INDUCTION OF MUTATIONS IN COWPEA
(*Vigna unguiculata* (L.) Walp.)

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Induction of mutations in cowpea (*Vigna unguiculata* (L.) Walp.)" 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 title of any other University or Society.

Vellayani,
6th August, 1980.

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CERTIFICATE

Certified that this thesis, entitled "Induction of mutations in cowpea (*Vigna unguiculata* (L.) Walp.)" is a record of research work done independently by Shri. SUNNY K. COMMEN under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

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The significance of artificially induced mutations in cultivated plants has long been a point of controversy among plant breeders. Today, mutation breeding is one of the accepted methods available to the breeder to improve his crop. This is amply demonstrated by the relatively large and steadily increasing number of mutant varieties which have been commercially released from different countries all over the world.

For the induction of mutations in plant materials, two groups of mutagenic agents, namely, physical and chemical mutagens are available to the breeder. The former has been used for many decades, whereas the use of chemicals is relatively recent.

Studies on induced mutagenesis in plants are being conducted since the beginning of the present century to have a clear understanding of the mode of action of various mutagenic agents in biological systems and the reaction of plant species subjected to such treatments. With physical and chemical mutagens, three types of effects of special interest in genetics and breeding which are easy to measure are produced. They are (1) gene mutations (2) chromosome aberrations and (3) physiological disturbances. The chromosome aberrations and physiological disturbances produce undesirable damaging effects leading to reduced

germination, decreased survival, seedling injury and reduced fertility in the M_1 generation. Application of different mutagens as well as different doses of each has been reported to induce varying degrees of gene mutations and damaging effects. For the economic use of mutagens in plant breeding, high mutation rates as well as high proportions of mutations to damage are essential. An understanding of the effects of mutagenic treatments in a biological system will help in identifying the most efficient dose-range of mutagens.

Induced mutagenesis among crop plants has been extensively studied in cereals, especially wheat and barley. These studies have enabled the choice of efficient doses of effective mutagens for the purpose of inducing a very high frequency of useful and recoverable mutations. But the theoretical studies concerning mutation induction in legumes are meagre.

Cowpea is widely grown in India as a highly esteemed grain legume and as a fodder crop. It is a diploid, naturally self-pollinated leguminous species offering only limited scope for improvement through conventional plant breeding methods due to the low genetic variability existing in natural populations. Hence mutation breeding is suggested as an attractive proposition to improve this crop.

The present investigation was undertaken to study the effects of gamma rays and ethyl methanesulphonate in the

M_1 and M_2 generations of cowpea and to estimate the effectiveness and efficiency of different doses of the two mutagens. The results obtained are presented and discussed in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The idea of producing mutations artificially and using them for breeding was clearly stated as early as 1901 by De Vries. In the first twenty five years of this century, numerous investigators tried to induce mutations in many different organisms by physical and chemical agents of varied nature. Though these efforts were successful in some cases, the methods used were not sufficient to provide clear and convincing results (Saul, 1964). Muller in 1927 demonstrated the induction of sex-linked recessive lethal mutations in Drosophila melanogaster by X-irradiation and formulated strict methods of analysis. This was closely followed by the successful experiments of Stadler (1928a,b) in inducing mutations in barley and maize. Since then all kinds of radiations have been examined.

Chemical mutagenesis was tried by Schiemann in 1912 with some encouraging results. Auerbach discovered the mutagenisity of mustard gas (Auerbach and Robson, 1947). The studies of Kaypport in 1948 with mutagenic compounds of epoxide and epimine types were of a pioneering nature. A considerable number of chemical mutagens were soon discovered and the conceptual bases of point mutagenesis were rapidly formulated by Freese (1959), Brenner et al. (1961) and Crick et al. (1961). Recently the number of chemicals shown to possess mutagenic properties has greatly increased.

Fishbein et al. (1970) listed 114 chemical mutagens with literature citations regarding point mutations in transforming DNA, phages, other microorganisms or insects and chromosome aberrations in plants, insects, mammals or mammalian cells. Freese (1963) classified chemical mutagens as inhibitors, base-analogue substitutes, dyes, acids, metals and alkylating agents. In higher plants the latter group, especially ethyl methanesulphonate has proved to be very effective. The relatively low toxic and high genetic effects of ethyl methanesulphonate (Gaul, 1961) and its high mutagenic effectiveness as well as efficiency in higher plants (Konzak et al., 1965) demand attention for enhanced practical application.

The investigation of induced mutations for improving plants started immediately after the demonstration of the mutating effect of X-rays. The first true cases of induced positive mutations in plants were obtained in the thirties by Nilsson-Ehle and Gustafsson when they isolated erectoid mutants in barley (Gustafsson, 1969). As a result of the progress in understanding the role of induced mutations in breeding and of the mutation process, a number of important mutant crop varieties have been commercially released.

The progress in mutation research achieved in the last half century is great. Detailed reviews on the various aspects relating to induction of mutations have been presented by various investigators (Gaul, 1961, 1964; Konzak et al., 1965;

Gregory, 1966; Gustafsson, 1969; Milan et al., 1969; Sigurbjornsson and Micke, 1969; Brock, 1971; Davies, 1971). Hence, no attempt is made here to present an elaborate review of mutation research in crops other then legumes.

I. Effects of mutagens in the M_1 generation.

The mutagen treatment produces genetic effects and undesirable physiological effects in biological systems. The physiological effects result in a general damage measured as reduced germination, decreased survival, seedling injury and reduced fertility. These parameters are taken as measures of mutagen sensitivity.

1. Germination of seeds

Mutagenic treatment affects the germinability of seed. Bilquez and Martin (1961) reported a reduction in seed germination in groundnut following X-irradiation at 40 krad. Van Huystee and Cherry (1967) also got similar results, but at still higher dosages. Louis and Kadambavanasundaram (1973a) following gamma-irradiation of cowpea seeds, reported a reduction in germination percentage and an increase in time for germination. Ojomo and Chheda (1971) found that the germination of cowpea seeds was not affected by treatment with X-rays upto 30 krad.

Stimulatory effect of ionising radiations at relatively low exposures on seed germination has been reported by several investigators. Swarup and Gill (1963), in french beans, noted

better germination in 7 and 14 krad treatments. Mujeeb (1975) reported earlier germination in Cicer arietinum gamma irradiated upto 10 krad.

Seed treatment with chemical mutagens significantly reduces the germination percentage. Narsinghani and Kumar (1970) observed a reduction in seed germination, in cowpea, following ethyl methanesulphonate treatment. Similar results were obtained in pea with ethyl methanesulphonate (Wellensiek, 1965; Selim et al., 1974), N-nitroso N-ethylurea (Migacheva, 1972) and ethylene imine (Bhojwani and Kaul, 1976).

2. Survival of plants

A significant reduction in plant survival with high doses of X-rays and neutrons was reported by Ojomo and Chheda (1971) in cowpea. Similar result has been obtained by Louis and Kodambavanasundaram (1973a) with gamma rays and found the LD_{50} to be around 40 krad. Jaranowski (1970) obtained a sharp fall in survival rate with gamma ray doseages above 25 krad in Pisum arvense and Vicia sativa. Mujeeb and Greig (1972), in Phaseolus vulgaris, following gamma irradiation observed a progressive reduction in survival with the increase in dose. However, there are reports of high survival rate even after relatively large exposures of ionising radiation. Such results have been obtained by Silquez and Martin (1961) in groundnut with X-rays and Constantin and Love (1964) in cowpea with neutrons.

Wellensiek (1965) following ethyl methanesulphonate treatment of pea seeds found that the percentage of healthy seedlings and full grown plants decreased rapidly with the increase in concentration. Santos (1969) observed a reduction in survival of mungbean with ethyl methanesulphonate treatment. Migacheva (1972) found that the survival of pea plants decreased with increase in concentration of ethylene imine and N-nitroso-N-ethylurea. Narsinghani and Kumar (1970) found a reduction in survival percentage in cowpea with ethyl methane-sulphonate and methyl methanesulphonate treatments. Constantin et al. (1976) reported reduction in survival of field grown populations of soybean treated with fission neutrons, gamma rays, ethyl methanesulphonate and diethyl sulphate.

3. Plant height

Wellensiek (1965) found that the average height of pea plants decreased with increasing doses of X-rays, gamma rays, neutrons and ethyl methanesulphonate. Reduction in plant height following gamma irradiation was reported in pea (Monti and Donini, 1968; Selim et al., 1974), french bean (Bajaj et al., 1970), and soybean (Constantin et al., 1976). Though the height reduction was conspicuous at seedling stage, Louis and Kadambavanasundaram (1973a) found the plant height at maturity to be uniform over different treatments after gamma irradiation of cowpea seeds at different doses. In french bean, Swarup and Gill (1968) found the growth and vigour of M_1 plants to be normal at 7 and 14 krad X-ray treatments.

Jones (1965) found some stimulatory effect on seedling height in cowpea at low doses of X-rays and thermal neutrons, but increased doses of both mutagens resulted in marked reduction in seedling vigour. Similar result was obtained by Mujeeb (1975) in Cicer arietinum with gamma rays.

Narsinghani and Kumar (1970) following ethyl methanesulphonate treatment reported reduction in plant height in cowpea. So was the result obtained by Santos (1969) in mungbean. In pea, Dhojwani and Kaul (1976) reported that the plant height was significantly reduced following ethylene imine treatment. Magri-Allegra and Zannone (1965) found, in comparison with ethyl methanesulphonate, ethylene imine produced much less growth reduction in vetch. An inverse relationship between soybean seedling height and doses of ethyl methanesulphonate and diethyl sulphate was reported by Constantin et al. (1976).

4. Fertility

The mutagenic treatments generally result in reduced fertility. The sterility, mostly caused by chromosome aberrations, can be quantitatively determined by counting sterile pollen or missing seed-setting.

Vasileva and Mekhanshiev (1972) reported an increase in chromosome aberrations with the increase in gamma irradiation doses in different varieties of pea. Structural chromosome rearrangements were observed in mitosis and

meiosis after treatment of pea seeds with gamma rays and ethyl methanesulphonate by Rekhmatulla and Gostimskii (1976). They also found that the frequency of chromosome aberrations to be considerably greater after treatment with gamma rays. Khvostova et al. (1973) suggested that chemical mutants involve either point mutations or small intra-chromosomal rearrangements since the examination of pea M_1 microsporocytes after treatments with chemical mutagens failed to show the presence of translocation rings and inversion bridges which were normally present in irradiated material. The elimination of chemical mutagen induced aberrations during ontogeny in pea was reported by Akhun-Zade (1977). In cowpea, Ojomo and Ohhoda (1971) found that X-rays produce fairly high levels of chromosome aberrations which ranged from 18 per cent in 10 krad treatment to 41 per cent in 30 krad treatment.

Zamnone (1965) found that average fertility was highly reduced in Vicia sativa by ethyl methanesulphonate and ethylene imine treatments. Wellensiek (1965) got similar results in pea with X-rays, gamma rays, neutrons and ethyl methanesulphonate. Reduction in plant fertility in cowpea following physical and chemical mutagen treatments has been observed by several investigators (Sharma, 1969; Narasinghani and Kumar, 1969; Louis and Kadambavanasundaram, 1973a). Selim (1974) reported of the occurrence of relatively high degree of sterility in pea by ethyl methanesulphonate than gamma rays. Nerker (1977a) observed that the pollen and seed sterility increased with

increasing doses of gamma rays, ethyl methanesulphonate and nitrosomethylurea in Lathyrus sativus and were highest with nitrosomethylurea treatments.

5. Chlorophyll chimeras

The incidence of chlorophyll deficient spots on the leaves of M_1 generation plants in legumes after mutagenic treatment of seeds has been observed by various workers (Speckman, 1964; Blixt and Gelin, 1965; Ojomo and Chheda, 1971). Blixt and Gelin (1965) found a close correlation between leaf spotting and mutation rate and advocated to use it as a criterion for selecting in M_1 generation for plants giving higher yield of mutations in the M_2 generation. They also found that the frequency of sectors of different colours from ethyl methanesulphonate treated peas showed good agreement with ethyl methanesulphonate induced chlorophyll mutation spectrum.

