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X RAY- AND FAST NEUTRON - INDUCED MUTATIONS IN *ARABIDOPSIS THALIANA* AND THE EFFECT OF DITHIOTHREITOL UPON THE MUTANT SPECTRUM

Proefschrift

ter verkrijging van de graad van

doctor in de landbouwwetenschappen,

op gezag van de rector magnificus,

dr. H.C. van der Plas,

hoogleraar in de organische scheikunde,

in het openbaar te verdedigen

op vrijdag 1 februari 1980

des namiddags te vier uur in de aula

van de Landbouwhogeschool te Wageningen.

STELLINGEN

1. Naarmate er meer gebruik gemaakt wordt van röntgenstralen voor de inductie van mutaties in vegetatief vermeerderde gewassen zal de behoefte aan een mutagens, dat een ander mutatie-spectrum induceert, toenemen.

2. Wanneer de grootte van de M_1-populatie een beperkende rol speelt bij de omvang van mutatie-proeven zijn er redenen om af te wijken van de veelal gepropageerde "single seed descent" methode.
C.F. Konzak en K. Mikaelsen (1977) in: Manual on mutation breeding, 2nd ed., Technical Reports series No 119, IAEA, Vienna: 125-138.
C.P. Rédei (1975) in: L. Ledoux, Genetic manipulations with plant material, Plenum Press: 329-350
Y. Yoshida, M. Tsuru en S. Kitara (1969) Radiat.Bot. 9: 15-20.

3. De door Li en Rédei aangeprezen methode voor de schatting van de mutatie-frequentie per cel heeft slechts een beperkte toepasbaarheid.
S.L. Li en G.P. Rédei (1969) Radiat.Bot. 9: 125-131.

4. Gerst is een minder geschikt gewas voor mutatieonderzoek dan men uit de veelheid van verslagen zou kunnen opmaken.

5. Bij generatief vermeerderde gewassen wordt het uiteindelijk resultaat van een mutatie veredelingsprogramma bepaald door de mate waarin de selectie methodiek toelaat om begeleidende ongewenste mutaties te elimineren.
Dit proefschrift.

6. Plantenveredeling is de beste werkwijze om, zonder drastisch in te grijpen in locale landbouwsystemen, vooruitgang te boeken in de voedselvoorziening, mits het juiste veredelingsdoel gekozen wordt.

7. De gevonden typen van waardplant-resistentie tegen biotrofe schimmels doen vermoeden dat d.m.v. selectie op celniveau geen resistente planten kunnen worden verkregen tegen deze schimmels.

8. In tegenstelling tot selectie voor partiële resistentie bevordert selectie voor volledige resistentie van de waardplant tegen biotrofe schimmels het ontstaan van virulente schimmel-populaties.

9. Het streven naar verlaging van het gehalte aan nitraat in groentegewassen is toe te juichen; het is echter onwerkelijk om te streven naar een gehalte van maximaal 1500-2000 mg NO_3 per kg vers produkt in alle gewassen.
H.J. Mol (1979) Bedrijfsontwikkeling, 10e jaargang: 948-954.

10. De rechtzoekende is meer gebaat bij een inzicht in hetgeen niet bereikbaar is dan bij de bereidheid in ieder geschil naar een juridische overwinning te streven.

11. Planmatige woonkernen voor specifieke bevolkingsgroepen leiden tot getto-vorming.

12. Er bestaat een aanzienlijk verschil tussen de maximum toegestane snelheid en de toegelaten maximum snelheid.

Lidwine M.W. Dellaert

X-ray- and fast neutron-induced mutations in Arabidopsis thaliana and the effect of dithiothreitol upon the mutant-spectrum.

1 februari 1980

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CONTENTS

GENERAL INTRODUCTION

HISTORY OF MUTATION RESEARCH

The Dutch botanist Hugo de Vries introduced the term 'mutation' for sudden hereditary changes in *Oenothera lamarckiana*, the evening primrose (de Vries, 1901). Although it was later proved that these changes were not due to mutations but to polyploïdy, polysomy or rare recombination events in a very unusual karyotype, the term has been preserved for changes in the quality, quantity or arrangement of genes.

The first clearly effective mutagen discovered was X-rays. In 1927, at the third International Congress of Genetics in Berlin, Muller presented data that demonstrated unambiguously the capacity of X-rays to produce mutations in *Drosophila*. In 1928 Stadler showed the same for maize. Since then many radiation types and chemicals have been listed as being mutagenic.

Comparisons between the types of mutants induced by radiation and different chemicals showed that the mutant spectra, i.e. the proportions of different mutant phenotypes or mutations at different loci, were not always the same. This was first discovered in plants where the proportions of different types of chlorophyll mutants were shown to depend on the mutagen used (Gustafsson and McKey, 1948). It was subsequently confirmed for reverse mutations in micro-organisms, from a specific biochemical requirement to independence, where the very large numbers screened confirmed

the reality of the phenomenon beyond doubt (Demerec, 1953; Kølmark, 1953).

The analysis of mutagen specificity, i.e. all cases in which mutagens differ from each other in the proportions of the effects they produce, became a tool for studying mutagenesis as a process. In this thesis the term mutagen specificity is applied to specificity with respect to the proportions of different mutant phenotypes or mutations at different loci. This type of mutagen specificity is of interest for the production of commercial mutants in plants.

MUTAGEN SPECIFICITY

Research into mutagen specific effects, in terms of mutant spectra, has mainly concentrated on the comparison of the effects of different types of chemical mutagens and UV in prokaryotes. These studies, reviewed by Drake (1970), revealed that some cases of specificity can be attributed to the reaction between DNA (base or base sequence) and the mutagen. However, even closely related mutagens, which presumably act by very similar mechanisms, may yield specific mutant spectra (Chevalier, 1964). Such specificities may be due to treatment effects on the fixation of potential mutations and on the viability of the mutant, especially in haploid cells. Direct evidence for the origin of at least some of the mutagen specificity at later steps of the mutation process was obtained in experiments in which specificity was modified by experimental conditions, mutagen dose, genetic background or cell phase (examples will be found in the reviews by Auerbach, 1976 and Auerbach and Kilbey, 1971).

In some plant species significant differences between the spectra of various chlorophyll mutants induced with ionizing radiation and alkylating chemicals were found, such as in barley (Ehrenberg et al, 1959; Gustafsson, 1963; Nilan and Konzak, 1961), in rice (Rao and Gopal-Ayengar, 1964) and in *Arabidopsis* (Jacobs, 1969; Röbbelen, 1962). Differences in radiation- and chemical--induced morphological mutants were observed in barley (Lundqvist, 1975; Lundqvist and Wettstein, 1962; Lundqvist et al, 1968; Persson and Hagberg, 1969), in *Arabidopsis* (McKelvie, 1963) and in peas (Monti, 1968). Some of the observed specificity can be explained by the specific effects of the mutagen on DNA. In general, however, the interpretation of specificity in higher plants is more difficult than in micro-organisms, because usually the number and location of genes, proportions of deletions, and chromosome rearrangements among the mutants are not known. In barley (Lundqvist, 1975; Lundqvist and Wettstein, 1962; Lundqvist et al, 1968) X-rays, fast neutrons and various chemical mutagens yielded different spectra of *eceriferum* mutants, many of which involved known loci. Among possible explanations the authors consider the presence of viability loci in the vicinity of some of the *eceriferum* genes. In maize no significant difference between ionizing radiation and ethyl methanesulfonate (EMS) in the proportions of mutations at the *I*, *Sh* and *Wx* loci was observed. However, it was found that single--locus mutations were frequently and almost exclusively induced with EMS, while irradiation produced a high proportion of "multiple-locus mutations", i.e. closely linked genes were "mutated" simultaneously, presumably due to deficiencies (Amano and Smith, 1965).

In *Drosophila*, allelism tests were performed between X-ray and EMS--induced lethals that were located on the X-chromosome in a region covered by the $Y.ma\text{-}l^{+}$ translocation (Lifschytz and Falk, 1968 and 1969). A map of

34 functional units could be constructed. The distribution of the mutations in the affected units was highly non-random and was different for X-ray- as compared to EMS-induced mutations. Furthermore, it was observed that 80 per cent of the EMS-induced lethals comprised single units, while most X-ray--induced lethals covered several functional units, and were presumably small deficiencies. Lifschytz and Falk (1969) suggested that mutagen specificities may arise from differences between genes in the number of "essential sites" in their code message. Mutagens that produce base changes will yield more mutations in those genes with many essential sites than in those with only a few; mutagens that produce frameshifts or deletions will be less selective.

The preferential action of some mutagens on specific chromosomes or chromosome regions, as observed in *Oenothera* (Krautblatter and Arnold, 1967; Kressel and Arnold, 1967), in *Vicia faba* (Schubert and Rieger, 1976) and in *Drosophila* (Pimpinelli et al, 1977) may also explain the observed differences in mutant spectra.

X-ray- and fast neutron-induced mutant spectra

Differences between X-ray- and fast neutron-induced mutant spectra in higher plants have been demonstrated in barley (1) for the different types of chlorophyll mutants (Conger and Constantin, 1974; Dellaert, unpublished; Ehrenberg et al, 1959), (2) for different types of viable mutants (Dellaert, unpublished; Ehrenberg et al, 1959), and (3) for the *erectoides* and *ecériferum* mutant spectra, which indicate differences in mutant frequency of specific loci (Lundqvist, 1975; Lundqvist and Wettstein, 1962; Lundqvist et al, 1968; Persson and Hagberg, 1969). Unfortunately, the mutants were induced at various radiation doses and under various conditions, and were collected by different

observers over many years. Therefore, the observed differences might have been due, at least partly, to differences in experimental conditions or mutagen dose. However, my experiments under standardized conditions, resulted in X-ray- and fast neutron-induced *ecoriferum* mutant spectra phenotypically similar to the spectra described by Lundqvist and Wettstein in 1962 (Dellaert, unpublished). Apart from these observations in barley, very little evidence of a qualitative difference between mutations induced by X-rays (low-LET) and fast neutrons (high-LET) is on record in the literature.

In the mouse, there is evidence that more *d-se* deficiencies are induced by fast neutrons than by X- or γ-irradiation. On the other hand, the spectrum of specific locus mutations (apart from the *d-se* mutation) in the original 7-locus stock is very similar after both types of radiation (Batchelor et al, 1966; Russell, 1965; Russell and Russell, 1959).

In *Neurospora*, it was observed by de Serres (1970) that, compared to X-rays, high-LET irradiation with 40 MeV helium or 108 MeV carbon ions is more efficient in inducing recessive lethal mutations by chromosome deletions than by point mutations. However, comparison of the spectra of allelic complementation among X-ray-, helium-ion- and carbon-ion-induced *ad-3B* mutants in *Neurospora* did not indicate differences in the spectra of induced genetic alterations at the gene level resulting in point mutations (Malling and de Serres, 1967a and b).

Very little relevant work has been reported in bacteria or other prokaryotes. This may be partly due to the masking of the mutations by associated lethal effects as the LET increases. In the bacteriophage T_4 as well as in *E. coli* a decrease in the effectiveness for the production of gene

mutations with increasing LET has been observed (Bridges and Munson, 1968; Munson and Bridges, 1969 and 1973).

Molecular mechanisms of X-ray and fast neutron mutagenesis

The molecular mechanisms of radiation mutagenesis is very complex. Chemical changes in nucleic acid bases, breakage of hydrogen bonds in the double helix, single- and double-strand breakage in DNA, cross-linking between the two strands of DNA, between different molecules of DNA and between DNA and protein - all these and more indirect ones, such as the release of endonuclease from lysosomes - have been reported (Auerbach, 1976; Box, 1977).

X-rays and fast neutrons differ from each other in linear energy transfer (LET), i.e. the ion density along the tracks they produce. X-rays (except very soft ones) produce mainly sparse ionizations (low-LET), while fast neutrons yield densely ionized tracks caused by recoil protons (high-LET). Recent studies concerning the efficiency by which various types of lesions were produced by fast neutrons compared to γ-rays (≈ X-rays) in bacteriophage DNA indicated that neutron-induced double strand-breaks sometimes occur in clusters of more than 100 in the same phage and that the relative efficiency with which double strand-breaks form is about 50 times that of γ-induced double strand-breaks (Hawkins, 1979).

In a recent review (Traut, 1978) the molecular nature of gene mutations produced by ionizing radiation is discussed. Studies with phage (ΦX 174, T_4) revealed that ionizing radiation is able to induce true gene mutations, i.e. chemical changes in nucleic acid bases. Furthermore, a remarkable observation is that, irrespective of the organism studied, most radiation-induced gene mutations are characterized by "double-stranded segregation", i.e. they pro-

duce mainly mutant daughter cells in unicellular organisms and "whole body mutants" in multicellular organisms. A discussion about the origin of the "double-strandness" of the radiation-induced gene mutations is beyond the scope of this chapter (For a review the reader is referred to Auerbach, 1976).

Relative biological effectiveness of fast neutrons compared to X-rays

The difference between X-rays and fast neutrons in the proportions of various types of induced lesions, i.e. fast neutrons produce relatively more double strand-breaks (Hawkins, 1979), has already been indicated by studies on the relative biological effectiveness (RBE) of fast neutrons compared to X-rays for different types of genetic damage (Anonymus, 1972). Two peculiarities of fast neutrons as opposed to X-rays were observed; a higher RBE for translocations than for point mutations and, in general, absence of an oxygen effect. A differential effect of the irradiation condition (dose; dose rate; oxygen and moisture content; concentration of sulphydryl compounds) on the RBE for chromosomal aberrations and point mutations has been reported (Broertjes, 1968; Malvarez et al, 1965; Smith, 1969; Smith and Combatti, 1967; Smith et al, 1968; Underbrink et al, 1970). Besides, it has recently been shown that at the molecular level the relative proportion of radiation-induced strand-breaks is modified by changing the oxygen concentration or the concentration of sulphydryl compounds (Christensen et al, 1972; de Jong et al, 1972; van der Schans et al, 1979). These observations suggest that manipulation of the irradiation conditions, in terms of oxygen concentration or concentration of sulphydryl compounds, may affect the differences between X-ray- and fast neutron-induced mutant spectra, provided that they are due to differences in the proportion of various lesions, such as double strand-breaks, single strand-breaks and molecular changes in nucleic acid bases. To see to what

extent sulphydryl compounds influence X-ray and fast neutron specificity, various effects of both types of radiation were compared, with and without dithiothreitol pre-irradiation treatment, in *Arabidopsis*.

X-RAY- AND FAST NEUTRON-INDUCED MUTANT SPECTRA IN *ARABIDOPSIS*

The aim of the present investigation was to determine whether, in addition to the results obtained in barley, a difference between X-ray- and fast neutron-induced mutant spectrum could also be observed in *Arabidopsis*. Furthermore, to find out whether the induced mutant spectra are influenced by radiation dose or pre-irradiation treatment with the sulphydryl compound dithiothreitol (DTT).

It is of interest to see whether X-ray- and fast neutron-induced mutants differ in their general performance, due to differential "pleiotropic" effects of the mutation. To study "pleiotropic" effects one needs to isolate mutants in an otherwise undisturbed background, since direct selected mutants might carry, in various amounts, other mutations in their genetic background. With a view to research concerning this aspect, a study on the efficiency of various selection methods in self-fertilizing crops for the isolation of mutants in an undisturbed genetic background was made. Besides, for the production of commercial mutants in agricultural crops, mutants in an undisturbed genetic background are to be preferred.

The results of these studies are reported in seven articles, presented as different chapters. Chapters I and II are devoted to irradiation effects, with or without a DTT pre-treatment, measured with Müller's embryo test (Müller, 1961) in the M_1-inflorescence of *Arabidopsis*. In chapter III the

spectra of X-ray- and fast neutron-induced morphological mutants are described, and the effects of radiation type, radiation dose and a DTT pre-treatment on the induced spectra are discussed. In chapter IV the results of a study on the genetic behaviour of the radiation-induced mutants are discussed. The emphasis is laid on gametophytic and sporophytic selection of mutant genotypes. The results are discussed with reference to the transformation of the observed mutant frequency to mutation frequency per cell. In chapter V the frequencies and morphology of a specific group of mutants, namely the *ecériferum* mutants, is described in detail. Finally, chapter VI makes a (theoretical) comparison of selection methods for specified mutants in self fertilizing crops.

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CHAPTER I

DOSE RESPONSE CURVES AFTER IRRADIATION OF *ARABIDOPSIS* SEEDS: A POSSIBLE EXPLANATION FOR THE "SATURATION" IN MUTANT FREQUENCY AT HIGH RADIATION DOSES.*

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ABSTRACT

Tests were done to see whether the "saturation" in mutant frequency in *Arabidopsis*' "main" inflorescence, after fast neutron or X-ray treatment of seeds, was found because cell populations with heterogeneous radiation-sensitivities were studied. Mutant frequencies were determined in (1) morphologically normal and conspicuous plants, (2) main and lateral inflorescences, and (3) different M_1-fertility classes. At moderate radiation doses (47 Gy fast neutrons and 233 Gy X-rays) significant differences in mutant frequencies between different M_1-fertility classes were observed. This suggests that cell populations with heterogeneous sensitivities were studied. No significant differences were observed either between the mutant frequencies in main and lateral inflorescences or between the ones in inflorescences of morphologically normal and conspicuous plants. This suggests that these inflorescences were formed by cell populations with similar but heterogeneous sensitivities.

* Research carried out jointly at the Institute for Atomic Sciences in Agriculture, Wageningen and at the Department of Genetics, Agricultural University, Wageningen, The Netherlands.

INTRODUCTION

To compare the effect of different mutagenic treatments (type of mutagen, dose) only cell populations uniform in mutagen sensitivity should be studied. When the mutagenic treatment does not interfere with normal histogenesis, the scored M_2-progenies can be traced back to the M_1-main inflorescence cells, the apical initial cells present in the L_2 layer of the embryonic shoot apex at the time of seed-treatment. The "overall" sensitivity for X-rays, EMS and MNU treatment of these initial cells in a given seedlot of *Arabidopsis* is said to be uniform (18, 23, 30).

Although an increase in mutant frequency with increasing dose is expected (2, 6, 17) for *Arabidopsis* progenies of the main inflorescences, no further increase in chlorophyll mutant frequency after relatively high radiation doses was found (Fig. 1). This could be explained for instance in terms of a deviating histogenesis after the applied relatively high doses (i.e. 20, 33, 47 and 60 Gy fast neutrons; 140, 233, 327 and 420 Gy X-rays). Since irradiation may severely damage the embryonic shoot apex, a new apex may be formed either from one or a few surviving cells within the apex, or the whole apical function may be taken over by axillary or adventitious buds (1, 5, 12, 13, 14, 15, 26, 29, 32). This "replacement" of the apical initial cells will reduce the mutation frequency only when the cells which form the new apex are less radiation-sensitive.

An increase in dose increases the number of plants *without* apical dominance of the main inflorescence (this report). It could be that in these plants the original shoot apex is severely damaged. The decrease in the mutant frequency at relatively high radiation doses could then be due to the presence of these plants. Also, at higher doses the main inflorescence cannot easily be distinguished with the naked eye from equally vigorous rosette lateral inflorescence(s). Partial replacement of the main inflorescences by lateral ones during scoring can lead to reduced mutant frequencies if cells forming the lateral inflorescences are less sensitive to radiation.

To explain the differences in observed and "expected" mutant frequencies, the effect of X-ray and fast neutron irradiation on M_1-plant

development was studied. The mutant frequencies in plants with *normal* apical dominance, with *reduced* apical dominance, or *without* apical dominance were compared, as were the mutant frequencies in main and lateral inflorescences. The mutant frequencies in different M_1-fertility classes were also compared. Partial replacement of the apical initial cells by cells which are less radiation-sensitive would produce M_1-"initial" cell populations with heterogeneous sensitivities. After a given dose M_1-sporophytic tissues that had descended from sensitive cells would then produce a higher sterility and a higher mutant frequency than those, that had descended from less sensitive cells. Therefore, the partial replacement of the initial cells would produce within-treatment dependence of mutant frequency and M_1-fertility class.

MATERIAL AND METHODS

Arabidopsis seeds used in the experiment were mutant *erecta* of the ecotype "Landsberg" (27). To break dormancy, seeds were kept on moist filter paper at 2 °C for 5 days and re-dried (24 °C, 24 hr). Approximately 300 re-dried seeds per treatment per replication were submerged into 22 °C tap water 3 hr before irradiation. To standardize environmental conditions, the irradiation was applied to seeds submerged in 22 °C tap water, because it can then be assumed that oxygen concentration and water content was equal in all seeds. X-radiation (60 Gy, 233 Gy and 327 Gy) was applied with an MG 301 X-ray machine with an MCN-420 tube, operating at 320 kVp and 10 mA with an additional 0.25 Cu and 1.0 Al filter and a 4 Gy/min. dose rate. Fast neutrons (33 Gy, 47 Gy and 60 Gy) were given in the irradiation room of the BARN (Biological Agricultural Reactor Netherlands, Wageningen) with a 1 Gy/min. dose rate and a γ-contamination of approximately 3 percent on a Gy basis. Seven treatments (including the control, 0 Gy) were given in two replications with a two month interval.

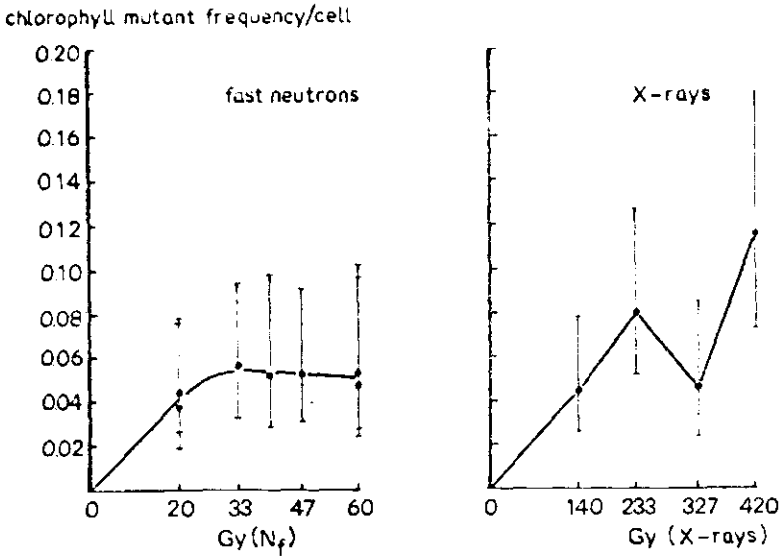

Fig. 1 The chlorophyll mutant frequency in *Arabidopsis* "main" inflorescence after fast neutron or X-ray treatment of presoaked (3 hr, 22 °C) seeds.
(DELLAERT, details to be published elsewhere)

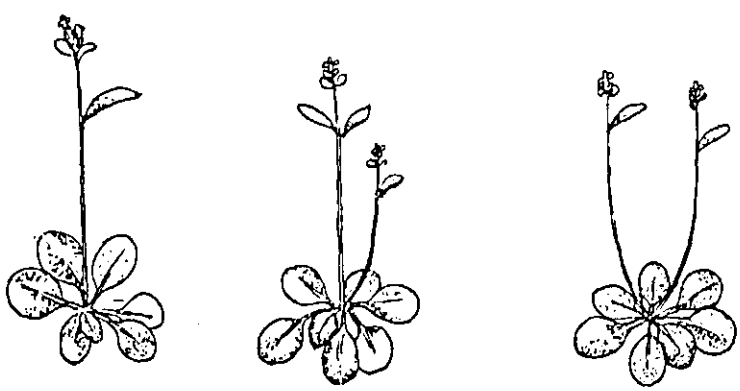

Fig. 2 The three different development classes distinguishable at flowering time after irradiation of *Arabidopsis* seeds.

After irradiation the seeds were sown (equally spaced) in portions of 30 in a petri dish on a standard mineral medium and put to germinate at 24 °C under continuous illumination by fluorescent light tubes, 8000 lux/cm^2. After 8 days 265 seedlings per treatment per replication were transplanted into soil in an air-conditioned greenhouse. The culture medium and culture conditions used were as described by Feenstra (9) and Oostindiër-Braaksma and Feenstra (25). To have equal conditions for the plants of all treatments within one replication, randomization on a single plant basis was done at transplanting.

At flowering, the plants were grouped into three classes depending on the degree of main inflorescence apical dominance (Fig. 2). A subdivision was made into plants with or without main inflorescence fasciation. The main inflorescence was marked. In doubtful cases, i.e. when two (or more) of the first flowering inflorescences flowered simultaneously, one of them was taken at random.

For scoring mutants, Müller's embryo test (21, 22) was applied to silique number 5 or 6 of the main inflorescence and of the first flowering lateral inflorescence (formed in the rosette leaf axils). For ease of handling scoring in a silique was done at one side of the septum.

The frequency of non-fertilized ovules in the M_1-siliques was expressed as a percentage of the total number of ovules, and the frequency of embryonic lethals in the M_1-siliques was expressed as a percentage of the total number of fertilized ovules. The chlorophyll mutant frequency, m, was expressed as the chlorophyll mutation frequency per cell (10, 11);

$$m = \frac{m'}{f}, \text{ where}$$

m = the number of mutated cells among the total number of cells,

m' = the number of mutants among the total number of non-lethal M_2-embryos,

f = the segregation frequency of recessive mutants, i.e. the probability that a non-lethal M_2-embryo descending from a heterozygous flower is a homozygous mutant.

In applying Müller's embryo test, a cluster sampling was used. With this method the silique is the sampling unit. Within each sampling unit the individual ovules (fertilized and non-fertilized ovules) or embryos (normal, lethal and "chlorophylls") were classified. Therefore, in estimating the percentage of non-fertilized ovules, the total number of ovules scored per silique formed the cluster and in estimating the percentage of embryonic lethals, the number of fertilized ovules per silique formed the cluster. In the case of chlorophyll mutants, the cluster was the number of non-lethal embryos scored per silique. The clusters varied in size. For the calculation of the standard error, Sp, of the mean proportion of non-fertilized ovules and embryonic lethals, Snedecor's (28) formula was used:

$$Sp = \frac{S}{\sqrt{N}}, \text{ where}$$

$S = \frac{1}{\bar{n}} \sqrt{\frac{1}{(N-1)} \{\Sigma a_i^2 - 2p\Sigma a_i n_i + p^2 \Sigma n_i^2\}}$, the sample standard deviation,

N = number of siliques scored,

n_i = size of cluster i,

$\bar{n}$ = $\Sigma n_i / N$, the mean clustersize in the sample,

a_i = the number of units within cluster i that belong to a specified class,

p = $\Sigma a_i / \Sigma n_i$, their overall proportion in the sample.

For chlorophyll mutants it is possible to estimate the mutation frequency per cell (m), as the segregation frequency of recessives (f) can be used. A confidence interval for m is calculated using Stam's formula (pers. comm.). This formula is based on the assumption that;

- each M_2-line (M_1-silique in our case) originates from a single cell,
- the segregation frequency (f) in segregating M_2-lines is equal for all types of recessive mutants,
- the total number of mutants (K), is normally distributed.

