INDUCED MUTATIONS IN COWPEA
(*Vigna unguiculata* cultivar Kuruthola-payar)

BY

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF
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DECLARATION

I hereby declare that this thesis, entitled "Induced mutations in cowpea (Vigna unguiculata cultivar Kurutholа-payar)" is a bonafide record of the research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar titles of any University or Society.

(MINI. V.)

College of Agriculture,
Vellayani, 15th April, 1989.

CERTIFICATE

Certified that this thesis, entitled "Induced mutations in cowpea (*Vigna unguiculata* cultivar Kurothola-payar)", is a record of research work done independently by Smt.MINI.V., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Mutations are the sole source of allelic differences, the raw material for genotype alternatives. They provide nature with inherited variability and are the key to natural selection success. Following the rediscovery of Mendel's work, interest increased in genetic variability and the potential for altering this variability at will. Later work with Drosophila demonstrated the scientific nature and feasibility of increasing mutation rates with artificial treatments. With the rapid growth in the theoretical understanding of the processes relating to the induction and recovery of mutations, the stage is set for a more dynamic phase in the use of induced mutations as a tool for striking new paths in Agriculture. While induced mutations, in general, will continue to be a supplement rather than a substitute to other methods of breeding, its major role hereafter will have to be sought in the development of altogether new cropping patterns and plant characteristics.

Induced mutations can be generated with two principal types of treatments, viz., energy and chemical. Included in the energy category are X-rays, gamma rays, beta rays, fast and slow neutrons, alpha particles, deuterons, and ultraviolet light. X-ray is by far, the most commonly used.

The chemical group includes several forms of methane sulphonate, ethylene-imine, diepoxybutane, nitrogen mustards, sulphur mustards, epoxides, and ethylene oxide. The ethyl form of methanesulphonate (EMS) and ethylene-imine (EI) have been the most widely used.

Mutation rates vary with mutagen dosage. The higher the dose of mutagen, the more frequent the mutations and the greater the associated possibility of undesirable chromosome damage and lethality. A level commonly utilised is the dose at which 50 per cent of the treated material is killed. This is called 50 per cent lethal dose or LD_{50}. The first step in a mutation-induction programme is to establish methods of treatment appropriate to the plant material and the chosen agent, and to estimate a suitable dose.

The production of induced mutations appears to be nondirectional. This means that a mutagen treatment will alter alleles in a random pattern throughout the genotype. The frequency and severity of induced gene change depends on the mutagen dosage, tissue type and age, and physical factors including moisture and temperature. Occasionally, the desired spectrum of genetic variability can be obtained by exposing several generations of plant material to mutagen treatment.

Cowpea is a major pulse crop of South India and forms one of the important vegetables of the homestead gardens in the state. It is a diploid, naturally self-pollinated leguminous species and the amount of natural variability available is limited. In a breeding programme, the relative worth of any artificially induced mutation depends on the amount of natural variability available. Since cowpea is a self-pollinated crop, the probability of producing desirable mutations and genetic variability by artificial means is higher. Hence mutation breeding is suggested as an attractive proposition to improve this crop.

The present work was undertaken to study the mutagenic effects produced by gamma rays and EMS on cowpea cultivar Kuruthola-payar in the M_1 and M_2 generations. The cultivar, though a high yielder, has the great disadvantage of a trailing nature, which necessitates provision of supports, thereby increasing the cost of cultivation. The possibility of isolating height mutants with bushy, erect or shy-trailing nature was also examined. The effectiveness and efficiency of the different doses of gamma rays and EMS were estimated. The methods employed and the results obtained are presented and discussed in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Mutation breeding has clearly been established as one of the potent tools for improving plant type and yield of crop plants. De Vries proposed the idea of producing mutations artificially as early as 1901 and X-ray was the first radiation to be applied to cells and chromosomes.

When the effects of X-rays and other radiations on living cells were announced, a large number of tests on plant cells were carried out. Muller (1927) found that X-irradiation can induce sex-linked recessive lethal mutations in *Drosophila melanogaster*. This was followed by induction of mutations in barley and maize by Stadler (1928). Their findings paved the way for rapid progress in this field. The potential of artificially induced mutations in plant breeding was recognised with Gustafsson's (1947) elaborate work on the effect of X-rays on agricultural crops.

Successful experiments were carried out in chemical mutagenesis by Schiemann (1912). The experiments of Auerbach and Robson (1947) with Drosophila using sulphur and nitrogen mustards paved the way for intensive research in this field. Freese (1963) classified chemical mutagens as inhibitors, base-analogue substitutes, dyes, acids, metals and alkylating agents. The relatively low toxic and high genetic effects of Ethyl Methane Sulphonate (Gaul, 1961) and its high mutagenic effectiveness as well as efficiency in higher plants (Konzak *et al.*, 1965) demand attention for enhanced practical application.

Extensive research has been done in mutation breeding by various investigators and detailed reviews relating to the induction and recovery of mutations in numerous crops have been presented. Here, the review of mutation research is mainly restricted to leguminous crops.

I. Effect of mutagens in the M_1 generation.

The effects can be classified under six heads.

1. Reduced germination
2. Reduction in survival
3. Inhibition of growth
   a) Shoot length and root length
   b) Plant height
4. Reduced pollen and seed fertility
5. Chlorophyll chimeras
6. Other morphological and developmental abnormalities.

1. Germination of seeds

Irradiation of seeds of *Cicer* with X-rays resulted in delayed germination, as observed by Athwal (1963). The percentage of total germination decreased and the number of days taken to germinate increased in the higher doses of irradiation. Similar results were reported by Bajaj and Saettler (1970) in *Phaseolus* following gamma ray treatment and by Alikhan *et al.*(1973) in *Cajanus cajan* with gamma rays and EMS. Louis and Kadambavanasundaram (1973a) conducted studies on cowpea variety Meshed and found that germination of seeds was drastically affected by high doses of gamma rays and that germination was completely suppressed in 60 krad treatment and above. The effect of different doses of gamma radiation on mung bean was studied by Dahiya (1973) and it was observed that among the different gamma radiation treatments, 30 and 70 krad treatments decreased germination and high doses of gamma radiation did not have any drastic effect on the viability of seeds. Khanna and Maherchandani (1980) reported that the germination of the L 144 variety of gram seedling decreased with increasing doses of gamma irradiation, while the variety C 214 showed no reduction and Hima, yet another variety, showed slight reduction at higher doses.

Seed treatment with chemical mutagens significantly reduces the germination percentage. Wellensiek (1965) reported a rapid decrease in germination with increase in concentration of EMS in pea. Similar results were also obtained by Narsinghani and Kumar (1976) in cowpea following EMS and MMS treatments and by Bhojwani and Kaul (1976) in pea using EI. Experiments conducted by Nadarajan *et al.*(1985) in red gram using gamma rays and diethyl sulphate, revealed that germination gradually reduced with the increase in doses of the mutagens and the reduction was more with gamma rays than with DES.

The stimulatory effect of irradiation on germination has also been reported. Swarup and Gill (1968) conducted studies in French bean using X-rays and they observed that the seeds germinated better in 7 and 14 krad X-ray treatments, than in the control. Mujeeb (1974) also reported earlier germination and higher germination rates in all treatments in *Cicer arietinum* with gamma irradiation

2. Survival of plants:

Wellensiek (1965) reported that the percentage of healthy seedlings and full grown plants decreased rapidly with increasing concentrations of EMS in pea. Ojomo and Chheda (1971) conducted studies in cowpea using X-rays and neutrons and reported that the number of seedlings which survived after being transplanted was found to be inversely proportional to exposure. The survival of plants from seeds treated with low radiation exposure was reported to be more than the survival of plants from seeds treated with high radiation exposure. Louis and Kadambavanasundaram (1973a) observed a significant reduction in the survival of cowpea plants with the increase in dosage of gamma rays and the LD_{50} was found to be around 40 krad. Narsinghani and Kumar (1976) found a reduction in survival percentage in the M_1 in cowpea following EMS and MMS treatments. Similar results were obtained by Constantin _et al_. (1976) in soybean under field conditions using physical and chemical mutagens. They observed that under green house conditions, survival was unaffected by neutron and gamma irradiation doses less than 70 krad, but decreased with an increase in dose of EMS. Krishnaswami _et al_. (1977) reported that there was 50 per cent reduction in survival in green gram on treatment with 140 to 160 krad of X-rays and there was no survival of plants in doses above 100 krad of gamma rays. Oommen (1980) employed very low doses of EMS and found that the LD_{50} was much above the doses employed.

Zannone (1965) observed that the M_1 plant survival was unaffected except at 0.2 per cent EMS, 0.06 per cent EI and 28 krad X-rays in _Vicia sativa_.

Nadarajan *et al.*(1985) carried out experiments with red gram using gamma rays and diethyl sulphate and observed that the reduction in survival was more with gamma rays than with diethyl sulphate.

3. Plant growth

a) Shoot-root ratio

Oommen (1980) reported that the growth of the shoot and primary root in cowpea was affected by gamma irradiation and EMS. The shoot was reported to be more affected in the gamma ray treatments while there was a higher growth inhibition for the root with EMS.

b) Plant height

Wellensiek (1965) observed that the average height of pea plants decreased with increasing doses of X-rays, gamma rays, neutrons and EMS. Swarup and Gill (1968) reported normal growth in all treatments with X-rays in French bean except in 21 krad where the growth was poor. The seedling height was significantly reduced under 20 and 30 krad gamma ray treatments in cowpea, as observed by Louis and Kadambavanasundaram (1973a). They also observed that the plant height at maturity was uniform over different treatments at different doses. Sreerangaswamy *et al.*(1973) reported that the green gram plants treated with gamma rays were shorter than the parents and those treated with 60 krad were the shortest. Reduction in seedling height was also reported by Constantin et al. (1976) in soybean, following treatment with neutrons, gamma rays, EMS and DES. Similar results were obtained by Appa Rao and Jana (1976) in black gram, following X-irradiation and EMS treatment. Bhojwani and Kaul (1976) conducted studies using EI in pea and reported a reduction in seedling height and height of plants at maturity. They also reported a significant increase in seedling height when the EI treated seeds were post treated with cysteine, particularly in 0.03 per cent EI treated material. Khanna and Maherchandani (1980) reported seedling height reduction in gram, following gamma irradiation. Similar results were obtained by Nadarajan et al. (1985) in red gram, following gamma ray and DES treatments.

An increase in plant height was reported by Mujeeb (1974) in *Cicer* following gamma irradiation.

4. Pollen and seed fertility

Mutagen treatment generally result is reduced fertility. This is mostly caused by chromosome aberrations and can be quantitatively determined by counting sterile pollen or missing seed setting.

Heringa (1964) reported low fertility, following EMS and MMS treatments in pea. Zannone (1965) observed that average fertility decreased with increasing doses of EMS, EI and X-rays in *Vicia sativa*. Wellensiek (1965) obtained similar results in pea with X-rays, gamma rays, neutrons and EMS. Sato and Gaul (1967) reported that the low fertility cannot be attributed to chromosomal aberrations but to other reasons including genic alterations. Ojomo and Chheda (1971) reported fairly high levels of chromosome aberrations, which ranged from 18 per cent in 10 krad treatment to 41 per cent in 30 krad treatment in cowpea, following X-ray treatment. Louis and Kadambavanasundaram (1973a) conducted experiments with cowpea using different doses of gamma rays and observed that the pollen fertility and dosage of gamma rays were inversely related and the LD_{50} for pollen sterility was around 25 krad. Narsinghani and Kumar (1976) reported that the pollen fertility was reduced in cow pea on treatment with 0.25 per cent EMS and 0.025 per cent MMS. Bhojwani and Kaul (1976) reported a significant reduction in pollen fertility in pea, following treatment with EI and this reduction was associated with an increase, both in the concentration of EI and the frequency of chromosomal aberrations. Nerkar (1977a) observed that chemical mutagens induce greater pollen sterility and seed sterility than radiations. He conducted experiments with gamma rays, EMS and NMU in *Lathyrus sativus*. At comparable survival doses, chemical mutagens induced greater pollen sterility and seed sterility due to increased sensitization of seeds as a result of presoaking, and decreased intrasomatic selection. Radiation induced sterility might be the result of chromosome aberrations while sterility induced by chemicals might be attributed to cryptic deletions and specific gene mutations. Appa Rao and Jana (1979) observed that cowpea treated with combined and single doses of X-rays and EMS showed varying degrees of sterility in the M_1 generation and the mutation frequency was high in the medium sterility group of materials from the X-ray or combined treatment and in the low sterility group after the EMS treatment. Nadarajan et al.(1985) conducted experiments with gamma rays and DES on red gram and observed more reduction in pollen fertility with DES in SA-1 and the reverse was the case in CO-2. It was observed that DES is more potent in reducing the seed fertility than gamma rays.

5. Chlorophyll chimeras

Narsinghani and Kumar (1969) observed that with EMS and MMS treatment in cowpea, not a single chlorphyll mutant could be spotted in the M_1 generation. Kiang and Halloran (1975) reported that no chlorophyll mutants were obtained in the M_1 generation of soybean, following EMS treatment. They reported that this was to be expected as chlorophyll mutants are normally recessive and could be expressed only through segregation in the M_2 and later generations.

Nair (1964) reported chimeric plants characterised by chlorophyll deficient alterations in anthocyanin pigmentation and pollen sterility in cowpea, following X-ray treatment. Swarup and Gill (1968) reported that most of the mutants obtained in French bean following X-ray treatment were in chimeral form. Gohal et al. (1970) reported two chlorophyll deficient plants among the M_1 plants grown from seeds of Cyamopsis tetragonoloba treated with EMS. Ojomo and Chheda (1971) observed chlorophyll deficient spots in the M_1 generation of cowpea treated with X-rays. Appa Rao and Jana (1979) observed M_1 cowpea seedlings with 'deviation areas' on the leaves, following treatment with X-rays and EMS, their frequency being greater following EMS treatment than X-ray treatment.

6. Morphological variations

Mutagenic treatment may lead to abnormalities in stems, buds, leaves, branches, flowers and fruits. The type of response depends upon the duration of exposure, age and condition of the plant and the environment during and after exposure. The most common variation seen in legumes is leaf abnormalities.

Gunckel and Sparrow (1961) cited reports in which leaf anomalies were attributed to chromosome breakages and induced physiological changes. Sjodin (1962) reported morphological variations in M_1 generation in *Vicia*. Athwal (1963) reported leaf abnormalities such as narrow and broad leaves in *Cicer* following X-ray treatment. Constantin and Love (1964) reported relatively small increases in the frequency of leaf abnormalities at low doses, rapid increase at intermediate doses and either a plateau or decrease at higher doses following gamma and neutron irradiations in cowpea.

Louis and Kadambavanasundaram (1973a) reported that the number of pods per plant in cowpea decreased with the increase in dose of gamma rays. The highest number of pods per plant was recorded under 10 krad (31.96) which was on par with the control. Narsinghani and Kumar (1976) also reported a reduction in the number of pods per plant with increasing doses of EMS and MMS. Similar results were also reported in soybean by Constantin *et al*. (1976).

Ashri and Goldin (1965) reported the occurrence of non-heritable morphological changes in the M_1 generation, following DES treatment in groundnut. In *Cajanus cajan*, Chopde (1970) observed a number of morphological variants in the M_1 generation, following X-irradiation. Louis and Kadambavanasundaram (1973b) observed multicarpellate nature in 40 per cent of the M_1 progenies of cowpea seeds treated with 40 krad dose of gamma rays. Narsinghani and Kumar (1976) conducted mutation studies in cowpea using EMS and MMS and observed a few mutants with longer enlarged pods with less number of seeds, and leaf mutants affecting size and shape of leaves in the M_1 generation. Rafiev and Gasanov (1981) obtained mutants of Vigna unguiculata with altered testa and seed colour, seed size, growth rate and yield, following EMS treatment.

