MUTAGENIC EFFICIENCY OF GAMMA IRRADIATION IN SESAMUM

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THESIS

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(N.RAMACHANDRAN NAIR)

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C E R T I F I C A T E

Certified that this thesis is a record of research work done independently by Sri. N. Ramachandran Nair, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

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Nearly five decades have passed since Muller and Stadler made the first report on the genetic effects of X-rays in Drosophila and maize respectively. The reports made available immediately after this discovery were mostly on the cytological and morphological effects of radiations. Since then a number of papers have been published on the effects of radiations on biological systems. These results were reviewed by various authors (Gustaffson, 1944; Hammons, 1953; Konzak et al. 1965; Gregory, 1968) and all of them have stressed the importance of radiation as a tool in mutation breeding of crop plants. With the rapid growth in the theoretical understanding of the process of induction and recovery of mutations, the stage is now set for a dynamic phase in the use of this tool in plant breeding. While mutation breeding in general will supplement rather than substitute the other methods of breeding, its major role will be in the development of altogether new characteristics.

It was noticed in as early as 1944 that seeds which contain a high degree of fats and fatty acids are relatively resistant to ionising radiations (Gustaffson, 1944). It is interesting to know whether the inherent capacity of these plants species to resist radiation effects is due to their ability to resist initial damage or by virtue of having damage repairing mechanisms. According to Gregory (1968) modern techniques involving radiation treatments form an integral part in the genetic improvement of oil seed crops.

Induced gene mutations being mostly recessive will make thoir appearance normally in the M_2 generation. Hence the study of mutagenic effectiveness and efficiency can be made only in the second generation. The effects of mutagens observable in the first generation are lethality, injury, sterility, chimerism, etc. A proper knowledge of these parameters would help us to understand the nature and magnitude of changes that may appear in the subsequent generations.

In Kerala, Sesamum is the second important oilseed crop. Yot the reports available on the efforts made towards tho genetic improvement of this crop are very few. Attempts have to be made for evolving new varieties by employing modern techniques like mutation breeding. In view of the need to study the radio-biological effects and explore tho scope for mutation breeding, the present investigation was undertaken in Sesamum employing gamma rays.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The principal objective of irradiating plant material is the production of mutations of economic importance. However, most of the mutagens are found to induce morphological effects such as lethality, injury, chromosomal aberrations and sterility in the M_1 generation. With the knowledge acquired on the action of radiations on genes it is possible to increase mutation frequencies without a corresponding increase in physiological disturbance or chromosome damage. Many studies on effectiveness of mutagens are carried out with chlorophyll mutations, because the other types of mutations are induced with frequencies parallel to that of chlorophyll mutations. These mutations can be scored during the early seedling stage because they can be easily detected. Since highly efficient mutagens have been found, the problem of controlling the mutation spectrum is also attracting attention.

A great majority of the literature available on mutation research relates to monocotyledonous plants such as barley, rice and wheat. In contrast to this, only much less work has been done in dicotyledonous plants. Since elaborate reviews have already been made by various authors, no attempt has been made in this study to review the voluminous literature available on monocots. This review is thus restricted to mutation research in dicotyledonous plants.

I. Effects of mutagens in the M_1 generation

1) Germination

Bilquez and Martin (1961) reported in *Arachis* that irradiation with X-rays at 8000 R stimulated germination. De Nettancourt and Contant (1966) reported in *Lycoporsicon esculentum* and *Lycoporsicon pimpinellifolium* a slight stimulatory effect on germination after chronic gamma irradiation at the dose rate of 1.9 r/hr. Vishnuswarup and Gill (1968) observed that in french beans, seed germination was better in treatment with X-rays at 7 kR and 14 kR. According to Rajan (1969), the effect of gamma irradiation at lower doses on germination was stimulatory in *Sesamum* and *Brassica*.

Ankineedu *et al*. (1968) studied the effect of fast neutrons and gamma rays on castor. They observed that at the lowest dose (25 kR) there was no appreciable reduction in sprouting, but the treated seeds took longer time to sprout. Sinha and Godward (1972) reported that germination was unaffected in *Lens escularis* by gamma irradiation at lower doses of 4 and 8 kR. Joshua et al. (1972) also reported in jute that irradiation with gamma rays and fast neutrons did not affect germination.

Reduction in germination was reported by a number of investigators consequent to treatment with physical and chemical mutagens at varying doses. Bilquez and Martin (1961) reported in *Arachis* a reduction in seed germination following X-irradiation at 40000 R. According to De Nettancourt and Contant (1966) a higher dose rate of gamma irradiation could slightly decrease germination in *Lycopersicon esculentum* and *Lycopersicon pimpinellifolium.* Das and Mukherjee (1968) in grapes following gamma irradiation and ethyl methane sulphonate treatment, Witherspoon (1968) in *Liriodendron tulipifera* following gamma irradiation and Narsinghani and Sudhirkumar (1969) in *Vigna sinensis* following ethyl methane sulphonate treatment have also observed reduction in seed germination with an increase in doses. Similar results were obtained by Magassy and Farkas (1970) in sugar beet following X-irradiation, Dudek (1970) in *Nicotiana tabaccum* and *Nicotiana rustica* and Mujeeb and Greig (1973) in *Phaseolus vulgaris* following gamma irradiation.

2) Survival

Bilquez and Martin (1961) reported a high survival rate after the application of high dose (40,000 R) of X-rays in Arachis. Constantin and Love (1967) observed that the highest neutron dose employed by them did not affect survival in Vigna sinensis. However a decrease in survival at higher doses of gamma rays was recorded. Magassy and Farkas (1970) in Sugarbeet following X-irradiation, Joshua et al. (1972) in jute following gamma ray and thermal neutron irradiation and Mujeeb and Greig (1973) in Phaseolus vulgaris following gamma irradiation also observed reduction in survival in M_1 with increase in dose.

Ankineedu et al. (1968) in their studies on the effects of fast neutrons and gamma rays on castor reported that the mortality percentage before flowering decreased slightly. According to Sinha and Godward (1972) the percentage of survival at maturity remained unaffected in two varieties of Lens escularis at doses of 4 and 8 kR.