The 90 per cent confidence limits of K for given m are

$$\varepsilon(K) \pm 1.645\sqrt{\text{var}(K)},$$

where both $\varepsilon(K)$ and var(K) are functions of m, i.e.
$\varepsilon(K) = mf\Sigma n_i$, and $\text{var}(K) = f^2m(1-m)\Sigma n_i^2 + mf(1-f)\Sigma n_i$.
The confidence limits of m are the values of m that satisfy

$$K-\varepsilon(K) = \pm 1.645\sqrt{\text{var}(K)}.$$

The solutions of this quadratic equation in m are asymmetric with respect to the estimate $\hat{m}$.

RESULTS AND DISCUSSION

A. *The effect of fast neutrons or X-rays on M_1-plant development.*

The distribution of the M_1-plants over the different development categories after irradiation is given in Table 1. For analysis of the data a computer program for discrete multivariate analyses was used (tests of independence in a multi-way contingency table; see appendix). Significant effects ($p < 0.01$) were found for the two-factor--interactions; treatment x development class, treatment x fasciation and development class x fasciation, and for the three-factor-interaction treatment x replication x development class. This indicates that:

a) The fasciation of the M_1-plants was influenced by the irradiation and by the main inflorescence development.
b) The main inflorescence development was affected by the irradiation. The extent of this effect was dependent on the growing season, i.e. was different in the two replicates.

Fasciation mainly occurred in those plants with *normal* main inflorescence apical dominance. The observed proportion of fasciated plants in this group is given in Fig. 3. Fasciation results from the inhibition of cell differentiation which leads to a broad apex with forks (12). Thus it may be assumed that fasciation only occurs when inhibition of

Table 1. *The effect of X-ray and fast neutron irradiation on plant development in Arabidopsis.*

Irradiation		Repli-	Number of plants per development class							
type	dose (Gy)	cation	dead	normal a.d.[a] -	normal a.d.[a] +	reduced a.d.[a] -	reduced a.d.[a] +	without a.d.[a] -	without a.d.[a] +
control	0	1	21	240	0	1	0	0	0
		2	17	241	0	4	0	0	0
N_f[b]	33	1	25	181	28	19	0	6	1
		2	52	73	29	80	3	23	0
N_f	47	1	42	95	28	81	1	17	1
		2	43	68	20	101	10	22	0
N_f	60	1	61	88	18	71	1	22	0
		2	52	54	8	105	7	32	0
X-rays	60	1	24	232	2	2	0	0	0
		2	43	211	4	5	0	1	0
X-rays	233	1	29	149	29	47	3	4	0
		2	43	111	27	59	5	16	0
X-rays	230[c]	1	36	170	10	35	0	9	0
	327	2	58	92	26	69	4	13	0

a) a.d. = apical dominance of the main inflorescence; - indicates no fasciation + indicates fasciation of the "main" inflorescence.

b) N_f = fast neutrons.

c) In the first replication, when applying a dose of 327 Gy, the X-ray apparatus failed after 230 Gy.

----- Partitioning of the table used for analysis of the data; above ----- is sub-table *I*, below ----- is sub-table *II*.

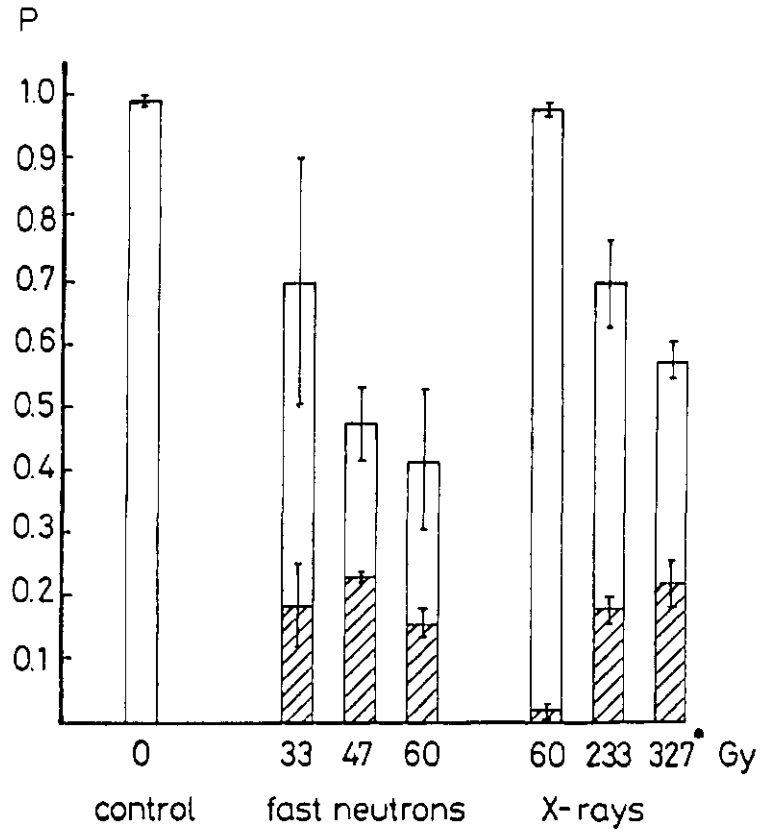

Fig. 3 The overall proportion (p) (after seed irradiation) of M_1-plants with;

□ *normal* apical dominance (including fasciation) of the total number of M_1-plants,

▨ fasciation of the total number of M_1-plants with *normal* apical dominance.

(*For 327 Gy X-rays, the proportions found in replication 2 are given)

cell differentiation takes place in multicellular apices. In the mature *Arabidopsis* embryo the apex is about 6 cells in diameter (20). The primordia of the first two leaves, present in the seed (1, 12, 19) probably reduce the shoot apex to 2 or 3 cells in diameter. No pre-formed axillary buds are present. The fact that almost no fasciated plants were found in the group of plants *without* main inflorescence apical dominance suggests that the shoot apex was severely damaged in these plants and that new apices were formed by one or more surviving cells after the irradiation.

The proportion of plants with *normal* main inflorescence apical dominance decreased with increasing dose (Fig. 3). It was of interest, therefore, to determine whether the reduction in mutant frequency at relatively high radiation doses could be attributed to

1) an increased proportion of plants with *reduced* or *without* main inflorescence apical dominance, because these plants might be connected with cell replacement, or
2) a partial replacement of the main inflorescences by lateral ones during scoring, due to an increased frequency of plants in which the main inflorescence could not easily be distinguished from laterals.

These two points will be discussed in B and C, respectively.

B. *The mutant frequency in plants of the different development categories*

For the analyses of the mutant frequencies (in B, C and D resp.) the data from the two replications were pooled, since within dose no significant difference in mutant frequency between the replications was found. Table 2 gives the data obtained in the "main" inflorescence of plants from each development category, excluding those with fasciation. It can be seen from the table that plants with *reduced* apical dominance generally tended to have higher mutant frequencies than those with *normal* apical dominance. It is concluded that the reduction in mutant frequency at relative high doses cannot be attributed to the increased proportion of plants with *reduced* apical dominance. Plants *without* apical dominance

Table 2. *Mutant frequency in different M_1-plant development classes after treatment with fast neutrons (N_f) or X-rays.*

I Plants with normal apical dominance; II Plants with reduced apical dominance; III Plants without apical dominance of the main inflorescence.

Irradiation		No. of siliques			non-fertilized ovules (%)			embryonic lethals (%)			chlorophyll mut. freq. x 10^{-2}/cell†		
type	dose (Gy)	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
control	0	481	5*		7.6±0.5			1.0±0.2			0.01<0.11< 0.87		
N_f	33	254	99	29	34.2±1.9	33.6±3.2	41.7±6.1	4.4±0.6	8.8±1.7	3.8±1.3	1.18<2.56< 5.49	0.59<2.55< 8.23	0<14.97
	47	163	182	39	50.6±2.7	50.9±2.7	50.9±2.7	7.4±1.1	9.9±1.3	10.0±2.9	3.08<6.23<12.27	5.16<9.04<15.44	0.35<2.64<17.91
	60	142	176	54	54.8±3.1	61.3±2.5	54.2±4.4	9.9±1.5	9.2±1.5	10.0±2.6	1.55<4.21<11.03	0.65<2.24< 7.50	0.08<1.05<12.19
X-rays	60	443	7*	1*	13.8±0.9			2.3±0.3			0.06<0.28< 1.25		
	233	430	141	29	34.4±1.5	46.0±3.0	49.4±6.6	6.4±0.6	9.4±1.6	4.8±1.7	0.96<1.93< 3.86	1.66<4.14<10.05	1.91<7.81<27.78
	327	92	69	13	40.4±3.3	45.6±3.0	45.4±8.9	6.5±1.4	11.7±2.7	4.2±2.8	1.78<4.74<12.10	1.57<5.23<16.33	5.10<18.91<53.33

† For segregation frequency recessive mutants f = 0.20; 90% confidence interval, z = 1.645.

* Items with number of siliques < 10 have been omitted.

had a lower frequency of embryonic lethals than plants with *normal* or *reduced* apical dominance after some irradiations. However, it can be seen from Table 2 that this effect was not consistent for all doses (not after 47 and 60 Gy fast neutrons for instance). Because a "saturation" of mutant frequency was also indicated in plants with *normal* and *reduced* apical dominance, it is concluded that the reduction in mutant frequency can at the most only partly be ascribed to the plants without apical dominance.

C. *The mutant frequency in main and lateral inflorescences*

In comparing the mutant frequency in the main inflorescence with that in the lateral inflorescence, the data of the following M_1-plants were not included:

1) those *without* apical dominance, since they have main inflorescences not distinguishable from the lateral one(s),
2) fasciated ones, because of their small number,
3) plants of which the data were obtained from either the main or the lateral inflorescence.

No significant differences were found between the frequencies of non-fertilized ovules, embryonic lethals and chlorophyll mutants in the main and in the lateral inflorescence (Table 3). The lateral inflorescence had the same, or perhaps slightly higher, mutant frequency as the main inflorescence after a given dose. It can be concluded therefore, that a (partial) replacement of the main inflorescences by lateral ones will not affect the mutant frequency.

The data indicate that in the irradiated seeds of the plants considered (i.e. without fasciation and with *normal* or *reduced* main inflorescence apical dominance), the cells which formed the sporophytic tissue of the lateral inflorescences had the same radiation-sensitivity as the ones which formed the sporophytic tissue of the main inflorescence.

Table 3. *Ovule sterility, embryonic lethality and chlorophyll mutant frequency in main and lateral inflorescences of Arabidopsis after treatment with fast neutrons (N_f) or X-rays.**

Irradiation		No. of siliques	ovule sterility (%)		embryonic lethals (%)		chlorophyll mutant freq. x 10^{-2}/cell†	
type	dose (Gy)		main infl.	lateral infl.	main infl.	lateral infl.	main infl.	lateral infl.
control	0	470	7.55±0.46	6.15±0.48	1.04±0.16	1.51±0.28	0.01< 0.11< 0.89	0.03< 0.16< 0.98
N_f	33	335	33.89±1.66	29.38±1.69	4.92±0.58	6.14±0.73	1.30< 2.56< 5.01	2.28< 3.99< 6.89
	47	308	51.46±1.88	55.19±1.95	7.92±0.88	7.53±0.96	3.70< 6.21<10.26	2.61< 4.84< 8.86
	60	259	58.74±2.15	61.02±2.08	9.40±1.17	10.30±1.36	1.21< 2.93< 6.99	2.06< 4.36< 9.05
X-rays	60	420	14.09±0.94	11.85±0+93	2.30±0.30	2.64±0.36	0.08< 0.34< 1.39	0.59< 1.29< 2.78
	233	516	37.17±1.41	36.10±1.48	7.05±0.64	6.77±0.64	1.17< 2.17< 4.00	1.41< 2.54< 4.52
	327	138	42.48±2.67	48.06±3.01	8.82±1.46	10.64±1.62	1.53< 3.83< 9.35	7.18<12.41<20.77

† For segregation frequency recessive mutants f = 0.20; 90% confidence interval, z = 1.645.

* The data are obtained from plants with *normal* apical dominance and *reduced* apical dominance (excluding fasciation).

D. *The mutant frequency in different M_1-fertility classes*

Having excluded the effects under B and C as possible causes for the "saturation", a comparison of the mutant frequency in different M_1-fertility classes was considered. M_1-fruits were divided into three fertility classes, namely 0-10, 11-20, and > 20 embryos per fruit half. The frequency of embryonic lethals and chlorophyll mutants were calculated for each fertility class. The data were analysed inclusive (Table 4a) and exclusive of lateral inflorescences (Table 4b). From Table 4 it can be seen that at a given dose the frequency of embryonic lethals decreased with increasing M_1-fertility. Thus, with fast neutron and X-ray exposures embryonic lethals are strongly correlated with the degree of M_1-sterility. Similar results were obtained by Müller (23) and van der Veen (30) for X-ray or EMS-treated *Arabidopsis* seeds. Müller and van der Veen concluded that this correlation indicated a common causal factor, i.e. maternal effects. Because no correlation between chlorophyll mutants and degree of M_1-sterility was found within-treatment, M_1-"initial" cell heterogeneity in mutagen sensitivity was excluded.

In the present experiment similar results were found for relatively low and high radiation doses. However, with moderate doses (47 Gy fast neutrons and 233 Gy X-rays) significant differences in chlorophyll mutant frequency between the M_1-fertility classes were found. The chlorophyll mutant frequency decreased with increasing M_1-fertility. Hence, there was a within-treatment dependence of mutant frequency and M_1-fertility class. This suggests that the M_1-progenies scored for these treatments originated from M_1-embryo cells which were heterogeneous in radiation-sensitivity. This was also observed when only M_1-progenies of the main inflorescence were scored (Table 4b).

Oftedal (24) has theoretically demonstrated that mutagenic and cell killing effects on cell populations with heterogeneous sensitivities result in a "humped" dose-response curve for mutant frequency, if there is coincidence of the sensitivities for both effects. Therefore, it is concluded that the "saturation" of mutant frequency in *Arabidopsis* at relatively high doses, may be attributed to a more frequent scoring of M_1-progenies originating from M_1-embryo cells which were less sensitive to irradiation.

Table 4a. *Mutant frequency in different M_1-fertility classes after treatment with fast neutrons (N_f) or X-rays.**
Fertility classes are: 0 to 10 embryos per ½ silique (0-10); 11 to 20 embryos per ½ silique (11-20); more than 20 embryos per ½ silique (> 20).

Irradiation		No. of siliques			No. of fertilized ovules per ½ silique			embryonic lethals (%)			chlorophyll mutant freq. x 10^{-2}/cell†		
type	dose (Gy)	0-10	11-20	> 20	0-10	11-20	> 20	0-10	11-20	> 20	0-10	11-20	> 20
control	0	41	54	877	4.2	16.8	28.9	16.8±4.1	6.5±2.1	1.0±0.1	0	0	0.03< 0.14< 0.60
N_f	33	310	241	337	5.0	15.2	26.4	9.9±1.1	8.2±0.9	4.3±0.5	1.68< 3.58< 7.54	2.00< 3.73< 6.86	1.77< 3.06< 5.25
	47	518	221	149	3.9	15.0	24.8	12.3±1.1	8.9±1.0	6.5±0.9	5.82< 8.87<13.37	4.83< 7.61<11.84	0.66< 1.21< 2.21
	60	555	165	94	3.4	14.9	24.7	16.9±1.3	11.5±1.4	5.2±1.0	3.32< 5.75< 9.85	0.52< 1.61< 4.87	0.93< 2.72< 7.73
X-rays	60	89	132	693	5.0	15.7	27.8	9.6±1.9	4.7±1.1	2.3±0.2	0.94< 3.71<13.88	0.02< 0.25< 3.11	0.38< 0.80< 1.66
	233	525	371	454	4.3	15.1	26.4	14.0±1.1	7.5±1.4	6.2±0.6	4.82< 7.51<11.57	1.50< 2.71< 4.85	1.14< 2.04< 3.63
	327	214	198	86	3.9	15.4	24.9	13.1±1.7	11.6±1.6	7.2±1.4	6.10<10.99<19.30	2.96< 6.12<12.33	3.14< 6.55<13.23

† For segregation frequency recessive mutants f = 0.20; 90% confidence interval, z = 1.645.

* All data obtained per treatment are combined, i.e. data obtained from the main and lateral inflorescences of plants with *normal* apical dominance and *reduced* apical dominance and the data obtained from plants *without* apical dominance (including fasciation).

Table 4b. *Mutant frequency in the main inflorescence in different M_1-fertility classes after treatment with fast neutrons (N_f) or X-rays.** *Fertility classes are: 0 to 10 embryos per ½ silique (0-10); 11 to 20 embryos per ½ silique (11-20); more than 20 embryos per ½ silique (> 20).*

Irradiation		No. of siliques			No. of fertilized ovules per ½ silique			embryonic lethals (%)			chlorophyll mutant freq. x 10^{-2}/cell†		
type	dose (Gy)	0-10	11-20	> 20	0-10	11-20	> 20	0-10	11-20	> 20	0-10	11-20	> 20
control	0	9	18	459	5.4	16.3	28.9	10.2±7.9	1.7±0.8	1.0±0.2	0.00	0.00	0.01< 0.11< 0.89
N_f	33	120	130	164	5.4	15.1	25.9	8.0±1.5	8.6±1.3	3.6±0.6	1.91< 5.00<12.65	1.90< 4.20< 9.06	1.03< 2.44< 5.69
	47	205	114	85	4.3	14.9	24.5	9.4±1.3	9.8±1.6	6.7±1.1	6.99<11.98<20.06	6.57<11.07<18.19	0.19< 1.03< 5.53
	60	227	73	52	3.7	14.6	24.9	16.8±2.1	12.4±2.2	3.6±1.0	2.09< 4.98<11.55	0.97< 3.21<10.14	0.28< 1.60< 8.66
X-rays	60	30	64	362	8.3	16.1	27.6	2.0±0.1	3.8±1.0	2.2±0.3	0.78< 4.08<19.49	0.00	0.07< 0.31< 1.41
	233	214	186	246	5.1	15.0	26.2	11.3±1.6	7.9±0.9	5.8±0.7	3.50< 6.78<12.88	1.82< 3.70< 7.39	0.60< 1.48< 3.64
	327	88	51	52	4.8	15.2	25.0	9.7±2.1	11.1±2.1	6.5±1.8	7.95<15.63<29.24	0.00	0.74< 2.88<10.69

† For segregation frequency recessive mutants f = 0.20; 90% confidence interval, z = 1.645.

* Data obtained from the main inflorescence of plants with *normal* apical dominance and with *reduced* apical dominance (including fasciation).

GENERAL DISCUSSION AND CONCLUSION

No significant difference in chlorophyll mutant frequency among the different fertility classes was found, neither at relatively low doses (33 Gy fast neutrons and 60 Gy X-rays) nor at relatively high doses (60 Gy fast neutrons and 327 Gy X-rays). Therefore, the M_1-"initial" cell heterogeneity suggested by the data from Table 4 (at 47 Gy fast neutrons and 233 Gy X-rays) cannot be due to M_1-seed heterogeneity since, in that case, a correlation between chlorophyll mutant frequency and M_1-fertility should occur with *all* doses, at least if there is coincidence of the sensitivities for mutant induction and induction of sterility. This was however not observed. For instance at 33 Gy fast neutrons the mutant frequencies were the same for all M_1-fertility classes (Table 4a). A significant difference was observed in the transition area of the applied dose range (at 47 Gy fast neutrons and 233 Gy X-rays). Therefore, the data indicate that heterogeneity in radiation-sensitivity of the M_1-"initial" cells is dependent on dose. This was also found for the "initial" cells of "pre-formed" barley tillers after X-ray and thermal neutron treatment (31) and for the "initial" cells of the "main" shoots of tomato after fast neutron irradiation (7).

The effect of dose upon the heterogeneity of the M_1-"initial" cell population can be explained by differential effects of various irradiations on the development of the shoot apex. It is postulated that in *Arabidopsis* an M_1-"initial" cell population with homogeneous sensitivity is found after specific mutagenic treatments. Firstly, for treatments that do not influence the stability of the apices, i.e. where no replacement of the few stable initial cells in the shoot apex occurs (for instance at 33 Gy fast neutrons and 60 Gy X-rays in the present experiment). Secondly, for treatments that severely damage the shoot apices, i.e. replacement occurs by cells that are homogeneously less sensitive (for instance at 60 Gy fast neutrons and 327 Gy X-rays). The M_1-cell heterogeneity in radiation-sensitivity - e.g. due to differences in mitotic activity between and within cell layers in the embryo (13, 16) - is then expected to be found after mutagenic treatments where partial replacement of the original initials

occurs. This hypothesis agrees with our experimental results and explains the "contrasting" results between our experimental data and the data obtained by Müller (23) and van der Veen (30).

Müller (23) found a within-treatment independence of chlorophyll mutant frequency and fertility class after 200 and 250 Gy X-irradiation of presoaked (18 hr) *Arabidopsis* seeds. These irradiations induced 52 and 56 per cent M_1-sterility, respectively. At these high doses the shoot apices may have been severely damaged and, therefore, the original initials could have been replaced by cells that were homogeneous but less sensitive. Van der Veen (30) and Müller (23) observed within-treatment independence of chlorophyll mutant frequency and fertility class after EMS treatment of *Arabidopsis* seeds. The EMS treatments applied [8.3 mM EMS, 24 hr at 24 °C and 20 mM EMS, 18 hr at 22 °C, respectively] may not have influenced the stability of the shoot apices.

In conclusion, the "saturation" in mutant frequency at high doses is explained by M_1-"initial" cell heterogeneity in "overall" mutagen sensitivity. It is suggested that this heterogeneity is induced by random cell killing of the initial cells with higher doses.

An M_1-"initial" cell heterogeneity is also indicated in plants with *normal* apical dominance. Hence, it is concluded that the meristematic cell replacement in the shoot does not necessarily manifest itself in the morphology of the plant, i.e. does not only occur in the morphologically conspicuous plants, the plants *without* apical dominance.

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APPENDIX

Discrete multivariate analysis of the distribution of M_1-plants over the different development categories.

The distribution of M_1-plants over the different development categories after the irradiation is given in Table *I*. It can be seen from the table that the data form an incomplete multidimensional contingency table. Firstly, the dead plants could not be classified as plants with or without fasciation, and secondly, the dose of 327 Gy X-rays was only applied in the second replication.

Because the main points were to test whether or not the distribution of the surviving plants was associated with the irradiation and whether or not there was a replication effect, the data were divided into two sub-tables (-- in Table 1). Each sub-table formed a complete multidimensional contingency table. The variables which may have affected the distribution of M_1-plants over the distinguished classes are given in Table 5. To test whether these variables were associated, a test for independence (G-test) was applied by discrete multivariate analysis of the data in each sub-table. A computer program was used for the calculations and an attempt was made to find the best log-linear model to fit the data (4, 8).

The variables u_1 (irradiation) and u_4 (replication) are explanatory variables, which classify in this case the plant, according to the experimental conditions. Therefore, the log-linear model describing the data of sub-table *I* should include the variables u_1 and u_4 and their interaction u_{14} (8). The variables u_2 (plant development) and u_3 (fasciation) are response variables, which describe what happens to the plant during the experiment (3, 8).

To cover the range of all possible models, the fitting of models with terms of uniform order was tested in the initial computations. The goodness of fit (G-test) of these models is given in Table 6. It can be seen from this table that for the data of sub-table *I*, a log-linear model including three-factor-interactions gave a well-fitting description ($G^2 = 2.940$; d.f. $10 \rightarrow p > 0.975$), but a log-linear model including only main effects and two-factor-interactions did not give a fitting description ($G^2 = 49.215$; d.f. $37 \rightarrow p < 0.25$).

Table 5. *The variables which may have affected the observed distribution of M_1-plants into the distinguished classes.**

sub-table	variable	category types	No. of categories
I	u_1 - irradiation	control; 33, 47 and 60 Gy fast neutrons; 60 and 233 Gy X-rays	6
	u_2 - plant development	*normal* apical dominance ; *reduced* apical dominance; *without* apical dominance	3
	u_3 - fasciation	no fasciation (-); fasciation (+)	2
	u_4 - replication	replication 1 ; replication 2	2
II	u_1 - irradiation	230 and 327 Gy X-rays	2
	u_2 - plant development	*normal* apical dominance ; *reduced* apical dominance; *without* apical dominance	3
	u_3 - fasciation	no fasciation ; fasciation	2

* see Table 1.

The next step was confined to the "intervening models", i.e. models with all first and second order terms and one or more third order terms. The best-fitting "intervening model" found for sub-table *I* was a model including the three-factor-interaction u_{124} (model 2.1 in Table 7). The effects of the parameters in the models were then measured as the difference between the goodness of fit of the models that provided a well-fitting description and the models that did not include the parameter(s) (4). From Table 7 it is apparent that of the three-factor-interactions, the interaction U_{124} (treatment x development class x replication) had the largest effect, and that u_{13} (treatment x fasciation) and u_{23} (development class x fasciation) were the most important ones among the two-factor-interactions.

The data of sub-table *II*, were well described by a log-linear model including all two-factor-interactions (Table 6). From Table 7 it can be seen that the two-factor-interactions u_{12} (treatment x development class) and u_{13} (treatment x fasciation) were the most important ones.

Table 8 gives the expected cell frequencies and the Freeman-Tukey deviates of the data in Table 1 according to the models that provided a well-fitting description (i.e. *I* 2.1 and *II* 3.1 in Table 7).

Table 6. *Models with terms of uniform order for data in Table 1.*

sub-table	model	G^2 a)	d.f.	Test of:
I	$u + u_1 + u_2 + u_3 + u_4 + u_{14}$	986.138**	57	independence
	$u + u_1 + u_2 + u_3 + u_4 + u_{14} + u_{12} + u_{13} + u_{23} + u_{24} + u_{34}$	49.215*	37	two-factor-interaction
	$u + u_1 + u_2 + u_3 + u_4 + u_{14} + u_{12} + u_{13} + u_{23} + u_{24} + u_{34} + u_{123} + u_{124} + u_{134} + u_{234}$	2.940	10	three-factor-interaction
II	$u + u_1 + u_2 + u_3$	42.685**	7	independence
	$u + u_1 + u_2 + u_3 + u_{12} + u_{13} + u_{23}$	0.000	2	two-factor-interaction

a) significant value for G^2 implies that the model does not fit the data; ** significant for $p < 0.005$; * significant for $p < 0.25$.

Table 7. *The effect in Table 1 of the different parameters, measured as differences between goodness of fit of models 1.1°, 2.1† and 3.1°, respectively, and the models not including parameter(s) as indicated.*

sub-table	code model j.i	model	G^2_{j1} a)	d.f.	parameter	$G^2_{ji}-G^2_{j1}$ b)	d.f.
I	1.1°	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34} + u_{123} + u_{124} + u_{134} + u_{234}$	2.940	10			
	1.2	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34} + \quad + u_{124} + u_{134} + u_{234}$	9.752	20	u_{123}	6.812	10
	1.3	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34} + u_{123} + \quad + u_{134} + u_{234}$	43.022*	20	u_{124}	40.082*	10
	1.4	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34} + u_{123} + u_{124} + \quad + u_{234}$	10.078	15	u_{134}	7.138	5
	1.5	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34} + u_{123} + u_{124} + u_{134}$	8.600	12	u_{234}	5.660	2
II	2.1†	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34} + \quad + u_{124}$	16.785	27			
	2.2	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + \quad + \quad + u_{124}$	23.683	28	u_{34}	6.898*	1
	2.3	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + \quad + u_{24} + u_{34} + \quad + u_{124}$	85.008*	29	u_{23}	68.223*	2
	2.4	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + \quad + u_{14} + u_{23} + u_{24} + u_{34} + \quad + u_{124}$	133.257*	32	u_{13}	116.472*	5
	2.5	$u + u_1 + u_2 + u_3 + u_4 + u_{12} + u_{13} + u_{14} + u_{23} + u_{24} + u_{34}$	49.215	37	u_{124}	32.430*	10
III	3.1°	$u + u_1 + u_2 + u_3 + \quad + u_{12} + u_{13} + \quad + u_{23}$	0.000	2			
	3.2	$u + u_1 + u_2 + u_3 + \quad + u_{12} + u_{13}$	10.623*	4	u_{23}	10.623*	2
	3.3	$u + u_1 + u_2 + u_3 + \quad + u_{12} + \quad + u_{23}$	17.949*	3	u_{13}	17.949*	1
	3.4	$u + u_1 + u_2 + u_3 + \quad + u_{13} + \quad + u_{23}$	31.500*	4	u_{12}	31.500*	2

o models with terms of uniform order that give a fitting description for resp. sub-table *I* and sub-table *II*.