II. Mutations in the M_2 generation

1. Chlorophyll mutations

(a). Frequency

The methods commonly employed for estimating the frequency of chlorophyll mutations are:

i) Mutation per hundred M_1 plants

ii) Mutations per hundred M_1 spikes or inflorescences.

iii) Mutations per hundred M_2 plants .

Athwal (1963) observed several kinds of mutations in the X_2 generation of *Cicer*, but the most frequent were those affecting chlorophyll development.

Zannone (1965) reported a higher frequency of chlorophyll mutants in Vicia sativa with EMS than with EI and X-rays. Wellensiek (1965) reported that EMS yielded approximately seven times as many chlorophyll mutants in cowpea as X-rays, gamma rays and neutrons, which equalled each other. Swarup and Gill (1968) reported both viable and lethal chlorophyll mutations in French bean in all the treatments of X-rays. Sharma (1969), isolated two types of mutations in the M_2 following treatment of the seeds of Vigna sinensis with EMS, DMS and NMU. Alikhan and Veeraswamy (1974) conducted studies in red gram subjected to gamma irradiation and EMS treatment and reported that chlorophyll mutations were maximum at 24 krad and 70mM treatments respectively. Chlorophyll mutations were also reported in cowpea by Narsinghani and Kumar (1976) following treatment with EMS and MMS and by Subramanian (1979), following gamma irradiation.

Nerkar (1976) reported an increase in the frequency of chlorophyll mutations with an increase in the dosage of gamma rays in Lathyrus sativus, but with NMU, the mutation frequency was considerably reduced as the dosage increased. Vardanyan (1976) observed that there is no correlation between the frequency of chlorophyll mutations and that of morphological mutations in French bean, after treatment with chemical mutagens.

Krishnaswami *et al.* (1977) observed maximum number of chlorophyll mutations in 80 krad dose of X-rays and gamma rays in green gram. Chlorophyll mutation frequency was found to be the highest after high doses of gamma radiation in peas, by Ghosh *et al.* (1979). Oommen and Gopimony (1984) observed that the chlorophyll mutation frequency, estimated on M_1 plant basis, increased with an increase in the mutagen dose in cowpea and the mutagens used were gamma rays and EMS.

(b) Spectrum

Zannone (1965) observed that the spectrum of chlorophyll mutations was wider in *Vicia sativa* with EMS than with X-rays and EI. Jones (1965) conducted experiments using X-rays and thermal neutrons in peas and obtained albina mutants. Swarup and Gill (1968) observed that the chlorophyll mutants obtained in French bean following X-ray treatment were generally yellow or yellowish green in colour. Dahiya (1973) reported that in mung bean, following gamma irradiation, the chlorophyll mutation spectrum was confined to four types, viz., albina, xantha, viridis and maculata, and there was a conspicuous absence of tigrina, chlorina and striata types. Louis and Kadambavanasundaram (1973b) reported the occurrence of albina, xantha and viridis mutants in cowpea by gamma irradiation. Das and Kumar (1974) reported four chlorophyll mutants in pea, following gamma ray treatment, which had light yellow, light green, white spotted and orange-yellow spotted leaves. Nerkar (1976) reported a wider spectrum of chlorophyll mutations such as albina, xantha, albo-virescens, xantha-virescens, chlorina, chlorina-virescens, chlorotica and chlorotica-virescens in *Lathyrus sativus*, following gamma irradiation and EMS treatment. Narsinghani and Kumar (1976) observed that treatment with EMS resulted in albina, xantha, chlorina and striata mutants, and treatment with MMS resulted in albina, xantha and chlorina mutants. Vardanyan (1976) conducted a study on the frequency and spectrum of chlorophyll mutations in the M_2 generation of French bean treated with chemical mutagens. The main types of chlorophll mutants obtained were viridis, xantha, xantha-viridis and striata. Venkateswarlu *et al.* (1978) obtained viridis, chlorina and xantha mutants in pigeon pea, following gamma irradiation. Subramanian (1980) reported that the most common chlorophyll mutant was albina and that more mutants were produced by higher than by lower radiation doses.

(c) Segregation ratio

Patil and Bora (1961) reported one xantha and one virescent mutant after X-irradiation in groundnut. 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 is possibly controlled by more than on locus. Santos (1969) found the frequency of mutants in M_2 rows segregating for xantha, chlorina and albino mutants in mung bean to be 5.4 per cent, 7.3 per cent and 4.0 per cent respectively. Sur (1970) experimented with gamma and neutron irradiations in black gram and obtained different chlorophyll mutants which gave in the M_2 generation, segregation ratios ranging from 6.2 to 16.1 per cent, with an average of 9.1 per cent. Vardanyan (1976) reported that, following chemical mutagen treatment in French bean, viable and fertile striata mutants segregated in the 3:1 ratio.

2. Viable mutations

Gunckel and Sparrow (1961) reported that leaf abnormalities are commonly observed after irradiation and apparently the youngest leaf will exhibit the highest frequency of leaf alterations. Athwal (1963) reported several kinds of mutations in the X_2 generation of X-ray treated *Cicer* seeds, viz., flat stemmed, single leaved, bushy, narrow leaved and small leaved mutations. Constantin and Love (1964) observed many trifoliate leaves with one or two lateral leaflets missing in cowpea, following irradiation with gamma rays and neutrons. Jones (1965) reported a dwarf, a wrinkle leaf, a brown eye and an extra long peduncle type mutant in Southern peas, following treatment with X-rays and neutrons. Swarup and Gill (1968) observed that the maximum frequency of viable mutations were obtained in the 7 krad treatment in French bean using X-rays. The colour of pods and seeds was reported to be greatly affected, particularly the latter. Several polygenic and economic characters like number and size of pods, number of seeds per pod, seed yield and hundred seed weight were also affected. Leaf mutants of varying types with crumpled leaves, very small and large-sized leaves were reported. They also observed a surculus mutant in the 21 krad treatment, where the growth of the plant ceased after two cotyledonary leaves were formed, and a seed coat colour mutant. Santos (1969) obtained unifoliate and multifoliate leaf mutants following mutagen treatment in mung bean. Sharma (1969) reported a giant type late mutant in the M_2 generation of chemically treated cowpea seeds, which had large leaves, thick peduncles and fruits, and large seeds with mottled testa, and a trailing habit. The mutant maintained high fertility and was high yielding and was reported to constitute 11.3 per cent of all the mutants recorded. Louis and Kadambavanasundaram (1973b) observed a large number of morphological variants in the M_2 generation of cowpea, following gamma irradiation. Alikhan and Veeraswamy (1974) reported that the percentage of viable mutations was greater for gamma ray treatments than that for EMS treatments in red gram. Saini et al.(1974) obtained dwarf plants in Phaseolus aureus treated with 50 krad gamma rays, which had smaller leaves, stipules, flowers an anthers. Louis and Kadambavanasundaram (1975) obtained a mutant in cowpea which was devoid of anthocyanin in all parts, in the M_2 generation of 40 krad gamma irradiation, and a mutant with white seeds in 20 krad gamma treatment. Leaf mutants affecting size and shape were obtained by Narsinghani and Kumar (1976) in cowpea, following treatment with EMS and MMS. Sharma and Sharma (1979), isolated in the M_2 generation of lentil, treated with 10 krad gamma rays, a mutant with crinkled leaves which showed twisting, folding, shrinking of leaves, and irregular serrations on the leaf margins. Onim (1983) reported large variations for morphological and physiological traits in cowpea, following gamma irradiation.

In cowpea subjected to X-ray treatment, Pokle (1972) detected a white flowered mutant. Crumpled petal mutants were obtained by Appa Rao and Reddy (1975) in the

M_2 generation of X-ray irradiated and EMS treated seeds of bengal gram and cowpea. Chaturvedi and Sharma (1978a) reported that EMS was more efficient than NMU in inducing floral mutants in red gram where all the M_2 mutants lacked papilionaceous corolla due to an alteration in aestivation or due to an increase in petal number.

Narsinghani and Kumar (1969) obtained early flowering types with large pods in Vigna sinensis, following treatment with EMS and MMS. A true breeding mutant induced by gamma rays was obtained by Bhatt et al. (1972) in green gram, which matured earlier, and was shorter with longer pods and larger seeds than the parents. Krishnaswami et al. (1977) reported dwarf mutants at a high frequency in 80 krad of X-rays and 100 krad of gamma rays. Sheriff et al. (1977) obtained many mutants with terminal clusters of pods in red gram treated with EMS. Two mutants showed determinate growth coupled with profuse pod bearing habit. Subramanian (1979) reported dwarf mutants in Vigna unguiculata and Macrotilium lathyroides following 20 and 30 krad treatment of gamma rays, which proved high yielding compared with control plants.

Abdulomonov and Nigmatullin (1978) conducted studies in chick pea using chemical mutagens and obtained six mutants which exceeded the initial variety in seed yield, thousand seed weight, number of pods and the number of seeds per pod and reported that Mutant 18 was the most promising. Chaturvedi and Sharma (1978 b) obtained six male sterile mutants from EMS treated red gram seeds which were grouped under two heads, viz., the TSM type, which did not produce any fruit due to a high degree of pollen sterility, and the SSM type, which exhibited 95-100 per cent pollen fertility.

3. Variation in economic characters

Swarup and Gill (1968) conducted studies in French bean using X-rays and reported that the variance was enlarged in the treated progenies in M_2 although the mean was shifted in the negative direction. Ojomo and Chheda (1972) reported that the variation in yield, number of pods per plant, and time to flower was greater in the irradiated population of cowpea than among the untreated plants, following treatment with ionizing radiations. Prasad (1976) suggested that variation for pod number per plant and growth pattern in green gram could be brought about by induced mutations. He isolated ten mutants showing a higher number of pods and yield than the parents, using EMS. The mean values for the number of pods per plant, number of seeds per pod, and the seed weight per plant were reported to be less in mutagen treated populations than in the untreated populations by Virupakshappa *et al.* (1980) in cowpea. Lawhale (1982) conducted studies on the genetic variability in quantitative characters of cowpea and reported that the 22 kR dose was the most effective for inducing improvements. Khan (1984) reported that the variances were higher in the M_2 in the characters, height, days to maturity, number of productive branches, number of pods per plant and length of pods, number of seeds per pod, hundred seed weight, and seed yield, indicating the effectiveness of gamma rays in inducing mutations in polygenic characters in mung bean.

III. Mutagenic effectiveness and efficiency

Mutagenic effectiveness indicates the relationship of effect to dose (Ehrenberg, 1960). Konzak *et al.* (1965) reported that EMS seems to possess many properties favourable to high mutagenic effectiveness as well as efficiency. The greater efficiency of the lower concentration of an agent is due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration at a faster rate than mutations. Zannone (1965) reported that in *Vicia sativa*, comparison of the extent of chlorophyll mutations emphasised the greater mutagenic efficiency of chemicals, compared to X-rays and within chemicals, EMS was more effective than EI. Kawai (1969) reported that the studies of mutagenic effectiveness and efficiency are made on the basis of chlorophyll mutations, on the assumption that other types of mutations are induced with frequencies parallel to that of chlorophyll mutations. Sharma (1969) conducted experiments with DMS, EMS and NMU in cowpea and reported that on the basis of mutation frequency in the M_2, DMS and EMS showed almost equal effectiveness and NMU is almost twice as effective. Nerkar (1976) observed in *Lathyrus sativus* that EMS and NMU were more efficient than radiations, in producing chlorophyll mutations. Nerkar (1977b) also observed that the order of the mutagens based on effectiveness measured by the frequency of mutations induced by unit dose of mutagen, was NMU > EMS > gamma rays. The order of the mutagen based on efficiency measured by the proportion of mutations in relation to undesirable effects was gamma rays > EMS > NMU. Both mutagenic effectiveness as well as efficiency were higher at lower doses of the mutagens. Chaturvedi and Sharma (1978a) reported that EMS is more effective in inducing floral mutations than NMU in red gram. Subramanian (1980) conducted experiments in cowpea using gamma rays and reported that mutagenic effectiveness increased with increasing dose in all species, but mutagenic efficiency increased similarly in *Vigna radiata* alone. Oommen and Gopimony (1984) reported that effectiveness of gamma rays increased with increasing dose only upto 15 krad and the most efficient dose estimated on the basis of lethality and sterility was 10 krad, and on injury basis, 30 krad. They also observed that EMS at 0.4 per cent was the best treatment of its kind, irrespective of the measurement criterion.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was undertaken in the Department of Agricultural Botany, College of Agriculture, Vellayani, during the period 1983-1985.

A. MATERIALS

1. Biological Material

The crop selected for the present study was Kuruthola-payar variety of cowpea (*Vigna unguiculata*) which was subjected to induced mutagenesis. The cultivar has been evolved through selection from local varieties and is an excellent vegetable type recommended by the K.A.U. It has high yield (3.5-4.5 tons per hectare), trailing habit and 85-95 days duration. Seeds of the cultivar were obtained from the College of Agriculture, Vellayani.

2. Mutagens

The physical mutagen employed was gamma rays. The gamma irradiation facilities available at the Department of Botany, Kerala University, Kariavattom were utilised. Cobalt 60 gamma chamber was employed for irradiation. The source was operating at an intensity of 60 krad per hour.

The chemical used was Ethyl Methane Sulphonate (EMS:CH_3SO_2-O-C_2H_3), having a molecular weight of 124.16, of Sigma Chemical Company, U.S.A. The chemical has a specific gravity of 1.18 at 20°C.

B. <u>METHODS</u>

I. <u>Mutagen treatments</u>

(a) Gamma irradiation

Seeds of uniform size were sorted out. The moisture content of the seeds was approximately 12 per cent. Four samples of 230 seeds each were irradiated at four doses of gamma rays, viz., 10, 20, 30 and 40 krad.

(b) Application of EMS

Four samples of 230 seeds each were selected and presoaked in single distilled water for two hours. This was followed by treatment with E M S at concentrations of 0.5, 1.0, 1.5 and 2.0 ml per cent prepared in double distilled water. The duration of treatment was six hours. The volume of the mutagen solution was approximately ten times the volume of dry seeds (100 ml per 100 seeds). The treatment was conducted at the room temperature of 27 ± 1°C and intermittent shaking was given to maintain uniform concentration. After treatment, the seeds were washed in double distilled water three to four times, followed by running water for about an hour to remove all traces of the chemical from the seeds.

II. Study of the M_1 generation

i) Pot experiments

Samples of 30 seeds per dose in both the mutagen treatments were raised in pots, replicated three times and the following observations were recorded.

1. Germination of seeds

Germination counts were made on the tenth day of sowing to estimate the percentage of germination.

2. Shoot length and root length of seedlings

The seedlings raised in pots were carefully uprooted on the fifteenth day of sowing and the length of the primary shoot and root of each was measured. The mean shoot length and the mean root length for each treatment were estimated and expressed as percentage of the respective control. The shoot-root ratio was worked out from the data on mean length of primary shoot and root.

ii) Field studies

The gamma irradiated seeds were sown in the field on the eighth day after treatment along with the unirradiated control. The experiment was laid out in

Randomised Block Design with five treatments and five replications. The seeds were sown at the rate of 100 seeds per plot at a spacing of 30 cm x 15 cm. The plot size was 3.0 m x 1.5m.

Another field experiment was laid out in Randomised Block Design with five treatments and five replications for sowing the seeds treated with EMS. The seeds were sown in the plots immediately after treatment. The number of seeds per plot, spacing and plot size were the same as in the case of gamma irradiated seeds.

