New Method for the Estimation of the Mutation Rate in Acute and Chronic gamma-ray Irradiation of Growing Plants

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In the case of irradiation of seeds of self-pollinated and disomic plant materials, the frequency of mutated plants appearing in the M_2 generation divided by the Mendelian ratio is an index, as proposed by Gaul (1960), that enables the direct estimation of the mutation rate, representing the ratio of the number of mutated cells to the number of survived cells after treatment of mutagen. In the case of chronic irradiation of growing plants throughout the entire life cycle from germination to maturity, on the other hand, the mutations induced at the stage preceding the differentiation of the male and female organs appear at the M_2 generation, the but mutations induced after this critical time are observed in the M_2 plants in the heterozygous condition and are detected only at the M_3 generation. Therefore, the mutation rate can not be correctly estimated only on the basis of the frequency of mutants either in the M_2 or M_3 generation. A new method for the estimation of the mutation rate by combining the data of the frequency of mutants and the M_2 and M_3 generation, which is comparable to Gaul's method for seed irradiation, is proposed. Application of this method to the data of acute and chronic gamma-ray irradiation of growing barley revealed that a much higher mutation rate was observed after irradiation at a higher daily exposure rate, when the plants were treated with the same accumulated dose.

KEY WORDS : Mutation rate, chlorophyll mutation, barley, chronic irradiation, dose rate effect, chimera.

Introduction

Estimation of the mutation rate is a prerequisite to a comparison of genetic effects of several mutagenic treatments. The conventional method for the estimation of the mutation rate, after treatments applied to seeds of barley, consists of scoring the frequency of M_1 spike progenies segregating for mutants among all the M_1 spike progenies tested. The method, initiated by Stadler (1930), has long been applied in mutation works in barley. However, the frequency does not give a direct estimation of the frequency of mutated cells among the initial cells that were treated and survived, because a M_1 spike usually develops from more than one initial cell and the spikelets carrying mutation are chimerically recovered in some part of the spike. This consideration is supported by the fact that the observed segregation frequency of mutants in each mutated M_1 spike progeny is often significantly lower than the Mendelian ratio expected from a single recessive mutation. A more direct evidence was brought out by experimental results showing that mutated pollens from normal to waxy types of barley appeared chimerically in a spike after seed irradiation (Eriksson 1965, Lindgren *et al.* 1970). Gaul (1960) proposed a method for scoring the frequency of mutated M_2 plants among total tested M_2 plants to replace the conventional method. The mutation rate per

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initial cell can be estimated by the frequency divided by the mean particular segregation ratio of the recessive mutation. Gaul's method enables to overcome the error in the mutation rate associated with the chimeric nature of the mutated sector. In the case of chronic irradiation throughout the growing period, the frequency of mutated M_3 plant (CONGER *et al.* 1974), M_2 ear branch progeny carrying mutation (YAMAGUCHI 1963), and M_2 spike progeny segregating for mutants (HERMELIN 1970, NYBOM *et al.* 1956), has been accounted for the mutation rate. However, the values are often different from the mutation rate because the materials used in the count viz, M_2 ear branch, M_2 spike do not always originate from a mutated single cell, as illustrated later in this paper. The present paper will describe a method which makes it possible to estimate the mutation rate after irradiation applied to growing plants based on the number of mutated M_2 and M_3 plants in omitting information on the chimeric nature of the mutated sector, and is comparable to Gaul's method for seed irradiation.

Theoretical considerations

1) Life cycle in relation to chimeric state of mutated sector

In taking into account the chimeric state of the mutated sector after mutagenic treatment, the life cycle can be divided into two essential periods, one extending from the stage of fertilized egg, through the dormant seed stage, to the stage of differentiation of the male and female organs in a floret, and the other covering the following stage until the period immediately preceding fertilization.

