https://doi.org/10.29321/MAJ.10.A03563

Induced Mutagenesis in Rice - I. Sensitivity and Chlorophyll Mutation Rate

by

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Introduction: Radiogenetical studies in rice received a fairly wide importance, as early as 1934, from the pioneering studies of Ramiah and his associates in India and those of Ichijima in Japan. The increased application of physical mutagens, attained prominance in the 'Atom Era' of forties and fifties, contributing to isolation of a large number of mutants in various crops, including rice. In the present studies, an attempt has been made to understand the relative mutagenic effects caused by ionizing radiations, namely, X-rays and gamma rays, on rice by (i) evaluating the relative sensitivity and (ii) the rate of chlorophyll mutation in M_2 generation.

Material and Methods: A short duration rice strain, Co. 13, evolved by pureline selection was chosen for the study. Well filled seeds with 11 per cent moisture content were X-irradiated with Philips C. T. apparatus, operated at 50 KV 2 Ma without filter at a distance of 4 cm from the source, at Agricultural College and Research Institute, Coimbatore. Gamma irradiation was done at the Atomic Energy Establishment, Trombay. One hundred seeds each were irradiated at six levels of doses, 10, 20, 30, 40, 50 and 60 Kr. in both the mutagens, namely, X-rays and gamma rays.

The treated seeds were sown replicated four times in shallow trays. At 30th day, seedlings (M_1) were transplanted in the field. In each M_1 plant, the first formed three spikes were selected and each spike was selfed. Seeds from the first, second and third spike from each of the M_1 plants under different treatments were sown in separate seed beds and the chlorophyll mutations were scored from sixth to fifteenth day in M_2. The chlorophyll mutations were classified according to the nomenclature adopted by Gustafsson (1940).

Results: *Germination and survival in M_1:* The germination in the control and treated seeds did not differ significantly and ranged from 96.6 per cent from control to 91.6 per cent in treated seeds. The survival of the seedlings on 30th day recorded highly significant differences between the different treatments of the same mutagen and also between X-rays and gamma rays. The survival of seedlings from X-ray treated seeds showed a gradual reduction from 100 per cent in control to 52.8 per cent in case of 60 Kr. in respect of X-ray treatment. The reduction in survival was very much

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reduced from 100 per cent in the control to 26.4 and 3.3 per cent respectively at 40 and 50 Kr. and no survival at 60 Kr. treatment. The mean survival of seedlings was more in the case of X-rays than that in gamma rays at doses above 20 Kr. The LD. 50 was found to be around 60 Kr. for X-rays and between 20 and 30 Kr. for gamma rays as evident from the survival of seedlings under different treatments. At maturity, the survival of plants was less in gamma rays as compared to X-rays treatment. At doses of 50 Kr. and above, plants could not be reared to maturity with gamma rays.

In case of X-ray treatment, there was a gradual reduction in height of plant while in gamma ray treatment the reduction was pronounced and proportionate to the doses applied. The actual reduction in height was manifest even from 10th day onwards and the same trend was also maintained at different stages, 10th, 15th, 20th, 25th and 30th day, after sowing. The