+ intervening model that gives a fitting description for sub-table *I*.

a) significant value of G^2_{ji} implies that the model does not fit the data.

b) significant value of $G^2_{ji}-G^2_{j1}$ implies that the parameter tested has a significant effect.

* significant for $p < 0.05$.

Table 8. *Estimations of the expected "cell" frequencies of Table 1 for model I.2.1 and II.3.1 (see Table 7) and their Freeman-Tukey deviate.*

Irradiation		Repli-	Estimated "cell" frequency per development class					
			normal a.d.[a]		reduced a.d.[a]		without a.d.[a]	
type	dose (Gy)	cation	-	+	-	+	-	+
control	0	1	240.0+0.02	0.0	1.0+0.18	0.0	0.0	0.0
		2	241.0+0.02	0.0	4.0+0.11	0.0	0.0	0.0
N_f[b]	33	1	175.5+0.43	33.5-0.94	19.0+0.06	0.0	6.7-0.18	0.3+0.92
		2	79.6-0.72	22.4+1.34	78.2+0.23	4.8-0.75	23.0+0.05	0.0
N_f	47	1	99.1-0.39	23.9+0.85	77.9+0.37	4.1-1.74	17.0+0.06	1.0+0.19
		2	64.9+0.41	23.1-0.61	103.1-0.18	7.9+0.75	22.0+0.05	0.0
N_f	60	1	91.8-0.38	14.2+1.01	69.7+0.19	2.3-0.80	22.0+0.05	0.0
		2	50.5+0.52	11.5-1.03	106.7-0.14	5.3+0.78	31.3+0.17	1.7-0.39
X-rays	60	1	231.5+0.05	2.6-0.20	2.0+0.15	0.0	0.0	0.0
		2	211.6-0.02	3.5+0.39	5.0+0.10	0.0	1.0+0.18	0.0
X-rays	233	1	149.7-0.04	28.3+0.18	48.0-0.11	2.0+5.76	4.0+0.11	0.0
		2	107.9+0.32	30.1-0.53	60.4-0.14	3.7+0.74	16.0+0.06	0.0
X-rays	230	1	170.0+0.02	10.0+0.08	35.0+0.04	0.0	9.0+0.08	0.0
	327	2	92.0+0.03	26.0+0.05	69.0+0.03	4.0+0.11	13.0+0.07	0.0

a) a.d. = apical dominance of the main inflorescence; - indicates no fasciation
+ indicates fasciation of the "main" inflorescence.

b) N_f = fast neutrons.

----- partitioning of the table; above ----- is sub-table *I*, below ----- is sub-table *II*.

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CHAPTER II

THE EFFECT OF DITHIOTHREITOL ON RADIATION-INDUCED GENETIC DAMAGE IN *ARABIDOPSIS THALIANA* (L.) HEYNH.*

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SUMMARY

A study was made on the effect of dithiothreitol (DTT; present during irradiation) on M_1-ovule sterility, M_2-embryonic lethals, M_2-chlorophyll mutants and M_2-viable mutants induced with fast neutrons or X-rays in *Arabidopsis thaliana*. It was found that DTT provides considerable protection against both fast neutron and X-ray-induced genetic damage. However, a higher protection was observed against M_1-ovule sterility, than against embryonic lethals, chlorophylls and viable mutants. This implies a significant DTT-induced spectrum shift ($0.01<p<0.05$), i.e. a shift in the relative frequencies of the different genetic parameters. This spectrum shift is explained on the basis of a specific DTT protection against radiation-induced strand-breaks, and by differences in the ratio strand-breaks/base damage for the genetic parameters concerned i.e. a higher ratio for ovule sterility than for the other parameters.

The induction of the genetic damage by ionizing radiation, either with or without DTT, is described by a mathematical model, which includes both strand-breaks and base damage. The model shows that the resolving power of a test for a "mutation" spectrum shift depends on the relative values of the strand-break reduction factor of -SH compounds and on the ratio strand-breaks/base damage of the genetic parameters. For each genetic parameter the DTT damage reduction factor (DRF) is calculated per irradiation dose, and in addition the average (over all doses) ratio strand-breaks/base damage.

INTRODUCTION

Since the radioprotective action of cysteine was first observed [26], many other sulphydryl compounds have been reported to act as radioprotectors when present during X- and γ-[2, 3, 16] or fast neutron irradiation [2, 6, 33]. One of the most effective SH-protector reported is dithiothreitol (DTT, Cleland's reagent) [4, 10, 13, 28, 29, 35, 41].

It has been suggested [5, 11, 41] that -SH compounds primarily protect by increasing the concentration of radical-reducing species (i.e. hydrogen donating compounds), resulting in radical scavenging and in an enhanced repair of free-radical damage in the targets. This hypothesis is supported by the data of (1) Chapman et al. [11], describing the radioprotection by cysteamine of Chinese hamster cell inactivation and sensitizer binding to DNA in vitro, (2) van der Veen and Sree Ramulu (pers. comm.), who studied the radioprotection by dithiothreitol of genetic damage in *Arabidopsis* in relation to the oxygen concentration, and (3) of others who described the radioprotection of bacteria by -SH compounds [17, 30].

A few studies on sulphydryl protection against radiation-induced damage show that the extent of the protective effect is, amongst others, dependent on the endpoint studied. Malvarez et al. [19] concluded for barley that cysteamine protects seeds against X-rays by improving M_1-seedling growth and adult survival, and reducing the frequency of chromosomal aberrations, without changing the frequency of chlorophyll mutations. Van der Veen et al. [39] found for *Arabidopsis* that DTT, present during X-irradiation, gives considerable protection against induced M_1-lethality. The protective effect for induced M_1-ovule sterility and M_2-embryonic lethality was less. These studies suggest that -SH compounds preferentially protect against chromosome breaks, at least when one assumes that M_1-lethality is to a greater extent caused by gross chromosomal aberrations, and M_2-mutants (embryonic lethals and chlorophylls) to a greater extent by gene mutations.

Thus, a spectrum shift towards a higher ratio gene mutations/chromosome breaks might be obtained when -SH compounds are present during irradiation [6, 39]. Recently, the same hypothesis was formulated by van der Schans et al. [31], based on studies on sulphydryl protection against radiation-induced damage on the molecular level under anoxic conditions. De Jong et al. [18] found that γ-irradiation in the presence of -SH compounds enhances the ratio of the number of biological hits (single strand-breaks + nucleotide damage) to the number of single-strand-breaks from approximately 2.5 to 16 when cysteamine was added to a solution of single-stranded DNA of the bacteriophage IX 174 before γ-irradiation.

Van der Schans et al. [31] found for Chinese hamster ovary cells that cysteamine protects against γ-ray induced single strand-breaks (and alkali-labile sites), whereas it did not significantly affect the number of endonuclease-susceptible sites. If the number of induced endonuclease-susceptible sites corresponds to the number of nucleotide impairments which gives rise to gene mutants, then the number of radiation-induced gene mutations will not be affected by -SH compounds. The result then, is a spectrum shift towards an increased mutant frequency at a given level of survival after irradiation in the presence of -SH compounds [31].

Since in higher plants (barley and *Arabidopsis*) irradiation in the presence of -SH compounds resulted in a spectrum shift in favour of more "mutants" (M_1-ovule sterility, M_2-embryonic lethals and M_2-chlorophyll mutants) at a given level of survival [19, 39], a similar effect of -SH compounds at the molecular level can be expected in higher plant cells, i.e. preferential protection against strand-breaks. Studies on the effect of -SH compounds, present during irradiation, on the ratio between different genetic parameters, such as M_1-ovule sterility, M_2-embryonic lethals, M_2-chlorophyll mutants and M_2-viable mutants, may then increase our knowledge concerning the kind of radiation-induced damage responsible

for these genetic changes ("mutants"). Only when the ratio strand--breaks/nucleotide damage for these genetic parameters is different, can it be expected that irradiation in the presence of -SH compounds will alter the ratio between these genetic parameters, e.g. at a given level of ovule sterility more embryonic lethals, chlorophylls and viable mutants.

The following comparison between the effect of DTT on M_1-ovule sterility, M_2-embryonic lethals, M_2-chlorophyll mutants and M_2-viable mutants in *Arabidopsis* induced by X-rays or fast neutrons, is presented to extend the results concerning sulphydryl protection against induced mutations, and to obtain more information about the effect of DTT on the ratio between different genetic parameters. This study may also reveal differences in the mode of action of X-rays and fast neutrons, since differences in the protective effect for a given genetic parameter might reflect a different action at the molecular level, i.e. differences in the ratio of the number of biological hits to the number of strand-breaks for this parameter. A theoretical model, based on sulphydryl protection against strand-breaks, is constructed in order to see how sensitive a test for an induced shift in mutation spectrum by -SH compounds is for a given difference in the ratio strand-breaks/nucleotide damage between two genetic parameters.

MATERIALS AND METHODS

Plant material

Seed stocks used in the experiments were of the mutant *erecta* of the ecotype "Landsberg" [27]. In experiment I a control line was used (C = MsMs), while in experiment II seeds of a male sterile line were used (maintained as msms cross-pollinated with Msms → msms + Msms).

Dithiothreitol treatment

Van der Veen et al. [39] and Sree Ramulu and van der Veen [33] tested a series of DTT concentrations (0 to 2.4%) and pre-treatment durations (0.5 to 4 h) in *Arabidopsis*. Based on their results 1.2 per cent (= 78 mM) given 3 h before irradiation was selected as the optimum concentration and duration for DTT treatment. Notably higher concentrations reduced the germination speed, the final germination percentage and the seedling survival, as a result of the toxic effect of DTT.

Radiation treatment

In order to break dormancy, seeds were kept on moist filter paper at 2 °C for 5 days, and re-dried (24 °C, 24 h). The re-dried seeds were then submerged in tap water or DTT-solution (1.2%), at 22 °C, 3 h before irradiation.

X-irradiation was carried out with an MG 301 X-ray machine with an MCN-420 tube, operating at 320 kVp and 10 mA, with an additional filter of 0.25 Cu and 1.0 Al and with a dose rate of 4 Gy/min. (= 400 rad/min). Fast neutrons were given in the irradiation room of the BARN (Biological Agricultural Reactor Netherlands, Wageningen) with a dose rate of 1 Gy/min. The γ-contamination was only approximately 3 per cent on Gy basis.

Preliminary studies indicated that under the conditions used fast neutrons were 7 times more effective than X-rays in inducing M_1-ovule

sterility in *Arabidopsis* (Dellaert, unpublished). Therefore, to obtain comparable levels of induced genetic damage, the doses of X-rays applied to seeds submerged in tap water (140, 233, 327 and 420 Gy) were 7 times the dose of fast neutrons (20, 33, 47 and 60 Gy) applied, in experiment I. In the same experiment the doses applied to seeds submerged in 1.2 per cent DTT were twice the dose applied to seeds submerged in tap water (i.e. 280, 467, 653, 840 Gy X-rays and 40, 67, 93, 120 Gy fast neutrons), because for DTT a dose reduction factor of approximately 2 has been found for the induction of M_1-ovule sterility in *Arabidopsis* [33, 39]. The maximum duration of irradiation was 3.5 h.

To standardize the effect of environmental conditions, the seeds for all irradiations were kept submerged for 6.5 h at 22 °C, and subsequently rinsed with tap water (5 min.) and sown. In total 18 different treatments (including the controls; 0% and 1.2% DTT at 0 Gy) were given in experiment I (Table I). In experiment II, different fast neutron doses, such as 0, 20, 40 and 60 Gy were applied to seeds submerged in tap water, likewise 0, 33, 67 and 100 Gy were given to seeds submerged in 1.2 per cent DTT. Other treatments were as in experiment I.

Culture medium and culture conditions

The seeds were sown (equally spaced) in portions of 30 in a petri dish on a standard mineral medium and put to germinate at 24 °C under continuous illumination by fluorescent light tubes, 8000 lux/cm^2. After 8 days the seedlings were transplanted to soil in an air-conditioned greenhouse. The culture medium and culture conditions used were as described by Feenstra [14], and Oostindiër-Braaksma and Feenstra [25].

Scoring for genetic damage

For scoring the genetic parameters M_1-ovule sterility, M_2-embryonic lethals and M_2-chlorophyll mutants in experiment I, Müller's embryo test [21]

was applied to M_1-silique number 5 or 6 of the main inflorescence. For ease of handling, scoring in a silique was done at one side of the septum. Viable mutants were scored in the M_2-generation by testing the progeny of one silique per M_1-plant from the top of the main inflorescence (to avoid chimerism). In the M_1 only well filled siliques (indicating good fertility) were harvested, in order to (1) increase the germination frequency of the M_2-seeds, (2) increase the fertility in the M_2-generation, and (3) decrease the number of deviant M_2-plants caused by chromosomal aberrations [20, 22]. There might be an induced shift in the ratio M_1-ovule sterility/M_2 viable mutants by selection for fertility in the M_1-generation, since within-dose a correlation between M_1-ovule fertility and M_2-mutant frequency was found [12]. However, this correlation is relatively small and only occurred at intermediate dose levels [12]. Viable mutants in the M_2-generation were defined as flowering plants showing deviations from wild type in plant morphology or leaf colour. Thus, pre-flowering lethal mutants were not included.

In experiment II, male sterile plants (msms) were selected at flowering time. The flower numbers 5 to 10 of the main inflorescence were crossed with the non-irradiated control (C). Müller's embryo test was applied to M_1-silique number 9 or 10 of the main inflorescence, both from msms plants crossed with C and Msms self-pollinated plants. The siliques 5 to 8 from the msms x C plants, were used for the indication of the right time to apply Müller's test to silique number 9 or 10. Within a given treatment, the percentage of embryonic lethals in msms crossed with C (i.e. non-recessive lethals) was compared with the percentage of embryonic lethals in Msms self-pollinated (i.e. non-recessive and recessive lethals). M_1-ovule sterility in the two groups of plants was compared to obtain (1) a measure for mechanical damage in crossing within the control (0 Gy, 0% and 1.2% DTT), and (2) a measure for the availability and quality of Msms-

pollen (for relatively high radiation doses).

Calculations

The frequency of non-fertilized ovules and embryonic lethals is expressed as a percentage of the total number of ovules and as a percentage of the total number of fertilized ovules, respectively. Snedecor's formula [12, 34] was used to calculate the standard error. The chlorophyll mutant frequency (embryo test) and the viable mutant frequency is expressed as the mutation frequency per cell [15]; i.e.

$m = \frac{m'}{f}$, where

m = the frequency of mutated initial cells,

m' = the number of embryonic chlorophyll mutants per total number of non-lethal embryos, or the number of viable M_2-mutants per total number of (flowering) M_2-plants,

f = the segregation frequency of recessive mutants, i.e. the probability that a plant (or embryo) descending from a heterozygous flower is a homozygous mutant.

For chlorophylls and viable mutants a realistic value for f, f= 0.20, was used (assuming non chimeric fruits). This makes it possible to calculate a 90 per cent confidence interval for the mutation frequency per cell using Stam's formula [12].

RESULTS

A. *The effect of DTT on radiation induced M_1-ovule sterility, M_2-embryonic lethals, M_2-chlorophyll mutants and M_2-viable mutants.*

The results of experiment I are given in Table 1. It should be noted that in this experiment the effects of the fast neutron dose range and the X-ray dose range are approximately the same. Thus, in *Arabidopsis* and under the treatment conditions used, fast neutrons on a Gy basis are

Table 1. THE EFFECT OF IRRADIATION (X-RAYS OR FAST NEUTRONS), IN THE PRESENCE AND IN THE ABSENCE OF DITHIOTHREITOL (DTT), ON THE FREQUENCY OF M_1-OVULE STERILITY, EMBRYONIC LETHALS, CHLOROPHYLL MUTANTS AND VIABLE MUTANTS IN *ARABIDOPSIS THALIANA*.

Irradiation type	dose (Gy)	DTT %	† n_1	No ovules per ½ silique	ovule sterility %	embryonic lethals %	chlorophyll mutant frequency $x10^{-2}$/cell*	† n_2	viable mutant frequency $x10^{-2}$/cell*	segregation frequency of viable mutants
Control	0	0	499	31.6 ± 4.7	3.9 ± 0.2	0.7 ± 0.1	0.00 <0.63	649	2.9 < 4.6 < 7.2	0.081 ± 0.025
	0	1.2	442	31.1 ± 4.4	4.4 ± 0.3	1.0 ± 0.1	0.00 <0.71	546	1.4 < 3.7 < 6.4	0.088 ± 0.024
X-rays	140	0	246	28.5 ± 5.5	23.0 ± 1.6	5.5 ± 0.7	2.6 < 4.5 < 7.8	398	20.7 <26.0 <32.4	0.193 ± 0.013
	233	0	245	26.9 ± 5.5	35.6 ± 2.0	7.3 ± 0.9	5.1 < 8.1 <12.7	285	25.1 <32.1 <40.4	0.169 ± 0.018
	327	0	249	25.6 ± 5.3	42.8 ± 2.3	8.9 ± 1.1	2.5 < 4.7 < 8.7	339	31.4 <38.2 <46.0	0.195 ± 0.015
	420	0	247	23.8 ± 4.9	57.9 ± 2.3	13.2 ± 1.5	7.3 <11.6 <18.0	155	37.8 <49.5 <62.9	0.187 ± 0.024
	280	1.2	248	28.2 ± 5.0	17.7 ± 1.4	5.8 ± 0.8	2.0 < 3.7 < 6.7	389	15.2 <20.0 <25.9	0.167 ± 0.017
	467	1.2	247	25.9 ± 4.8	41.8 ± 2.1	11.0 ± 1.1	4.5 < 7.4 <11.9	341	29.3 <36.0 <43.7	0.190 ± 0.015
	653	1.2	246	23.7 ± 4.5	56.7 ± 2.2	15.0 ± 1.4	10.6 <15.6 <22.5	190	46.1 <56.7 <68.1	0.185 ± 0.017
	840	1.2	173	22.3 ± 5.3	70.9 ± 2.1	17.3 ± 2.6	7.9 <14.6 <25.5	41	41.8 <64.9 <91.2	0.160 ± 0.044
fast neutrons	20	0	252	29.1 ± 4.7	15.6 ± 1.4	3.3 ± 0.5	2.6 < 4.4 < 7.6	389	14.1 <18.6 <24.4	0.184 ± 0.018
	33	0	248	26.4 ± 5.5	31.9 ± 1.9	6.3 ± 0.8	3.2 < 5.6 < 9.4	340	19.7 <25.4 <32.4	0.166 ± 0.018
	47	0	250	26.6 ± 4.8	39.5 ± 2.1	7.8 ± 1.8	3.0 < 5.3 < 9.2	339	23.4 <29.5 <36.6	0.162 ± 0.015
	60	0	245	25.6 ± 5.4	56.6 ± 2.2	12.8 ± 1.5	2.7 < 5.3 <10.2	307	42.4 <50.4 <59.1	0.214 ± 0.015
	40	1.2	248	27.3 ± 5.7	21.5 ± 1.7	5.8 ± 0.7	3.1 < 5.3 < 8.8	344	20.8 <26.5 <33.3	0.180 ± 0.017
	67	1.2	248	25.6 ± 5.6	42.4 ± 2.1	11.1 ± 1.1	8.9 <13.0 <18.6	326	33.1 <40.3 <48.5	0.165 ± 0.016
	93	1.2	238	22.0 ± 5.8	67.9 ± 2.1	16.7 ± 1.9	8.2 <13.6 <21.9	161	45.9 <57.6 <70.4	0.198 ± 0.022
	120	1.2	209	22.1 ± 5.2	76.7 ± 2.2	22.4 ± 2.9	4.3 < 9.6 <20.2	99	51.4 <65.8 <80.7	0.218 ± 0.022

† n_1 = No of siliques scored with Müller's embryo test; n_2 = No of M_2-lines, i.e. M_1-progenies, scored for viable mutants.

* For segregation frequency of recessive mutants $f = 0.20$; 90% confidence interval, $z = 1.645$.

roughly 7 times more effective than X-rays in the induction of ovule sterility, embryonic lethals, chlorophyll mutants and viable mutants. This agrees with the results of preliminary experiments (Dellaert, unpublished), Furthermore, it can be concluded from Table 1 that DTT provides considerable protection against both fast neutron and X-ray - induced genetic damage. However, we will see that the extent of the protective effect depends on the irradiation as well as on the genetic parameter studied. Finally, it is observed that there is a general tendency for a decrease in the number of ovules per silique with increasing dose (Table 1).

In Fig. 1 the percentage M_1-ovule sterility has been plotted as a function of fast neutron dose (Fig. 1A) and X-ray dose (Fig. 1B), either with or without 1.2 per cent DTT. It is calculated (by interpolation) that DTT gives a damage reduction factor (DRF) of about 2 at relatively low doses, which agrees with the results obtained by van der Veen et al. [39] and Sree Ramulu and van der Veen [33]. At relatively high doses (60 Gy fast neutrons and 420 Gy X-rays) the damage reduction factor for the induction of ovule sterility is about 1.6 (Table 4; DRF_1-values). Thus, there is a tendency for a decrease in the protective effect of DTT with increasing dose.

To see whether DTT has an effect on the spectrum of the different genetic parameters, the percentage of embryonic lethals (Fig. 2), chlorophyll mutant frequency per cell (Fig. 3) and viable mutant frequency per cell (Fig. 4) are plotted against the induced percentage of M_1-ovule sterility, for treatments with or without DTT. It appears that there is a tendency towards more embryonic lethals (Fig. 2) and chlorophyll mutants (Fig. 3) at a given level of M_1-ovule sterility in the presence of DTT.

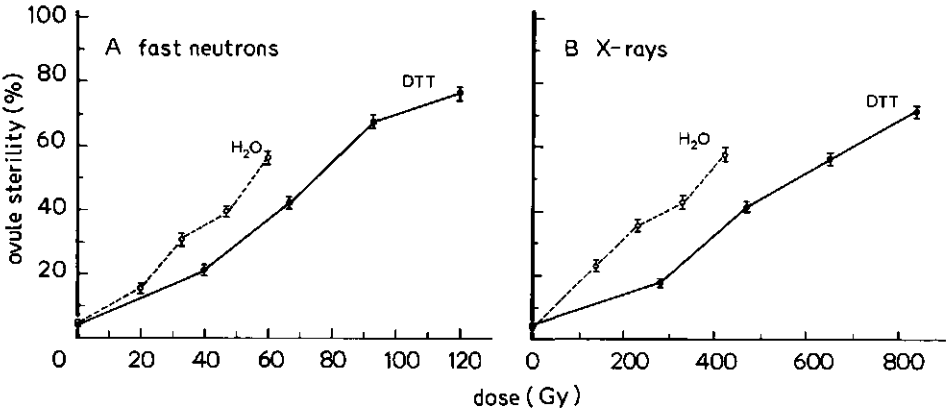

Fig. 1 The effect of fast neutron and X-irradiation of seeds, either with or without dithiothreitol (DTT), on the percentage of M_1-ovule sterility in *Arabidopsis*.

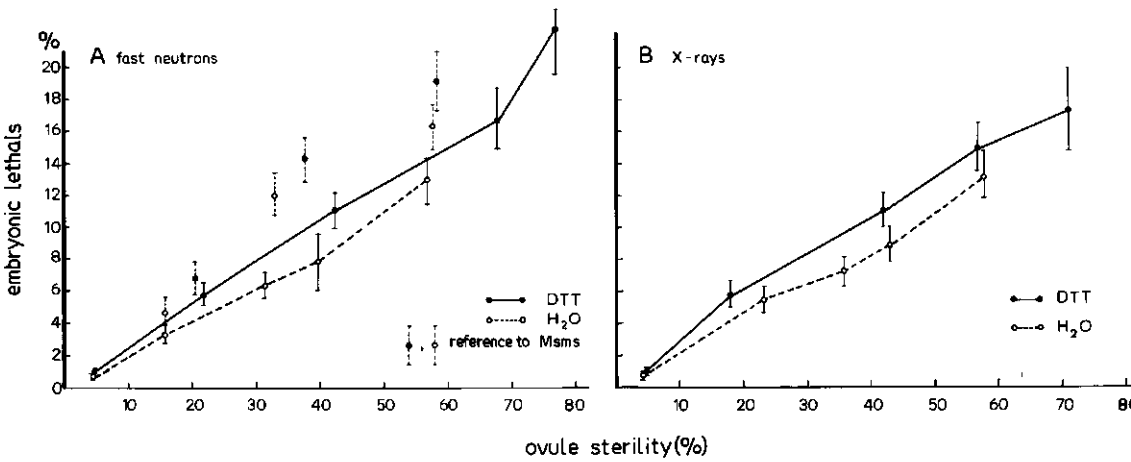

Fig. 2 The ratio embryonic lethals/ovule sterility, induced in *Arabidopsis* with fast neutrons and X-rays, either with or without dithiothreitol (DTT).

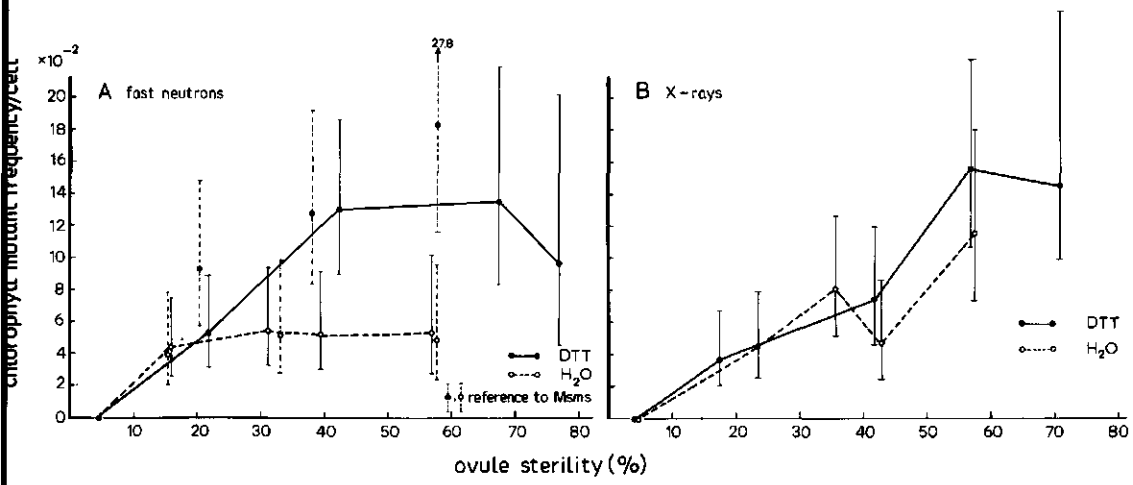

Fig. 3 The ratio chlorophyll mutants/ovule sterility, induced in *Arabidopsis* with fast neutrons and X-rays, either with or without dithiothreitol (DTT).

