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The Multiple-Cluster Mutation Complex in Mutagenesis with Higher Plants

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Summary. After treatment of dry and pre-soaked seeds of barley with gamma-rays, EMS, NEU and EI, the frequency of multiple mutations (multimutations) was higher with EMS and NEU treatment, while cluster mutations appeared in greater numbers following treatment with gamma rays and NEU. Pre-soaking the seeds led to a reduction in the frequency of total mutations, cluster mutations and multimutations. This has been explained as a result of the application of lower doses and the induction of mutations at a relatively later stage in ontogenetic development in the case of pre-soaked seeds.

Some new mutation types in barley have been described and some of the old types have been given names representing the mutation characters more precisely.

The compound mutation frequency of different seedling mutation types, when taken separately, was found to be independent of the mutagen employed and the stage of treatment. The size of mutated chimeras in M_1 plants, as indicated by the segregation ratio of mutants in M_2, was largest in *albina, xantha, chlorina, albina-tigrina, chl-terminalis* and *eceriferum*, and lowest in *viridis, viridoalbina* etc. This could be expected if the unstable premutations induced by mutagenic treatment are resolved into mutations at different intervals after their initiation, or it can be explained by the induction of dominant mutations, or lethal changes together with visible mutations.

Introduction

The primary aim in mutation research with agricultural plants is to increase the mutation rate, accompanied by a broader spectrum. Chlorophyll mutations in barley, as classified by Gustafsson (1940), have been widely used for studies on the mutation process in higher plants (Nybom, 1954; Gaul, 1964; Holm, 1954; Natarajan and Shivasankar, 1965). Using chlorophyll mutations, significant progress has been made in understanding the complexities of the mutation process, including the induction of multiple mutations (or multimutations), cluster mutations (Sharma and Orav, 1965; Sharma, 1965), the determination of the ontogenetic relationship of different spikes (Jacobsen, 1966), and the chimeral constitution of the spikes in the M_1 generation (Gaul, 1959, 1964, 1965; Eriksson, 1965; Frydenberg and Jacobsen, 1966; Frydenberg *et al.*, 1964). Very often a number of different mutations are obtained in a single plant or spike progeny (multimutations). On the other hand, more than one spike progeny may share one and the same mutational event, as a result of which a single mutation appears as a cluster of segregating spike progenies (cluster mutations). Because a large number of genes, when mutated, give rise to phenotypically similar mutations, it is often difficult to distinguish between different, although phenotypically similar, multimutations. Similarly, in a plant progeny carrying a cluster mutation the different mutated spike progenies tend to be considered as having different mutational events. These phenomena can therefore contribute significantly to the error in the determination of mutation frequency. However, by careful observation of the mode of appearance of mutations (colour of plants, lethality, change in colour, segregation ratio etc.) it is possible to make a fairly good categorization of different cases of multimutations and cluster mutations. These phenomena should be taken into account when determining the significance of the factors affecting mutagenic efficacy. It may be seen that multimutations and cluster mutations are antagonistic to each other in their mode of appearance, and it is essential to know the factors leading to their occurrence.

Material and Methods

All treatments were carried out with the barley (*Hordeum vulgare*, $2n = 14$) variety N. P. 104. Treatment with varying doses of gamma-rays (10—50 *kR*), *N*-nitrosoethyl urea (NEU: 0.01 to 0.03%, 2—6 hr), and ethyl methane-sulphonate (EMS: 0.05 to 0.4%, 6—24 hr) was given to air dried and to pre-soaked (8—12 hr) seeds. The results of one experiment with ethylene-imine (EI: dry seeds, 0.025 and 0.0125% — 4 hr) are also included in the present study for comparison. Application of a large range of dosages to dry and pre-soaked seeds resulted in complete mortality of pre-soaked seeds when treated with higher doses. Therefore, only sub-lethal doses were selected for a comparative study in the two lots of dry and pre-soaked seeds. The data presented here concern two sets of experiments — in 1968 and 1969. Since no marked difference was noted between the results of the two years' experiments, the data were pooled from the two experiments and doses for the different categories of seeds, i. e. dry and pre-soaked. The data on dry and pre-soaked seeds was statistically evaluated using the *d test*, *d* being the normal variable having zero mean and unit standard deviation.

