MUTAGENICITY OF GAMMA RAYS AND EMS ON WINGED BEAN [*Psophocarpus tetragonolobus* (L.)]

By

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THESIS

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1995

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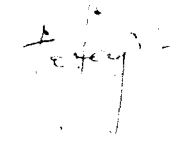

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INTRODUCTION

I. INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus* (L.)] is an under exploited nutritious legume with a great many positive attributes. The winged bean is highly productive in tropical environments and is an excellent source of protein. The winged bean produces an array of econonomically important products including pods, seeds, tuberous roots and animal fodder. Virtually all organs are edible and generally multiple organs are consumed. (Haq, 1982, NAS, 1975). Seeds of this crop is nutritionally equivalent to soyabean.

The report of National Academy of Sciences in 1975 on the potential of the winged bean as a protein and oil crop generated interest among the agriculturalists throughout the world. Inspite of its importance little attention has been paid to improve the genetic potential of the crop. Now also several wild characters exist in most of the winged bean accessions and only very little variability in maturity and other characteristics are available. It is obvious that for effective crop improvement a diverse genepool with genetic variability is necessary to select better plant type which

will make winged bean a successful crop. An intensive search for such plant types should be needed, otherwise mutagenesis can be adopted. (Chomechalow, et al. 1982). The inherent low genetic variability caused by long periods of cultivation as a backyard crop and perpetuated by self-pollination have imposed limitations on the use of conventional plant breeding methods for the crop improvement. In winged bean, being a self pollinated crop induced mutation technique can be successfully employed as in many other crops including pulses to create and expand the genetic variability that is different from the kind obtainable through gene recombination. Mutation breeding refers to induction of mutation with mutagens at suitable doses. Since the induced changes occur randomlyin desired and undesired direction the treated material has to be screened and the mutation yield has to be assessed. Hence in the present investigation an attempt was made to assess the effect of two mutagens, gamma rays and EMS in M2 generation of winged bean.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Mutation research in the past years has added valuable information on various aspects of mutation breeding. Hence a review of literature, is presented, on some important studies of mutation research.

Since the discovery of Muller (1927) in Drosophila and Stadler (1928) in plants that ionizing radiations can induce hereditary changes (mutation), there by increasing the rate of mutation many times than that exists in nature, we are no longer limited to the store of natural mutations only, for crop improvement, that occur at a very slow rate. Among the first researchers who used mutagenesis strictly for plant breeding, Freisleben and Lein (1942) succeeded in obtaining mildew resistance in Haisa barley following a treatment with X-rays. A chemical substance, β-β dichloro ethyl sulfide (mustard gas) was first used for mutation induction by Auerbach and Robson (1946). After these initial discoveries it was found that all ionizing radiations and also ultraviolet rays, were capable of inducing mutations. Similarly a wide range of chemical substances was soon found

to cause mutation. The particular characteristics of radiation have been described in radio-biological literature by Bacq and Alexander (1961) and Casarett (1968) and the types of special interest of crop improvement by Ahnstroem (1977). Literature on alkylating agents and its effects has been reviewed by Loveless (1966) and Freeze (1971). Mutagenic chemicals of potential use for crop improvement were described by Heslot (1977). Now the main emphasis is on gathering experiences with established mutagens regarding treatment procedures for a wide range of plant species such as leguminous and oil seed crops, vegetables, tropical fruits and tuber crops. (Donini, 1977, Micke 1984 and Donini et al. 1984).

INDUCED MUTATIONS IN THE M_2 GENERATION.

From the analysis of induced barley mutants Schloz and Lehman (1958; 1959; 1961; 1962) and Gustafsson (1963) showed that properties such as productivity, earliness, lodging resistance, protein formation and quality were conditioned by numerous genes and the mutations of qualitative or of the quantitative type can be employed to improve on these characters.

From practical point of view the phenotypes of mutations can be divided into macro and micro mutations (Gaul, 1964). Macro mutations can easily be recognized on a single plant and can easily be selected in the M_2, but micromutations can only be detected in a group of plants by statistical methods. The procedure with micromutations used by Gaul (1965) was to select at random the M_2 plants which appeared to be non-mutated and atleast as vigorous as normal plants

According to Ganguli (1993) the successful utilization of induced mutations as a method of plant breeding is dependent on various factors like choice of parents, characters to be improved, the type of mutagen and its doses used, experimental procedures chosen and the efficiency of detection of mutations.

Published works on mutagenesis in winged bean is scarce. Hence literature related to the study in major pulse crops are also briefly reviewed under the following sections.

2.1. Chlorophyll mutations

Gustafsson (1947) reported that chlorophyll mutants in the M_2 generation would provide a good guide line for estimating efficient mutagen treatments and leafspots or leafstreaks occurring after mutagen treatment would apparently be dose dependent. The occurrence of chlorophyll deficient mutant might be attributed to change in gene or a set of genes in recessive forms in M_1 generation and exposed in M_2 generation onwards (Gottschalk and Wolff, 1983). Shik (1991) reported after studying cytogenetic, physiological and biochemical characteristics of chlorophyll mutations that the chlorophyll mutants were cytologically unstable with a high frequency of structural chromosome rearrangement and with a reduced rate of photosynthesis.