The mutated sector developed after irradiation during the former period may involve a whole M_1 plant, or several spikes or a part of spike, or can be limited to only one floret. The size of the mutated sector is determined by the time of irradiation either at the stage of early proembryo, dormant seeds, or later vegetative stage immediately before the differentiation of male and female initials. Though the mutated sector may display such a range of sizes, the mutated sector involves both the male and female organs of the respective floret, in other words, pollen and egg cell in each floret are derived from the same initial cell and recessive homozygotes will appear in the next generation. A best estimation of the mutation rate in omitting the bias associated with the size of the mutated sectors can be expressed by the mutant frequency (a_2) divided by the mean particular segregation ratio (f_{aa}) for induced mutations

$$\frac{a_2}{f_{aa}}$$

On the other hand, in the later period which extends from the differentiation of the male and female organs to the stage immediately preceding fertilization, mutations are induced independently in the germ line of either the male or female organ in a respective floret. Since the probability that the same locus would undergo mutation simultaneously in both reproductive organs of the same floret is extremely low, a fertilized embryo after selfing will always have the mutated gene in the heterozygous state and induce non-chimeric M_2 plant. The frequency of M_2 plant progenies segregating for mutants is a direct estimate of the mutation rate per initial cell. Mutant frequency observed in the M_3 generation (a_3) can be converted to the mutation rate by dividing the frequency

with the mean segregation ratio (f_{aa}).

$$\frac{a_3}{f_{aa}}$$

Thus, the methods for estimating the mutation rate as well as the generation of the appearance of mutants differ from one another between these two stages of mutation induction. The essential cause of the difference depends on whether a M_1 floret, from which a M_2 plant arises as the smallest unit for screening mutation, is covered with the same mutated sector or with two independent sectors of male or female origin, respectively. In chronic irradiation throughout the growing period, the irradiation is applied from germination and not immediately after fertilization. Therefore, the life cycle of an annual plant can be divided into the following three stages in taking into account the chimeric nature of the mutated sectors. Stage I: From germination to the differentiation of male and female organs. mutants appear in the M_2 generation as recessive homozygotes and the mutation rate can be estimated by Gaul's method based on the mutant frequency among the plants tested. Stage II: From the differentiation of male and female organs to immediately before fertilization. Mutants appear in the M_2 plant progeny originating from a M_1 embryo fertilized by an egg cell or pollen with a mutated gene. M_2 plant heterozygous for the mutated gene is not chimeric and segregates for mutants with a Mendelian ratio in the M_2 plant progeny. The frequency of M_2 plant progenies segregating for mutants can be considered as a direct reflection of the mutation rate, which can also be deduced from the frequency of mutated M_3 plants by dividing the value of the frequency by the mean segregation ratio of mutant. Stage III: From fertilization to maturity. The chimeric nature of the mutated sector, arising from the irradiation at this stage, is essentially similar to that of M_1 plant in seed irradiation. In most cases mutants appear among one or a few spike progenies of a M_2 plant carrying mutation, because the developing embryo is multicellular except for the stage that immediately follows fertilization (Mericle 1962). For the same reason, the mutated sector does not involve the whole spike. The frequency of mutated M_3 plants divided by the mean particular segregation ratio, is the best means to estimate the mutation rate.

In case of chronic irradiation going through these three stages, there are three types of mutant appearance, 1) recessive homozygotes in the M_2 generation, 2) M_2 plant progenies segregating for mutants with a Mendelian ratio and 3) M_2 spike progenies segregating for mutants with a frequency lower than the Mendelian ratio. These types of mutation appearance are responsible for the events occurring at stages I, II, and III, respectively. However, at present it is difficult to determine the relative importance of these three stages because information on the number of initial cells at the respective stages is not available.

2) Derivation of formulae

Here, self-pollinated material and mutant characters controlled by a single recessive gene will be exclusively considered. The following assumptions are made, 1) only one mutation is induced in the same germ line through the whole succession of cell generation from germination to maturity throughout the irradiation period, 2) there is no compe-

tition between cells with and without mutant gene, and also no interplant competition at all in the diplo-phase during which the mutated gene is being transmitted successively from the induction to the appearance of the mutant as recessive homozygotes, and 3) the average segregation ratio of heterozygotes and recessive homozygotes recovered from a whole heterozygous plant is equal in all mutations irrespective of their origin.