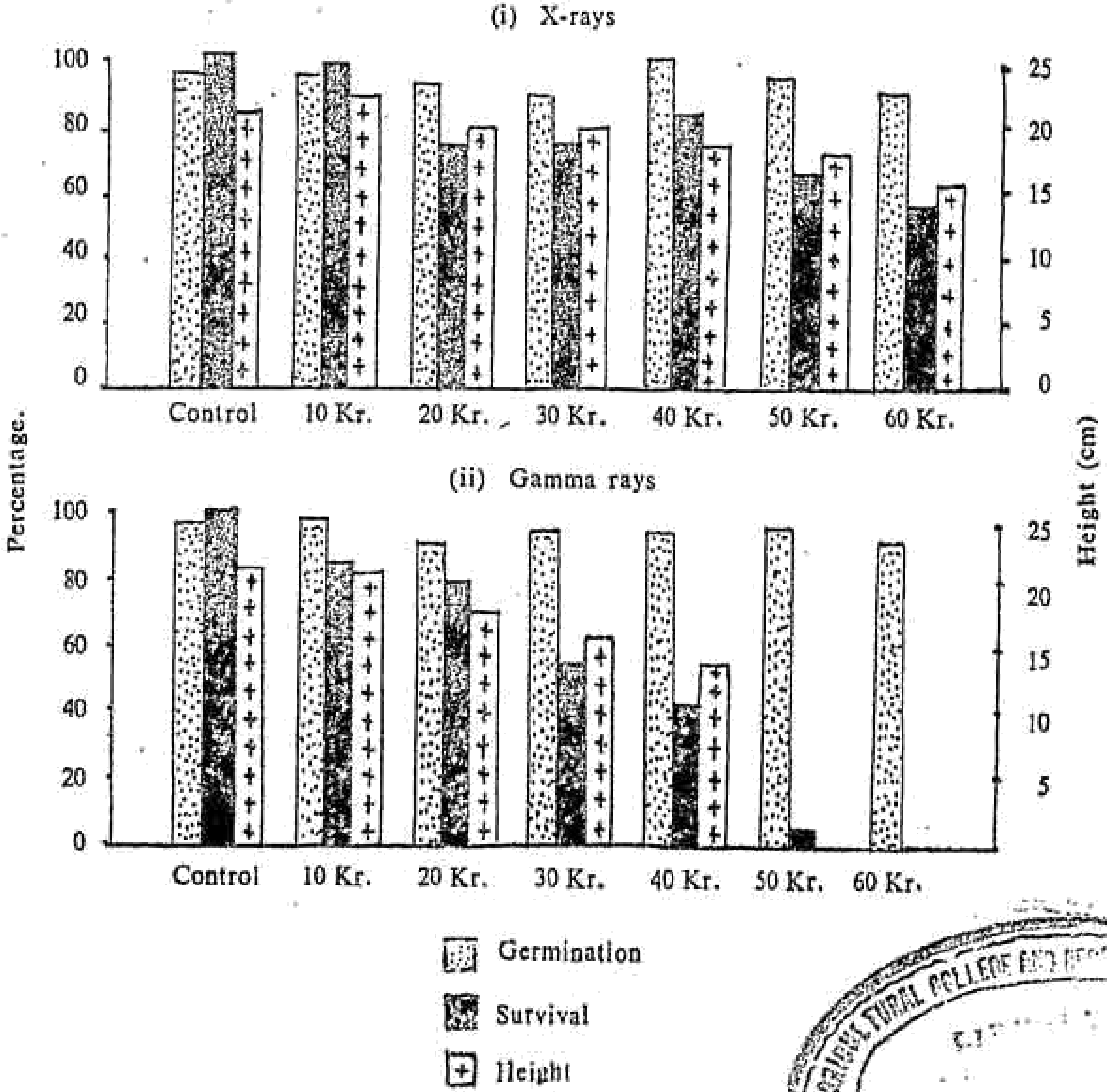

Fig. 1. Germination, survival and height of M_1 seedlings

LD. 50 for height of seedlings was 40 Kr. in gamma ray treatments. The differences in reduction of height in gamma rays was significantly greater than that caused by treatments with X-rays for a given dose. The comparative effects of germination, survival and height of seedlings of X- and gamma rays are shown in Fig. 1.

The percentage of germination ranged from 66.6 to 82.4 in seeds of M_1 plants treated with X-rays and from 74.4 to 85.2 in those treated with gamma rays. No consistent relationship was observed between the mean percentage of germination and survival of M_2 seedlings under different dosages of both the mutagens. The chlorophyll mutations were scored upto fifteenth day after sowing. Four types of mutations commonly noticed were *albino*, *xantha*, *viridis* and *albo-viridis*. The frequency of chlorophyll mutations scored in the first formed three spikes are given in Table 1. The data on chlorophyll mutations from the first formed three spikes, were pooled together to arrive at the rate of mutation. The mutations that occurred independently or together in the first, second and third spikes were considered together to compute the number of segregating families. The mutation rate expressed on the basis of M_1 family, M_1 spikes and M_2 seedlings is given in Table 2 in comparison with the rate of mutation in the first formed three spikes in the development sequence.