3) Plant height

Vishnuswarup and Gill (1968) in french beans observed that growth and vigour of M_1 plants were normal at all doses of X-irradiation except at 21 kR.

Arsagova (1972) reported a stimulation of growth and increase in yield in two varieties of cucumber in the M_1 following treatment with chemical mutagens.

Das and Mukherjee (1968) in grapes following gamma irradiation and ethyl methane sulphonate treatment, Ankineedu *et al*. (1968)in castor after fast neutron and gamma ray treatment, Monti and Donini (1968) in *Phaseolus* following chronic gamma irradiation and Narsinghani and Sudhirkumar (1969) in *Vigna sinensis* after ethyl methane sulphonate treatment have reported reduction in plant height. Rukmanski (1969) observed greater variability in plant height and stem length among the M_1s in french beans after gamma rays and fast neutron treatment. A reduction in plant height was also reported by Bajaj *et al*. (1970) in *Phaseolus* following gamma irradiation, Joshua *et al*.(1970) in jute following gamma ray and fast neutron irradiation and George and Nayar (1973) in linseed following gamma irradiation.

4) *Fertility*

Ankineedu *et al*. (1968) observed in castor following gamma ray and fast neutron irradiation, an increase in sterility with increase in dose. Sinha and Godward (1972) also observed similar effects in Lens escularis following gamma irradiation.

Ciurdarescu et al. (1968) reported that in lucerne following thermal neutron irradiation at various doses chromosome aberrations, chromosome bridges, laggards, fragments and combinations of these irregularities. Bridges were seen in about 42% of the cells examined.

Scumpu and Ionescu (1968) studied the effect of gamma irradiation in some field pea varieties. Chromosome aberrations in mitosis showed an incidence of 4-5% at low doses rising to 16 to 51% at 15 kR. Aberrations in meiosis showed a similar incidence at low doses but reached 40 - 66% at 15 kR. Dudek (1970) found various chromosome abnormalities in the root tip and pollen mother cells of Nicotiana tabaccum and Nicotiana rustica following gamma irradiation. Kuliev (1970) in his studies on mutagenic effects of colchicine on cotton reported polyploids, bridges and fragments at meiosis in the M_1 generation.

5) Chlorophyll chimeras

De Nettancourt and Contant (1966) reported color sectors on the first leaves of Lycopersicon esculentum and *Lycopersicon pimpinellifolium* following chronic gamma irradiation. Further investigations showed that the frequency of plants with visible sectors increased linearly with exposure rate and was higher always in *Lycopersicon pimpinellifolium*.

Radiation induced chlorophyll deficiencies in M_1 plants were reported by Ankineedu *et al*. (1968) in castor following irradiation with fast neutrons and gamma rays and Bankowska and Rymsza (1969) in *Phaseolus vulgaris* following gamma irradiation at doses 3000 to 12000 R.

6) *Morphological abnormalities*

Sinha and Godward (1972) reported in *Lens escularis* a stimulation in morphological developments at 4 and 8 kR treatments of gamma rays. However, Nayar and George (1969) observed no morphological changes in the M_1 generation after irradiating *Sesamum* with gamma rays at doses 25 kR to 100 kR.

De Nettancourt and Contant (1966) observed in *Lycorpersicon esculentum* a remarkable twisting of the tip of one or more of the lower leaves after chronic gamma irradiation. This was found to occur in frequencies which also increased linearly with dose. In *Arachis*, a number of investigators reported changes in growth habit, stem thickness and other morphological characteristics following irradiations (Bhatt *et al*. 1961, Sinha and Roy, 1969, Arzumanova, 1970). Das and Mukherjee (1968) observed in grapes that ethyl methane sulphonate treated seeds yielded some bushy branched seedlings but without any marked deviation in later stages of growth. Ankineedu *et al*. (1968) in castor following gamma ray and fast neutron irradiation reported that deformed leaves develop slowly giving rise to irregularly lanceolate, oblique, thick and dark leaves with wavy margin without any regular shape and outline. Gupta (1968) studied morphological changes induced by X-irradiation in *Brassica campestris*. Tetraploid plants having closed petals and dark yellow flowers were also found. Nazarov and Egorova (1969) in peas following gamma irradiation, Moh (1970) in beans following cycasin treatment and Paucheva and Dirkovos (1972) in vetch following treatment with chemical mutagens also reported a number of morphological changes. In jute, following irradiation with gamma rays and fast neutrons, Joshua *et al*. (1968) reported deformed leaves, suppression of terminal bud and appearance of tendril like structures on the upper surface of leaves. Rajan (1969) reported in Brassica and Sesamum several types of abnormalities such as cleft cotyledons, tricotyledons and unicotyledons after treatment with gamma rays and fast neutrons.

Morphological changes were also reported by Constantin and Love (1967) in Vigna sinensis following neutron irradiation, by Dudek (1970) in Nicotiana tabaccum and Nicotiana rustica following gamma irradiation and by Schroter (1972) in sugarbeet following X and gamma irradiation.

7) M_1 mutations

Raut (1969) reported that hydrazine induces recessive mutations in M_1 generation in tomato. Bankowska and Rymsza (1969) isolated a number of chlorophyll mutants and some dwarf mutants with supernumery leaflets in the M_1 generation in Phaseolus vulgaris following treatment with gamma rays. Hangildin (1970) in his studies on X and gamma ray irradiation in pea observed a mutant in the M_1 having three pods on the lower peduncles and a bush like habit. Bansal (1973) isolated in Capsicum annuum a mutation in the M_1 generation in which the reproductive parts were transformed to vegetative ones, after treatment with ethyl methane sulphonate.