6. Morphological abnormalities

Ashri and Goldin (1965) reported the occurrence of nonheritable morphological changes in M_1 generation following diethyl sulphate treatment in groundnut. There are also reports of changes in growth habit, stem thickness and other morphological characters in groundnut following seed irradiations (Sinha and Roy, 1970; Arzumanova, 1970). In Cajanus cajan, Chopde (1970) observed a number of morphological variants in M_1 generation following X-irradiation.

Leaf abnormalities following mutagen treatment are commonly observed in legumes (Speckman, 1964; Ojomo and Chheda, 1971). Constantin and Love (1964) following gamma and neutron seed irradiations in cowpea found 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. They also found that in many cases the early secondary leaves developed with one or two lateral leaflets absent. Gunckel and Sparrow (1961) cited reports in which leaf anomalies were attributed to chromosome breakages and induced physiological changes.

II. Mutations in the M_2 generation

1. Chlorophyll mutations

(a) Frequency

Semmons (1965) reported of a higher frequency of chlorophyll mutants in vetch with ethyl methanesulphonate than with ethylene imine and X-rays. Wellensiek (1965) found that in pea ethyl methanesulphonate yielded approximately seven times as many chlorophyll mutants as X-rays, gamma rays and neutrons which equalled each other. Pipic (1967) observed that the incidence of chlorophyll mutations in pea showed a positive linear correlation with the dose of diethyl sulphate. Cheicalin (1977) in his studies on chlorophyll mutations induced by chemical mutagens and gamma rays, in Lathyrus sativus, found 0.012 per cent N-nitroso-N-ethylurea to be the most effective treatment. He also found a high correlation

between the frequency of chlorophyll mutations and the frequency of economically useful mutations. Vardanyan (1976) could not get any correlation between the frequency of chlorophyll mutations and morpholigical mutations in french bean after treatment with ethylene imine and dimethyl sulphate. Allikhan and Veerasamy (1974), following gamma irradiation and ethyl methanesulphonate treatment in redgram, found that chlorophyll mutations were maximum at 24 krad and 70 mM treatments respectively. Vasileva and Mekhanzhiev (1972) in their experiment with eleven varieties of pea found varietal differences in response to the dose inducing highest frequency of chlorophyll mutations. They also found that the irradiation intensity had no noticeable effect on induction of chlorophyll mutations.

(b) Spectrum

Zannone (1965) found the spectrum of chlorophyll mutations in Viola sativa to be wider with ethyl methane-sulphonate than with X-rays and ethylene imine. The chlorophyll mutations not represented in X-ray and ethylene imine treatments were those which were also less frequent in ethyl methanesulphonate treatment. Swarup and Gill (1968) observed several chlorophyll deficient mutations such as yellow, chlorina and those with yellow leaf tips or yellow margin in french bean following X-irradiation. Louis and Kadambavanasundaram (1973b) reported of the occurrence of albino, xantha and viridis mutants in cowpea following gamma irradiation. In Phaseolus mungo,

Appa Rao and Jana (1975) obtained viable chlorophyll mutants such as viridis, chlorotica, chlorina-terminalis, chlorina-virescens, albo-virescens and aureo-virescens after treatment with X-rays, ethyl methanesulphonate or both. Vardanyan (1976) following chemical mutagen treatments in french beans obtained viridis, xantha, xantha-viridis and striata types of which last one was viable and fertile. Chekalin (1977) obtained a wide spectrum of chlorophyll mutations in Lathyrus sativus, the most frequent was chloroviridis, after gamma irradiation and treatments with different chemical mutagens.

(c) Segregation ratio

Patil and Bora (1963) described the origin of 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 one 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) following gamma and neutron irradiations in black gram 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) following chemical mutagen treatment in french bean obtained viable striata mutants which segregated in the 3:1 ratio.

2. Viable mutations

In Cicer, Athwal (1963) obtained several mutants such as flat-stemmed, simple leaved, narrow leaved, small leaved, bushy and sterilles following X-irradiation. Santos (1969) following mutagen treatment in mungbean got unifoliate and multifoliate leaf mutants. In groundnuts, Ashri and Goldin (1965) obtained mutants having deviating growth habit and pod shape after diethyl sulphate treatment. Swarup and Gill (1968) reported of X-ray induced seed coat colour mutants and sterile mutants in french bean. Seed coat colour mutants induced by ethyl methanesulphonate were reported in french bean by Moh (1971).

Gottschalk (1969) described interesting flower structures and leaf structures produced by mutant genes in different species of leguminosae family. Some mutants developed leaf structures not known elsewhere in the family. Appa Rao and Jana (1976), following X-ray and ethyl methanesulphonate treatments, reported of different types of leaf mutations in black gram.

Popova (1972, 1977) reported of the production of various economically useful characters in pea following treatments with different chemical mutagens. The useful ones obtained included determinate forms, forms with altered growth habit, compact forms, lodging resistant types, early ripening types and mutants with open flowers, narrow stipules and larger number of pods per peduncle. Following ethyl methanesulphonate treatment in Phaseolus radiata, Prasad (1976) isolated ten

mutants with higher number of pods per plant. Chaturvedi and Sharma (1976a,b) following ethyl methanesulphonate treatment in redgram observed different types of floral and other morphological mutations.

In cowpea, several investigators have reported of various types of mutations. Sharma (1969) got a late, giant, trailing mutant with larger leaves, peduncles and fruits, thick stem and changed seed coat colour. Pohle (1972) obtained an X-ray induced white flower mutant. Louis and Kolambavenacunderam (1975b) observed a number of interesting morphological mutants in the M_2 generation of cowpea following gamma irradiation.

Pipic (1967) found that ethyl methanesulphonate induced greater frequency of mutation and more varied morphological and physiological mutations than diethyl sulphate in pea. Following gamma irradiation in pea, Jarenowski (1970) found that 2.3 per cent of the M_2 population was comprised of detectable mutants. Allchan and Veeraswamy (1974) reported that in redgram the percentage of viable mutations was greater for gamma ray treatments than ethyl methanesulphonate treatments. Nerker (1977b) found, in Lathyrus sativus, that the frequency of mutation induced per unit dose to be higher with nitrosomethylurea than ethyl methanesulphonate and gamma rays.

III. Mutagenic effectiveness and efficiency

The mutation frequency estimated as the number of

mutations per 100 M_1 plants is dependent on the surviving and seed bearing M_1 plants. So Walther (1969) proposed that the factor of effectiveness should be calculated as the number of mutations per 100 treated seeds. To express the mutagenic effectiveness Konzak et al. (1965) advocated the use of the ratio of mutation frequency to dose. According to them the usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness, but also on its mutagenic efficiency. Efficient mutagenesis is the production of desirable changes free from association with undesirable changes such as lethality, injury and sterility. They defined efficiency as mutations/damage. Gaul et al. (1972) defined efficiency as the ratio of chlorophyll mutations to biological damage where the criteria for measuring damage are lethality, injury, sterility or chromosome mutations. The studies of mutagenic effectiveness and efficienty on the basis of chlorophyll mutations are made on the assumption that the other types of mutations are induced with frequencies parallel to that of chlorophyll mutations (Kawai, 1969).

Ashri and Goldin (1965) found diethyl sulphate to be an efficient mutagen in groundnut. According to Sharma (1969), dimethyl sulphate and ethyl methanesulphonate showed almost equal effectiveness in cowpea. He also found that nitroso-methylurea was twice as effective as the above mentioned mutagens. Ushiki (1972) reported that ethyl methanesulphonate

was more efficient than gamma rays in Lens esculenta. Akinn-zede (1977) found that alkylating compounds were more effective than gamma rays in inducing chlorophyll mutations in pea.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was undertaken in the Department of Agricultural Botany, College of Agriculture, Vellayani, during the period 1978-80.

A. Materials.

The biological material used in the present study was the variety 'New Era' of cowpea, Vigna unguiculata (L.) Walp. It is a grain cum fodder variety of 75-80 days duration with bushy growth habit (Plate 1).

The gamma irradiation was done at the School of Genetics, Tamil Nadu Agricultural University, Coimbatore, utilising Cobalt 60 gamma chamber installed by BARC, Bombay. The source was operating at an intensity of 0.3×10^6 rads per hour.

The chemical mutagen used was methane sulphonic acid ethyl ester (ethyl methanesulphonate) of Sigma Chemical Company, U.S.A. The chemical has a density of 1.16 gram per c.c. at 20°C*.

B. Methods

I. Mutagen treatments

1. Gamma rays

Seeds of uniform size were selected out. The moisture content of the seeds was approximately 12 per cent. Six

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*Personal communication from Brown, E.M., Sigma Chemical Company, U.S.A.

samples of 400 seeds each were exposed to gamma radiation at doses 5, 10, 15, 20, 25 and 30 krad.

2. Ethyl methanesulphonate

For ethyl methanesulphonate treatments, eight samples of 400 seeds each were selected out. The seeds were soaked for two hours in double distilled water. The treatment was done by immersing the seeds for six hours at 27±1°C in solutions of ethyl methanesulphonate at concentrations 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml per cent prepared in double distilled water. The volume of mutagen solutions used was 100 ml per 100 seeds (approximately ten times the volume of dry seeds). During the treatment intermittant shaking was given. The seeds after treatment were thoroughly washed in tap water.

II. Study of the M_1 generation

The gamma irradiated seeds were sown in the field on the eighth day after treatment along with the unirradiated control. The field experiment was laid out in Randomised Block Design with seven treatments and three replications. 100 seeds were sown in each plot at a spacing of 30 cm x 15 cm. The plot size was 3.0 m x 1.5 m.

Another field experiment was laid out in Randomised Block Design with eight treatments and three replications for sowing the seeds treated with ethyl methanesulphonate. The number of seeds per plot, spacing and plot size were the

same as in the case of gamma irradiated seeds. The seeds were sown in the plots immediately after the post-treatment washing.

From both mutagen treatment series, a sample of 30 seeds per dose was sown in metal trays filled with a 1:1 mixture of soil and sand at a spacing of 5 cm x 5 cm and was replicated thrice.

The following observations were made for both gamma ray and ethyl methanesulphonate treatments.

1. Germination of seeds
2. Shoot length and root length of seedlings
3. Survival of plants
4. Plant height
5. Pollen fertility
6. Chlorophyll chimeras
7. Morphological abnormalities

The first two observations namely, germination of seeds and shoot length and root length of seedlings were taken from tray experiments, while the remaining observations were taken from the experiments laid out in the field.

1. Germination of seeds

The emergence of plumule from the soil was taken as the criterion for germination. Germination counts were taken at six hour intervals. The percentage of germination and the average time taken for germination were estimated.

2. Shoot length and root length of seedlings

The seedlings raised in the trays in the gamma ray and ethyl methanesulphonate treatment series were carefully uprooted on the tenth day of sowing and the length of shoot and of root of each were measured. The mean shoot-length, root-length and shoot-root ratio were estimated and expressed as percentage of the respective control.

3. Survival of plants

The total number of plants surviving in each treatment was counted on the 30th day of sowing and the survival data estimated on the basis of the number of seeds sown are expressed as percentage of the respective control.

4. Plant height

From each treatment, thirty plants per replication were selected at random and the plant height was measured on the 6th, 15th, 30th and 45th day of sowing. The height was measured from soil surface to the terminal bud. The mean plant height was estimated and expressed as percentage of the respective control.

5. Pollen fertility

Pollen fertility was studied in 30 plants in each of the treatments. Mature flower buds produced during the early part of the flowering period were selected. Anthers were taken and the pollen grains were stained in a 1:1 proportion of glycerine acetocarmine solution. Fertility counts were taken after two hours of staining. The well stained and

properly filled pollen grains were scored as fertile and the others as sterile. In each of the slides 30 microscopic fields were scored and the data recorded. Fertility of each plant was estimated as 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 the percentage of the respective control.

