BIOLOGICAL EFFECTS OF GAMMA RAYS AND EMS IN THE M_2 GENERATION OF RED GRAM (*Cajanus cajan* L.)

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By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Biological effects of gamma rays and EMS in the M_2 generation of red gram (Cajanus cajan L.)" is a bonafide record of research work done by me during the course of research work 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.

Vellanikkara,
17--6--1987.

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CERTIFICATE

Certified that this thesis entitled "Biological effects of gamma rays and EMS in the M_2 generation of red gram (Cajanus cajan L.)" is a record of research work done independently by Smt. Brenda Valentina Aranha 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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CERTIFICATE

We, the undersigned, members of the Advisory Committee of Smt. Brenda Valentina Aranha, a candidate for the degree of Master of Science in Agriculture with major in Agricultural Botany, agree that the thesis entitled "Biological effects of gamma rays and EMS in the M_2 generation of red gram (*Cajanus cajan* L.)" may be submitted by Smt. Brenda Valentina Aranha in partial fulfilment of the requirement for the degree.

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Introduction

INTRODUCTION

Mutation breeding, as a tool for altering the genetic structure of the population and also as a means to step up the level of expression of yield and other economic attributes, besides providing newer and additional gene pools, has played a significant role in crop improvement work. Breeding techniques have, as their objective, maximisation of crop production both qualitative and quantitative. Breeding behaviour of individual crop with its inherent genetic structure, decides the realisation of the breeder's objective. The extent of natural variability, if low, limits the scope of crop improvement by the conventional breeding techniques. In this respect, mutation, as a means of artificially creating variability, comes to the aid of the plant geneticists.

The term mutation was introduced by De-Vries in 1900. The discovery of the possibility of enhancing the mutation frequency through radiation treatments in *Drosophila melanogaster* by Muller in 1927 followed by the demonstration of the effectiveness of radiation in crop plants such as maise and barley by Stadler in 1928, led many plant scientists to adopt mutagenesis in crop improvement efforts. With the advent of this method, the genotype and phenotype of living organisms are under human control and can be changed at times according to the needs. Soon after the discovery of physical mutagens, a rapid effort was made in the identification of a wide array of potent chemical mutagens. The attractive potential and relative ease of application of chemical mutagens have caused an unprecedented surge of interest in the artificial induction of mutations with these agents. Ionising radiations like X-rays, gamma rays and fast neutrons still remain the most potent tools for inducing variability. Among the chemical mutagens tested widely, ethyl methane sulphonate (EMS) seems to possess many properties favourable to high mutagenic effectiveness as well as high mutagenic efficiency.

The legume seeds or pulses, sometimes termed 'grain legumes', are second only to the cereals as a source of human food and provide the much needed proteins to our predominantly vegetarian population. Following the recent advances in the enhanced production of cereals in our country, pulses have also been receiving more and more attention. One of the main reasons for the stagnation in in pulse production in our country, and more so in our state, is the non-availability of suitable varieties possessing high yielding potential both in terms of grain yield and protein content as well as earliness and synchronised flowering. Further, natural variability in the germplasm is found to be a limiting factor to evolve varieties of superior performance. In view of the limited amount of variability which is presently available in pulses in general and in red gram in particular, it is considered essential to undertake methods for inducing new genetic variability in this crop.

Red gram is probably a native of Africa where it is often found naturalised. This is one of the most important among pulses from the point of view of per capita consumption. India is the largest producer accounting for about 1.8 million tonnes. It is the second most important pulse in India, being grown on an area of 2.5 million hectares. It is an excellent source of protein (22.3%) and starch (40%). Present yield level, which is very low, could marginally be improved and stabilised through scientific crop management. Eventhough it is widely cultivated, its importance as a rich source of protein and vitamins has not been fully recognised.

Long duration of many of the existing cultivars and their photosensitivity are some of the bad aspects which require immediate attention. In order to overcome these problems, the plant must be restructured both morphologically and physiologically.

The present work was, therefore, undertaken for fulfilling the following objectives:

1. To investigate the effects produced by gamma rays and EMS in the M_2 generation of red gram.

2. To study the chlorophyll mutation such as frequency, spectrum, effectiveness and efficiency of different doses of gamma rays and EMS in the M_2 generation.

3. To isolate the different types of viable mutants produced with reference to growth habit such as stature, branching habit, duration etc. in the M_2 generation.

The results obtained are presented, discussed and valid conclusions drawn in the following pages.

Review of Literature

REVIEW OF LITERATURE

In recent years, induced mutagenesis has become one of the potent tools in the hands of the plant breeders to restructure the crop plants according to the needs of Agriculture. With his pioneering work, Gustafsson, as early as in 1947, established a very useful and sound basis for mutation breeding. Refinement of technique in the later period and some of the notable and spectacular achievements have given confidence and encouragement to this line of research in plant breeding.

Induced mutagenesis has become handy not only in the detection of mutants but also direct utilization of mutants isolated as well as in employing these mutants as useful parents in the recombination breeding. NC. 4X in groundnut (Gregory, 1955); Aruna in castor (Kulkarni, 1968); MCU-7 in cotton (Narayanan et al., 1973) and Co-3 in red gram (Sheriff et al., 1977) are some of the economic mutants that are now under large scale cultivation.

Mutations in general

X-ray was the first radiation to be applied to cells and chromosomes. Subsequently the installation of cobalt and cesium sources made gamma rays also available. Horlacher and Killough (1931) observed that in cotton X-rayed seeds gave reduced germination and a number of dwarf plants were produced. There also resulted various abnormalities of the cotyledons. Kraievoi (1934) in a study of the influence of increasing doses of X-ray on seed production and on the meiotic processes in pear and vetch, arrived at the conclusion that with increasing dosage, meiotic irregularities became more pronounced, and the seed production fell proportionately. Krajevoj (1935) reported that X-ray treatment of young seedlings of a pureline of *Pisum sativum* produced a number of abnormalities in the resulting plants.

In addition to several ionizing radiations, a number of chemical mutagens also produced mutations in plants when applied singly, combined with other chemicals and in succession or simultaneously with radiation (Ehrenberg *et al*., 1961; Konzak *et al*., 1965).

Chemical mutagenesis was tried by Schiemann (1912) with some encouraging results. The experiments of Auerbach and Robson (1947) with *Drosophila* using sulphur and nitrogen mustards brought to light the usefulness of chemicals for the induction of mutations. Freese (1963) classified chemical mutagens as base analogue substitutes, dyes, acids, metals and alkylating agents. In higher plants, the last group has proved to be very effective. The relatively low toxic and high genetic effects of EMS (an alkylating agent) (Gaul, 1961) and its high mutagenic effectiveness as well as efficiency in higher plants (Konzak et al., 1965) led to its enhanced practical application.

I. Mutations in the M_2 generation

1. Chlorophyll mutations

(a) Frequency

Among the three methods of estimating chlorophyll mutation frequency, viz., (1) mutations per 100 M_1 plants, (2) mutations per 100 M_1 spikes or inflorescences and (3) mutations per 100 M_2 plants, the last method was found to be the best index as it was proportional to the initial rate of mutation and was independent of variation in progeny size and size of mutated sector (Gaul, 1960; Blixt, 1966)b.

Genter and Brown (1941) in their studies on field bean using X-rays, observed that 67% of the mutant types showed chlorophyll abnormalities. Gottschalk and Scheibe (1960) obtained three X-ray induced chlorophyll variants in

Pisum sativum upon X-irradiation of dry seeds with doses of 5,000 to 15,000 r. Athwal (1963) observed several kinds of mutations in the X_2 generation of *Cicer*, but the most frequent were those affecting chlorophyll development. Blixt *et al*. (1963) conducted studies on induced mutations in pear and they reported that scoring mutations in the X_2 gave a good approximation of the frequency of chlorophyll mutations. Akhun - zade and Hvostova (1966) observed the greatest number and range of chlorophyll mutations after treatment with EMS and the least after neutrons in pea. They found that there was a correlation between chlorophyll and morphological mutation frequencies. The frequency of chlorophyll mutations recoverable in a mutagenic experiment formed a good indication of the effectiveness and efficiency of mutagenic treatment (Monti, 1968). Swarup and Gill (1968) observed both viable and lethal chlorophyll mutations in French bean in all the treatments of X-rays. Bankowska and Rymsa (1969) reported that following treatment of dry seeds of *Phaseolus vulgaris* L. with four doses of gamma rays, a number of chlorophyll mutants were isolated. Vlk and Kupec (1970) observed that when seeds of the M_2 generation of peas after chronic gamma irradiation were stored for four years at constant temperature and humidity, the frequency of chlorophyll mutations was found to be 0.74 to 11.6 per cent. These values did not differ greatly from those obtained immediately after irradiation, i.e. 2 to 14 per cent. Vassileva and Mekhandzhiev (1971) in their experiment with 11 varieties of pea, found varietal differences in response to the dose inducing highest frequency of chlorophyll mutations. Debeley and Ptashenechuk (1973) observed a number of chlorophyll mutants in the M_2 plants of Vicia sativa after treatment with various doses of gamma rays and chemical mutagens in which the number of chlorophyll mutations was higher in the progenies from the lower inflorescence. Ghosh et al. (1973) showed that the frequency of chlorophyll mutations induced by gamma irradiation in pea was linearly related to dose. Illieva-Staneva (1973) showed that in French bean the mutation frequency was higher in treatments with EMS than in those with fast neutrons and EMS mostly induced chlorophyll mutations. Alikhan and Veeraswamy (1974) found that in red gram subjected to gamma irradiation and EMS treatment, the chlorophyll mutations were maximum at 24 krad and 70 mM treatments respectively. Hussein and Abdalla (1974) reported that in Vicia faba L. chlorophyll mutation frequency was directly proportional to the EMS concentration applied, while the combined treatment of

EMS and gamma rays doubled the frequency obtained with EMS alone. Uhlik and Romer (1974) treated <u>Vicia faba</u> with neutrons and obtained chlorophyll mutations in 6.6 per cent of segregating M_2 progenies, at the rate of 0.67 mutants per 100 seeds from M_1 plants. Levy and Ashri (1975) observed that in <u>Arachis hypogaea</u>, EMS induced chlorophyll mutations most frequently and plant size mutation next. Mekhandzhiev and Vassileva (1975) in their studies with <u>Pisum sativum</u>, observed that neutrons gave a greater frequency and wider range of mutations than gamma rays. The higher the gamma ray dose, the greater was the frequency of mutations but the lower was the range. In cowpea, Narasinghani and Kumar (1976) obtained a large number of chlorophyll mutants in the M_2 following EMS and MMS treatments. Narkar (1976) showed that there was an increase in the frequency of chlorophyll mutations with an increase in the dosage of gamma rays in <u>Lathyrus sativus</u>, but with NMU the, mutation frequency was considerably reduced as the dosage increased. Vardanyan (1976) found no correlation between the frequencies of chlorophyll mutations and morphological mutations in French bean following EI and DMS treatments. Vassileva and Mekhandzhiev (1976) in their studies with peas, reported that highest mutation frequency was obtained using EMS and the lowest with DES. The greatest range of mutations was obtained using low concentrations of EMS and EI. Agarkova and Yakovlev (1977) reported that in Phaseolus vulgaris with certain doses and concentrations, the percentage of mutations induced by combined treatment with gamma rays and ethyleneimine was greater than the sum of the mutations induced by separate treatment. However, the range of mutations induced was narrower. Chekalin (1977) observed that the total frequency of chlorophyll mutations was 0.012 per cent with NEU in Lathyrus sativus and a high correlation was found between the frequency of chlorophyll mutations and that of economically useful mutations. Krishnaswami et al. (1977) reported that the maximum occurrence of chlorophyll mutations was noticed in 80 krad dose of X-rays and gamma rays in green gram. Kulkarni and Shivasankar (1978) observed that in horse gram, the frequency of chlorophyll mutations increased with increasing concentrations of EMS upto 1.2 per cent and then decreased. In the M_2 generation of seeds treated with gamma rays there was an increase in frequency as the dose increased from 5 to 15 krad followed by a decrease. Subramanian (1979) observed in Vigna unguiculata sub sp cylindrica, various chlorophyll mutants at the seedling stage in the M_2.