The following observations were recorded.

1. Germination counts
2. Survival counts
3. Plant height
4. Pollen fertility
5. Seed fertility
6. Chlorophyll chimeras
7. Number of pods per plant
8. Morphological variations.

1. Germination counts

Counts of germinated seeds for each treatment were made on the fifth, tenth and fifteenth days after sowing and the percentage of germination estimated. The emergence of plumule from the soil was taken as the criterion for germination.

2. Survival counts

The total number of plants surviving in each treatment was counted on the twentieth day after sowing and the survival data, estimated on the basis of the number of seeds sown, is expressed as percentage of the respective control.

3. Plant height

Ten plants were selected at random from each treatment and the plant height was measured on the 40th day after sowing and at the time of harvest. The height was measured from the soil surface to the terminal bud. The mean plant height was estimated and expressed as percentage with respect to the control.

4. Pollen fertility:

Pollen fertility was assessed using stainability with 1:1 glycerine-acetocarmine solution as a criterion. Ten plants from each treatment were selected at random and mature flower buds produced during the early part of the flowering period were selected. The uniformly stained, properly filled pollen grains were scored as fertile, and the unstained, under sized, partially stained and shrivelled pollen grains were scored as sterile. The counts were made after two hours of staining. In each of the slides, fifteen microscopic fields were scored and the data recorded. The fertility of each plant was estimated as the percentage of the number of fertile pollen grains to the total number of pollen grains scored. The mean fertility of each treatment was estimated and expressed as percentage with respect to the control.

5. Seed fertility

Pods were collected from ten plants selected at random from each treatment, and the fertile seeds were counted. The fertility of each plant was estimated as the percentage of the number of fertile seeds to the total number of seeds, and the mean fertility of each treatment was estimated and expressed as percentage with respect to the control.

6. Chlorophyll chimeras

The M_1 plants were examined at periodical intervals for chimeric plants exhibiting chlorophyll deficient patches or sectors on their leaves.

7. Number of pods per plant

Ten plants in each of the treatments were selected at random and the number of pods per plant counted, and the mean was estimated and expressed as percentage with respect to the control.

8. Morphological variations

The M_1 population was examined at regular intervals to locate plants with morphological variations such as dwarf plants and plants with alterations in number, size and shape of leaflets in the early formed secondary leaves.

III. Study of the M_2 generation

Mature fruits were collected from M_1 plants selected at random from each treatment and the seeds were used to raise the M_2 generation. The number of M_1 plants carried forward to M_2 generation varied from 10-100. The M_2 generation was raised as M_1 plant progenies. Fifty seeds from each M_1 plant formed a progeny row in the M_2 with a spacing of 30 cm between rows and 15 cm between plants in a row.

The following observations were recorded.

1. Chlorophyll mutations
2. Viable mutations
3. Flowering
4. Plant height
5. Number of pods per plant
6. Pod length
7. Seed fertility
8. Hundred seed weight

1. Chlorophyll mutations

The M_2 seedlings were examined in the early morning hours from the third day of sowing upto the twentieth day and chlorophyll mutations were scored. The progeny rows segregating for chlorophyll mutations were first counted and the chlorophyll mutation frequencies on M_2 progeny row (M_1 plant) basis were estimated as the number of plants segregating per hundred M_2 progeny rows. The total number of mutants and normal seedlings were counted from both segregating and non-segregating M_1 families to compute the mutation frequency per 100 M_2 seedlings.

The different types of chlorophyll mutants in each of the segregating progeny rows were counted separately and their relative percentages estimated. The segregation ratios were estimated as the percentage of the number of mutants to the total number of plants scored in segregating M_1 families.

2. Viable mutations

The M_2 plants were observed periodically upto harvest to score viable mutations. The viable mutation frequency was estimated on the basis of the number of mutations per 100 M_2 progeny rows. The viable mutants were described with respect to the deviations from the normal plants.

3. Flowering

The number of days taken for flowering to commence in individual plants was noted. The mean value for each treatment was estimated and expressed as percentage with respect to the control.

4. Plant height

Twenty plants were selected at random from each treatment and the plant height was measured at harvest. The mean plant height was estimated and expressed as percentage with respect to the control.

5. Number of pods per plant

Twenty plants in each treatment were selected at random and the number of pods per plant counted. The mean value for each treatment was estimated and expressed as percentage with respect to the control.

6. Pod length

Pods were collected from twenty plants which were selected at random from each treatment, and the length of pods was measured. The mean length was estimated and expressed as percentage with respect to the control.

7. Seed fertility

Pods were collected from twenty plants selected at random from each treatment and the fertile seeds were counted. The fertility of each plant was estimated as before, and the mean fertility of each treatment calculated and expressed as percentage with respect to the control.

8. Hundred seed weight

Seeds were taken from pods collected from twenty plants selected at random from each treatment, and the hundred seed weight was determined. The mean value for each treatment was estimated and expressed as percentage with respect to the control.

IV. Estimation of mutagenic effectiveness and efficiency

The effectiveness and efficiency of the mutagens in inducing chlorophyll mutations were estimated adopting the formulae suggested by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness} = \frac{M \times 100}{krad} \text{ or } \frac{M \times 100}{c \times t}$$

$$\text{Mutagenic efficiency} = \frac{M \times 100}{L} \quad \frac{M \times 100}{I} \text{ or } \frac{M \times 100}{S}$$

Where M = Mutation frequency on M_2 progeny row basis.

t = Time of chemical mutagen treatment in hours.

c = Concentration of chemical mutagen in ml per cent.

L = Percentage of lethality on the basis of survival reduction.

I = Percentage of injury or plant height reduction.

S = Percentage of pollen sterility.

RESULTS

RESULTS

The effect of gamma irradiation and EMS treatment on cowpea in the M_1 and M_2 generations was studied and the results are presented below.

I. Effect of mutagens in the M_1 generation

1. Germination of seeds

The data on the percentage of germination in the pot experiment and under field conditions are given in Table - 1 and Table - 2 respectively and the former is graphically represented in Fig. 1. The germination percentage was affected by gamma rays as well as by EMS, although the effect was more severe in the latter. A progressive decrease in the germination percentage was observed with increasing doses of both mutagens. The treatment 40 krad exhibited a low germination percentage in the case of gamma rays, while with EMS, all the treatments exhibited drastic reduction in germination. Even the lowest dose of EMS, viz. 0.5 per cent recorded as low a germination percentage as 15.00 in the field study. The highest dose reduced the germination percentage to 5.50.

Gamma irradiation did not appear to delay the germination of seeds at low doses, and maximum germination for the 10 krad and 20 krad treatments was attained by the

Table 1. Effect of mutagens on total seed germination (Pot experiment)

Treatment	Germination percentage	Relative percentage
<u>Gamma rays</u>		
Control	83.33	100.00
10 krad	80.00	96.00
20 krad	73.33	88.00
30 krad	66.67	80.00
40 krad	46.67	56.00
<u>EMS</u>		
Control	80.00	100.00
0.5%	33.33	41.66
1.0%	10.00	12.50
1.5%	6.67	8.34
2.0%	3.33	4.16

Table 2. Effect of mutagens on the percentage of total seed germination (field condition).

Period	Control	Gamma rays - dose				Control	EMS dose			
		10 krad	20 krad	30 krad	40 krad		0.5%	1.0%	1.5%	2.0%
5th day	82.50	74.50	70.00	58.00	30.00	78.50	7.50	0.00	0.00	0.00
10th day	82.50	74.50	70.00	60.00	35.00	83.50	14.00	5.50	3.00	2.00
15th day	82.50	74.50	70.00	61.00	36.50	83.50	15.00	8.50	7.50	5.50

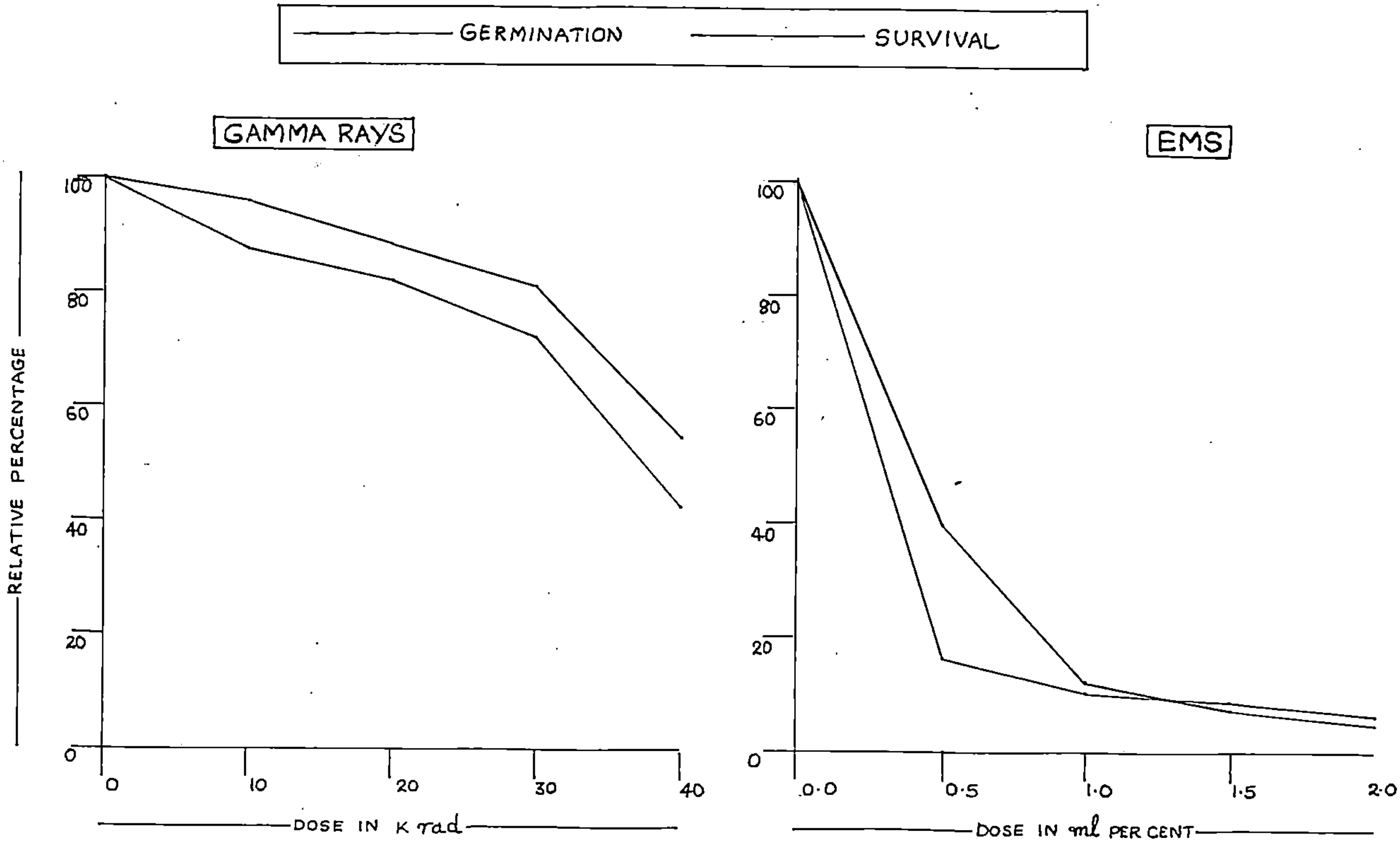


FIG: 1. EFFECT OF MUTAGENS ON THE GERMINATION AND SURVIVAL fifth day. At higher doses, ie., 30 krad, a slight delay was noticed. Delay in germination was observed in the case of all EMS treatments, especially at higher concentrations.

2. Survival of plants

The data on the survival of plants in the field on the twentieth day after sowing are given in Table 3 and are graphically represented in Fig.1. The percentage of survival was observed to decrease with increase in the dose of both mutagens. The lethality percentage showed an increase in value with higher doses, and this was more pronounced in the case of EMS. The reduction in survival was very drastic at higher doses of EMS.

3. Shoot-root ratio

The data on the mean length of shoot and that of the primary root measured on the fifteenth day of sowing are presented in Table 4 and are graphically represented in Fig. 2. The growth of shoot and root was drastically reduced by both mutagens. The shoot-root ratios were less than that of the control in the gamma ray treatments, indicating that the growth of the shoot was more affected than that of the root. In the case of EMS, the shoot-root ratios were found to be greater

Table 3. Effect of mutagens on the survival of plants on the 20th day.

Treatment	20th day		Survival reduction (Lethality %)
	Survival percentage	Relative percentage	
Gamma rays			
Control	82.50	100.00	0.00
10 krad	72.00	87.27	12.73
20 krad	68.00	82.42	17.58
30 krad	59.50	72.12	27.88
40 krad	35.00	42.42	57.58
EMS			
Control	83.50	100.00	0.00
0.5%	13.50	16.17	83.83
1.0%	8.50	10.18	89.82
1.5%	7.50	8.98	91.02
2.0%	5.50	6.59	93.41

Table 4. Effect of mutagens on shoot/root (Pot experiment) ratio

Treatment	Shoot length (cm)	Relative percentage	Root length (cm)	Relative percentage	Shoot/root ratio
Gamma rays					
Control	99.83	100.00	27.00	100.00	1.00
10 krad	92.42	92.58	26.93	99.74	0.93
20 krad	58.29	58.39	25.26	93.56	0.62
30 krad	51.23	51.32	24.43	90.00	0.57
40 krad	26.53	26.58	16.22	60.07	0.44
EMS					
Control	99.26	100.00	26.72	100.00	1.00
0.5%	59.56	60.00	13.59	50.86	1.18
1.0%	37.23	37.51	9.82	36.75	1.02
1.5%	34.74	35.00	9.08	33.98	1.03
2.0%	30.05	30.27	8.02	30.01	1.01

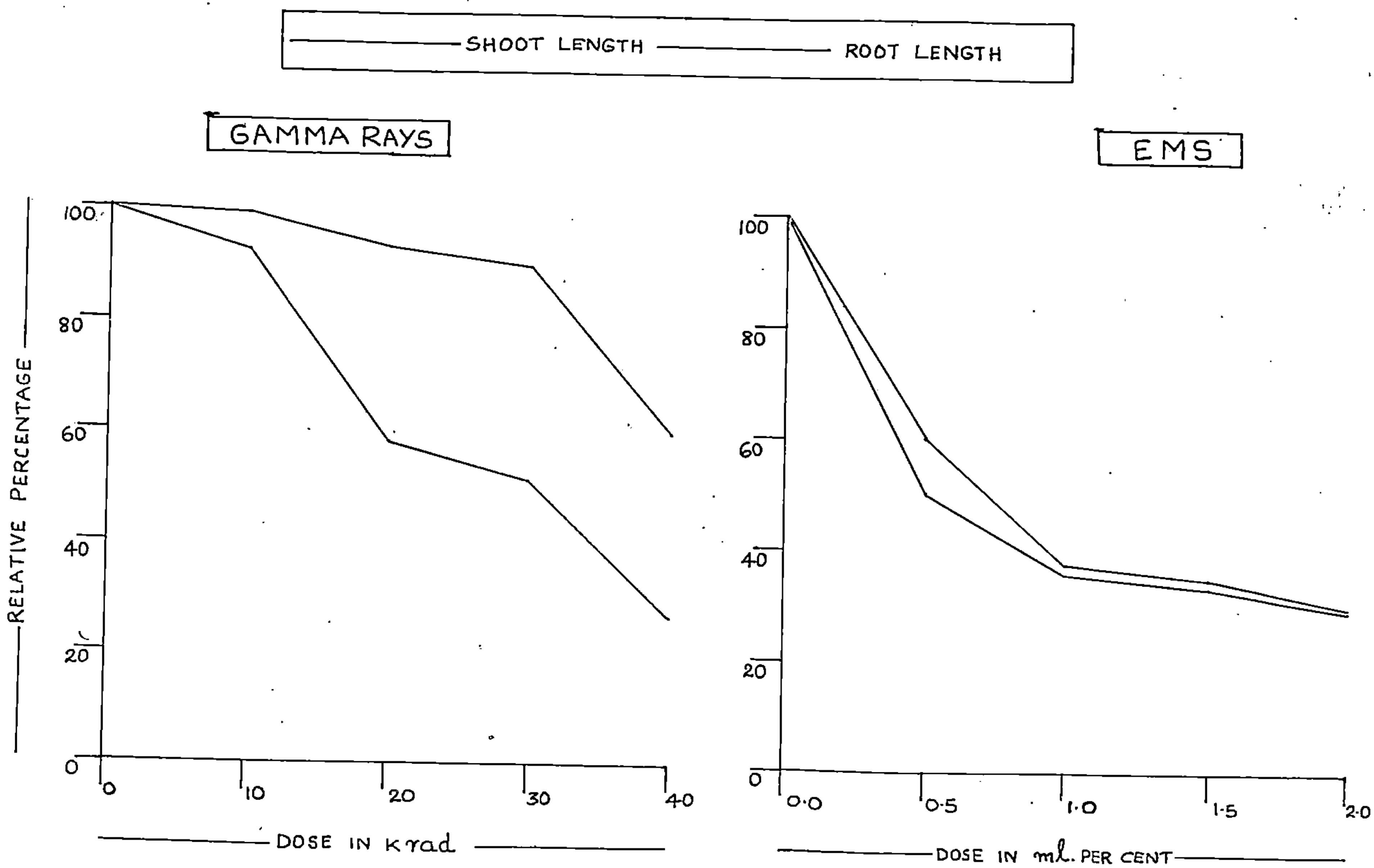


FIG: 2. EFFECT OF MUTAGENS ON THE SHOOT LENGTH AND ROOT LENGTH than that of the control, which indicates a higher growth inhibition for the root than for the shoot, eventhough both shoot and root growths were adversely affected.