II. Mutations in the M_2 generation

1) Chlorophyll mutations

i) Frequency

Pipie (1967) in his studies on mutagenic effects of diethyl sulphate and ethyl methane sulphonate on peas observed that the incidence of leaf spotting and chlorophyll mutations after treatment showed a positive linear correlation with the dose of diethyl sulphate but not with that of ethyl methane sulphonate. He also observed that ethyl methane sulphonate induced a greater frequency of mutations than diethyl sulphate. Vishnuswarup and Gill (1968) reported that following X-irradiation in french beans, the frequency of mutation was maximum at 7 kR. Chekalin (1968), in *Lathyrus sativus* and *Lathyrus tingitanus* reported that the frequency of mutation depends more on the mutagen and the species than on the mutagen dose. According to Vasileva and Mekhandzhievm (1971), radiation intensity had no noticeable effect on chlorophyll mutation frequency and spectrum in peas. Kustarev (1972) observed in tomato that mutation frequency was highest after treatment with N-nitroso-N-methyl urea and dimethyl sulphate. According to Paponova (1969) the frequency of chlorophyll mutations was greater in the diploid radish than in the tetraploids and increased with increasing dose of nitroso ethyl urea. Ushlik (1972) based on a comparative study of mutational activity of ethyl methane sulphonate and gamma rays in Lens esculenta observed that the highest proportion of segregating M_2 progenies comprising chlorophyll mutants and chimeric plants were 29.7 and 20% for ethyl methane sulphonate and gamma rays respectively.

ii) Spectrum

Das and Mukherjee (1968) reported albino seedlings in grapes following gamma irradiation and ethyl methane sulphonate treatment. Sur (1969) observed in blackgram following irradiation with gamma rays and thermal neutrons a large variety of chlorophyll mutations such as chlorina, albina, xantha, chloritica etc. Bankowska and Rymsza (1969) observed a number of chlorophyll mutations in Phaseolus vulgaris following gamma irradiation. Paponova (1969) observed chlorophyll deficit types such as albina (lethal), chlorina (lethal), Xantha (lethal) and viridis (more viable than the other mutants) in radish following nitroso ethyl urea treatment. Joshua et al.(1972) found in jute following thermal neutron and gamma irradiation that chlorophyll mutants seldom grow beyond the cotyledonary stage and the predominant types of mutations were xantha and viridis with thermal neutrons and xantha with gamma rays. Bianu and Marki (1970) reported in flax following treatment with some alkylating agents and irradiation with gamma rays that the spectrum of chemically induced mutations was quite distinct from that resulting from irradiation. There were more albina and fewer xantha and viridis mutants in the radiomutant population than among those induced chemically. According to Beard (1971) chlorophyll mutations in flax following recurrent X-irradiation could be assigned to six classes viz., light green 29%, yellow green 35%, yellow virescent 12%, yellow 14%, albinovirescent 7% and albino 3%. Yellow and albino mutants usually died in the seedling stage.

iii) Segregation ratio

Segregation ratio: Bora (1963) described the origin of one xantha and one virescent mutant after X-irradiation in

Arachis. The segregation ratio of the virescent type was not clear. The ratio ranged from 1:1 to 15:1 indicating that the development of chlorophyll in groundnut was possibly controlled by more than one gene locus.

De Nettancourt and Contant (1966) observed in *Lycopersicon esculentum* and *Lycopersicon pimpinellifolium* after chronic gamma irradiation that two fruits of the former and one fruit of the latter species carried a chlorophyll mutant which segregated in the 3:1 ratio. According to Sur (1969), the segregation ratios obtained in blackgram following thermal neutron and gamma irradiation were different. For gamma rays the ratio ranged from 3.5 to 10.0% whereas for thermal neutrons it varied from 1.2% to 25.0%.

2) Viable mutations

Hammons (1953 a, b) reported a cup mutant in *Arachis* following X-ray treatment of seeds with 18500 R. This mutant was characterised by a complex of morphological features which were ascribed to pleiotropic effects of a single gene. Nayar (1969) in *Sesamum* reported a mutant with green cotyledon but deficient in chlorophyll from the seedling stage to maturity following X-irradiation. The plant had few short internodes, weak branches and retarded growth and flowered late producing small few seeded fruits. In tomato, following chemical treatments Raut (1969) reported dwarf, dilute, sufflava, and wooly mutants. Bankowska and Rymsza (1969) reported in Phaseolus vulgaris following gamma irradiation some dwarf mutants with supernumery leaflets. In common bean (Phaseolus vulgaris), a number of seed cot color mutants were reported by Moh (1969), following ethyl methane sulphonate treatment. These mutants had white, olive brown and yellow brown seed color. Dudek (1970) observed dwarfism,albinism, and male sterility in Nicotiana tabaccum and Nicotiana rustica following gamma irradiation. Wichertkobus (1971) observed mutants with reduced growth in the M_2 in Nicotiana tabaccum following gamma irradiation.

Bansal (1973) in Capsicum annuum following treatment with ethyl methane sulphonate and nitroso methyl urea reported mutants in which the reproductive parts were transformed to vegetative ones. Thakare et al. (1973) found in jute following thermal neutron and gamma irradiation some drastic mutations with completely altered morphology exhibiting transgeneric and transpecific variations.

George and Nayar (1973) isolated four plants in linseed following gamma irradiation which flowered about 25 days earlier than the control. These plants were relatively short and had sparsely arranged long narrow leaves.

3. Mutagenic effectiveness and Efficiency

According to Konzak et al. (1965) the usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. Efficient mutagenesis is the production of desirable changes free from association with undesirable changes. Assuming that the desirable changes are mutations, two agents may be equal in mutagenic effectiveness because they induce the same frequency of a given mutation at a given dose. But when they differ in the production of undesirable changes such as gross chromosome aberration such as sterility and lethality, they may be said to differ in mutagenic efficiency. The most effective agent or treatment may not be the most efficient one. The apparent mutagenic efficiency as well as the effectiveness of any two agents may differ for different organisms and tissues because the comparable test conditions may not permit the expression of the true potential of the agents. Efficient treatments are essential for economical use of mutagens as tools for direct improvement or for the induction of certain qualitative or quantitative traits.