The experiments were carried out in field conditions. All the spikes of M_1 plants were harvested and sown separately. The mutations were scored at weekly intervals, starting at germination and continuing until the seedlings were one month old. This allowed a careful comparison of the behaviour of various chlorophyll mutants, and made possible distinction between different phenotypically similar (or almost similar) mutations. In this way, some new types of mutations not specified earlier in the literature were discovered.

Mutation Types

If it is intended to find whether the mutations appearing in different spike progenies of the same plant, or even all the mutants within the same spike progeny, belong to one and the same mutation or owe their origin to different mutational events, it is essential to distinguish different types according to the mode of manifestation of the mutant character.

It was observed that there were three major colour changes according to Gustafsson's system of classification: (i) pure white (*albina*); (ii) yellow-bright and lethal (*xantha*) or dull and sometimes viable (*chlorina*); (iii) light green, viable (*viridis*). The complex "bi-colour" mutations are well known: *xanthalbina*, *xanthoviridis*, *alboxantha*, *alboviridis*, *viridoalbina* and *viridoxantha*. The two striped mutation types (transverse — *tigrina* and longitudinal — *striata*) have been sub-divided according to the three major colour changes, viz. *al* — white, *xa* — yellow, and *vir* — light green stripes (see Table 3).

A new group of chlorophyll mutations was recognized in barley. In repeated observations of growing seedlings it was noticed that in some cases the seedlings were normally green at the time of germination, but after about 15—20 days their newly appearing leaves were yellow or white. The change in leaf-colour usually started in the third to fifth leaf and, although the lower leaves remained green, the change of colour in the growing point could be lethal and such mutants did not survive if the new colour was white or deep yellow. The stage of colour-change was uniform in all the mutants of one segregating spike progeny, although it could be different in other spike or plant progenies. Analogous mutations are known in *Pisum sativum*, and therefore the names *albina-terminalis*, *xantha-terminalis* and *chlorina-terminalis* (cf. Blixt, 1961) have been used for these mutations in barley, and abbreviated to *al*, *xa* and *chl-terminalis*, respectively.

The mutations with spotting on the leaves (group *maculata*) can be further sub-divided into *al-maculata* (white), *xa-maculata* (yellow) and *chl-maculata* (dull yellow) etc. according to the colour of spotting (Table 3).

As the seedlings were observed until the age of one month, three types of morphological mutations were also noticed: onion leaf (very narrow tubular), *eceriferum* (waxless) and dwarf (probably *compactum* etc.).

Results

Mutation rate: The mutation frequency calculated by all five known methods is presented in Table 1. It can be seen that the treatment of dry seeds of barley with EMS and NEU produced more than twice (5.7 and 6.7% mutants) the mutations produced by gamma-ray doses up to 50 kR (2.5% mutants). The treatment of pre-soaked seeds with all three mutagens (gamma rays, EMS and NEU) resulted in a reduction in mutation rate. In the treatment using *E* I, the rate of mutation induction was almost equal (3.3%) to that in the gamma-ray treatment of dry seeds. These conclusions hold true, in general, for all the methods of estimating mutation frequency, although there were some variations in the magnitude of difference depending upon the method of calculation used. The percentage of mutational events showed a greater increase in mutation rate with more effective treatment (EMS — dry seeds) than did the

Table 1. *Mutation frequency in M_2 generation*

Treatment	No. of plant progenies	No. of spike progenies	No. of M_2 plants	% mutated plant progenies	% mutated spike progenies	% mutants	% mutations mut. events per M_1 plant	% mutations mut. events per M_1 spike	d value for comparing % mutants (dry vs pre-soaked)
Control	103	469	10558	0.9	0.2	0.01	0.9	0.2	
Gamma-rays (dry-seed)	274	1334	36063	54.05	18.5	2.5	77.4	16.9	9.00 ($P > 0.01$)
Gamma-rays (pre-soaked)	373	1717	41731	48.5	16.1	1.6	76.0	12.3	
EMS (dry-seed)	72	257	5583	84.7	42.0	5.7	193.1	54.1	9.97 ($P > 0.01$)
EMS (pre-soaked)	73	247	6591	49.3	21.9	2.2	82.2	24.3	
NEU (dry-seed)	81	275	6606	88.9	47.6	6.7	150.6	46.8	1.24 ($P < 0.05$)
NEU (pre-soaked)	470	1513	36257	78.3	45.7	6.3	106.8	30.3	
EI (dry-seed)	186	651	20031	61.8	32.9	3.3	102.2	29.2	