The following parameters for the derivation of formulae were selected:

p^I, p^{II}, p^{III} : Rate of mutations occurring at stages I, II, III

$a_2^I, a_2^{II}, a_2^{III}$: Frequency of mutants which appear in the M_2 and are responsible for the mutational event at stages I, II, III

$a_3^I, a_3^{II}, a_3^{III}$: Frequency of mutants which appear in the M_3 and are responsible for the mutational events at stages I, II, III

p : Total mutation rate throughout the stages

a_2 : Frequency of mutants observed in the M_2 generation

a_3 : Frequency of mutants observed in the M_3 generation

f_{aa} and f_{Aa} : Mean frequency of recessive homozygotes and heterozygotes from a full heterozygote

Then $p = p^I + p^{II} + p^{III}$, $a_2 = a_2^I + a_2^{II} + a_2^{III}$, $a_3 = a_3^I + a_3^{II} + a_3^{III}$

Since mutations induced at stages II and III are included in the M_2 plants in the heterozygous state, as explained in the previous section, a_2^{II} and a_2^{III} equal zero. It is also assumed that all the mutants appearing in the M_2 generation are thoroughly selected and recessive homozygote is not transmitted to the M_3 generation (in case of lethal chlorophyll mutation). The next relationship can be inferred.

$$\begin{aligned} a_2^I &= f_{aa} \times p^I & a_3^I \times (1 - a_2) &= f_{aa} \times f_{Aa} \times p^I \\ a_2^{II} &= 0 & a_3^{II} \times (1 - a_2) &= f_{aa} \times p^{II} \\ a_2^{III} &= 0 & a_3^{III} \times (1 - a_2) &= f_{aa} \times p^{III} \end{aligned}$$

The factor $(1 - a_2)$ is the correction factor for the decrease of M_3 population size due to the selection of mutants appearing in the M_2 generation. From these nine equations parameters with suffixes I, II and III can be eliminated and p can be obtained by the next equation.

$$p = \frac{1 - f_{Aa}}{f_{aa}} a_2 + \frac{1}{f_{aa}} (1 - a_2) a_3 \quad (1)$$

When 0.25 and 0.50 are taken as the value of f_{aa} and f_{Aa} , the equation can be simplified as follows:

$$p = 2 a_2 + 4 a_3 (1 - a_2) \quad (2)$$

In cases of irradiation involving only stages I and II, the frequency of M_2 plant progenies segregating for mutants (b_3) will be equal to $4 a_3$ and thus the equation can be written as:

$$p = 2 a_2 + b_3 (1 - a_2) \quad (3)$$

When mutants are viable and not selected in the M_2 generation two kinds of M_2 plant progeny will be observed, one which is the segregating progeny from heterozygous M_2 plant and the other is the fixed progeny from recessive homozygous M_2 plant. Then, the frequency of the latter type of progenies is expressed by a_2 , and the mutation rate per cell can be obtained by the following equation:

$$p = 2 a_2 + b_3 \quad (4)$$

The significance of Equ. 4 is self-evident. The value of $2a_2$ represents the frequency of the sum of "aa" and "AA" plants which are derived from the mutated sectors responsible for the mutational events before the differentiation of the male and female organs, and the residual half of plants "Aa" from the same mutated sectors are included in the value b_3 , together with those for mutations induced after the differentiation of the male and female organs. Therefore this equation is equivalent to Gaul's formulae, when all of the mutations occurred exclusively at stage I as in the case of dormant seed irradiation, because $b_3=2a_2$. On the other hand, when the mutational events are limited to the stage which follows the differentiation of the male and female organs, the equation can be simplified and the conventional formulae $p=b_3$ (because $a_2=0$) applied to the cases of irradiation at the reproductive stage can be used.