6. Chlorophyll chimeras

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

7. Morphological abnormalities

The M_1 population was periodically examined to locate plants with morphological variations. The alterations in the number and shape of leaflets in the early formed secondary leaves were recorded. The plants with altered leaf digit number in the first formed secondary leaf in each of the treatments were counted and their frequency estimated as percentages of the number of surviving plants. The plants with bifoliate and unifoliate secondary leaves were counted separately and their frequencies were also estimated in a similar manner.

III. Study of the M_2 generation

1. Chlorophyll mutations - Frequency, spectrum and segregation ratios

Seeds obtained from mature fruits collected from

randomly selected M_1 plants were used to raise the M_2 generation. The number of M_1 plants carried forward to M_2 generation to study chlorophyll mutations varied from 30 to 100. The seeds were sown in progeny rows 30 cm apart giving a spacing of 15 cm between plants in the row. The M_2 seedlings were observed in early morning hours from the third day of sowing upto the fifteenth day and chlorophyll mutations were scored. The progeny rows segregating for chlorophyll mutations were first counted and the chlorophyll mutation frequencies on M_1 plant basis were estimated as the number of plants segregating per 100 M_1 plants. The chlorophyll mutants and normal plants in each segregating progeny row were counted separately. The plants in the non-segregating progeny rows were also counted. The mutation frequencies on M_2 plant basis were estimated as the number of mutants per 100 M_2 plants. The different types of chlorophyll mutants in each of the segregating progeny rows were counted separately and their relative percentages were estimated. The segregation ratios were estimated as percentages of the number of mutants to the total number of plants scored in segregating M_1 progenies.

2. Viable mutations

The number of M_1 plant progeny rows studied for viable mutations in different treatments varied from 21 to 34. The M_2 plants were observed periodically during their

entire life period and viable mutations were scored. All visible changes were scored. Viable mutation frequencies were estimated as the number of mutations per 100 M_1 plants. The viable mutants were described with respect to the deviations from normal plants. The viable mutants were labelled and harvested separately.

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).

Mutagenic effectiveness = M/krad or M/tc

Mutagenic efficiency = M/L, M/I or M/S,

where M = Mutations per 100 M_1 plants

t = time of chemical mutagen treatment in hours

c = Concentration of chemical mutagen in ml percentage

L = Percentage survival reduction on the 30th day of sowing

I = Percentage plant height reduction on the 30th day of sowing

S = Percentage reduction in pollen fertility.

RESULTS

RESULTS

The effects of gamma irradiation and ethyl methanesulphonate treatment on cowpea in M_1 and M_2 generations were studied and the results are presented below.

I. Effects in the M_1 generation.

1. Germination of seeds

The percentage of germination and the mean time for germination are presented in Table 1. The germination was not affected by gamma irradiation, whereas a progressive decrease in the germination percentages with increasing doses of ethyl methanesulphonate was observed (Figure 1). A retardation in germination was evident from the increase in mean period for germination with increase in doses of both mutagens. The effect was only slight in the case of gamma rays, but much pronounced with ethyl methanesulphonate.

2. Survival of plants

The percentages of survival estimated on the 30th day of sowing are given in Table 1. The survival was effected by gamma rays as well as ethyl methanesulphonate. But a progressive decrease with increasing doses could be observed only in the case of ethyl methanesulphonate (Figure 1). The reduction in survival was very drastic at higher doses of ethyl methanesulphonate.

3. Plant growth

The data on the mean length of shoot and that of primary

Table 1. Effect of mutagens on seed germination and survival of plants in the M_1 generation.

Mutagen and dose	Germination percentage	Period ofor germination		Survival on the 30th day	
		Mean period in hours	Percentage of control	Survival percentage	Percentage of control
Gamma rays					
Control	100.00	66.20	100.00	94.67	100.00
5 krad	100.00	66.20	100.00	93.33	98.58
10 "	100.00	67.20	101.51	92.67	97.89
15 "	100.00	67.97	102.67	86.67	91.55
20 "	100.00	68.80	103.93	86.67	91.55
25 "	100.00	69.40	104.83	87.33	92.25
30 "	100.00	71.00	107.25	88.33	93.30
EMS					
Control	100.00	65.20	100.00	94.00	100.00
0.1 per cent	100.00	77.60	119.02	91.09	96.90
0.2 "	96.67	94.96	145.64	89.33	95.03
0.3 "	93.33	109.86	168.50	88.00	93.62
0.4 "	90.00	129.33	198.36	82.00	87.23
0.5 "	86.67	147.23	225.81	80.67	85.82
0.6 "	76.67	159.52	244.66	74.66	79.43
0.7 "	46.67	179.63	275.51	42.00	44.68

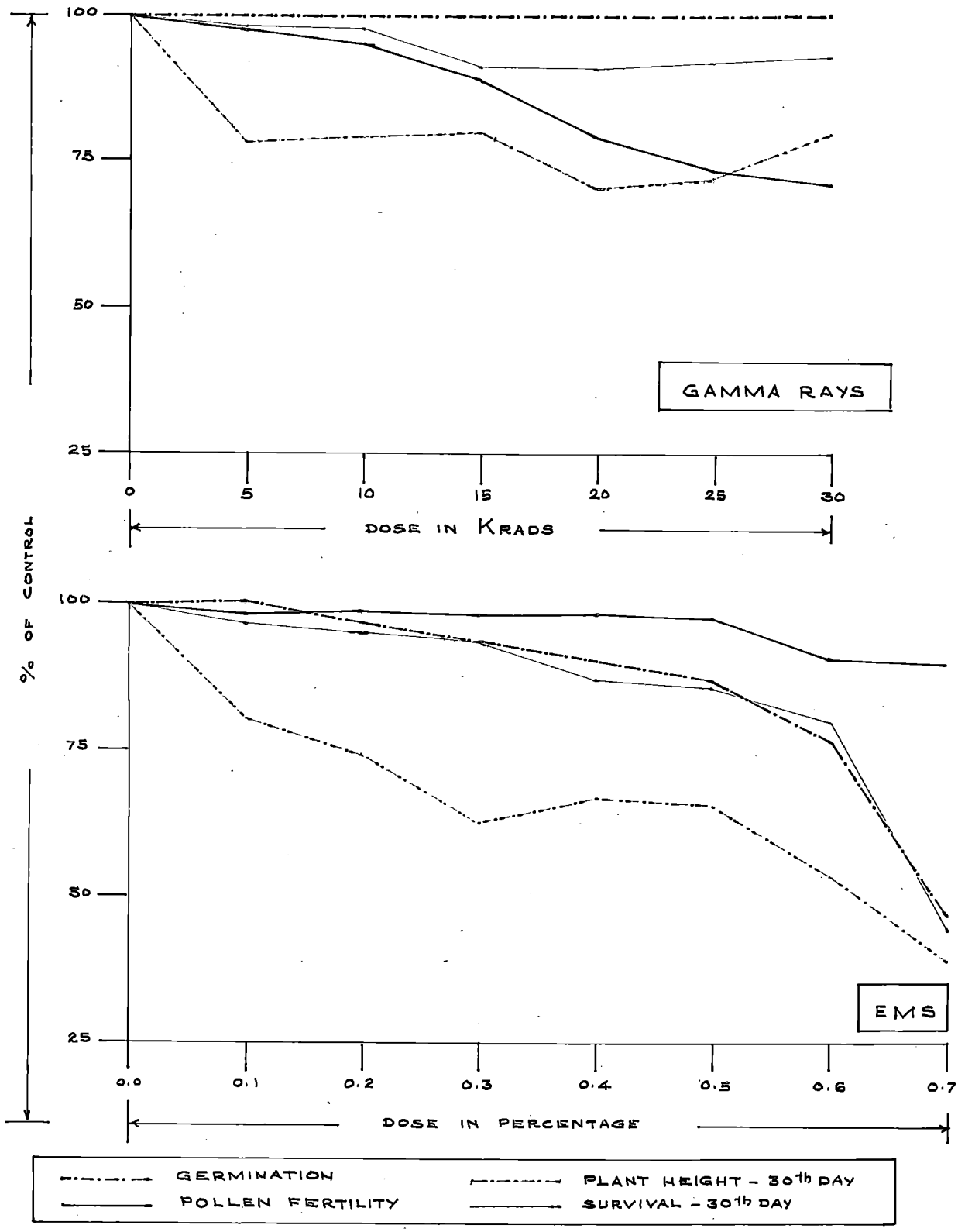

FIG. 1. GERMINATION, SURVIVAL, PLANT HEIGHT AND FERTILITY IN THE M_1 GENERATION

root measured on the tenth day of sowing expressed as percentages of control are presented in Table 2. The growth of shoot and root was reduced by both mutagens. The shoot-root ratios were less than that of the control in the gamma ray series, indicating that the growth of shoot was more affected than the growth of root. But with ethyl methane-sulphonate, the shoot-root ratios indicated a higher growth inhibition for the root than for the shoot.

The mean values for plant height measured on the 6th, 15th, 30th and 45th days were converted into the percentages of control and are presented in Table 2. The plant height estimates showed reduction with gamma ray and ethyl methane-sulphonate treatments at all the stages at which measurements were taken. The latter one has caused more severe reduction in growth. The reduction in plant height on the 30th day was found to be more drastic than that on the 45th day (Figure 2).

4. Pollen fertility

The percentages of pollen fertility were found to be decreasing with increasing doses of gamma rays and ethyl methanesulphonate (Table 2). The patterns of fertility reduction with increasing doses were different for the two mutagens (Figure 1). The pollen fertility showed an almost uniform reduction with increasing doses of gamma rays while with ethyl methanesulphonate smaller decreases at lower doses and larger decreases at higher doses could be observed.

Table 2. Effect of mutagens on plant growth and fertility in the M_1 generation

Mutagen and dose	Percentage of control							Pollen fertility	
	Shoot length	Primary root length	Shoot/ primary root	Plant height				Percentage	Percentage of control
				6th day	15th day	30th day	45th day		
Gamma rays									
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.92	100.00
5 krad	79.96	94.88	84.45	94.20	89.29	78.81	95.67	97.16	98.22
10 "	95.49	111.58	85.71	94.20	92.01	79.48	95.07	94.92	95.96
15 "	87.62	98.30	89.08	92.61	87.78	79.71	84.27	88.05	89.01
20 "	80.39	94.72	84.87	89.45	89.74	70.27	78.20	78.45	79.31
25 "	87.33	99.83	87.39	93.40	86.88	72.28	85.46	72.94	73.74
30 "	71.44	93.52	76.47	88.71	85.52	79.98	90.53	70.73	71.50
EMS									
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.01	100.00
0.1 per cent	99.29	85.60	116.11	92.74	86.79	80.66	81.23	97.98	98.96
0.2 "	96.49	83.94	114.98	87.08	84.32	74.20	76.75	97.40	98.37
0.3 "	83.73	76.56	109.42	73.81	77.00	62.95	63.13	97.12	98.09
0.4 "	65.35	59.59	109.73	70.27	74.65	67.33	69.42	97.09	98.06
0.5 "	44.17	41.83	105.55	68.14	73.58	65.80	66.87	96.31	97.27
0.6 "	38.08	33.94	100.45	58.41	55.19	52.57	52.83	89.67	90.57
0.7 "	33.66	32.65	103.11	40.00	45.17	39.29	41.60	88.65	89.54