Frequency

Among the three methods of estimating chlorophyll mutation frequency, viz., 1) mutations per 100 M_1 plants. 2) Mutations per 100 M_1 inflorescences and 3) Mutations per 100 M_2 plants, the last method gave good approximation of the frequency of chlorophyll mutations and it was proportional to

the initial rate of mutation and was independent of variation in progency size and size of the mutated sector (Gaul, 1960, Blixt et al. 1963, Blixt, 1966). Yankulov et al. (1979) studied sensitivity of gamma rays and EMS on bean. They related M_1 generation survival to frequency of M_2 chlorophyll mutations and found that there was an inverse relationship for the two mutagens between percentage survival and percentage chlorophyll mutations and a direct correlation between dose and percentage of chlorophyll mutations. Varadanyan (1976) found no correlation between the frequencies of chlorophyll mutations and morphological mutations in french bean following EMS and DMS treatments. Venkateswaralu et al. (1980) observed that chlorophyll mutation rate showed linearity at low to medium doses, saturation and erratic behaviour at high doses of the mutagens and on comparing the frequencies, it was found that gamma rays followed by EMS and then HA was the order in inducing chlorophyll mutations. A sample seed lot obtained from M_1 plant survivals of a treatment, corresponding to three times the number of initially treated seeds proved to be effective for high chlorophyll mutation frequency and other mutants obtained in M_2 population (Tulman Neto 1990)

Spectrum

Chlorophyll mutations were classified into various groups by Gustafsson (1940) and Blixt (1961). The differential mode of chemical mutagens with regard to appearance of chlorophyll mutation was reported by Ehrenberg et al (1962). 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. Rukmanski (1972) found that the spectrum induced by gamma rays was wider than that induced by chemical mutagens. Vishnoi and Gupta (1980) assessed the chlorophyll content of the chlorophyll mutants which was in the order of viridalba, chlorina, striata, viridis, alboxantha, xantha and albina, Sinha and Himanshu (1984) reported the effect of various dosages of gamma irradiation on chlorophyll metabolism in greengram and other species of vigna, such physiological variability resulting in chlorophyll deficient mutants. Turishcheva et al. (1987) described the structure and function of chloroplasts in chlorophyll mutants. In chlorina type, lamellar system was irregular in structure, differing in this respect from that found in chloroplast of parent

lines. One mutant showed a 25% lower content of ribosomes and also lower chlorophyll content and reduced photosynthetic activity

Frequency of multiple chlorophyll mutations

The range of mutations was lower at higher doses of gamma rays eventhough high frequency was obtained (Mekhandzhiev and Vassileva, 1975). Similarly the greatest range of mutations was obtained using low concentration of EMS and ET. Packairaj (1988) reported that the frequency of M_1 plants segregating for two or more mutations decreased with dose.

Segregation ratio

Meono (1975) observed that the segregation ratios of chlorophyll mutations obtained by gamma irradiation were always lower than expected Mendelian segregation ratio. Manju (1981) reported that the segregation ratios of chlorophyll mutations didnot have any dose dependence with gamma irradiation, while with EMS treatment she reported definite dose relationship.

Winged bean

Kesavan and Khan (1978) treated two genotypes, UPS-31 and UPS-122 with both gamma rays and EMS. In case of gamma rays LD 50 was found to be between 15 - 20 kR and doses over 30 kR reduced germination drastically in M_1. Twenty per cent height reduction was reported in a treatment with of 0.05% of EMS. Many chlorophyll mutations were also reported.

Armachevilo and Bernardo (1981) observed yellowish variegation and leaf streaking in winged bean as an effect of treatment with EMS and Co^{60} gamma irradiation.

Veeresh and Sivasankar (1986) identified the spectrum of chlorophyll mutations in M_2 after gamma ray irradiation (10-30 kR) including five types of chlorophyll mutations, viz., albina, xantha, chlorina, viridis and albo-xantha. Chlorina was the most frequent type followed by xantha.

Blackgram

Jana (1964) identified chloro-xantha, chloroviridis, viridis, albo-xantha and chlorina types in blackgram. Maximum chlorophyll mutation frequency was observed in 60kR gamma ray treatment.

A wide spectrum of chlorophyll mutations, albina, chlorina, xantha, viridis and alboviridis was obtained and maximum frequency was recovered in 60kR gamma ray (Ramaswamy, 1973). It was also reported that the specificity for the mutants and mutagen was evident in higher doses only, albina being specific for gamma rays and xantha and viridis for EMS.

Kundu and Singh (1982a) reported that at LD 50 dose of gamma rays (50kR) the frequency of chlorophyll mutations was highest in the M_2 generations. The chlorophyll mutation frequency was highest by M_1 plant basis followed by M_1 cluster basis while it was lowest in M_2 seedling basis. Three types, albina, chlorina and alboviridis types were found in Type 9 and UPU-2 of blackgram after gamma ray irradiation. Viridis type was found only in Type 9 at higher dose (50kR).

In ADT-3 and Vamban-1 blackgram varieties, when subjected to gamma irradiation at the doses of 20-100kR and EMS treatments of 20-70mM, multiple chlorophyll mutants like albina, chlorina, viridis and albomaculata were reported Albina occured at low frequency, but higher proportion of chlorina was produced by both the mutagens in both the

varieties. Next to chlorina, xantha mutants were found to be frequent after gamma irradiation in the variety ADT-3 (Vaniarajan et al. 1993).

Cowpea

Louis and Kadambavanasundaram (1973) identified chlorophyll mutant, viridis in gamma irradiated M_2 seedlings. Albina was found in low frequency in 20kR gamma ray dose.

Large number of mutations was reported in the M_2, eventhough not a single chlorophyll mutant in M_1 could be spotted by Narasinghan and Kumar, 1976. Treatment with EMS produced albina, xantha, chlorina and striata, while treatment with MMS produced albina, xantha and chlorina types.

Palaniswamy et al. (1978) isolated chlorophyll mutations in cowpea after gamma irradiation. The spectrum suggested the occurrence of four types, of which viridis was the most frequent. Albina and chlorina were observed only at higher doses, while xantha appeared at intermediate doses.