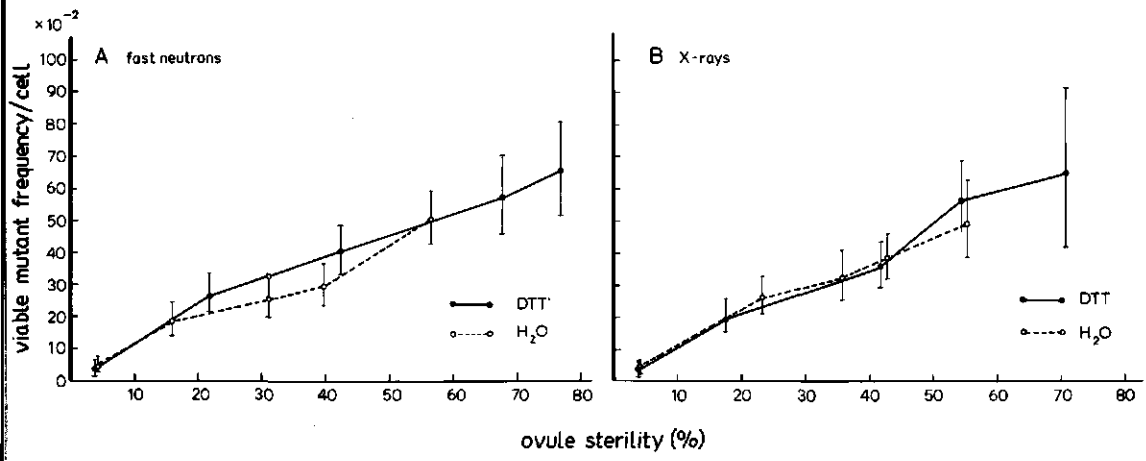

Fig. 4 The ratio viable mutants/ovule sterility, induced in *Arabidopsis* with fast neutrons and X-rays, either with or without dithiothreitol (DTT).

A rank-test [40] shows that for the data of experiment I, there is a significant increase ($p<0.01$) in embryonic lethals, but the increase in chlorophylls is not significant. If in addition the data obtained in experiment II (Table 2) are taken into consideration, then a significant increase is found with DTT ($0.01<p<0.05$), not only in embryonic lethals but also in chlorophyll mutants at a given level of M_1-ovule sterility (Table 3). For the frequency of viable mutants no significant increase is found. However, the ovule sterility is scored in silique no 5 or 6 of the M_1-main inflorescence, while the viable mutants are scored in M_2-progenies of "well-filled" siliques from the top of the M_1-main inflorescence. Therefore, the ratio viable mutants/ovule sterility might be influenced by the effect of selection on fertility in the M_1-generation. This of course is not the case for the ratio's embryonic lethals/ovule sterility and chlorophyll mutants/ovule sterility since these parameters are all scored in the same M_2-population. Because the possible effect of selection on fertility in the M_1-generation on the frequency of viable mutants in the M_2, and because no significant effect of DTT was found on the relative frequency of these mutants, the viable mutants are not longer taken into consideration.

The DTT induced "spectrum shift" can be seen directly in tables 1 and 2, when the genetic effects at the approximately equal fast neutron doses of 60 Gy (0% DTT) and 67 Gy (1.2% DTT) are compared. Without DTT the induced percentage of M_1-ovule sterility is significantly higher, the percentage of embryonic lethals is equal, and the chlorophyll mutant frequency is lower than with 1.2 per cent DTT.

The DTT induced "spectrum shift" suggests that the ratio strand-breaks/nucleotide damage is less for chlorophyll mutants and embryonic lethals than for ovule sterility (cf. introduction). Therefore, the data support the

Table 2. COMPARISON OF FAST NEUTRON INDUCED GENETIC DAMAGE, MEASURED AFTER NATURAL SELFING (Msms SELF-POLLINATED PLANTS) AND AFTER POLLINATION WITH POLLEN OF THE UNTREATED CONTROL (msms x C), AFTER IRRADIATION OF *ARABIDOPSIS* SEEDS IN THE PRESENCE OR IN THE ABSENCE OF 1.2 PER CENT DTT.

Irradiation		DTT	Number of siliques		% non-fertilized ovules		% embryonic lethals		chlorophyll mutant frequency x 10^{-2}/cell*	
type	Dose (Gy)	%	Msms	msms x C	Msms	msms x C	Msms	msms x C	Msms	msms x C
Control	0	0	186	170	5.3 ± 0.6	11.0 ± 1.3	1.4 ± 0.4	0.9 ± 0.2	–	–
fast neutrons	20	0	166	184	15.5 ± 1.6	20.8 ± 1.7	4.8 ± 0.9	1.7 ± 0.3	1.9 ≤ 3.9 < 7.8	–
	40	0	188	174	33.0 ± 2.3	34.6 ± 2.3	12.1 ± 1.4	3.9 ± 0.6	2.8 < 5.2 < 9.8	–
	60	0	229	262	57.6 ± 2.0	57.7 ± 2.0	16.2 ± 1.5	5.7 ± 0.6	2.4 < 4.8 < 9.6	–
Control	0	1.2	158	170	6.5 ± 0.7	9.9 ± 1.1	1.4 ± 0.3	0.8 ± 0.2	–	–
fast neutrons	33	1.2	154	206	20.1 ± 2.1	19.8 ± 1.7	6.7 ± 1.0	1.8 ± 0.3	5.7 < 9.3 <14.8	–
	67	1.2	186	156	37.9 ± 2.4	39.7 ± 2.4	14.2 ± 1.4	4.9 ± 0.7	8.4 <12.8 <19.2	–
	100	1.2	156	210	58.0 ± 3.0	63.0 ± 2.4	19.1 ± 1.9	7.6 ± 1.1	11.6 <18.3 <27.8	–

*For segregation frequency of recessive mutants $f = 0.20$; 90% confidence interval, $z = 1.645$.

Table 3. RANK-TEST [42], FOR UNPAIRED MEASUREMENTS IN THE PRESENCE OR ABSENCE OF DTT;
A. THE RATIO PERCENTAGE OF EMBRYONIC LETHALS/PERCENTAGE OF M_1-OVULE STERILITY,
B. THE RATIO CHLOROPHYLL MUTANT FREQUENCY PER CELL/PERCENTAGE OF M_1-OVULE STERILITY,
C. THE RATIO VIABLE MUTANT FREQUENCY PER CELL/PERCENTAGE OF M_1-OVULE STERILITY.

DTT %	Test	X-rays				fast neutrons				fast neutrons †			T*	T 0.05	T 0.01
1.2	A. ratio	32.66	26.39	26.44	24.39	27.09	26.15	24.64	29.20	33.58	37.38	32.99			
	rank	*18*	*12*	*13*	*9*	*14*	*11*	*10*	*16*	*20*	*22*	*19*	*164*		
0	ratio	23.95	20.39	20.80	22.85	21.30	20.34	19.77	22.70	30.88	36.62	28.15			
	rank	*8*	*3*	*4*	*7*	*5*	*2*	*1*	*6*	*17*	*21*	*15*	*89*	*96*	*87*
1.2	B. ratio	20.85	17.69	27.57	20.53	24.63	30.70	20.01	12.49	46.19	33.90	31.58			
	rank	*13*	*7*	*17*	*12*	*15*	*19*	*10*	*4*	*22*	*21*	*20*	*160*		
0	ratio	19.57	22.87	10.88	20.07	28.42	17.89	13.31	9.33	25.39	15.90	8.40			
	rank	*9*	*14*	*3*	*11*	*18*	*8*	*5*	*2*	*16*	*6*	*1*	*93*	*96*	*87*
1.2	C. ratio	1.13	0.86	1.00	0.92	1.23	0.95	0.85	0.86						
	rank	*14*	*5.5*	*12*	*10*	*16*	*11*	*3.5*	*5.5*				*77.5*		
0	ratio	1.11	0.90	0.89	0.85	1.19	0.82	0.75	0.89						
	rank	*13*	*9*	*7.5*	*3.5*	*15*	*2*	*1*	*7.5*				*58.5*	*49*	*43*

† Msms line (heterozygous for male sterility factor ms) ; exp. II.

* T is the sum of ranks, the smaller sum of ranks is used to determine significance. If this sum is less then T 0.01 or T 0.05, the null hypothesis is rejected with $p<0.01$ or $p<0.05$, respectively.

hypothesis of van der Veen et al. [39] and van der Schans et al. [31] that -SH compounds induce a spectrum shift towards more "gene" mutations.

The results of experiment II are listed in Table 2. It is seen that within the control treatments (0% and 1.2% DTT) there is a small difference in ovule sterility between Msms self-pollinated plants and msms plant crossed with C. This difference is attributed to mechanical damage during crossing. After relatively high doses such a difference is not observed. This is probably due to the reduced availability and quality of the Msms-pollen. [37]

Comparison of the data of experiment II (Table 2; columns of Msms) with those of experiment I (Table 1; all plants MsMs) shows that for the genetic parameters equal results were obtained with equal irradiations. Therefore, it is concluded that there were no differences induced by treatment conditions or the material (i.e. Msms versus MsMs) so that the results of the two experiments could be pooled for analysing the DTT effect on the induced spectrum, as was done in the rank-test mentioned above.

In Fig. 5 the percentage embryonic lethals observed in msms plants crossed with C, i.e. the fraction due to non-recessive mutations, has been plotted against the percentage of embryonic lethals in Msms self-pollinated plants. It is observed that for all irradiations, either with or without DTT, the percentage of embryonic lethals in msms plants crossed with C is about 30 per cent of the lethals in Msms self-pollinated plants. Thus, DTT does not cause a spectrum shift towards more *recessive* embryonic lethal mutations. The results suggest that for both types of embryonic lethals, the ratio strand-breaks/nucleotide damage is similar. After 10 mM EMS treatment (24 h, 24 °C, which induces about 45% M_1-ovule sterility) the same percentage of non-recessive embryonic lethals of

the total embryonic lethals was found [37]. Van der Veen [38] showed by a crossing program in the M_2-generation, that the EMS induced non-recessives were mainly caused by maternal effects. It is remarkable that the fast neutron-induced non-recessives are the same proportion of the total embryonic lethals as in the case of EMS-induced non-recessives. However, the present data do not permit any conclusion about similarity in origin, i.e. whether also with irradiation there is no substantial contribution of (1) dominant embryonic lethals mutations (2) gene-dose effects in the triploid endosperm, or (3) effects of the embryonic cytoplasm.

B. *Theoretical model for a shift in mutation spectrum in the presence of -SH compounds.*

It is of interest to test the hypothesis [31] that DTT causes a spectrum shift towards a relatively higher number of gene mutations. The present experiments were focused on a test for differences between genetic parameter j (for instance embryonic lethals, chlorophyll mutants, viable mutants), induced either with or without DTT at an equal level of genetic parameter i (for instance ovule sterility, dominant embryonic lethals). The underlying idea was that where for genetic parameter j the ratio strand-breaks/nucleotide damage is smaller than the ratio strand-breaks/nucleotide damage for genetic parameter i, a spectrum shift will occur in the presence of DTT, i.e. the ratio parameter j/parameter i will become larger.

A model has, therefore, been constructed to see if the test is indeed sensitive for the detection of a spectrum shift towards a relatively increase of gene mutations.

Definitions and formulae

K refers to the type of induced genetic damage protected by DTT (i.e. strand breaks and alkali-labile sites)

O refers to the type *not* protectable by DTT (.i.e. endonuclease-susceptible sites, which cause gene mutations)

Consider a given dose N:

K_N and $\frac{1}{b} K_N$ are the overall amount (e.g. per cell) of K type damage at dose N, in the absence and the presence of DTT, respectively. So b is the corresponding damage reduction factor.

O_N is the overall amount of O type damage at dose N, both in the absence or presence of DTT.

I_N and J_N are the mutational yield (e.g. per cell) for genetic parameter i resp. j.

Thus $I_N = i_k K_N + i_o O_N$

and $J_N = j_k K_N + j_o O_N$, where i_k, i_o, j_k and j_o are the fractions of the two types of damage (K and O) of which I_N resp. J_N are composed (in the absence of DTT).

At a *fixed* dose N:

$$\frac{I_N}{J_N} = \frac{i_k K_N + i_o O_N}{j_k K_N + j_o O_N} \text{ without DTT}$$

$$\frac{I^+_N}{J^+_N} = \frac{\frac{1}{b} i_k K_N + i_o O_N}{\frac{1}{b} j_k K_N + j_o O_N} \text{ with DTT}$$

$$\text{now } \frac{DRF_{IN}}{DRF_{JN}} = \frac{I_N}{I^+_N} \times \frac{J^+_N}{J_N}$$

$$= \frac{i_k K_N + i_o O_N}{\frac{1}{b} i_k K_N + i_o O_N} \times \frac{\frac{1}{b} j_k K_N + j_o O_N}{j_k K_N + j_o O_N}, \text{ where}$$

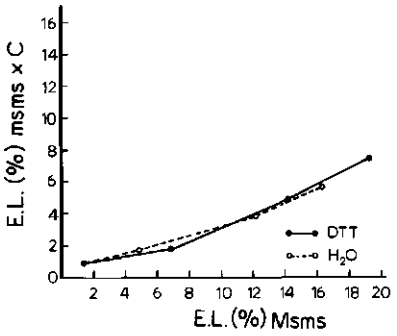

Fig. 5 The percentage of embryonic lethals in msms plants crossed with C (i.e. non-recessive lethals) plotted against the percentage of embryonic lethals in Msms self-pollinated plants (i.e. non-recessive + recessive lethals), induced in *Arabidopsis* with fast neutrons, either with or without dithiothreitol.

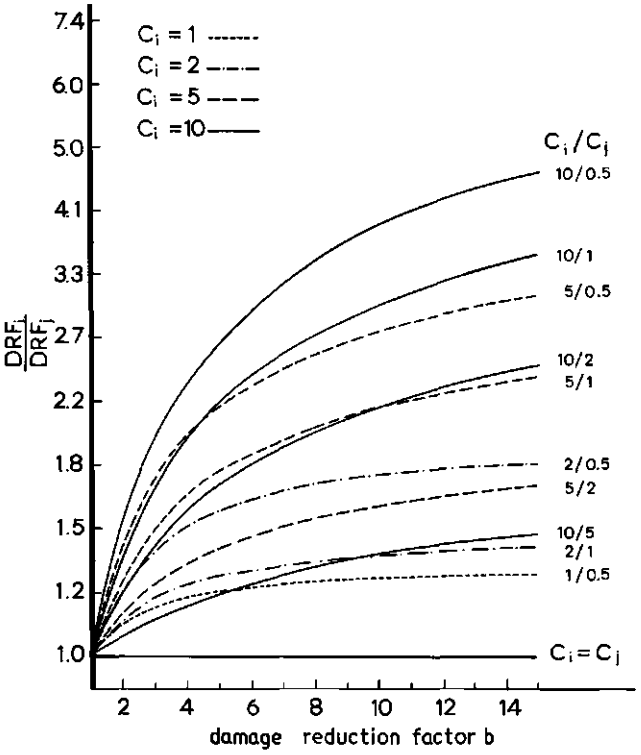

Fig. 6 The expected spectrum shift $\frac{DRFi}{DRFj}$, due to dithiothreitol (DTT), for different values of b, C_i and C_j.

DRF_{iN} and DRF_{jN} are the damage reduction factor in the presence of DTT for i_N and J_N, respectively.

Denote $\frac{i_k K_N}{i_o O_N}$ by C_{iN} and $\frac{j_k K_N}{j_o O_N}$ by C_{jN}

one obtains;

$$\frac{DRF_{iN}}{DRF_{jN}} = \frac{(C_{iN} + 1)\ (\frac{1}{b} C_{jN} + 1)}{(\frac{1}{b} C_{iN} + 1)\ (C_{jN} + 1)}$$

This ratio is unity (i.e. no spectrum shift due to DTT) not only if b = 1, but also if $C_{iN} = C_{jN}$, i.e. $\frac{i_k}{i_o} = \frac{j_k}{j_o}$

Conversely $\frac{i_k}{i_o} \neq \frac{j_k}{j_o}$, i.e. $C_{iN} \neq C_{jN}$, i.e. $DRF_{iN} \neq DRF_{jN}$, implies a spectrum shift (when applying DTT at dose N).

The ratio DRF_{iN}/DRF_{jN}, i.e. the resolving power of the test, depends on the values b, C_{iN} and C_{jN}.

It is implicitely assumed that i_k, i_o, j_k, j_o and b are independent of dose; hence the omission of subscript N. If moreover no *relative* change of damage types K and O with dose is assumed, we may also omit N from K_N and O_N. And consequently from C_{iN} and C_{jN}. One then obtains:

$$\frac{DRF_i}{DRF_j} = \frac{(C_i + 1)\ (\frac{1}{b} C_j + 1)}{(\frac{1}{b} C_i + 1)\ (C_j + 1)}$$

This has been done in Fig. 6, where the value of $\frac{DRF_i}{DRF_j}$ is given for different values of b, C_i and C_j. It is shown that for certain values of C_i and C_j (and $C_i \neq C_j$) the resolving power of the test for a difference between C_i and C_j is small, i.e. $\frac{DRF_i}{DRF_j} \approx 1$.

Table 4. THE AVERAGE VALUE FOR THE RATIO STRAND-BREAKS/ENDONUCLEASE-SUSCEPTIBLE SITES ($\bar{C}_i$) FOR THE GENETIC PARAMETERS; M_1-OVULE STERILITY ($i = 1$), EMBRYONIC LETHALS ($i = 2$), AND CHLOROPHYLL MUTANTS° ($i = 3$), BASED ON THE DAMAGE REDUCTION FACTOR OF DTT (DRF_{iN}) FOR GENETIC PARAMETER i, I.E. $DRF_{iN} = \frac{C_{iN} + 1}{\frac{1}{b}C_{iN} + 1}$, FOR DIFFERENT VALUES OF b^+.

Irradiation		DRF_{1N}*	$\bar{C}_1$				DRF_{2N}*	$\bar{C}_2$				DRF_{3N}*
type	dose (Gy)		b = 2	b = 3	b = 5	b = 10		b = 2	b = 3	b = 5	b = 10	
fast neutrons	33	1.72					1.26					1.46
	47	1.46	2.65	1.20	0.83	0.66	1.06	0.52	0.35	0.27	0.24	0.72
	60	1.54					1.30					0.48
X-rays	233	2.33					1.44					2.71
	327	1.71	15.14	2.37	1.42	1.09	1.29	1.17	0.68	0.51	0.43	1.01
	420	1.61					1.38					1.61

+ b = damage reduction factor of DTT for strand-breaks

* By linear interpolation

° Because the chlorophyll mutant frequency data obtained per treatment have large confidence intervals, the estimation of the DRF_{iN} values are unreliable, therefore the calculation of C_i is omitted for this parameter.

DISCUSSION

The damage reduction factor of DTT for the genetic parameter i at a given dose N (DRF_{iN}), i.e. the ratio between the value of parameter i in the absence of DTT and the value of parameter i in the presence of DTT at a given dose N, is estimated from the data of Table 1 (by interpolation) for M_1-ovule sterility, M_2-embryonic lethals and M_2-chlorophyll mutants (Table 4). It is found that the value of DRF_{iN} is dependent on the irradiation as well as on the genetic parameter studied. In general a tendency for a decrease in DRF_{iN} with increasing dose is observed. This decrease in DRF_{iN} can be explained by:

1. a decrease in the reduction factor b (i.e. dose reduction factor for type K damage) due to a limited concentration of DTT [28, 29]
2. formation of two-hit types of chromosome aberrations, which due to elimination results in a decrease of the $k_N/0_N$ ratio, or by
3. cell replacement of the initial cells by cells which are less radiosensitive [12]. In the case of irradiation in the absence of DTT, this may occur at lower levels of induced genetic damage, since an increased cell survival after DTT treatment would lead to the inclusion of a larger number of sensitive (initial) cells in the population sampled.

For the chlorophyll mutants some values of DRF_{iN} are <1 (at 47 and 60 Gy fast neutrons). These values can only be explained by point 3, i.e. DTT protects more effectively against cell killing than against induced mutations and therefore cell replacement (which explains the plateau in mutation frequency [12]) occurs at a higher level of mutation frequency in the presence of DTT. However, it is noticed that the estimates of the DRF_{Ni} values for chlorophylls might be unreliable, because of the large confidence intervals of the mutant frequency data.

The DRF with fast neutrons is less than with X-rays for all genetic parameters studied. This might be due to a relative decrease in the participation of K type damage (strand-breaks) in the induction of the genetic parameters. This suggestion is in agreement with postulations formed in the early days of radiation genetics [35] and with the observed decrease in effectiveness of different types of ionizing radiation with increasing LET for the production of gene mutations in *E. coli* and in the bacteriophage T 4 [7, 23, 24]. This is to be expected from target hypothetical considerations if the primary lesion is caused by a single energy loss event [36].

In Table 4 the calculated average value for the ratio strand-breaks/endonuclease-susceptible sites ($C_i = i_k K / i_o 0$) for the genetic parameters M_1-ovule sterility and embryonic lethals are given for different values of b. Because the chlorophyll mutant frequency data obtained have large confidence intervals, an estimation of the C_i value for chlorophylls from these data will be unreliable. Therefore this C_i value is omitted from Table 4.

For the explanation of the DTT induced spectrum shift, observed in the present experiments, it is assumed that both radiation-induced strand-breaks and base damage (=endonuclease-susceptible sites) play a significant role in the generation of the genetic parameters studied. The role of base damage is suggested by the following facts on record in the literature;

1. base damage is a major component of damage induced by ionizing radiation in prokaryotic as well as eukaryotic systems and is subjected to excision repair [8, 9, 31]
2. true gene mutations, i.e. intragenic changes (presumably the result of base damage), are induced by ionizing radiation in micro organisms and higher organisms (for reviews see 32, 36).

CONCLUSIONS

In the present experiments it is found that with both X- and fast neutron irradiation of *Arabidopsis* seeds, the presence of DTT results in:

1. a reduction of the induced genetic damage, such as M_1-ovule sterility and embryonic lethals, and
2. a slight spectrum shift in favour of more mutants at a given level of ovule sterility, i.e. with DTT the ratio mutant frequency/M_1-ovule sterility percentage is significantly higher than without DTT (Figs. 2 and 3; Table 3). In other words the DRF is higher for ovule sterility than for mutants.

In terms of our model, this DTT-induced spectrum shift suggests that the ratio strand-breaks/base damage is higher for M_1-ovule sterility than for mutants. It is known [31] that -SH compounds protect against radiation-induced strand-breaks, whereas they do not affect the number of induced base damage (i.e. endonuclease-susceptible sites). Unfortunately however, no conclusive data about the involvement of radiation-induced base damage in mutation induction in higher organisms ara available in literature.

The extent of the DTT protection depends not only on the genetic parameters studied but also on the irradiation (dose *and* type). In spite of the fact that in the experiments the effects of X-ray and fast neutron doses were approximately the same, it is found that the average DTT damage reduction factor is higher with X-rays than with fast neutrons. This is in agreement with other data in literature [1, 7, 23, 24, 35, 36] which suggest a different action on the molecular level between both types of radiation.

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INDEX WORDS

Arabidopsis thaliana, X-rays, fast neutrons, mutagen specificity, mutant spectrum, -SH radioprotector, dithiothreitol, mutation breeding.

SUMMARY

Arabidopsis seeds were irradiated with X-rays or fast neutrons, in the presence or absence of dithiothreitol. Well-filled siliques were selected in the M_1-generation, resulting in a good M_2-fertility. In the M_2-generation, where specific mutants were used as parameter, a significant difference ($p<0.005$) was found between X-ray- and fast neutron-induced mutant spectra. X-rays, for instance, induced more mutants with closely packed broad leaves with short petioles, while fast neutrons induced more mutants with loosely packed leaves with long petioles as well as more *ecerifera* mutants. This difference between the mutability of certain characters by X-rays and fast neutrons was consistent over several doses.

The -SH radioprotector dithiothreitol did not significantly influence the mutant spectra induced. Although certain mutant types, notably those more frequently induced by fast neutrons, seem to be less frequent after irradiation in the presence of DTT.

INTRODUCTION

Research into the specific effects of mutagens in higher plants indicates that particular mutants are more frequently or even exclusively obtained with a specific mutagen (EHRENBERG et al, 1959; GUSTAFSSON, 1963; McKELVIE, 1963; NILAN and KONZAK, 1961; RAO and GOPAL-AYENGAR, 1964; RÖBBELEN, 1962). Differences in mutability of certain chlorophyll

characters by X-rays and fast neutrons were found in barley (EHRENBERG et al, 1959; CONGER and CONSTANTIN, 1974), and significant mutagen specificity of X-rays or γ-rays and fast neutrons for some individual loci was revealed by genetic analysis of *erectoïdes* and *ecériferum* mutants in barley (LUNDQVIST, 1975; LUNDQVIST and von WETTSTEIN, 1962; LUNDQVIST et al, 1968; PERSSON and HAGBERG, 1969). Although CONGER and CONSTANTIN (1974), GAUL (1964) and NILAN (1964) showed that both experimental conditions and mutagen dose affected the mutant spectrum, these factors have not usually been considered in work on mutagen specificity.

The present study about the effect of X-rays or fast neutrons on the mutant spectrum in *Arabidopsis* was carried out to extend the data on X-ray and fast neutron specificity and to study more closely the effect of dose on mutant spectrum. Moreover, irradiation was applied with or without a dithiothreitol (DTT) pre-treatment.

Fast neutrons induce relatively more chromosome breaks and less base damage than X-rays (AHNSTRÖM, 1977 and 1979; HAWKINS, 1979). This could possibly explain the difference in induced mutant spectrum. Since the data recorded in the literature (MALVAREZ et al, 1965; van der SCHANS et al, 1979) indicate that irradiation in the presence of -SH compounds reduces the ratio strand-breaks/base damage, the effect of DTT on X-ray and fast neutron-induced mutant spectra could illucidate whether the specificity of radiation type is influenced by its relative frequency of induced strand-breaks.

MATERIAL and METHODS

Plant material. Arabidopsis thaliana (L.) Heynh. is a small, fast growing, self-fertilizing crucifer. Seed stocks used in the experiment were of the mutant *erecta* of the ecotype "Landsberg" (REDEI, 1962).

Table 7. The mutant frequency per mutant group in *Arabidopsis* after X-ray or fast neutron irradiation, with or without 1.2% dithiothreitol (DTT) pre-treatment.