Venkateswarlu et al. (1980) observed that in pigeon pea, the chlorophyll mutation rate showed linearity at low to medium doses, saturation and erratic behaviour at higher doses of the mutagen. Gamma rays followed by EMS and then by HA was the order in the magnitude of the frequency of chlorophyll mutations in the M_2. Yankulov et al. (1980) reported that gamma irradiation and EMS treatment of French bean resulted in a direct correlation between dose and percentage of chlorophyll mutants in the M_2. Armacheulo and Bernardo (1981), in winged bean, reported that chlorophyll mutations occurred as yellowish emergent seedlings with EMS treatments and yellow variegations and leaf streaking associated with leaf deformations with irradiation. Khan (1981) reported that in mungbean the mutation rates were highest in the combination treatments of EMS and hydrazine (HZ) with gamma rays. Mashkin et al. (1981) observed in soybean treated with different chemical mutagens that the highest frequency and widest range of mutations were obtained with N-nitroso N-ethyl urea (NEU). Chaturvedi et al.(1982) reported that treatment of presoaked seeds of Cajanus cajan with 0.03 per cent N-nitroso-N-methyl urea (NMU), resulted in the highest rate of chlorophyll mutation on the basis of mutated M_2 families and plants.

(b) **Spectrum**

Zannone (1965) reported that the spectrum of chlorophyll mutation was wider in <u>Vicia sativa</u> with EMS, showing 11 different types (alboxantha, xanthalba, xantha, xanthaviridis, viridis, variegate, albomarginata, lutescens, light green, green and alboviridis) followed by X-rays with eight types and EI with five types. Zoz et al.(1965) in their studies on experimental mutations in pea observed that EI and its two derivatives produced similar M_2 mutation spectra but the NEU M_2 spectrum was different in several aspects. Akhun-zade and Hvostova (1966) showed that EMS produced a relatively high proportion of xantha mutants and EI-a high proportion of chlorina, in peas. Sharma (1966) conducted mutation studies in <u>Pisum sativum</u> employing some physical and chemical mutagens and observed that of all the chemical mutagens, NMU produced the widest spectrum of mutants exhibiting six mutant types, not ordinarily produced by gamma rays, fast neutrons or other chemicals. Dudits (1967) obtained albina and xantha mutants showing increased phenotypic variability in peas treated with EMS or X-rays. In pea, Popova (1968) found that NEU gave a wider mutation spectrum than NMU. Sidorova (1968) showed that EMS and NEU induced wider mutation spectra compared to that by gamma rays in pea. In French bean, Swarup and Gill (1968) observed that the chlorophyll mutants were generally yellow or yellowish green in colour, following X-ray treatment, and a few were of chlorina types. Mekhandzhiev (1969) reported that the combined action of gamma rays and diethylsulphate gave a greatly increased spectrum of chlorophyll mutations in the M_2 of garden peas. In mungbean Santos (1969) reported that there were differences in mutation spectrum among mutagenic agents and that there might be differences in mutation spectrum between the different gamma ray dose levels and/or the different types of heat treatment. Rukmanski (1972), in a study of the spectrum of chlorophyll mutations in French bean, found that the spectrum induced by gamma rays was wider than that induced by chemical mutagens, xantha mutants being the most common and virio-maculata the least. Dahiya (1973) reported that with gamma irradiation in mung bean, the chlorophyll mutation spectrum was confined to four types, albina, xantha, viridis and maculata and there was a conspicuous absence of tigrina, chlorina and striata types. Illieva-Staneva (1973) found that the mutation spectra from treatments with EMS and fast neutrons were narrow in French bean. Louis and Kadambavanasundaram (1973) reported the occurrence of albino, xantha and viridis mutants in cowpea by gamma irradiation. In pea, Das and Kumar (1974) observed four chlorophyll mutant types induced by gamma rays which had light yellow, light green, white spotted and orange-yellow spotted leaves. Hussein and Abdalla (1974) reported that only EMS and gamma rays induced viridis, xantha and albina mutations in the M_2 of Vicia faba L. Iyyangar and Subramanian (1974) obtained albino and xanthophyll mutants in Phaseolus following 10 to 40 krad gamma radiation. Uhlik and Romer (1974) found that with thermal neutrons, most of the mutants in Vicia faba were of xantho-viridis type and viridis mutants were only below 8 per cent. Apparao and Jana (1975) obtained chlorophyll mutants such as viridis, chlorotica, chlorina-terminalis, chlorina-virescens and albo-virescens in Phaseolus mungo with X-rays, EMS treatment or both. Moone (1975) obtained five type of mutant, all lethal, viz., albino, cream, yellow, yellow-green and light green in Phaseolus vulgaris L. by gamma irradiation. In cowpea, Narasinghani and Kumar (1976) observed that treatment with EMS resulted in albino, xantha, chlorina and striata mutants and treatment with MMS resulted in albino, xantha and chlorina mutants. Rekhmatulla and Gostimskii (1976) reported that in pea, the spectrum of mutation was wider in treatments with EMS than with gamma rays. Nerkar (1976) observed 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 ray and EMS treatments. Vardanyan (1976) obtained viridis, xantha, xantha-viridis and striata types in French bean following EI and DMS treatments. In Lathyrus sativus, Chekalin (1977) obtained chlorophyll mutations with chemical mutagens and gamma rays, the most frequent of which was chloroviridis, followed by chlorina, atrovirens, lutea, albina, xantha, terminalis, maculata, marginata and albolutea. Venkateswarlu et al. (1978) irradiated pigeon pea with gamma rays and obtained viridis, chlorina and xantha mutants, with xantha occurring most frequently and chlorina the least. Grover and Tejpaul (1979) reported that when seeds of Phaseolus aureus were treated with maleic hydrazide and gamma rays separately and in combination, the mutation spectrum was found to be widest following maleic hydrazide treatment. Sichkar (1979) reported that with chemical mutagens and gamma rays, 16 different types of chlorophyll mutants were induced in soybean, of which, the most frequent were xantha, lutescens and xantho-terminalis, Kozera and Roszko (1980) observed that in Phaseolus vulgaris L. treated with fast neutrons, chlorophyll mutants of the types chlorotica, xantha, epicalis, costata, maculata and marginata were obtained. Subramanian (1980) observed eight types of chlorophyll mutation in the M_2 of Vigna upon gamma irradiation. More were produced by high than by low radiation dose and more were albina than any other type. Varma et al. (1980) observed chlorophyll mutants of the type albina, xantha, chlorina and viridis, the last two being most frequent in Vigna marina, following gamma irradiation Venkateswarlu et al. (1980) observed that in pigeon pea the spectrum for gamma rays was xantha >viridis > chlorina, while for EMS and HA it was viridis > chlorina > xantha. Vishnoi and Gupta (1980) reported that in Vicia faba L. the chlorophyll mutation frequency in the M_2 was highest for xantha and lowest for striata, and more mutations were caused by chemicals than by gamma rays. The chlorophyll content of the mutants was in the following decreasing order of viridalba > chlorina > xanthoalba > striata > viridis > alboxantha > xantha > albino. Manju (1981) in horse gram obtained a wide chlorophyll mutation spectrum which included albina, xantha, chlorina, viridis, maculata, alboviridis and viridalba. Al-Rubeai (1982) observed that when seeds of Phaseolus vulgaris L were treated with five different acute gamma radiation doses, the frequency of albino, xantha and viridis chlorophyll mutations in the M_2 was broadly dependent on dose, atleast upto 7 kR. Kundu and Singh (1982) reported that in black gram irradiated with 10 to 50 kR gamma rays, albina, chlorina and alboviridis types of chlorophyll mutations were found.

(c) Segregation ratio

Patil and Bora (1961) noticed the occurrence of one xantha and one virescent mutant in groundnut after X-irradiation. The segregation ratio of the virescent type was not clear. The ratio ranged from 1:1 to 15:1 indicating that the development of chlorophyll in groundnut was possibly controlled by more than one locus. Blixt (1966) studied the segregation of an albina mutant in peas in the M_2 generation in the seeds where the white cotyledons could be distinguished from the green. In mung bean, Santos (1969) found the frequency of mutants in M_2 rows segregating for xantha, chlorina and albino mutants to be 5.4, 7.3 and 4.0 per cent respectively. Sur (1970) reported that in black gram following gamma and neutron irradiations, different chlorophyll mutants appeared in the M_2 generation at frequencies ranging from 6.2 to 16.1 per cent with an average of 9.1 per cent. Uhlik (1971) reported that in

Lens esculenta, the highest percentage of segregating progenies in chlorophyll mutants and chimeras was 22.0 in doses of 1.6 and 2.0 x 10^{12} ncm^{-2} of thermal neutrons. The greatest proportion of progenies segregating into viridis mutants only was 10 per cent at a dose of 1.6 x 10^{12} ncm^{-2}, the maximum percentage of other types of mutants was much lower. In *Phaseolus vulgaris* L. Meono (1975) observed that the segregation ratios of chlorophyll mutants obtained by gamma irradiation were always lower than expected for Mendelian segregation. Vardanyan (1976) found that chemical mutagen treatments with EI and DMS in French bean gave viable striata mutants which segregated in the 3:1 ratio. Singh *et al*. (1979) reported that in green gram treated with gamma rays, chlorophyll mutants of the albina, xantha and chlorina types segregated in the ratio 1 mutant: 15 normal, indicating control by two recessive genes. In horse gram, Manju (1981) reported that the segregation ratios for chlorophyll mutations did not have any dose-dependence with gamma irradiation, while with EMS treatment, there was a definite dose-relationship.

2. Viable mutations

Rosen and Von (1942) in their studies of mutation in *Pisum sativum* employing X-rays, observed that plants from the irradiated seeds were somewhat smaller with the germination percentage, the number of seeds per pod and the number of plants that flowered to be in inverse proportion to the dose. Down and Anderson (1956) obtained an early bush mutant of agronomic use in beans upon X-irradiation. Zacharias (1956) observed a number of mutants of economic importance of soybeans after treatment with X -rays. They included plants maturing upto three weeks earlier, seeds germinating at low temperatures and resistant to frost and plants with dense foliage protecting the flowers and so able to set a large number of pods even under unfavourable climatic conditions. Lamprecht (1958) obtained a fruticose X-ray mutant in Pisum which had an average of 10 stems per plant, whilst normal plants had only 3 or 4. Wellensiek (1959) in peas treated with neutrons obtained mutants of the type, bushy, with several short, small leaved branches arising from the base of the plant Priadcencu (1961) in French bean treated with ionising radiations obtained mutants with morphological aberrations, dwarf and tall forms, early and late types and forms with increased yield, protein content and disease resistance. Teodoradze (1961), obtained mutants in soybean characterised by improved earliness and yield and in phaseolus mutants with 8 to 10 days earliness and 20 to 31 per cent more productivity. The viable mutations observed by Athwal (1963) in Cicer using X-rays were designated as flat stemmed, simple leaved, bushy narrow leaved, small leaved, bold and steriles. Jana (1964) observed in the M_2 of Phaseolus mungo L. after treatment with X-rays, mutants of interest for breeding purposes which included forms with a compact bush-type growth habit and forms with improved earliness or higher productivity. Moh and Alan (1964) obtained a dwarf bean mutant in the R_2 after treatment of seed with a 2000 r gamma ray dose from ^{137}Cs source. Sokolov (1966) isolated pea plants in the M_2, which showed changes in height, earliness, shape of leaves and stipules, leaf colour, seed size, shape and colour when treated with EI, EMS and DES. Silva and Marinho (1967) observed a large number of morphological abnormalities in gamma irradiated French bean, with the frequent occurrence of two or more of them in the same plant. Swarup and Gill (1968) obtained mutants in French bean with increase in number and size of pods, number of seeds per pod, seed yield and 100 seed weight after X-ray treatment. Pipie (1969)b reported that in peas EMS induced a greater frequency of mutations and more varied morphological and physiological mutations than