Ashri and Goldin (1965) observed that diethyl sulphate is an efficient mutagen in peanut despite its amphidiploid nature. Monti (1968) reported in peas that diethyl sulphate is 3 to 4 times more efficient than X-rays at comparable levels of M_1 plant survival and fertility. According to Sharma (1969), dimethyl sulphate and ethyl methane sulphonate showed almost equal effectiveness in cowpea. Nitrosomethyl urea was about twice as effective as those mutagens. Ushlik (1972) reported that ethyl methane sulphonate was more efficient than gamma rays in Lens esculenta producing a higher percentage of mutants and more types of mutants per progeny. Thakare et al. (1973) observed that thermal neutrons were 1.5 times more effective than gamma rays in inducing viable mutations in jute.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was undertaken in the Division of Agricultural Botany, College of Agriculture, Vellayani, during the period 1973-1975.

A. Materials

The biological material used was the variety Kayamkulam-1 of Gingelly (*Sesamum indicum*), a strain evolved through pureline selection from the Onattukara local variety, at the Oil Seeds Research Station, Kayamkulam. It is a short duration variety of about 80 days duration.

Gamma irradiation was done at the Department of Botany, Kerala University Centre, Karyavattom, Trivandrum, utilising the Cobalt 60 " Gamma shine 1000 curies" source installed by the BARC, Bombay. The source was operating with a dose rate of 32.81 krad per hour.

B. Methods

1. Irradiation with gamma rays

Gingelly seeds of uniform size and maturity were selected out. Six samples of air dry seeds (1000 each) were exposed to gamma radiation at doses of 5, 10, 15, 20, 25 and 30 krad.

2. Study of the M_1 generation

The seeds were sown in the field on the third day after irradiation adopting a Randomised Block Design, replicated thrice. A total of 270 seeds per treatment were sown in each replication. The plot size was 4M x 1.5M and the spacing given between plants and between rows was 15 cm.

The following observations were made.

i) Germination of seeds.
ii) Seedling emergence.
iii) Survival of plants.
iv) Plant height.
v) Fertility.
vi) Chlorophyll chimeras.
vii) Morphological abnormalities.

i) Germination of seeds

A germination test was carried out under laboratory conditions. One hundred seeds from each treatment were kept in three replications in petridishes lined with moist filter paper. Germination counts were taken from the third day onwards and continued upto the seventh day. Germination percentage was estimated based on the total count upto the

7th day.

ii) Seedling emergence

A set of 20 seeds each in two replications per treatment were kept in petridishes under laboratory conditions. The observation on radiole and plumule emergence was made at intervals of 8 hours from 24 hours to 104 hours. The average time taken for radicle and plumule emergence was calculated.

iii) Survival of plants

The total number of plants surviving per treatment per replication were counted 30 days after sowing and the percentage of survival calculated on the basis of the total number of seeds sown. The survival data is expressed as a percentage.

iv) Plant height

20 plants each per treatment per replication were selected from the field at random and the height of these plants were measured at 30 days after sowing. The height was measured in centimeters from the soil surface to the terminal bud. The mean plant height was estimated.

v) Fertility

Pollen fertility was studied in 30 plants in each of the treatments. Flowers produced during the early part of the flowering period were selected. Anthers were taken from mature flower buds and the pollen grains were stained in glycerine acetocarmine. The well stained and properly filled pollen grains were counted as fertile and the others as sterile. 50 microscopic fields in each of the slides were scored and the data recorded. Fertility was estimated as a percentage of the number of fertile grains to the total number of grains scored.

vi) Chlorophyll chimeras

The M_1 population was examined at regular intervals and plants exhibiting chlorophyll deficit patches on their leaves were recorded as chimeras.

vii) Morphological abnormalities

The M_1 population was periodically examined to locate plants with morphological variations. Such plants were labelled and brief description of each type made.

3. Study of the M_2 generation

A total number of 50 M_1 plants per treatment were carried forward to the M_2 generation. Mature fruits were collected from the main shoot and the M_2 generation was raised as M_1 progenies.

The seeds were sown in one meter square plots. The M_2 seedlings were observed in the early morning from the 7th day onwards upto the 20th day, and the chlorophyll mutations were scored. The progenies segregating for mutations were first counted and the chlorophyll mutation frequency on M_1 plant basis was estimated as the number of plants segregating per 100 M_1 plants. The number of mutants in each segregating progeny was then counted separately. The number of normal plants in each of the segregating and non segregating progenies were also counted. These data were utilised for estimation of mutation frequency on M_2 plant basis ie; number of mutants per 100 M_2 plants. The different types of chlorophyll mutants in each of the segregating progenies were counted separately and the mutation spectrum was calculated as the relative percentage of the different types of mutants. The segregation ratio was estimated as a percentage of the number of mutants to the total number of plants scored in the segregating M_2 progenies.

The progenies segregating for other types of mutations were also scored by making observations at regular intervals. Viable mutations were scored until flowering and fruit setting. The different types of viable mutations observed were described.

Mutagenic effectiveness was calculated as a function of the mutation fréquency (M_1 plant basis) in relation to the radiation doses employed and mutagenic efficiency was calculated as a function of the mutation frequency in relation to M_1 damage such as lethality injury and sterility as suggested by Konzak et al. (1965).

RESULTS

RESULTS

The effects of gamma irradiation on dry seeds of Sesamum in the M_1 and M_2 generations were studied and data presented below.

I. Effects in the M_1 generation

1. Germination of seeds

Table (I) gives the data on germination of seeds under laboratory conditions. The germination percentages did not show any significant difference between the various treatments indicating that the doses employed had no effect on germination of seeds.

The mean period for radicle emergence (Table I) also did not show any difference between the treatments. However, the maximum period for radicle emergence was recorded at the 25 krad treatment. Similarly, the period for plumule emergence in the different treatments also did not show any significant difference.