Chlorophyll mutation frequency increased with increasing mutagen dose in cowpea (Sunny and Gopimony 1984).

Thirugunakumar (1986) identified albina, viridis chlorina and xantha types in the treatments with gamma rays and EMS separately and in combination. Albina was seen only in higher doses of gamma ray treatment.

Greengram

Dahiya (1973) identified four types of chlorophyll mutations, viz, xantha, albina, viridis and maculata with gamma irradiation in greengram, of which maculata appeared less frequently.

Rathnaswamy et al. (1978) reported that among the four types isolated chlorina and viridis were more than albina and xantha.

Grover and Virk (1984) observed a spectrum of chlorophyll mutations including xantha, chlorina, maculata, viridis, and xantha-chlorina. Maximum frequency of

chlorophyll mutants was seen in MNNG treated plants. xantha types were more frequent in X-ray irradiated treatments and chlorina in chemical treatments.

Kamini et al. (1988) reported four types of chlorophyll mutations, albina, xantha, viridis and striata in M_2 generation of gamma irradiation in mungbean. The higher dose level of 20kR to 25kR induced comparatively wider spectrum of chlorophyll mutants. Among the total of 1859 seedling mutants observed 15.91% were albina, 11.7% xantha, 18% viridis and 9.5% striata types.

Redgram

Venkateswaralu et al. (1978) undertook radiosensitivity studies and found that frequency was relatively low in all treatments when seven varieties of early, medium and late maturity groups were exposed to 5-40 KR Co^{60} gamma rays. Among the three types of chlorophyll mutations observed xantha was the most frequent and chlorina the least.

Following treatments with gamma rays, EMS and NMU separately five types of chlorophyll mutations appeared in M_2

generation. The occurrence of chlorina was more followed by viridis, xantha, tigrina and albina. The rate of chlorophyll mutation in M_2 generation was associated with the increase in the dosage of the chemical mutagens (Chaturvedi et al. 1982).

Brenda (1987) reported different spectrum of chlorophyll mutations including xantha, chlorina, viridis, maculata, viridalba and alboviridis.

Gram

Athwal (1963) identified lethal and sublethal mutations of variable chlorophyll deficiency after x-ray irradiation.

2.2 Viable mutations

According to Gaul (1961) the phenotypes of mutations can be recognised and divided into macro and micro mutations. During the past two decades, research in mutagenesis had expanded rapidly and large number of mutants in pulses with favourable characters were isolated.

2.2.1. **Macro mutations**

Viable macromutants recovered in various studies included leaf mutants, tall mutants, dwarf mutants, flower mutants, high yielding and early maturing types etc. According to Gottschalk and Wolff (1983) tall and bushy mutants represent the alternation in shoot system, the early maturing mutants in flowering and fruiting time and the leaf mutants in the number, size, shape and location of leaves.

Winged bean

Karikari (1981) found significant reduction in vegetative parts, seed yield and tuber yield when the seeds of genotypes were treated with 15 and 20kR gamma rays. The report also shows that by making use of irradiation bushy type plants requiring shorter staking could be developed.

Jugran et al. (1986) isolated three dwarf variants (height <38 cm.) in M_2, of which only one flowered and set seeds, from the gamma irradiated population of seventeen cultivars.

Savithramma (1987) found that 0.6% EMS was optimum for inducing mutations based on four characters in the genotypes SL-86 and Mysore local. M_2 mutants isolated included dwarf with compressed internodes, bushy, early and determinate types.

Veeresh and Sivasankar (1987) identified eleven early mutants in the M_2 generation from seeds exposed to gamma rays, which matured 27-34 days earlier than control plants and which had similar yield potential.

Blackgram

Tallmutant (Jana 1963), dwarf insensitive mutants (Rao et al. 1975 a) in X-ray irradiated population and dwarf mutants (Ignacimuthu and Babu, 1988) and dwarf early maturing mutants (Sinha, 1988) in gamma ray irradiated population were reported as growth habit mutants.

Leaf mutants reported include crinkled leaf, waxy leaf, narrow leaf and unifoliate leaf in X ray and EMS treated population (Rao and Jana 1976) and unifoliate, bifoliate and tetrafoliate types in sodium azide treated population (Khan, 1987a).

Early flowering (Bandyopadhyay and Bose, 1980) and early maturing (Khan 1988a) types were isolated after mutagenic treatments.

Bhadra and Jain (1986) isolated black seeded, bold seeded and shrunken seeded types after EMS and gamma ray treatment in T9 and S-1 varieties.

Among pod variants, mutants with different podlengths (Bhamburkar and Bhalla 1987, Khan 1988a) and mutants with different number of pods per cluster (Khan 1987a) were reported in black gram.

A large number of viable mutants of plant height, maturity, pod number, seed size and sterility and other morphological variations were isolated in the M_2 generation after EMS and gamma rays treatments (Vanniarajan et al. 1993)

Cowpea

Chowdhari (1983) isolated a bold seeded mutant with dwarf stature, early duration, increased pod and seed yield per plant following irradiation of seeds with 40kR gamma rays.

Mutations involving changes in plant height, cotyledon abnormalities, stem and leaf modifications, pod modifications and seed colour variation were noticed by Thirugunakumar (1986)

Greengram

Seth et al. (1983) reported that the use of low radiation doses (5,10 and 20 kR gamma rays) was effective in inducing earliness. Among a few mutants isolated for earliness in M_2 generation by Pawar et al. (1984) one of them gave higher yield. Following mutagenesis with gamma rays and EMS eight mutants for seed weight, pod weight and number of seeds per pod were isolated by Khan (1986)

Sumanggono (1987) isolated tall, determinate and semidwarf plant types and variants with large, erect, slender glaborous and cream coloured pods in M_2 generation after gamma irradiation (0.1 - 0.4 k Gy) of mungbean cv. manyar.