DES. In groundnut, Sanjeeviah _et al_. (1969) observed abnormal and dwarf plants in varying frequencies under different dosages. Such mutants were more frequent in the case of gamma irradiation at higher levels than with X-rays indicating that the locus or loci concerned were more sensitive to gamma rays. Bankowska and Rymsza (1970) obtained some dwarf mutants with supernumerary leaflets in _Phaseolus vulgaris_ following gamma irradiation. Jaranowski (1970) reported that in pea treated with gamma rays, 2.3 per cent of the population comprised of detectable mutants, which included dwarf and gigas forms and variations in leaf and flower form. Sachanski (1970) obtained mutants with short stems and short internodes, tall habit and early or late maturity in _Pisum sativum_ using X-and gamma rays. A true breeding mutant induced by gamma rays was obtained by Bhatt _et al_. (1972) in green gram, which matured earlier, was shorter, had longer pods and larger seeds than the parents. Kalinina (1972) obtained short mutants in pea with various mutagenic treatments, which were classed as compact, dwarf or fasciated. Ghosh _et al_. (1973) in pea obtained viable mutants affected by maturation period, seed type and pod number which were of practical value. Louis and Kadambavanasundaram (1975) obtained in cowpea, a mutant devoid of anthocyanin in all parts, in the M_2 generation of 40 krad gamma irradiation. They could also obtain a mutant with white seeds in 20 krad gamma ray treatment. Pande and Seth (1975) isolated seven early flowering mutants with fleshy pods following treatment of dry seeds of Phaseolus vulgaris with EMS and MMS. The maximum frequency of mutations was obtained with 0.3 per cent EMS. Prasad (1976) obtained 10 mutants with a higher number of pods per plant than the parent in the M_2 generation of green gram after treatment of seeds with 0.2 per cent or 0.3 per cent EMS. Isasi and Bhusto (1977) obtained tall, compact, high-yielding and erect mutants in French bean upon gamma irradiation. In green gram, Krishnaswami et al. (1977) found dwarf mutants at a high frequency in 80 krad of X-rays and 100 krad of gamma rays. Leaf mutants occurred in population from X-irradiated seeds and grain colour mutant was observed in gamma ray treatment only, where the grain colour changed from green to yellow. 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. Zelenskaya and Pomogaibo (1977) detected a mutant in Vicia sativa with vigorous growth and large leaves in M_2 by 2.5 krad gamma irradiation. Kulkarni and Shivasankar (1978) isolated two types of macromutations (dwarfness and testa colour) in horse gram after gamma ray treatment. Nerkar and Mote (1978) observed that the highest frequency of viable mutants in the M_2 of Bengal gram (Cicer arietinum L.) was obtained from the 20 kR treatment of which the leaf size and shape mutants were the most common. Abdul Shakoor et al. (1979) observed four early dwarf, determinate, synchronously maturing mutants in the M_2 of mungbean following treatment with gamma rays. Grover and Tejpaul (1979) reported that when seeds of Phaseolus aureus were treated with maleic hydrazide and gamma rays separately and in combination, the mutation obtained, affected height, stem diameter, leaf morphology and chlorophyll content. Krishnaswami and Rathinam (1980) observed that in green gram the frequency of viable mutations increased with mutagen dose. EMS and gamma ray combined treatments resulted in the increase in frequency of viable mutants. Among the viable mutants, those affecting maturity and height predominated. Singh et al. (1980) reported in mungbean that gamma irradiation of dry seeds resulted in the increase of clusters per plant and pods per plant. Manju (1981) in horse gram obtained viable mutants with respect to growth habit, leaf size and shape and time of flowering upon gamma ray treatment while EMS induced changes in growth habit and leaf size and shape. Mashkin et al. (1981) in soybean, treated with different chemical mutagens obtained many useful mutant forms with high insertion of the lowest pods, many pods per plant and a short growth period. Al-Rubeai (1982) in Phaseolus vulgaris L. treated with five different acute gamma irradiation doses, observed morphological mutant forms designated dwarf, stiff stem, shiny small leaf, narrow leaf and green giant. Chowdhury (1983) obtained a bold seeded dwarf mutant in the M_2 of cowpea following irradiation of dry seeds with 40 kR gamma rays. The mutant was 50 per cent shorter than the parent, but had increased values for pods per plant, seeds per plant, 100 seed weight and seed yield with an earlier maturity period of 10 to 12 days. Shivasankar (1983) obtained mutants with a bushy habit and varying degrees of earliness in winged bean following exposure to 10 to 15 krad gamma rays. The plants produced 5 to 6 pods with 5 to 6 seeds each. Rao and Reddy (1984) in pigeon pea treated with different doses and concentrations of different mutagens, obtained promising mutants with reduced stature, profuse branching, prolific pod clusters and bold seeded pods.

Vasudevan *et al.* (1984) obtained two non-twining, erect and two semierect mutants in the M_2 of *Vigna unguiculata* (L) Walp following 20 krad gamma irradiation of the seeds. Nagata and Basselt (1985) discovered seven plants with a dwarf mutant phenotype in the M_2 following treatment of the *Phaseolus vulgaris* with 10 kR gamma rays.

With respect to leaf alterations, Gunckel and Sparrow (1961) reported that leaf abnormalities were commonly observed after irradiation and apparently the youngest leaf exhibited the highest frequency of leaf alteration. In cowpea following irradiation with gamma rays and neutrons, Constantin and Love (1964) observed that many of the trifoliate leaves had one or two lateral leaflets missing. Sidorove *et al.* (1967) in the M_2 generation of pea obtained plants with variations in the length and shape of leaflets. Swarup and Gill (1968), following X-ray treatment in French bean, observed leaf mutants of varying types with crumpled leaves, very small leaves and large sized leaves. They also observed a aurculus mutant in 21 krad treatment, where the growth of the plant ceased after two cotyledonary leaves were formed; and a seed coat colour mutant. Kasprzyk (1970) detected mutations with changes in leaf width, flower colour, seed colour, seed size and shape in broad bean following gamma irradiation. Saini et al. (1974) obtained dwarf plants in *Phaseolus aureus* treated with 50 krad gamma rays which had smaller leaves, stipules, flowers and anthers. In soybean, Singh et al. (1974) obtained narrow, crinkled and curved leaved mutants by gamma irradiation. Four new leaf mutants - crinkled leaf, waxy leaf, narrow leaf and unifoliate were observed by Apparao and Jena (1976) following treatment of black gram with X-rays and EMS. In cowpea, Narasinghani and Kumar (1976) observed leaf mutations with EMS and MMS treatments, which included those with leaflets of varying size and shape of leaf blade and with altered leaf apex. Chaturvedi and Sharma (1978 b) obtained three mutants in the M_2 of red gram after EMS treatment, all with a modified leaflet shape. Kesavan and Khan (1978) noted mutants for leaf colour, leaf shape, reduced internodes and early flowering in winged bean treated with gamma rays and EMS. Sharma (1978) observed in the M_2 generation of lentil, leaf mutations with changes in leaf, rachis and leaflet width after treatment with gamma rays or N-nitro N-methyl urea. Sharma and Sharma (1979)b) 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. Varman *et al.* (1980) observed mutations in habit, leaf type, leaf size, leaf shape and growth period, in *Vigna marina* following gamma ray and EMS treatments.

Considering the floral mutants, Deshmukh and Phirke (1962) obtained plants with larger flowers and seeds and flattened pods in *Cajanus cajan* after treatment with chemical mutagens. Chandola *et al* (1965) obtained a floral mutant following neutron treatment, with one normal and one abnormal flower borne at each axil in pea. Sidorova *et al.* (1967) in the M_2 generation of pea obtained plants with variations in the colour of corolla and size and number of seeds. Rudy and Leznaja (1968) obtained a productive white flowered plant in *Vicia sativa* following EI treatment. In cowpea subjected to X-ray treatment, Pokle (1972) detected a white flowered mutant. Hussein and Disouki (1976) obtained 26 testa and flower colour mutants in the M_2 of *Phaseolus vulgaris* L. after four gamma ray and two EMS treatments of the seeds of five varieties. Chaturvedi and Sharma (1978a) reported that EMS was more efficient than NMU in inducing floral mutations in red gram where all the M_2 mutants lacked papilonaceous corolla due to an alteration in aestivation or to an increase in petal number. Sharma and Sharma (1979b) observed that in lentil, seed coat colour mutants were induced with 0.01 per cent N-nitroso-N-methyl urea at a frequency of 0.3 per cent among M_2 plants, whereas gamma irradiation in 10 kR doses produced only one seed colour mutant in a single M_2 generation of 1885 individuals. Tagaki and Hiraiwa (1980) obtained a number of mutants with changes in the colour of the seed coat and hilum in the M_2 generation following mutagenic treatment of seed of soybean. Kazanzhi et al. (1984) obtained white seeded mutants in the M_2 of French bean after treatment with 10 and 15 kR gamma ray doses.

3. Mutagenic effectiveness and efficiency

Mutagenic effectiveness indicated the relationship of effect of dose (Ehrenberg, 1960). Konzak et al. (1965) calculated the mutagenic effectiveness as the ratio of mutation frequency to dose. They were of opinion that the usefulness of any mutagen in plant breeding depended not only on its mutagenic effectiveness but also on its mutagenic efficiency. Efficient mutagenesis referred to the production of desirable changes free from association with undesirable changes and they defined efficiency as mutations per damage. Monti (1968) noticed that in pea, after DES and X-ray treatments, the proportion of chlorophyll to morphological mutations was 2:1 and came to the conclusion that the frequency of chlorophyll mutations recoverable in a mutagenic experiment might be taken as a good indication of the effectiveness and efficiency of the mutagenic treatments. Gaul et al. (1972) defined efficiency as the ratio of chlorophyll mutations to biological damage where the criteria for measuring damage were lethality, injury and sterility or chromosome mutations.

Makarova (1965) showed that in Pisum sativum EI and its derivatives had a similar mutagenic activity but NMU was twice as effective in inducing 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. Sharma (1966)b conducted mutation studies in Pisum sativum employing some physical and chemical mutagens and observed that irradiation with gamma rays proved the least effective. However, the proportion of useful mutants produced was highest with this mutagen, the next best being neutrons. Maslov and Stepanova (1967) treated pea seeds with gamma rays and several chemical mutagens and found that the most effective mutag ns were EMS and EI. Carapkin and Carapkina (1969) reported that high intensity gamma rays was less effective than low intensity radiation for inducing all types of chromosomal aberrations in pea, probably because the use of gamma rays at high dose resulted in a lowering of the "effective dose". Pipie (1969b) reported that in peas EMS was more eff ctive than DS in inducing a range of mutation types. Sharma (1969) reported that in cowpea, on the basis of mutation fre u ncy in the M_2, DMS and EMS showed almost equal effectiveness and NMU was about twice as effective (18.2 per cent mutations). Borejko (1970) treated soybean with various chemical mutagens of which NMU and NEU were found to be the most effective. Ivannikov et al. (1970) in chickpea observed the greatest mutagenic effect after tr atm nt with ethyleneimine. In mungo, Soriano and Baula (1970) found that the mutagenic efficiency was higher for fast neutrons than gamma rays. Gerasimenko et al. (1971) observed that gamma rays were more effective than EI or EMS in inducing mutations in the black Russian variety of broad bean. In pea, Tarasenkov (1973) observed that fast neutrons and gamma rays were less effective in inducing mutations than NMU, NEU, EI, DES and EMS and similar results were obtained by Sobchuk (1973) in pea. Constantin et al. (1974) reported that in soybean, fast neutrons and EMS were the most efficient inducers of chlorophyll deficiencies and morphological mutants compared to gamma rays and DES. In 1976, Nerkar observed that EMS and NMU were more efficient than radiations in producing chlorophyll mutants in *Lathyrus sativus* and similar results were also obtained by Akhun-zade (1977) in pea. Chaturvedi and Singh (1978) observed that dimethyl sulphoxide enhanced the effectiveness of EMS as indicated by the frequency and spectrum of chlorophyll and viable mutations induced in the M_2 of *Vigna radiata*. Birhman and Gupta (1980) treated dry seeds of green gram with 30 - 70 kR gamma rays. Data on lethality and sterility in M_1 and chlorophyll mutation frequency in the M_2 indicated that mutagenic effectiveness and efficiency were greatest at 30 kR. Kozera and Roszko (1980) observed that in *Phaseolus vulgaris* L. treated with fast neutrons, mutagenic efficiency was highest at 17J/kg radiation and mutagenic effectiveness was highest at 6 and 17 J/kg. Khan (1981) reported that in mung bean, the mutation rates were highest in the combination treatments of EMS and hydrazine (HZ) with gamma rays. Gamma rays caused most seedling injury and seed sterility, while HZ produced most lethals. Nadarajan and Ramalingam (1982) found that of the two mutagenic agents tested on red gram, gamma rays were more effective than diethylsulphate in inducing both chlorophyll and viable mutations when estimated on the basis of lethality. Diethylsulphate was more efficient than gamma rays in inducing both types of mutation when estimated on an injury basis. Rao and Reddy (1983) reported that in pigeon pea the mutant types differed from varieties and treatments of the mutagens, gamma rays being the most effective and hydrazine - the least. Filippetti and Marzaro (1984) treated dry seeds of <u>Vicia faba</u> L. with different doses of gamma rays and EMS, and found that the mutagenic effect of EMS was 2 to 3 times greater than that of gamma rays.