TABLE 1. *The rate of chlorophyll mutations in M_2 and the comparison of the rate of chlorophyll mutations in the spikes in the development sequence*

Doses Kr.	Mutation rate (%)			Mutation rate in the spike (%)					
				Primary		Secondary		Tertiary	
	M_1 family basis	M_1 spike basis	M_2 seedlings basis	M_1 spike	M_2 seedling	M_1 spike	M_2 seedling	M_1 spike	M_2 seedling
X-rays									
10	12.2	9.1	2.3	12.2	2.3	11.1	3.4	—	—
20	12.5	7.8	1.5	6.3	0.8	50.0	5.4	—	—
30	15.1	11.1	0.9	12.1	1.0	—	—	25.0	1.3
40	7.7	3.8	1.3	7.7	1.3	—	—	—	2.3
50	19.0	13.5	1.7	9.4	1.3	—	—	60.0	7.4
60	21.4	14.2	5.5	21.4	8.2	—	—	—	—
Gamma rays									
10	20.5	8.6	1.2	2.3	0.2	16.1	2.0	9.7	1.6
20	26.5	15.5	4.5	17.7	5.5	10.0	0.4	14.2	5.5
30	38.1	18.1	3.0	28.5	3.5	10.0	4.2	8.5	1.0
40	—	—	—	—	—	—	—	—	—

The rate of mutatation was in general higher when expressed as percentage on M_1 family basis than on M_1 spike basis (Fig. 2). The gamma ray treatments yielded higher rate of mutations than X-rays at similar doses, the highest percentage being 38.1 in the 30 Kr. of gamma rays. The rate of mutations on M_1 spike basis ranged from 3.8 to 18.1 per cent, the highest being in 30 Kr. of gamma rays. The mutation rate on M_2 seedling basis was, in general, low in both the mutagens, the range being from 0.9 to 5.5 per cent. However, the greater potency of gamma rays over X-rays in inducing high frequency of chlorophyll mutation is expressed in all cases.