Table 2. *Frequency of multiple and cluster mutations and segregation ratio of M_2 mutants*

Treatment Dry seed = (d—s) pre-soaked = (p—s)	Compound segregation ratio of mutants (% mutants in mutated spike progenies)	Average No. of mutational events per mutated spike	Multiple mutational plant progenies (% of mutated plants progenies)	Cluster mutations	
				% of mutated plant progenies	% of mutated spike progenies
Gamma-rays (d—s)	12.3	1.0	32.4	20.1	39.3
Gamma-rays (p—s)	9.0	0.9	28.2	19.1	29.6
EMS (d—s)	14.7	1.3	67.2	19.1	23.1
EMS (p—s)	10.8	1.1	41.8	11.1	20.4
NEU (d—s)	11.0	1.2	59.6	33.1	43.2
NEU (p—s)	9.1	1.0	57.8	27.7	38.1
EI (d—s)	8.8	0.9	40.0	39.1	54.6

percentage of mutated M_1 plants and spikes as well as M_2 mutants.

Frequency of multiple and cluster mutations: As is evident from the data in Table 1, the increase in mutation frequency after the more effective EMS and NEU treatments was not only the result of mutation-induction in a higher number of treated seeds (percent mutated plant progenies). A part of this increase was caused by the induction of more than one mutation within the embryonic tissue of each seed. This is supported by the data in Table 2, which show that the average number of mutations per mutated spike was always lower in pre-soaked treatments (gamma-rays — 0.9, EMS — 1.1, NEU — 1.0) than treatment of dry seeds (gamma-rays — 1.0, EMS — 1.3, NEU — 1.2).

The data of Table 2 also show that EMS and NEU treatments induced a higher number of multimutations per mutated M_1 plant than did gamma-rays. An interesting feature of these findings is that if the frequency of multimutational plant progenies is determined by a relatively crude method which overlooks phenotypically similar multimutations and cluster mutations, EMS (pre-soaked) treatments could appear to be inferior to gamma-irradiation; after making adjustments for these phenomena, it turned out that the two treatments had equal effectiveness in this respect also (41.2 and 41.8% respectively). Further, the adjustment for phenotypically similar multimutations and cluster mutations resulted in an increased rate of multimutations for EMS, while lowering the previously calculated frequency for gamma-ray treatment from 47.1 to 41.2% multimutations. Therefore, only corrected values have been shown in Table 2.

The overall segregation of mutants in M_2 was higher in dry seed treatments (gamma-rays — 12.3%, EMS — 14.7%, NEU — 11.0%). Treatment of pre-soaked seeds gave a lower number of mutants for all the three mutagens studied (gamma-rays — 9.0%, EMS — 10.8%, NEU — 9.1%).

The occurrence of mutated sectors covering more than one spike in M_1 plants (cluster mutations) showed a definite trend: their frequency was higher with gamma-ray treatment (19.1 & 20.1% plants and 29.6 & 39.3% spikes) than with EMS; and with all the mutagens a higher number of mutated M_1 plants or spikes showed cluster mutations when the dry seeds were treated compared with the treatment of pre-soaked seeds. A larger frequency of cluster mutations was obtained with NEU and EI than with EMS (Table 2).

Mutation spectrum: In these studies it was possible to score almost all the expected types of chlorophyll abnormalities, and also three morphological mutations were noticed, viz. onion leaf, *eceriferum* and dwarf (probably *compactum, compactoid* etc.). Out of the twenty-two mutation types listed in Table 3, one did not appear in these experiments, i.e. *alboxantha*. The other mutation types were induced with varying frequency. In a total of 1683 mutational events analyzed, the most common type was *viridis* (31.0%), followed by *albina* (21.8%), *chlorina* (9.0%); *xantha* (7.1%), *al-tigrina* and *eceriferum* (4.8% each) and *chl-terminalis* (4.6%) constituted the next group of mutations induced with more or less similar frequency, while *xanthalbina, alboviridis, viridoalbina, al-terminalis, al-striata*, and *xa-tigrina* belong to the third major group appearing with a low frequency (1.1 to 3.2%). The remaining mutations appeared only as individual cases.