2) Survival of plants

Table (II) provides the data on survival of plants at 30 days. With an increase in the dose of irradiation

Table I

Seed germination and seedling emergence in the M_1 generation

Doses	Germination		Emergence			
			Radicle		Plumule	
	%	% on control	Mean period (hrs.)	% on con-trol	Mean period (hrs.)	% on control
Control	94	100	27.9	100	62.1	100
5 krad	94	100	27.4	98.2	66.0	106.3
10 krad	98	104.25	28.8	103.22	65.4	105.3
15 krad	97	103.20	28.5	102.15	62.1	100.0
20 krad	98	104.25	27.9	100.00	61.5	99.0
25 krad	98	104.25	32.2	115.41	65.5	105.5
30 krad	96	102.12	29.0	103.94	61.9	99.7

there was a steady decrease in the percentage of survival. The reduction in survival (lethality) at 30 days was thus maximum at 30 krad followed by 25, 20, 10, 15 and 5 krad treatments in that order.

3) Plant height

The plant height measured on the 30th day presented in Table (II) indicates the effect of irradiation on the growth of M_1 plants. There was no relationship between the mean plant height and doses employed. A slight increase in height was observed in the 5 and 20 krad treatments. In the 10, 15 and 30 krad treatments there was a slight decrease. However, for height of individual plants there was wide variation within the treatments.

4) Fertility

The percentage of pollen fertility (Table II) decreased with increase in doses. In the treated population, fertility was maximum at 10 krad and minimum at 30 krad.

5) Chlorophyll chimeras

Radiation induced chlorophyll deficit sectors were observed in the leaves of plants at 30 krad treatment only. These chimeric plants had a few round yellow patches on

Table II

Survival, plant height and fertility in the M_1 generation

Doses	Survival at 30 days		Plant height at 30 days		Fertility (Pollen)	
	%	% on control	cms	% on control	%	% on control
Control	23.3	100	5.8	100	93.30	100
5 krad	17.3	74.24	6.0	103.44	70.50	75.6
10 krad	12.2	52.40	5.7	98.30	74.40	79.7
15 krad	14.9	63.94	5.5	94.80	71.10	76.2
20 krad	11.5	49.35	6.0	103.44	65.80	70.5
25 krad	8.5	36.50	5.8	100.00	58.60	62.8
30 krad	7.0	30.00	5.5	94.80	51.90	55.6

leaves which persisted till maturity.

6) Morphological abnormalities

Irradiation induced a few morphological variations in the M_1 population. The morphological abnormalities observed are presented below.

i) Dwarf plants

Plants were very short in stature. The different plant parts were also much reduced in size.

ii) Early branching

Branches developed 10 - 15 days earlier than the normal plants.

iii) Forking of main stem

These plants presented a forked appearance of their main stem.

iv) Leaf variations

Leaf variations included forked leaves, crinkled leaves, mottled leaves and leaves with palmately lobed lamina.

II. Effects in the M_2 generation

1. Chlorophyll mutation

i) Frequency

Mutation frequencies calculated on M_1 plant basis and M_2 plant basis (Table III) did not show any defenite relationship with the dose. On M_1 plant basis, the lowest dose, viz., 5 krad yielded the lowest mutation frequency and the dose 15 krad produced the highest mutation frequency. However, the frequencies of mutations estimated on M_2 plant basis were the highest at 10 krad treatment followed by 15, 30, 5, 20 and 25 krad in that order.

ii) Spectrum (relative %) of chlorophyll mutation

Two types of chlorophyll mutations viz., yellow (xantha) and yellow green (chlorina) were seen in all the treatments. But the relative percentages of these mutations (Table IV) varied with different doses. The frequency of yellow (xantha) was highest at 25 krad and lowest at 10 krad. Maximum frequency of yellow green (chlorina) was observed at 10 krad and minimum at 25 krad. The frequency of xanthas and chlorinas had no relationship with dose, but the dose 25 krad produced the highest percentage of xanthas

Table III

Frequency of chlorophyll mutations in the M_2 generation

Doses	M_1 plant basis			M_2 plant basis		
	No. of M_1 plant progenies		No.of mutations per 100 M_1 plants	No.of M_2 plants scored.	No.of mutants.	No.of mutants per 100 M_2 plants.
	Scored	Segregating				
5 krad	44	7	15.91	9941	61	0.613
10 krad	43	8	18.60	7752	97	1.251
15 krad	43	9	20.93	5434	54	0.993
20 krad	46	8	17.39	5791	23	0.397
25 krad	41	8	19.51	5043	19	0.376
30 krad	46	9	19.56	1934	19	0.982

Table IV

Spectrum of chlorophyll mutants in the M_2 generation

Doses	Total No. of mutants.	No.of mutants in each type		Relative percentage	
		Yellow (xantha)	Yellow green (chlorina)	Yellow (x)	Yellow green (c)
5 krad	61	47	14	77.00	23.00
10 krad	97	4	93	4.12	95.88
15 krad	54	8	46	14.81	85.19
20 krad	23	9	14	39.13	60.87
25 krad	19	15	4	78.94	21.06
30 krad	19	1	18	5.26	94.74
Total	273	84	189	30.76	69.24

and the lowest percentage of chlorinas and 10 krad produced the highest percentage of chlorinas and the lowest percentage of xanthas.

iii) Segregation ratio

Segregation ratio is the percentage of the number of mutants to the total number of plants in segregating M_2 progenies. The ratio provided in Table V did not show any relationship with the dose. Segregation ratio was maximum (5.62) at 10 krad treatment and minimum (1.71) at 25 krad.

2) Viable mutations

Several types of viable mutants were scored in the M_2 generation. They were very few in number and hence a frequency estimate was not attempted. Brief descriptions of these types are presented below.

i) Dwarfs

Plants were very short in stature. Some of them had normal leaves and some others had very narrow leaves. Such dwarfs could be noticed in all the treatments.

ii) Lanky mutant

Plants were very tall growing and thin stemmed. These plants were very slender and had very long internodes

Table V

Segregation ratio of chlorophyll mutants in the M_2 generation.