Multiracemose inflorescence mutant isolated in var 744, induced by recurrent gamma irradiation by Singh et al. (1988) was found to be higher yielder than the parent.

A wide range of mutants affecting leaf morphology, plant habit, maturity and fertility were isolated in M_2 after treatment with gamma rays, EMS and hydrazine hydrate in mungbean var G-65 and PS-16 (Khan 1989).

Kulkarni et al. (1990) identified high yielding types in M_2 after gamma ray irradiation and EMS treatment of varieties PIM 51 and Pusa baisakhi and reported that some of the selections produced M_3 progenies which outyielded the control.

The mutation spectrum was observed for plant morphology and yield characters such as plant height, branching pattern, leaf morphology, venation, pigmentation, peduncle length, pod characters and grain yield in M_2 generation. These induced mutations were related to the studies on mutagenesis in greengram cv. T44 with gamma rays (5-40 kR), EMS (0.01-0.05m), combination treatments (5-40 kR gamma doses followed by 0.02m EMS) and recurrent doses of gamma rays (5-40 kR gamma rays accompanied by respective gamma doses in the following generation (Singh and Yadav, 1991).

Redgram

A number of morphological flower variants (Chaturvedi and Sharma 1978 a, 1978b) and inflorescence variants (Venkateswaralu et al. 1976) in EMS treated population were isolated. Rao et al. (1975b) reported that in cultivars S-5 and S-8 of pigeonpea after treatment with gamma rays induced mutations were found to be effective in generating variability for the improvement of plant type. From variety S-5, early maturing with improved plant type suitable for multiple cropping and from variety S-8, medium tall, highly compact but not clustered and tall mutants were isolated.

A wide range of mutants were obtained of which mutants with different forms of reduced stature and habit were most common in pigeonpea after gamma irradiation (Rao, 1984).

Bhalla (1989) reported the effect of gamma rays, EMS and magnetic fields on two early maturing cultivars of pigeonpea and observed. Several mutants of plant height, seed and pod characters and yield

Bhatia et al. (1991) isolated large seeded pigeonpea mutant contributing to higher yield.

Chickpea

Kharkwal (1983) noticed forty five different types of morphological mutations affecting all plant parts after treating two desi (G130, H214) and culinary (L345, C104 Kabuli) types with gamma rays (40,50, 60kR) fast neutrons (0.5,1,1.5 kR) NMU (0.01,0.02%) and EMS (0.1, 0.2%).

Haq et al. (1989) identified very early flowering photoperiod insensitive induced mutant from 20 kR gamma ray irradiation.

Mutant line CM 1918 selected after exposure of a blight susceptible variety to 10 kR gamma radiation, was reported to be semi-erect, maturing two weeks earlier and moderately resistant to blight. (Hassan and Khan 1991).

2.2.2 Micro mutations

The mutational changes which can be isolated and fixed only through the adoption of biometrical procedure in a

group of plants are called as micro mutations (Gaul 1961, Swaminathan, 1964). Gregory (1965) reported that the number of plus and minus mutations in the polygenic system was equal and it is the magnitude of the phenotypic effects which is influenced by mutation. Increased variability for all characters was reported by Dahiya (1973) in greengram.

Rajput (1974) treated the seeds of greengram cv. 6601 with gamma ray doses ranging from 10-40 kR and observed that in the M_2 generation the change in the mean values of treated material for all the phenotypic traits occurred towards either positive or negative direction except for mean pod length.

Significant changes in the mean values of a large number of economically useful characters were noticed due to the mutagenic effect of Ethylene imine and Diethyl sulphate vapour in pea (Agarkova et al. 1976).

Maleic hydrazide (MH) and gamma rays individually and in combination produced a large number of macro and micro mutation in greengram (Grover and Tejpal 1980).

In blackgram, it was observed that the mean values of quantitative characters didnot change except for plant height in EMS treated material but the variability had increased for all the characters. In gamma irradiated population mean values for number of seeds per pod and seed size didnot change in M_2 and M_3 generations, however the variability had increased. The mean values of pods per plant and yield per plant had shifted in a positive direction (Kundu and Singh 1982b).

Khan (1983) studied mutations in greengram to explore the possibility of inducing micro mutations in quantitative characters after treating with hydrazine hydrate and gamma rays individually and in combined treatments. The report showed that the mean value of treated populations shifted towards the positive direction for days to maturity and plant yield in M_2 generation and the co-efficient of variability increased for these characters indicating the effectiveness of mutagenic treatments in inducing polygenic mutations. Increase as well as decrease in mean values of several yield characters were reported in greengram by Khan (1986, 1987b, 1988b).

Neutron irradiation (6-15 J/kg) of bean seeds increased the range of variations for seed yield per plant, 1000 seed weight, pod length, pods/plant, plant shape coefficient and branch number in the M_2 (Kozera et al. 1986).

Chary and Bhalla (1988) reported that 0.3% EMS was effective in increasing the number of branches and pods per plant in pigeon pea.

2.3 Mutagenic Efficiency and Effectiveness

The usefulness of any mutagen in plant breeding depends not on the mutagenic effectiveness, the relation of mutagenic frequency to dose but on mutagenic efficiency also, the production of desirable changes free from association with undesirable changes (Konzak et al. 1965).

Effectiveness means the rate of mutation induction as dependent upon the mutagenic dose and efficiency refers to the mutation rate in relation to biological effects, usually a measure of damage (Nilan et al. 1965).