Materials and Methods

MATERIALS AND METHODS

The investigations reported herein on the "Biological effects of gamma rays and EMS in the M_2 generation of red gram (<u>Cajanus</u> <u>cajan</u> L.)" were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the period 1984-'86.

A. Materials

This study formed a continuation of a project already in progress in the Department of Agricultural Botany. Pure seeds of SA-1 variety of red gram received from the Director, School of Genetics, Tamil Nadu Agricultural University, Coimbatore, were subjected to five doses of gamma rays viz., 10, 20, 30, 40 and 50 krads and five doses of EMS viz., 0.3, 0.4, 0.5, 0.6 and 0.7 percent gas for a period of six hours and the M_1 generation was studied during 1983-'85 by my predecessor.

Seeds gathered from M_1 plants formed the material for raising the M_2 generation. In the case of gamma rays, 35 M_1 plants selected at random per dose were carried forward to the M_2 generation and 50 seeds from each M_1 plant formed a progeny row in the M_2. In the case of EMS, since the number of M_1 plants which reached to maturity was limited, all the M_1 plants were carried forward to M_2, each M_1 plant being represented in the M_2 by 50 seeds. The details regarding the number of M_1 plants that reached to maturity in each dose of the two mutagens, number of M_1 plants that were carried forward to M_2, number of seeds per plant used for raising an M_2 progeny row etc. are given in Table 1 below.

Table 1. Details of progenies studied

Treatment	No.of M_1 plants		No.of seeds per plant carried to M_2	Total No. of seeds per dose carried to M_2
	Reached to maturity	Carried to M_2		
Gamma rays				
10 krads	137	35	50	1750
20 "	105	35	50	1750
30 "	133	35	50	1750
40 "	78	35	50	1750
50 "	77	35	50	1750
EMS				
0.3 per cent	25	25	50	1250
0.4 "	8	8	50	400
0.5 "	6	6	50	300
0.6 "	5	5	50	250
0.7 "	-	-	-	-

B. Methods

The M_2 generation was raised as M_1 plant progenies during July 1985 to February 1986.

The seeds collected from M_1 plants selected at random were used to raise the M_2 generation and 50 seeds from each M_1 plant forming progeny row in the M_2, were sown adopting a spacing of 1 m between rows and 50 cm between plants in a row. Untreated controls were repeated after every twenty progeny rows. The layout of the experiment was non-replicated progeny rows.

The cultural, manurial and plant protection measures were done as per the Package of Practices Recommendations 1987 of the Kerala Agricultural University. The following observations were made in the M_2 generation.

1. Chlorophyll mutations

The M_2 seedlings were examined from the 3rd day of sowing upto the 20th day to score the chlorophyll deficient seedlings. The mutation frequencies on M_2 progeny row (M_1 plant) basis were estimated as the number of progenies segregating for mutants per 100 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 were scored separately for calculating the spectrum or relative percentage of different types of mutants. The spectrum of chlorophyll mutants was classified as albina (chlorophyll deficient white), Xantha (yellow), viridis (light green leaves), chlorina (yellow green leaves), maculata (irregular patches of chlorophyll deficient spots on the leaves), alboviridis (green in the proximal region of the leaf and white at the distal end), and viridalba (white in the proximal region of the leaf and green at the distal end). In the segregating M_1 families, the number of mutants and number of normal plants were counted to calculate the segregation ratio, i.e., percentage of mutants to the total number of plants in the family.

2. Viable mutations

Thirtyfive progenies per dose of gamma rays and all the available progenies of EMS were scored for viable mutations. All the plants in each progeny were periodically examined for morphological deviations from normal progenies and were classified into segregating and non-segregating.

Observations were continued upto harvest stage of the crop to score viable mutants. They were scored as the number of mutations per 100 M_2 progeny rows. The viable mutants were also described in detail with respect to the deviations from the normal ones, with special reference to duration, stature, branching pattern, floral characters, pod and seed characters.

Utilizing the data thus collected, mutagenic effectiveness and efficiency were calculated using the formulae suggested by Konzak et al. (1965).

3. Estimation of mutagenic effectiveness and efficiency

The formulae suggested by Konzak et al. (1965) for estimating the effectiveness and efficiency of both physical and chemical mutagens were follows.

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

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

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 seedling height reduction.

S = Percentage of pollen sterility

Seeds from all the viable mutants were separately collected for confirmation in M_3. From the non-segregating progeny rows, seeds of 25 plants selected at random were separately collected for the study of micromutations in the next generation.

Results

RESULTS

Results of observations on the "Biological effects of gamma rays and EMS in the M_2 generation of red gram" based on the study conducted during 1984-'86 are presented in this chapter.

The five concentrations of EMS tried in the present investigation appeared to be slightly on the higher side, as indicated by the comparatively few M_1 plants which reached to maturity, producing very few seeds for raising the M_2 generation. The highest dose of EMS tried here viz., 0.7 per cent appeared to be lethal, since no M_1 plant could produce seeds for studying the M_2 lines from this concentration.

The data collected on the frequency, spectrum and segregation ratio of chlorophyll mutations, viable mutations, mutagenic effectiveness and efficiency in respect of the different doses of gamma rays and EMS were subjected to suitable analyses and the mean values are presented in Tables 2 to 11.

1. **Chlorophyll mutations**

(a) Frequency

The data on the frequency of chlorophyll mutants induced by different doses of gamma rays, after calculating the frequencies on the M_2 progeny row and M_2 seedling basis are given in Table 2.

(TABLE 2)

The results presented in the above table have shown the following: The frequencies estimated on M_2 progeny row basis have decreased when the dose increased from 10 krads to 20 krads. Again it is found to increase from 20 to 30 krads after which the frequency is seen to be decreasing with increasing doses of the mutagen from 30 to 40 and from 40 to 50 krads.

The frequency of chlorophyll mutations estimated on M_2 seedling basis has also shown an almost similar trend. The frequencies are found to decrease from 10 to 20 krads after which it is seen to increase from 20 to 40 krads following a reduction in the frequency from 40 to 50 krads.

Table 2. Frequency of chlorophyll mutations in the M_2 generation (gamma rays)

Dose (in krads)	M_2 progeny row basis			M_2 seedling basis		
	No.of M_2 progeny rows		No.of mutants per 100 M_2 progeny rows	No.of M_2 seed-lings scored	No.of mutants	No.of mutants per 100 M_2 seedlings
	Scored	Segregating				
Control	9	0	0.00	357	0	0.00
10	35	29	82.86	1042	155	14.88
20	35	24	68.57	1411	99	7.02
30	35	32	91.43	1306	283	21.67
40	35	30	85.71	1289	309	23.97
50	35	29	82.86	1418	224	15.80

The data on the frequency of chlorophyll mutations induced by different doses of EMS are given in Table 3.

(TABLE 3)

As indicated by the results presented in the above table, the mutation frequencies calculated on M_2 progeny row basis have shown an increase from the dose 0.3 to 0.4 per cent after which it is found to be decreasing rapidly to 0.5 per cent from where the frequency is found to increase again to 0.6 per cent concentration of EMS.

The changing pattern of the frequencies of chlorophyll mutations estimated on M_2 seedling basis is observed to be the same as seen above. From the dose 0.3 per cent of the mutegen, the frequency is seen to shoot up rapidly to 0.4 per cent of the mutegen from where a steep fall is seen to the next dose viz., 0.5 per cent after which a slight increase of the frequency to the next dose is observed.

A comparison of the two mutegens viz., gamma rays and EMS based on the frequency of chlorophyll mutations in the M_2 generation has revealed the following. The distribution pattern of the frequencies in respect of both the mutegens either on M_2 progeny rows or on M_2 seedling basis

Table 3. Frequency of chlorophyll mutations in the M_2 generation (EMS)

Dose (in %)	M_2 progeny row basis			M_2 seedling basis		
	No.of M_2 progeny rows		No.of mutants per 100 M_2 progeny rows	No.of M_2 seedlings scored	No.of mutants	No.of mutants per 100 M_2 seedlings
	Scored	Segregating				
Control	2	0	0.00	72	0	0.00
0.3	25	14	56.00	505	89	17.62
0.4	8	5	62.50	75	29	38.67
0.5	6	1	16.67	10	1	10.00
0.6	5	4	80.00	45	8	17.78

is almost the same. In either case, the frequencies do not seem to have any dose dependence of the mutagen, either physical or chemical.

(b) Spectrum

The data pertaining to the spectrum of chlorophyll mutations induced by gamma rays are given in Table 4.

(TABLE 4)

Different types of chlorophyll mutants were obtained which were classified as xantha, chlorina, viridis, maculata, alboviridis and viridalba (Plate 1). The relative percentages of the different types of chlorophyll mutants at each of the doses of the mutagen are given in the table. The frequencies of the different types chlorophyll mutants are found to vary with the different doses of the mutagen and are not showing any dose dependence. Viridis mutants are found in all the doses of the mutagen, the maximum being in 40 krads and the minimum in 20 krads. Maculata types are found to occur in all the doses with the exception of 10 krads, the maximum occurring in 20 krads and minimum in 40 krads of the mutagen. Chlorina types occur in 10, 20 and 40 krad doses of the mutagen, the maximum being in 10 krads and minimum in 40 krads. Alboviridis and viridalba types

Table 4. Spectrum of chlorophyll mutations (gamma rays)

Dose (in krads)	Total No.of mutants	Relative percentage						
		Albina	Xantha	Viridis	Chlorina	Maculata	Albovi-ridis	Viridalba
Control	0	-	-	-	-	-	-	-
10	155	-	-	96.13	3.87	-	-	-
20	99	-	3.03	58.59	3.03	35.35	-	-
30	283	-	1.77	77.39	-	20.85	-	-
40	309	-	-	97.09	0.32	1.94	0.32	0.32
50	224	-	-	94.20	-	5.80	-	-

Plate 1. Chlorophyll mutation spectrum

1. Normal
2. Maculata
3. Viridis
4. Viridalba
5. Alboviridis
6. Xantha
7. Chlorina

Plate 2. Variation in the number of leaflets

1. Normal
2.]
3.] Mutants
4.]

Plate 1.

Plate 2.

constitute a very small percentage and are found only in 40 krad dose of the mutagen. Xantha mutants are seen in 20 and 30 krad doses, following a decreasing pattern. Albina types are totally absent.

Data on the spectrum of chlorophyll mutations induced by EMS are given in Table 5.

(TABLE 5)

The conclusions obtained from the above table are the following: Only three types of mutants are observed as a whole from the different doses of the mutagen. Albina, xantha, chlorina and viridalba types are completely absent. Viridis mutants are seen in all the doses of the mutagen, the frequency of which shows a direct dose relationship up to 0.5 per cent of EMS and thereafter remaining constant. Maculata types occur only in 0.3 per cent dose and alboviridis in 0.4 per cent dose of EMS.

A comparison of gamma rays and EMS based on the relative percentages of the different types of chlorophyll mutants is attempted here. It is seen that gamma rays have produced a wider mutation spectrum than EMS. Viridis

Table 5. Spectrum of chlorophyll mutations (EMS)

Dose (in %)	Total No.of mutants	Relative percentage						
		Albina	Xantha	Viridis	Chlorina	Maculata	Albovi-ridis	Viri-dalba
Control	0	-	-	-	-	-	-	-
0.3	89	-	-	86.52	-	13.48	-	-
0.4	29	-	-	96.55	-	-	3.45	-
0.5	1	-	-	100.00	-	-	-	-
0.6	8	-	-	100.00	-	-	-	-

types are found to occur most frequently in all the doses of gamma rays and EMS, the frequencies being dose dependent in the latter case to some extent.

(c) Segregation ratio

The observations on the segregation ratio of chlorophyll mutants obtained by gamma irradiation are presented in Table 6.

(TABLE 6)

Segregation ratios 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 mutagen, do not show any definite dose relationship. There is a sudden fall in the ratio from 10 krads to 20 krads from where it rises to 30 krads and reaches a maximum at 40 krads and then tends to decrease at 50 krads

The data on the segregation ratio of chlorophyll mutants obtained from the different doses of EMS are given in Table 7.