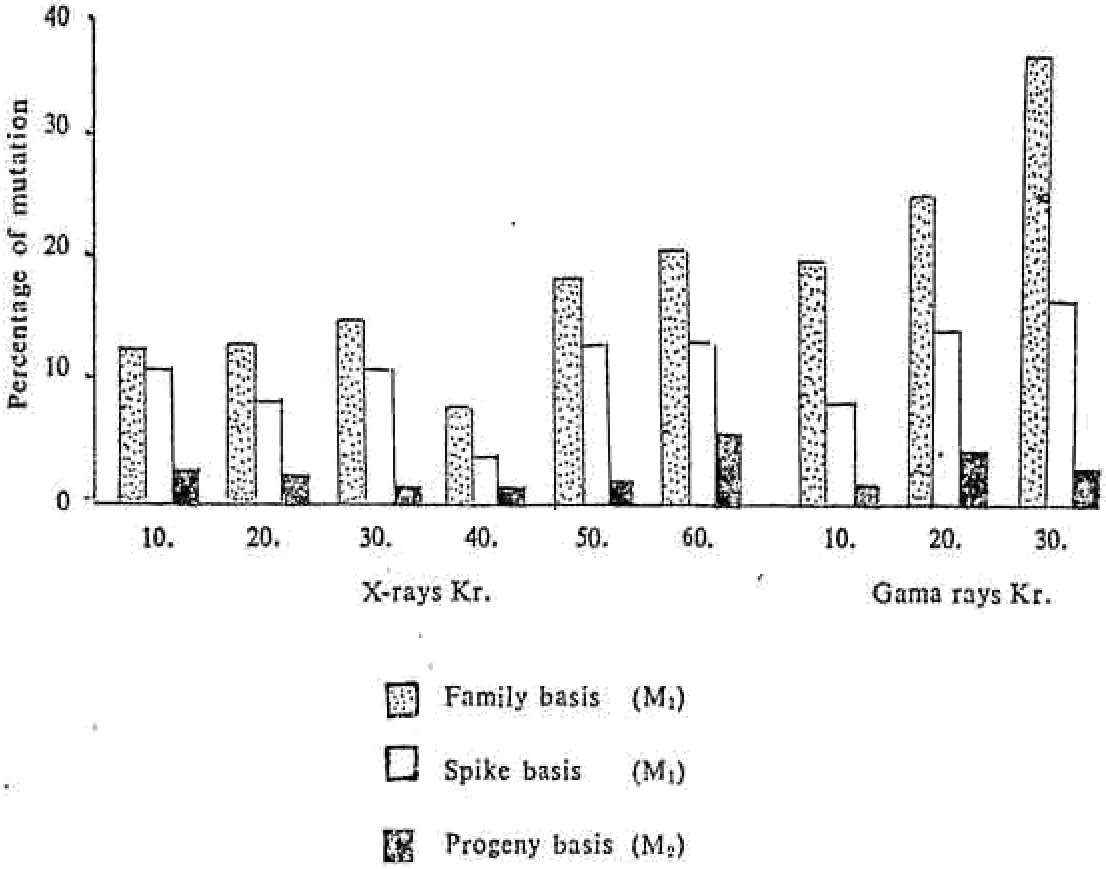

FIG. 2. Camparative rate of chlorophyll mutation (Percentage)

The chlorophyll mutations occurred in the main tillers in all the dose while in a few doses only the mutations were recorded in the second and third tillers. The rate of mutations, when compared between the three tillers in their developmental sequence revealed no consistent relationship between the occurrence of mutations, dose rate and the formation of tillers. There was an increase in the rate of mutations with the increase in levels of doses, the increase being distinct and high with gamma rays and somewhat proportionately low in X-rays. The curves depicting the rate of mutations was exponential with gamma rays and sigmoid with X-rays (Fig. 3).

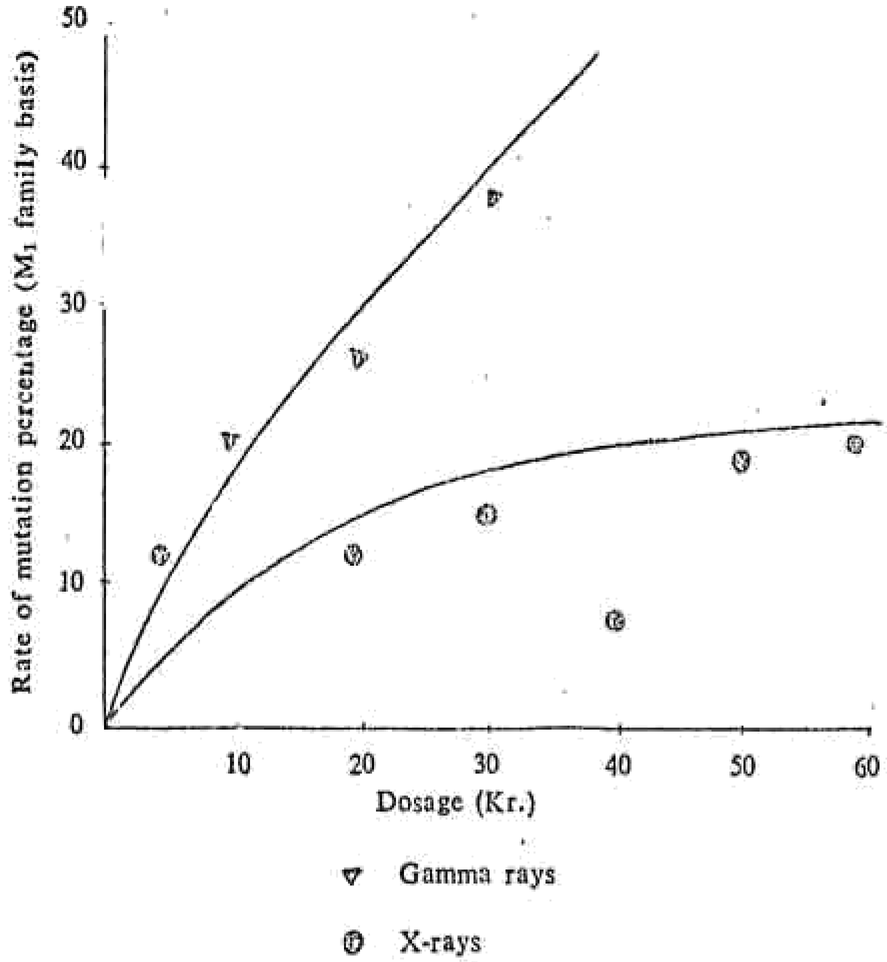

FIG. 3. Rate of Chlorophyll mutation

Spectrum of mutations: The four kinds of chlorophyll mutations, namely, *albina*, *xantha*, *viridis*, and *albo-viridis* were noticed in all treatments with X-rays as well as gamma rays. There was no relationship between the type of mutations and doses of mutagens. Among the mutations, *albina* was more in proportion than the rest. The spectrum of mutations expressed on percentage basis under each dose is shown in Fig. 4.