The present method enables to estimate the relative importance of the mutational events occurring at stage I compared to the mutations induced throughout the irradiation period extending from stage I to stage III. The mutation rate responsible for stage I is

$$\frac{a_2}{f_{aa}} (=4a_2, \text{ when } f_{aa}=0.25)$$

and the relative importance can be expressed as Follows:

$$\frac{4a_2}{2a_2+4a_3(1-a_2)} \quad \text{or} \quad \frac{4a_2}{2a_2+b_3(1-a_2)} \quad (5)$$

Examples illustrating the application of the present method

As a demonstration of the present method, the results of previous experiments (Yamashita 1967b) will be revised here. The experiments had been carried out to study the differences between the mutagenic effects of chronic and acute gamma-ray irradiation on growing barley at the late vegetative stage (stages I and II). The experiments consisted of five treatments as follows: chronic irradiation at 17 R/day for 64 days from Jan. 16th to Apr. 4th, semi-acute irradiation at 125 R/day for 8 days from Jan. 16th and Feb. 23rd, and acute irradiation at 1,000 R/day for one day on Jan. 16th and Feb. 23rd, respectively. All spikes of irradiated plants (M_1) were sampled separately for raising progenies in the next generation (M_2), in which the number of chlorophyll mutants was scored, and all the normal plants were grown as M_1 spike progenies. In the M_3 generation, the number of M_2 plant progenies segregating for mutants was counted. Based on the segregation data in the M_2 and M_3 generation, the number of recessive homozygotes and heterozygotes within each mutated M_1 spike progeny was determined.

As shown in Table 1, the results enabled to demonstrate that there were three types of M_1 spike progenies with regard to appearance of mutation. The 1st of which, expressed as $M_2M_3(++)$, segregated at least for one recessive homozygote and one heterozygote, respectively. In the 2nd type of progeny, $M_2M_3(+-)$, only recessive homozygotes appeared in the M_2 generation and none of the remaining normal plants were heterozygotes. The progeny of type $M_2M_3(-+)$ did not give rise to recessive homozygotes and produced only heterozygotes. In the semi-acute irradiation experiments, the number of mutated progenies detected as $M_2M_3(++)$, $M_2M_3(+-)$ and $M_2M_3(-+)$ types was 1, 4 and 8 in the treatment from Jan. 16th and 2, 5 and 16 in the treatment

Table 1. Number of recessive homozygote (aa), heterozygote (Aa) and normal type (AA) in M_1 spike progeny segregating for lethal chlorophyll mutation after acute and chronic gamma-ray irradiations on growing barley

Treatment	1,000 R/day						125 R/day						17 R/day		
	Jan. 16			Feb. 23			Jan. 16			Feb. 23			Jan. 16		
Genotype	aa	Aa	AA	aa	Aa	AA	aa	Aa	AA	aa	Aa	AA	aa	Aa	AA
M_2M_3	2	6	3	2	6	2	1	2	6	1	2	15	1	1	16
(++)	1	4	6	1	3	4				1	2 ²⁾	16			
	2	8	4	5	10	4									
				1	7	2									
total	5	18	13	9	26	12	1	2	6	2	4	31	1	1	16
M_2M_3							1	0	7	1	0	9	1	0	25
(+-)							1	0	8	1	0	13	1	0	17
							1	0	5	1	0	18			
							1	0	16	1	0	14			
										1	0	15			
total							4	0	36	5	0	69	2	0	42
M_2M_3	0	1	3	0	1	15	0	1	9	0	1	9	0	1	12
(-+)	0	1	9	0	2	12	0	1	1	0	1	6	0	1	6
				0	4	6	0	1	1	0	1	14	0	1	12
				0	1	2	0	1	10	0	1	17	0	1	14
							0	1	3	0	1	22	0	1	23
							0	1	9	0	1	12	0	1	11
							0	1	20	0	1	17	0	1	8
							0	4	7	0	1	13	0	1	13
										0	1	9	0	1	13
										0	1	11	0	1	7
										0	1	7	0	1	8
										0	1	9	0	1	15
										0	2	5	0	1	21
										0	2	10	0	1	13
										0	2 ²⁾	6	0	1	4
										0	3 ²⁾	14	0	1	16
													0	1	10
													0	1	9
													0	1	20
													0	1	44
													0	1	14
													0	1	20
													0	2 ²⁾	23
total	0	2	12	0	8	35	0	11	60	0	21	184	0	24	336