(TABLE 7)

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Table 6. Segregation ratio of chlorophyll mutants in the M_2 generation (gamma rays)

Dose (in krads)	Total No.of plants scored in segregating M_2 progeny rows	No.of mutants	Segregation ratio
Control	0	0	0.00
10	865	155	17.92
20	940	99	10.53
30	1188	283	23.82
40	1122	309	27.54
50	1176	224	19.05

Table 7. Segregation ratio of chlorophyll mutants in the M_2 generation (EMS)

Dose (in %)	Total No.of plants scored in segregating M_2 progeny rows	No.of mutants	Segregation ratio
Control	0	0	0.00
0.3	441	89	20.18
0.4	75	29	38.67
0.5	5	1	20.00
0.6	44	8	18.18

Segregation ratios are estimated in the same manner as that in gamma ray treatment. Here too the ratios do not show any dose dependence. The maximum ratio is met with at 0.4 per cent EMS treatment. There is a rise in the segregation ratio from 0.3 to 0.4 per cent from where it starts decreasing and reaches to a minimum at 0.6 per cent of EMS.

A comparison of the two mutagens viz., gamma rays and EMS with respect to the segregation ratios of chlorophyll mutants in the M_2 generation reveals that, the ratios do not show any dose relationship in either case of the mutagens.

2. Viable mutations

Results of observations on the frequency of viable mutations obtained by gamma irradiation are given in Table 8.

(TABLE 8)

The viable mutation frequency estimated as the number of mutations per 100 M_2 progeny rows is found to show a direct dose dependence upto 40 krads from where it is seen to decrease to 50 krads.

Table 8. Frequency of viable mutations in the M_2 generation (gamma rays)

Dose (in kreds)	No.of M_2 progeny rows: Scored	No.of M_2 progeny rows: Segregating	No.of mutations per 100 M_2 progeny rows
Control	9	0	0.00
10	35	3	8.57
20	35	11	31.43
30	35	15	42.86
40	35	16	45.71
50	35	13	37.14

The SA-1 variety of pigeon pea selected for the study is a woody, perennial shrub, 1 to 4 m tall, but is generally grown as an annual crop. Many deviations from the normal growth pattern of the plant were observed in the M_2 population of gamma ray treatment. The different types of viable mutants produced by gamma irradiation were with respect to growth habit, leaf size and shape. Leaf variations such as alterations in the number and size of leaflets were noticed. These leaves lacked one or two lateral leaflets thereby appearing as bifoliate or unifoliate leaves instead of the normal trifoliate leaf (Plate 2). The dwarf mutants possessing short stature and the profuse branching capacity gave them a bushy appearance thereby deviating from the normal SA-1 plant. The total number of leaves per branch in these mutants was normal, but had a much shorter internode than that of normal plants (Plates 3 and 4). A small leaved mutant obtained with 40 krad gamma ray treatment had a sparse branching habit (Plate 5). Dark green leaved mutant with reduced height and very few branches was obtained in 50 krad treatment of gamma rays (Plate 6). Mutants with round leaves having blunt ends were also observed in 20 krad gamma ray treatment (Plate 7).

Plate 3. SA.1 Variety of pigeon-pea-Normal plant.

Plate 3

Plate 4. Dwarf bushy mutant (gamma ray 50 krad)

Plate 4

Plate 5. Sparsely braching small leaved mutant (gamma ray 40 krad)

Plate 6. Dark green leaved mutant (gamma ray 50 krad)

Plate 5

Plate 6

The data pertaining to the frequency of viable mutations obtained by EMS treatment are given in Table 9.

(TABLE 9)

The mutation frequency calculated on M_2 progeny row basis does not show any definite dose dependence. The frequency of viable mutants tends to increase at first from 0.3 to 0.4 per cent, from where it falls to 0.5 per cent and there again increases to 0.6 per cent.

The different types of viable mutants produced by EMS treatment were with respect to growth habit, time of flowering, leaf size and shape and seed colour. Mutants with crinkled primary leaves were observed (Plate 8) A dwarf mutant with reduced branching habit and short internodes was observed with 0.5 per cent of mutagen treatment (Plate 9). With respect to the time of flowering, two early flowering mutants were observed in 0.5 per cent and 0.6 per cent EMS treatment. The former flowered 30 days earlier and the latter 35 days earlier than the control (Plate 10). Mutants with variation in seed coat colour was also observed in 0.3 and 0.4 treatments of EMS. Control had a dark brown seed coat colour, while the mutants possessed light brown and purple seed coats (Plate 11).

Table 9. Frequency of viable mutations in the M_2 generation (EMS)

Dose (in %)	No.of M_2 progeny rows		No.of mutations per 100 M_2 progeny rows
	Scored	Segregating	
Control	2	0	0.00
0.3	25	4	16.00
0.4	8	2	25.00
0.5	6	1	16.67
0.6	5	1	20.00

Plate 7. Round leaved mutant (gamma ray 20 krad)

Plate 7

Plate 8. Modification of the primary leaves

Plate 8.

Plate 9. Sparsely branching dwarf mutant (EMS 0.5%)

Plate 10. Early flowering mutant (EMS 0.5 & 0.6%)

Plate 9.

Plate 10

Plate 11. Variation in seed coat colour.

1. Normal (control)
2. EMS - 0.3%
3. EMS - 0.4%

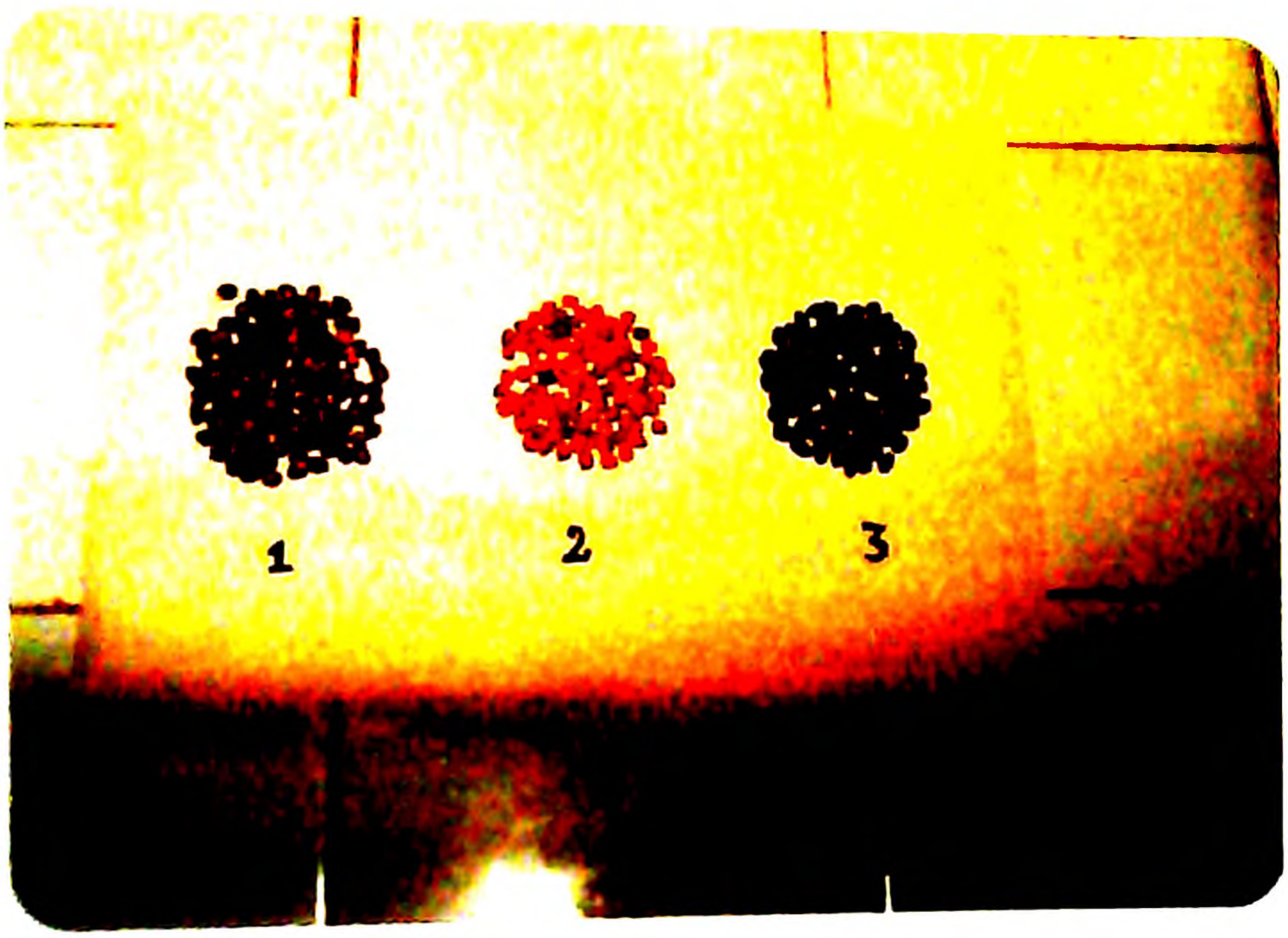

Plate 11

A comparison of the frequency of viable mutations induced by gamma rays and EMS has revealed the following: The distribution pattern of frequencies with respect to gamma rays is found to show a dose dependence to certain extent, where as in the case of EMS no such linearity is seen.

3. Mutagenic effectiveness and efficiency

The mutagenic effectiveness and efficiency of the different doses of gamma rays in inducing chlorophyll mutations were estimated and are presented in Table 10.

(TABLE 10)

It is seen that the percentage of lethality does not have a linear relationship with dose. At first it rises from 10 to 20 krads from where it shows a decline and thereafter again it increases. Considering the injury and sterility percentages, increasing doses of the mutagen, always increase the percentages of injury and sterility.

Mutagenic effectiveness follows a decreasing pattern with the dose of the mutagen i.e. it has an inverse relationship with dose.

Table 10. Mutagenic effectiveness and efficiency (gamma rays)

Dose (in krads)	No. of mutants per 100 M_2 progeny rows	Lethality (L)	Injury (I)	Sterility (S)	Mutagenic effectiveness $\frac{M \times 100}{krad}$	Mutagenic efficiency $\frac{M \times 100}{L}$	$\frac{M \times 100}{I}$	$\frac{M \times 100}{S}$
Control	0.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00
10	82.86	8.40	5.10	5.10	828.57	986.39	1624.65	1624.65
20	68.57	27.90	12.80	9.90	342.86	245.78	538.71	692.64
30	91.43	19.50	18.40	15.20	304.76	468.86	496.89	601.50
40	85.71	23.50	24.50	19.10	214.29	364.74	349.85	448.77
50	82.86	48.70	26.90	24.50	165.71	170.14	308.01	338.19

Considering the efficiency of gamma rays on the basis of lethality, injury and sterility, 10 krad treatment is found to be the most efficient and 50 krad treatment the least.

The data pertaining to the mutagenic effectiveness and efficiency of the different doses of EMS in inducing chlorophyll mutations are given in Table 11.

(TABLE 11)

Considering the lethality, injury and sterility percentages, all the three are found to follow an increasing pattern of distribution with increasing dose of the mutagen i.e. they have a direct dose dependence.

Mutagenic effectiveness seems to be high at the lowest doses of EMS, thereby showing an inverse relationship with dose.

Mutagenic efficiency of EMS on the basis of lethality, injury and sterility is found to be highest at the lowest dose of the mutagen (0.3%). The efficiency follows a decreasing pattern with increasing dose upto 0.5 per cent wherein, it suddenly shoots up at 0.6 per cent.

Table 11. Mutagenic effectiveness and efficiency (EMS)

Dose (in %)	No. of mutants per 100 M_2 progeny rows	Lethality (L)	Injury (I)	Sterility (S)	Mutagenic effectiveness $\frac{M \times 100}{c \times t}$	Mutagenic efficiency		
						$\frac{M \times 100}{L}$	$\frac{M \times 100}{I}$	$\frac{Mx 100}{S}$
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.3	56.00	18.30	21.80	13.90	3113.11	306.01	256.88	402.88
0.4	62.50	26.20	32.80	19.60	2604.17	238.55	190.55	318.88
0.5	16.67	36.60	37.70	25.20	555.67	45.54	44.21	66.14
0.6	80.00	43.30	41.40	34.70	222.22	184.76	193.24	230.55

A comparison of the two mutagens for their effectiveness and efficiency is attempted here. In the case of mutagenic effectiveness, it is seen that EMS is more effective than gamma rays in producing chlorophyll mutations. With respect to efficiency of the mutagen, it can be said that gamma rays have a higher efficiency compared to EMS as judged by the magnitude of lethality, injury and sterility caused by the two mutagens.