Seed fertility of M_1 plants and chlorophyll mutation in M_2: The fertility of all the M_1 plants in each dose showed a wide range. The fertility of the individual mutated plants also exhibited a wide range in all the doses and the frequency of the mutated M_1 plants in the different seed fertility groups did not indicate any consistant relationship. However, the mean seed fertility of M_1 population and the rate of mutations, under each dose revealed a trend of increase in mutation rate with corresponding increase in seed sterility, the trend being more apparent with gamma rays, than X-ray treatments. (Table 2). The occurrence of different types of chlorophyll mutations with reference to the seed fertility of M_1 plants showed no relationship whatsoever between the M_1 fertility and kind of chlorophyll mutations,

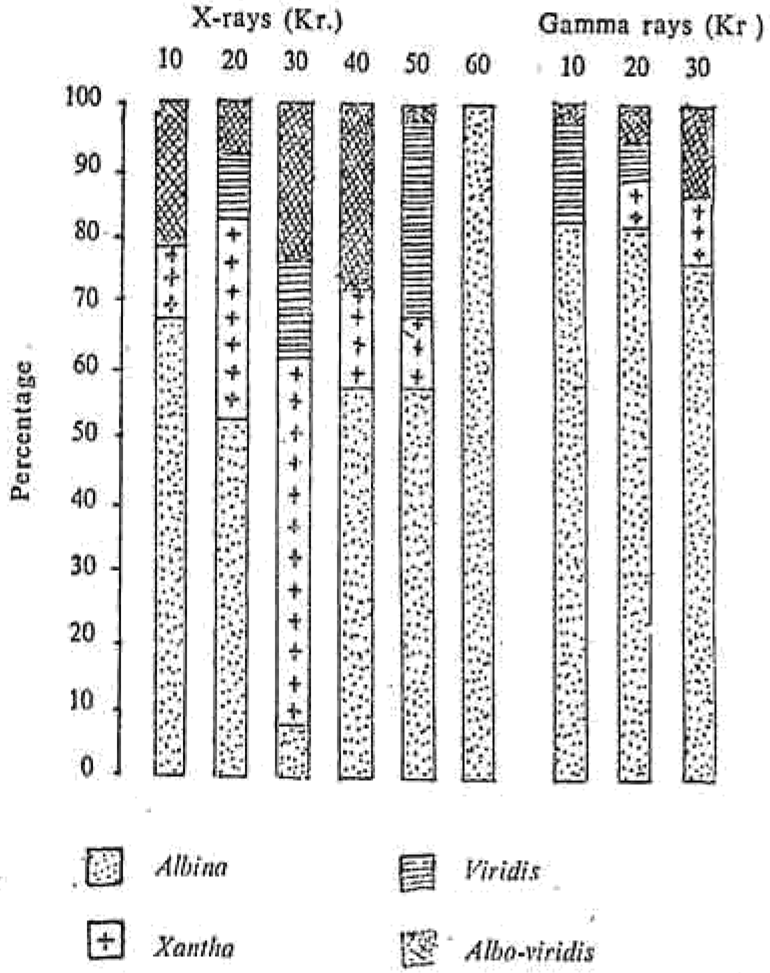

Fig. 4. Spectrum of mutants

TABLE 2 *Seed fertility in M_1 and number of M_1 plants showing chlorophyll mutations (First spike)*

Dosage (Kr.)	Frequency distribution in percentage										Mean seed fertility (percentage)	Mutation (percentage)
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100		
X-rays												
10	—	1(1)	—	—	1	4	2(1)	3	12	18(3)	82.9	12
20	—	—	1	3(1)	3	—	6(1)	8	5	7	72.9	6
30	1	4(1)	1	2	1(1)	2	2	4	5	12	68.9	6
40	2	2	1	3	5	—	4(1)	—	1(1)	8	60.0	8
50	5	2	—	1	2(1)	2	2(1)	2	1	4	49.7	10
60	1	1	2	2	3(1)	1	—	—	1(1)	3(1)	49.3	21
Gamma rays												
10	5(1)	—	5(1)	5(2)	6(1)	4	4	2	7(1)	6(2)	53.3	18
20	5(1)	11(2)	2(1)	2	2(1)	2	1	2	3	5(4)	42.3	26
30	9(3)	3(2)	—	2	2	1(1)	1	—	2(1)	3(1)	36.6	35
40	3	—	—	2	1	—	1	—	1	—	27.9	—

The number of mutated plants under the frequency class is indicated within paranthesis.