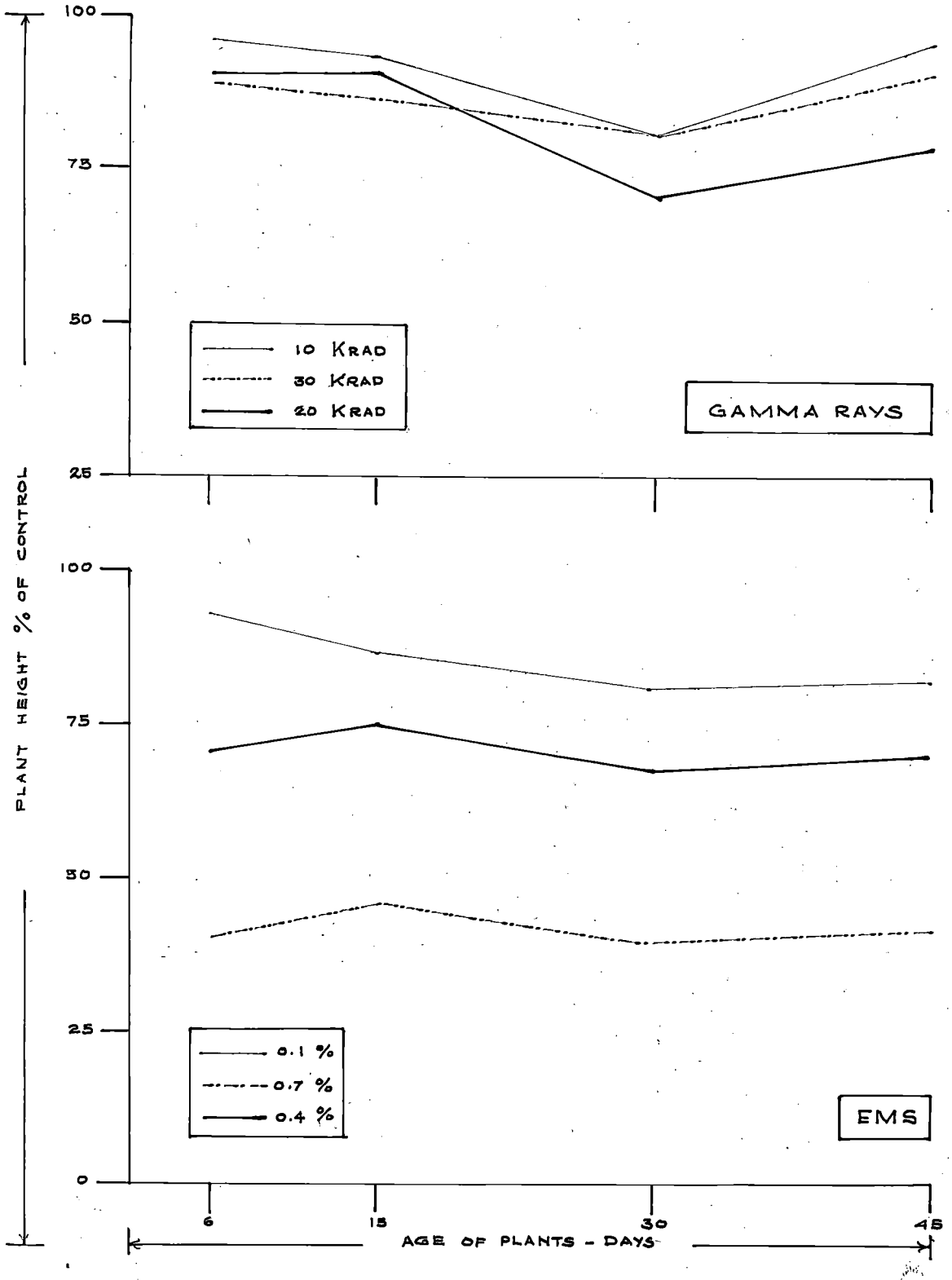

FIG: 2. EFFECT OF MUTAGENS ON PLANT GROWTH IN THE M_1 GENERATION

5. Chlorophyll chimeras

Chlorophyll deficient yellow patches on the leaves of M_1 plants were observed in gamma ray treatments as well as ethyl methanesulphonate treatments. The frequency of such chimeric plants was very low. The chlorophyll deficient patches were found on the leaves of one plant each in 5, 20 and 25 krad gamma ray and 0.1, 0.5 and 0.7 per cent ethyl methanesulphonate treatments.

6. Morphological abnormalities

In the present study, the morphologically visible changes were restricted to the alteration in the number, size and shape of leaflets in the first formed secondary leaves of the M_1 plants. Frequently, 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 (Plate 2). In some cases, the terminal leaflets became expanded and contorted in the absence of lateral leaflets. However, the plants recovered and produced normal leaves afterwards. The data provided in the Table 3, clearly indicated an increase in leaf anomalies with increase in the doses of both mutagens. Relatively smaller increases in the frequency of leaf anomalies at lower doses and larger increases at higher doses were observed with both mutagens. Another interesting feature noted was that the

Table 3. Frequency of plants with leaflet number changes in the first formed secondary leaf.

Mutagen and dose	Percentage of plants with changed leaf digit number	Percentage of plants with bifoliate leaf	Percentage of plants with unifoliate leaf
Gamma rays			
Control	0.00	-	-
5 krad	0.78	0.78	-
10 "	1.58	0.79	0.79
15 "	1.64	1.64	-
20 "	2.29	2.29	-
25 "	4.92	3.28	1.64
30 "	20.68	8.68	12.20
EMS			
Control	0.00	-	-
0.1 per cent	2.29	2.29	-
0.2 "	8.96	8.21	0.75
0.3 "	23.88	15.67	8.21
0.4 "	33.60	23.20	10.40
0.5 "	46.80	25.40	21.40
0.6 "	60.51	16.06	44.45
0.7 "	75.00	7.50	67.50

proportion of bifoliate and unifoliate leaves did not remain similar for all doses of the mutagens. As the doses increased, it showed a tendency to develop higher proportions of unifoliate leaves.

II. Mutations in the M_2 generation.

1. Chlorophyll mutations

a. Frequency

Chlorophyll mutations were scored at seedling stage and their frequencies estimated as the number of mutations per 100 M_1 plants and the number of mutants per 100 M_2 plants are presented in Table 4. The frequencies estimated on M_1 plant basis increased with increasing doses of gamma rays and ethyl methanesulphonate. On M_2 plant basis, the same dose-frequency relationship was obtained for gamma rays, while for ethyl methanesulphonate the frequency decreased slightly at the 0.5 and 0.6 per cent treatments (Figure 3).

b. Spectrum

The chlorophyll mutant types obtained were albino (no visible pigment), xantha (yellow), chlorina (yellowish green), viridis (light green) and those with yellow leaf tips or margins. Of these the first four types are shown in Plate 3. The spectra of chlorophyll mutations induced by gamma rays and ethyl methanesulphonate were found to be similar. The relative percentages of different types of mutants at each dose of the mutagens are presented in Table 5. The relative

Table 4. Frequency of chlorophyll mutations in the M_2 generation

Mutagen and dose	M_1 plant basis			M_2 plant basis		
	No. of M_1 plant progenies		No. of mutations per 100 M_1 plants	No. of M_2 plants scored	No. of mutants	No. of mutants per 100 M_2 plants
	Scored	Segregating				
Gamma rays						
Control	50	0	0.00	2280	0	0.00
5 krad	79	3	3.80	3028	9	0.30
10 "	80	8	10.00	3307	14	0.42
15 "	79	12	15.19	3161	32	1.01
20 "	83	16	19.28	3362	35	1.04
25 "	78	16	20.51	2399	33	1.38
30 "	52	13	25.00	1662	38	2.29
EMS						
Control	30	0	0.00	1312	0	0.00
0.1 per cent	80	1	1.25	2856	2	0.07
0.2 "	85	2	2.35	2656	4	0.15
0.3 "	62	2	3.23	1738	3	0.17
0.4 "	91	8	8.79	2614	30	1.15
0.5 "	67	6	8.96	1572	15	0.95
0.6 "	76	7	9.21	2193	18	0.82
0.7 "	32	4	12.50	873	12	1.37

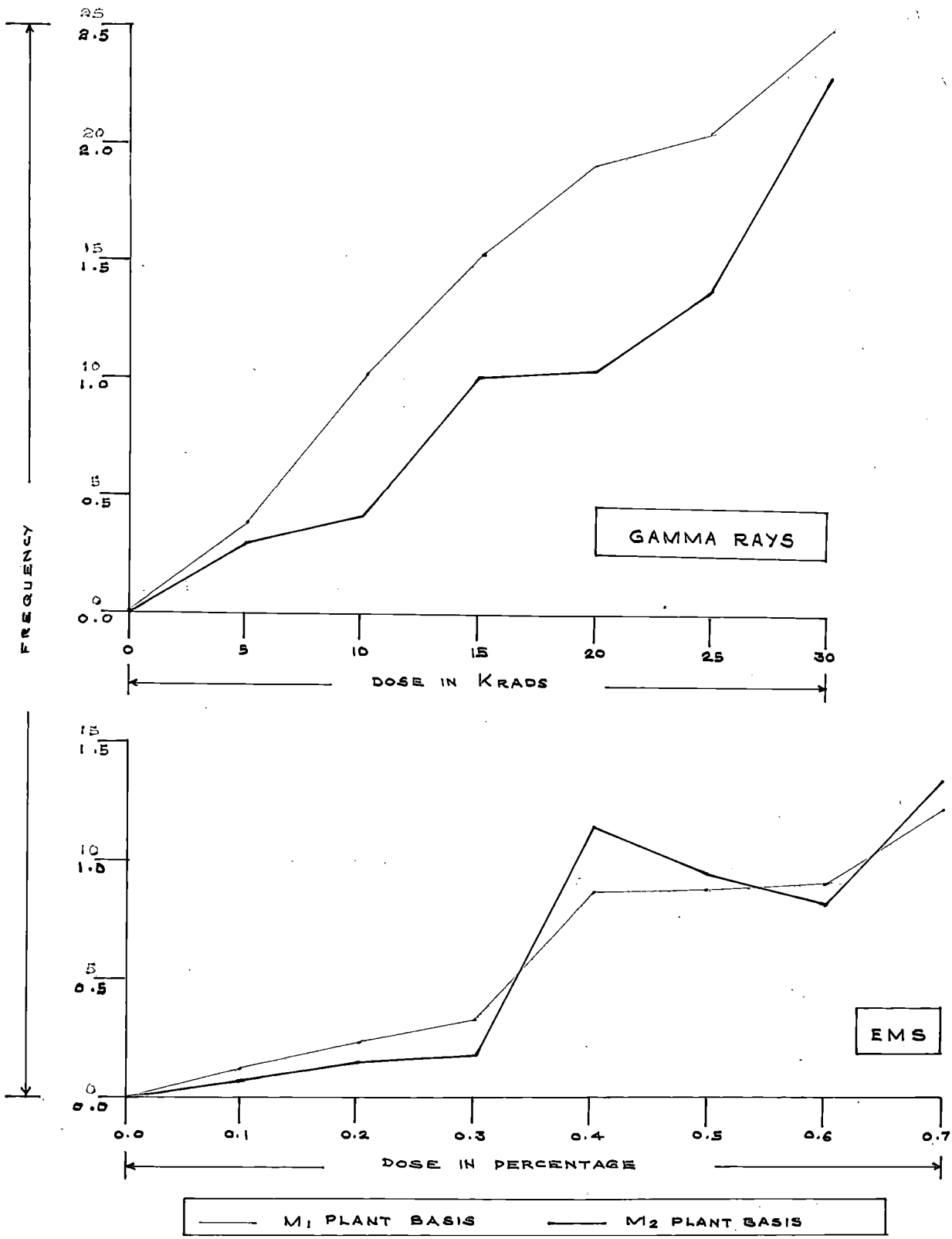

FIG: 3. CHLOROPHYLL MUTATION FREQUENCY IN THE M_2 GENERATION

Table 5. Relative percentages of different types of chlorophyll mutants in the M_2 generation.

Mutagen and dose	No. of chloro-phyll mutants	Relative percentages of chlorophyll mutant types				
		Albino	Xantha	Chlorina	Viridis	Others
Gamma rays						
5 krad	9	-	33.33	-	66.67	-
10 "	14	-	50.00	14.29	21.43	14.29
15 "	32	3.13	21.87	-	46.87	28.13
20 "	35	14.29	51.43	25.71	8.57	-
25 "	33	-	9.09	30.30	51.52	9.09
30 "	38	-	68.42	10.53	15.79	5.26
Total	161	3.72	39.75	15.53	31.06	9.94
EMS						
0.1 per cent	2	-	-	100.00	-	-
0.2 "	4	-	-	25.00	75.00	-
0.3 "	3	-	-	-	100.00	-
0.4 "	30	33.33	33.33	23.33	10.00	-
0.5 "	15	-	46.67	33.33	20.00	-
0.6 "	18	-	22.22	38.89	38.89	-
0.7 "	12	16.66	-	41.67	-	41.67
Total	84	14.29	25.00	32.14	22.62	5.95

percentages of mutants varied with different doses and the frequencies of different types had no relationship with the doses. The xantha and viridis types were obtained in all doses of gamma rays, while none occurred simultaneously at all doses of ethyl methanesulphonate.