4. Plant height

The height of the plants in the field was measured on the fortieth day of sowing and at harvest and the mean values were estimated. The data are presented in Table 5 and are graphically represented in Fig.3. The plant height values showed reduction with both gamma irradiation and EMS treatment, at both the stages, compared with that of the control. The reduction in plant height increased with increase in doses of both the mutagens. However, EMS at the highest dose induced severe reduction in plant height.

5. Pollen fertility

The data on pollen fertility are presented in Table 6 and are graphically represented in Fig. 4. The percentage of pollen fertility was found to decrease with increase in dose of gamma rays and EMS. The patterns of fertility reduction with increasing doses were found to be different for the two mutagens. Greater reduction in pollen fertility was noted with gamma rays than with EMS treatment. Higher doses of gamma rays

Table 5. Effect of mutagens on plant height under field conditions

Treatment	Plant height				Height reduction on 40th day (Injury %)
	40th day height (cm)	Relative percentage	Height at harvest (cm)	Relative percentage	
Gamma rays					
Control	205.96	100.00	384.50	100.00	0.00
10 krad	201.63	97.90	380.62	98.99	2.10
20 krad	192.71	93.57	362.88	94.38	6.43
30 krad	172.92	83.96	336.73	87.58	16.04
40 krad	134.50	65.30	302.07	78.56	34.70
EMS					
Control	204.44	100.00	381.30	100.00	0.00
0.5%	197.97	96.84	369.05	96.79	3.16
1.0%	163.80	80.12	331.38	86.91	19.88
1.5%	159.70	78.12	309.50	81.17	21.88
2.0%	115.14	56.32	243.17	63.77	43.68

Table 6. Pollen fertility

Treatment	Pollen fertility (%)	Relative percentage	Pollen sterility (%)
Gamma rays			
Control	94.38	100.00	0.00
10 krad	87.50	92.71	7.29
20 krad	73.85	78.25	21.75
30 krad	62.51	66.23	33.77
40 krad	49.63	52.59	47.41
EMS			
Control	94.62	100.00	0.00
0.5%	90.48	95.62	4.38
1.0%	87.16	92.12	7.88
1.5%	68.16	72.04	27.96
2.0%	70.55	74.56	25.44

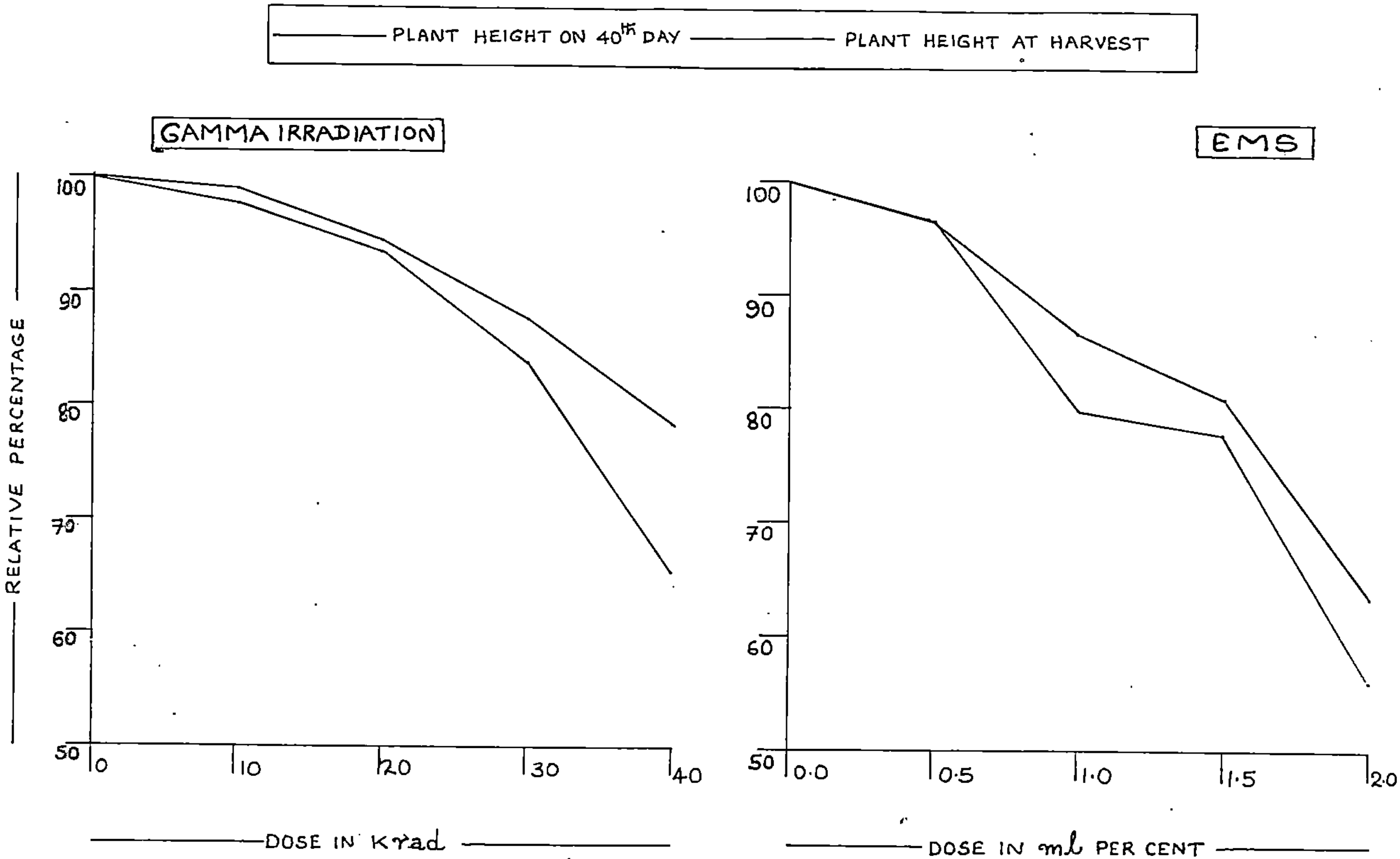


FIG: 3. EFFECT OF MUTAGENS ON THE RATE OF GROWTH OF PLANTS induced greater sterility compared to the control. Even though higher doses of EMS also recorded higher sterility compared to the control, the sterility percentage at the highest dose was much less than that inflicted by the higher doses of gamma rays.

6. Seed fertility

Table - 7 shows the data on seed fertility and Fig.4, the graphical representation. The percentage of seed fertility decreased linearly with increase in doses of both the mutagens and the pattern of decrease was more or less the same for both the mutagens over the doses tested.

7. Chlorophyll chimeras

Plants with chlorophyll deficient patches on the leaves were noticed in the 30 krad and 40 krad doses of gamma rays, while chimeric plants were completely absent in the M_1 generation in all the EMS treatments used in the present study.

8. Number of pods per plant

The data on the number of pods per plant are given in Table 8. The number of pods per plant was found to decrease with an increase in dose of both mutagens.

Table 7. Seed fertility

Treatment	Seed fertility (%)	Relative percentage	Seed sterility (%)
Gamma rays			
Control	92.34	100.00	0.00
10 krad	75.64	81.91	18.09
20 krad	66.23	71.72	28.28
30 krad	66.17	71.66	28.34
40 krad	60.84	65.89	34.11
EMS			
Control	92.25	100.00	0.00
0.5%	83.27	90.27	9.73
1.0%	74.56	80.82	19.18
1.5%	66.13	71.69	28.31
2.0%	64.85	70.30	29.70

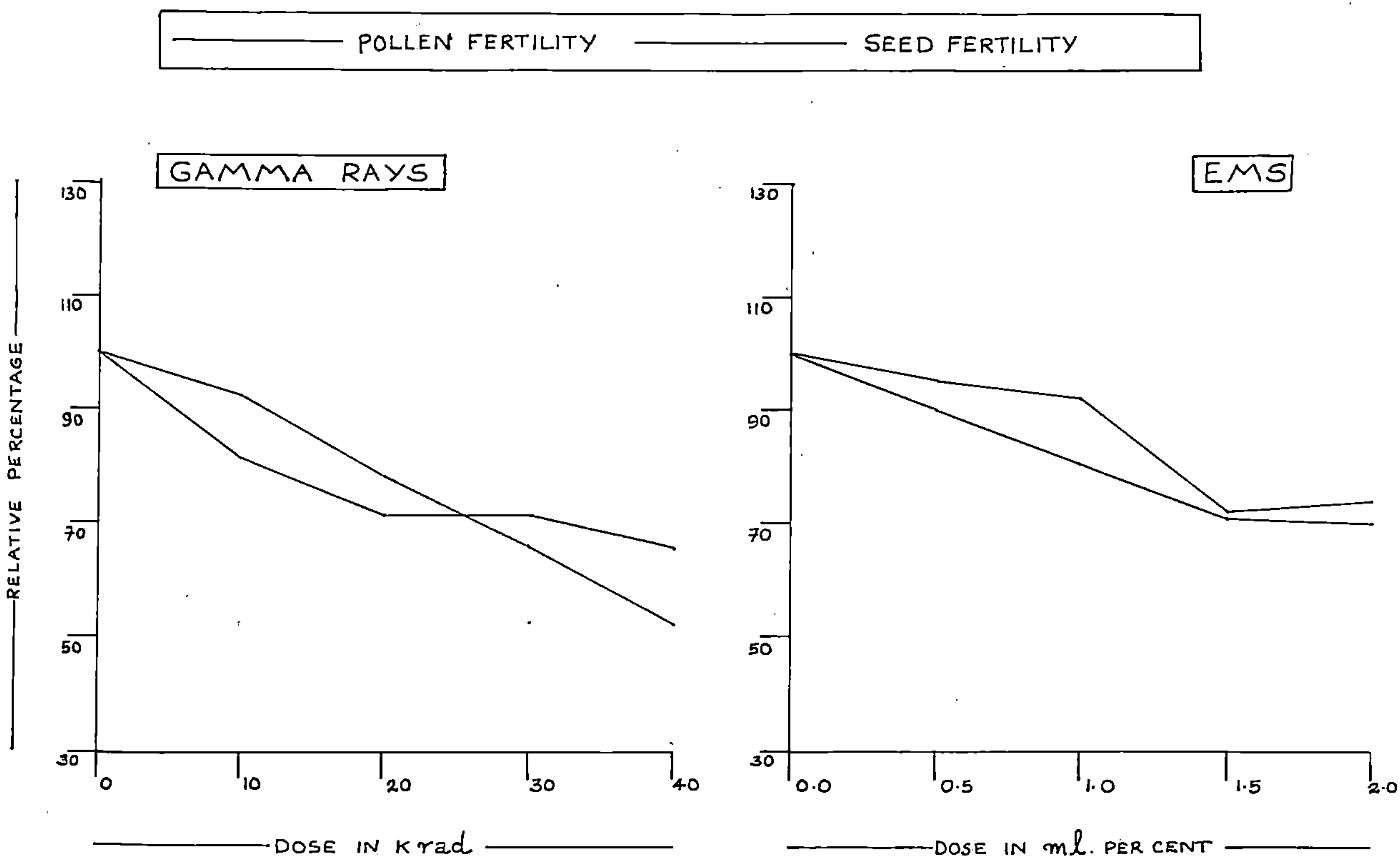


FIG:4. EFFECT OF MUTAGENS ON POLLEN FERTILITY AND SEED FERTILITY

Table 8. Number of pods per plant

Treatment	Number of pods per plant	Relative percentage
Gamma rays		
Control	12.50	100.00
10 krad	8.25	66.00
20 krad.	8.00	64.00
30 krad	7.50	60.00
40 krad	6.00	48.00
EMS		
Control	11.75	100.00
0.5%.	8.25	70.21
1.0%	7.25	61.70
1.5%	7.00	59.57
2.0%	6.50	55.32

9. Morphological variations

In the present investigation, the morphological abnormalities observed in the M_1 generation were restricted to the alteration in number, size and shape of leaflets in the first formed secondary leaves of the M_1 plants. In some cases, the first formed secondary leaf lacked one or two lateral leaflets, thereby appearing as a bifoliate or unifoliate leaf instead of the normal trifoliate leaf. However, these plants recovered and produced normal leaflets afterwards.

Small and narrow leaflets were observed in one plant in 40 krad of gamma irradiation and in one plant in 2.0 per cent of EMS treatment.

II. Effect of mutagens in the M_2 generation

1. Chlorophyll mutations

(a) Frequency

The data on the frequency of chlorophyll mutations are presented in Table 9 and are graphically represented in Fig.5. The mutation frequencies are estimated on the M_2 progeny row and M_2 seedling basis. The frequencies estimated on M_2 progeny row basis increased with increasing doses of gamma rays, except in the 40 krad treatment, which showed a slight decrease.