Doses	Total No.of plants scored in segregating M_2 progenies.	No. of mutants	Segregation ratio
5 krad	2391	61	2.55
10 krad	1723	97	5.62
15 krad	1023	54	5.28
20 krad	1123	23	2.05
25 krad	1111	19	1.71
30 krad	511	19	3.72

in comparison to normal plants. Lanky mutants were observed at the 25 krad treatment only.

iii) <u>Mutant with flattened stem</u>

Plant with very flat and forked stem. This mutant was observed at the 15 krad treatment only.

iv) <u>Early branching mutant</u>

Plants exhibited an early branching habit. These mutants were seen at the 5, 15, 20 and 25 krad treatments.

v) <u>Twisted leaf mutant</u>

The cotylendonary leaves were twisted near the tip. This mutant was observed at all the six treatments.

vi) <u>Curly leaf mutant</u>

They presented a curly appearance of the leaves. Such mutants were observed at the 15, 20, 25 and 30 krad treatments.

vii) <u>Little leaf mutant</u>

Normally growing plants with very small leaves. This was observed in the 20 krad treatment.

viii) Cluster leaf mutant

Mutants with their leaves arranged in a whorl or in a cluster. This was noticed in the 5, 15, 20 and 25 krad treatments.

ix) Forked leaf mutant

These mutants had forked leaves. They were seen in all the treatments except 25 krad.

x) Abnormal flower mutant

Plants which had their flowers modified into vegetative parts. Such mutants were observed in the 25 and 30 krad treatments.

3) Mutagenic effectiveness and efficiency

The mutagenic effectiveness and efficiency of gamma rays in inducing chlorophyll mutations were estimated and presented in Table VI. Effectiveness decreased progressively with increasing doses of radiation. This indicated that the increase in the frequency of mutations was not proportional to the increase in the dose of the mutagen. Mutagenic effectiveness was high at the lowest dose of 5 krad (318.2) and low at the highest dose 30 krad (65.2).

Table VI

Mutagenic Effectiveness and Efficiency

Doses	Mutation frequency (M_1 plant basis)	M_1 damage			Mutagenic effectiveness.	Mutagenic efficiency		
		Lethality	Injury	Sterility				
	(M)	(L)	(I)	(S)	$\frac{Mx100}{krad}$	$\frac{Mx100}{L}$	$\frac{Mx100}{I}$	$\frac{Mx100}{S}$
5 krad	15.91	25.76	0	24.4	318.20	61.76	∝	65.20
10 krad	18.60	47.60	1.7	20.3	186.00	39.07	1094.11	91.62
15 krad	20.93	36.06	5.2	23.8	139.53	58.04	402.50	87.94
20 krad	17.39	50.65	0	29.5	86.95	34.33	∝	58.95
25 krad	19.51	63.50	0	37.2	78.04	30.72	∝	52.45
30 krad	19.56	70.00	5.2	44.4	65.20	27.94	376.15	44.05

Mutagenic efficiency estimates based on lethality and sterility were found to decrease with increasing doses of radiation. However, efficiency in relation to injury did not show any such relationship. In relation to lethality, efficiency was highest at the lowest dose of 5 krad. Efficiency in relation to injury was very high at doses 5, 20 and 25 krad and in relation to sterility it was highest at 10 krad.

DISCUSSION

DISCUSSION

The effect of gamma radiation at various doses in the M_1 and M_2 generations of Sesamum is discussed below.

I. Effects in the M_1 generation

i) Germination and survival

Germination of irradiated seeds remained unaffected irrespective of the doses employed in the present study. This is in confirmity with the observations of Sinha and Godward (1972) in Lens escularis and Joshua et al. (1972) in Jute. Ankineedu et al. (1968) reported in castor that gamma rays at 25 kR did not affect germination. Bilquez and Martin (1961) in Arachis, De Nettancourt and Contant (1966) in Lycopersicon esculentum and Lycopersicon pimpinellifolium, and Vishnuswarup and Gill (1968) in french beans, reported that the lower doses of gamma and X-irradiation hastened germination. The increase in the percentage of germination at lower doses was attributed to the activity of enzymes involved in the synthesis of auxins (Casarett, 1968). An inverse relationship between dose and germination was reported by a number of investigators (Das and Mukherjee, 1968; Narsinghani and Sudhirkumar, 1969; Dudek, 1970). Reduced germination percentage at moderate and high doses can be due to varied response of irradiation on the chromosome compliments or initiation of cell divisions or due to greater lapse between irradiation and planting (Curtis et al. 1958).

The relationship between the percentage of survival and dose of radiation was inverse, ie. with an increase in the dose of radiation there was a decrease in the percentage of survival, except at 15 krad. Similar findings were reported by Constantin and Love (1967) in *Vigna sinensis*, Magassy and Farkas (1970) in sugarbeet, Joshua et al. (1972) in jute and Mujeeb and Greig (1973) in *Phaseolus vulgaris*. The reduction in survival percentage with increased doses of radiation can be explained in cytological terms. Mitotic abnormalities due to irradiation results in structural changes in the chromosomal compliments. This interfers with the normal growth and development of organisms which might have led to the fall in survival percentage with increased doses (Radhammal, 1972). According to Bilquez and Martin (1961) the application of 40,000 R of X-rays in *Arachis* did not affect survival. Constantin and Love (1967) also observed similar results in *Vigna sinensis* following highest dose of neutron irradiation. This indicates that at higher doses there is a tendency for recovery or an increased resistance of plants to the drastic conditions of treatments. It implies a direct connection between damage to the vascular connective tissue and the acute response of the radio-sensitive parenchymal cells of certain specific organ systems (Caserett, 1968).

ii) Plant height

It is well known in radiation experiments with higher plants that plant height is a good indicator of radiation damage. The data on plant height indicates that there is no relationship between the mean plant height and the doses employed. The plant height recorded on the 30th day did not show significant variation between the different treatments. However, a greater variability in plant height was observed within treatments similar to the findings of Rukmanski (1969) in french beans. According to Gaul (1970) the larger variability after treatment with gamma rays is apparently the result of modifying factors and can be eliminated by post-irradiation treatments. Arsagova (1972) reported a stimulation of growth in cucumber following chemical mutagen treatments. The stimulatory effect on plant growth at lower doses was explained to be due to the destruction of inhibitory substances and an increase in physiologically active substances like auxin, giberellin, etc.