Mutant group [a]	Number of loci [c]	Mutant frequency x 10^{-3}/cell [d]			
		X-rays		fast neutrons	
		0% DTT	1.2% DTT	0% DTT	1.2% DTT
1. vital yellow-green mutants	7 + (42)	54.5<67.9<84.4	45.4<59.0<76.3	48.8<61.0<76.1	54.6<70.1<89.7
2. hypocotyl elongated (long hypocotyl, *hy*)		3.4< 6.8<13.6	0.1< 0.6< 5.2	3.0< 6.0<12.2	2.5< 5.8<13.7
3. closely packed leaves with short petioles		1.3< 3.4< 8.9	0.6< 2.3< 8.1	0.0< 0.4< 4.0	1.5< 4.1<11.2
4. closely packed broad leaves with short petioles		5.0< 9.2<16.8	6.4<11.6<20.8	0.7< 2.1< 6.8	0.6< 2.3< 8.6
5. loosely packed leaves with long petioles		1.3< 3.4< 8.9	1.2< 3.5< 9.9	6.3<10.7<18.3	6.3<11.7<21.5
6. more rosette leaves and late flowering		7.5<12.6<21.0	6.0<11.0<20.1	2.7< 5.6<11.6	7.1<12.8<23.0
7. roundish or broad leaves		15.9<23.3<33.9	14.3<22.0<33.7	27.7<37.0<49.2	27.4<38.6<54.0
8. narrow leaves		22.7<31.5<43.6	16.6<24.9<37.1	19.0<26.6<37.4	25.5<36.2<51.3
augustifolia (*an*) [b]	1 (3)	0.0< 3.4	0.0< 4.2	0.0< 3.1	0.9< 2.9< 9.5
9. glabrous or weakly hairy leaves (*glabra, gl*)	3 (10)	0.6< 1.9< 6.7	2.5< 5.8<13.2	0.9< 2.6< 7.4	0.9< 2.9< 9.5
10. compact dwarfs, inflorescence short		26.8<36.4<49.1	36.4<48.6<64.6	22.9<31.4<42.8	28.9<40.3<56.0
11. shiny stalks and/or siliques (*ecériferum, cer*)	14 + (53)	2.7< 5.8<12.3	0.7< 2.3< 8.1	9.6<15.0<23.6	11.0<18.1<29.6
12. hanging pods, pod-stalks short		0.6< 1.9< 6.7	0.1< 0.6< 5.2	1.6< 3.9< 9.3	1.2< 3.5<10.4
13. club-shaped pods (*clavata, clv*)	4 + (12)	2.1< 4.8<11.0	5.2< 9.8<18.6	7.6<12.5<20.4	4.3< 8.8<17.7
14. no petals or dwarfed petals		5.4< 9.7<17.4	14.7<22.5<34.4	7.6<12.5<20.4	9.7<16.4<27.5
apetalous (*ap-1*) [b]	1 (6)	1.3< 3.4< 8.9	0.1< 0.6< 5.2	0.5< 1.7< 6.1	0.0< 4.8
15. other changes of the flowers		0.2< 1.0< 5.2	0.0< 4.2	0.3< 1.3< 5.4	1.2< 3.5<10.4
agamous (*ag*) [b]	1 (3)	0.0< 3.4 [e]	0.0< 4.2 [e]	0.0< 3.1 [e]	0.1< 0.6< 5.7 [e]
16. yellow seeds, *transparent testa* (*tt*)	5 (11)	5.8<13.9<33.0	0.0<10.5	3.7<10.5<29.6	0.2< 1.8<16.3
transparent testa + glabra (*ttg*) [b]	1 (7)	0.0<11.2	0.0<10.5	2.0< 7.0<24.3	0.0<12.9
17. gibberellin-sensitive mutants (*ga*)	3 (45)	0.8< 2.4< 7.5	0.0< 4.2	1.6< 3.8< 9.3	3.6< 7.6<16.5

a) Between brackets the description or gene symbols used by REDEI (1965) or KOORNNEEF and van der VEEN (1978) for mutants which were tested in genetic experiments.

b) Within the mutant groups 8, 14, 15 and 16 the specific mutants *angustifolia* (REDEI, 1965), *apetala* KOORNNEEF and van der VEEN, 1978), *agamous* (van der VEEN, pers. comm.) and *transparent testa + glabra* (KOORNNEEF and van der VEEN, 1978) were distinguished.

c) The number of loci per mutant class were determined by KOORNNEEF (pers. comm.); between brackets the number of mutants included in the complementation test for genetic analysis are given; + indicates that the actual number of loci involved might be more, because the mutants were not tested in a complete diallel.

d) The mutant frequency (m) was calculated from the pooled data (over doses); for the segregation frequency of recessive mutants, f = 0.20 was used; the 90% confidence interval for m was calculated with STAM'S formula (DELLAERT, 1979 b). The average radiation doses applied were 280 Gy X-rays (0% DTT); 560 Gy X-rays (1.2% DTT); 40 Gy fast neutrons (0% DTT), and 80 Gy fast neutrons (1.2% DTT).

e) The mutant frequency for yellow seed (*transparent testa*) mutants is based on the numner of harvested M_2 plants, because only in this group were mutants with yellow seeds identified.

P_I mutants was less with a DTT pre-treatment than without, irrespective of radiation type by which the mutants were induced, suggests that P_I mutants more often originate from strand-break damage than the P_{II} and P_{III} mutants. Therefore, the data indicate that the difference between X-ray-and fast neutron-induced mutant spectra is caused by the relatively higher frequency of breaks after fast neutrons than after X-rays. In this respect it is worth mentioning that SCHUBERT and RIEGER (1976) found differences between segmental response of chromosomes *I* and *V* in *Vicia faba* for the induction of aberrations by X-rays and fast neutrons. This indicates that certain parts of the chromosomes have a better ability to 'repair' induced strand-breaks than others. This specific 'repair' ability might be the basis for differences between X-ray-and fast neutron induced mutant spectra.

Induced mutations are widely used in commercial breeding of vegetatively propagated plants. Irradiation is generally more effective than chemicals for the induction of mutants in these plants, possibly because of limited penetration of chemicals into vegetative tissues (BROERTJES and van HARTEN, 1978) because of/or "elimination" of undesirable dominant genes in a given heterozygous genotype by the induction of chromosome deficiencies. The present study, as well as other data on record in literature, have shown that fast neutrons are more efficient for the induction of mutants of specific groups. This specificity seems of importance especially for mutation breeding of vegetatively propagated crops.

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CHAPTER IV

SEGREGATION FREQUENCIES OF RADIATION-INDUCED VIABLE MUTANTS IN *ARABIDOPSIS THALIANA* (L.) HEYNH.*

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SUMMARY

The segregation of viable mutants derived from various fast neutron and X-ray treatments of *Arabidopsis* seeds was studied in the M_2- and M_3-generation. An equal segregation frequency in the M_2- and M_3-generation was observed. This indicates that the M_2-lines, each of which descended from a single silique from the top of the main inflorescence of an M_1-plant, originated from non-chimeric tissue. Furthermore, it was found that neither radiation type nor radiation dose affected the segregation frequency of the mutants. The average segregation frequency of the mutants was 21.5 per cent and significantly below the Mendelian expectation of 25 per cent. It was found that the mutant deficit was mainly due to reduced transmission of the mutant gene through the gametophyte. These findings are discussed with reference to the transformation of mutant frequency scores to mutation frequency per cell.

KEYWORDS

Arabidopsis thaliana, radiation-induced mutants, haplontic and zygotic selection, chimerism, mutation frequency.

INTRODUCTION

The scoring of recessive mutants, e.g. chlorophyll mutants or other specific mutants, is extensively used for evaluating the genetic effects of a mutagenic treatment of plant material. For the comparison of various treatments, within and among species, the mutation frequency should be expressed as mutation frequency per cell

or genome (Li and Rédei, 1969). As has been pointed out by Frydenberg (1963) and Yonezawa and Yamagata (1975), transformation of mutant frequency scores to mutation frequency per cell requires knowledge about the genetic behaviour of the mutants.

When the mutation frequency is expressed as the number of segregating M_1-progenies among the total number of progenies tested, an estimate of the number of meristematic cells (initials) contributing to the formation of the progeny is needed (Li and Rédei, 1969) as well as information about the number of M_2-plants per M_1-progeny (Frydenberg, 1963; Yonezawa and Yamagata, 1975). With respect to the estimation of the number of initial cells, the segregation frequency of the mutants in the offspring of their heterozygotes should be ascertained. This frequency is also needed to convert mutant frequencies, expressed as the number of mutant plants among the total number of M_2-plants (Gaul, 1957), into mutation frequencies per cell. In addition, the transformation actually requires estimates of the following selection variables (Frydenberg, 1963; Yonezawa and Yamagata, 1975);

- the relative multiplication ability of the initial cells carrying a mutated gene, compared to normal initial cells belonging to the same primordium (δ; in the absence of diplontic selection $\delta = 1$),
- the relative viability of mutant gametes compared to gametes carrying the normal allele (β; in the absence of haplontic selection $\beta = 1$),
- the viability of the mutant plants relative to normal plants in the interval between fertilization and scoring (γ).

Induced chlorophyll mutants often segregate with a frequency below the Mendelian expectation of 25 per cent in the offspring of heterozygotes (Avanzi et al., 1960; Moh and Smith, 1951). This may be due to disturbances acting during meiosis and at the gametophyte stage

(β <1), or after fertilization (γ <1). Moh and Nilan (1956) and Doll (1968) concluded from a study on the segregation frequency of radiation-induced chlorophyll mutants in barley, that the deviation was mainly due to reduced transmission of the mutant gene through the male gametophyte, i.e. β <1. With regard to the segregation frequency of a number of radiation-induced chlorophyll mutants in *Arabidopsis* it was found that the deficit was due to reduced transmission through the male and the female gametophyte as well as to decreased viability of the mutant plants, i.e. β <1 and γ <1. (Batalov et al., 1972). Furthermore, in *Triticum durum* a larger deficit was found for the *albina* and *striata* mutants than for the *xantha*, *tigrina*, *viridis* and *chlorina* mutants (D'Amato et al., 1962). In *Arabidopsis*, the average segregation frequency of the *chlorina* and *viridis* mutants was significantly less than that of the *albina* and *xantha* mutants (Ivanov, 1971). These data suggest that the segregation frequency depends on the mutant phenotype and the plant species. However, from these data it could not be determined whether or not the observed difference in segregation frequency was due to a heterogeneity between the individual mutants which by chance happened to have a different phenotype.

A number of data indicate that the segregation frequency is affected by mutagen type and dose (D'Amato et al., 1962). However, the data and conclusions are derived from M_2-segregation frequencies, and thus influenced by the number of initial cells contributing to the M_1-progeny. This number of initial cells is itself affected by mutagen type and dose (Eriksson, 1965). Ivanov (1971) and Moh and Smith (1951) did not observe an effect of mutagen type or dose on the mutant segregation frequency in the M_3-generation.

Observations on the progressive loss of chimerism in *Arabidopsis* along the flowering stem showed that this was (with a few exceptions) a random process in which the lost tissue did not preferentially contain the observed (chlorophyll or embryonic lethal) mutation (Balkema, 1972; Grinikh et al., 1974; Müller, 1963). Therefore, it can be assumed that, in general, the relative multiplication ability of mutated initial cells equals that of non-mutated ones, i.e. $\delta = 1$.

This paper presents a study of the segregation frequency of radiation-induced viable mutants in *Arabidopsis thaliana* in the M_2- and M_3-generation. The objective of this investigation was to determine the factors which influence the segregation, i.e. chimerism, the viability of mutant gametes (β) and the viability of mutant plants (γ). In addition, the effect of radiation type, i.e. X-rays or fast neutrons, and radiation dose on the segregation frequency was studied.

MATERIAL AND METHODS

Plant material

Arabidopsis thaliana (L.) Heynh. is a small, fast growing, self-fertilizing crucifer. Seed stocks used in the experiments were of the mutant *erecta* of the ecotype "Landsberg" (Rédei, 1962).
X- and fast neutron-irradiation were applied to seeds submerged in water (0 per cent) or in 1.2 per cent dithiothreitol (DTT), 3 h before irradiation. A detailed description of the radiation treatment and the handling of the M_1-generation is given elsewhere (Dellaert, 1979). Since chimerism is progressively lost upwards along the stem (Balkema, 1971) and within-flower chimerism does not occur frequently in *Arabidopsis* (Ivanov, 1973), chimerism was in the majority of cases

avoided by harvesting a single silique from the top of the main inflorescence per M_1-plant for progeny testing. Five to twelve seeds were sown per M_1-progeny. A random sample of lines from the M_2-lines, which segregated for specific viable mutants, as described by Bürger (1971), Dellaert (1979) and Kranz (1978), was harvested per plant. These M_2-plants were progeny tested by germinating, when available, 20 seeds per plant.

Culture medium and culture conditions

Seeds were sown in petri dishes on perlite saturated with a standard mineral solution. The culture medium was as described by Oostindiër-Braaksma and Feenstra (1973). To break dormancy, the dishes were kept at 2 °C for 5 days, and subsequently placed at 24 °C under continuous illumination by fluorescent light tubes, 8000 lux/cm^2, for germination. After 8 days, the seedlings were transplanted into soil and cultivated in an air-conditioned greenhouse.

Segregation frequency

In the M_2- and M_3-generation the number of plants as well as the number of segregating mutants were recorded for each tested plant progeny. This was done either in the seedling stage, e.g. long hypocotyl mutants, at the start of flowering, e.g. vital chlorophyll deficient mutants, *Rosetomut* and *Foliomut* mutants, or approximately two weeks after flowering, e.g. *Florimut*, *Flosculimut* and *Semine-coloremut* mutants. Segregating M_2-progenies (each with approximately 20 plants) were considered to descend from heterozygous plants, while the parent plants of non-segregating progenies were regarded as wild-type homozygotes.

In order to exclude dominant mutants, plasmatic mutants, extreme cases of haplontic or zygotic selection and "mutants" resulting from residual instabilities (Auerbach, 1976) from the data, the recorded mutants were chosen so that they met the following criteria:

1. A mutant observed in the M_2 also appears in the M_3.
2. At least one of the progeny-tested normal M_2 plants yields a segregating M_3 line.

Lines containing mutants not fulfilling these criteria were *a posteriori* excluded from the random sample of progeny-tested M_2-lines. It will be clear that this procedure may lead to the exclusion of some "true recessive" mutants from the data because they did not happen to meet the above criteria. Therefore, the estimation of the mutant segregation frequency in the M_2 and M_3 (p_1 and p_3, respectively) and the estimation of the frequency of heterozygous M_2-plants (p_2) necessitates a proband correction.

The probability that a plant descending from an M_1-flower with a number, in the case of within-flower chimerism, or with all heterozygous sporocytes, is a recessive mutant is denoted by p_1. Estimation of p_1 was done by the method proposed by Mantel and Li (1968) which, by discarding the singletons, corrects for the ascertainment of a heterozygous parent when family sizes are small.

$$\hat{p}_1 = \frac{R - S}{T - S}, \text{ where}$$

$\hat{p}_1$ = the estimate of p_1

R = the total number of mutants in the segregating M_2-lines

S = the number of M_2-lines with a single mutant (i.e. singletons)

T = the total number of plants in the segregating M_2-lines.

The probability that a plant in the offspring of a heterozygous M_2-plant is a recessive mutant is denoted by p_3. In M_3 the line sizes

were not so small. The maximum likelihood method (see Lejeune, 1958) was employed to estimate p_3 from the total number of segregating lines of which the average size is known.

$$R = \hat{p}_3 \Sigma (n_e \frac{e.\hat{p}_3}{1-(1-\hat{p}_3)^e}), \text{ where}$$

$\hat{p}_3$ = the estimate of p_3

R = the total number of mutants in the segregating M_3-lines

e = the size of segregating M_2 progenies

n_e = the number of M_2 progenies with size e.

The probability that a non-mutant plant in a segregating M_2-line is heterozygous is denoted by p_2. A first estimate of p_2 was obtained by the method of Mantel and Li, mentioned above. This estimate was then corrected for the ascertainment of a heterozygous parent on the basis of $\hat{p}_3$ ($(1-\hat{p}_3)^e$ being the probability that a heterozygous parent has no mutants among e offspring).

The procedure employed for the estimation of p_1 actually needs a second proband correction for the exclusion of some "true segregating" M_2-lines from the data because they did not happen to have a segregating progeny in the M_3 ($\{(1-p_2)+\hat{p}_2(1-\hat{p}_3)^e\}^N$ being the probability that a segregating M_2-line has no segregating progeny among the N progeny-tested M_2 plants). However, in our range of N values this correction factor is so small that it can be neglected. (Stam, pers. comm.).

Factors affecting the segregation frequency

The mutant segregation frequency in the M_2, i.e. p_1, and the frequency of heterozygous plants among the non-mutant M_2 plants, i.e. p_2, may *a priori* be influenced by;

a) the degree of chimerism in the M_1 sub-epidermal cell-layer; chimerism may result in a deviation from the 1:1 ratio of normal and mutant

Table 1. The fraction of wild-type (genotype AA), heterozygous plants (genotype Aa) and mutants (genotype aa) in a segregating M_2-line

1.A. in case of random union of gametes per flower

1.B. in case only pollen of heterozygous anthers participate in the fertilization

	M_1-gametes			M_2-sporophyte	
	sex	genotype	fraction*	genotype	fraction*
1.A.	♀	A	1	AA	1
	♀	a	$\alpha\beta_1$	Aa	$\alpha(\beta_1+\beta_2)$
	♂	A	1	aa	$\alpha^2\beta_1\beta_2\gamma$
	♂	a	$\alpha\beta_2$		
1.B.	♀	A	1	AA	1
	♀	a	$\alpha\beta_1$	Aa	$\alpha\beta_1+\beta_2$
	♂	A	1	aa	$\alpha\beta_1\beta_2\gamma$
	♂	a	β_2		

*For the definitions of α, β_1, β_2 and γ see text.

Table 2. The fraction of wild-type (genotype AA), heterozygous plants (genotype Aa) and mutants (genotype aa) in a segregating M_3-line.

M_2-gametes			M_3-sporophyte	
sex	genotype	fraction*	genotype	fraction*
♀	A	1	AA	1
♀	a	β_1	Aa	$\beta_1+\beta_2$
♂	A	1	aa	$\beta_1\beta_2\gamma$
♂	a	β_2		

*For the definitions of β_1, β_2 and γ see text.

alleles in the M_1-sporocytes,

b) haplontic selection; difference in viability of normal and mutant gametes may further disturb the 1:1 ratio of normal and mutant gametes at the moment of fertilization,

c) zygotic selection; differential viability of non-mutant and mutant zygotes will change the theoretical 3:1 ratio of phenotypes

Table 1 gives a parameterization of the factors. Notice that absence of chimerism corresponds to $\alpha = 1$; absence of haplontic and zygotic selection corresponds to $\beta_1 = \beta_2 = 1$ and $\gamma = 1$, respectively.

In the case of random union of gametes per flower, the mutant frequency in the segregating M_2-lines is (Table 1.A);

$$p_1 = \frac{\alpha^2\beta_1\beta_2\gamma}{1 + \alpha(\beta_1 + \beta_2) + \alpha^2\beta_1\beta_2\gamma} \qquad (1)$$

However, the possibility cannot be excluded that pollen clustering occurs (Müller, 1961; van der Veen, pers. comm.). If only pollen of one anther participates in fertilization and no within-anther chimerism is assumed, p_1 is (Table 1.B);

$$p_1 = \frac{\alpha\ \beta_1\beta_2\gamma}{1 + \alpha\beta_1 + \beta_2 + \alpha\beta_1\beta_2\gamma}\ , \qquad (2)$$

since segregating progenies arise only if pollen of a heterozygous anther participates in the fertilization.

The frequency of heterozygous plants among the non-mutant M_2 plants, p_2, is either

$$p_2 = \frac{\alpha(\beta_1 + \beta_2)}{1 + \alpha(\beta_1 + \beta_2)}\ , \qquad (3)$$

in the case of random union of gametes, or

$$p_2 = \frac{\alpha\beta_1 + \beta_2}{1 + \alpha\beta_1 + \beta_2}\ , \qquad (4)$$

if only pollen of a heterozygous anther participates in the

fertilization. Note that for $\alpha = 1$ and $\beta_1 = \beta_2 = 1$; $p_2 = 0.6667$.

Segregating M_3-lines originate of course from non-chimeric heterozygous M_2-plants. Thus, the mutant segregation frequency in M_3, i.e. p_3, may *a priori* be influenced by haplontic selection and zygotic selection. Table 2 gives a parameterization of the factors. It can be seen from this table that

$$p_3 = \frac{\beta_1\beta_2\gamma}{1 + \beta_1 + \beta_2 + \beta_1\beta_2\gamma} \qquad (5)$$

In the case of $\beta_1 = \beta_2 = \beta$, or $\beta_1 = 1$ and $\beta_2 = \beta$ (or the reverse), the values α, β and γ are obtained when the frequencies p_1, p_2 and p_3 are known, by solving either equations (1), (3) and (5), or (2), (4) and (5). With respect to the value of α it is noted that $\alpha = 1$ for $p_1 = p_3$ and α is <1 for $p_1 < p_3$.

In M_3 the survival frequency, i.e. the number of plants at the time of scoring per number of seeds sown, of the offspring of mutant heterozygotes (segregating M_3-lines) was compared with the survival frequency of the offspring of homozygous normal plants (non-segregating M_3-lines) by means of a sign test. Differences in survival frequency indicate differences in viability of the mutant sporophyte compared to the wild-type. It should be noted that early zygotic selection is not detected in this way, because only "good" seeds were sown.

RESULTS

Descents disregarded for the determination of the segregation frequency

In total 467 segregating M_2-lines were progeny tested. Excluded from the data were 32 M_2-lines which yielded no mutants in the offspring of M_2 wild-type plants. From these 32 descents at least 22 contained a dominant mutation, because segregation was observed in

the progeny of M_2 mutant plants (assuming no cross pollination of mutant plants in M_2). Dominant mutations were indicated for roundish or broad leaf (5x), narrow leaf (5x), chlorophyll deficiency (5x), compact dwarfness (4x), late flowering (1x), *eceriferum* (1x) and club-shaped pods (1x). The mutants in the remaining 10 descents might have resulted from either a dominant mutation, a plasmatic mutation, an induced residual instability in the DNA, or the mutant might have an extreme disadvantage, compared to wild-type, in the gametophytic or sporophytic stage (haplontic or zygotic selection, respectively). Of course, some descents might not have had any mutants in the offspring of M_2-wild type plants (i.e. either no heterozygous M_2-plants or no mutants in the offspring of heterozygous M_2-plants).

The segregation frequencies p_1, p_2 and p_3

The estimates of the mean segregation frequencies of mutants in the segregating M_2-lines ($\hat{p}_1$) and in the segregating M_3-lines ($\hat{p}_3$), as well as the estimate of the frequency of heterozygous M_2-plants among the non-mutant plants in segregating M_2-lines ($\hat{p}_2$) are presented in Table 3. It can be seen from the table that the frequencies $\hat{p}_1$, $\hat{p}_2$ and $\hat{p}_3$ fluctuate with radiation dose. However, a tendency for a consistent change with increasing dose is not observed either in the M_2 or in the M_3-generation. The data from Table 3 also indicate that there is no difference between X-ray- and fast neutron-induced mutants with respect to their segregation frequencies. Thus, neither an effect of radiation type nor of radiation dose on the mutant segregation frequency is found. These findings agree with observations made by Ivanov (1971) and Moh and Smith (1951) concerning the segregation frequency of

Table 3. The survival frequencies of the offspring of mutant heterozygotes (segretating M_3-lines) and wi segregating M_3-lines), and the average mutant segregation frequencies in the M_2 (i.e. p_1) and M_3 (i.e. p_3) as well as the mean frequency of heterozygous mutant carriers among the non-mutant plants in segregating M_2-lines (i.e. p_2) per radiation treatment by which the mutants were induced.

Irradiation[a]		DTT[a]	No. of segr.	Mean no. of plants[c]		Survival %		Segregation frequencies (%)[d]		
type	dose (Gy)	%	M_2-lines[b]	per M_2-line	per M_3-line	non-segr. M_3-lines	segr. M_3-lines	$\hat{p}_1$	$\hat{p}_2$	$\hat{p}_3$
X-rays	140	0	22	8.0 ± 2.6	17.0 ± 2.2	83.3	87.0	25.9 ± 3.7	65.0 ± 3.2	24.5 ± 2+3
	223	0	23	7.6 ± 3.0	16.5 ± 2.9	81.8	84.9	22.6 ± 3.7	68.4 ± 4.3	18.3 ± 2.1
	327	0	27	7.0 ± 2.8	16.4 ± 2.5	80.0	81.7	23.3 ± 3.6	73.1 ± 3.8	20.2 ± 1.9
	420	0	25	6.5 ± 2.5	15.3 ± 3.4	78.1	78.7	24.3 ± 4.0	62.1 ± 5.1	21.4 ± 2.2
	Σ	0	97	7.2 ± 2.7	16.2 ± 2.9	80.4	82.9	24.0 ± 1.9	67.0 ± 2.3	21.1 ± 1.0
	280	1.2	36	7.6 ± 3.0	15.6 ± 2.7	78.8	79.7	21.4 ± 2.8	60.0 ± 3.4	19.5 ± 1.7
	467	1.2	43	7.8 ± 3.0	15.6 ± 3.1	81.3	81.0	21.6 ± 2.6	63.1 ± 3.1	22.5 ± 1.6
	653	1.2	26	7.3 ± 3.3	15.6 ± 2.4	77.8	78.9	14.8 ± 3.2	62.5 ± 4.3	20.4 ± 2.1
	840	1.2	8	5.8 ± 1.8	14.6 ± 4.9	77.8	80.4	14.6 ± 7.0	53.9 ± 9.7	16.0 ± 3.6
	Σ	1.2	113	7.5 ± 3.0	15.5 ± 2.9	79.4	80.1	19.7 ± 1.6	61.4 ± 2.0	20.8 ± 1.0
Fast neutrons	20	0	18	8.2 ± 3.1	18.4 ± 1.4	89.2	91.4	21.1 ± 3.9	60.5 ± 4.9	18.9 ± 2.2
	33	0	27	8.0 ± 3.1	17.4 ± 1.7	90.7	88.1	17.7 ± 3.1	67.3 ± 3.8	22.0 ± 1.9
	47	0	33	8.8 ± 2.7	16.1 ± 2.7	86.1	83.5	20.2 ± 2.8	61.3 ± 3.4	19.8 ± 1.8
	60	0	31	6.7 ± 2.4	16.0 ± 2.8	82.9	81.4	25.8 ± 3.5	63.9 ± 4.2	19.6 ± 1.9
	Σ	0	109	7.9 ± 2.9	16.7 ± 2.6	86.5	85.0	21.1 ± 1.6	63.3 ± 2.0	20.3 ± 1.0
	40	1.2	22	8.3 ± 2.6	15.8 ± 3.6	81.4	77.9	25.9 ± 3.7	49.3 ± 4.7	21.9 ± 2.5
	67	1.2	48	7.4 ± 2.8	14.7 ± 3.5	76.2	75.1	18.5 ± 2.5	60.2 ± 3.1	20.1 ± 1.6
	93	1.2	28	7.9 ± 2.9	15.7 ± 3.0	76.5	79.9	24.8 ± 3.3	71.4 ± 3.7	24.5 ± 2.1
	120	1.2	18	9.6 ± 2.6	13.7 ± 3.5	72.9	77.4	25.8 ± 3.7	59.3 ± 4.6	22.1 ± 2.8
	Σ	1.2	116	8.0 ± 2.8	15.0 ± 3.4	77.1	77.3	22.8 ± 1.6	60.5 ± 2.0	22.1 ± 1.1

a) The irradiation was applied to seeds submerged in 0% or 1.2% dithiothreitol (DTT), 3 h before irradiation. A detailed description of the radiation treatments is given elsewhere (Dellaert, 1979).

b) The number of segregating M_2-lines used for determining the segregation frequencies, i.e. M_2-lines that had at least one mutant and at least one heterozygous mutant carrier among the non-mutant plants.

c) Per M_2-line 5-12 seeds and per M_3-line a maximum of 20 seeds were sown.

d) For the methods used to estimate the segregation frequencies see text.

chlorophyll deficient mutants in *Arabidopsis*, barley and durum wheat (cf. introduction). Besides, dithiothreitol (DTT) pre-irradiation treatment does not seem to influence the mutant segregation frequency.

