Theor. Appl. Genet. 57, 137-143 (1980)

THEORETICAL AND APPLIED GENETICS

© by Springer-Verlag 1980

Segregation Frequencies of Radiation-induced Viable Mutants in *Arabidopsis thaliana* (L.) Heynh.*

L.M.W. Dellaert
ITAL, Wageningen (the Netherlands)

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 indicated 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.

Key words: *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 ($\beta < 1$), or after fertilization ($\gamma < 1$). 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 gameto-

* Research carried out jointly at the Foundation ITAL and at the Department of Genetics, Agricultural University, Wageningen (the Netherlands)

0040-5752/80/0057/0137/£1.40

phyte, i.e. $\beta < 1$. 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. $\beta < 1$ and $\gamma < 1$. (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 object 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 either water (0 per cent) or 1.2 per cent dithiothreitol (DTT) for 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/cm², for germination. After 8 days, the seedlings were transplanted into the 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 as descending 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 Li and Mantel (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 estmate 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.

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

$\hat{p}_3$ = the estimate of p_3
R = the total number of mutants in the segretating 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 Li and Mantel, 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 ($\{(1-\hat{p}_2) + \hat{p}_2(1-\hat{p}_3)^e\}^N$ 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 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);

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

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);

$$p_1 = \frac{\alpha \beta_1 \beta_2 \gamma}{1 + \alpha\beta_1 + \beta_2 + \alpha\beta_1\beta_2\gamma}, \tag{2}$$

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[a]	Genotype	Fraction[a]
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		

[a]For the definitions of α, β_1, β_2 and γ see text

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

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

in the case of random union of gametes, or

$$p_2 = \frac{\alpha\beta_1 + \beta_2}{1 + \alpha\beta_1 + \beta_2}, \tag{4}$$

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

$$p_3 = \frac{\beta_1\beta_2\gamma}{1 + \beta_1 + \beta_2 + \beta_1\beta_2\gamma} \tag{5}$$

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 Eqs. (1), (3) and (5), or (2), (4)

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[a]	Genotype	Fraction[a]
♀	A	1	AA	1
♀	a	β_1	Aa	$\beta_1+\beta_2$
♂	A	1	aa	$\beta_1\beta_2\gamma$
♂	a	β_2		

[a] For the definitions of β_1, β_2 and γ see text

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 32M_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 since 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).

Table 3. The survival frequencies of the offspring of mutant heterozygous (segregating M_3-lines) and wild-type (non 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

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 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 Eq. (3), discussed in material and methods. With $\alpha = 1$, Eq. (1) becomes equal to Eq. (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. $\gamma\hat{p}_1$ minimum and $\gamma\hat{p}_3$ minimum, respectively. In the same way a 'maximum value' of γ was obtained, i.e. $\gamma\hat{p}_1$ maximum and $\gamma\hat{p}_3$ 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.

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 Eqs. (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,

Table 4. Factors affecting the segregation frequency of radiation-induced mutants. $\beta_1 + \beta_2$ is the fraction of ♀ and ♂ mutants 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		$\gamma\hat{p}_1$ [d]		$\gamma\hat{p}_3$ [d]	
Type	Dose (Gy)	%	$\beta_1 + \beta_2$ [c]	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	20	0	1.5336	1.1543	1.2718	1.0008	1.1030
neutrons	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	1.0097	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 obatined 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 $\beta_1 \rightarrow 1$, $\beta_2 \rightarrow < 0$, or the other way round

i.e. $\hat{p}_3$, directly from the M_2-data, since $p_1 = p_3$ in the absence of chimerism.

Acknowledgements

I wish to thank Prof. J.H. van der Veen and Dr. P. Stam for their critical and stimulating discussions while preparing this paper, and Mrs. J. Rus-Gootjes for her technical assistance.