Note (°) Two kinds of mutant were segregated, but in different M_2 plant progenies in the M_3 generation (see text).

from Feb. 23 rd, respectively. In the acute irradiation treatments, the values were 3, 0 and 2 in the treatment on Jan. 16 th and 3, 0 and 4 in the treatment on Feb. 23 rd respectively. On the other hand, in the chronic irradiation experiment, only one progeny belonged to type $M_2M_3(++)$, and two to type $M_2M_3(+-)$ among all the 26 mutated progenies.

It is usually recognized that the relative frequency of these three types of progenies depends on the mean size of the mutated sector. Undoubtedly, the progeny of type $M_2M_3(++)$ is likely to be found more frequently when the mean sector size is larger. And the mean size of the mutated sector seems to be limited to either the male or female organ when all the mutations were detected in type $M_2M_3(-+)$. Theoretical considerations on the relation between the relative frequency of the three types of M_1 spike progenies and the size of the mutated sector have been presented in a previous report (YAMASHITA 1967 a).

Moreover, the number of recessive homozygotes and heterozygotes in a mutated M_1 spike progeny was larger in case of irradiation with higher daily exposure rate. The pooled data for four M_1 spike progenies, which belonged to type $M_2M_3(++)$ after the acute irradiation of Feb. 23 rd, show that the number of recessive homozygotes, heterozygotes and dominant homozygotes was 9, 26 and 12 respectively, and fitted well to the 1:2:1 expected ratio ($X^2=0.7021$, $P>0.05$), suggesting that each of these M_1 spike progenies originated from a single initial cell. Also two among the three progenies of the same type induced following acute irradiation on Jan. 16 th, seemed to be derived from a single initial cell. However, in four progenies belonging to type $M_2M_3(++)$, which appeared after semi-acute and chronic irradiation, the number of recessive homozygotes was far lower than the number expected from the Mendelian ratio, indicating that the size of the mutated sectors was larger than one floret but did not include the whole M_1 spike.

Segregation data pooled for the respective treatments show that the ratio of aa, Aa and AA was 14%, 50%, 36% and 19%, 55%, 26% in acute irradiation on Jan. 16 th and Feb. 23 rd, respectively. The values of the ratio in the semi-acute irradiation from Jan. 16 th and Feb. 23 rd were 11%, 22%, 67% and 5%, 11%, 84% respectively, whereas in the chronic irradiation, the number of recessive homozygotes and heterozygotes was only one among 18 plants of the mutated progeny belonging to type $M_2M_3(++)$. Thus, it is concluded that in this experiment the chimeric nature of the mutated sector induced in M_1 spike differed between the chronic and acute irradiation experiments as well as among different mutated spikes in each treatment. It is noteworthy that two types of M_1 spike progenies were induced, with different mutated sectors covering either both reproductive organs or only one part of them, even though the size of the mutated sector varied considerably.

The mutation rate estimated by the present method is indicated in Table 2. In the acute irradiation experiment, the frequency of mutants in the M_2 generation (a_2) was 1.02% and 1.27% for the irradiation of Jan. 16 th and Feb. 23 rd, and the frequency of M_2 plant progenies segregating for mutants in the M_3 generation (b_3) was 4.15% and 4.85%. Based on Equ. 4, the mutation rate per initial cell was found to be 6.15% and 7.33% respectively, while the mutation rate for the semi-acute irradiation at two stages and chronic irradiation for 64 days at 17 R/day was estimated at 3.37%, 2.27% and 1.49% respectively. From these results it can be concluded that irradiation at a higher exposure rate resulted in a higher mutation rate per exposure unit and that there was no significant difference in the mutation rate between two irradiation stages. The relative

Table 2. Estimation of the chlorophyll mutation rate in acute and chronic gamma-ray irradiation of growing plants of barley, based on the frequency of mutants appeared in M_2 and the frequency of M_2 plant progenies segregating for mutants in M_3