Mutagenic effectiveness and efficiency of gamma rays, EMS and hydrazine hydrate in greengram was studied and found that mutagenic effectiveness and mutagenic efficiency were highest at lower doses of mutagens (Khan and Hashim 1978).

Nadarajan and Ramalingam (1982) reported that gamma rays were more effective and efficient than DES in inducing chlorophyll and viable mutations when estimated on the basis of lethality and DES was more efficient on injury basis.

Combined treatment of gamma rays and EMS produced higher mutation rate and efficiency than individual treatments in greengram (Bahl and Gupta 1982).

Varietal×mutagen interactions have also been reported in different pulse crops. Kharkwal (1983) reported the sensitivity of two types, desi and culinary types of chickpea to the treatment with four mutagens, namely gamma rays, fast neutrons, NMU and EMS, desitypes being more resistant to mutagens.

In greengram effective doses were 40 kR for X-rays (16-60 kR) and 2 x 10^{12} n cm^2/s for thermal neutrons (10-60 x 10^{12} n cm^2/s) (Song et al. 1988).

Mutagenic effectiveness and efficiency of gamma rays at 20, 40 and 60 kR, magnetic fields at 3000, 4000 and 5000 Gy, Sodium azide at 1 x 10^3, 2 x 10^3 and 3 x 10^3M and a combination of treatments were studied in cluster bean by Badani and Bhalla (1992). They found that the higher levels of each treatment were more efficient in inducing mutations and sodium azide was the most efficient mutagen.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

The present investigation on "Mutagenicity of gammarays and EMS on winged bean (Psophocarpus tetragonolobus (L.))" was conducted in the Department of Plant breeding and Genetics, College of Agriculture, Vellayani during the year 1992-1994.

A. MATERIALS

This study formed a continuation of a concluded project in the Department of Plant breeding namely "Morphological effect of gamma rays and EMS on winged bean (Psophocarpus tetragonolobus (L.))". Seeds of PT-62, variety of winged bean received from the instructional farm, vellayani was selected as the source material for the induction of mutationswith gamma rays and EMS in the previous study. Dry seeds of uniform size (12% moisture) were treated with five doses of gamma rays viz., 100, 200, 300, 400 and 500 Gray units and five doses of EMS viz., 40, 80, 120, 160 and 200 Millimoles for a period of '6' hours and the M_1 generation was studied by my predecessor.

Seeds collected individually from the surviving plants in the first generation (M_1) constituted the seed material for the present study (M_2 generation). Since none of treatments in 500 gray units germinated in M_1 generation, the M_2 generation studies didnot include the plants from that treatment. Each treatment in gamma rays and EMS, was represented by twenty M_1 plants selected at random and each M_1 plant by maximum of fourty seeds. Wherever the number of M_1 plants which reached to maturity was limited, all the M_1 plants were carried forward to M_2. The details regarding the number of M_1 plants carried forward to M_2 and the number of seeds in each dose of the mutagens are given in table I.

B. METHODS

The M_2 generation was raised as M_1 plant progenies during September 1993 to March 1994. Seeds collected individually from each M_1 plant were selected at random and were sown as a progeny row in the M_2 at a spacing of 75cm between rows and 50cm between plants in non-replicated progeny row trial. Different progeny rows of a particular dose were sown continuously as a single line of 20M length. After every such ten lines there was a control line which was

Table 1. Details of progenies studied

Treatments	Number of M_1 plants carried to M_2	Number of seeds carried to M_2
Control	20	400
Gamma rays		
100 gray units	20	615
200 "	20	404
300 "	12	249
400 "	3	60
EMS		
40 millimoles	17	332
80 "	18	357
120 "	18	364
160 "	12	262
200 "	11	280

raised from the seeds of untreated plants. The cultural, manurial and plant protection measures were done as per the package of partices recommendations of the Kerala Agricultural University.

Observations made are detailed below

3.1. CHLOROPHYLL MUTATIONS

Plants in each progeny row were observed from germination to spot out chlorophyll deficient mutants. All the seedlings which exhibited deviations from the normal ones by colour differences or by the presence of white spots, streaks or patches on leaves were considered as chlorophyll mutants.

3.1.1. Mutation Frequency

The number of M_2 progeny rows showing segregation were noted to find out the mutation frequency. Total number of normal seedlings and mutants were also counted from both segregating and non-segregating progeny rows. The mutation frequency was calculated as the number of mutants per 100 M_2 rows and 100 M_2 plants basis.

3.1.2. Spectrum

Observed chlorophyll mutants were classified according to the system proposed by Gustafsson (1940) and followed by Brenda (1987). Different types of chlorophyll mutation observed in each progeny row were scored separately to find out the spectrum of different types of mutants. Relative per cent of each type of mutations was calculated.

3.1.3. Single and multiple chlorophyll mutation frequency

The number of M_2 progeny rows segregating for single and two or more mutational events were counted separetely for the segregating progeny rows in each dose. Frequency of single or multiple mutations was calculated as ratio of the number of segregating progeny rows for single or multiple mutations to total number of segregating rows and expressed in percentage.

3.1.4. Segregation ratio

In segregating M_2 progeny rows, the number of mutants and normal seedlings were counted separately to

calculate the segregation ratio. Segregation ratio was expressed as the per cent number of mutants to total number of seedlings in segregating rows of a treatment.

3.2. VIABLE MUTATIONS

3.2.1. Macro mutations

All available plant progenies were observed through out their life period. Any plant which exhibited morphological deviation from the normal one with special emphasis on plant height, duration, branching pattern, floral, pod and seed characters were marked as a viable macro mutants. Individual visual scoring method was done to identify the viable mutants of such types.