The data in Table 3 also include the segregation ratio of mutants belonging to various mutation types (expressed as percentage of segregants in mutated spike progenies). It was observed that certain mutation types showed, on average, a higher ratio of mutant-segregation than others. Considering only the cases with a larger number of mutational events, it may be seen that one group of mutations, i. e. *albina, xantha, chlorina, al-tigrina, chl-terminalis* and *eceriferum* segregated about half (11.5 to 15.9%), and *viridis, viridoalbina, al-striata* and *xa-tigrina*, only one fourth, the mutants expected on the basis of a 3:1 segregation ratio (or 25% of the spike progeny).

It should be mentioned here that in many cases the spike progenies produced a markedly higher number of mutants than 25%. In this experiment one progeny of the rare examples had 11 mutants and only one normal plant in the spike progeny to which it belonged.

Table 3. *Spectrum of mutations appearing in M_2*

Mutation type	Total number of mutation events	Segregation ratio, % mutants in the mutated spike progenies	Relation frequency of appearance, %				
			Total of the experiments	Gamma rays	EMS	NEU	EI
1. *Albina*	367	11.5	21.8	25.2	21.0	20.0	22.1
2. *Xantha*	119	12.0	7.1	8.1	7.5	6.6	5.9
3. *Chlorina*	151	14.3	9.0	10.7	5.4	3.8	9.1
4. *Viridis*	521	8.9	31.0	30.4	27.4	32.0	31.2
5. *Alboxantha*	0	—	—	—	—	—	—
6. *Alboviridis*	24	10.8	1.4	1.2	1.6	1.0	3.9
7. *Xanthalbina*	26	12.3	1.6	1.4	2.7	1.5	0.7
8. *Xanthaviridis*	5	22.6	0.3	0.4	0.0	0.4	0.0
9. *Viridoalbina*	42	9.5	2.5	1.8	1.6	3.0	3.2
10. *Viridoxantha*	15	6.8	0.9	1.2	1.1	0.8	0.0
11. *Al-striata*	53	6.8	3.1	1.6	6.5	3.5	1.9
12. *Xa-striata*	9	12.9	0.5	0.4	0.6	0.8	0.0
13. *Al-tigrina*	81	12.0	4.8	3.4	15.6	3.5	3.2
14. *Xa-tigrina*	54	9.6	3.2	3.8	0.0	3.7	2.6
15. *Al-maculata*	4	9.1	0.2	0.0	0.0	0.4	0.7
16. *Xa-maculata*	15	11.1	0.9	1.4	0.0	0.6	1.9
17. *Al-terminalis*	19	8.9	1.1	0.4	2.7	1.2	1.3
18. *Xa-terminalis*	13	10.0	0.8	0.2	1.1	0.9	1.3
19. *Chl-terminalis*	78	13.8	4.6	3.4	4.8	5.5	3.2
20. *Eceriferum*	81	15.9	4.8	4.4	0.5	5.4	7.8
21. Onion leaf	5	28.8	0.3	0.4	0.0	0.4	0.0
22. Dwarf	1	40.0	0.1	0.0	0.5	0.0	0.0
Total:	1683	100	100	100	100	100	100

Discussion

Maximization of mutation frequency: The detailed study of the mode of appearance of seedling mutations, most of which were lethal, provides an opportunity to understand the process which influences the ultimate production of mutations. The significance of methods of calculating mutation frequency has been stressed by several workers (Frydenberg, 1963; Gaul, 1960, 1961; Sharma, 1966). To increase the total mutation rate is perhaps the most important object of mutation research in higher plants. Usually the frequency of M_2 seedling mutants after EMS treatments has been below 15% in barley (Eriksson, 1965; Froese-Gertzen *et al.*; 1963; Konzak *et al.*, 1965; Frydenberg and Jacobsen, 1966; Rao and Natarajan, 1965; Savin *et al.*, 1968), although Gaul (1962, 1964) succeeded in raising it above 30% by using very high doses of EMS combined with diplontic selection through close planting. The mutant frequency induced by EMS in the present experiment was not very high (highest 5.7%). This low frequency may be partly attributed to the dilution effect caused by inclusion of all the spikes of M_1 plants, as shown by Bekendam (1961), Gaul (1960, 1961), Frydenberg *et al.* (1964) and Osone (1963). As expected, NEU gave the highest rate of mutations (6.7% mutants in dry seed treatments).