Daily exposure rate start of irradiation	1,000 R/day		125 R/day		17 R/day
	Jan. 16	Feb. 23	Jan. 16	Feb. 23	Jan. 16
M_2 Total number of tested plant	487	710	697	1,726	2,028
Number of mutated plants	5	9	5	7	3
Frequency of mutants (a_2) %	1.02	1.27	0.72	0.41	0.14
M_3 Total number of tested progenies	482	701	669	1,711	2,078
Number of segregating progenies	20	34	13	25	25
Frequency of segregating progenies (b_3)	4.15	4.85	1.95	1.46	1.21
Estimated mutation rate : $2a_2 + b_3(1-a_2)$	6.15	7.33	3.37	2.27	1.49
Mutation rate per R unit	67×10^{-6}		28×10^{-6}		15×10^{-6}
Relative importance of event at stage I	66%	69%	85%	72%	38%

Note : Total exposure was 1,000 R in all treatments.

importance of mutational events at stage I is also indicated in Table 2. In the acute and semi-acute irradiation experiments, 66%–85% of the mutated M_1 spike progenies was attributable to the mutational events that took place at stage I, whereas in the chronic irradiation experiments the amount of mutations induced at stage II was much larger than that of stage I.

Discussion

In taking into account the chimeric nature of the mutation induced by chronic irradiation throughout the growing period, Nybom *et al.* (1956) and Hermelin (1970) considered that there were two critical periods, one extending from germination to the time immediately preceding fertilization, the other extending from fertilization to maturity. However, they did not divide the vegetative stage into two parts, namely before and after the differentiation of two germ lines for the male and female organs. In their studies on chronic gamma-ray irradiation, mutants in the M_2 generation were observed in only a few instances and the frequency of mutants was as low as the spontaneous rate, indicating that most of the mutations were induced at a more advanced stage than the differentiation of the male and female organs in a floret. Similar results were reported by Mikaelson (1956) in barley and by Donini *et al.* (1968) in durum wheat. However, in chronic irradiation experiments throughout the growing period of rice (YAMAGUCHI 1968) and winter barley (YAMASHITA unpubl), a considerable number of mutants appeared in the M_2 generation. To obtain such mutants, the dose accumulated during stage I should be as large as that required to induce a sufficient number of mutations in the germ line cells at stage I. The long vegetative period of winter barley and the low radiosensitivity of rice plants to chronic irradiation at a high daily exposure rate (TANAKA and SEKIGUCHI 1966) seem favourable for the germ line cells to accumulate a large amount of radiation in this stage. In contrast, when the crops are sown in the spring, the duration of stage I seems to be too short for the germ line cells to accumulate enough dose for producing mutants in the M_2 generation.

The occurrence of mutations induced in the period covering stages I and II, is not limited to long-term chronic irradiation but is also observed when plants are subjected to acute irradiation during the period when two kinds of initial cells coexist in stages I and stage II, due to the asynchronized development of sexual organs of different ears in a plant and different florets in a ear. Acute irradiation applied only at stage II, especially at a semi-lethal dose, often destroys some of the differentiated reproductive organs and promotes the alternative development of new shoots, in which both reproductive organs can be formed from a common initial cell (YAMAKAWA and SEKIGUCHI 1968). In the progenies derived from these spikes, mutants can be found in the next generation. In this case, it can be said that the stage of initial cells of the germline sector regressed from stage II to stage I, or that the initial cells at stage II are replaced by the initial cells at stage I.

Actually, in the experiments referred to as an example, the developing spikes with fully differentiated male and female organs at the time of irradiation, were prevented from developing further, and were replaced by new spikes which segregated for mutants with the expected Mendelian ratio in the next generation. This result indicates the single cell origin of the new spikes. However, it must be emphasized that not all of the differentiated spikes are destroyed and some of them remain affected to a lesser extent because the proportion of mutational events at stage I was estimated to be about 60%-70% based on Equ. 5 in case of acute irradiation and the remaining 30%-40% of the mutations seemed to have taken place at stage II, as shown in Table 2.