Discussion

DISCUSSION

The success of any breeding programme mainly depends on the extent of genetic variability available in the breeding population. Induced mutagenesis has been recognised as one of the means by which genetic variability can be created in the population. This variability can further be exploited by the breeder and superior genotypes can be picked up by adopting suitable selection procedures.

The term mutation was introduced into biology by Hugo De-Vries in 1900. He suggested the concept of inducing artificial mutations and utilizing them by the breeder in his attempts on crop improvement. However, artificial induction of mutations as an approach in plant breeding was recognized only after the classical works of Muller (1927) in drosophila and Stadler (1928) in barley and maize who successfully employed X-rays and induced mutations artificially. These discoveries of Muller and Stadler paved the way for further mutation breeding research. Following this, Gustafsson (1947) with his pioneering work in some agricultural crops like barley, wheat, oats, rye, pea etc., recognized the practical utilisation of radiation to induce useful mutations.

Among the various types of radiations, gamma irradiation is one of the widely used physical mutagens of the present day. Different doses of gamma irradiation, ranging from 0 to 100 krads have been tried and reported to be effective in various pulse crops.

Besides radiations, a large number of other chemical substances are also reported to have mutagenic properties and have been widely used to induce mutations in plants (Ehrenberg et al. 1961; Konzak et al., 1965). Freeze (1963) classified chemical mutagens as base analogue substitutes, dyes, acids, metals and alkylating agents. In higher plants, the last group viz., alkylating agents especially EMS has been reported to be very effective. The relatively low toxic and high genetic effects of EMS (Gaul, 1961) and its high mutagenic effectiveness as well as efficiency in higher plants (Konzak et al., 1965) are factors which favour for its wider practical application.

Red gram is an important pulse crop of peninsular India. Based on per capita consumption it ranks first among the pulses. Since it contains a fairly high amount of protein (22.3%), it can along with other pulses very well be considered as a protein substitute in the vegetarian kitchen. Because of the limited amount of variability which is presently available in pulses in general and in red gram in particular, it is considered necessary to undertake means of inducing genetic variability through induced mutagenesis.

In the light of the facts mentioned above, the choice of the problem is fully appropriate and justifiable. The inclusion of gamma rays in a dose range from 10 to 50 krads and EMS in a dose range from 0.3 to 0.7 per cent is also justified as supported by information gathered from allied pulse crops.

The present study, a continuation of an ongoing project in the department, was taken up with the objective of finding out the biological effects of gamma rays and EMS in the M_2 generation of red gram. These biological effects produced in the M_2 and later generations are determined by the mode of action of the mutagens and the interaction of many factors in the organism subjected to mutagen treatment. Among the various parameters employed in measuring these biological effects of mutagens in the M_2

generation, chlorophyll mutations including frequency, spectrum and segregation ratio are reported to have been widely employed for assessing the effectiveness of mutagenic treatments in higher plants (Gaul, 1964; Nilan et al., 1965; Kawai, 1969). From the utility point of view, the success of a mutagen treatment depends upon its ability to throw apparently different, but useful segregants capable of producing viable seeds, thereby enabling their maintenance, in the progeny. An estimate of viable mutations is capable of giving a correct picture in the magnitude of such abnormal segregants. According to Konzak (1965), mutagenic effectiveness gives a measure of gene mutations in relation to dose and mutagenic efficiency provides an estimate of the biological effects such as lethality, injury and sterility. As such the parameters viz., chlorophyll mutations including frequency, spectrum and segregation ratio, viable mutations, mutagenic effectiveness and efficiency etc. observed in the present investigation are capable of giving satisfactory answers to the problems contained in the objectives of the study.

The results obtained in the present study with reference to the effect of the mutagens viz., gamma rays and EMS are discussed in the following lines so as to draw valid and reliable conclusions.

1. **Chlorophyll mutations**

(a) **Frequency:** The mutations produced in the M_2 and subsequent generations are determined as a result of the mode of action of the mutagens and also of interaction of many associated factors in the organism subjected to mutagen treatment. In higher plants it has been reported that chlorophyll mutations give a correct estimate for the effectiveness of mutagen treatments (Gaul, 1964; Nilan et al., 1965 and Kawai, 1969). According to Gaul (1964) chlorophyll mutations form the most frequent gene mutations which can be clearly recognized and classified and they can also be studied in a limited space under semi-controlled green house conditions and they provide a rapid information since the seedlings alone need be raised.

In the present investigation, chlorophyll mutants were classified as the number of mutants per 100 M_2 progeny rows and the number of mutants per 100 M_2 seedlings, for both gamma rays and EMS. In either case, viz., the frequencies estimated on M_2 progeny row basis and on M_2 seedling basis, the pattern of change in the frequency in relation to dose was the same not only for gamma rays but also for EMS. In case of both the mutagens, the frequency did not appear to exhibit any relationship with dose (Fig.1). Such erratic behaviour of the frequencies in relation to dose of the mutagen has earlier been reported by Varadanyan (1976) in French bean, Kulkarni and Shivasankar (1978) in Horse gram and Venkateswarlu *et al*. (1980) in pigeon pea.

In the present investigation, a comparison of the two mutagens, gamma rays and EMS regarding their efficiency in inducing chlorophyll mutations cannot be considered to be absolute because of the limited number of M_2 progeny rows as well as M_2 seedlings scored under EMS treatment in comparison with the same under gamma ray treatment. However, barring the above discrepancy, based on the results obtained it has been found that gamma ray is more efficient in inducing chlorophyll mutations as compared to EMS, since the different doses of gamma rays have yielded higher frequencies not only on the basis of M_2 progeny rows but also on M_2 seedlings. These results are in agreement with that of Venkateswarlu *et al*.(1980) in pigeon pea. However, the results obtained in this study do not agree with those of Zannone (1965) in *Vicia sativa*, Akhun-sade and Hvostova (1966) in pea,

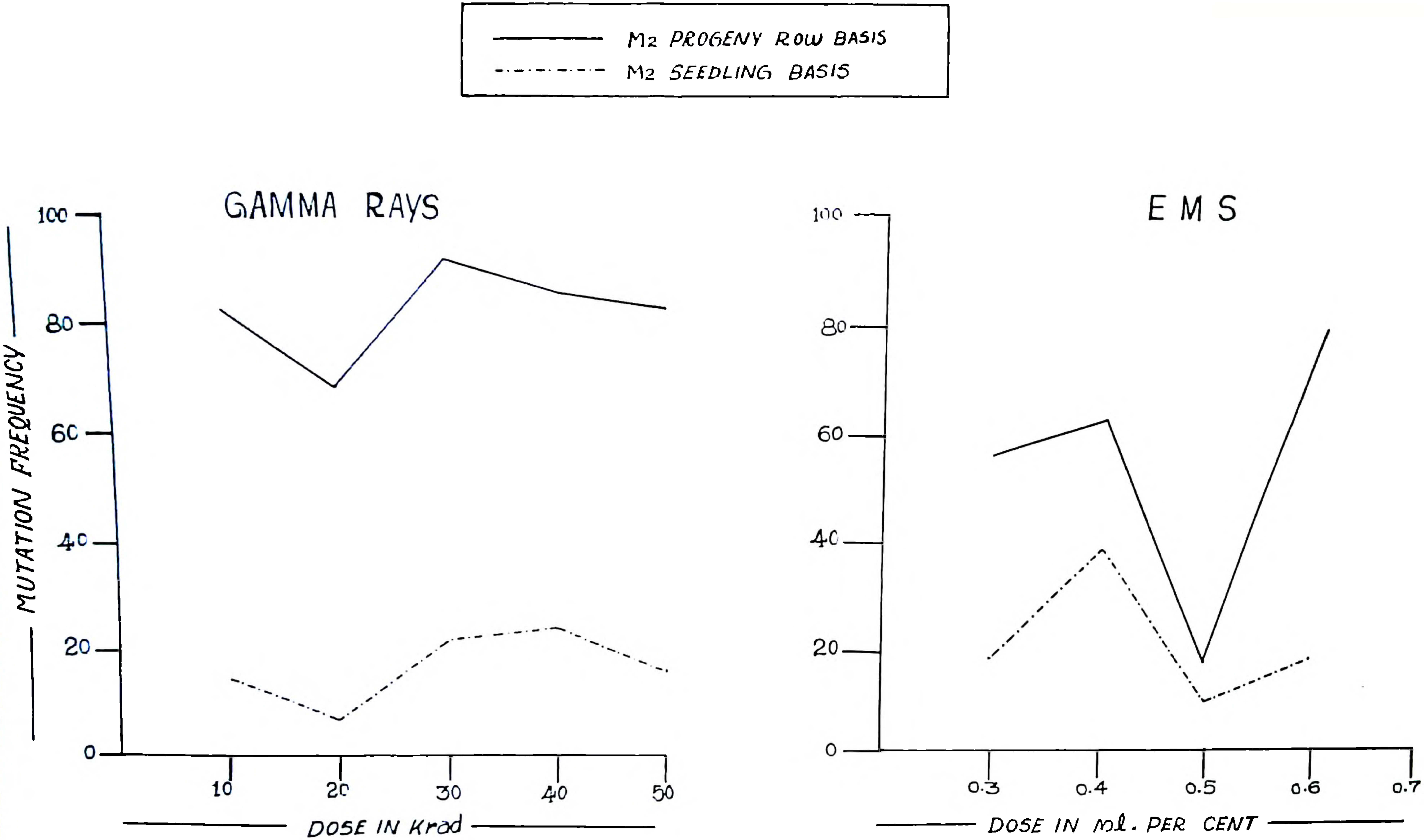

FIG. 1. FREQUENCY OF CHLOROPHYLL MUTATIONS IN THE M2 GENERATION (IN %)

Illieva-Staneva (1973) in French bean and Manju (1981) in Horse gram. These dásparities observed in the present case require further confirmation.

(b) <u>Spectrum</u>: In the present investigation, the spectrum of chlorophyll mutants obtained included xantha, viridis, chlorina, maculata, alboviridis and viridalba. This is in agreement with the results of previous investigators who have reported a fairly wide spectrum of chlorophyll mutations in the leguminous crops (Akhun-zade and Mvostova, 1966; Sidorova, 1968; Swarup and Gill, 1968; Rukananski, 1972; Dahiya, 1973; Illieva-Staneva, 1973; Iyyengar and Subramanian, 1974; Apparao and Jana, 1975; Nerkar, 1976; Vardanyan, 1976; Chekalin, 1977; Venkateswarlu *et al.*, 1978; Sichkar, 1979 and Manju, 1981).

The albino mutants were strikingly absent in all the doses of gamma rays and EMS. Xantha mutants were, however, present in 20 and 30 krads of gamma rays but absent in all the doses of EMS. Viridis were observed in all the doses of gamma rays and also EMS, its frequencies showing an erratic behaviour with reference to the five doses of gamma rays tried. However, frequencies of viridis mutants were found to increase along with an increase in the concentration of EMS. Chlorina and maculata mutants were observed in three and four out of the five doses of gamma rays tried, having no relationship with dose. In the case of EMS, chlorina types were totally absent in all the doses while maculata was realised in the lowest concentration. Alboviridis and viridalba mutants could be observed in only 40 krad dose of gamma rays while in the case of EMS, alboviridis could be spotted in one out of the four doses of the mutagen with viridalba types occurring in none of the doses (Fig.2 and 3).

In general gamma rays induced a wider spectrum of chlorophyll mutants, with six types, compared to EMS where only three types could be seen. Similar results regarding the ability of gamma rays to induce a wider spectrum of chlorophyll mutants have been reported by Rukmanski (1972), in French bean. Lack of sufficient population under EMS treatment might perhaps be the reason for the narrow spectrum of chlorophyll mutants realised here. However, this needs confirmation.