Table 9. Frequency of chlorophyll mutations in the M_2 generation

Treatment	M_2 progeny row basis			M_2 seedling basis		
	Number of M_2 progeny rows		No. of mutants per 100 M_2 progeny rows	Number of M_2 seedlings scored	No. of mutants	No. of mutants per 100 M_2 seedlings
	Scored	Segretating				
Gamma rays						
Control	50	0	0.00	2066	0	0.00
10 krad	87	9	10.34	3363	18	0.54
20 krad	78	15	19.23	2656	34	1.28
30 krad	75	16	21.33	2238	37	1.65
40 krad	69	14	20.29	1208	29	2.4
EMS						
Control	30	0	0.00	1253	0	0.00
0.5%	27	4	14.81	183	7	3.83
1.0%	16	2	12.50	73	3	4.11
1.5%	15	2	13.33	52	3	5.77
2.0%	10	1	10.00	28	2	7.14

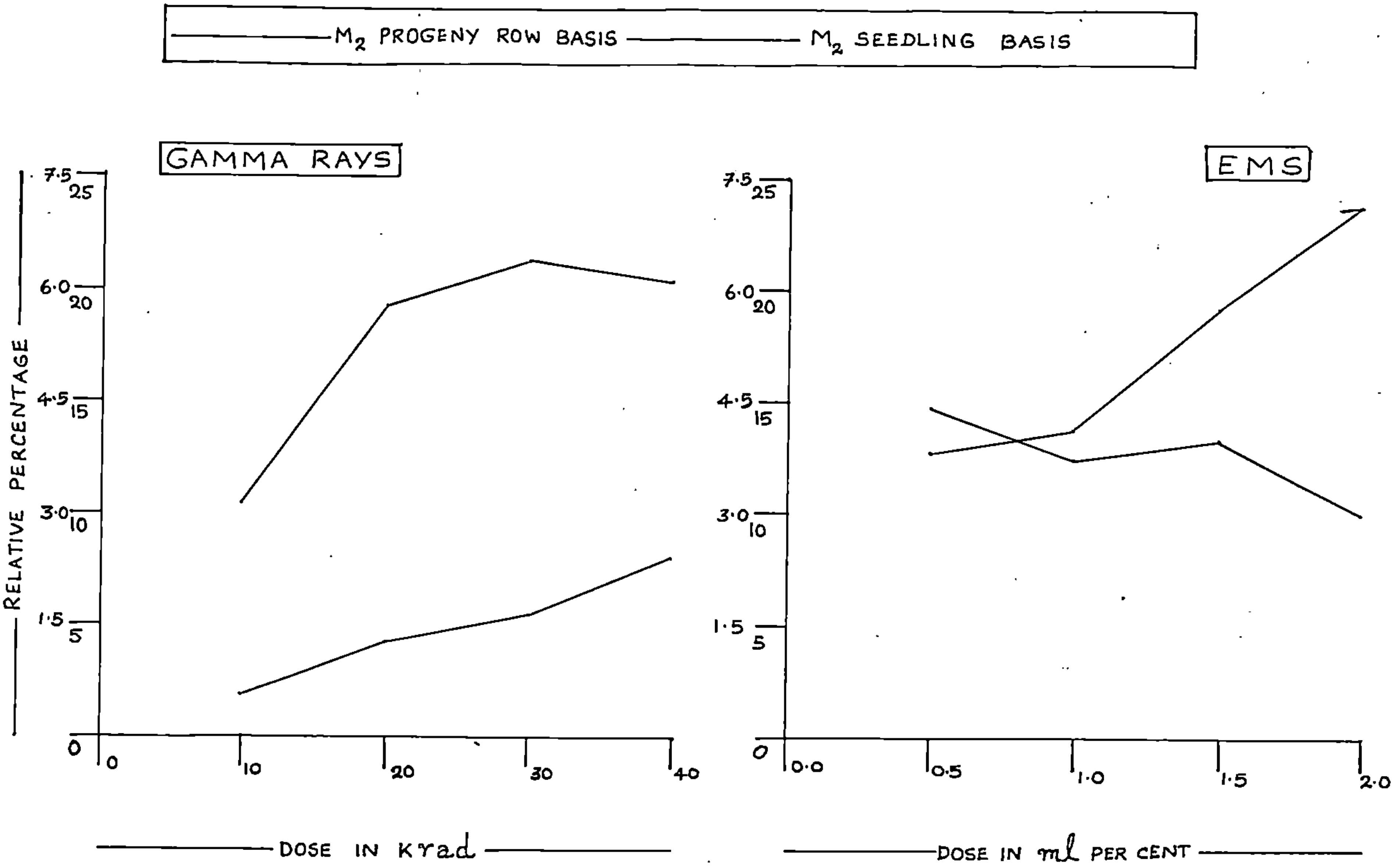


FIG:5. FREQUENCY OF CHLOROPHYLL MUTATIONS IN THE M_2 GENERATION

In the case of EMS, the maximum number of mutants were obtained at the lowest dose, ie., 0.5 per cent. The frequencies of chlorophyll mutations estimated on M_2 seedling basis showed dose dependence and increased with increasing doses of both mutagens.

(b) Spectrum

The chlorophyll mutants obtained were albina (no visible pigment), xantha (yellow), chlorina (yellowish-green), viridis (light green) and those with irregular chlorophyll deficient patches or spots on the leaves (plate 1). The relative percentages of different type of chlorophyll mutants with gamma rays and EMS are presented in Table 10 and are graphically represented in Fig.6 (a) and Fig. 6(b) respectively.

The albina mutants were observed only in the medium doses of gamma rays, viz., 20 krad and 30 krad treatments and in the lowest dose of EMS treatment. The relative percentages of chlorophyll mutants varied with different doses and the frequencies of the different types of mutants did not show any dose dependence. The xantha, viridis and chlorina types occurred in all the treatments of gamma rays. The xantha and viridis types occurred in all the treatments of EMS, while chlorina types were absent in the 2.0 per cent treatment. In gamma ray treatments, viridis

Plate 1. Types of chlorophyll mutants induced

1. Xantha

2. Viridis

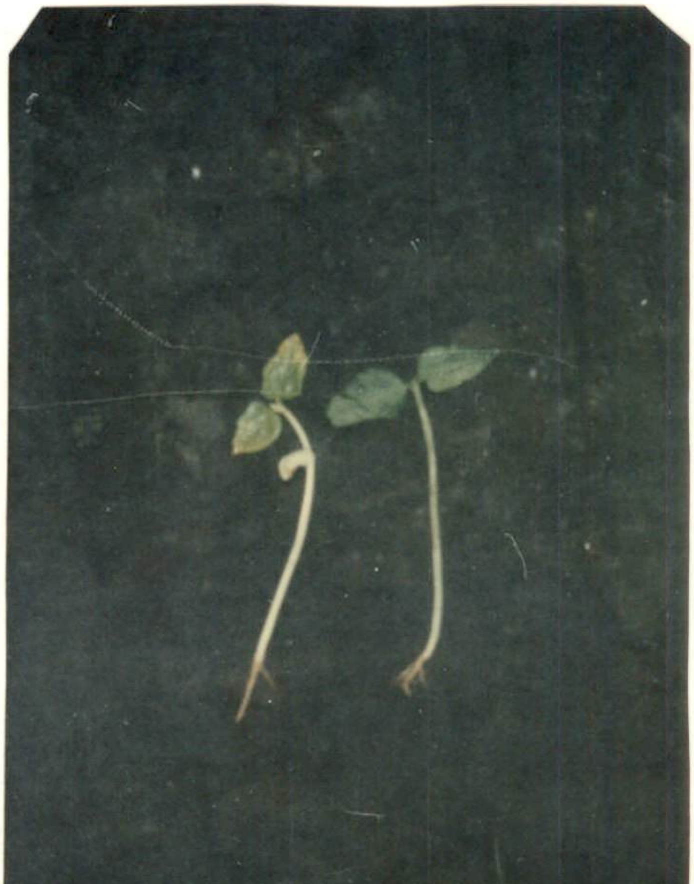


Plate 1.

Table 10. Spectrum of chlorophyll mutations

Treatment	Total No.of mutants	Relative Percentage			
		Albina	Xantha	Viridis	Chlo
Gamma rays					
Control	0	-	-	-	-
10 krad	18	-	50.00	27.78	5.5
20 krad	34	2.94	20.59	38.24	23.5
30 krad	37	8.11	5.41	37.84	27.0
40 krad	29	-	13.79	41.37	20.6
Total	118	2.76	22.45	36.30	19.2
EMS					
Control	0	-	-	-	-
0.5%	7	14.29	28.57	28.57	28.5
1.0%	3	-	33.33	33.33	33.3
1.5%	3	-	33.33	33.33	33.3
2.0%	2	-	50.00	50.00	-
Total	15	3.57	36.31	36.31	23.8

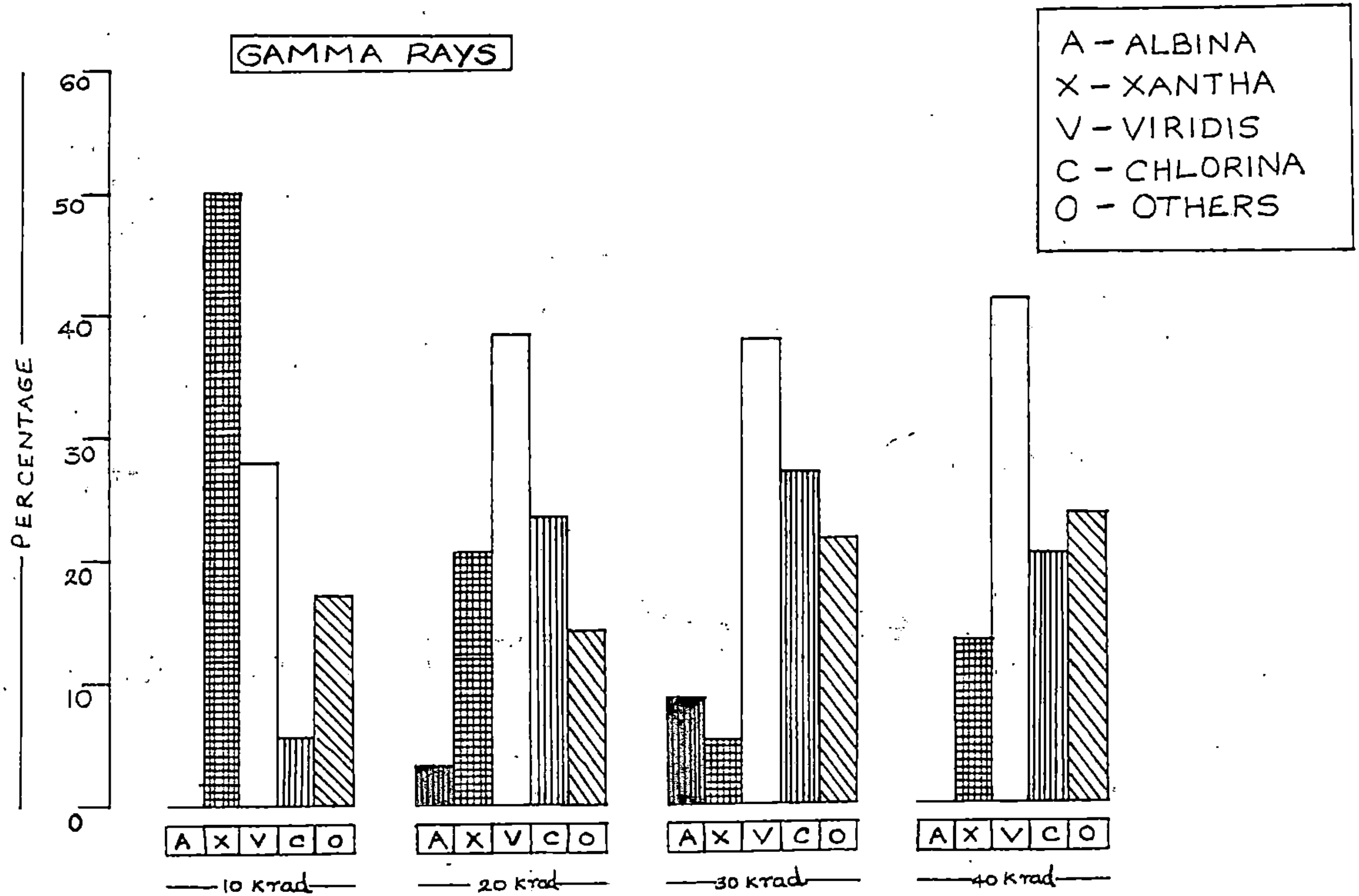


FIG:6(a) CHLOROPHYLL MUTATION SPECTRUM

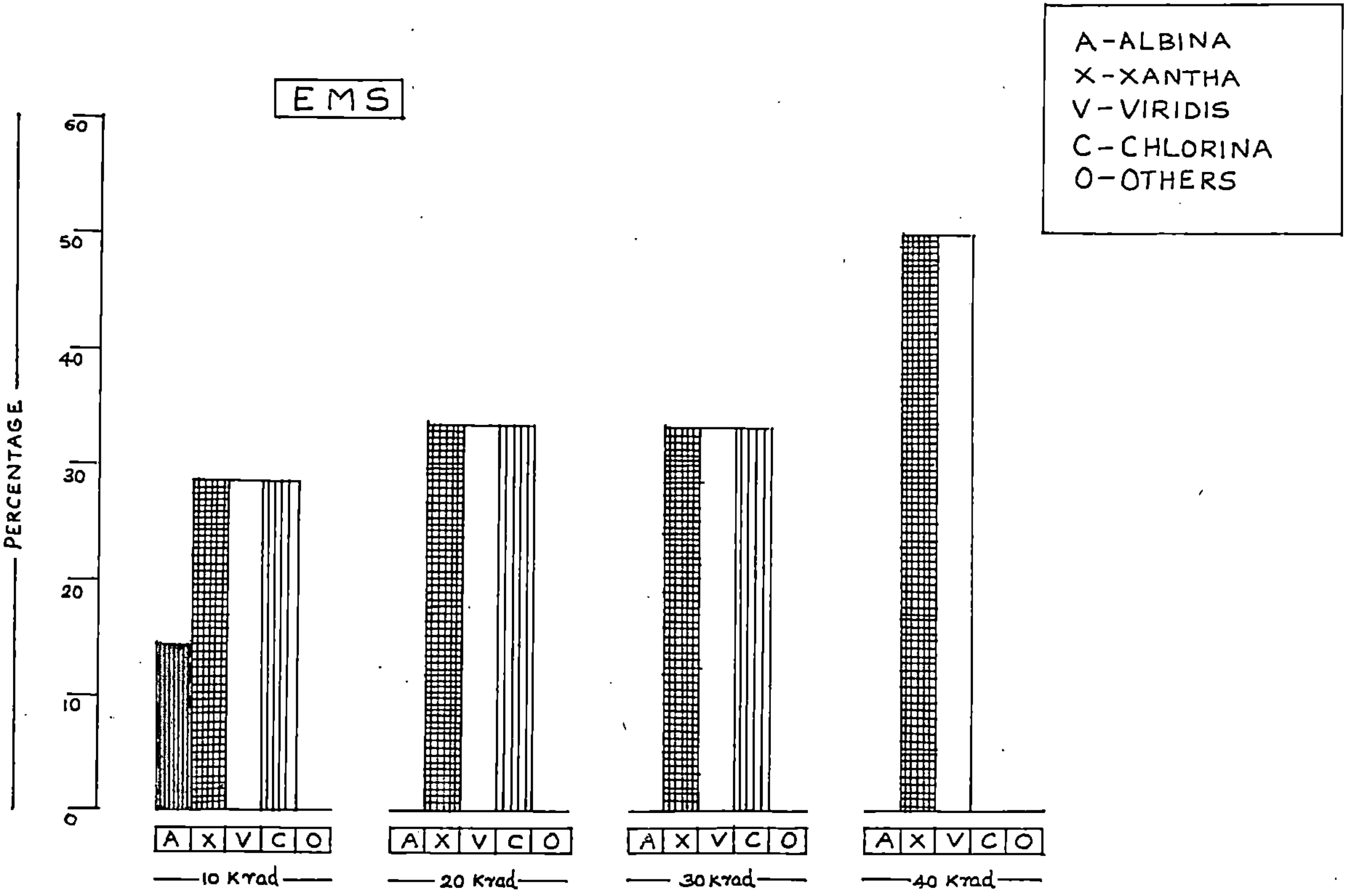


FIG: 6(b) CHLOROPHYLL MUTATION SPECTRUM

was the most frequently occurring type (36.30 per cent), while in EMS treatments, it was xantha and viridis (36.31 per cent).