3.2.1.1. Mutation frequency

The number of M_2 progeny rows showing segregation for viable mutants and the number of viable mutants in each treatment were noted. The frequency was estimated by the same method used in case of chlorophyll mutations.

3.2.1.2. Spectrum and segregation ratio

The number of particular type of mutations in each treatment was counted separately to find the spectrum of mutation in each dose. The number of mutants and normal seedlings were counted separately to find the segregation ratio. Spectrum and segregation ratio were expressed as that in chlorophyll mutations.

3.2.2. Micro mutations

Apart from visual scoring fifteen plants were selected randomly in each line of particular treatment and observations on plant height at 45 and 75 days after sowing, days to flower, number of pods at first harvest, pod length, number of seeds per pod and 100 seed weight were noted.

Height

The height of the plant was measured from the base of the main shoot to the tip of leaf bud on 45th and 75th day after sowing.

Days to flower

Number of days from sowing to flowering was taken as days to flower.

Number of pods per plant

The number of pods per plant at first harvest was noted.

Pod length

Length of first harvested fruits of each plant was noted and average length was estimated.

Number of seeds per pod

Number of seeds per pod in each plant was noted and the mean was calculated.

100 seed weight

Weight of 100 seeds in each plant was taken as 100 seed weight.

Frequency distribution of variants and comparison of mean performance.

Plant progenies were categorised into three groups, negative variants, control group and positive variants for seven characters based on the control performance. Mean values of the characters in different treatments were compared with control by applying student's 't' test.

3.3. ESTIMATION OF MUTAGENIC EFFICIENCY AND MUTAGENIC EFFECTIVENESS

Mutagenic efficiency and effectiveness were estimated using the formula proposed by Konzak et al. (1965) in both physical and chemical mutagens.

Mutagenic effectiveness

$$= \frac{MF \times 100}{K} \quad \text{or} \quad \frac{MF \times 100}{c \times t}$$

were MF = Mutation frequency

K = irradiation units (K.rad)

c = Concentration (%)

t = time (hr)

Mutagenic efficiency

$$= \frac{MF \times 100}{L} \text{ or } \frac{MF \times 100}{I} \text{ or } \frac{MF \times 100}{S}$$

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

I = Percentage of injury on seedling height reduction.

S = Percentage of seed sterility.

3.4. Isolation of viable mutations

Individual plant harvest was done to easily isolate the macro mutants. Ten plants looking normal were selected in the segregating rows to study micro mutations in M_3.

RESULTS

IV. RESULTS

The mutagenicity of gamma ray and EMS treatments on winged bean in the M_2 generation was studied and the results are presented below:

The highest dose of gamma ray exposure viz. 500 Gray units appeared lethal in M_1 generation since no M_1 plant could survive.

4.1 CHLOROPHYLL MUTATIONS

A total number of 1574 M_2 seedlings were examined from the date of germination to spot out chlorophyll mutations.

4.1.1. Mutation frequency

The effect of different exposures of gamma rays on chlorophyll mutation frequency on M_2 progeny row and M_2 seedling basis are presented in Table 2.

The chlorophyll mutation frequency on M_2 progeny row basis ranged from 33.33 to 42.11 per cent. The

Table 2. Frequency of chlorophyll mutants (gamma rays)

Treatments (Gray units)	Number of M_2 progeny-rows scored	Number of M_2 seedlings scored	Chlorophyll mutatation frequency			
			M_2 progeny row basis		M_2 seedling basis	
			No. of segregating rows	%	No. of mutants	%
0 (Control)	9	90	0	0	0	0
100	20	280	7	35.00	12	4.29
200	19	149	8	42.11	10	6.71
300	12	79	4	33.33	5	6.33
400	3	34	1	33.33	2	5.88

Table 3. Frequency of chlorophyll mutants (EMS)

Treatments (Millimoles)	Number of M_2 progeny-rows scored	Number of M_2 seedlings scored	Chlorophyll mutatation frequency			
			M_2 progeny row basis		M_2 seedling basis	
			No. of segregating rows	%	No. of mutants	%
0 (Control)	9	90	0	0	0	0
40	17	208	4	23.53	7	3.37
80	18	169	5	27.78	6	3.55
120	17	221	6	35.29	10	4.52
160	12	124	5	41.67	7	5.65
200	11	130	4	36.36	6	4.62

chlorophyll mutation frequency showed an increase from 35 to 42.11 per cent when the dose increased from 100 to 200 Gray units and above which it decreased to 33.33 per cent at 300 and 400 Gray units.

The frequency of chlorophyll mutation estimated on M_2 seedling basis ranged from 4.29 to 6.71 per cent. The mutation frequency showed an increase from 4.29 to 6.71 per cent when the dose increased from 100 to 200 Gray units and after which, the frequency decreased with increasing doses of gamma ray exposures upto 400 Gray units.

The frequency of chlorophyll mutations on M_2 progeny row and M_2 seedling basis were maximum at the same dose (200 Gray units).

The effect of different concentrations of EMS on the chlorophyll mutation frequency are depicted in Table 3.

The chlorophyll mutation frequency on M_2 progeny row basis had a range from 23.53 to 41.67 per cent. The frequency increased with increase in dose, reached maximum (41.67 per cent) at 160 millimoles and thereafter decreased to 36.36 per cent at 200 mM.

The range of chlorophyll mutation frequency on M_2 seedling basis was from 3.37 to 5.65 per cent. The chlorophyll mutation frequency increased with the increase in concentration upto 160mM (5.65 per cent) following a reduction at 200 mM.