A reduction in plant height was reported by a number of investigators following chemical and physical mutagen treatments (Anikneedu et al., 1968; Monti and Donini, 1968; Bajaj et al. 1970; Joshua et al. 1970). The inhibition of growth at higher doses of mutagens can be interpreted in physiological, biochemical and anatomical view points, such as upsetting of oxidation reduction system in the cells (Forssberg and Nybom, 1953), inhibition in the rate of assimilation and consequent changes in the nutrient level of plants (Ehrenberg, 1955) and inactivation of vital enzymes especially those concerned with respiration (Lea, 1947; Casarett, 1968).

iii) Fertility

Pollen fertility is indicative of the percentage of viable pollen grains which reflects the normal functioning of reproductive system. The present investigation indicated an inverse relationship between pollen fertility and radiation doses. This observation is in line with the findings of Ankineedu et al.(1968) in castor and Sinha and Godward (1972) in *Lens escularis*.

The reduced pollen fertility with increased doses of gamma rays may be explained on cytological terms. According to Ciurdarescu et al. (1968) irradiation at various doses induces chromosome aberrations, chromosome bridges, laggards and combination of these irregularities in lucerne. Scumpu and Ionescu (1968) observed in peas chromosome aberrations in mitosis at doses from 5 to 15 kR. The incidence of such aberrations were only 4-5% at low doses and increased upto 51% at 15 kR. Aberrations in meiosis also showed a similar incidence.

Dudek (1970) also observed various chromosome abnormalities in the root tip and pollen mother cells of Nicotiana tabaccum and Nicotiana rustica. The induction of semi and complete sterile plants in higher doses tend to indicate the incidence of similar chromosomal aberrations. Mutagen induced sterility may be caused by chromosome mutations, factor mutations, cytoplasmatic mutations and physiological effects. Chromosome mutations are probably the major origin of all mutagen induced sterility (Gaul 1970). As the treatment dose enhanced, the deleterious effects of irradiation were more marked in the chromosome compliment which were conclusively proved by cytological observations. Thus meiotic abnormalities including bridges, laggards, fragments, univalents etc. interrupt the normal development of fertile microspores. Nilan (1960) in barley and Yeh and Henderson (1968) in rice reported segmental interchanges and various other chromosomal aberrations as causes of semi sterility.

iv) Chlorophyll chimeras

Chlorophyll deficit plants observed in the M_1 generation had round yellow patches on their leaves. The occurrence of such chimeras had no relationship with the dose. Similar chlorophyll chimeras were reported by Ankineedu *et al.* (1968) in castor, and Bankowska and Rymsza (1969) in *Phaseolus vulgaris*.

According to Erikson and Lindgren (1970) the M_1 plant following seed irradiation will carry an induced mutation in certain part of the shoot, the other parts being normal or differently mutated. The part containing the mutation is frequently referred to as a mutated sector of the plant, but the plant may be as well a sectorial or a periclinal or mericlinal type of chimera. In mutation research it is of great interest to reveal mutated sectors already in the M_1 plants. Although, the genetic origin of the leaf spots is not exactly known, Kaplan (1954) has suggested that the number of leafspots increased exponentially with the dose and concluded that chromosomal aberrations were responsible for the induction of the spots.

v) Morphological abnormalities

Morphological abnormalities were observed in the irradiated population even in the M_1 generation. Among the treated population, several dwarf plants were observed. Nazarov and Egorova (1969) in peas, Moh (1970) in beans, Paucheva and Dirkovos (1972) in vetch also reported the occurrence of dwarfs in the irradiated population. Production of dwarfs may be due to the inactivation of respiratory enzymes by the radiation.

A few leaf variations obtained during the present study included forked leaf, mottled leaf, crinkled leaf, etc. Such variations were also reported by Ankineedu et. al (1968) in castor and Joshua et al. (1968) in jute. Athwal (1963) attributed such variations to the level and duration of exposure, age, physiological condition of plants and environmental condition during and after the exposure. Another morphological variation observed was early branching of the seedlings and plants with forked main stems. The specific changes which lead to the initiation of such changes are still unknown. It is probable that physiological disturbances have played a major role in this phenomenon.

II. Mutations in the M_2 generation

i) Chlorophyll mutations

a) Frequency

Mutation frequency calculated on M_1 plant basis and M_2 plant basis followed no defenite relationship with the doses. The frequêncies estimated as number of mutations per 100 M_1 plants gave higher values than the other estimate at each of the doses employed. This evidently was an over estimation of the mutation event in consideration of the differentiated nature of the embryo.

Pipie (1967) observed that in peas the incidence of chlorophyll mutations after treatment with chemical mutagens showed a positive linear relationship with the dose of diethylsulphate but not with that of ethyl methane sulphonate. He reported that the differential response is because of the fact that ethyl methane sulphonate induced a higher frequency of mutations than diethyl sulphate.

The present observation that the frequency of mutation depended to a lesser extent on mutagen dose was in agreement with that of Mekhandzhiev (1971) in peas. According to Chekalin (1968) the frequency of mutation in *Lathyrus sativus* and *Lathyrus tingitanus* depends more on the mutagen and the species than on the dose. Ushlik (1972) observed that in Lens esculenta 20% of the segregating M_2 progenies following gamma irradiation yielded chlorophyll mutants.

b) Spectrum

Only two types of chlorophyll mutants were scored, viz., yellow (xantha) and yellow green (chlorina) in the present investigation. The mutation spectrum reveals that chlorina was more than xantha. Das and Mukherjee (1968) reported albino seedlings in grapes following gamma irradiation and ethyl methane sulphonate treatment. According to Paponova (1969) the spectrum of chlorophyll mutations in radish following nitroso ethyl urea treatment included albina (lethal), chlorina (lethal) xantha (lethal) and viridis (more viable than the other mutants). However, Bianu and Marki (1970) reported that the spectrum of chemically induced mutations was quite distinct from that resulting from irradiation. The chlorina and xantha mutants observed in the present study were lethal and perished in the cotyledonary leaf stage. Joshua et al.(1972) also reported that chlorophyll mutants in jute, following thermal neutron and gamma irradiation seldom grew beyond the cotyledonary leaf stage. Beard (1971) reported lethal yellow and albino seedlings in flax.