(c) Segregation ratio

The data on segregation ratios are presented in Table 11. Segregation ratios were estimated as the percentages of the number of chlorophyll mutants to the total number of plants in the segregating M_2 progeny rows in each dose of the mutagens. The segregation ratios did not show any definite relationship with the doses. In the gamma ray treatments, the maximum segregation ratio was observed in 30 krad (9.23 per cent) and the minimum in 10 krad (6.12 per cent) treatments. With EMS, the segregation ratio was maximum at 0.5 per cent (14.29 per cent) and minimum at 2.0 per cent (10.53 per cent). EMS treatments showed higher segregation ratios than gamma irradiation.

2. Viable mutations

The viable mutation frequency estimated on the basis of the number of mutations per 100 M_2 progeny rows is presented in Table 12. The frequencies did not show any definite relationship with the doses in both the mutagen treatments. A higher percentage of segregating M_2 progeny rows was obtained in the 40 krad treatment of gamma rays and in the 1.0 per cent EMS treatment.

Table 11. Segregation ratio of chlorophyll mutants in the M_2 generation.

Treatment	Total no.of plants scored in segregating M_2 progeny rows	No.of mutants	Segregation ratio
Gamma rays			
Control	0	0	0.00
10 krad	294	18	6.12
20 krad	370	34	9.19
30 krad	401	37	9.23
40 krad	326	29	8.90
EMS			
Control	0	0	0.00
0.5%	49	7	14.29
1.0%	26	3	11.54
1.5%	22	3	13.64
2.0%	19	2	10.53

Table 12. Frequency of viable mutations in the M_2 generation.

Treatment	Number of M_2 progeny rows		No.of mutations per 100 M_2 progeny rows.
	Scored	Segregating	
<u>Gamma rays</u>			
Control	50	0	0.00
10 krad	93	5	5.38
20 krad	78	11	14.10
30 krad	75	14	18.67
40 krad	69	13	18.84
<u>EMS</u>			
Control	30	0	0.00
0.5%	27	1	3.70
1.0%	16	2	12.50
1.5%	15	1	6.67
2.0%	10	1	10.00

The different types of viable mutants produced by gamma irradiation were with respect to leaf size and shape, length of pods, time of flowering and seed coat colour, while EMS induced changes in leaf size and shape, length of pods, time of flowering and size of seeds.

Viable mutants, with changes in leaf size and shape, comprised of those with large leaves, small and narrow leaflets, long and narrow leaflets and round leaflets, in the gamma ray treatments (plate 2). In EMS treatments, mutants with small and round leaflets as well as mutants with large and long leaflets were observed (Plate 3).

Early and late flowering types as well as early and late maturing types were obtained from the M_2 population of both mutagen treatments.

Another interesting group of mutants obtained was the seed coat colour mutants (Plate 4). From the M_2 population of gamma ray treatments, plants were isolated, which had seed coat colours that were cream, brown and a shade of red.

From the M_2 population of EMS treatments, mutants which had different sizes of seeds were also isolated (Plate 5).

Plate 2. Variation in leaf size and shape induced by gamma rays

Plate 3. Variation in leaf size and shape induced by EMS

Plate 2.

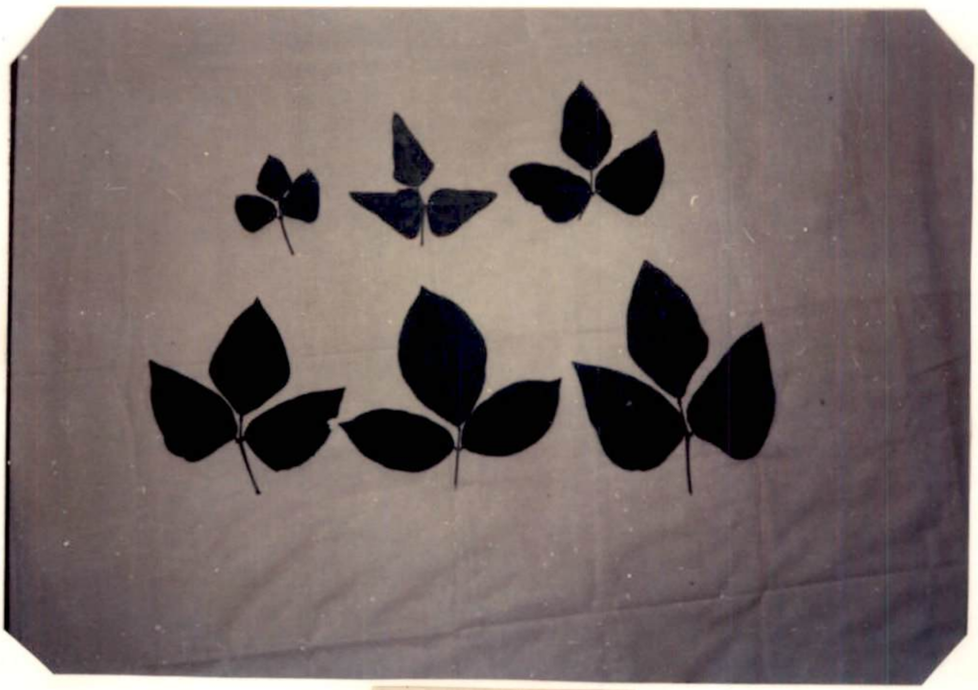


Plate 3.

Plate 4. Seeds of the gamma ray-induced seed coat colour mutants

Plate 5. Variation in size of seeds induced by EMS

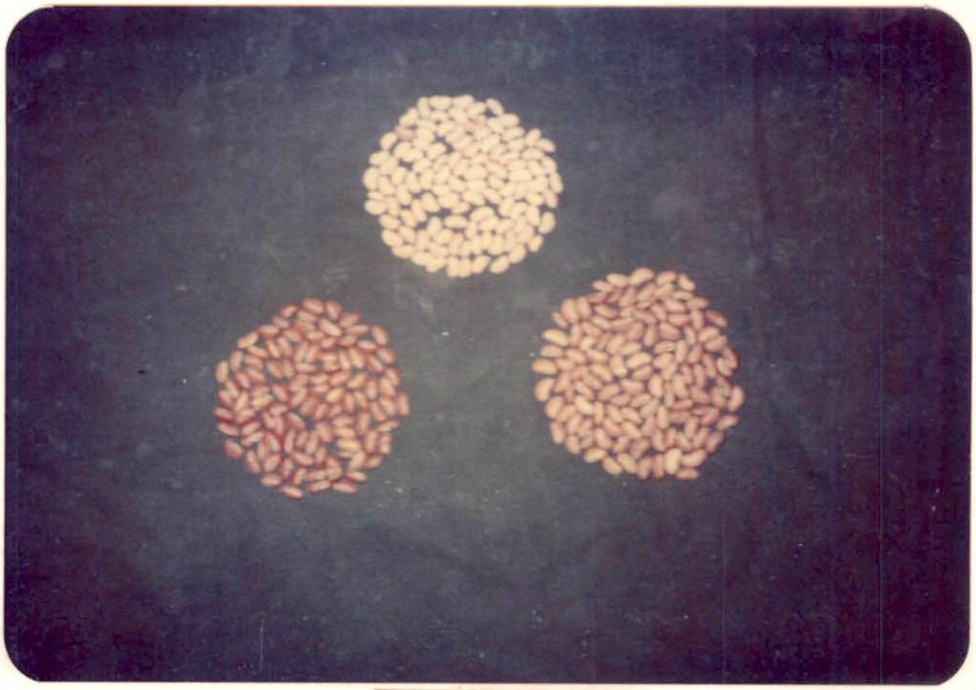


Plate 4.

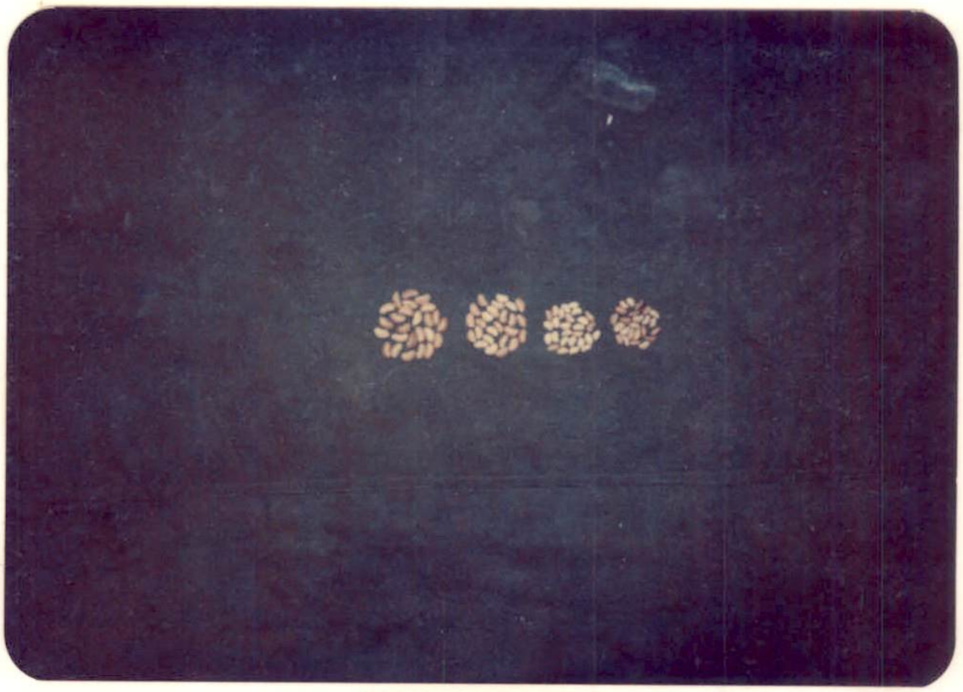


Plate 5.

In addition to the above, mutants with long as well as very short pods were obtained with both the mutagen treatments (Plates 6 and 7).

3. Flowering and plant height

The data on the number of days taken for flowering to commence and the plant height at flowering are given in Table 13. Delay in the initiation of flowering was noted in both the mutagen treatments, but the delay was not significant. Decrease in height was noted with increase in dose of both mutagens. The height reduction was found to be significant in gamma ray treatment and EMS treatment.

4. Number of pods per plant, pod length and hundred seed weight

The data on the number of pods per plant, pod length and hundred seed weight are presented in Table 14. The number of pods per plant, pod length and hundred seed weight were found to decrease with an increase in dose of both the mutagens. This reduction in number and length of pods and hundred seed weight was found to be significant in both gamma ray treatment and EMS treatment.

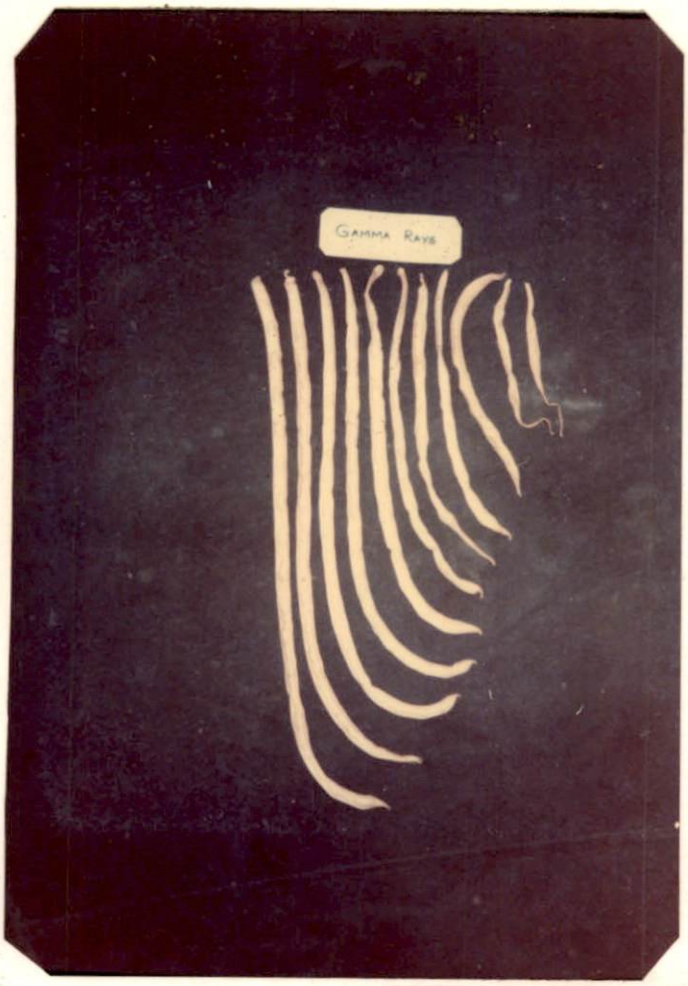
5. Seed fertility

The data on seed fertility are given in Table 15. The percentage of seed fertility was observed to decrease with increase in dose of both mutagens.

Plate 6. Variation in pod length induced by gamma rays

Plate 7. Variation in pod length induced by EMS

Plate 6.



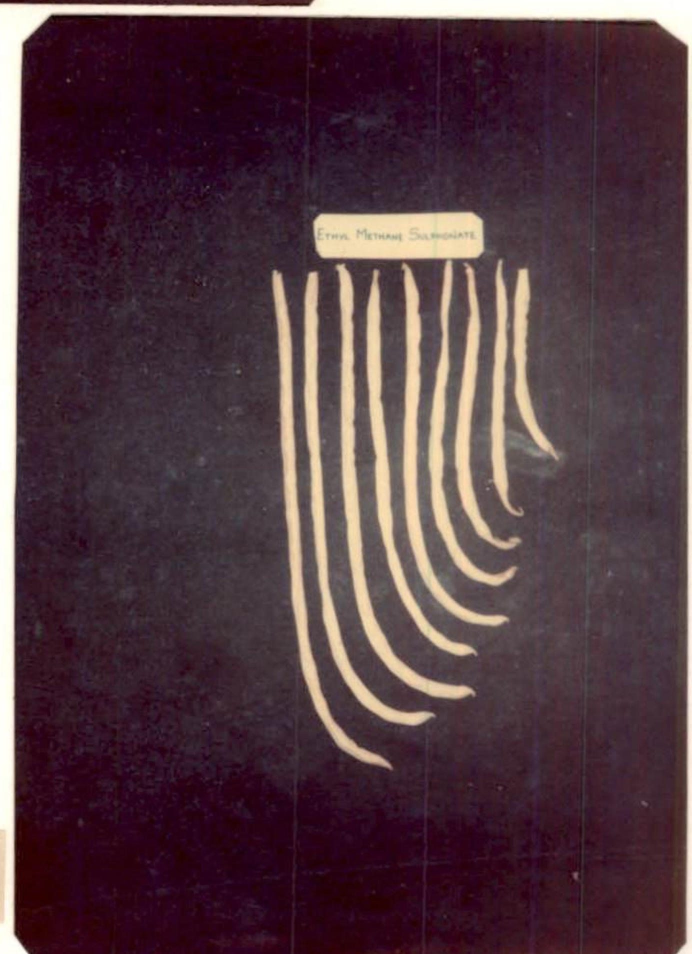


Plate 7.