KAWAI and INOSHITA (1965) presented a complete set of data for the frequency of chlorophyll mutants in the M_2 generation and the frequency of M_2 plant progenies segregating for mutants in the M_3 generation after acute irradiation at four different ontogenetic stages in the rice plant. When irradiation was applied at the late tillering stage 45 days before heading, a_2 and b_3 were 0.15% and 1.00% in 2 kR, 0.65% and 2.50% in 4 kR, 2.11% and 5.70% in 8 kR and 4.21% and 6.30% in 16 kR, respectively. From these data, based on Equ. 5, it is evident that proportion of mutational events at stage I increased from 46% to 100% with the increase of the dose applied. This indicates that male and female organs have undergone differentiation in half of the florets at the time of irradiation, and that the semi-lethal dose of gamma-ray damaged the meristematic region, resulting in more important mutational events at stage I. As shown in these examples, in many instances, after acute or chronic irradiation of growing plants, two kinds of mutated germ line tend to coexist, which are responsible for the events occurring at stage I and stage II. Thus, the mutation rate can not be correctly estimated only on the basis of the mutant frequency either in the M_2 or M_3 generation, but should be calculated by applying the present method which combines data for both generations.

In this regard, the author wishes to emphasize that the designation of the generation of chronically irradiated plants may give rise to a controversy, as to whether the irradiated generation (HERMELIN 1970, NYBOM *et al.* 1956) or the next generation (CONGER *et al.* 1974) should be referred to as M_1 . The latter is based on the fact that mutations are generally masked as a heterozygous condition in the plants of the 2nd generation, as in the case of M_1 plants derived from seeds subjected to irradiation.

However, as seen in these experiments, the plants irradiated at stages I and II have always the possibility of segregating for mutants in the next generation and the possibility depends on how large the mutated sectors can be further enlarged. TANAKA and SEKIGUCHI (1967) succeeded in screening most of the induced mutations in the next generation after chronic irradiation extending from germination to heading, by applying the "stubbling method" which is essentially the same as the "cutting back method" (ZWINTSCHER 1955) widely applied in mutation breeding of vegetatively propagated crops. After acute irradiation of growing tomato plants with a semi-lethal dose, many mutants were screened in the next generation by the "internal disbudding method", in which the fruits on the recovered shoots are used as materials for the next generation (YAMAKAWA and SEKIGUCHI 1968). In cross-pollinated species, recessive homozygotes never appear until a chance combination of gametes with the gene mutated at the same locus occurs, even in case of seed irradiation. Thus, the fact that the appearance of recessive homozygotes in the next generation depends on the dose of radiation, the treatment to enlarge the mutated sector and on the reproductive nature of the materials, seems to support the opinion that the 1st generation in which the plant became heterozygous for mutant genes(s) should be designated as M_1 .

The value of f_{Aa} and f_{aa} is one of the major variables in the present method of estimation of the mutation rate per cell. NYBOM (1954) reported that the mean value of f_{aa} and f_{Aa} for chlorophyll mutants of barley was 0.23 and 0.43, respectively and MOH (1951) listed a value of 0.22 for f_{aa} in barley. DOLL (1968) reported values of f_{aa} ranging from 11.2% to 35.0% with a mean of 23.6%. When converting the values reported by Nybom to the present data for an acute irradiation of 1,000 R at the daily exposure rate of 1,000 R/day on Feb. 23rd, the estimated value must be revised from 7.33% to 7.93%. However, when the mean value of f_{Aa} and f_{aa} is identical in mutants induced by different mutagenic treatments, the cause of error associated with the different mutagenic treatment becomes negligible.