For a long time pre-soaking of seeds has been recommended as one of the techniques to increase mutation rate. But the findings of this experiment have shown that mutation induction is lowered by presoaking. These findings concur with the results obtained in the pea (Sharma, 1966). The low mutation frequency in pre-soaked seed treatments may be caused by a marked reduction in the mutagenic dosage. Thus, in the present case dry seeds could resist up to a dose of 50 kR gamma-rays, while for the seeds presoaked for 12 hours even a dose of 10 kR proved to be completely lethal. A dosage below 10 kR has been shown to yield a mutation rate as low as 0.6% (Frydenberg *et al.*, 1964). It is possible that presoaked seeds might give a higher mutation rate if equal doses were applied to both types of seed. However, these doses have to be very low and this may not be a rational approach to the maximization of mutation frequency.

Determination of mutagenic efficiency: The mutation frequency has been calculated by five different methods reported in the literature. Due to the various types of 'errors' discussed by Gaul (1961), conflicting results can be obtained depending upon the method of calculation. As can be seen from Table 1, the mutation rate with EMS treatment of pre-soaked seeds was higher than with gamma-rays when calculated on the basis of M_1 spike progenies, while the position was reversed if the comparison was made on the M_1 or M_2 plant basis. One way of avoiding this difficulty is to estimate the mutation rate on the basis of independently induced mutational events in the treated embryo. Such an evaluation is, however, complicated by the fact that, in many cases, more than one mutation in the same plant gives rise to similar phenotypic changes (multimutations), or one and the same mutation spreads over several spike progenies (cluster mutation). The situation has been somewhat simplified from the finding that those

spikes whose primordia are differentiated before the time of the mutation induction do not share a cluster mutation (Jacobsen, 1966), and it will be further simplified by making precise distinctions between various mutational phenotypes. The segregation ratio in M_2 and the behaviour of mutated plants are other criteria for finding affinity between similar mutations recorded in different spike progenies of the same plant. If there are several, independently induced, phenotypically similar, mutations in a genome (giving rise to a spike) the ratio of mutants may rise above 25% due to an "overlapping effect" in the case of recessive mutations.

The overall segregation ratios of mutants obtained in this experiment were the highest with EMS — dry seeds (14.7%). This could be due to the larger size of mutated-tissue chimeras in M_1 and induction of multimutations. An increase in the size of M_1 chimeras should also be represented by a greater number of cluster mutations. This was found to be so in treatments with all the mutagens, i.e. the frequency of cluster mutations was always higher in those treatments which gave a higher segregation ratio of mutants in M_2 (Table 2). Similar results were obtained in rice (Sharma, 1968). The frequency of multimutations was higher in EMS (dry seed) and NEU (dry and pre-soaked) treatments. The average size of M_1 chimera per mutational event could be smaller as a result of less killing of initial cells by low doses of the mutagens or slow penetration and late mutation induction by chemical mutagens. In accordance with the results obtained in rice (Sharma, 1968), pre-soaking of barley seeds also resulted in a lower number of cluster mutations. Since the penetration of mutagen should be easier in pre-soaked seeds, this reduction can be attributed to the onset of ontogenetic development due to pre-soaking before the mutations were induced. Considering that EMS induced a lower number of cluster mutations than did gamma-rays even when dry seeds were treated, it seems plausible to assume that this agent induced relatively more mutations as "late effects" than did the ionizing radiation. Probably, a prolonged mutagenic effect of EMS continuing over several cycles of cell division adds to the number of mutations initially induced, thereby increasing the number of multimutations.

The other two chemical mutagens, NEU and EI, induced more multiple as well as cluster mutations than did gamma-irradiation (Table 2). This suggests that these agents have either quicker penetration than EMS or induce mutations relatively quicker after coming into contact with the genetic material.

Mutagenic specificity towards mutation spectrum: Several authors (Arnason *et al.*, 1962; Ehrenberg *et al.*, 1961; Gustafsson, 1938; Konzak *et al.*, 1965; Ramanna and Natarajan, 1965; Swaminathan, Chopra and Bhaskaran, 1962) have reported differences in the mutation spectra induced by chemical and irradiation treatments. The results of the present investigation, however, do not indicate any remarkable differences in the rate with which the different mutation types were induced by EMS and gamma-rays (Table 3). This confirms the results previously obtained in the garden pea (Sharma, 1965).