(c) <u>Segregation ratio</u>: In the present investigation, segregation ratios of chlorophyll mutants did not show dose dependence, either for gamma irradiation

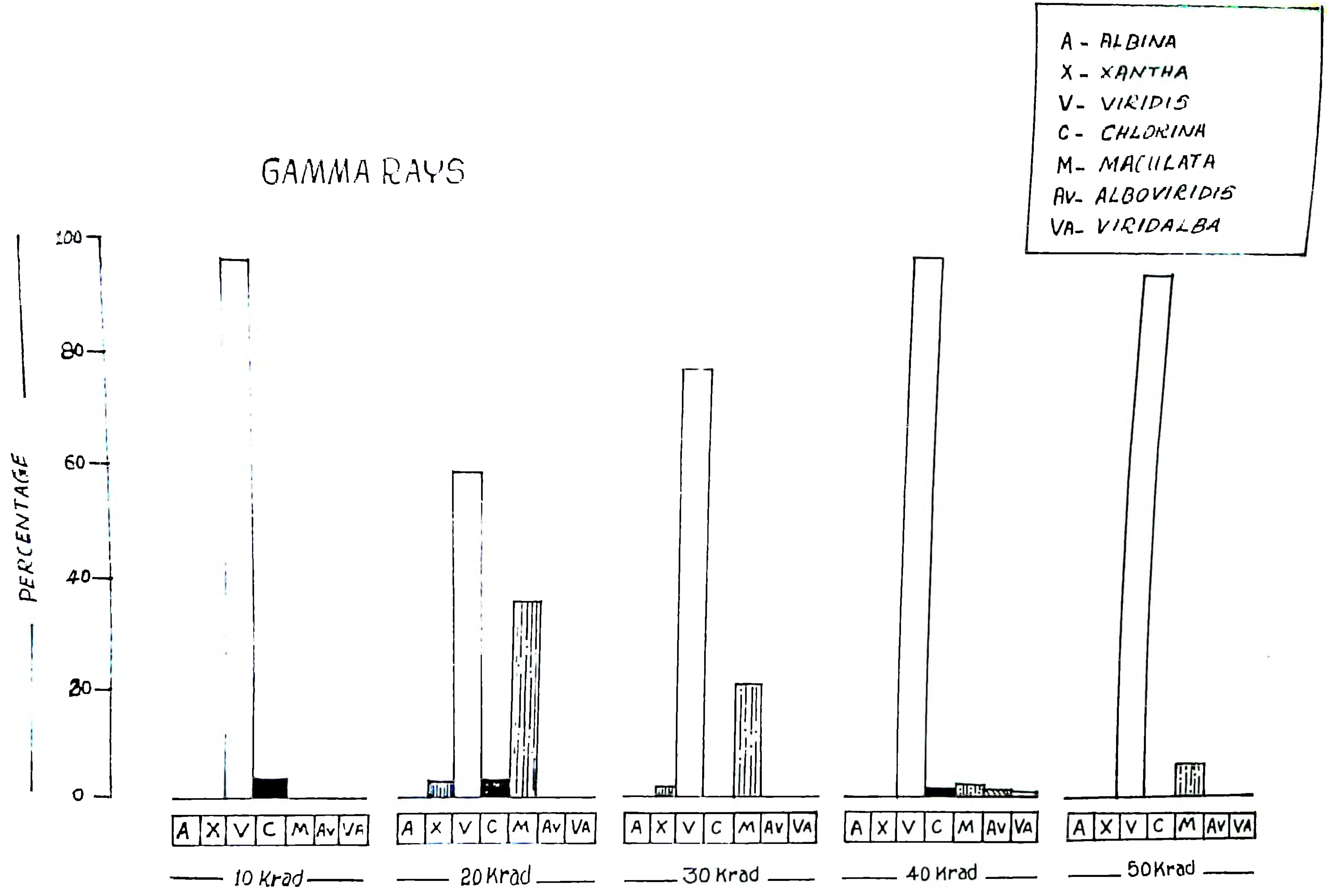

FIG:2 CHLOROPHYLL MUTATION SPECTRUM

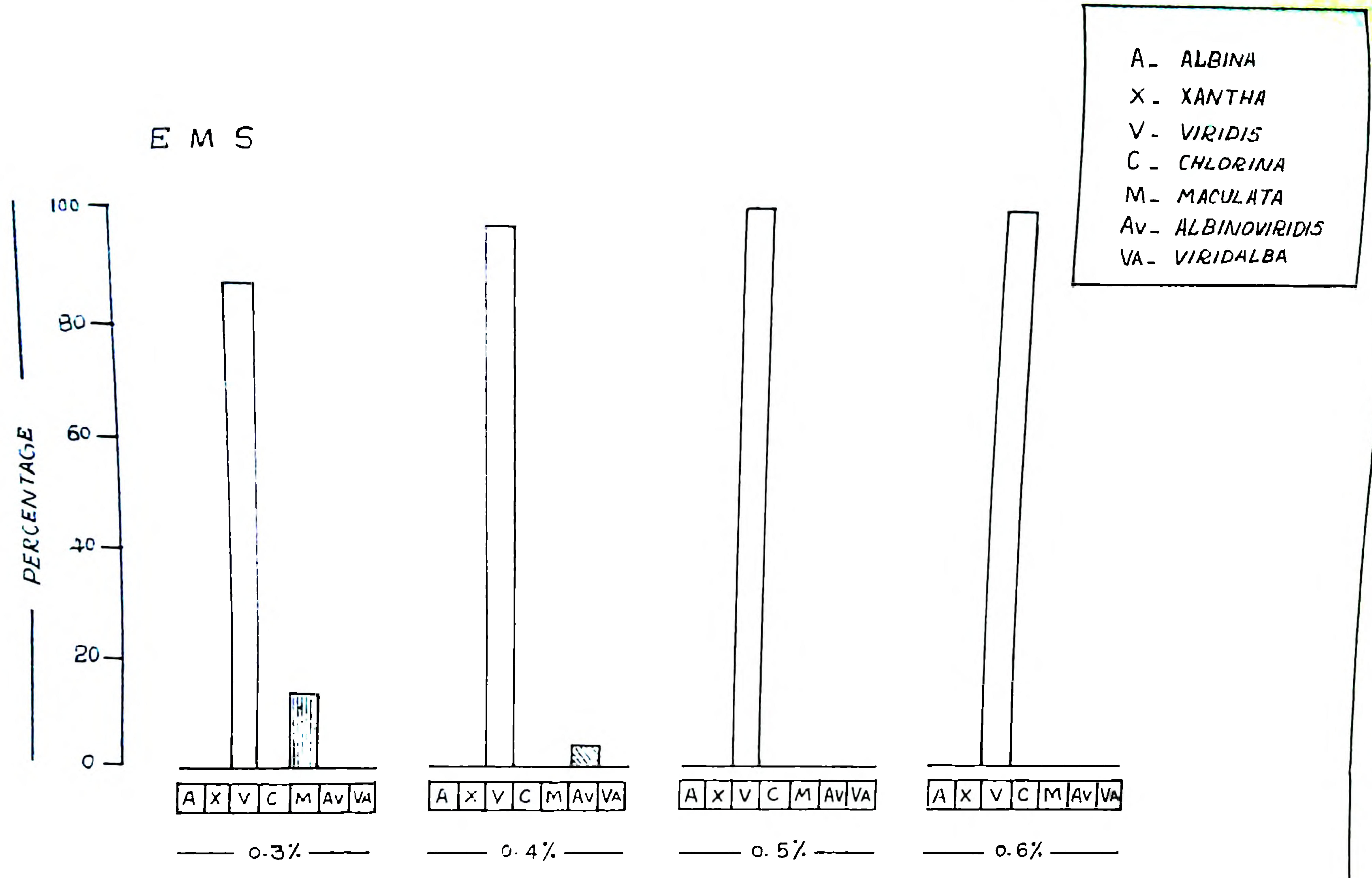

FIG.3 CHLOROPHYLL MUTATION SPECTRUM.

for EMS treatment. Manju (1981) had obtained similar results in horse gram in case of gamma irradiation. However, in case of EMS treatment, she had reported a direct relationship with dose. A comparison of the two mutagens based on the values of segregation ratios is not attempted here, since it cannot give any valid and reliable information, because of the difference in population size under the two mutagen treatments.

2. Viable mutations

The viable mutation frequency was estimated as the number of mutations per 100 M_2 progeny rows. Viable mutants produced by gamma rays showed a direct dose dependence upto 40 krads from where it decreased to 50 krads. In the case of EMS, the viable mutation frequency did not show any linear relationship with dose. With respect to gamma irradiation, this result obtained is in partial agreement with those obtained by Krishnaswami and Rathinam (1980) in green gram, where the frequency of viable mutations increased with mutagen dose. However the results obtained in the present study did not agree with the findings of Tavcar (1965) in certain cereals and Manju (1981) in horse gram.

The different types of viable mutants produced by gamma irradiation were with respect to growth habit, leaf size and shape, while EMS induced changes in growth habit, time of flowering, leaf size and shape and seed colour. Recovery of various viable mutants has been reported by Athwal (1963), Athwal *et al.* (1970) in Cicer; Pipie (1969)b and Kalinina (1972) in pea, Benkowska and Rymsza (1970) in *Phaseolus vulgaris*, Bhatt *et al.* (1972), Saini *et al.* (1974), and Krishnaswami *et al.* (1977) in green gram, Constantin and Love (1964), Louis and Kadambavanasundaram (1975) and Narsinghani and Kumar (1976) in cowpea, Kesavan and Khan (1978) in winged bean, Kulkarni and Srivasankar (1978) in horse gram; Sharma and Sharma (1979)b in lentil and Kazanzhi *et al.* (1984) in French bean.

In the present study, many deviations from the normal growth pattern of the plants were observed in the M_2 populations of gamma rays and EMS treatments. This included bushy, reduced branching, dwarf, small leaved, dark green leaved, round leaved and varying seed coloured types. Such mutant types were reported by Priadeencu (1961), Moh and Alan (1964) in French bean, Sanjeeviah *et al.* (1969) in groundnut, Jaranowski (1970) and

Sachanski (1970) in pea, Bankowska and Rymsza (1970), Pande and Seth (1975) and Al-Rubeai (1982) in *Phaseolus vulgaris* L.

Some of the dwarf mutants found in the M_2 population possessed short stature and profuse branching capacity giving them a bushy appearance. It might be possible that in such plants the main shoot apex might have stopped growth and differentiation very early in the ontogeny while the axillary buds might have carried on further growth as suggested by Joshua and Rao (1972). Moreover, the number and length of internodes in such individuals were also reduced. Dwarf mutants with reduced branching habit were also observed. Sanjeeviah *et al*.(1969) observed varying frequencies of dwarf plants in groundnut, under different dosages of radiations but such mutants were more frequent with gamma rays than with X-rays, indicating that the locus or loci concerned were more sensitive to gamma rays than to other radiations. Athwal *et al*. (1970) observed short statured mutants in *Cicer* using gamma rays which showed profuse branching. Mutants with round leaves were also detected in the population under the present study. Such mutant types showing variation in leaf shape were observed by Sobolev (1966), Jaranowski

(1970) in pea, Grover and Tejpaul (1979) in *Phaseolus aureus* and Manju (1981) in horse gram.

Viable mutants with changes in leaf morphology were noticed in the present study, both under gamma ray and EMS treatments. Leaf variations such as alteration in the number and size of leaflets, were noticed. These leaves lacked one or two lateral leaflets, thereby appearing as bifoliate or unifoliate leaves instead of the normal trifoliate leaf. Gunckel and Sparrow (1961) reported that leaf abnormalities were commonly observed after irradiation and apparently the youngest leaf exhibited the highest frequency of leaf alteration. In cowpea following irradiation with gamma rays and neutrons, Constantin and Love (1964) observed that many of the trifoliate leaves had one or two lateral leaflets missing. Plants with crinkled primary leaves were observed in the population with both mutagen treatments. Small leaved mutants with sparse branching habit were also obtained in the present study. Swarup and Gill (1968) observed leaf mutants showing crumpled leaves, very small leaves and large leaves in French bean, following X-ray treatment. Similar leaf mutants were observed in other leguminous crops also, such as in soybean by Singh *et al*. (1974), in black gram by Apparao and Jana (1976) and in green gram by Krishnaswami et al. (1977). A dark green leaved mutant with reduced height and very few branches was obtained in 50 krad treatment of gamma rays. Similar mutants for leaf colour and leaf shape were obtained by Kesavan and Khan (1978) in Winged bean.