Table 13. Number of days taken for flowering to commence and height at flowering

Treatment	Number of days for flowering to commence	Relative percentage	Height at flowering (cm)	Relative percentage
Gamma rays				
Control	49.50	100.00	217.00	100.00
10 krad	51.00	103.03	199.72	92.04
20 krad	51.25	103.54	209.08	96.35
30 krad	52.75	106.57	189.00	87.00
40 krad	54.00	109.09	172.00	79.26
EMS				
Control	49.00	100.00	218.10	100.00
0.5%	51.25	104.59	202.60	92.89
1.0%	54.50	111.22	164.38	75.37
1.5%	53.50	109.18	158.00	72.44
2.0%	55.00	112.24	100.82	46.23

Table 14. Number of pods per plant, Length of pods and 100 seed weight

Treatment	Number of pods per plant	Relative percentage	Length of pods (cm)	Relative percentage	Hundred seed weight(gm)	Relative percentage
Gamma rays						
Control	37.50	100.00	36.87	100.00	56.91	100.00
10 krad	35.00	93.33	35.50	96.28	43.50	76.44
20 krad	29.25	78.00	33.51	90.89	37.33	65.60
30 krad	21.50	57.33	30.96	83.97	21.34	37.48
40 krad	11.00	29.33	30.19	81.88	20.71	36.38
EMS						
Control	36.25	100.00	36.43	100.00	56.63	100.00
0.5%	31.00	85.52	34.70	95.25	55.25	97.56
1.0%	24.00	66.21	33.65	92.37	51.22	90.45
1.5%	21.75	60.00	30.31	83.20	49.74	87.83
2.0%	13.50	37.24	29.70	81.53	31.80	56.15

Table 15. Seed fertility

Treatment	Seed fertility (%)	Relative percentage	Seed sterility (%)
Gamma rays			
Control	91.24	100.00	0.00
10 krad	84.60	92.72	7.28
20 krad	72.70	79.68	20.32
30 krad	67.90	74.42	25.58
40 krad	62.73	68.75	31.25
EMS			
Control	91.70	100.00	0.00
0.5%	87.47	95.39	4.61
1.0%	81.24	88.59	11.41
1.5%	77.79	84.83	15.17
2.0%	72.61	79.18	20.82

Greater reduction was noted with gamma rays than with EMS. This reduction in fertility was found to be significant in both cases.

III. Mutagenic effectiveness and efficiency

The mutagenic effectiveness and efficiency of the different doses of gamma rays and EMS in inducing chlorophyll mutations were estimated and are presented in Table 16. A graphical representation of the effect of various doses of the mutagens on lethality, injury and sterility is given in Fig.7.

Mutagenic effectiveness was high at the lowest doses of both the mutagens, viz., 10 krad of gamma rays and 0.5 per cent EMS treatments. It was found to have an inverse relationship in all other doses of both the mutagens.

The mutagenic efficiency estimated on the basis of lethality, injury and sterility, did not show similar trends with increasing doses of gamma rays and EMS. Among the different doses of gamma rays employed, 20 krad was the most efficient when estimated on the basis of lethality, while on the basis of injury and sterility, 10 krad was the most efficient. With EMS treatments, 0.5 per cent was the most efficient treatment on the basis of lethality, injury and sterility.

Table 16. Mutagenic effectiveness and efficiency

Treatment	No. of mutants per 100 M_2 progeny rows	Lethality L	Injury I	Sterility S	Mutagenic effectiveness $\frac{M \times 100}{krad/ct}$	Mutagenic efficiency $\frac{M \times 100}{L}$	$\frac{M \times 100}{I}$	$\frac{M \times 100}{S}$
Gamma rays								
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10 krad	10.34	12.73	2.10	7.29	103.40	81.23	492.38	141.83
20 krad	19.23	17.58	6.43	21.75	96.15	109.39	299.07	88.41
30 krad	21.33	27.88	16.04	33.77	71.10	76.51	132.98	63.16
40 krad	20.29	57.58	34.70	47.41	50.73	35.24	58.47	42.80
EMS								
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5%	14.81	83.83	3.16	4.38	247.00	17.67	234.49	169.18
1.0%	12.50	89.82	19.88	7.88	208.33	13.92	62.88	158.63
1.5%	13.33	91.02	21.88	27.96	148.11	14.65	60.92	47.68
2.0%	10.00	93.41	43.68	25.44	83.33	10.71	22.89	39.31

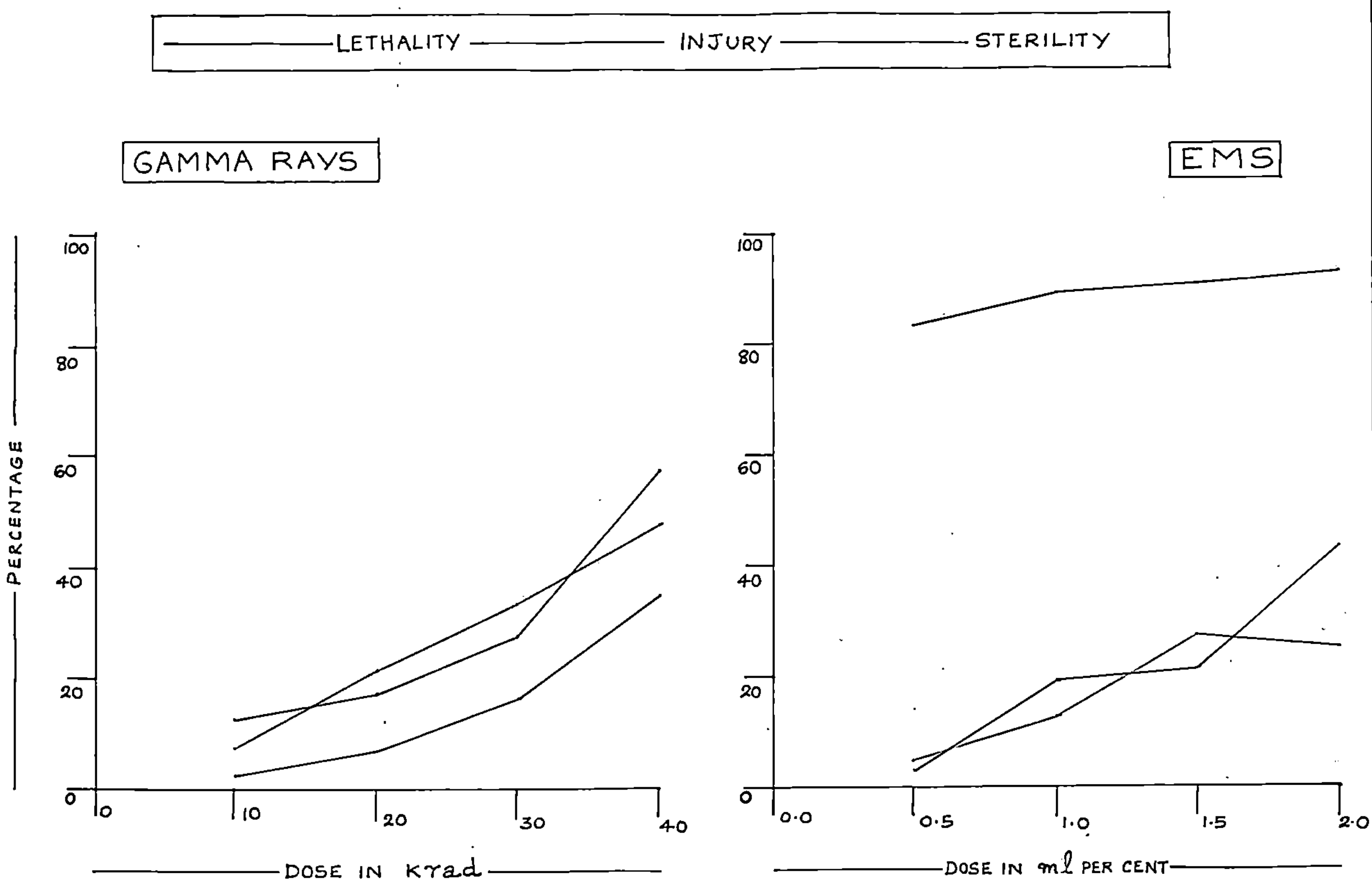


FIG: 7. EFFECT OF VARIOUS DOSES OF MUTAGENS ON LETHALITY, INJURY AND STERILITY

In general, the mutagenic efficiency was higher for gamma rays than for EMS, estimated with respect to lethality and injury, while on the basis of sterility, EMS proved to be more efficient than gamma rays.

DISCUSSION

DISCUSSION

Mutation breeding has opened a new era in plant breeding and is now widely used for crop improvement. Recent developments in this field suggest that the greatest mutagenic efficiency may be achieved by the selection of a mutagen with characteristics suited to the biological material, and by using an appropriate treatment (Konzak et al., 1965). The undesirable changes produced by mutagens result from chromosome aberrations and toxicity and are manifested as M_1 damages, such as injury, lethality and sterility. Efficient treatments, producing greater proportions of mutations to damages, are essential for bringing about crop improvement.

The present investigation was undertaken to study the effects of gamma rays (10 to 40 krad) and EMS (0.5 to 2.0 per cent) in the M_1 and M_2 generations of cowpea and to study the relative effectiveness and efficiency of the mutagens in inducing mutations. The results obtained are discussed in the following sections.

I. Effect of mutagens in the M_1 generation

1. Germination of seeds

Ojomo and Chheda (1971) defined seed germination as the ability of seeds to produce plumule and radicle under optimum growth conditions. In the present study, the percentage of total germination was found to decrease with increase in dosage of both gamma rays and EMS. Similar results have been reported by Athwal (1963) in Cicer; Wellensiek (1965), Louis and Kadambavanasundaram (1973a), Narsinghani and Kumar (1976) and Bhojwani and Kaul (1976) in pea; Bajaj and Saettler (1970) in Phaseolus; Alikhan et al. (1973) in Cajanus cajan; Venkateswarlu et al.(1978) in pigeon pea; Khanna and Maherchandani (1980) in gram and Nadarajan et al. (1985) in red gram. Contrary to this, Swarup and Gill reported a stimulatory effect on germination following irradiation with X-rays. Mujeeb (1974) also reported this effect in Cicer arietinum with gamma irradiation. However, no stimulatory effect was noticed on germination in any of the gamma ray treatments in this study.

With regard to EMS treatment, the germination percentage was found to decrease drastically with increasing doses. Konzak et al. (1965) reported that the alkyl alkane sulphonates and alkyl sulphates form strong acids, upon hydrolysis, occurring externally in the treatment solution, as well as inside the cells during the treatment. These acids which are produced in significant amounts cause toxicity. The drastic reduction in germination percentage observed in the present study might be due to the toxic properties of EMS.

Delay in germination was observed at all doses of EMS, while in the case of gamma rays, slight delay was observed only at higher doses. Mujeeb (1974) reported earlier germination in gram at low doses of gamma rays. According to Casarett (1968), the delay in germination might be due to the influence of mutagens on plant hormones and plant growth regulators, which affects initiation of germination.

2. Survival of plants

The survival percentage was found to decrease with increase in the dose of gamma rays. Similar results have been obtained following seed irradiation by Ojomo and Chheda (1971), and Louis and Kadambavanasundaram (1973) in cowpea; Constantin et al. (1976) in soybean; Krishnaswami et al. (1977) in green gram and Nadarajan et al. (1985) in red gram. The reduction in survival is an index of post-germination mortality in the treated plants as a result of cytological and physiological disturbances due to radiation effect. Structural changes in the chromosomes are brought about by cytological abnormalities caused by irradiation. This interferes with the normal growth and development of organs which lead to a decrease in survival percentages with increasing doses of radiation. Sato and Gaul (1967) reported that the reduction in survival could be attributed to chromosome caused reduction in fertility or physiological disturbances.

The percentage of survival was seen to decrease drastically with increase in concentration of EMS. Similar results were obtained by Wellensiek (1965) in pea; Narsinghani and Kumar (1975) in cowpea and by Constantin et al.(1976) in soybean. Contrary to this Zannone (1965) reported that the M_1 plant survival was unaffected except at 0.2 per cent EMS, 0.06 per cent EI and 28 krad X-rays in *Vicia sativa*. A comparison of the germination and survival data showed that the reduction in survival was due to reduction in germination resulting from the treatment. This indicates that post-germination mortality does not occur to any appreciable extent following EMS treatment. This finding is in agreement with the results obtained by Oommen (1980) in cowpea.

3. Shoot-root ratio

In the present investigation, the growth of shoot and primary root was found to be reduced by both mutagens. The growth of shoot was more affected than that of root in the gamma ray treatments, while a higher growth inhibition for the root was observed with EMS, eventhough both shoot and root growths were adversely affected. Similar results have been reported by Oommen (1980), in cowpea.

Inhibition of root length can be attributed to the amount of radiation scattered from the region of elongation to the meristem (Casarett, 1968). This shows that the root inhibition arises primarily from the effects on meristems, by arresting the synthesis of growth stimulating auxins and consequent inhibition of cell division.

4. Plant height

The plant height measured on the 40th day of sowing and at harvest showed reduction at all doses of gamma rays and EMS, at both the stages. These observations are in accordance with the findings of Wellensiek (1965) and Bhojwani and Kaul (1976) in pea; Swarup and Gill (1968) in French bean; Louis and Kadambavanasundaram (1973a) in cowpea; Sreerangaswamy et al.(1973) in green gram; Constantin et al.(1976)

in soybean; Appa Rao and Jana (1976) in black gram; Khanna and Maherchandani (1980) in gram and Nadarajan et al. (1985) in red gram. The height reduction on the 40th day was more than that at harvest, at all doses of gamma rays and EMS. This indicates an apparent recovery of M_1 plants from injury at later stages of growth. This finding is in agreement with the results obtained by Louis and Kadambavanasundaram (1973a) in cowpea. The recovery may be due to the elimination of damaged zones by inhibition of cell division, and the growth of uninjured meristematic cells which replaced the injured ones as growth proceeded.

Growth of plants is governed by the internal metabolism of the system and the external conditions which bear a direct or indirect influence on the former. The presence of an eletrostatic field or a toxic chemical has been reported to influence plant growth (Ehrenberg, 1960). The innate capacity of tissues for growth differs greatly. The biological responses to mutagen treatments may vary with the chemical composition of the tissue at the time of treatment because of the competition between biochemical reactions and mutation-yielding reactions.

The inhibition of growth at higher doses of both mutagens might be due to cytological, morphological and physiological changes in the cell. Gunckel and

Sparrow (1961) have reported changes in auxin levels and biological and physiological activities, inactivation of vital enzymes and disturbances in ascorbic acid content.

5. Pollen and seed fertility

Pollen fertility and seed fertility were observed to decrease with increasing dose of gamma rays and EMS. Decreased fertility with increasing doses of mutagens was reported by Zannone (1965) in *Vicia sativa*; Ojomo and Chheda (1971), Louis and Kadambavanasundaram (1973a), Narsinghani and Kumar (1976) and Appa Rao and Jana (1979) in cowpea; Bhojwani and Kaul (1976) in pea; Nerkar (1977a) in *Lathyrus sativus* and Nadarajan *et al*. (1985) in red gram.

Radiation induced sterility may be the result of chromosome aberrations while sterility induced by chemicals may be due to cryptic deletions and specific gene mutations (Nerkar, 1977a). It was also reported by Sato and Gaul (1967) that chemical induced sterility cannot be attributed to chromosomal aberrations, but to other reasons including genic alteration.

It was observed that gamma rays induced greater pollen and seed sterility than EMS. Inactivation of respiratory enzymes due to irradiation and a high degree of chromosomal disturbances may be the cause for increased sterility. Reduced sterility with EMS treatments might be due to the elimination of chromosome aberrations induced by the chemical to a large extent, during ontogeny and also due to cryptic deletions which may not involve essential genes.

6. Chlorophyll chimeras

In the present investigation, chlorophyll deficient patches were observed on the leaves in the 30 krad and 40 krad doses of gamma rays. Similar results have been obtained by Ojomo and Chheda (1971) and Appa Rao and Jana (1979) in cowpea.

