

[a] $P(n{:}1)$ = probability of hypothesis of n:1 segregation of lu marker.

[b] $P(F_n/F_{n+1})$ = probability of hypothesis of similarity between F_n and F_{n+1} segregation; $P(n{:}1)$ and $P(F_n/F_{n+1})$ probabilities were estimated by χ^2 method.

[c] lth was also inherited, F_{n+1} progenies were harvested from F_n plants expressing lth.

[d] abs was inherited, F_{n+1} progenies were harvested from F_n plants expressing abs.

homozygous deficiency results in zygotic or embryonic lethality, the F_2 will show a segregation of 2:1 wild-type:recessive marker phenotype. In the case when a deficiency results in partial or complete inviability of either the male (pollen certation) or female gametophyte, the F_2 will approach a ratio of 1:1 wild-type:recessive marker phenotype. In the case of incomplete linkage and varying degrees of viability of the affected gametophyte, these idealized 2:1 and 1:1 ratios will be modified. If both male and female gametophyte are reduced in viability, or when incomplete linkage of non-transmissible mutations and markers occurs, plants with the marker phenotype will exceed 50% ($1/N{:}1$) of the F_2. Complete linkage of markers with non-transmissible deficiencies in male and female gametophyte will result in 100% (0:1) of F_2 plants showing the marker phenotype.

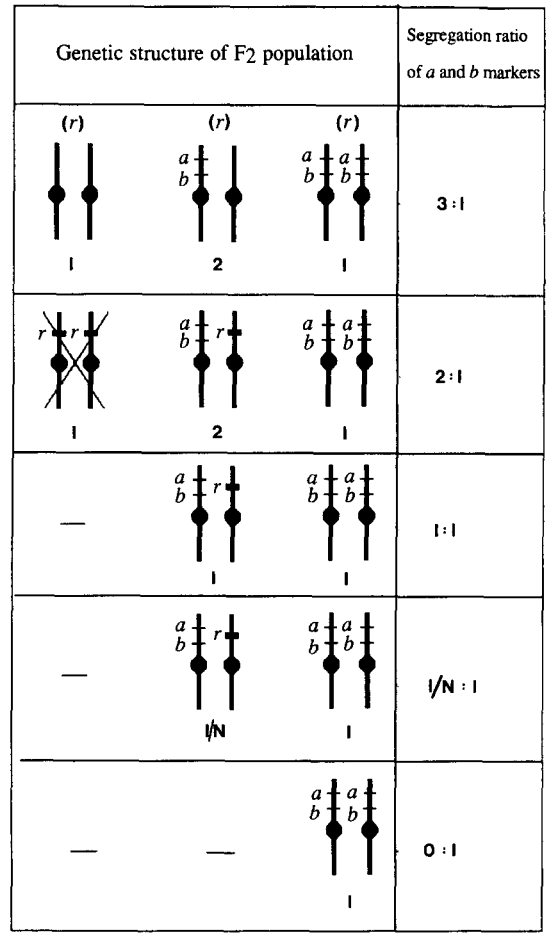

FIGURE 4.—Models to explain the observed range of distorted segregation of genetic markers caused by deficiencies. As discussed in the text, these idealized segregation ratios will be modified according to the degree of linkage of induced mutations and the marker loci and the degree of gamete inviability. For an explanation of symbols, see the legend to Figure 1.

Translocations induced in the pollen will not be apparent at fertilization of marker lines and phenotypically normal embryos will develop. During gametophyte formation, however, any male and female gametes carrying unbalanced chromosome sets will be inviable. Viable gametes will carry either a balanced pair of nonhomologous chromosomes, carrying the reciprocal translocations, or a corresponding pair of nonhomologous chromosomes derived from the unirradiated female gametes of the original multimarker line.

Selfing of an F_1 plant producing such gametes will result in an F_2 with a normal 3:1 segregation of wild-type: recessive marker phenotype.

Inversions, similarly, will have no effect on Mendelian segregation of marker phenotypes. When induced inversions are large, some gametophyte lethality is expected due to crossing over and resulting chromosome breakage in the inverted region (SUZUKI *et al.* 1989). However, since, in this situation, an equal number of viable male and female gametes will be found carrying either normal (unirradiated) chromosomes or viable inversions from the irradiated parent, there will be no effect on the 3:1 F_2 segregation of any linked or unlinked recessive marker phenotype. The possibility of such events in Arabidopsis is small, since, in maize, induced large inversions are rare events (CARLSON 1988). In the case of complex events such as deficiencies associated with inversions/translocations, the distortion of segregation characteristic of simple deficiencies will still be observed.

Another possible cause of aberrant F_2 segregation is the phenomenon of meiotic drive. To judge from work in other organisms, this is a rare consequence of particular mutations (LITTLE 1993). The significance, if any, of this phenomenon in Arabidopsis is unknown.

The range of frequencies of mutation observed for the loci analyzed in the present work can be explained by (i) differential mutability of chromosomal regions and (ii) the genetic distance, close or far, of essential genes (dominant lethal) from the vicinity of analyzed loci. For example, in the case of the *bp* locus, the relatively high frequency of deficiencies with embryo lethal effects suggests that deficiencies in top arm of chromosome *4* can be transmitted. The latter conclusion agrees with the previous result that gametes carrying an unbalanced chromosome *4* (part of the top arm replaced by part of the top arm of chromosome *3*) were functional (PETROV and VIZIR 1988; I. Y. VIZIR, unpublished). In contrast, the region of chromosome *3* nearby *ap3* may contain a mutationally silent region or alternatively contain genes which cause lethality in the hemizygous state.