Early flowering mutants were observed in treatments with EMS. Among the two observed, one flowered 30 days earlier and the other 35 days earlier than the control. Such early flowering mutants were also observed by Sobolev (1966) in pea and by Bhatt et al. (1972) in green gram.

Mutants with variation in seed coat colour were obtained with EMS treatments. Control had a dark brown seed coat colour, while the mutants possessed light brown and purple seed coats. The results obtained by Sharma and Sharma (1979) in lentil, Tagaki and Hiraiwa (1980) in soybean and Kazanshi et al. (1984) in French bean are in support of the findings of the present study.

3. Mutagenic effectiveness and efficiency

Konzak et al. (1965) proposed the term effectiveness as a measure of gene mutations in relation to dose and efficiency as estimate of biological effects induced such as lethality, injury and sterility. To obtain high efficiency, the mutagenic effect must greatly surpass other damaging effects in the cells such as chromosomal aberrations and toxic effects. Gaul *et al.* (1972) was of the opinion that the effectiveness of a mutagen was of theoretical importance but did not have any immediate practical implication, while for practical purposes the aim was to get high efficiency.

The effectiveness and efficiency of mutagenic agents depend on the nature and characteristics of the organism as a whole, as well as on the specific properties of the tissue treated, in addition to the properties of the mutagenic agent. The greatest efficiency in mutation experiments depends not only on the selection of a mutagen with characteristics suited to the biological material but also on appropriate treatment regime (Konzak *et al.*, 1965) Efficient treatments are essential for economical use of mutagens as a tool for direct improvement or for the induction of certain changes in qualitative and quantitative traits.

Gaul (1964) employed chlorophyll mutations for assessing the effectiveness and efficiency of mutagenic treatments in higher plants. Kawai (1969) stated that the chlorophyll mutations were taken as a basis for estimating the effectiveness and efficiency, on the assumption that the other types of mutations were induced with frequencies parallel to that of chlorophyll mutations.

An important aim of mutation breeding research is to increase the efficiency of induction of genetic changes. Increased efficiency may be expressed in two ways (Nilan et al., 1965) viz. 1) as higher ratios of mutations to chromosome aberrations or to surviving plants where beneficial mutations are sought; and 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 investigation effectiveness was maximum at the lowest doses of both the mutagens, thereby showing an inverse relationship with dose. Konzak et al (1965) obtained similar results in the barley.

The mutagenic efficiency was determined taking into consideration the biological parameters such as lethality, injury and sterility. The percentage of lethality did not have a linear relationship with the doses of gamma rays where as in the case of EMS, it showed a direct relationship with dose. Injury and sterility percentages with respect to both the mutagens showed a definite dose relationship (Fig.4).

Considering the efficiency of gamma rays and EMS on the basis of lethality, injury and sterility, it was found that among the various doses of gamma rays employed, 10 krad treatment was the most efficient and 50 krad treatment - the least. Among EMS treatments, 0.3 per cent was found to be the most efficient. The efficiency followed a decreasing pattern with the increasing dose up to 0.5 per cent from which, it suddenly increased at 0.6 per cent. The efficiency was higher at lowest doses of both the mutagens. Konzak et al. (1965) reported higher mutagenic efficiency in barley at low doses and it decreased as the dose increased. The reason for greater efficiency at low doses of mutagens seemed to be related to the fact that lethality, injury and sterility increased with dose at a rate faster than the occurrence of mutations.

In the present study, EMS was found to be more effective than gamma rays in producing chlorophyll mutations. With respect to efficiency of the mutagen, gamma rays had a higher efficiency than EMS as judged by the magnitude of lethality, injury and sterility caused by the two mutagens.

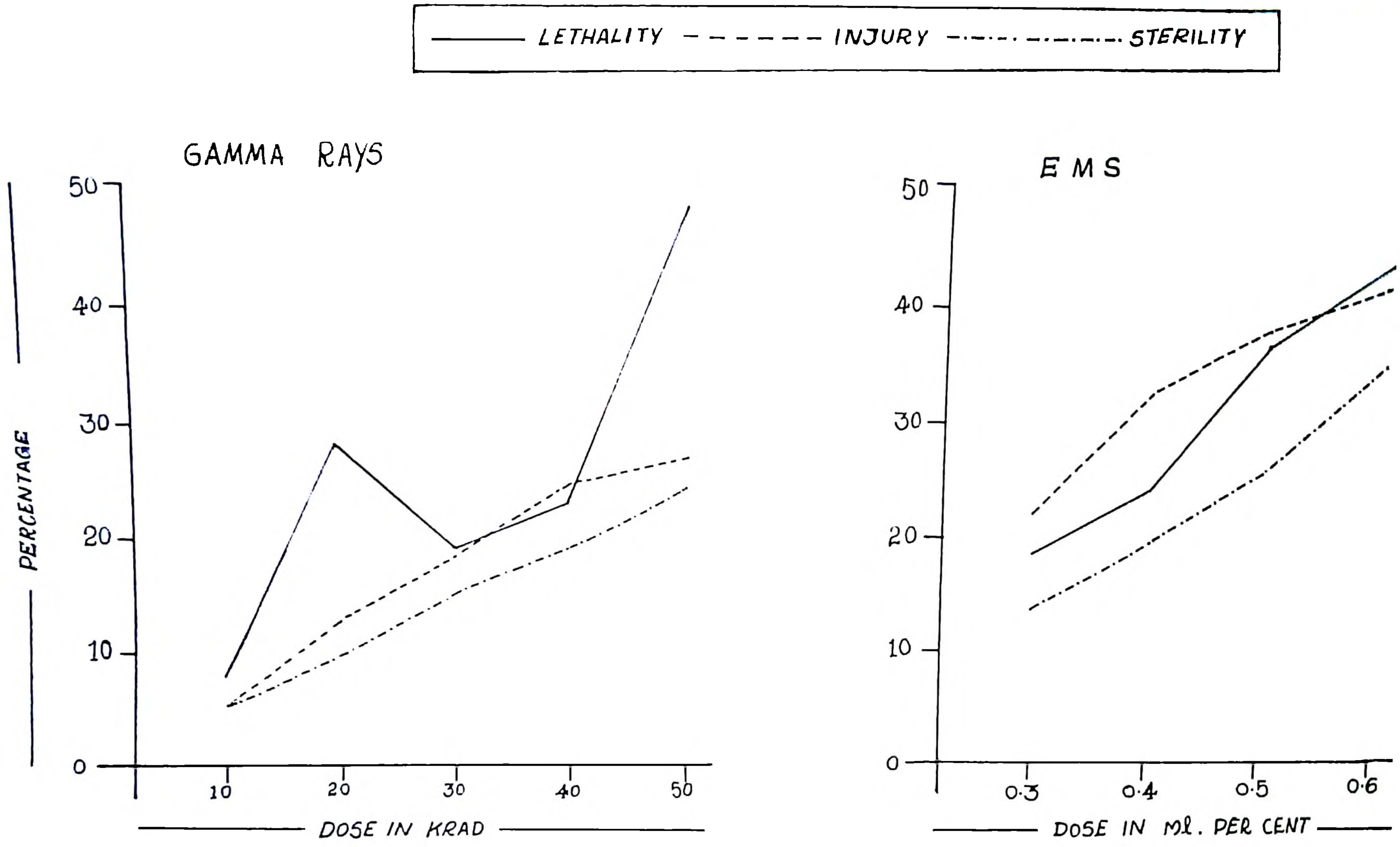

FIG.4. EFFECT OF VARIOUS DOSES OF MUTAGENS ON LETHALITY INJURY AND STERILITY

Summary

SUMMARY

Studies were undertaken on the biological effects of five doses of gamma rays (10, 20, 30, 40 and 50 krads) and EMS (0.3, 0.4, 0.5, 0.6 and 0.7 per cent) on SA-1 variety of red gram, in the M_2 generation. The five concentration of EMS included in the present investigation yielded comparatively few M_1 plants that reached to maturity, resulting in the production of very few seeds for raising the M_2 generation. The highest dose of EMS viz., 0.7 per cent proved to be lethal, since no M_1 plant could produce seeds for studying the M_2 lines representing this concentration. The effectiveness and efficiency of different doses of the two mutagens in inducing chlorophyll mutations including their frequency, spectrum and segregation ratio and also the different types of viable mutations were determined and the following conclusions have been drawn.

The pattern of change in the frequency of chlorophyll mutations estimated on M_2 progeny row basis and M_2 seedling basis, in relation to dose, was found to be similar for both gamma rays and EMS. In case of both the

mutagens, the frequency did not appear to exhibit any dose relationship.

The spectrum of chlorophyll mutants obtained included xantha, viridis, chlorina, maculata, alboviridis and viridalba. The frequency of different types of chlorophyll mutants varied with different doses of the mutagen with no visible dose relationship.

Gamma rays induced a wider spectrum of chlorophyll mutants of six types while EMS produced only three types.

The segregation ratios of chlorophyll mutants did not show any dose dependence either for gamma irradiation or for EMS treatments.

The frequency of viable mutants estimated on 100 M_2 progeny row basis increased along with the dose of gamma rays upto 40 krads after which it decreased. In the case of EMS, the frequencies did not show any relationship with dose.

Both gamma rays and EMS produced viable mutants involving changes in growth habit, leaf size and shape. In addition to this, EMS induced changes in time of flowering and seed colour.

The mutagenic effectiveness in inducing chlorophyll mutations was the highest at the lowest dose and lowest at the highest dose of both the mutagens, thereby showing an inverse relationship with dose.

Mutagenic efficiency of gamma rays on the basis of lethality, injury and sterility was highest at the lowest 10 krad dose and lowest at the highest 50 krad dose, thereby showing an inverse relationship with dose. In the case of EMS, 0.3 per cent was the most efficient, the efficiency being decreased correspondingly along with the increase in the concentration of the mutagen upto 0.5 per cent of the mutagen after which it suddenly increased.

EMS proved to be more effective than gamma rays in producing chlorophyll mutations. However, gamma rays were more effective than EMS as judged by the magnitude of lethality, injury and sterility caused by the two mutagens

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BIOLOGICAL EFFECTS OF GAMMA RAYS AND EMS IN THE M_2 GENERATION OF RED GRAM (*Cajanus cajan* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The studies reported herein were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara, during the period 1984-'86.

Seeds of the M_1 generation of SA-1 variety of red gram (<u>Cajanus cajan</u> L.) which were initially subjected to induced mutagenesis using five doses of gamma rays (10, 20, 30, 40 and 50 krad) and five doses of EMS (0.3, 0.4, 0.5, 0.6 and 0.7 per cent) were made use of to raise the M_2 generation and their biological effects were studied.

It was observed that in the chlorophyll mutation frequency estimated on M_2 progeny row basis and M_2 seedling basis, the pattern of change in the frequency in relation to dose was found to be the same not only for gamma rays but also for EMS. In case of both the mutagens the frequency did not appear to exhibit any dose relationship.

The spectrum of chlorophyll mutants obtained, included xantha, viridis, chlorina, maculata, alboviridis and viridalba. The frequency of the different types of

chlorophyll mutants was found to vary with the different doses of both the mutagens and did not show any dose relationship in majority of the cases. In general, gamma rays induced a wider spectrum of chlorophyll mutants with six types compared to EMS where only three types could be seen.

The segregation ratios of chlorophyll mutants did not show any dose dependence either for gamma irradiation or for EMS treatment.

The viable mutation frequency was estimated on 100 M_2 progeny row basis. Viable mutants produced by gamma rays showed a dose dependence upto 40 krads from where it decreased to 50 krads. In the case of EMS, the frequencies did not show any dose relationship.

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

The mutagenic effectiveness in inducing chlorophyll mutations was high at the lowest dose of both the mutagens viz., 10 krad of gamma rays and 0.3 per cent EMS treatment, thereby showing an inverse relationship with dose.

Considering the efficiency of gamma rays and EMS on the basis of lethality, injury and sterility, 10 krad of gamma rays and 0.3 per cent EMS was found to be the most efficient, showing that the efficiency was highest at the lowest doses of both the mutagens.

The mutagenic effectiveness of EMS was found to be higher than that of gamma rays in producing chlorophyll mutations. With respect of efficiency of the mutagens, gamma rays have a higher efficiency than EMS as judged by the magnitude of lethality, injury and sterility caused by the two mutagens.