Chlorophyll mutants were not observed in the M_1 generation in the case of EMS treatments. Narsinghani and Kumar (1976) reported the absence of chlorophyll mutants in the M_1 generation of chemical mutagen treated cowpea.

7. Number of pods per plant

In the present study, the number of pods per plant was found to decrease with an increase in the dose of both mutagens. Similar results have been reported by Louis and Kadambavanasundaram (1973a) and Narsinghani and Kumar (1976) in cowpea, and by Constantin et al. (1976) in soybean.

8. Morphological variations

The type of morphological abnormalities induced by mutagens depends upon the age and condition of the plant, duration of the exposure, and the environment during and after exposure. In the present investigation, plants with alterations in number, size and shape of leaflets were obtained in both gamma ray treated and EMS treated population. Leaf abnormalities following mutagen treatment were reported by Athwal (1963) in *Cicer*; Constantin and Love (1964) and Narsinghani and Kumar (1976) in cowpea; and Chopde (1970) in *Cajanus cajan*. Gunckel and Sparrow (1961) reported that these leaf anomalies may be attributed to chromosome breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism and accumulation of free amino acids.

II. Effect of mutagens in the M_2 generation

1. Chlorophyll mutations

(a) Frequency

Chlorophyll mutations have been widely employed for assessing the effectiveness of mutagenic treatments in higher plants (Gaul, 1964; Nilan et al., 1965 and Kawai, 1969). According to Gaul (1964) (1) they are the most frequent gene mutations (2) they can be clearly recognised and classified (3) they can be studied in a small space under semi-controlled green house conditions and (4) they provide rapid information, as only seedlings need be grown.

In the present investigation, the chlorophyll mutation frequencies were estimated as the number of mutations per 100 M_2 progeny rows and the number of mutations per 100 M_2 seedlings. The frequencies estimated as the number of mutations per 100 M_2 progeny rows gave higher values than the other estimate at each of the doses, for both the mutagens. This evidently is an over estimation of the mutation event. The frequencies estimated on M_2 progeny row basis increased with increasing doses of gamma rays except in the 40 krad treatment, while in the case of EMS, the maximum number of mutants was obtained at the lowest dose. The recovery of less number of mutants in the highest dose of gamma rays and in the doses above 0.5 per cent of EMS, might be due to the fact that many of the mutations at those higher doses might have been eliminated due to lethality. However, the frequencies estimated on M_2 seedling basis increased with increasing doses of both mutagens.

Generally, the mutation frequencies increase with the increase in doses of the mutagens. This type of dose-frequency relationship was reported by Nerkar (1976) in *Lathyrus sativus*; Krishnaswami *et al*.(1977) in green gram; Ghosh *et al*. (1979) in pea, and Oommen and Gopimony (1984) in cowpea.

When the frequencies estimated on M_2 seedling basis were compared, EMS treatments were found to yield more chlorophyll mutations compared to gamma rays. Swaminathan *et al*.(1962) reported that, in the evolution of gene placement along the chromosome arms, it is likely that, linkage groups of genes which do not need recombination are located near the centromere, and thus, would have a selective advantage. According to them, the location of genes relating to chlorophyll development in the proximal segment and the high susceptibility of such regions to EMS action may perhaps be factors involved in the induction of a large number of chlorophyll mutations in EMS treated material. Zannone (1965) also reported a higher frequency of chlorophyll mutants in *Vicia sativa* with EMS.

(b) Spectrum

In the present investigation, the spectrum of chlorophyll mutants obtained included albina, xantha, chlorina, viridis and those with irregular chlorophyll deficient patches or spots on the leaves. The occurrence of a wide spectrum of chlorophyll mutations following mutagen treatments was reported by Zannone (1965) in *Vicia sativa*; Swarup and Gill (1968), and Vardanyan (1976) in French bean; Dahiya (1973) in mung bean; Louis and Kadambavanasundaram (1973 b), and Narsinghani and Kumar (1976) in cowpea; Das and Kumar (1974) in pea; Nerkar (1976) in *Lathyrus sativus*, and Venkateswarlu *et al.* (1978) in pigeon pea.

The albina mutants were observed only in the medium doses of gamma rays and in the lowest dose of EMS treatment. The xantha, viridis and chlorina types occurred in all the treatments of gamma rays, while chlorina mutants were absent in the 2.0 per cent EMS treatment. In the gamma ray treatments, viridis was the most frequently occurring type, while in the EMS treatments, xantha and viridis types occurred frequently. Contrary to this, Subramanian (1980) reported in cowpea that albina mutants were the most frequent types found following gamma irradiation.

(c) Segregation ratio

Several workers have studied the segregation ratios of various types of chlorophyll mutants in the M_2 generation of different leguminous crops (Patil and Bora, 1961; Santos, 1969; Sur, 1970 and Vardanyan, 1976).

In this study, the segregation ratios were estimated as the percentages of the number of chlorophyll mutants to the total number of plants in the segregating M_2 progeny rows in each of the doses. The segregation ratios did not show any definite relationship with the doses. The maximum segregation ratios were observed in 30 krad of gamma rays and in 0.5 per cent EMS treatment. The segregation ratios were higher with EMS treatment than with gamma irradiation. The high segregation ratio relates to high frequency of mutants in the M_2 generation and is of great value in breeding programmes.

2. Viable mutations

The viable mutation frequency was estimated on the basis of the number of mutations per 100 M_2 progeny rows. The frequencies did not show any definite relationship with the doses in both the mutagens.

Gamma irradiation resulted in a viable mutation spectrum for leaf size, leaf shape, length of pods, time of flowering and seed coat colour, while EMS induced changes in leaf size and shape, length of pods, time of flowering and size of seeds. Recovery of various viable mutants has been reported by Athwal (1963), Athwal *et al.*(1970) in *Cicer*; Constantin and Love (1964), Sharma (1969), Louis and Kadambavanasundaram (1973 b), Narsinghani and Kumar (1976), and Onim (1983) in cowpea; Jones (1965) in Southern peas; Swarup and Gill (1968) in French bean; Santos (1969) in mung bean; Alikhan and Veeraswamy (1974) in red gram, and Sharma and Sharma (1979) in lentil.

Viable mutants with changed leaf size and shape, seed coat colour, size of seeds and length of pods, and those which were early as well as late in flowering, were noticed in the present study. Leaf mutations were reported by Gunckel and Sparrow (1961), Santos (1969) and Narsinghani and Kumar (1976). Changes in colour of seeds and size of seeds and length of pods were reported by Swarup and Gill (1968) in French bean; Bhatt *et al.* (1972) and Prasad (1976) in green gram.

The percentage of viable mutations was greater for gamma ray treatments than that for EMS treatments. This observation is in agreement with the results obtained by Alikhan and Veeraswamy (1974) in red gram.

Some of the viable mutants observed in the present study had several characters simultaneously changed. Thakare *et al.*(1973) reported that the mutants which exhibit a wider spectrum of phenotypic changes could either be the result of pleiotropic gene action or of cryptic chromosome deletions. According to Patil (1966), the loss of a segment may also result in such effects.

3. Flowering and plant height

In the present study, a delay was noted for initiation of flowering with the increase in dose of both mutagens. The plant height decreased linearly with increase in doses of both mutagens. Similar results have been reported by Louis and Kadambavanasundaram (1973 a) in cowpea and by Khan (1984) in mung bean.

4. Number of pods per plant, pod length and hundred seed weight

All the three characters recorded a decrease with increase in doses of both mutagens. This might be due to the delay in the initiation of flowering, growth inhibition and reduced fertility. This decrease was found to be significant. Similar reports have been made by Louis and Kadambavanasundaram (1973a) and Narsinghani and Kumar (1976) in cowpea, and Swarup and Gill (1968) in French bean. Virupakshappa *et al.* (1980) reported that the mean values for the number of pods per plant and seed weight in mutagen treated populations were lesserthan that in untreated populations. The negative shift in the means of the treated population from the mean of the control agrees with this finding.

5. Seed fertility

The seed fertility was found to decrease with increasing doses of both mutagens and a greater reduction was noted with gamma rays. Similar reports have been made by Narsinghani and Kumar (1976) in cowpea. The variation in fertility was found to be significant among the different treatments.

III. Mutagenic effectiveness and efficiency

Konzak *et al.* (1965) proposed the terms "effectiveness" as a measure of mutations in relation to dose, and "efficiency" as an estimate of mutation rate in relation to biological effects induced, such as lethality, injury and sterility. The greatest efficiency in mutagen experiments depends not only on the selection of a mutagen with characteristics suited to the biological material but also on appropriate treatment regime. This efficiency is essential for successful utilization of mutagens in crop improvement.

Kawai (1969) stated that the chlorophyll mutations are taken as a basis for estimating the effectiveness and efficiency on the assumption that the other types of mutations are induced with frequencies parallel to that of chlorophyll mutations. Nilan et al. (1965) reported that increased efficiency might be expressed in two ways, viz.,

1) as higher ratios of mutations to chromosome aberrations or to surviving plants where beneficial mutations are sought; or
2) as higher ratios of chromosome aberrations to surviving plants where these aberrations are to be used in various ways in plant breeding.

In the present study, effectiveness was maximum at the lowest doses of both the mutagens. Similar results have been obtained by Konzak et al. (1965) in barley.

The mutagenic efficiency determined on the basis of lethality, sterility and injury did not show a similar dose-effect relationship. Among the different doses of gamma rays used, 20 krad was the most efficient when estimated on the basis of lethality. On the basis of injury and sterility, 10 krad was found to be the most efficient. With EMS treatments, 0.5 per cent was found to be the most efficient on the basis of all the three biological parameters used. Konzak et al. (1965)

reported a higher mutagenic efficiency in barley, atlow doses. The reason for this seemed to relate to the fact that lethality, injury and sterility increased with dose, at a rate faster than the occurrence of mutations.

In the present investigation, on the basis of lethality and injury, gamma rays was found to be more efficient than EMS, while on the basis of sterility, EMS was more efficient than gamma rays.

SUMMARY

SUMMARY

Studies were undertaken on the effect of four doses of gamma rays (10 to 40 krad) and four doses of EMS (0.5 to 2.0 per cent) on cowpea cultivar Kuruthola-payar, in the M_1 and M_2 generations. The effectiveness and efficiency of different doses of the two mutagens in inducing chlorophyll mutations and also the frequencies and spectrum of viable mutations were estimated.

The germination percentage was affected by gamma rays as well as by EMS, although the effect was more severe in the latter. The reduction in percentage was progressive with increasing doses.

The survival of plants based on the number of seeds sown was reduced by both mutagens. The higher concentration of EMS showed a drastic reduction in survival.

The growth of shoot and root was reduced by both the mutagens. The shoot-root ratios indicated that the growth of the shoot was more affected than that of the root with gamma rays, while EMS produced a higher growth inhibition for the root than for the shoot.

The plant height was reduced by both mutagens and the reduction in plant height increased linearly with increase in doses of both the mutagens. Severe reduction in plant height was noticed with higher doses of EMS.

An inverse relationship was observed between pollen fertility and dose of both mutagens. The patterns of fertility reduction with increasing doses were found to be different for the two mutagens. Drastic reduction in pollen fertility was noted in gamma rays than in EMS treatments. Higher doses of gamma rays induced greater sterility than that induced by higher doses of EMS.

The seed fertility percentage was found to decrease with increase in doses of both the mutagens and the pattern of decrease was more or less the same for the mutagens, over the doses tested.

Chlorophyll chimeras were observed only in the 30 krad and 40 krad gamma ray treatments, in the M_1 generation.

Leaf variations such as alteration in number, size and shape of leaflets in the first formed secondary leaves were noticed in the M_1 generation with both the mutagens. However, these plants recovered and produced normal leaves afterwards.

In the M_2 generation, the chlorophyll mutation frequencies estimated on M_2 progeny row basis showed an increase with increasing doses of gamma rays except in the 40 krad treatment, which showed a slight decrease. In the case of EMS, maximum mutants were obtained at the lowest dose. The chlorophyll mutation frequency estimated on M_2 seedling basis showed dose-dependence.

The chlorophyll mutant types obtained were albina, xantha, chlorina, viridis and those with irregular patches and spots on the leaves. The frequencies of the different types of chlorophyll mutants did not show any dose-relationship.

The segregation ratios for chlorophyll mutations did not have any dose-dependence with gamma irradiation and EMS treatments. EMS treatments showed higher segregation ratios than gamma irradiation.

The viable mutation frequencies did not show any definite relationship with the doses, in both the mutagen treatments.

The different types of viable mutants produced by gamma irradiation were with respect to leaf size and shape, pod length, time of flowering and seed coat colour, while EMS induced changes in leaf size and shape, pod length, time of flowering and seed size.

The number of pods per plant, pod length and hundred seed weight were found to decrease with an increase in dose of both the mutagens.

The mutagenic effectiveness in inducing chlorophyll mutations was high at the lowest dose of both mutagens, viz., 10 krad of gamma rays and 0.5 per cent EMS treatment. The effectiveness was found to have an inverse relationship in all other doses of both the mutagens.

Among the gamma ray doses employed, 20 krad was the most efficient when estimated on the basis of lethality. But on the basis of injury and sterility, 10 krad was the most efficient. With EMS, the most efficient treatment was 0.5 per cent, irrespective of whether the criterion adopted for estimation was lethality, injury or sterility.

In conclusion, it can be stated that mutagenic efficiency was higher for gamma rays with respect to lethality and injury, while on the basis of sterility, EMS was found to be more efficient than gamma rays.

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* Original not seen

INDUCED MUTATIONS IN COWPEA
(*Vigna unguiculata* cultivar Kuruthola-payar)

BY

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ABSTRACT OF A THESIS
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ABSTRACT

Seeds of cowpea (*Vigna unguiculata*) cultivar Kuruthola-payar were subjected to induced mutagenesis using four doses of gamma rays (10 to 40 krad) and four doses of EMS (0.5 to 2.0 per cent) and their effects in the M_1 and M_2 generations were studied.

The germination percentage was observed to decrease progressively with the increase in dose of both mutagens although the reduction was more drastic with EMS.

Reduction in survival percentage was observed with increase in the dose of gamma rays and EMS.

The growth of shoot and root and plant height were reduced by both the mutagens, although gamma rays showed greater shoot inhibition and EMS showed greater root inhibition.

The pollen fertility as well as seed fertility decreased linearly with increase in doses of both the mutagens.

Chlorophyll chimeras were observed only in the 30 krad and 40 krad gamma ray treatments. Morphological variations noticed included plants with alterations in the number, size and shape of leaflets.

The chlorophyll mutation frequency estimated on M_2 progeny row basis showed an increase with increasing doses of gamma rays, except in the 40 krad treatment, which showed a slight decrease. In the case of EMS, maximum mutants were observed at the lowest dose. The chlorophyll mutation frequency estimated on M_2 seedling basis showed dose-dependence.

The frequencies of the different types of chlorophyll mutants did not show any dose-relationship. The segregation ratio of chlorophyll mutants was higher for EMS than gamma irradiation.

The viable mutation frequencies did not show any definite relationship with the doses in both the mutagen treatments.

The mutagenic effectiveness in inducing chlorophyll mutations was high at the lowest dose of both mutagens. On the basis of lethality, 20 krad of gamma rays was the most efficient, while on the basis of injury and sterility, 10 krad was the most efficient. With EMS, the 0.50 per cent treatment was the most effective as well as the most efficient treatment on the basis of lethality, injury and sterility.

The mutagenic efficiency in inducing chlorophyll mutations was higher for gamma rays with respect to lethality and injury, while on the basis of sterility, EMS proved to be more efficient than gamma