The numbers of chlorophyll mutants of each type obtained from different doses of each mutagen were added together and the corresponding relative percentages estimated and are presented in Table 5. In gamma ray treatment xantha was the most frequently occurring type (39.75 per cent), while in ethyl methanesulphonate treatment it was chlorina (32.14 per cent). In both cases, albino had relatively low frequencies (Figure 4).

c. Segregation ratio

The segregation ratios, estimated as the percentages of the number of chlorophyll mutants to the total number of plants in the segregating M_1 progenies, in each dose of the mutagens are given in Table 6. The segregation ratios did not show any definite relationship with the doses. In the gamma ray treatment series, the ratio was maximum at 30 krad (8.41 per cent) and minimum (3.63 per cent) at 10 krad treatments. With ethyl methanesulphonate, the segregation ratio was maximum (12.82 per cent) at 0.4 per cent and minimum (4.17 per cent) at 0.2 per cent treatments. The segregation ratios were comparatively higher with ethyl methanesulphonate treatment than with gamma irradiation.

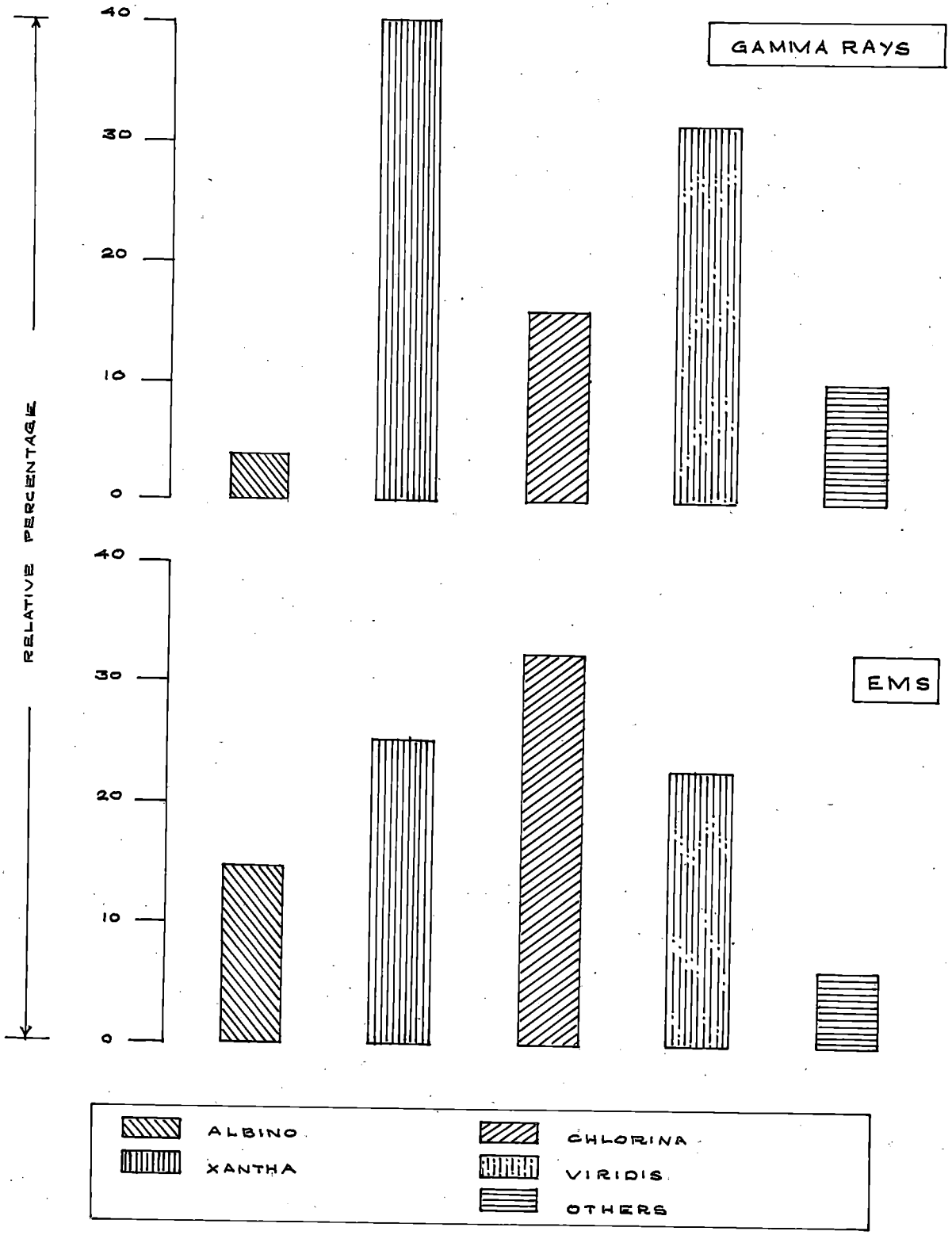

FIG : 4. RELATIVE PERCENTAGES (SPECTRUM) OF CHLOROPHYLL MUTATIONS IN THE M_2 GENERATION

Table 6. Segregation ratio of chlorophyll mutants in the M_2 generation.

Mutagen and dose	Total number of plants scored in segregating M_1 progenies	Number of mutants	Segregation ratio
Gamma rays			
5 kred	112	9	8.04
10 "	386	14	3.63
15 "	504	32	6.35
20 "	638	35	5.49
25 "	579	33	5.70
30 "	452	38	8.41
EMS			
0.1 per cent	22	2	9.09
0.2 "	96	4	4.17
0.3 "	36	3	8.33
0.4 "	234	30	12.82
0.5 "	123	15	12.20
0.6 "	178	18	10.11
0.7 "	115	12	10.43

2. Viable mutations

a. Frequency

The viable mutation frequency estimated as the number of mutations per 100 M_1 plants is presented in Table 7. The frequencies did not show any definite relationship with the doses of gamma rays. The highest percentage of segregating M_1 plants was observed in the 20 krad treatment. With ethyl methanesulphonate, an increase in frequency with the increase in dose was obtained except for the 0.6 per cent treatment.

b. Spectrum

Gamma irradiation resulted in a viable mutation spectrum for growth habit, time of flowering, duration, leaf shape, leaf size and seed coat colour while ethyl methanesulphonate induced changes in growth habit, time of flowering, duration, peduncle length, flower colour and seed coat colour. Some of the viable mutants are described with respect to deviations from the normal plants.

The 'New Era' variety has an erect, bushy semicompact and indeterminate growth habit. Many deviations from normal growth pattern were noted in the M_2 populations of both gamma ray and ethyl methanesulphonate treatments. This included twining, compact, divergent branching, sparsely branching, dwarf and gigantic types and those with stiff stem and determinate growth pattern. From the stand point of plant breeding, many of these growth habit mutants deserved special attention due to their newly induced attributes and in some

Table 7. Frequency of viable mutations in the M_2 generation

Mutagen and dose	Number of M_1 plant progenies		Number of mutations per 100 M_1 plants
	Scored	Segregating	
Gamma rays			
Control	23	0	0.00
5 kred	23	1	4.35
10 "	23	3	13.04
15 "	23	3	13.04
20 "	23	5	21.74
25 "	23	3	13.04
30 "	23	4	17.39
EMS			
Control	34	0	0.00
0.1 per cent	34	1	2.94
0.2 "	34	3	8.82
0.3 "	34	4	11.76
0.4 "	34	5	14.71
0.5 "	30	5	16.67
0.6 "	28	4	14.29
0.7 "	21	4	19.05

cases several characters were simultaneously changed. The 'compact stiff stemmed mutant', the 'twining mutant' and the 'giant mutant' obtained from the gamma ray treatments are worthy of special mention in this regard. The 'compact stiff stemmed mutant' had short stature and bushy growth pattern (Plate 4). The stiff stem of the mutant conferred resistance against lodging to the plant, inspite of the very high top weight. It had also cream seed coat colour as different from the unattractive mottled brown colour of the parent variety (Plate 5). The 'twining mutant' had smaller leaves, divergent branching nature and prolonged growth period (Plate 6). The 'giant mutant' was late flowering and had few branches, larger leaves and thick stem (Plate 7).

Early and late flowering as well as early and late maturing types were obtained from the M_2 populations of both mutagen treatments. The ethyl methanesulphonate treatment yielded a 'white flower mutant' (Original flower colour was violet) and a 'long peduncled mutant' (Plates 8 and 9). Divergent branching types (Plate 10) and sparsely branching types (Plate 11) were commonly observed in the M_2 population of ethyl methane sulphonate treatment.

Another interesting group of mutants obtained was the seed coat colour mutants. From the M_2 population of gamma ray treatments, plants were isolated which had seed coat colours that were cream, brown, different shades of red and

variously mottled and spotted (Plate 12). The seed coat colour mutants recovered from the ethyl methanesulphonate treatments were relatively fewer and were mostly cream or red (Plate 13).

III. Mutagenic effectiveness and efficiency.

The mutagenic effectiveness and efficiency of different doses of gamma rays and ethyl methanesulphonate in inducing chlorophyll mutations were estimated and are presented in Table 8.

The effectiveness was found to increase upto 15 krad gamma ray treatment after which there was a reduction. With ethyl methanesulphonate treatments, the effectiveness varied widely with the doses. The most effective one was the 0.4 per cent treatment.

The mutagenic efficiency estimated on the basis of lethality, sterility and injury did not show similar trends with increasing doses of gamma rays. Among the radiation doses employed, 10 krad was the most efficient when estimated on the basis of lethality or sterility, while on the basis of injury 30 krad was the most efficient. With ethyl methanesulphonate, the efficiency estimated on the basis of lethality and sterility increased with increase in doses upto the 0.4 per cent treatment. On injury basis, the 0.2 per cent treatment had slightly higher efficiency then the 0.3 per cent one. However, among the various doses of ethyl methanesulphonate employed, the 0.4 per cent treatment had the highest mutagenic efficiency, irrespective of whether the

Table 8. Mutagenic effectiveness and efficiency in inducing chlorophyll mutations.

Mutagen and dose	No. of mutations per 100 M_1 plants	M_1 damage			Mutagenic effectiveness. $\frac{M \times 100}{Krad\ or\ tc}$	Mutagenic efficiency		
		Lethality (L)	Injury (I)	Sterility (S)		$\frac{M \times 100}{L}$	$\frac{M \times 100}{I}$	$\frac{M \times 100}{S}$
Gamma rays								
5 krad	3.80	1.42	21.19	1.78	76.00	267.61	17.93	213.48
10 "	10.00	2.11	20.52	4.04	100.00	473.93	48.73	247.52
15 "	15.19	8.45	20.29	10.99	101.27	179.76	74.86	138.22
20 "	19.28	8.45	29.73	20.69	96.40	228.17	64.85	93.19
25 "	20.51	7.75	27.72	26.26	82.04	264.65	73.99	78.10
30 "	25.00	6.70	20.02	28.50	83.33	373.13	124.88	87.72
EMS								
0.1 per cent	1.25	3.10	19.34	1.04	208.33	40.32	6.46	120.19
0.2 "	2.35	4.97	25.80	1.63	195.83	47.28	9.11	144.17
0.3 "	3.23	6.36	37.05	1.91	179.44	50.63	8.72	169.11
0.4 "	6.79	12.77	32.67	1.94	366.25	68.83	26.91	453.09
0.5 "	8.96	14.18	34.20	2.73	298.67	63.19	26.20	328.21
0.6 "	9.21	20.57	47.43	9.43	255.83	44.77	19.42	97.67
0.7 "	12.50	55.32	60.71	10.46	297.62	22.60	20.59	119.50

criterion adopted for estimation was lethality, injury or sterility.