A sign test showed that the mutant segregation frequency in the M_2 ($\hat{p}_1$) and M_3 ($\hat{p}_3$) was significantly below the expected 25 per cent ($p = 0.021$ and $p < 0.004$, respectively). With the same test it was observed that the frequency of heterozygous M_2-plants ($\hat{p}_2$) was also significantly below the expected 66.67 per cent ($p = 0.077$).

The distribution of the individual mutant segregation frequencies in the M_3-generation was very skew with a marked tail below the expected 25 per cent, and with a significant heterogeneity of segregation frequencies between individual mutants (χ^2 test; $p < 0.005$). The deviation of these frequencies from the expected 25 per cent was tested (per independent mutant) on binomial paper (Mosteller and Tukey, 1949); 5 per cent limits of significance were used. It was found that 18.1 per cent of the mutants had a segregation frequency significantly lower than 25 per cent, 2.8 per cent of the mutants segregated with a frequency significantly higher than the expectation.

Factors affecting the segregation frequency

In the case of within-flower chimerism in the M_1-plants the mutant segregation frequency in the M_3 should be higher than in the M_2, i.e. $\hat{p}_3 > \hat{p}_1$. From Table 3 it can be seen that in the present investigation these frequencies do not differ significantly. This indicates that there was no chimerism, i.e. $\alpha = 1$. Thus, each of the tested M_1-progenies, descending from a single flower from the top of the M_1-main inflorescence, originates from genetically

homogeneous tissue and can, therefore, be traced back to a single cell in the irradiated embryo. This result is in agreement with the observations from Balkema (1971) and Ivanov (1973). Balkema found that chimerism is progressively lost upwards along the stem, and Ivanov concluded from the segregation frequency of chlorophyll embryos in M_1 flower progenies that within-flower chimerism does not occur frequently in *Arabidopsis*.

To find out to what extent the deficit in the mean segregation frequency of the mutants was associated with haplontic selection (i.e. $\beta_1 + \beta_2 < 2$) and the viability of mutant plants compared to wild-type (i.e. $\gamma < 1$), the quantities $\beta_1 + \beta_2$ and γ were calculated. Since $\alpha = 1$, the value of $\beta_1 + \beta_2$ could be directly obtained by solving equation (3), discussed in material and methods. With $\alpha = 1$, equation (1) becomes equal to equation (5). Assuming that $\beta_1 = \beta_2$, a minimum value of γ could be calculated from the mutant segregation frequency in the M_2 (p_1) as well as from the mutant segregation frequency in the M_3 (p_3), i.e. $\gamma\bar{p}_1$ minimum and $\gamma\bar{p}_3$ minimum, respectively. In the same way a "maximum value" of γ was obtained, i.e. $\gamma_{\bar{p}_1}$ maximum and $\gamma_{\bar{p}_3}$ maximum, respectively, assuming that $\beta_1 = 1$ or $\beta_2 = 1$. The computed values of $\beta_1 + \beta_2$ and the minimum and maximum values of γ are given in Table 4. From the table it can be deduced (sign test) that $\beta_1 + \beta_2$ is, in general, less than 2 ($p = 0.077$), but neither the minimum nor the maximum value of γ differs significantly from 1 ($p > 0.25$). A comparison of the survival rates of segregating M_3-lines with those of non-segregating lines also indicated no difference in viability of mutant plants compared to wild-type ($p > 0.50$). Therefore, it is concluded that γ is approximately 1 and that the deficit in the mutant segregation frequency can be attributed to haplontic selection.

Table 4. Factors affecting the segregation frequency of radiation-induced mutants;
- $\beta_1 + \beta_2$ is the fraction of ♀ and ♂ mutant gametes from a heterozygous flower, participating in the fertilization
- γ is the viability of mutant plants relative to wild-type in the interval from fertilization to scoring.

Irradiation		DTT	$\beta_1 + \beta_2$ [c]	$\gamma_{\hat{p}_1}$ [d]		$\gamma_{\hat{p}_3}$ [d]	
type	dose (Gy)	%		min.	max.	min.	max.
X-rays	140	0	1.8571	1.1570	1.1639	1.0765	1.0830
	233	0	2.1676	0.7856	0.7903	0.6049	0.6085
	327	0	2.7120	0.6133	0.6587	0.5113	0.5492
	420	0	1.6392	1.2640	1.3283	1.0678	1.1222
	Σ [a]	0	2.1125	0.9452	0.9771	0.8051	0.8320
	280	1.2	1.5019	1.2058	1.3549	1.0775	1.2107
	467	1.2	1.7093	1.0196	1.0498	1.0739	1.1057
	653	1.2	1.6638	0.6670	0.6954	0.9876	1.0296
	840	1.2	1.1711	1.0852	2.1741	1.2035	2.4112
	Σ	1.2	1.5947	1.0024	1.1451	1.0644	1.2141
	ΣΣ		1.8339	0.9760	1.0675	0.9446	1.0376
fast neutrons	20	0	1.5336	1.1543	1.2719	1.0008	1.1030
	33	0	2.0618	0.6175	0.6180	0.8121	0.8129
	47	0	1.5860	1.0430	1.1190	1.0141	1.0881
	60	0	1.7724	1.2255	1.2460	0.8577	0.8722
	Σ	0	1.7482	1.0079	1.0563	0.9174	0.9610
	40	1.2	0.9728	2.9086		2.3358	
	67	1.2	1.5113	0.9949	1.1111	1.1065	1.2357
	93	1.2	2.4928	0.7403	0.7704	0.7296	0.7592
	120	1.2	1.4552	1.6083	1.8705	1.3142	1.5284
	Σ	1.2	1.6374	1.3916		1.2809	
	ΣΣ		1.6911	1.2057		1.1048	
	Σ [b]	1.2	1.7929	1.0365	1.1550	1.0340	1.1498
	ΣΣ [b]		1.7689	1.0211	1.1020	0.9714	1.0484

a) Calculated as weighted average, according to the number of segr. M_2-lines used for determining the segregation frequencies (see Table 3).

b) Weighted average if the observations at 40 Gy fast neutrons + 1.2% DTT are omitted.

c) $\beta_1 + \beta_2$ is obtained by solving the equation $\hat{p}_2 = \frac{\beta_1+\beta_2}{1+\beta_1+\beta_2}$

d) $\gamma_{\hat{p}_1}$ is obtained by solving the equation $\gamma_{\hat{p}_1} = \frac{\hat{p}_1(1+\beta_1+\beta_2)}{(1-\hat{p}_1)\beta_1\beta_2}$;

$\gamma_{\hat{p}_1}$ is minimal for $\beta_1 = \beta_2$ and maximal for either $\beta_1 = 1$ or $\beta_2 = 1$.
For the calculation of $\gamma_{\hat{p}_3}$, $\hat{p}_1$ is substituted by $\hat{p}_3$. The value γ_{max} at 40 Gy fast neutrons + 1.2% DTT is omitted, because the value $\beta_1 + \beta_2 < 1$ and thus for $\beta_1 \to 1$, $\beta_2 \to < 0$, or the other way round.

DISCUSSION AND CONCLUSIONS

It has been shown that the segregation frequency of radiation-induced viable mutants in *Arabidopsis* is, in general, below the Mendelian expectation of 25 per cent. The average mutant segregation frequency in the M_2- and M_3-generation is 21.5 per cent. The extent of the deficit in segregation frequency is not affected by radiation type or dose, by which the mutants were induced. Because the viability of the mutants was, in general, similar to the viability of non-mutant plants (i.e. $\gamma \approx 1$), the deviation in mutant segregation frequency from 25 per cent can be ascribed to haplontic selection. The average viability of female and male mutant gametes (i.e. $\beta_1 + \beta_2$) compared to normal ones was 1.7625/2. It was calculated (for $\gamma = 1$) that $\beta_1 = 1.0229$ and $\beta_2 = 0.7396$ or vice versa. Thus, haplontic selection mainly occurs between either the female or the male gametes. In all likelihood selection occurs between the male gametes, because very few deficiencies can survive the haplophase as microspores and besides, male gametes are involved in certation.

The fact that the viability of mutant plants was similar to the viability of non-mutant plants implies that the estimated segregation frequency of the mutants in the M_3-generation, i.e. $\hat{p}_3$, can be used directly to convert the mutant frequency expressed as the frequency of mutant M_2-plants among the total number of M_2-plants, i.e. m', into the mutation frequency per cell, i.e. m, using the formula

$$m = \frac{m'}{\hat{p}_3}$$ (Gaul, 1957, Frydenberg, 1963).

Besides, because either $\beta_1 \approx 1$ or $\beta_2 \approx 1$, $\hat{p}_3$ can be used to estimate the degree of chimerism in the M_1 sub-epidermal cell-layer from which the tested M_2-lines descended, using equations (1) and (5)

(or (2) and (5) in the case of non-random union of gametes) described in material and methods, and the estimate $\hat{p}_1$.

In the present investigation no indication of within-flower chimerism was found. This means that when the progeny of one silique from the top of the main inflorescence per M_1-plant is tested in the M_2, as was done in this study, one can obtain the estimate of the mutant segregation frequency in the offspring of a heterozygous parent, i.e. $\hat{p}_3$, directly from the M_2-data, since $p_1 = p_3$ in the absence of chimerism.

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CHAPTER V

ECERIFERUM MUTANTS IN *ARABIDOPSIS THALIANA* (L) HEYNH: 1. INDUCTION BY X-RAYS AND FAST NEUTRONS.

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ABSTRACT

The effect of fast neutrons and X-rays (in the presence or absence of dithiothreitol) on the absolute and relative frequency of eceriferum mutants in Arabidopsis is studied. It is found that there are significant differences ($p < 0.01$) in 'group' mutability between the mutant spectra induced by X-rays and fast neutrons. It is shown that fast neutrons induce relatively more eceriferum mutants than X-rays, and that this difference is consistent over several irradiation doses. For the -SH radioprotector DTT, no effect on the relative frequency of eceriferum mutants is found.

INTRODUCTION

Research into the specific effects of mutagens in higher plants, in terms of mutant spectra, has mainly concentrated on the comparison between ionizing radiation and alkylating agents. Extensive data on chlorophyll mutants as well as viable mutants demonstrated significant differences in 'group' mutability between the two types of agents in Arabidopsis (MCKELVIE, 1963; RÖBBELEN, 1962), in barley (EHRENBERG et al, 1959; GUSTAFSSON,1963; NILAN and KONZAK, 1961) and in rice (RAO and GOPAL-AYENGAR, 1964). Differences in 'group' mutability between X-ray and fast neutron induced mutant spectra have been demonstrated in barley for the different phenotypes of chlorophyll mutants (EHRENBERG et al, 1959; CONGER and CONSTANTIN, 1974) and for the relative frequency of erectoides mutants among the total number of viable mutants (EHRENBERG et al, 1959). Genetic analysis of induced erectoides and eceriferum mutants in barley revealed, w i t h i n these mutant classes, specificity in the mutability of individual loci, and a significant mutagen specificity of sulfonates, ethyleneimine, X-rays, γ-rays and fast neutrons for some loci (PERSSON and HAGBERG, 1969; LUNDQVIST, 1975; LUNDQVIST and WETTSTEIN, 1962; LUNDQVIST et al, 1968).

Although GUSTAFSSON (1963) and MCKELVIE (1963) reported that the difference in mutant spectrum between alkylating chemicals and radiation in barley and Arabidopsis was similar for several 'mutagen dose' levels, other data show that apart from experimental conditions,the mutagen dose also differentially affects the (chlorophyll) mutant spectrum (CONGER and CONSTANTIN, 1974; GAUL, 1964; NILAN, 1964). These factors have not usually been taken into consideration in work on mutagen specificity.

As in barley (LUNDQVIST and WETTSTEIN, 1962), mutants with reduced or absent wax coating are common among the induced viable mutants in Arabidopsis (DELLAERT; unpublished). The present study into the effect of X-rays and fast neutrons - both in the presence or absence of dithiothreitol (DTT) - on the absolute and relative frequency of eceriferum mutants in Arabidopsis is executed in order to:

1. extend the data about X-ray and fast neutron mutagen specificity,
2. study the effect of mutagen dose on mutagen specificity,
3. study the effect of DTT on the induced mutant spectrum.

MATERIAL AND METHODS

After 5 days on wet filter paper at 2 °C (to break dormancy) and re-drying on filter paper (24 °C, 24 hrs), seeds of Arabidopsis thaliana ecotype Landsberg, mutant 'erecta', were submerged in tap water or DTT-solution (1.2%), at 22 °C, 3 hours before irradiation. Different X-ray doses, such as 140, 233, 327 and 420 Gy (10 Gy = 1 Krad) were applied to seeds submerged in tap water, likewise 280, 467, 653 and 840 Gy were given to seeds submerged in 1.2% DTT. The irradiation was carried out with an MG 301 X-ray machine with an MCN-420 tube, operating at 320 kVp and 10 mA, with an additional filter of 0.25 Cu and 1.0 Al and with a dose rate of 4 Gy/min. Fast neutrons (20, 33, 47 and 60 Gy to seeds submerged in tap water; 40, 67, 93 and 120 Gy to seeds submerged in 1.2% DTT) were given in the irradiation room of the BARN (Biological Agricultural Reactor Netherlands, Wageningen) with a dose rate of 1 Gy/min. The γ-contamination was only approximately 3 per cent, on a Gy basis. The maximum duration of irradiation was 3.5 hours. In order to equalize the environmental conditions, the seeds for all treatments were kept submerged for 6.5 hours at 22 °C. In total, 18 different treatments (including the controls) were given (Table 1). The X-ray and fast neutron doses applied - both in the presence or in the absence of DTT - induced approximately comparable levels of genetic damage, i.e. M_1-ovule sterility, M_2-embryonic lethality, frequency of chlorophylls and viable mutants (DELLAERT, 1979).

Subsequent to irradiation the seeds were rinsed with tap water (5 min.) and sown (equally spaced) on perlite saturated with a standard nutritional solution and put to germinate at 24 °C under continuous illumination by fluorescent light tubes, 8000 lux/cm^2. After 8 days the seedlings were transplanted into soil in an air-conditioned greenhouse. The culture medium and culture conditions used were as described by

FEENSTRA (1965), and OOSTINDIER-BRAAKSMA and FEENSTRA (1973).

In the M_1-generation MÜLLER's embryo test (MÜLLER, 1961 and 1963) was applied to silique number 5 or 6 of the main inflorescence, for scoring M_1-ovule sterility, M_2-embryonic lethals and chorophylls (details about the results will be published elsewhere). Viable mutants were scored in the M_2-generation, by testing the progeny of one silique per M_1-plant from the top of the main inflorescence (to avoid chimerism). In the M_1 only well-filled siliques were harvested, in order to (1) increase the germination frequency of the M_2-seeds (2) increase fertility in the M_2-generation, and (3) decrease the number of deviant M_2-plants caused by chromosomal aberrations (MÜLLER, 1966; MESKEN and van der VEEN, 1968). Viable mutants in the M_2-generation were defined as plants showing deviations from wild type in plant morphology and/or leaf colour. Among these, plants with visually reduced or absent epicuticular wax from siliques and/or stems were defined as eceriferum mutants. Initially, plants with glossy rosette leaves were also included in this class. However, because ultrastructural analysis of the wax coating on leaves of the wild type revealed very limited wax deposition (DELLAERT et al, 1979) it was decided to classify mutants with glossy rosette leaves in a separate mutant class.

The mutant frequency (m) for chlorophyll mutants, eceriferum mutants and total number of viable mutants, has been expressed as the mutation frequency per cell (GAUL, 1957). For the segregation ratio of recessives (f), the value of 0.20 has been used. A confidence interval for m has been calculated by means of a formula derived by STAM (DELLAERT, 1979).

RESULTS AND DISCUSSION

In total 36 M_2-lines segregated for eceriferum mutants (i.e. 16 induced with neutrons; 11 induced with neutrons + 1.2% DTT; 5 induced with X-rays; and 3 induced with X-rays + 1.2% DTT). In the M_3-generation selfed progenies of viable M_2-mutants and normal 'sister' plants were grown in order to confirm the mutant type. Among the latter, 5 more (independent) eceriferum mutants were isolated. These 5 mutants escaped detection in the M_2-generation (due to small progeny size) and are not included for the calculation of the mutation frequency. The phenotype description of the isolated eceriferum

Table 1. Comparison between irradiation treatments - X-rays and fast neutrons - in the presence or absence of dithiothreitol (DTT), on the frequency of chlorophyll mutants, viable mutants and eceriferum mutants in Arabidopsis.

Irradiation treatment		DTT	n_1 +	Chlorophyll mutant	n_2 +	Viable mutant	Eceriferum mutant	No. of independent
Source	Dose (Gy)	%		frequency/100 cells*		frequency/100 cells*	frequency/100 cells*	eceriferum mutants
control	0	0	499	-	649	2.90 < 4.59 < 7.23	0.009 <0.022 <0.054	1
	0	1.2	442	-	546	1.40 < 3.72 < 6.42	-	
X-rays	140	0	246	2.55 < 4.51 < 7.84	398	20.71 <26.03 <32.36	0.059 <0.276 <1.281	1
	233	0	245	5.14 < 8.14 <12.69	285	25.09 <32.08 <40.38	0.371 <1.015 <2.759	2
	327	0	249	2.46 < 4.66 < 8.71	339	31.36 <38.23 <46.01	0.074 <0.337 <1.539	1
	420	0	247	7.32 <11.62 <18.04	155	37.83 <49.48 <62.89	0.335 <1.184 <4.118	1
	280	1.2	248	2.00 < 3.69 < 6.72	389	15.21 <19.97 <25.91	0.113 <0.419 <1.543	1
	467	1.2	247	4.51 < 7.40 <11.93	341	29.25 <35.96 <43.66	0.024 <0.173 <1.262	2
	653	1.2	246	10.64 <15.64 <22.53	190	46.06 <56.66 <68.11	-	
	840	1.2	173	7.94 <14.56 <25.50	41	41.76 <64.94 <91.24	-	
fast neutrons	20	0	252	2.57 < 4.43 < 7.55	389	14.06 <18.61 <24.36	0.255 <0.710 <1.967	3
	33	0	248	3.23 < 5.55 < 9.39	340	19.72 <25.44 <32.41	1.043 <2.034 <3.945	4
	47	0	250	2.97 < 5.26 < 9.19	339	23.45 <29.50 <36.65	0.984 <1.935 <3.779	6
	60	0	245	2.68 < 5.28 <10.16	307	42.40 <50.40 <59.08	0.464 <1.169 <2.922	3
	40	1.2	248	3.12 < 5.29 < 8.85	344	20.83 <26.49 <33.28	0.439 <1.064 <2.564	2
	67	1.2	248	8.95 <13.02 <18.63	326	33.09 <40.33 <48.48	0.529 <1.249 <2.931	3
	93	1.2	238	8.17 <13.59 <21.94	161	45.89 <57.64 <70.40	1.656 <3.427 <6.998	5
	120	1.2	209	4.32 < 9.58 <20.16	99	51.43 <65.84 <80.74	0.873 <2.478 <6.888	1

+ n_1 = no. of siliques scored with Müller's embryotest; n_2 = No. of M_2-lines scored for viable mutants.

* For segregation ratio recessive mutants f = 0.20; 90% confidence interval, z = 1.645, for eceriferum mutants; 95% confidence interval, z = 1.96, for viable mutants.

mutants in Arabidopsis and the data about mutagen, dose and the conditions of the treatment, by which the mutations are induced, are given elsewhere (DELLAERT et al., 1979).

In Table 1 the calculated mutation frequencies per 100 cells (m) for chlorophylls, viable mutants and eceriferum mutants are presented. It is observed that in general the eceriferum mutant frequency is higher after fast neutrons than after X-rays. It has been calculated that with fast neutrons the average eceriferum mutation rate per Gy per cell is about 16 times higher than with X-rays. For chlorophylls and viable mutants, the average mutation rate per Gy per cell is only about 6.4 times higher with fast neutrons than with X-rays. A rank test (WHITE, 1952) shows that in the dose range studied the ratio eceriferum mutants/viable mutants and the ratio eceriferum mutants/chlorophyll mutants are significantly higher ($p < 0.01$) among fast neutron induced mutants than among X-ray induced mutants (Table 2). With a similar rank test no significant effect of DTT on these ratios is found.

Concerning the ratio eceriferum mutants/chlorophyll mutants, it is noted that the chlorophyll mutants are scored in silique numbers 5 and 6 of the M_1-main inflorescences, while the eceriferum mutants are scored in M_2-progenies of well- filled siliques from the top of the M_1-main inflorescences. Therefore, the ratio eceriferum mutants/chlorophyll mutants might be influenced by differences in mutagen sensitivity between the "initial" cells which form the sporophytic tissue of siliques number 5 or 6 and the "initial" cells which form the sporophytic tissue of the well- filled siliques at the top of the inflorescences. This does not apply to the ratio eceriferum mutants/viable mutants, since both types of mutants are scored in the same M_2-population.

The relatively low number of X-ray-induced eceriferum mutants, 8 independent mutants among the 659 X-ray-induced viable mutants, is in accordance with MCKELVIE's results (1963). MCKELVIE found no eceriferum mutants among 427 (independent) X-ray induced viable mutants. It should be noted that, seeing the phenotype description of the waxless mutants by MCKELVIE (1962), in his case only mutants with (visual) a b s e n c e of the expidermal wax coating of the stem are included in the waxless murant class in the experiment

Table 2. Rank-test (White, 1952) for unpaired measurements after fast neutron and X-ray irradiation;
A. the ratio eceriferum mutants/viable mutants,
B. the ratio eceriferum mutants/chlorophyll mutants.

Irradiation source	Test	0% DTT				1.2% DTT				T*	T 0.05	T 0.01
fast neutrons	A ratio	0.0376	0.0900	0.0656	0.0232	0.0402	0.0310	0.0595	0.0376			
	rank	9.5	14	13	5	11	7	12	9.5	81		
X-rays	ratio	0.0106	0.0316	0.0088	0.0239	0.0210	0.0048					
	rank	3	8	2	6	4	1			24	29	25
fast neutrons	B ratio	0.1603	0.3667	0.3679	0.2214	0.2011	0.0959	0.2522	0.2587			
	rank	8	13	14	10	9	4	11	12	81		
X-rays	ratio	0.0612	0.1247	0.0723	0.1019	0.1136	0.0234					
	rank	2	7	3	5	6	1			24	29	25

*T is the sum of ranks, the smaller sum of ranks is used to determine significance. Since this sum is less then T 0.01, the null hypothesis, i.e. no difference between fast neutrons and X-rays, is rejected with $p < 0.01$.

reported here, only 3 such X-ray induced mutants are found (DELLAERT et al., 1979). From the 27 fast neutron induced eceriferum mutants, 9 mutants have no (visual) epidermal wax coating on the stem. The relative frequency of these eceriferum mutants among the total number of fast neutron induced viable mutants (i.e. 9 per 684 viable mutants) is comparable to the relative frequency of eceriferum mutants observed after EMS treatment (i.e. 7 per 554 viable mutants) by MCKELVIE (1963).

KOORNNEEF (1979, pers comm.) has used criteria for the classification of the eceriferum mutants which are equal to the criteria described here. He observed for EMS induced eceriferum mutants in Arabidopsis (seed treatment; 10 mM EMS; 24 hrs, 24 ^{o}C) a mutant frequency of 2.3 per 100 cells. This mutant frequency is comparable to the mutant frequency observed after fast neutron irradiation in this experiment

In conclusion, the experimental data reported here demonstrate significant differences in 'group' mutability between the mutant spectra induced by X-rays and fast neutrons in Arabidopsis. It is shown that fast neutrons induce relatively more eceriferum mutants than X-rays, and that this difference in mutant spectra is consistent over several mutagen doses. The data conform with the results found in barley for differences in X-ray and fast neutron induced mutant spectra (EHRENBERG et al, 1958; CONGER and CONSTANTIN, 1974). Since phenotypic and genetic analysis of the eceriferum mutants in Arabidopsis (DELLAERT et al, 1979) indicate that there are at least 14 different cer loci in Arabidopsis, the number of mutants per locus per mutagen are still too low to pronounce upon mutagen specificity for individual loci.

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ECERIFERUM MUTANTS IN *ARABIDOPSIS THALIANA* (L.) HEYNH: II PHENOTYPIC AND GENETIC ANALYSIS.

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ABSTRACT

The phenotype of the wax coating of 53 eceriferum mutants of Arabidopsis thaliana (L.) Heynh is characterized by macroscopic and by scanning-electron microscopic techniques. Based on the macroscopic characterization of the wax coating on stems and siliques, and other morphological deviations of wild type (i.e. semi-sterility and dwarfness), the mutants are classified into 7 main phenotypic categories. Based on the results of the genetic analysis obtained so far, the minimum and maximum number of loci per main phenotypic category have been determined. The scanning-electron microscopic analysis of the wax coating on stems and siliques revealed that in wild type Arabidopsis the epicuticular waxes are phenotypically characterized by (1) a high proportion of rodlet-shaped crystals, (2) plate-like structures, and (3) an organized wax layer on the stem bases, decreasing in quantity towards the siliques. In the eceriferum mutants the rodlet-shaped crystals are reduced in size and/or frequency, or completely absent. The plate-like structures are reduced in size and/or frequency, or completely absent in mutants which are visually described as being waxless. In these mutants there is often, in contrast to the wild type, a thick organized wax layer present on the siliques and the stems.

INTRODUCTION

In a study about the effects of X-rays and fast neutrons [in the presence or absence of dithiothreitol (DTT)] on induced mutant spectrum in Arabidopsis thaliana (L.) Heynh, special attention is paid to the mutants with altered epidermal wax coating (DELLAERT, 1979). Like in barley (LUNDQVIST and WETTSTEIN, 1962), mutants with a reduced or without wax coating are common among the viable mutants in Arabidopsis. In Arabidopsis a b s e n c e of wax coating has been variously described as lucida (MCKELVIE, 1962), shiny stalk (BÜRGER, 1971), virescens (RÉDEI, 1965), and conform to the denotation in barley as erceriferum (KOORNNEEF and van der VEEN, 1978). The gene symbols lu_1 and lu_2, vc-1 and vc-2, and cer, respectively, have been used by these aythors for this character. The first (and only) mutant with r e d u c e d wax coating has been described as rhomboidea (gene symbol rh), based on the simultaneous morphological change of leaves, by MCKELVIE (1962). In order to have a generally acceptable name, we have adopted the word eceriferum for all mutants with visually reduced or absent wax coating, and we thus use the gene symbol cer, in analogy with the barley eceriferum mutants.