Discussion: The effect of physical mutagens on crop plants have been studied by many since the time of Stadler (1928). The immediate effect of these mutagens on dormant seeds were, in general, in the nature of inhibition of germination, increase in lethality, retardation of growth and reduction in fertility (Gustaffson, 1944; Gaul, 1958 and Natarajan, 1964). In the present study, it could be inferred that for similar biological effect with the same strain (Co. 13 rice) the dosages required were different for both mutagens, 60 Kr. and 30 Kr. of X- and gamma rays respectively for survival and inhibition of growth rate, thereby indicating the two fold effectiveness of gamma over X-rays. Likewise, at maturity gamma rays were found to be more drastic, since no plants survived at 50 Kr. and above. The LD. 50 for survival also showed in clear terms the biological effectiveness of gamma over X-rays. The LD. 50 estimates in the present report is in consonance with the previous reports for *indica* rice by Ota *et al* (1962), Nayar (1962) and Siddiq and Swaminathan (1966). The M_1 seed fertility also further confirmed the effectiveness of gamma rays, however, in a more drastic manner, as for example, the reduction of seed fertility by X-rays at 50 Kr. dose was biologically comparable with the dose of 10 Kr of gamma rays. A linear trend was noticed between the fertility and dosage in rice by Yamaguchi (1964), while Bekendam (1961) showed levelling off of fertility with X-rays and by Yeh and Henderson (1963) with gamma rays. In the present study, the saturation effect was evident only in respect of X-rays. The differences in the observations on seed fertility at different dosages might be due to the different genotype employed.

The comparison of chlorophyll mutation rate, in the present study, showed gamma rays to be more potent in inducing a higher frequency than X-rays (Table 2), as seen from the dosage requirement of gamma rays and X-rays to give a biologically similar effect. The effectiveness of gamma rays over X-rays has also been found by Masima and Kawai (1959) in rice, Jagadeesan (1960) in cotton, Gaul (1961) in barley, and Banerjee (1966) in wheat. The frequency of mutations showed an increase with increase in dosage giving an exponential curve with gamma rays and a sigmoid curve with X-rays. Nevertheless, the saturation effect was also evident with dosages upto 50 Kr in X-rays. In contrast, Yamaguchi (1964) showed mutation rate with X-rays to be high at middle doses and a decrease at higher doses.

However, a consideration of the chlorophyll mutation rate with reference to the ontogenic sequence revealed an altogether different trend and was of significance in adopting techniques in practical mutation breeding. The primary spikes yielded higher frequency of mutations than late formed spikes and they did not also carry mutations in many doses confirming the finding that main spikes carry more number of mutations than late formed ones and hence the expresssion of the mutation rate per M_1 family was higher than that on spike basis (Table 1). This is referred to as "Dilution of mutants" in rice by Matsuo and Onazava (1960), Osone (1963) and Siddiq and Swaminathan (1966) and also in other crop plants by Gaul (1961) and Gustafsson (1963) with physical, chemical and combination of physical and chemical mutagenic treatments.

In all the cases, the reduction in number of mutations in late formed spikes or the dilution of mutations was explained on the basis of intrasomatic selection, due to competition between healthy and affected cells. This phenomenon was termed as "Diplontic selection" by Gaul (1958). The diplontic selection is a limiting factor in induced mutagenesis to the realisation of mutations following mutagenic treatments and the problems arising out of diplontic selection are acute in seed propagated crops (Swaminathan, 1961). The reasons for the diplontic selection have been given by Gaul (1961). The presence of multicellular embryo and the chimeric nature of spikes from irradiated plants are put forth as responsible for intrasomatic selection. Bekendam (1961) has inferred that in rice, one to four embryo initial cells contribute to the formation of a spike. Osone (1963) has concluded the chimeric nature of X_1 spikes from their studies in X_2 and X_3 segregation. The present study also confirms the chimeric nature and multicellular embryo. However, Yamaguchi (1961) has also found that two kinds of spikes are formed in M_1, one type originating from single cells and the other from many cells, the former being more at higher doses than the latter. The present study also indicated the differential origin of main and late formed spikes and consequently the low mutation rate in the late formed spikes (Table 1) and also the independence in the occurrence of mutations in the main and late formed spikes.