In general, the efficiency was higher for gamma rays than ethyl methanesulphonate, when it was estimated on the basis of lethality or injury. But on the basis of sterility, ethyl methanesulphonate proved to be more efficient than gamma rays.

DISCUSSION

DISCUSSION

The induction of mutations by physical and chemical agents is invariably associated with the production of undesirable changes in the biological materials. The undesirable changes resulting from chromosome aberrations and toxicity are manifested as M_1 damages such as lethality, injury and sterility. For a particular mutagenic treatment there is a correlation between M_1 damage and M_2 mutation frequency (Gaul, 1959). Efficient treatments, producing greater proportions of mutations to damages, are essential for the economic use of mutagens in plant breeding. The present study was undertaken to investigate the effects of six doses of gamma rays (5 - 30 krad) and seven concentrations of ethyl methanesulphonate (0.1 - 0.7 per cent) in the M_1 and M_2 generations of cowpea and to find out the effectiveness and efficiency of the treatments in inducing mutations. The results obtained are discussed in the following sections in the light of informations already available.

I. Effects in the M_1 generation.

1. Germination of seeds

The seed germination was found to be unaffected by gamma irradiation even at the highest dose employed. It has been reported by various investigators that the seed germination is not affected by low doses of ionising radiations

(Sjodin, 1962; Wellensiek, 1965; Ojamo and Chheda, 1971). Sjodin (1962) advanced a physiological reason for this observation. The first phase of germination is the swelling of cells by hydration followed by enzymatic activation and metabolism. The materials and energy necessary for this initial growth are already available in the seed. So the young embryo has no need to form new substances but only to activate those already stored in the cotyledons. This stage of germination is unaffected by radiation, therefore damage to the embryo which might arise from ionising radiations result only in post-germination mortality.

For ethyl methanesulphonate, the percentage of germination decreased with increasing doses. This is in conformity with the observations of Narsinghani and Kumar (1970). Reduction in seed germination in other leguminous crops following treatments with chemical mutagens has been reported by various workers (Wellensiek, 1965; Migacheva, 1972; Selim et al., 1974; Bhojwani and Kaul, 1976). This might be due to the toxic properties of ethyl methanesulphonate. The alkyl alkanesulphonates form strong acids upon hydrolysis, occurring externally in the treatment solution as well as inside the cells during treatment, which cause toxicity (Konzak et al., 1965).

The period of germination increased with the increase in doses of gamma rays as well as ethyl methanesulphonate. This effect was much pronounced with ethyl methanesulphonate.

The delay in germination of cowpea seeds following mutagen treatment was reported earlier by Louis and Kadambavanasundaram (1973a). Contrary to this, Mujeeb (1975) reported earlier germination in gram at low doses of gamma rays.

2. Survival of plants

The survival of plants was much affected by gamma irradiation, though the germination was unaffected. There are numerous reports of reduction in plant survival following seed irradiation in various leguminous crop species (Jaranowski, 1970; Ojomo and Chheda, 1971; Louis and Kadambavanasundaram, 1973a; Mujeeb and Greig, 1972). The reduction in survival is an index of post-germination mortality in treated plants as a result of cytological and physiological disturbances due to radiation effect. The cytological abnormalities caused by irradiation may lead to structural changes in the chromosomes. This interferes with the normal growth and development of organs which might have led to the fall in survival percentages with irradiation.

The percentage of survival, estimated on the basis of number of seeds sown, progressively decreased with increasing doses of ethyl methanesulphonate. Similar results were reported in pea (Wellensiek, 1963; Migacheva, 1972), mungbean (Santos, 1969), cowpea (Narsinghani and Kumar, 1970) and soybean (Constantin et al., 1976). On comparing the germination and survival data, it became evident that the factor

contributing to decreased survival was the reduced germination resulting from the treatment. This indicates that the post-germination mortality do not occur to any appreciable extent following chemical mutagen treatment.

3. Plant growth

A reduction in growth of shoot and primary root following treatments with gamma rays and ethyl methanesulphonate was observed in the present investigation. The study of shoot-root ratios of plants in different treatments indicated that the growth inhibition was more for shoot than for root in gamma ray treatments and vice versa for ethyl methanesulphonate treatments.

The plant height measured on the 6th, 15th, 30th and 45th day of sowing indicated reduction in height with gamma ray and ethyl methanesulphonate treatments at all stages at which measurements were taken. These observations are in accordance with the findings of Wellensiek (1965) in pea and Constantin et al. (1976) in soybean. Ethyl methanesulphonate was found to be causing more severe growth reduction compared to gamma rays.

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 electrostatic field or toxic chemical has been reported to influence plant growth. The inhibition of growth by mutagens can be interpreted as due to

inhibition in the rate of assimilation and consequent changes in the nutrient level of plants (Ehrenberg, 1955). Pollard (1964) postulated that irradiation stops DNA transcription and leads to decrease in messenger RNA production which should cause a decrease in protein synthesis and growth. Evans (1963) found that the reduction in growth in an irradiated meristem is the cumulative expression of at least three different types of cytologically identifiable effects viz., (1) mitotic cycle delay (2) formation of chromosome aberrations and (3) loss of proliferative capacity of cells due to either premature differentiation or death of cells. They also found that the initial mitotic delay from which the cells later on recover and chromosome aberrations play only minor roles in bringing about growth depression. So the major cause of growth depression might be the loss of proliferative capacity due to premature differentiation or death of cells.

The plant height data showed that the percentage reduction in plant height on the 30th day was more drastic than that on 45th day at all doses of gamma rays and ethyl methanesulphonate. This indicates an apparent recovery of M_1 plants from injury at later stages of growth. Recovery from injury at later stages of growth of cowpea plants was reported by Louis and Kadambavanasundaram (1973a). The recovery might be due to the growth of uninjured meristematic cells which replaced the injured ones as growth

proceeded.

4. Pollen fertility

The present investigation revealed an inverse relationship between pollen fertility and doses of gamma rays as well as ethyl methanesulphonate. These observations are in line with the findings of Zannone (1965) in vetch, Wellensiek (1965) in pea and Louis and Kadambavanasundaram (1973a) in cowpea. The pollen fertility showed an almost uniform reduction with increasing doses of gamma rays, while with ethyl methanesulphonate smaller reductions at lower doses and larger decreases at higher doses could be observed.

Radiation induced sterility might be due to detectable chromosome aberrations and cryptic deficiencies, whereas sterility induced by ethyl methanesulphonate might be due to cryptic deficiencies and specific gene mutations (Gaul et al., 1966; Sato and Gaul, 1967).

5. Chlorophyll chimeras

The incidence of chlorophyll deficient spots on the leaves of M_1 generation plants in legumes after mutagenic treatment of seeds has been observed by various workers (Specknman, 1964; Blixt and Gelin, 1965; Ojomo and Chheda, 1971). Blixt and Gelin (1965) found a close correlation between leaf spotting and mutation rate and advocated to use it as a criterion for selecting in the M_1 generation for plants giving higher yield of mutations in the M_2 generation. In the present study, the chlorophyll chimeric M_1 plants were

observed in gamma ray and ethyl methanesulphonate treated populations. But their frequency was low and occurred only at certain doses of both mutagens.

6. Morphological abnormalities

In the present study, morphologically visible changes were restricted to the alteration in the number, size and shape of leaflets in the first formed secondary leaves of the M_1 plants. Leaf abnormalities in legumes following mutagen treatments were reported by Speckman (1964) and Ojomo and Ohheda (1971). Frequently the first formed secondary leaf lacked one or two lateral leaflets, thereby appearing as a bifoliate or unifoliate leaf instead of trifoliate leaf. In some cases, the terminal leaflets became expanded and contorted in the absence of lateral leaflets. Similar observations were made earlier by Constantin and Love (1964). In the present investigation, the frequency of leaf anomalies was found to increase with the increase in doses of both mutagens. Smaller increases in their frequency at lower doses and larger increases at higher doses could be observed with both mutagens. A similar pattern of response with respect to leaf abnormalities was observed by Constantin and Love (1964).

The abnormal secondary leaves produced by the M_1 plants were either unifoliate or bifoliate types, with a tendency to develop higher proportions of unifoliate leaves with increasing doses of the mutagens.

The exact genetic reason for the development of leaf anomalies following mutagen treatments is not known. Gunckel and Sparrow (1961) cited reports in which leaf anomalies were attributed to chromosome breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism and accumulation of free amino acids.

II. Mutations in the M_2 generation.

1. Chlorophyll mutations

a. Frequency

For theoretical studies concerning mutation induction, it is very important to have a reliable system for comparison of mutation rates. Chlorophyll mutations have been widely employed for assessing the effectiveness of mutagenic treatments in higher plants (Gaul, 1964; Nilan et al., 1964; Kawai, 1969). Gaul (1964) while stressing the reliability in using them as the basis for assessment of effectiveness stated that (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.

Generally, the mutation frequencies increase with the increase in doses of the mutagens. This type of dose-frequency relationship, in pea with different mutagens, was reported by Blixt and Gelin (1965), Wellensiek (1965) and Piple (1967).

In the present investigation, the chlorophyll mutation frequencies were estimated as the number of mutations per 100 M_1 plants and the number of mutants per 100 M_2 plants. The mathematical basis for the use of the M_2 plant basis for estimating mutation frequencies has been presented by Gaul (1960).

The frequencies estimated on M_1 plant basis increased with increasing doses of both mutagens. The same dose-frequency relationship was obtained for gamma rays when frequency was estimated on M_2 plant basis. But in the ethyl methanesulphonate treatment series, the frequencies on M_2 plant basis showed a slight decrease at the middle doses.

b. Spectrum

The occurrence of a wide spectrum of chlorophyll mutations following mutagen treatments in various leguminous crops has been reported by various workers (Zennone, 1965; Swarup and Gill, 1968; Louis and Kadambavanasundaram, 1973b; Appa Rao and Jana, 1975; Vardanyan, 1976; Chekalin, 1977). In the present study, the chlorophyll mutant types obtained were albino, xantha, chlorina, viridis and those with yellow leaf tips or margin. Louis and Kadambavanasundaram (1973b) reported of the occurrence of albino, xantha and viridis mutants in cowpea following gamma irradiation. Zennone (1965) reported of differences between physical and chemical agents in the spectrum of chlorophyll mutations induced in vetch. In this study, the spectrum of chlorophyll mutations induced

by gamma rays and ethyl methanesulphonate was found to be similar.

In the gamma ray treatments, xantha was the most frequently occurring type with a frequency of 39.75 per cent, while in the ethyl methanesulphonate treatments chlorina was the most frequently occurring type and had a frequency of 32.14 per cent. The albino had relatively low frequencies in both gamma ray and ethyl methanesulphonate treatments. Chekalin (1977) found chlorovirídis to be the most frequently occurring type in Lathyrus sativus after gamma irradiation and treatment with different chemical mutagens.

c. Segregation ratio

The segregation ratios of various types of chlorophyll mutants in the M_2 generations of different leguminous crop species have been studied by various workers (Patil and Bora, 1963; Santos, 1969; Sur, 1970; Vardanyan, 1976). In this study, the segregation ratios were estimated as the percentages of number of mutants to total number of plants scored in segregating M_2 lines in each of the doses. The dependence of M_2 segregation ratio on dose in crops other than legumes has been reported by various workers (Bekendam, 1961; Gaul, 1964; Kawai and Sato, 1966; Siddiq, 1968). However, the segregation ratios did not show any relationship with doses in this study. In the gamma ray treatment series, the segregation ratio was maximum at 30 krad treatment.

With ethyl methanesulphonate, 0.4 per cent treatment gave the highest segregation ratio. Higher segregation ratios relate to higher frequencies of mutants in M_2 populations and hence, are important in plant breeding.

The comparison of segregation ratios in gamma ray and ethyl methanesulphonate treatments showed, the latter to give higher proportion of mutants in segregating M_2 lines. The high segregation ratio obtainable with a change in the mutagen is of great value in plant breeding. It seems that ethyl methanesulphonate is more advantageous in securing a high segregation ratio of mutants in cowpea than gamma rays.