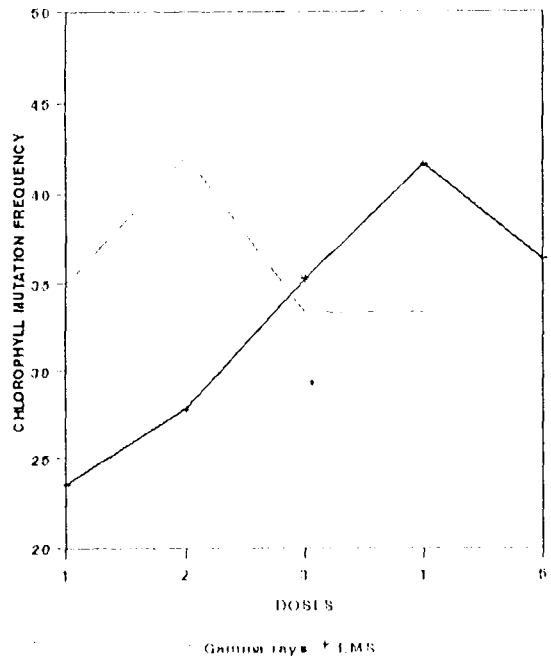
The frequency of chlorophyll mutations on M_2 progeny row and M_2 seedling basis was maximum at the same dose (160 mM).

By comparing gamma rays and EMS on the basis of chlorophyll mutation frequency it was found that maximum frequencies were recorded at the same dose for both the mutagens on M_2 progeny row and M_2 seedling bases and the the highest was for gamma rays, viz. at 200 Gray units (Fig. 1).

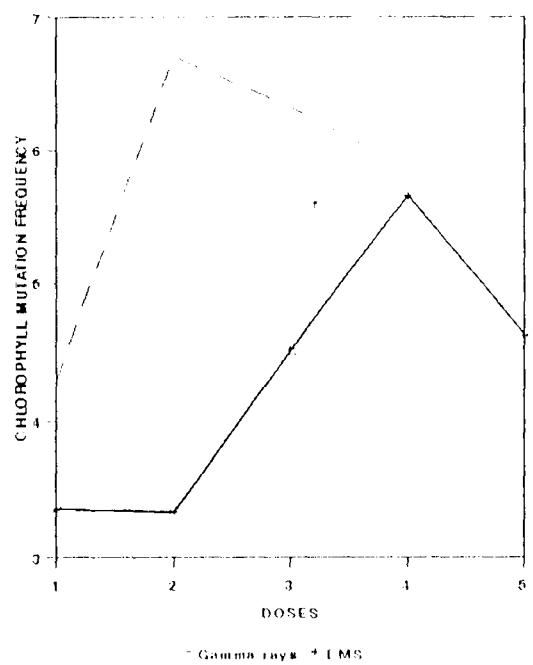
4.1.2 Spectrum

Different types of chlorophyll mutants observed during the period from germination to sixth leaf stage were classified as xantha (yellow), chlorina (yellowish green), viridis (light green); white streaked, (White streaks on the leaves), maculata (irregular patches of chlorophyll deficient

M2 PROGENY ROW BASIS



M2 SEEDLING BASIS



Treat-ments	Gamma rays	EMS
1	100 Gy	40 mM
2	200 Gy	80 mM
3	300 Gy	120 mM
4	400 Gy	160 mM
5	----	200 mM

Fig. 1. FREQUENCY OF CHLOROPHYLL MUTATIONS

Table 4. Spectrum of chlorophyll mutations (gamma rays)

Treatments (Gray units)	Number of chlorophyll mutants	Relative per cent					
		Xantha	Chlorina	Viridis	White streaked	Maculata	Viridalba
100	12	-	-	25	25	25	25
200	10	10	50	20	20	-	-
300	5	20	40	20	20	-	-
400	2	50	50	-	-	-	-

Table 5. Spectrum of chlorophyll mutations (EMS)

Treatments (Millimoles)	Number of chlorophyll mutants	Relative per cent					
		Xantha	Chlorina	Viridis	White streaked	Maculata	Viridalba
40	7	-	28.57	28.57	14.29	14.29	14.29
80	6	33.33	16.67	33.33	16.29	-	-
120	10	20.00	40.00	10.00	20.00	10.00	-
160	7	42.86	42.86	-	14.29	-	-
200	6	66.67	16.67	-	16.67	-	-

higher doses at an increasing rate (10, 20, 50) with increase in dose. Chlorina type was also seen in the three higher doses but not having any dose dependence, the maximum (50 per cent) being at 200 and 400 Gray units and minimum (20 per cent) at 300 Gray units. Viridis and white streaked types were seen with the same frequency in each dose, and not having any direct dose dependence. Maculata and viridalba types were present only in the lowest dose. Different types except xantha did not show any dose dependent change.

The effect of different types of chlorophyll mutations induced by different concentrations of EMS are presented in the Table 5.

Six different types identified were not occured together in any of the five doses of EMS. Five types viz. chlorina, viridis, white streaked, maculata and vorodalba types were seen in 40 mM, four types (xantha, chlorina, viridis and white streaked) in 80 mM, five types (xantha, chlorina, viridis, white streaked and maculata) in 120 mM and three types (xantha, chlorina and white streaked) in 160 and 200 mM of EMS. Chlorina type exhibited highest proportion in three doses, but along with viridis and xantha in 40 and 160

mM respectively. Xantha type showed highest proportion in 80 mM with viridis and in 200 mM. In total xantha and chlorina appeared as the most frequent types in EMS treated population.