In barley the large group of eceriferum mutants is characterized by a change in quantity or composition of the wax coating on different organs such as spike , stem, leaf-sheath or leaf-blade. LUNDQVIST (1975) has localized 988 induced and 4 spontaneous mutations at 59 loci by diallel crosses. Biochemical and phenotypic (visually and ultrastructurally) examination of the epidermal wax coating of the wild type and some cer mutants showed that there is a distinct correlation between the mutated loci involved and the phenotypic and biochemical characteristics of the mutants. In barley it is found that the eceriferum loci determine the synthesis and deposition of the epicuticular waxes (WETTSTEIN-KNOWLES, 1971, 1974a, 1974b, 1976). Based on the data of the biochemical analysis from the cer-mutants, the biosynthetic pathway of different wax components could be deduced (WETTSTEIN-KNOWLES, 1976).

As a means to obtain more information about the genetic control of the structure and deposition of the epicuticular waxes in Arabidopsis, 53 isolated

eceriferum mutants were phenotypically analysed (using macroscopic and scanning-electron microscopic techniques). Based on this analysis the mutants are classified into different phenotypic categories, and a start is made for genetic analysis by diallel crosses.

MATERIAL AND METHODS

The 30 fast neutron, 11 X-ray, 10 EMS induced and 1 spontaneous eceriferum mutants of Arabidopsis thaliana (DELLAERT, 1979; KOORNNEEF unpublished), and also vc-2 (RÉDEI, 1965) used in the present study are listed in Table 1.

Seeds of the eceriferum mutants and of the wild type A. thaliana ecotype Landsberg, mutant 'erecta', were sown on perlite saturated with a standard nutritional solution. After 5 days at 2 °C (to break dormancy) the seeds were put to germinate at 24 °C under continuous illumination. Two weeks after sowing the seedlings were transplanted into soil in an air conditioned greenhouse. The culture medium and culture conditions applied, were as described by FEENSTRA (1965a) and OOSTINDIËR-BRAAKSMA and FEENSTRA (1973).

In order to characterize the mutants phenotypically, the following two methods were adopted:

A. Macroscopic determination of the wax coating on siliques, stems, and leaf-blades:
   Under greenhouse conditions the wax coating on rosette leaves was visually determined at the start of flowering and approximately two weeks later on stems and siliques. In analogy to the method used for the phenotype description of the barley eceriferum mutants (LUNDQVIST and WETTSTEIN, 1962), the wax coating of the wild type was denoted by ++, reduced wax coating by +, and absence of wax coating by -. The signs -/+ and +/++ were used if seasonal variation was observed.

B. Scanning-electron microscopic determination of the wax coating on siliques, stems and leaf-blades:

For the scanning-electron microscopic determination of the wax coating, plants were harvested approximately two weeks after the start of flowering. Per plant various plant-parts, namely (1) silique no 4, (2) 1 cm stem at silique no 4, (3) 1 cm stem at the first cauline leaf, and (4) occasionally a part of one rosette leaf, were exposed to liquid N_2 (a few seconds) and freeze-dried. The dried plant parts were mounted on object trays with silver paste and coated with gold. The objects were examined in a Jeol JSM-U3 scanning-electron microscope.

For the characterization of the epicuticular wax of wild type Arabidopsis, the wax of a wild type plant was removed with chloroform. Wild type plant parts with and without wax were compared and the ultrastructure of the wax extrusions was determined. Subsequently, the waxes of the eceriferum mutants was compared with those of the wild type and specific differences were described.

The eceriferum mutants in Arabidopsis were classified into a number of phenotypic categories, based on the visual phenotypic analysis of the wax coating on siliques and stems and taking into account other morphological deviations of the wild type (probably due to pleiotropic effects of the particular cer genes). A start was made for the genetic analysis of the mutants by complementation tests. Crosses were made as described by FEENSTRA (1965b).

RESULTS AND DISCUSSION

In Table 1 the phenotype description - macroscopic and scanning-electron microscopic - of the mutant collection is given.

Macroscopic phenotype description

At the moment of visual determination of the wax coating of the rosette leaves it was found that (1) the rosette leaves of some mutant lines had become necrotic, and (2) in other mutant lines, the leaves which were originally described as glossy, showed no difference in wax coating with those of the wild type. We, therefore,

decided to classify the mutants according to the visual description of the wax coating on siliques and stems, without taking into consideration the description of the leaves. This reduced the possible number of phenotypic formulas for wax coating to 25 (including wild type; ++ ++). Since moreover, within the group of about 400 barley eceriferum mutants described by LUNDQVIST and WETTSTEIN (1962) and LUNDQVIST et al. (1968), no mutants were found which had fewer wax on stems than on spikes, and because so far no such mutants were observed in Arabidopsis (i.e. fewer wax on stems than on siliques), it is proposed to restrict the number of phenotypic formulas for wax coating in Arabidopis to 15. These 15 phenotypic formulas are given in Table 1.

Within the group of mutants lacking the wax coating on siliques and/or stems, a further distinction is made amongst them, based on other phenotypic deviations from the wild type, namely semi-sterility and/or dwarfness. This is done because, as far as is known, all mutants which are allelic to mutant F_4, the one with a mutation on locus cer-1 localized on chromosome 1 (KOORNNEEF and van der VEEN, 1978), are also semi-sterile. Moreover, this characteristic does not recombine with the waxless character (van der VEEN, 1976 pers. comm.). We consider this to be a true pleiotropic effect of some genes. This semi-sterility, both male and female, is dependent on environmental conditions. During the winter the sterility is almost complete, although fertility can always be restored by covering the plant with a polythene bag (FEENSTRA, 1977 pers. comm.).

At present we can distinguish 7 main phenotypic categories (categories A through G in Table 1).

Scanning-electron microscopic determination of the wax coating

The use of the scanning-electron microscope provides important details of wax depositions. In Fig. 1 the wild type phenotype of the stem of Arabidopsis (Fig. 1a) and for comparison the phenotype of the chloroform treated stem of the wild type (Fig. 1b) are reproduced. The latter is

characterized by a smooth surface and, therefore, all epicuticular structures seen in Fig. 1a are wax structures. The epicuticular waxes of the wild type (stem and silique) are characterized by

1. An organized wax layer, and on top of it the following surface structures,
2. irrigular shaped plate-like wax structures, and
3. a high proportion of rodlet-shaped crystals, varying in size, which might be associated with a high β-diketone or ketone content of the wax, similar to the tube-like wax crystals in Brassica, Poa, Eucalyptus and barley (HALL et al., 1965; LUNDQVIST et al., 1968).

In Table 1 the ultrastructural composition of the wax coating for each mutant (as far as available) is given. When the scanning-electron microscopic observations of the eceriferum mutants are compared, it is observed that there is a significant difference ($p < 0.005$) between the occurrence of rodlet-shaped crystals in mutants belonging to the phenotypic main categories A, B, C and D, and in mutants belonging to the phenotypic main categories E, F, and G (Table 1).

In almost all mutants belonging to the phenotypic main categories A, B, C and D (i.e. mutants with visually reduced or absent wax coating of the siliques, and with wild type or reduced wax coating of the stems) the rodlet-shaped crystals are reduced (in size or frequency) or rare, 15 and 5 mutants, respectively (Fig. 2a). This suggests that in these mutants, genes which regulate the quantity of the rodlet-shaped wax extrusions are affected. Exceptions are D_2, in which the rodlet-shaped crystals are absent, and A_3, in which the ultrastructure of the wax coating is similar to the wild type.

In contrast it is found that in the mutants belonging to the phenotypic categories E, F and G (i.e. mutants with visual absence of wax coating on the siliques and the stems) the rodlet-shaped crystals are absent or rare, 11 and 3 mutants, respectively. In these mutants, also, the plate-like structures are absent (Fig. 2b) or reduced in size and/or frequency. (Fig. 2c). In mutant G_7 the plate-like wax structures are erect and clearly crystalline with sharp borders (Fig. 2d), unlike all other plate-like structures observed in Arabidopsis so far. The ultrastructural composition of the wax coating of the mutants,

visually classified as -- types, suggest that in most cases structural genes, responsible for the formation of specific wax components, are affected (in analogy with barley (WETTSTEIN-KNOWLES, 1976)).

The organized wax layer decreases in quantity in the wild type from the stem base towards the siliques. In the eceriferum mutants it is found that some mutants show a thick organized wax layer on the stems and siliques (denoted by + in Table 1). This layer occurs mainly ($0.10 < p < 0.25$) on mutants in which the other surface wax structures are severely affected (Fig. 2e), i.e. in mutants which are visually classified as -- types.

When the phenotypic categories A + B are compared with the phenotypic categories C + D, it is observed that there are no significant differences in the descriptions of the ultrastructures of the wax coating of the mutants. Although in the latter categories there is a tendency towards an increasing number of mutants in which the rodlet-shaped crystals are rare. Also, between the phenotypic categories F and G, the observed ultrastructures of the wax coating in the mutants are similar.

In Fig. 2f the epicuticular wax of a wild type Arabidopsis leaf is reproduced. It can be seen that the wax extrusions on the leaf are different in composition and frequency compared to the wax extrusions on stem and silique. Wax structures on the leaf are rare and no rodlet-shaped crystals are present. This may be due in contrast to the wax structures on the leaves of maize (LORENZONI and SALAMINI, 1975; BIANCHI, 1978) and barley (LUNDQVIST et al., 1968), Brassica, Poa and Eucalyptus (HALL et al., 1965), to the relatively short period during which wax is produced on leaf-blades of wild type Arabidopsis before the leaves die-off (the life-span of rosette leaves of Arabidopsis under the greenhouse conditions used, is approximately 2 or 3 weeks). Comparison of the wax on the leaf of the wild type with the wax on the leaf of mutants being visually characterized by glossy leaf-blades did not show distinct differences. Therefore, it was decided to classify these mutants in a separate mutant class.

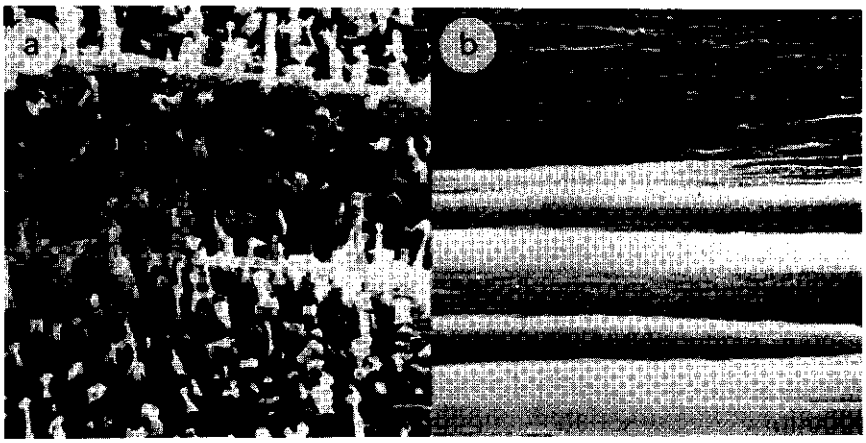

Fig. 1.

Fig. 1. Epicuticular wax structures on the stem of wild type (Fig. 1a) compared to the epicuticular surface of the stem of wild type after pretreatment with the wax solving chloroform (Fig. 1b) (enlargement; 1500 x).

Fig. 2. Epicuticular wax structures on different eceriferum mutants in Arabidopsis. (enlargement; 1500 x).

a Mutant D_4 (stem base); rodlet-shaped crystals are reduced in size and in frequency.

b Mutant vc-2 (G_5) (stem base); rodlet-shaped crystals and plate-like structures are absent, the wax layer is more developed than in the wild type.

c Mutant E_2 (stem base); rodlet-shaped crystals are absent, but not the plate-like structures.

d Mutant G_7 (silique); rodlet-shaped crystals are absent, the plate-like structures are erect and crystalline with sharp borders.

e Mutant F_5 (silique); thick organized wax layer on silique, other surface wax structures are severely affected.

f Wild type leaf; wax extrusions are rare in frequency and size, no rodlet-shaped crystals are present.

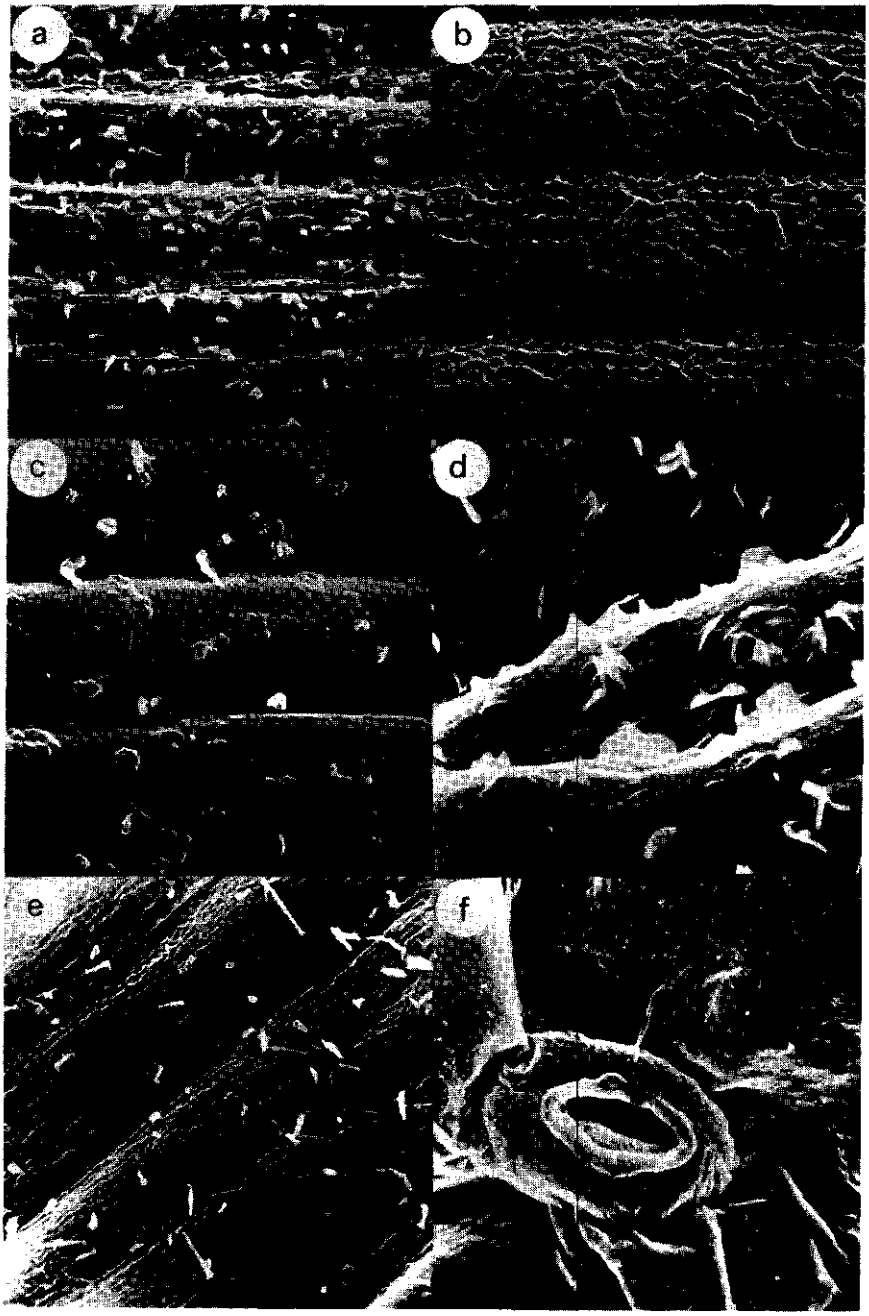

Fig. 2.

Genetic analysis

A start is made for genetic analysis of the eceriferum mutants by diallel crosses (Table 2, 3 and 4). Based on the results obtained so far, the minimum and maximum number of loci per main phenotypic category has been determined (Table 1). In order to do this, it has been assumed that mutants from the different main categories are non-allelic. The results of crosses between mutants of different main categories obtained so far (Table 5) support this assumption, since no cases of allelism were detected in the crosses, except the mutant line A_8, which did not complement D_3 and D_8. This mutant line A_8 carried several other mutations, e.g. lateness, curly leaves and siliques without petioles. It is suggested that these other mutations may have interfered with the scoring for wax coating. All eceriferum mutants used in the crossings were completely recessive, except D_2 which is semi-dominant. The complementation observed betwee the non-allelic mutants (within and between different phenotypic categories - Table 2, 3, 4 and 5) was always complete.
We do not take intragenic complementation into consideration since this is more often than not imcomplete.

Comparison of the wax composition in allelic mutants and in non-allelic mutants

It is found that allelic mutants have an identical composition of the epicuticular wax structures. There might, however, be some variation in the density of the different wax compounds (for example the allelic mutants A_1 and A_6 differ in frequency of the rods). It should be noted that the allelic mutants F_1, F_2 and F_4 have the same epicuticular wax structure (i.e. thick organized wax layer as well as absence of plate-like structures and rodlet-shaped crystals) as the allelic mutants G_5 and G_8. On the basis of semi-sterility these mutants are classified into different phenotypic categories (i.e. F and G) and it is proved that they are non-allelic. From the results of the scanning-electron microscopic analysis of the epicuticular wax structures of the stems and siliques of non-allelic mutants within a phenotypic category, it

Table 2. Results of the complementation tests[1)] between the mutants of the phenotypic categories A and B, i.e. eceriferum mutants with visually reduced wax coating on the siliques, and normal or reduced wax coating on the stems.

	A_1, A_6	A_2	A_3	A_4	A_9	A_{10}	B_1	B_2	B_3
A_1, A_6	0	+	+	+		+		+	
A_2	+	0	+		+	+		+	
A_3	+		0					+	
A_4	+			0				+	
A_9		+			0	+			
A_{10}	+	+			+	0		+	
B_1							0		+
B_2	+	+	+	+		+		0	
B_3							+		0

1) + = complete complementation

Table 3. Results of the complementation tests[1)] between the mutants of the phenotypic categories C and D, i.e. eceriferum mutants without visual wax coating on the siliques, and normal or reduced wax coating on the stems.

	C_1	C_2	C_3	D_1	D_2	A_8, D_3, D_8	D_4	D_5	D_6	D_7	D_9	D_{13}
C_1	0			+		+						
C_2		0		+		+			+			+
C_3			0			+						
D_1	+	+		0		+			+			+
D_2					0	±						±
A_8, D_3, D_8	+	+	+	+	±	0			+	+		+
D_4							0	+		+	+	
D_5							+	0		+		
D_6		+		+		+			0			
D_7						+	+	+		0		
D_9							+				0	
D_{13}		+		+	±	+						0

1) + = complete complementation
± = incomplete complementation (D_2 = partially dominant)

Table 4. Results of the complementation tests[1)] between the mutants of the phenotypic categories E, F and G, i.e. eceriferum mutants without visual wax coating on the siliques, and stems.

	E_2	F_1, F_2, F_4, F_6, F_7	F_3	F_5	G_1	G_3	G_5, G_8	G_6	G_7
E_2	0								
F_1, F_2, F_4, F_6, F_7		0		+		+	+	+	+
F_3			0		+			+	
F_5		+		0			+		+
G_1			+		0		+		+
G_3		+				0	+		+
G_5, G_8		+		+	+	+	0		+
G_6		+	+					0	+
G_7		+		+	+	+	+	+	0

[1)] + = complete complementation

Table 5. Results of the complementation tests between the phenotypic category D and the phenotypic categories A, B, E, F and G.
The mutants from phenotypic category D mentioned, showed complementation with the mutants referred to in this Table.
The cross $D_2 \times B_3$ showed incomplete complementation.
In addition, B_1 did not complement with C_4, E_2, F_3 and G_1, and B_3 did not complement with C_4.

Phenotypic categories					
D	A	B	E	F	G
D_1			E_2		
D_2		B_3			
A_3, D_3, D_8	A_1, A_6; A_2	B_1; B_3	E_2	F_1, F_2, F_4, F_6, F_7; F_5	G_5, G_8
D_4		B_2			
D_5		B_2			
D_6			E_2		
D_7	A_1, A_6; A_2				
D_9		B_1		F_3	
D_{13}				F_1, F_2, F_4, F_6, F_7	

follows that especially for mutants belonging to the phenotypic categories F and G, the scanning-electron microscopic analysis may reveal non-allelic mutants. However, the results also show that equality in epicuticular wax structures of mutants of a specific phenotypic category does not necessarily imply that the mutants are allelic. This seems especially true for mutants in which, compared to the wild type, the epicuticular wax structures are similar, but reduced in size and/or frequency.

CONCLUSIONS

The phenotypic analysis of the wax coating on stems and siliques of the 53 eceriferum mutants and the wild type, by macroscopic and by scanning-electron microscopic techniques, revealed that the eceriferum mutants in Arabidopsis are characterized by a change in composition (ultrastructural) and/or quantity (visual and ultrastructural) of the wax coating. In general, it has been observed that in mutants with visually reduced wax, the wax is ultrastructurally characterized by a change in quantity of the rodlet-shaped crystals. The wax coating of mutants with visually absent wax on the siliques and stems, is in general ultrastructurally characterized by a change in composition. The scanning-electron microscopic analysis of the composition of the wax coating of the latter mutants has shown that in these mutants specific wax "structures", such as rodlet-shaped crystals and/or plate-like structures and/or the organized wax layer, are severely affected. Therefore, it is suggested that especially within this mutant group, the mutants which differ in their composition of the wax coating, might be of interest for the biochemical examination of the epicuticular waxes in Arabidopsis.

The limited results of the genetic analysis of the eceriferum mutants obtained so far, indicate that the 53 eceriferum mutations are located at a minimum of 14 loci and at a maximum of 44 loci. This suggests that the number of eceriferum loci in Arabidopsis is in the same order of magnitude as the number of eceriferum loci in barley (LUNDQVIST, 1975).

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CHAPTER VI

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COMPARISON OF SELECTION METHODS FOR SPECIFIED MUTANTS IN SELF-FERTILIZING CROPS

Theoretical approach

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Abstract

COMPARISON OF SELECTION METHODS FOR SPECIFIED MUTANTS IN SELF-FERTILIZING CROPS: THEORETICAL APPROACH.

The most efficient method to extract lines, mutated for a specified character, fixed in an otherwise undisturbed genetic background is discussed. A mutation breeding programme, based on detection of M_1 cells heterozygous for the desired mutation by means of single-seed progeny testing, and the selection of the desired mutant line in the progeny of these M_1 cells (spare seed of M_2 is available) is proposed. The optimum mutation rate (m_{opt}) for detecting the desired mutation depends on the number of loci that, when mutated and in the homozygous state, do not allow expression of the desired mutation. For probability, p, to select in the progeny of an M_1 cell, heterozygous for the desired mutant, a mutant with desired phenotype, the mutation rate has an upper limit, m_{max}. This depends mainly on the number of loci that cannot mutate desirably (L_2) and on the progeny size N. In general $m_{max} < m_{opt}$, and thus the maximum mutant yield is determined by m_{max}. From M_2 onwards selection is for an undisturbed genetic background, within the M_1 progenies heterozygous for the desired mutation. For high values of L_2 the degree of heterozygosity in plants with desired phenotype in the M_2 will be high when the mutation rate per allele $\cong m_{max}$. In this case negative mass selection up to M_4 followed by line selection in M_5 for the desired genotype is proposed. Compared with the optimum mutation rate for selecting mutant plants with undisturbed genetic background direct in the M_2, the upper limit of the mutation rate for the proposed breeding programme is 9 to 10 times higher. The number of independent mutants with undisturbed genetic background obtained are 1 to 100 times (depending on M_2 line size) the number obtained when conventional mutation breeding procedures are used.

INTRODUCTION

Freisleben and Lein [1] were the first to construct theoretical models that could serve as a basis for the design of selection procedures in mutation breeding. These models were later modified, extended and generalized by various authors [2–13]. The selection procedures based on these models aim at isolating either a

$f(m)_A$ = the relative frequency of the plants in the progeny of an M_1 cell, heterozygous for the desired mutation, that are homozygous for desirable mutated alleles at one or more L_1 loci, and that have no negatively mutated allele(s) at the remaining L_1 loci, and no mutated alleles at the L_2 loci (which includes the L_3 loci)

$f(m)_B$ = the relative frequency of plants in the progeny an an M_1 cell, heterozygous for the desired mutation, that are heterozygous or homozygous for desirable mutated alleles at one or more L_1 loci, and that have no negatively mutated allele(s) in the homozygous state at the remaining L_1 loci, and no mutated alleles in the homozygous state at the L_2 loci (which includes the L_3 loci).

These symbols are similar to the symbols used by Yonezawa and Yamagata [12]. New are the definitions of the L_3 loci, $f(m)_I$, $f(m)_A$ and $f(m)_B$.

The L_3 loci are loci that can only mutate in an undesirable way and that when mutated and in homozygous state cause, for example, non-germination, lethality or sterility, through which the L_1 loci cannot come to phenotypic expression. The number of the L_3 loci thus depends on the selection criterion. The minimum number of the L_3 loci are the loci that affect germination and seedling growth.

The frequency $f(m)_I$ is related to the probability of detecting the desired mutations in the M_2 generation. The frequency $f(m)_{II}$ is the frequency of mutants for direct use in the M_2 generation, the frequency regarded as an index for efficiency of breeding by Yonezawa and Yamagata [12]. The frequencies $f(m)_A$ and $f(m)_B$ are directly related to the probability of selecting the 'ideal recombinant' in the progeny of an M_1 cell, heterozygous for the desired mutation.

Formulae

If the original allele A mutates to desirable allele a and undesirable allele a', with mutation rates $m\alpha$ and $m(1-\alpha)$ respectively, the relative genotype frequencies for this locus in the M_1 cells are:

Genotype:	AA	Aa	Aa'	aa	aa'	a'a'
Frequency:	$(1-m)^2$	$2\alpha m(1-m)$	$2(1-\alpha)m(1-m)$	α^2m^2	$2(1-\alpha)\alpha m^2$	$\{(1-\alpha)m\}^2$
For $m^2 \cong 0 \rightarrow$	$1-2m$	$2\alpha m$	$2(1-\alpha)m$	–	–	–

After self-fertilization the relative genotype frequencies in the M_2 for this locus are:

Genotype:	AA	Aa	Aa'	aa	a'a'
Frequency:	$1-\frac{3}{2}m$	αm	$(1-\alpha)m$	$\frac{1}{2}\alpha m$	$\frac{1}{2}(1-\alpha)m$

The relative frequencies of genotypes at the L_1 loci in the M_2 are:

Genotype	Frequency	$0 \leqslant \alpha \leqslant 1$	definition of f(m) for $\alpha = 1$	$\alpha = \frac{1}{2}$
AA+Aa+aa	$\lvert f_1(m)\rvert^{L_1}$	$\lvert 1-\frac{3}{2}(1-\alpha)m\rvert$	1	$1-\frac{3}{4}m$
AA+Aa	$\lvert f_2(m)\rvert^{L_1}$	$\lvert 1-(\frac{3}{2}-\alpha)m\rvert$	$1-\frac{1}{2}m$	$1-m$
AA+Aa+Aa'+aa	$\lvert f_3(m)\rvert^{L_1}$	$\lvert 1-\frac{1}{2}(1-\alpha)m\rvert$	1	$1-\frac{1}{4}m$
AA+Aa+Aa'	$\lvert f_4(m)\rvert^{L_1}$	$\lvert 1-\frac{1}{2}m\rvert$	$1-\frac{1}{2}m$	$1-\frac{1}{2}m$

In the same way the relative frequencies of genotypes at the L_2 loci (which cannot be changed desirably, i.e. $\alpha = 0$) are:

Genotype	Frequency
BB+Bb	$\lvert f_5(m)\rvert^{L_2} = \lvert 1-\frac{1}{2}m\rvert^{L_2}$
BB	$\lvert f_6(m)\rvert^{L_2} = \lvert 1-\frac{3}{2}m\rvert^{L_2}$

Similarly for L_3 loci.