2. Viable mutations

a. Frequency

The viable mutation frequency was estimated as the number of mutations per 100 M_1 plants. Any definite dose-frequency relationship was not observed in the gamma ray treatment. This might be due to the smallness of samples studied. The 20 kred treatment gave the highest frequency of viable mutations. With ethyl methanesulphonate treatment, an increase in mutation frequency with the increase in dose was observed except for a negligible decrease at the 0.6 per cent dose.

b. Spectrum

Gamma irradiation resulted in a viable mutation spectrum for growth habit, time of flowering, duration, leaf shape, leaf size and seed coat colour, while ethyl methanesulphonate induced changes in growth habit, time of flowering, duration,

peduncle length, flower colour and seed coat colour. There are numerous reports of the recovery of various viable mutant types in different leguminous crop species following mutagen treatments (Athwal, 1963; Ashri and Goldin, 1965; Pipie, 1967; Swarup and Gill, 1968; Santos, 1969; Sharma, 1969; Moh, 1971; Papova, 1972, 1977; Pokle, 1972; Louis and Kadambavanasundaram, 1973b; Alikhan and Veeraswamy, 1974; Appa Rao and Jana, 1976; Prasad, 1976; Chaturvedi and Sharma, 1976a,b). In the present investigation, many variations from normal growth pattern were observed in the M_2 populations of both gamma ray and ethyl methanesulphonate treatments. The growth habit mutants isolated from M_2 populations included twining, compact, determinate, divergent branching, sparsely branching, dwarf and gigantic types. In peanuts, Ashri and Goldin (1965) obtained mutants having deviating growth habits after diethyl sulphate treatment. Papova (1972, 1977) reported of the production of various economically useful types like determinate forms, forms with altered growth habits, compact forms and early ripening forms in pea following treatment with different chemical mutagens.

A few of the growth habit mutants obtained in the present study had several characters simultaneously changed. Such multiple phenotypic effects of mutation have been explained by many investigators to be due to pleotropic action of a single mutated gene. Gottschalk (1968, 1970) suggested three possible interpretations for the multiple effects in mutated organisms viz., (1) single mutated gene being responsible

for the whole complex of deviating characters, (2) a portion of a chromosome containing several genes being lost or (3) several closely linked genes having mutated. The first case is true pleotropism, whereas the other two events although simulate pleotropic effect of one gene, result from loss or alteration of several genes.

The major breeding objectives in pulses are to cut down excessive spreading vegetative growth, to improve harvest index and to reduce duration of maturity so as to increase the per day yield of crops and to make it possible to fit them in multiple cropping patterns (Jain, 1971). The 'compact stiff stemmed mutant' obtained from the gamma ray treatment in the present study had several attributes conforming to the ideal plant type concept in pulses. It had a compact, bushy, non-spreading growth pattern. The stiff stem of the mutant conferred resistance to lodging inspite of the high top weight. Moreover, it had cream seed coat colour as different from the unattractive mottled brown colour of the parent variety. From the M_2 populations early flowering and early maturing types were also isolated. Papova (1977) obtained early ripening types in pea following treatment with N-nitroso-N-ethylurea. The results of the present study indicated that mutation breeding would be helpful in attaining the above said objectives.

The 'twining mutant', obtained from gamma ray treatment was one with several characters simultaneously changed. In addition to the twining habit, it had smaller leaves, divergent

branching nature and prolonged growth period. The 'giant mutant' obtained was analogous to the 'late giant mutant' reported by Sharma (1969). It was late flowering and had fewer branches, large leaves and thick stem.

Chaturvedi and Sharma (1978a) observed different types of floral mutations in red gram following ethyl methanesulphonate treatment. In the present study, the only floral mutation observed was the one producing white flower colour obtained from ethyl methanesulphonate treatment. Feicle (1972) obtained a white flower mutant in cowpea following irradiation with X-rays.

The seed coat colour mutations in legumes following physical and chemical mutagen treatments have been reported earlier by Swarup and Gill (1968) and Moh (1971). In the present study, gamma radiation-induced cream, brown, different shades of red, variously mottled and spotted seed coat colour mutants were isolated. Ethyl methanesulphonate induced relatively fewer seed coat colour variants and these were mostly in shades of cream or red.

III. Mutagenic effectiveness and efficiency.

Konzak et al. (1965) presented a detailed treatment of the concepts of mutagenic effectiveness and efficiency. They proposed the terms 'effectiveness' as a measure of mutations in relation to dose and 'efficiency' as an estimate of mutation rate in relation to other biological effects induced such as lethality, injury and sterility. High

mutagenic effectiveness as well as efficiency are essential for successful utilisation of mutagens in plant breeding. To obtain high efficiency, the mutagenic effects must greatly surpass other effects in the cells such as chromosome aberrations and toxic effects which generally lead to damage.

Chlorophyll mutations have been widely employed for assessing the effectiveness and efficiency of mutagenic treatments in higher plants (Gaul, 1964; Nilan et al., 1964; Kawai, 1969). Gaul et al. (1972) defined efficiency as the ratio of chlorophyll mutations to biological damage. The chlorophyll mutations are taken as a basis for effectiveness and efficiency estimations on the assumption that the other types of mutations are induced with frequencies parallel to that of chlorophyll mutations (Kawai, 1969).

In the present study, effectiveness of gamma rays in inducing chlorophyll mutations was found to increase upto 15 kred dose. At higher doses there was a reduction in mutagenic effectiveness. With ethyl methanesulphonate, the effectiveness estimates varied widely with the doses. The highest mutagenic effectiveness was obtained in the 0.4 per cent treatment.

The mutagenic efficiency estimated on the basis of lethality, injury and sterility did not show similar trends with increasing doses of gamma rays. This is obviously due to the fact that the damaging effects estimated on the basis

of the three different criteria did not have any parallel dose-effect relationship. Among the radiation doses employed, 10 krad was the most efficient when estimated on the basis of lethality or sterility. On injury basis, the 30 krad dose was the most efficient one. With ethyl methanesulphonate the efficiency estimated on the basis of lethality and sterility increased with increase in doses upto the 0.4 per cent. On injury basis, the 0.2 per cent dose had a slightly higher efficiency than the 0.3 per cent one. It was found that the 0.4 per cent treatment had the highest mutagenic efficiency among the various doses of ethyl methanesulphonate employed, irrespective of whether the criterion adopted for the estimation was lethality, injury or sterility. The efficiency was lower at still higher doses. The reason for lower efficiency at higher doses appears to be due to the fact that lethality, injury and sterility increase with the doses at a faster rate than the mutations (Konzak et al., 1965).

Mutagenic agents vary widely in their capacity to induce mutations as well as damaging effects in a particular biological system. So selection of efficient mutagens with characteristics suited to a particular biological material is essential from a practical point of view. In the present investigation, on the basis of lethality and injury, gamma rays was found to be more efficient than ethyl methanesulphonate. But on the basis of sterility, ethyl methanesulphonate proved to be more efficient than gamma rays.

SUMMARY

SUMMARY

Studies were undertaken to obtain precise information on the effects of six doses of gamma rays (5-30 krads) and seven concentrations of ethyl methanesulphonate (0.1-0.7 per cent) in cowpea, using the variety 'New Era'. The effectiveness and efficiency of different doses of the two mutagens in inducing chlorophyll mutations were estimated.

1. The germination of cowpea seeds was not effected by gamma rays even at the highest dose employed, whereas ethyl methanesulphonate inhibited germination and the reduction in percentage was progressive with increasing doses.

2. The survival of plants based on the number of seeds sown was reduced by both mutagens. Post-germination mortality occurred to a certain degree with gamma irradiation, whereas it did not occur to any considerable extent with ethyl methanesulphonate treatment.

3. A differential effect on the growth of shoot and that of root was observed with both mutagens in the present study. The growth inhibition was more for the shoot with gamma rays, while ethyl methanesulphonate produced a higher degree of inhibition for the growth of root than for shoot.

4. The plant height was reduced by both mutagens, but the plants showed a tendency to recover from injury at later stages of growth.

5. The study revealed an inverse relationship between

pollen fertility and dose of both mutagens. The pollen fertility showed an almost uniform reduction with increasing doses of gamma rays, while with ethyl methanesulphonate smaller decreases at lower doses and larger decreases at higher doses were observed.

6. Chlorophyll chimeras were observed in both gamma ray and ethyl methanesulphonate treated M_1 populations.

7. The frequency of M_1 plants with reduced leaf digit number in the first formed secondary leaves increased with the increase in doses of both mutagens. A tendency to produce higher proportions of unifoliate leaves in comparison with bifoliate ones at higher doses was also evident.

8. The chlorophyll mutation frequency estimated on M_1 plant basis increased with increase in doses of gamma rays and ethyl methanesulphonate. With gamma rays, similar dose-frequency relationship was observed when frequency was estimated on M_2 plant basis. But with ethyl methanesulphonate, the frequency on M_2 plant basis showed a slight decrease at the middle doses.

9. The chlorophyll mutation spectrum was the same for gamma rays and ethyl methanesulphonate. The chlorophyll mutant types obtained were albino, xantha, chlorina, viridis and those with yellow leaf tips or margins.

10. The segregation ratios for chlorophyll mutations were higher for ethyl methanesulphonate than gamma rays.

11. The viable mutation frequency on M_1 plant basis did not show any definite relationship with the doses of gamma rays. With ethyl methanesulphonate treatment, an increase in frequency with the increase in dose was observed except for a negligible decrease at 0.6 per cent dose.

12. Gamma irradiation resulted in a viable mutation spectrum for growth habit, time of flowering, duration, leaf shape, leaf size and seed coat colour, while ethyl methanesulphonate induced changes in growth habit, time of flowering, duration, peduncle length, flower colour and seed coat colour. Several useful mutants were isolated from the M_2 populations.

13. The mutagenic effectiveness in inducing chlorophyll mutations was found to increase upto the 15 krad gamma ray treatment, higher doses reduced the effectiveness. With ethyl methanesulphonate no definite dose-effectiveness relationship was observed. The 0.4 per cent treatment was found to be the most effective among the various doses of ethyl methanesulphonate employed.

14. Among the gamma ray doses employed, 10 krad was the most efficient, when estimated on the basis of lethality or sterility. But on the basis of injury, 30 krad was the most efficient dose. With ethyl methanesulphonate, 0.4 per cent treatment had the highest mutagenic efficiency, irrespective of whether the criterion adopted for estimation was lethality, injury or sterility.

15. The mutagenic efficiency was higher for gamma rays than ethyl methanesulphonate when it was estimated on the basis of lethality or injury. On the basis of sterility, ethyl methanesulphonate was found to be more efficient than gamma rays.

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*Original not seen

PLATES

Plate 1

A typical cowpea plant of the variety 'New Era'.

Plate 2

Types of secondary leaves produced by the M_1 plants

1. trifoliate leaf (normal) 2. unifoliate leaf
3. bifoliate leaf.

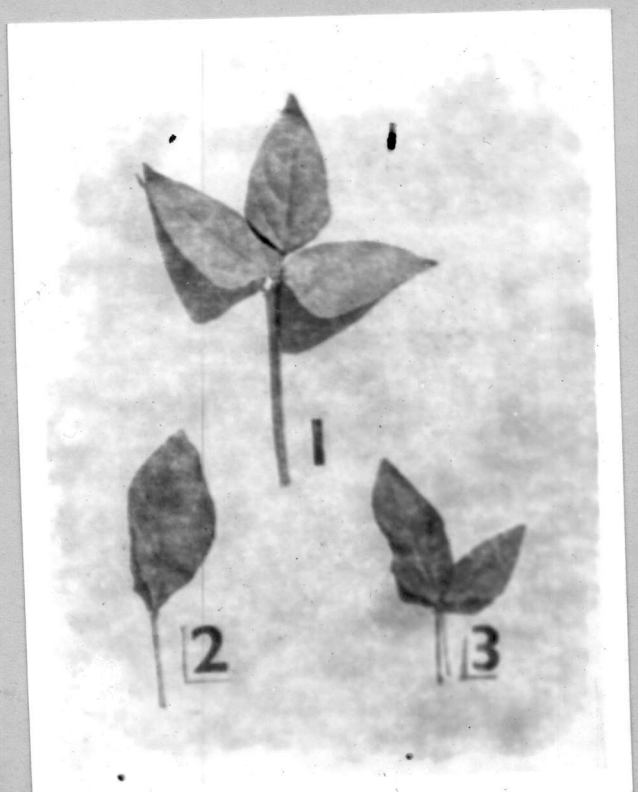


Plate 3

Types of chlorophyll mutants induced.