Literature

Auerbach, C. (1976): Mutation research problems, results and perspectives. London: Chapman and Hall

Avanzi, S.; D'Amato, F.; Donini, B.; Scarascia, G.T. (1960): Impiego di radiazioni ionizzanti nel miglioramento genetico dei grani italiani. Genet. Agrar. **12**, 231-250

Balkema, G.H. (1971): Chimerism and diplontic selection. Thesis, Landbouwhogeschool, Wageningen. Rotterdam-Kaapstad: Balkema

Balkema, G.H. (1972): Diplontic drift in chimeric plants. Radiat. Bot. **12**, 51-55

Batalov, R.C.; Kvitko, K.V.; Usmanov, P.D. (1972): On the nature of pigment mutations in *Arabidopsis thaliana* (L.) Heynh. Arabidopsis Inf. Serv. **9**, 25-26

Bürger, D. (1971): Die morphologischen Mutanten des Göttinger *Arabidopsis*-Sortiments, einschliesslich der Mutanten mit abweichender Samenfarbe. Arabidopsis Inform. Serv. **8**, 36-42

D'Amato, F.; Scarascia, G.T.; Monti, L.M.; Bozzini, A. (1962): Types and frequencies of chlorophyll mutations in durum wheat induced by radiations and chemicals. Radiat. Bot. **2**, 217-239

Dellaert, L.M.W. (1979): Comparison of X-ray- and fast neutron-induced mutant spectra. Experiments in *Arabidopsis thaliana* (L.) Heynh. Submitted

Doll, H. (1968): Segregation frequencies of induced chlorophyll mutants in barley. Hereditas **59**, 464-472

Eriksson, G. (1965): The size of the mutated sector in barley spikes estimated by means of waxy mutants. Hereditas **53**, 307-326

Frydenberg, W.J. (1963): Some theoretical aspects of the scoring of mutation frequencies after mutagenic treatment of barley seeds. Radiat. Bot. **3**, 135-143

Gaul, H. (1957): Die verschiedene Bezugsysteme der Mutationshäufigkeit bei Pflanzen angewendet auf Dosis-Effektkurven. Z. Pflanzenzücht. **38**, 63-76

Grinikh, L.I.; Shevchenko, V.V.; Grigorieva, G.A.; Draginskaya, L.Y. (1974): On the chimerism in generative tissue of *Arabidopsis* after X-ray-irradiation of seeds. *Arabidopsis* Inform. Serv. **11**, 13-14

Ivanov, V.I. (1971): Leaf colour mutants in *Arabidopsis* induced by gamma-irradation of dormant seeds. Arabidopsis Inform. Serv. **8**, 29-30

Ivanov, V.I. (1973): Estimation of induced mutation rate in *Arabidopsis*. Arabidopsis Inform. Serv. **10**, 31-32

Kranz, A.R. (1978): Demonstration of new and additional population samples and mutant lines of the AIS-seed bank. Arabidopsis Inform. Serv. **15**, 118-127

Lejeune, J. (1958): Sur une solution 'apriori' de la méthode 'a posteriori' de Haldane. Biometrics **14**, 513-520

Li, C.C.; Mantel, N. (1968): A simple method of estimating the segragation ratio under complete ascertainment. Amer. J. Human Genet. **20**, 61-81

Li, S.L.; Rédei, G.P. (1969): Estimation of mutation rate in autogamous diploids. Radiat. Bot. **9**, 125-131

Moh, C.C.; Nilan, R.A. (1956): Reduced gene transmission in radiation-induced mutant barley. J. Hered. **47**, 129-131

Moh, C.C.; Smith, L. (1951): An analysis of seedling mutants (spontaneous, atomic bomb-radiation- and S-ray-induced) in barley and durum wheat. Genetics **36**, 629-640

Mosteller, F.; Tukey, J.W. (1949): The uses and usefulness of binomial probability paper. J. Amer. Statistics Ass. **44**, 174-212

Müller, A.J. (1961): Zur Charakterisierung der Blüten und Infloreszenzen von *Arabidopsis thaliana* (L.) Heynh. Kulturpflanze **9**, 364-393

Müller, A.J. (1963): The chimerical structure of the M_1-plants and its bearing on the determination of mutation frequencies in *Arabidopsis*. In: Induction of mutations and the mutation process (eds.: Velemínský, J.; Gichner, T.), pp 46-52. Proc. Symp. Prague

Oostindiër-Braaksma, F.J.; Feenstra, W.J. (1973): Isolation and characterization of chlorate-resistant mutants of *Arabidopsis thaliana*. Mutation Res. **19**, 175-185

Rédei, G.P. (1962): Single locus heterosis. Z. Vererbungsl. **93**, 164-170

Yonezawa, K.; Yamagata, H. (1975): Comparison of the scoring methods for mutation frequency in self-pollinating disomic plants. Radiat. Bot. **15**, 241-256

Received December 29, 1979
Communicated by B.R. Murty

Dr. L. M.W. Dellaert
ITAL, P.O. Box 48
Wageningen (the Netherlands)