Another difficulty arises due to the occurrence of multiple mutations in the same mutated sector. The cell mutated at an earlier stage of development is likely to undergo further mutations in the later stages in case of chronic irradiation. In the present experiments, four of seventy five mutated M_1 spike progenies carried two kinds of chlorophyll mutations, but the mutations appeared in different M_2 plant progenies, suggesting that the two kinds of mutations did not share the same mutated sector. In case of highly mutagenic treatment, the possible occurrence of multiple mutations affects the segregation ratio and results in a lower estimate of the mutation rate, but the bias associated with the presence of multiple mutations is as low as about 2% of the mutation rate when the latter ranges from 10% to 20% (HÄNSEL 1968). The mutation rate observed in the present experiments was less than 7.3% and the highest value so far recorded in gamma-ray irradiation of growing plants does not exceed 20% (KAWAI and INOSHITA 1965). Thus, this factor plays a minor role in an actual estimate.

In somatic mutations occurring in plant materials, irradiation at a higher intensity of gamma-rays has been found to induce a higher rate of mutation per exposure unit in several plant species (CUANY *et al.* 1957, NAKAJIMA 1965, GUPTA and SAMATA 1963).

However, the effect of the intensity on the induction of chlorophyll mutation is poorly documented. In the irradiation of dormant seeds of wheat (NATARAJAN and MARIC 1961), rice and einkorn wheat (MATSUMURA and MABUCHI 1964), as well as growing rice plant (NIWA and KEREDY 1967), the frequency of mutated M_1 spike progenies increased with the increases in the intensity of irradiation. In these cases, especially in the experiments conducted in growing plants, it remains to be determined whether the increase of the frequency of mutated M_1 spike progenies can be ascribed to the increase in the mutation rate per initial cell, or to the increase in the values of relative importance of mutations appearing in the M_2 generation due to the enlargement of the mutated sectors. The present experiments enable to demonstrate unequivocally that the mutation rate per R unit increased by about five times from 14×10^{-6} to 67×10^{-6} with a sixty four fold increase of the daily exposure rate from 17 R/day to 1,000 R/day.

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ガンマー線生体照射における突然変異率推定の新しい方法

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突然変異率は突然変異誘発処理を行った後, 生存した細胞数に対する変異細胞数の比で表される。自殖性2倍体植物における種子照射の場合, 突然変異は大部分が1遺伝子の劣性方向への変化であるから, M_2 世代に表れる変異個体の頻度をメンデルで除した値が変異細胞の頻度——突然変異率——の推定値となる (GAUL, 1960)。

突然変異誘発処理が植物の分化発育の途中で行われる生体処理では, それが雌雄ズイ両器官の原基細胞が分化する前であれば, 種子照射と同様に扱うことができる。分化後の場合には変異遺伝子は次代 M_2 個体にヘテロに保有され変異体は3代目になってはじめて出現する。突然変異率は M_3 における変異個体率をメンデルで除した値である。したがって突然変異誘発処理が雌雄器官分化の前後にわたる場合には, M_2 世代あるいは M_3 世代の変異個体頻度のいずれか一方の値のみでは突然変異率の正しい推定はできない。本論文はこのような場合における突然変異率の推定法を明らかにしたものである。

M_2 および M_3 世代における変異個体率をそれぞれ, a_2 および a_3 , 変異遺伝子の固有の分離比の平均値を劣性ホモ個体について f_{aa} , ヘテロ個体について f_{Aa} とすれば突然変異率 p は次式によって表される。

$$p = \frac{1-f_{Aa}}{f_{aa}} a_2 + \frac{1}{f_{aa}} a_3 (1-a_2)$$

ただし M_2 世代に出現した変異体はすべて選抜されることを前提にしており, $(1-a_2)$ は M_3 世代の集団がそのため縮小することによる補正項である。

従来の生体処理による突然変異誘発研究では, M_3 世代あるいは M_2 世代の変異個体頻度にもとづいて変異率を推定していたが, 処理時における変異セクターの始源細胞が雌雄両器官の潜圧的分化前であったのか, 以降であったのか, 両時期にわたっていたのか, 不明確のまま推定していたので, いずれも突然変異率を過小評価していたと考えられる。

この推定方法を用いてオオムギの幼穂分化初期および後期に行ったガンマー線緩・急照射における葉緑素突然変異のデータを検討した結果, 総線量が同じであっても線量率の高い程突然変異率が高かった。