The mutations *albina, xantha, viridis, chlorina, al-tigrina, chl-terminalis* and *eceriferum* appeared more frequently than others, both in the gamma-ray and chemical mutagenic treatments. It has been reported by Heslot *et al.* (1961), Ehrenberg *et al.* (1961), Favret (1964), Nilan *et al.* (1964), and Gaul (1964), that the proportion of *albina* mutations is higher after gamma-irradiation than with EMS-treatments. Apparently, the differences in genotypic constitution of the treated varieties play an important role in such cases, as demonstrated by Swaminathan (1961) for wheat. Natarajan and Shivasankar (1965) obtained a higher ratio of *albina* mutations after EMS treatment in another Indian barley variety, N.P. 13, while, in the variety N.P. 104 Sharma (1968) reported *albina* mutations induced with a relatively lower frequency, similar to the findings of the present experiment.

The mutation types are named here according to the similarity in their phenotypic expression. However, it is now established that phenotypically similar mutations in barley may, in some cases, owe their origin to a few dozen loci (Gustafsson, 1960; Hagberg and Persson, 1964), which individually may have a different mutability and specific reaction to different mutagens and modifying factors (Auerbach, 1966). In the experiments where such mutations are pooled on the basis of phenotypic similarity, the mutagenic specificity cannot be established with the same precision as is possible in lower organisms (Demerec and Cahn, 1953; Kolmark, 1953; Loveless and Howarth, 1959). According to Eriksson (1965), the compound mutability of multigenic mutations, like those affecting plastid development, was almost the same as in the case of a mutation determined by one locus. Contrary to this, the present investigations have shown that different mutation types vary from 3.1% (*al-striata*) to 31.0% (*viridis*). Some of the recorded mutation types were represented only by single examples (Table 3).

The pre-soaking of seeds prior to mutagenic treatment was not found to influence the mutation induction of different types markedly. Gaul (1958) has shown that mutation spectrum was independent of factors such as the sterility of M_1 plants.

Segregation ratio and mutation-fixation: As discussed above, the ratio of segregating mutants in M_2 is affected by various factors influencing intrasomatic selection and ontogenetic development in the M_1 generation. The results obtained in these investigations show that different mutation types give varying ratios of mutants in M_2 (Table 3). Gustafsson (1938) found that *albina* mutants appeared in a greater

number of individuals than did other types. Swaminathan *et al.* (1968) reported the segregation ratio of *albina* mutants to vary from 6.28% to 22.45% in rice. In the present experiment, the average mutant segregation in *albina* was 11.6%, which was almost equal to *xantha* and *al-trigina*. It was also observed that several lethal mutations had a higher segregation ratio (e.g. *chlorina*, *chl-terminalis* etc.) while viable mutations like *viridis* gave a lower segregation ratio. Gustafsson (1938) interpreted the high segregation ratio of *albina* as a result of minor changes in the genotype leading to this mutation. Gaul (1957), on the other hand, believes *albina* mutations to be caused by greater damage. However, with the discovery of molecular mechanisms of mutation induction at the level of DNA, it should be expected that the basic changes caused at genic level will be the same, irrespective of the viability of the resulting mutation. Nevertheless, a difference in segregation ratio may be due to the differential ability of competition in ontogenetic development or the viability of gametes and zygote (Gustafsson and Nybom, 1950; Gustafsson *et al.*, 1950; Blixt, 1968). Besides, a smaller size of chimera in M_1 plants for certain mutational types, resulting in low segregation ratios, may be expected if there exists a differential pattern in time for resolving premutational instabilities into mutations, for mutations belonging to different phenotypic groups, as proposed by Demerec and Cahn (1953) for gene mutations.

The progenies giving a mutant ratio above 25% are specially interesting. The data in Table 3 show that the segregation ratio was 28.8% in onion leaf and 40% in dwarf. In one progeny of onion leaf, out of 12 plants in the spike progeny, 11 were mutants. Such deviations which may be caused partly by small progeny size, have also been observed by previous authors (Moh and Smith, 1952; Gaul 1965). Other reasons for high segregation ratios could be the induction of similar multimutations in the same genome with an overlapping effect of recessive mutations, induction of dominant mutations, or simultaneous induction of visible and lethal mutations where the latter causes death of individuals not affected by the former.

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