The average size of deficiencies (D) induced by pollen irradiation can be estimated on the basis of an average frequency of deficiencies per locus relative to the frequency of deficiencies induced over the whole genome (USPENSKY 1937):

$$D = kG \sum_{L=1}^{m} n_L \Big/ \sum_{L=1}^{m} N_L \qquad (1)$$

where n_L is the number of deficiencies obtained at a specific locus (L), N_L is the total number of deficiencies carried by the F_1 plants analyzed, m is the number of loci, k is a coefficient which takes into account the reduction in N_L due to F_1 seed abortion, and G is the genome size.

Assuming (i) the haploid size of the Arabidopsis genome is 100 Mb (MEYEROWITZ 1992), (ii) single rather than multiple deficiencies were induced, (iii) for a dose of 60 krad, $k = 0.2$ (see RESULTS), (iv) that the 12 loci analyzed (Table 1) reflect the average frequency of deficiencies throughout the genome and (v) that each mutant plant carries a deficiency, then the average size of deficiencies estimated from Equation 1 is 160 kb. In fact assumption (ii) is an oversimplification because multiple mutation events must be expected. Therefore, the average size of deficiencies must, in practice, be less than 160 kb.

In practice, selection against multiple deficiencies would be expected. Such events explain the complex phenotypes such as lth combined with abs and very low fertility or complete sterility in F_1. In the Equation 1, therefore, in the case of single events, N_L is equal to the number of mutant (sterile + abs + lth + (abs + lth)) F_1 plants analyzed. Of the F_1 plants showing abs, lth or abs with lth, 19.7% showed distorted *lu* segregation in F_2. Since distorted F_2 segregation in mutant lines is characteristic of deficiencies, the results presented in the present paper suggest that most induced mutations are deficiencies (Figure 3C).

Also relevant to this estimate is the fact that 45% of plants carrying induced mutations were sterile (Table 1) and could not be maintained. Therefore, the remaining set of mutations, including transmissible deficiencies, must represent average DNA losses of less than 90 kb. Further analysis of deficient lines is required to assess the accuracy of these estimates. Furthermore, while the estimates of frequency and size of deficiencies induced by 60 krad irradiation of pollen may be valid for the experimental conditions described in the present paper, other irradiation doses, genetic backgrounds and environmental conditions may produce different results. Pulsed field gel electrophoresis (ECKER 1990; BANCROFT *et al.*, 1992) and fluorescent *in situ* hybridization (PARRA and WINDLE 1993) are appropriate techniques for direct sizing of deficiencies.

In Drosophila, irradiation of mature sperm has been used to generate genome rearrangements (ROBERTS 1986; GRIGLIATTI 1986) at a saturation level of one break point per 10 kb (MERRIAM *et al.* 1991). Deficiencies covering the *b* locus can be more than 1000 kb in size (ALEXANDROV and ALEXANDROVA 1991). In Drosophila gametogenesis, the absence of mitotic divisions of haploid cells after meiosis is in contrast to the pathways of plant gametogenesis. In microsporogenesis, meiosis is followed by a mitotic division forming a vegetative and one generative nucleus, followed by a second division of the latter. In megasporogenesis, three mitotic divisions follow meiosis. In the haploid microspore, transcription of many genes is required for mitosis and pollen maturation (STINSON *et al.* 1987). Thus, it might be expected that haploid lethal mutations would be relatively more

common in plants than in Drosophila and that maintainable deficiencies would be relatively smaller. In this regard, complete sequencing of the yeast chromosome *3* and gene disruption analysis of 30% of coding sequences on this chromosome, indicated that the average distance between two neighboring essential genes in this haploid organism is about 30 kb (OLIVER *et al.* 1992). The recent demonstration that more than 15% of the genes in Arabidopsis are repeated and mostly unlinked (MCGRATH *et al.* 1993), suggests that in the event of certain deficiencies covering essential genes, second copies existing elsewhere in the genome may permit plant survival and increase the likelihood of transmission of larger deficiencies.

New methods currently under development aim to introduce deletions at specific regions of the plant genome. Such methods are based on the use of transposons carrying copies of recombination sites and genes for site specific exonucleases expressed under plant promoters (ODELL *et al.* 1990; OSBORNE *et al.* 1993). The remarkable feature of these methods is the precise control of location of simple deletion events, but at present such methods are not likely to be suitable for saturation deletion analysis of the Arabidopsis genome. The site-specific approach, therefore, complements the pollen irradiation method, first described for maize by STADLER (MCCLINTOCK 1984; COE *et al.* 1988), and which, as described here, can be used to generate a large collection of deficiencies relatively easily in Arabidopsis.

Deficiencies may prove useful in fine-scale deletional mapping of mutations (SUZUKI *et al.* 1989), linking physical maps based on YAC contigs (WARD and JEN 1990; GRILL and SOMERVILLE 1991; HWANG *et al.* 1991, MATALLANA *et al.* 1992), restriction fragment length polymorphism and random amplified polymorphic DNA linkage maps (NAM *et al.* 1989; CHANG *et al.* 1988; REITER *et al.* 1992) and a genetic map of visible markers (KOORNNEEF *at al.* 1983; PATTON *et al.* 1991; HAUGE *et al.* 1993; FRANZMANN *et al.* 1993) and may be a useful complement to molecular cytology (SCHWEIZER *et al.* 1988; MALUSZYNSKA and HESLOP-HARRISON 1993). In addition, crosses between plants carrying viable overlapping deficiencies may provide material suitable for gene cloning by subtractive procedures (STRAUS and AUSUBEL 1990; SUN *et al.* 1992) and for analysis of the functional importance of different chromosomal regions (SLATTER *et al.* 1991; SHAFFER *et al.* 1993).

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