c) Segregation ratio·

The segregation ratio ranged from 1.71 to 5.62. However, the ratio did not show any relationship with dose. It was highest at 10 krad and lowest at 25 krad treatments. Sur (1969) observed that in blackgram the segregation ratio ranged from 3.5 to 10.0 after irradiation with gamma rays whereas with thermal neutrons it varied from 1.2 to 25.0. The higher segregation ratio indicated severe elimination of initial cells in the seed primordia following irradiation. With high segregation ratios a high proportion of mutants will be observed facilitating their selection in segregating populations. The increase in segregation ratio obtainable with an increase in dose will be of great value in mutation breeding.

ii) Viable mutations

The viable mutations observed included, plants with completely changed growth habit such as dwarf, lanky, flat stem, early branches etc. Besides those, a few types with leaf variations and flower abnormalities were also recorded. The frequencies with which these mutants appeared in the different treatments were very low and did not depend on the dose.

Hammons (1953 a, b) reported a cup mutant in *Arachis* characterised by a complex of morphological features. According to him this was due to the pleiotropic effects of a single gene. Nayar (1969) reported in *Sesamum* following X-irradiation a mutant with few short internodes, weak branches and retarded growth. This mutant flowered late and produced small few seeded fruits. Dwarf mutants were reported by Raut (1969) in tomato, Bankowska and Rymsza (1969) in *Phaseolus vulgaris* and Dudek (1970) in *Nicotiana tabaccum* and *Nicotiana rustica*. Moh (1969) reported a number of seed coat colour mutants in *Phaseolus vulgaris*. Thakare *et al*. (1973) reported in jute some drastic mutations with completely altered morphology exhibiting transgeneric and transpecific variations, following thermal neutron and gamma irradiation. George and Nayar (1973) isolated mutants with leaf variations in linseed. In this study, 9 mutants were isolated from amongst the 25 and 30 krad populations which had their flowers modified into vegetative ones. This is similar to the findings of Bansal (1973) in *Capsicum annuum* following chemical mutagen treatments.

iii) Mutagenic Effectiveness and Efficiency

A detailed treatment of the concepts of mutagenic effectiveness and efficiency was presented by Konzak *et al*. (1965). They proposed the terms effectiveness as a measure of gene mutations in relation to doses and efficiency as an estimate of the mutation rate in relation to other biological effects induced, such as lethality, injury and sterility. To obtain high efficiency the mutagenic effect must greatly surpass other effects in the cell such as chromosomal aberrations and toxic effects which generally lead to damage.

In the present study, effectiveness was highest at the lowest dose employed and decreased with increasing doses. This inverse relationship could be explained as due to the failure of mutations to increase proportionately with increasing doses. Efficiency in relation to lethality was also found to be the maximum at the lowest dose employed and decreased with increasing doses. Similar result was obtained in case of efficiency estimation in relation to sterility also. However, efficiency in relation to injury was lowest at the highest dose (30 krad) employed. According to Konzak *et al*. (1965) the greater efficiency of low doses of mutagen relate to the fact that lethality, injury and sterility increased with increase in doses at faster rates than mutations.

The effectiveness and efficiency of various mutagens in a number of crops have been reported by many workers. Ashri and Goldin (1965) reported that diethyl sulphate is an efficient mutagen in peanuts. According to Sharma (1969) in cowpea, nitroso methyl urea is twice as effective as dimethyl sulphate and ethyl methane sulphonate. Ushlik (1972) observed that in Lens esculenta ethyl methane sulphonate was more efficient than gamma rays. Thakare et al. (1973) observed that thermal neutrons were 1.5 times more effective than gamma rays in inducing viable mutations in jute.

SUMMARY

SUMMARY

Seeds of the <u>Sesamum</u> variety Kayamkulam-I were treated with gamma rays at doses, 5, 10, 15, 20, 25 and 30 krad by using the ^{60}Co gamma source at the Kerala University Centre, Karyavattom, Trivandrum. The effects of radiation in the M_1 and M_2 generations are summarised below.

In the M_1 generation, the effects studied were germination, survival, plant height, pollen fertility, chlorophyll chimeras and morphological abnormalities. The different doses employed had no effect on germination and seedling emergence. With an increase in the dose there was a steady decrease in survival and pollen fertility. Survival was maximum at 5 krad and minimum at 30 krad. Fertility was maximum at 10 krad and minimum at 30 krad. There was no relationship between plant height and the doses employed. Chlorophyll deficit plants were observed in the 30 krad treatment only. A number of morphological abnormalities were also observed.

In the M_2 generation, the chlorophyll mutation frequency, spectrum, segregation ratio and mutagenic effectiveness and efficiency were estimated. The viable mutants observed were described. The frequency of chlorophyll mutations estimated on M_1 plant basis was higher than the estimate on M_2 plant basis. The mutation frequency did

not show any defenite relationship with dose.

The mutation spectrum included yellow (xantha) and yellow green (chlorina) types. The frequency of yellow was highest at 25 krad and lowest at 10 krad. The frequency of yellow green was highest at 10 krad and lowest at 25 krad.

The segregation ratio did not show any relationship with dose ie., the ratio did not increase proportionately with increasing doses or vice versa. Segregation ratio was minimum (1.71) at 25 krad and maximum (5.62) at 10 krad.

Mutagenic effectiveness was maximum at the lowest dose (5 krad) and decreased with increasing doses. Mutagenic efficiency estimates based on lethality and sterility decreased with increasing doses. However, in relation to injury such a relation was not observed. Thus the lower doses were found to be more effective and efficient in inducing mutation.

The observations made in the M_1 and M_2 generation following gamma irradiation thus indicate that gamma rays at low to medium doses can be successfully used for inducing mutations in <u>Sesamum</u>.

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