THE OPTIMUM MUTATION RATE FOR DETECTING THE DESIRED MUTATION

The relative frequency of mutants that can be detected in the M_2, $f(m)_I$, is defined as the frequency of mutants that are **homozygous** for desirable mutated alleles at one or more loci among the L_1, carrying no negatively mutated allele **homozygous** at the remaining L_1 loci and no mutated alleles in **homozygous** state at the L_3 loci. Thus:

$$\begin{aligned} f(m)_I &= \{|f_3(m)|^{L_1} - |f_4(m)|^{L_1}\}\ |f_5(m)|^{L_3} \\ &= \{|1-\tfrac{1}{2}(1-\alpha)m|^{L_1} - |1-\tfrac{1}{2}m|^{L_1}\}\ |1-\tfrac{1}{2}m|^{L_3} \\ &\cong \{e^{-\frac{1}{2}(1-\alpha)mL_1} - e^{-\frac{1}{2}mL_1}\}\ e^{-\frac{1}{2}mL_3} \end{aligned}$$

or

$$f(m)_I \cong e^{-\frac{1}{2}m|(1-\alpha)L_1+L_3|} - e^{-\frac{1}{2}m(L_1+L_3)}$$

$$\frac{df(m)_I}{dm} \cong -\tfrac{1}{2}|(1-\alpha)L_1+L_3|e^{-\frac{1}{2}m|(1-\alpha)L_1+L_3|} + \tfrac{1}{2}|L_1+L_3|e^{-\frac{1}{2}m(L_1+L_3)}$$

$f(m)_I$ maximum is obtained when $\frac{df(m)_I}{dm} = 0$

number of L_1 and L_2 loci. Calculating in the same way as before and using the approximations

$$\text{Ln}\,(1-\tfrac{3}{2}m) \cong -\tfrac{3}{2}m$$

and

$$\text{Ln}\,(1-\tfrac{3}{2}(1-\alpha)m) \cong -\tfrac{3}{2}(1-\alpha)m$$

one obtains

$$m_{\max A} \cong \frac{2\,\text{Ln}\,0.25 - 2\,\text{Ln}\,f'(m)_A}{3|(1-\alpha)(L_1-1)+L_2|}$$

$$m_{\max A} \cong \frac{2\,\text{Ln}\,0.25 - 2\,\text{Ln}\,f'(m)_A}{3\,L_2} \qquad \text{for } \alpha = 1$$

$$m_{\max A} \cong \frac{2\,\text{Ln}\,0.25 - 2\,\text{Ln}\,f'(m)_A}{3|(L_1-1)+L_2|} \qquad \text{for } \alpha \to 0$$

$$\frac{m_{\max A\,(\alpha=1)}}{m_{\max A\,(\alpha\to 0)}} = \frac{(L_1-1)+L_2}{L_2} \qquad \text{which is} \cong 1 \qquad \text{for } L_1 \ll L_2$$

The upper limit of the mutation rate per allele, $m_{\max A}$, is given in Fig.2. It is seen that $m_{\max A}$ is mainly determined by the value of L_2 and N.

As suggested in the introduction, apart from the genotypes called **desired genotypes** there is another important category of genotypes that can also be used for breeding purposes called **useful genotypes**. When in the progeny of useful genotypes selection is for an undisturbed genotypic background, the **desired genotypes** can be obtained.

The relative frequency of **desired + useful genotypes**, $f(m)_B$, in the progeny of an M_1 cell heterozygous for the desired mutation is the relative frequency of plants (in the progenies of these M_1 cells) that are heterozygous or homozygous for desirable mutated alleles at one or more L_1 loci and that have no negatively mutated allele(s) in homozygous state at the remaining L_1 loci and no mutated alleles in homozygous state at the L_2 loci.

$$f(m)_B = \tfrac{3}{4}|f_3(m)|^{L_1-1}|f_5(m)|^{L_2}$$

$$= \tfrac{3}{4}|1-\tfrac{1}{2}(1-\alpha)m|^{L_1-1}|1-\tfrac{1}{2}m|^{L_2}$$

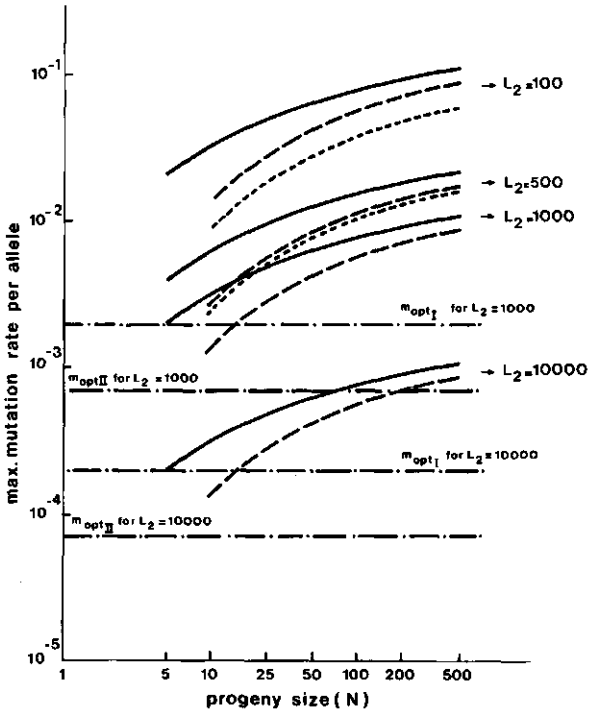

FIG.3. The upper limit of the mutation rate per allele for probability, p, to select the desired or useful genotype *in the M_1 progeny, size N, of an M_1 cell heterozygous for the desired mutation.* —— *$p = 0.80$, $\alpha = 1$;* – – – – *$p = 0.99$, $\alpha = 1$;* ····· *$p = 0.99$, $\alpha \to 0$, $L_1 = 50$.*

The probability, p, to select in the M_1 progeny of a cell heterozygous for the wanted mutation, **useful + desired genotypes**, is

$$p = 1 - |1 - f(m)_B|^N$$

Thus like $f'(m)_A$, $f(m)_B$ has a minimum value, $f'(m)_B$, depending on progeny size N and p.

TABLE I. THE RATIO $m_{max\ B}/m_{maxA} = 0.86 + 3\ Ln\ f'(m)/1.39 + Ln\ f'(m)$ (for $Ln\ f'(m) < -1.39$)

p \ N	10	25	50	100	200	500
0.80	9.35	5.37	4.61	4.20	3.96	3.76
0.99	–	11.51	6.15	4.94	4.38	4.00

And thus the mutation rate has an upper limit, $m_{max\ B}$, determined by $f'(m)_B$, α, L_1 and L_2. Again, calculating in the same way as before and using the approximations $Ln(1-\frac{1}{2}m) \cong -\frac{1}{2}m$ and $Ln(1-\frac{1}{2}(1-\alpha)m) \cong -\frac{1}{2}(1-\alpha)m$ one obtains

$$m_{max\ B} \cong \frac{2\ Ln\ 0.75 - 2\ Ln\ f'(m)_B}{(1-\alpha)(L_1-1)+L_2}$$

$$m_{max\ B} \cong \frac{2\ Ln\ 0.75 - 2\ Ln\ f'(m)_B}{L_2} \qquad \text{for } \alpha = 1$$

$$m_{max\ B} \cong \frac{2\ Ln\ 0.75 - 2\ Ln\ f'(m)_B}{L_1 - 1 + L_2} \qquad \text{for } \alpha \to 0$$

$$\frac{m_{max\ B\ (\alpha=1)}}{m_{max\ B\ (\alpha\to 0)}} = \frac{(L_1-1)+L_2}{L_2} \qquad \text{which is} \cong 1 \qquad \text{for } L_1 \ll L_2$$

Figures 2 and 3 give the values of $m_{max\ A}$ and $m_{max\ B}$ respectively, for increasing M_1 progeny size, N. It is seen that:

(a) There is a minimum in progeny size for probability, p, to select the desired genotype or the desired + useful genotypes
(b) m_{max} is mainly determined by the number of L_2 loci
(c) L_1 only has an effect on m_{max} when the number of L_2 loci is small
(d) The effect of an increase in N diminishes above a certain value of N
(e) The ratio $m_{max\ B}/m_{max\ A}$ increases with decreasing value of N (Table I). $M_{max\ B}$ is 4 to 10 times $m_{max\ A}$.

DISCUSSION

For values of $L_3 \ll L_2$ the mutation rate per locus that will give the maximum mutant yield is determined by the upper limit of the mutation rate, m_{max}, for the proposed breeding procedure, i.e. selection of M_1 plants by means of single-seed progeny testing, and M_2 spare seed per M_1 plant is kept in store.

When selection in M_2 is for the **desired genotype**, the suggested breeding procedure allows mutation rates 1 to 8.5 times ($N > 20$) the optimum mutation rate calculated by Yonezawa and Yamagata [12] for **direct** selection (m_{optII} in Fig.2). When selection in M_2 is for desired + useful genotypes, the mutation rate may increase 2.85 to 14.29 times ($N > 5$) m_{optII} (see Fig.3). Therefore, contrary to the conclusion of Yonezawa and Yamagata [12], to obtain mutant lines for direct use, mutagenic treatments are required that give effective mutation rates.

If L_3 is replaced by L_2 in formula $f(m)_I$, this formula gives the frequency of genotypes in the M_2 that are homozygous for the desired mutation at one or more loci of the L_1 and that have no negatively mutated allele(s) homozygous at the remaining loci of the L_1 and no mutated alleles in homozygous state at the L_2 loci. From these genotypes the **desired genotype** can be obtained, when from M_3 onwards selection is for an undisturbed genotypic background. Therefore m_{optI} (L_3 replaced by L_2) is the optimum mutation rate for **direct** selection of M_2 **mutant** (aa) plants **within** the group desired + useful genotypes. The mutation rate per allele may increase ($N > 10$) when selection in M_2 is for these genotypes and M_2 spare seed is available.

Then it follows that the mutation rates per allele may obtain a larger value than m_{optI} and m_{optII}, when M_2 spare seed is available (m_{max} depends on N and L_2 mainly).

However, we must realize that the degree of heterozygosity for unwanted alleles in the selected plants increases with increasing number of L_2 and with m, and thus the ease of selecting the desired genotype in their progenies decreases. With the mutagens and treatments used in mutation breeding mutation rates per allele of 10^{-4} to 10^{-3} and even higher are obtained [12, 18].

With a mutation rate of 10^{-3} per allele, the probability, p, to select a desired or useful genotype in the progeny of an M_1 cell heterozygous for the desired mutation is 80% for $L_2 \cong 10^4$ and $N \cong 100$. For mutation rate m, the mean number of mutations per cell, n, is

$$n = m(L_1 + L_2) \cong mL_2 \qquad \text{for } L_1 \ll L_2$$

thus

$$n = 10 \text{ for } m = 10^{-3} \text{ and } L_2 = 10^{f4}$$

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SUMMARY AND CONCLUSIONS

The genetic effects of X-ray and fast neutron seed-irradiation of *Arabidopsis thaliana* (L.) Heynh., and the influence of a pre-irradiation treatment with the radio-protector dithiothreitol (DTT), are the main subjects of this thesis.

Chapters I and II deal with the effects of radiation - with or without a pre-irradiation treatment - on M_1-plant development, and on ovule sterility, embryonic lethality and frequency of chlorophyll deficient embryos in the M_1-siliques. These investigations revealed that:

- with increasing radiation doses, no further increase in chlorophyll mutant frequency is found at relatively high doses, for *Arabidopsis* progenies of the "main" inflorescence;
- this observed "saturation" in chlorophyll mutant frequency is, at least partly, due to scoring of progenies from initial cells - forming the sub-epidermal cell layer - which have heterogeneous radiation-sensitivities;
- this heterogeneity, which was indicated after some of the X-ray as well after some of the fast neutron treatments, is most likely caused by replacement of the original initial cells by less sensitive cells;
- fast neutron-irradiation of pre-soaked (3 hours) *Arabidopsis* seeds is approximately seven times more effective than X-irradiation with respect to the induction of M_1-ovule sterility and M_2-embryonic lethality;
- dithiothreitol (DTT) provides considerable protection against X- and fast neutron-irradiation, using M_1-ovule sterility, and, to a lesser extent, the number of embryonic lethals and chlorophyll mutants as parameters. This, as compared to irradiation without DTT, leads to more mutants at a given level of M_1-ovule sterility.

To compare the genetic effects of different mutagens, a standardized mutagen dose with reference to a specific genetic effect is necessary. The applied doses of X-rays and fast neutrons as reported in chapter II - the X-ray doses were seven times the applied fast neutron doses - induced similar levels of ovule sterility and embryonic lethality in *Arabidopsis*. Therefore, these doses were applied in the analysis of the X-ray and fast neutron induced mutant spectra. In chapter III the results of a study on the effects of (1) radiation type, (2) radiation dose and (3) a DTT pre-irradiation treatment, on the spectra of X-ray- and fast neutron-induced morphological mutants are reported. These investigations demonstrated that;

- the X-ray- and fast neutron-induced spectra of different types of viable mutants vary significantly;
- throughout the studied dose range, some mutant types occur significantly more frequently with fast neutrons than with X-rays, i.e. mutants with loosely packed leaves with long petioles and the *ecriferum* mutants;
- mutants with closely packed leaves with short petioles were more frequently induced with X-rays. Other mutants, e.g. the vital chlorophyll deficient mutants, were induced with equal frequencies by X-rays and fast neutrons in the studied range of radiation doses;
- certain mutant types are relatively less frequent after irradiation in the presence of DTT. Since DTT preferentially protects against radiation-induced single and double strand-breaks, this observation suggests that these mutants more often than other mutants, originate from strand-break damage. It is striking that these mutant types were more frequently induced with fast neutrons than with X-rays.

In order to compare different mutagenic treatments, the mutation frequency should be expressed per cell. For the transformation of the

mutant frequency scores, expressed as the number of mutant plants among the total number of M_2-plants (or embryos in the case of Müller's embryo test), a mutant frequency of twenty per cent in the offspring of a heterozygous parent and an equal viability of mutant and non-mutant plants have been assumed for all treatments in chapters I, II and III.

In chapter IV, the segregation frequency of mutants induced by various X-ray and fast neutron doses, with and without DTT pre-treatment is studied.

From this investigation the following conclusions can be drawn:

- the average mutant segregation frequencies of X-ray- and fast neutron-induced mutants are equal;
- a DTT pre-irradiation treatment does not influence the segregation frequency of radiation-induced mutants;
- there is no consistent change in the mutant segregation frequency with increasing radiation dose;
- progenies from single flowers in the top of the M_1-main inflorescence originate, in general, from genetically homogeneous tissue;
- the average mutant segregation frequency is 21.5 per cent. Since no difference in viability of mutant and non-mutant plants is observed, the mutant deficit is due to reduced transmission of the mutant gene through (probably the male) gametophytes;
- the observations lead to the conclusion that the mutant segregation frequency in the offspring of a heterozygous parent can be estimated from the M_2-segregation frequencies, provided that progenies from a single flower from the top of the M_1-main inflorescence are tested. The obtained value can then be used directly for the calculation of the mutant frequency per cell.

Arabidopsis mutants with a reduced or absent wax coating, i.e. *eceriferum* mutants, are relatively more frequently induced with fast neutrons than with X-rays (chapters III and V). The phenotypic analyses - by macroscopic and scanning-electron microscopic techniques, reported in chapter V - of the wax coating on stems and siliques of the wild type and of 30 fast neutron -, 11 X-ray-, and 10 EMS-induced and of 1 spontaneous mutant, indicate that:

- in *eceriferum* mutants with visually reduced wax coating, the quantity of rodlet-shaped wax extrusions is less than in the wild type;
- in *eceriferum* mutants, with visually absent wax coating, the wax extrusions are affected in specific ways. Compared to the wild type, the rodlet-shaped crystals are absent or rare; the plate-like wax structures are absent or reduced, and often there is, in contrast to the wild type, a thick organised wax layer present on the siliques and stems.

Genetic analysis of the *eceriferum* mutants, reported in chapter V revealed that the mutants were conditioned by many different loci (minimum 14, maximum 44). The ultrastructural research of the *eceriferu* mutants with absent wax coating demonstrated that, at the level of resolution of the scanning-electron microscope, we can distinguish between differential effects of different loci. However, the number of mutants per locus was still too low to pronounce upon X-ray or fast neutron specificity for individual loci.

In chapter VI, various methods to select lines, mutated for a specific characteristic and with an otherwise undisturbed genetic

background, are discussed. A mutation breeding programme is proposed. It is based on the determination of M_1-heterozygous plants by means of single seed progeny testing. Subsequently, the desired mutant line is selected in the progeny of these M_1-plants of which M_2 spare seed is available. As compared to M_2-bulk testing, the mutation frequency can be increased considerably with the proposed method. Besides, the number of independent mutants is higher compared to M_1-progeny testing (without spare seed) and M_2-bulk testing. The mutant frequency and the number of spare seed determine to what extent.

SAMENVATTING EN CONCLUSIES

Het centrale thema van dit proefschrift omvat het onderzoek naar de genetische effecten van Röntgen en snelle neutronen bestraling van *Arabidopsis thaliana* (L.) Heynh. zaden en de invloed daarop van een voorbehandeling met de tegen straling beschermende stof dithiothreitol (DTT).

Het onderzoek naar de bestralings effecten, al dan niet met een DTT voorbehandeling, op de ontwikkeling van de M_1-plant en op de fertiliteit van de eicellen, de letaliteit van embryos en de frequentie van chlorophyl mutanten in de M_1-hauwen is beschreven in de hoofdstukken I en II. Dit onderzoek liet zien dat:

- bij hogere stralingsdoses de frequentie van chlorophyl mutanten in de hauwen van de M_1-hoofdas niet meer toeneemt bij verdere verhoging van de doses;
- deze "verzadiging" van de chlorophyl mutanten frequentie ten dele of geheel kan worden toegeschreven aan het scoren van nakomelingen afgeleid van (subepidermis vormende) initiaal cellen die verschillen in stralingsgevoeligheid;
- dit verschil in stralingsgevoeligheid waarschijnlijk zijn oorzaak vindt in vervanging van de oorspronkelijke initiaal cellen door minder gevoelige cellen, zowel na Röntgen als na snelle neutronen bestraling;
- betreffende de inductie van steriliteit en embryonaal letaliteit bestraling met snelle neutronen van voorgeweekte *Arabidopsis* zaden ongeveer zeven maal zo effectief is als bestraling met Röntgen stralen;

- dithiothreitol (DTT) een goede bescherming geeft tegen Röntgen stralen en snelle neutronen. DTT vermindert duidelijk de steriliteit, en in geringere mate ook de embryonaal letaliteit en de frequentie van chlorophyl mutanten. De grotere bescherming tegen de inductie van steriliteit is waarschijnlijk het gevolg van specifieke bescherming door DTT tegen chromosoom breuken.

Om de genetische effecten van verschillende mutagentia te kunnen vergelijken is het gebruik van een standaard mutagene dosis met een specifiek genetisch effect noodzakelijk. De toegepaste doses Röntgen stralen en snelle neutronen - de Röntgen doses waren zeven maal zo hoog als die van snelle neutronen - induceerden, zoals in hoofdstuk II is vermeld, vergelijkbare niveaus van steriliteit en embryonaal letaliteit bij *Arabidopsis*. Daarom werden deze doses gebruikt om het verschil tussen de door Röntgen stralen en snelle neutronen geïnduceerde mutanten spectra en de invloed hierop van een DTT voorbehandeling te onderzoeken. Uit dit onderzoek, beschreven in hoofdstuk III, bleek dat:

- er significante verschillen bestaan tussen de door Röntgen stralen en door snelle neutronen geïnduceerde mutanten spectra;
- sommige typen mutanten in groter aantal voorkomen na bestraling met snelle neutronen dan na Röntgen bestraling, te weten de mutanten met ver uit elkaar staande rozet bladeren met lange bladsteel en de *ecerifera* mutanten;
- mutanten met dicht opeenstaande brede rozet bladeren met korte bladsteel in grotere mate voorkomen na Röntgen bestraling. Andere mutanten, b.v. chlorophyl mutanten, worden relatief even frequent door snelle neutronen als door Röntgen stralen geïnduceerd;
- bepaalde typen mutanten relatief minder vaak voorkomen na een DTT voorbehandeling. Vermoedelijk zijn deze mutanten vaker door chromosoom of chromatide breuken ontstaan dan het geval is bij

andere mutanten, omdat DTT specifiek beschermt tegen inductie van breuken. Opmerkelijk is dat deze mutant typen vaker voorkomen na bestraling met snelle neutronen dan na Röntgen bestraling.

Ten einde verschillende mutagene behandelingen te vergelijken, dient de mutatie frequentie per cel weergegeven te worden. Bij het omrekenen van de mutanten frequentie, uitgedrukt in het aantal mutanten per totaal aantal M_2-planten (of embryos, in het geval van Müller's embryo toets), is in de hoofdstukken I, II en III aangenomen dat de mutanten frequentie in de nakomelingschap van een heterozygote ouder-plant twintig procent bedraagt en dat de levensvatbaarheid van de mutanten gelijk is aan die van niet mutanten.

In hoofdstuk IV zijn de resultaten van een onderzoek naar deze frequentie en levensvatbaarheid van de door Röntgen stralen en snelle neutronen geïnduceerde mutanten weergegeven. Uit dit onderzoek blijkt dat:

- er geen significant verschil is tussen de door Röntgen stralen en de door snelle neutronen geïnduceerde mutanten wat betreft de frequentie waarmee ze in de nakomelingschap van een heterozygote ouder-plant voorkomen;
- er geen systematische verandering in deze frequentie optreedt bij toenemende bestralingsdoses;
- er geen verschil is waargenomen in levensvatbaarheid tussen mutanten en niet mutanten;
- de gemiddelde frequentie van mutanten in de nakomelingschap van een heterozygoot 21.5 procent bedraagt. Het tekort aan mutanten is veroorzaakt door gameten selectie (certatie);
- nakomelingen van een bloem in de top van de M_1-hoofdas in het

algemeen voortkomen uit genetisch homogeen weefsel, m.a.w. dat er geen binnen-bloem chimaerie is.

Uit deze gegevens (hoofdstuk IV) blijkt dat de gemiddelde frequentie van mutanten in de nakomelingschap van een heterozygoot voor alle toegepaste bestralingen bepaald kan worden uit de splitsingsverhoudingen in de M_2, tenminste wanneer de nakomelingschappen van één hauw van de top van de M_1-hoofdas getoetst worden. De gevonden waarde kan dan gebruikt worden om rechtstreeks de mutatiefrequentie per cel te berekenen.

Arabidopsis mutanten met een gereduceerde waslaag of zonder waslaag op stengel en hauwen, de zgn. *eceriferum* mutanten, worden relatief vaker met snelle neutronen dan met Röntgen stralen geïnduceerd. Deze mutanten zijn met behulp van een rasterelektronenmicroscoop (REM) fenotypisch gekarakteriseerd (hoofdstuk V). Hieruit bleek dat:

- in *eceriferum* mutanten, met een op het oog gereduceerde waslaag, de staafvormige was structuren in het algemeen geringer in aantal of korter zijn dan in niet mutanten;
- wanneer men de waslaag van *eceriferum* mutanten, met een op het oog ontbrekende waslaag, vergelijkt met die van normale planten, bepaalde was structuren geheel ontbreken of anders zijn. Staafvormige structuren zijn zeldzaam of afwezig; plaatvormige structuren zijn geringer in aantal, omvang of afwezig en vaak is er, in tegenstelling tot normale planten, een dikke amorfe waslaag aanwezig op stengel en hauwen.

De kruisingsexperimenten, beschreven in hoofdstuk V, toonden aan dat de *eceriferum* mutanten door een groot aantal verschillende loci bepaald worden (minimaal 14, maximaal 44). Het aantal mutanten per

locus was te gering om een uitspraak te doen over verschil in specificiteit tussen Röntgen en snelle neutronen voor afzonderlijke loci. Het rasterelektronenmicroscopisch onderzoek van mutanten met een op het oog ontbrekende waslaag, toonde aan dat op deze wijze specifieke effecten van verschillende loci kunnen worden vastgesteld.

In hoofdstuk VI zijn verschillende selectie methoden bij zelfbevruchters besproken voor het verkrijgen van specifieke mutanten die, met uitzondering van de gewenste mutatie, genotypisch gelijk zijn aan het uitgangsmateriaal. Er is voorgesteld om eerst de M_1-planten op te sporen die heterozygoot zijn voor de gewenste mutatie d.m.v. het toetsen van een nakomeling per plant. Vervolgens kan men dan na uitzaai van M_2-reserve zaad van deze heterozygote M_1-planten de gewenste mutante M_3-lijnen selecteren. Op grond van een theoretisch model wordt aangetoond dat met deze selectie methode de mutatie frequentie per locus hoger kan zijn dan bij M_2-massa selectie. Bovendien is, bij een gegeven mutatiefrequentie en een bepaalde omvang van de M_2-populatie, het aantal onafhankelijke mutanten met het gewenste genotype dat zo verkregen wordt groter dan door middel van M_2-massa selectie of door middel van afzonderlijk toetsen (zonder reserve zaad) van M_1-nakomelingschappen. Hoeveel groter dit aantal is, is afhankelijk van de mutatiefrequentie en de hoeveelheid reserve zaad per M_1-plant.

CURRICULUM VITAE

De auteur werd geboren op 4 november 1946 te 's-Gravenhage en behaalde in 1965 het einddiploma HBS-B aan het St. Jan Lyceum te 's-Hertogenbosch.

In datzelfde jaar begon zij met de studie aan de Landbouwhogeschool te Wageningen, waar zij in januari 1972 afstudeerde in de richting plantenveredeling met de keuzevakken erfelijkheidsleer, algemene plantenziektenkunde en tropische landbouwplantenteelt.

Van 1 februari 1972 tot 1 november 1975 was zij in dienst van het "Centro Internacional de Mejoramiento de Maiz y Trigo" werkzaam in Nepal. Op 1 januari 1976 kwam zij in dienst van de Stichting Instituut voor Toepassing van Atoomenergie in de Landbouw, waarvoor zij tot 1 juli 1979 onderzoek verrichtte naar de mutageen specifieke werking van Röntgen stralen en snelle neutronen bij *Arabidopsis thaliana* en gerst.

Een groot deel van dit onderzoek werd op en in nauwe samenwerking met de afdeling erfelijkheidsleer van de Landbouwhogeschool uitgevoerd.