1. albino 2. xantha 3. chlorina
4. viridis 5. normal seedling.

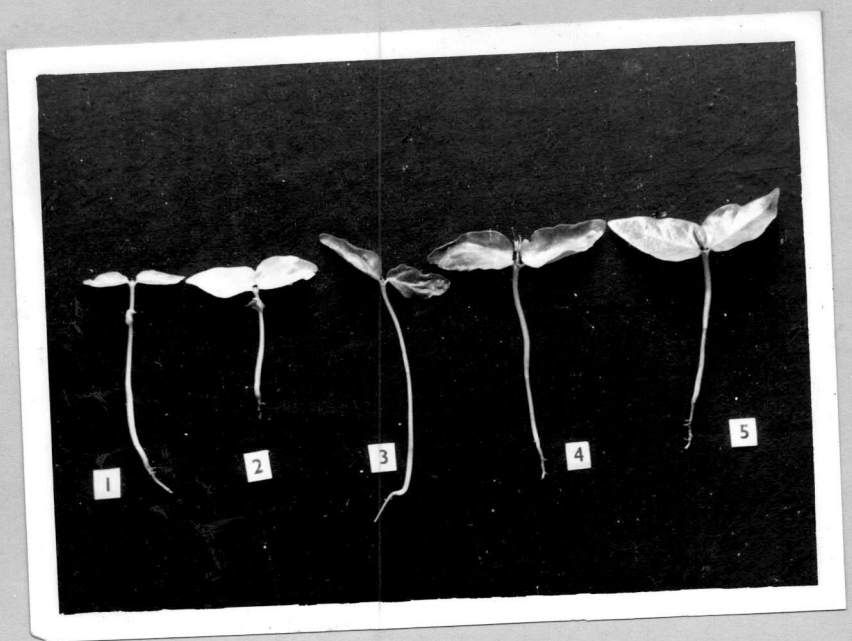


Plate 4

Gamma ray-induced 'compact stiff stemmed mutant'.

Plate 5

Seeds of the 'compact stiff stemmed mutant'.

1. Control (mottled brown) 2. Seeds of the mutant (cream)

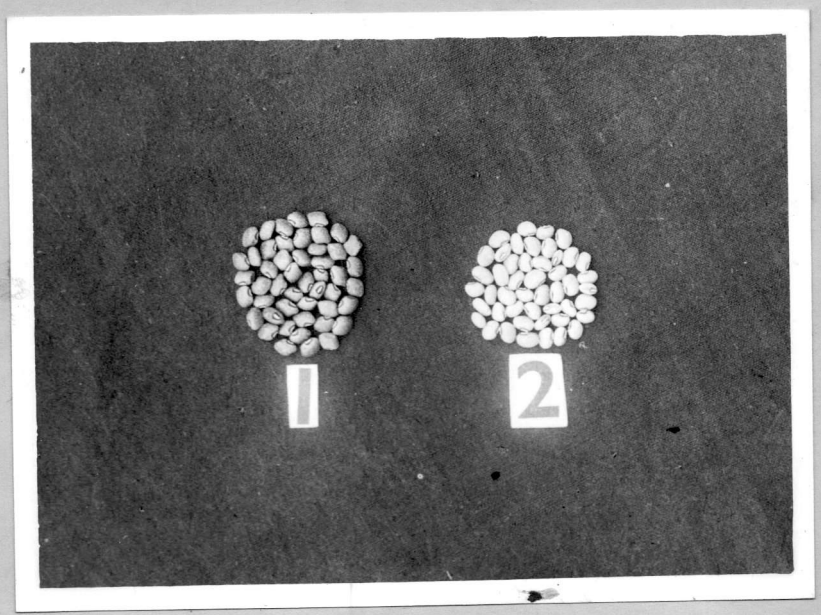


Plate 6

'Twining mutant' induced by gamma rays.

Plate 7

'Giant mutant' induced by gamma rays.

Plate 8

Flowers of the ethyl methanesulphonate-induced flower colour mutant.
1. Flowers of the control plant (violet)
2. Flowers of the mutant (white)

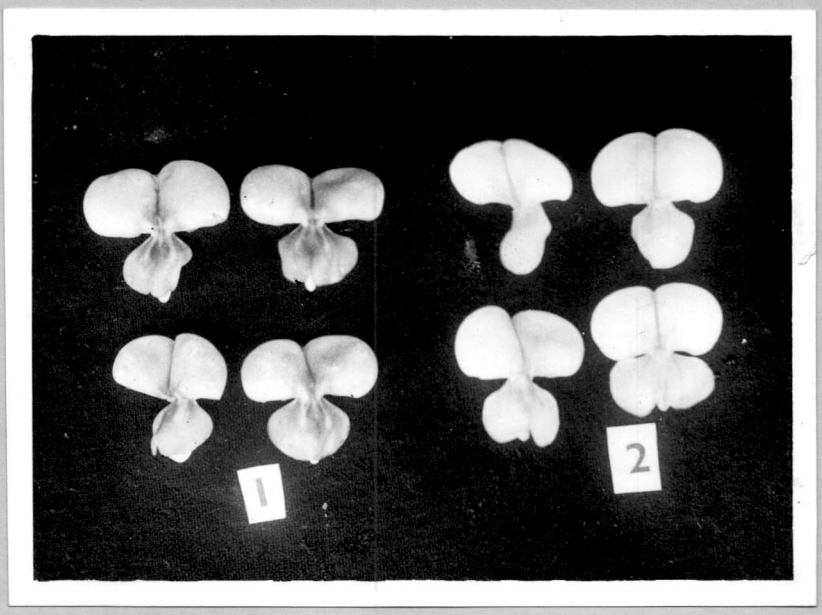


Plate 9

A mutant with long peduncle induced by ethyl methanesulphonate.

Plate 10

A divergent branching mutant induced by ethyl methanesulphonate.

Plate 11

A sparsely branching mutant induced by ethyl methanesulphonate.

Plate 12

Seeds of the gamma ray-induced seed coat colour mutants.
1. Control (mottled brown)
2. Cream
3. Cream with black spots
4. Brown
5. Light red with black mottlings
6. Medium red
7. Medium red with black mottlings
8. Dark red.

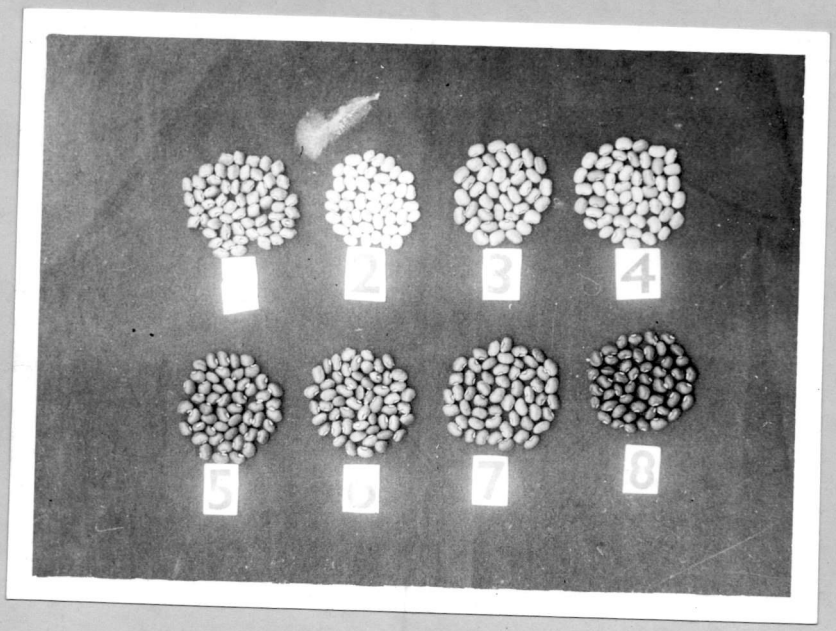
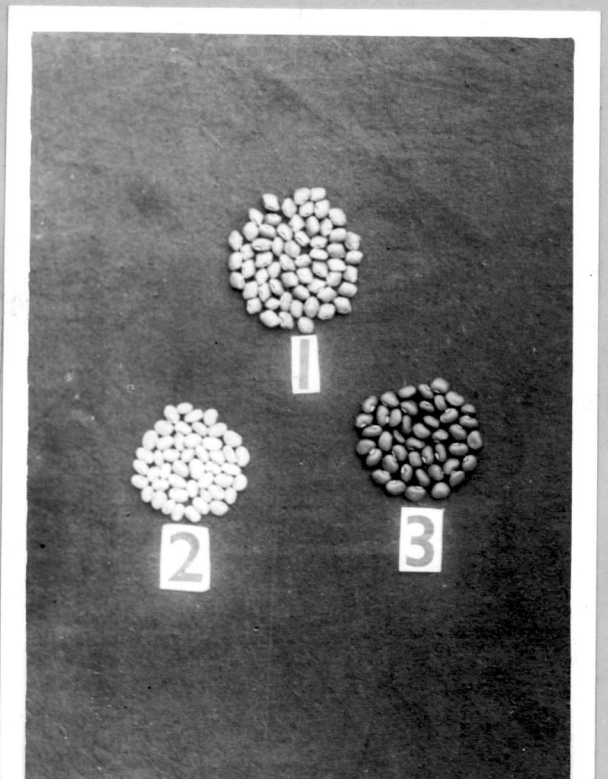


Plate 13

Seeds of the ethyl methanesulphonate-induced seed coat colour mutants.
1. Control (mottled brown)
2. Cream
3. Red



ABSTRACT

Seeds of 'New Era' variety of cowpea (Vigna unguiculata (L.) Walp.) were exposed to six doses of gamma rays (5-30 kreds) and seven concentrations of ethyl methanesulphonate (0.1-0.7 per cent, 6 hours) and their effects in the M_1 and M_2 generations were studied.

The seed germination was not affected even by the highest dose of gamma rays employed, while for ethyl methanesulphonate the percentage of germination decreased rapidly with increasing doses. Treatments with both mutagenic agents retarded the seed germination.

The survival of plants and plant growth were found to be effected by the treatments with gamma rays as well as ethyl methanesulphonate.

The pollen fertility showed an inverse relationship with the doses of gamma rays as well as ethyl methanesulphonate.

An increase in the frequency of leaf abnormalities with increasing doses of both mutagens was observed in the present study.

The chlorophyll mutation frequency expressed as the number of mutations per 100 M_1 plants clearly increased with increasing doses of gamma rays and ethyl methanesulphonate. The mutation frequency estimated on M_2 plant basis also showed a similar relationship with the doses of gamma rays. With ethyl methanesulphonate, the frequency on M_2 plant basis showed a slight decrease at the middle doses.

No difference in chlorophyll mutation spectrum occurred with the two mutagens employed. But the segregation ratios of chlorophyll mutants were higher for ethyl methanesulphonate treatments than gamma irradiation.

Among the gamma ray doses employed, the 15 krad dose was the most effective in inducing chlo rophyll mutations. The efficiency was maximum for the 10 krad dose when it was estimated on the basis of lethality or sterility, while on the basis injury, 30 krad proved to be the most efficient one. With ethyl methanesulphonate, the 0.4 per cent treatment was the most effective as well as efficient, irrespective of whether the criterion adopted for the estimation of efficiency was lethality, injury or sterility.

The mutagenic efficiency in inducing chlorophyll mutations was higher for gamma rays than for ethyl methanesulphonate, when it was estimated on the basis of lethality or injury. On the basis of sterility, ethyl methanesulphonate proved to be more efficient than gamma rays.