The difficulties in selection of M_1 plants for isolating mutants in M_2 will be obviated, if any relationship exists between the M_1 fertility and M_2 mutation rate. The frequency of the mutated plants to the fertility group (Table 2) revealed that M_1 plant seed fertility and M_2 mutation rate were independent to one another. However, the mean seed fertility of the M_1 population as a whole per dose revealed a positive relationship with the mutation rate. In other words, higher the mean sterility of M_1 population, higher the mutation rate, a relationship that evidently contradicts the independence of individual M_1 seed sterility and M_2 mutation rate. The fertility status and mutation rate have been dealt on different aspects by Gustafsson (1940), Mackey (1951) and Gaul (1961) in barley, by Bhatia and Swaminathan (1963) in wheat and by Hildering and Vender (1966) in tomato.

The four types of chlorophyll mutants had also been noted to bear association in respect of specificity of occurrence with none of the factors, such as, dosage, kind of mutation, fertility status of M_1 and the tillers from which they arose. The spectrum of chlorophyll mutants noticed in other cereals also essentially showed, similar behaviour (Bhatia and Swaminathan, 1963; Gustafsson, 1963; and Siddiq and Swaminathan, 1966).

Summary: The mutagenic efficiency of the two physical mutagens, X- and gamma rays and the rate of induced mutation were studied with reference to chlorophyll mutation. The application of the two mutagens, X- and gamma rays, at doses of 10, 20, 30, 40, 50 and 60 Kr. to dry dormant seeds of Co. 13 resulted in the differential survival of seedlings, without affecting the sprouting ability. The survival of seedlings was more drastically reduced at higher doses in treatments with gamma rays than with X-rays. The LD. 50 for survival of seedlings on 30th day was around 60 Kr. for X-rays and around 30 Kr. for gamma rays. The M_1 plants exhibited seed sterility to varying extent at maturity, the mean sterility increasing with increase in dosage. It was evident from the observations on M_1 generation that gamma rays have greater adverse effect than X-rays at similar doses, in respect of survival, growth rate of seedlings, and seed fertility.

The chlorophyll mutation rate was found to range from 7.7 to 21.4 per cent with X-rays and from 20.5 to 38.1 per cent with gamma rays, at doses ranging from 10 Kr. to 60 Kr. The rate of mutations exhibited an increase with increase of dosage, the trend being exponential with gamma rays and sigmoid with X-rays. The rate of chlorophyll mutations per M_1 family was found to be higher than that on M_1 spike basis.

The role of 'diplontic selection' as a limiting factor in realising fully the induced mutations in the seed propagated diploid, *O. sativa*, has been critically discussed and the practical implications have been pointed out. The early formed spikes showed higher rate of mutations than the late formed ones, but the spikes irrespective of the sequence of their emergence, showed a similarity in the type of mutations produced. The spectrum of chlorophyll mutations, comprising of *albino*, *xantha*, *viridis* and *albo-viridis*, remained unaltered, either by the dosage or by the type of mutagen. It was also evident from the present study that the mutation rate and spectrum were independent of M_1 plant seed fertility, indicating the desirability of selection of fertile M_1 plants for study of progenies.

Acknowledgements: The author expresses his deep gratitude to Dr. B. W. X. Ponnaiya, Dean, Agricultural College and Research Institute, for his guidance and encouragement. The help of Dr. P. Madhava Menon, Reader in Genetics and Paddy Specialist and his staff is gratefully acknowledged. His thanks are also due to Dr. S. R. Sree Rangasamy, for his valuable suggestions during investigation and help rendered in computing the data.

The studies formed a part of the dissertation submitted to the University of Madras in part fulfilment of the conditions for the M. Sc. Degree Examination in Agriculture held in May 1967.

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