The relative per cent of different types of chlorophyll mutations varied with dose of EMS. Xantha type occured in all the four higher doses with maximum (66.67 per cent) in the highest dose (200 mM). Chlorina type was seen in all the five doses and showed an initial decrease followed by increases in relative per cent upto 160 mM with maximum frequency in 160 mM and minimum in two doses (80 mM and 200 mM). Viridis type was having highest relative per cent in 80 mM and lowest in 120 mM. White streaked type was also seen in all the five doses having maximum occurence in 120 mM and minimum in 40 and 160 mM. Maculata type was present in 40 and 120 mM whereas viridalba in 40 mM of EMS. None of the different chlorophyll mutations showed any direct dose dependence.

On comparison of the effects of two mutagens, it was found that chlorina type was the most frequent type in case of gamma rays, xantha also in EMS and hence in total, chlorina appeared as the most frequent one (Fig. 2).

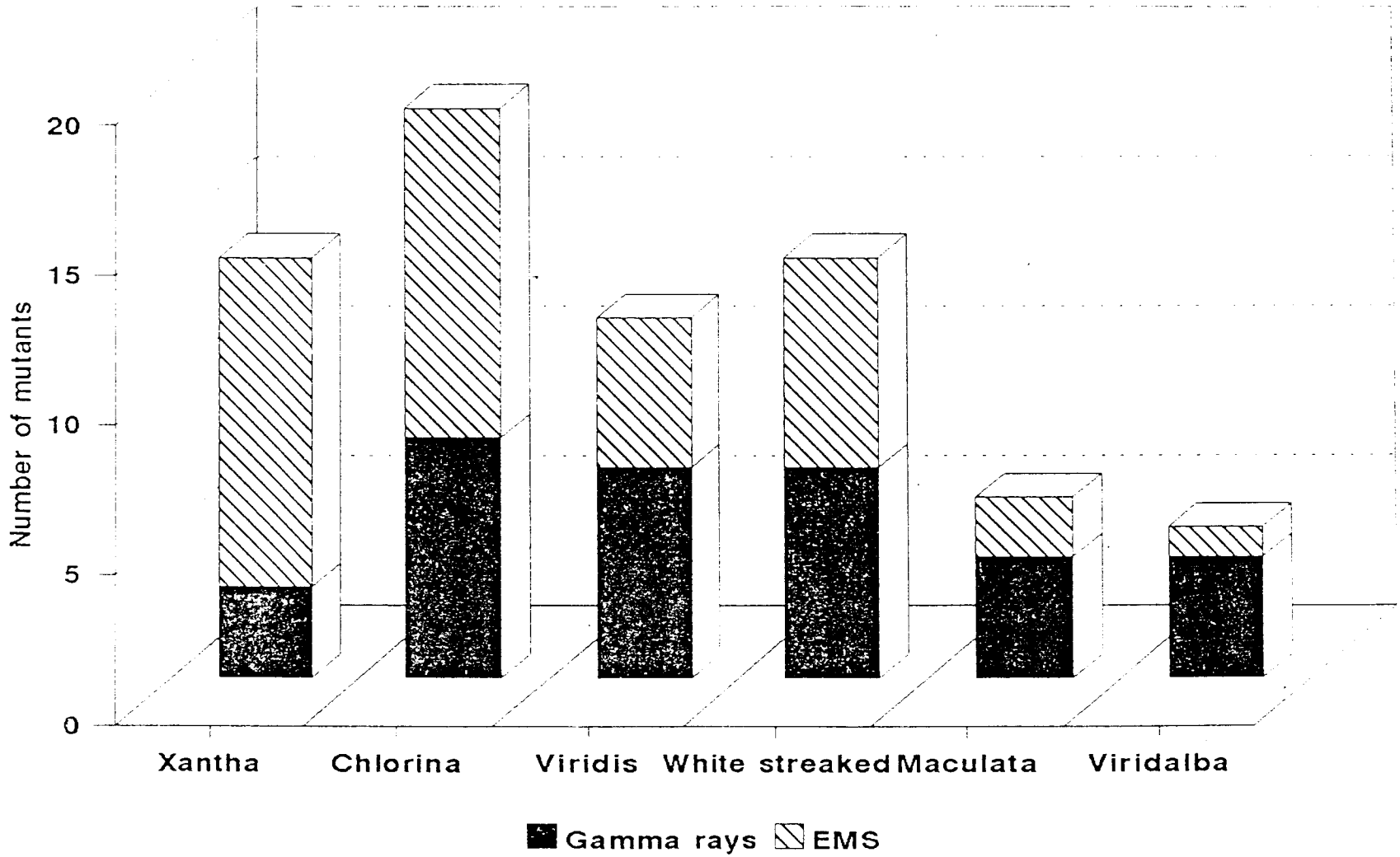


Fig. 2. FREQUENCY OF DIFFERENT CHLOROPHYLL MUTANTS

Different chlorophyll mutations did not show any direct relationship with dose except xantha in gamma rays. However it was found that xantha type was confined to higher doses with high frequency (Fig. 3 and Fig. 4).

4.1.3 Single and multiple chlorophyll mutation frequency

The effect of different exposures of gamma rays on single and multiple chlorophyll mutations are depicted in Table 6.

The relative per cent of progeny rows showing segregation for a single type of mutation was minimum at the lowest dose and then showed higher values except in 400 Gray units. Similarly the relative per cent of multiple chlorophyll mutations exhibited corresponding change in the reverse direction of single mutation frequency.

In respect of multiple mutations, the composition was either viridis and viridalba or maculata and white streaked types in 100 Gray units, chlorina and xantha types in 200 Gray units, chlorina and white streaked types in 300 Gray units and Chlorina and xantha in 400 Gray units. Chlorina

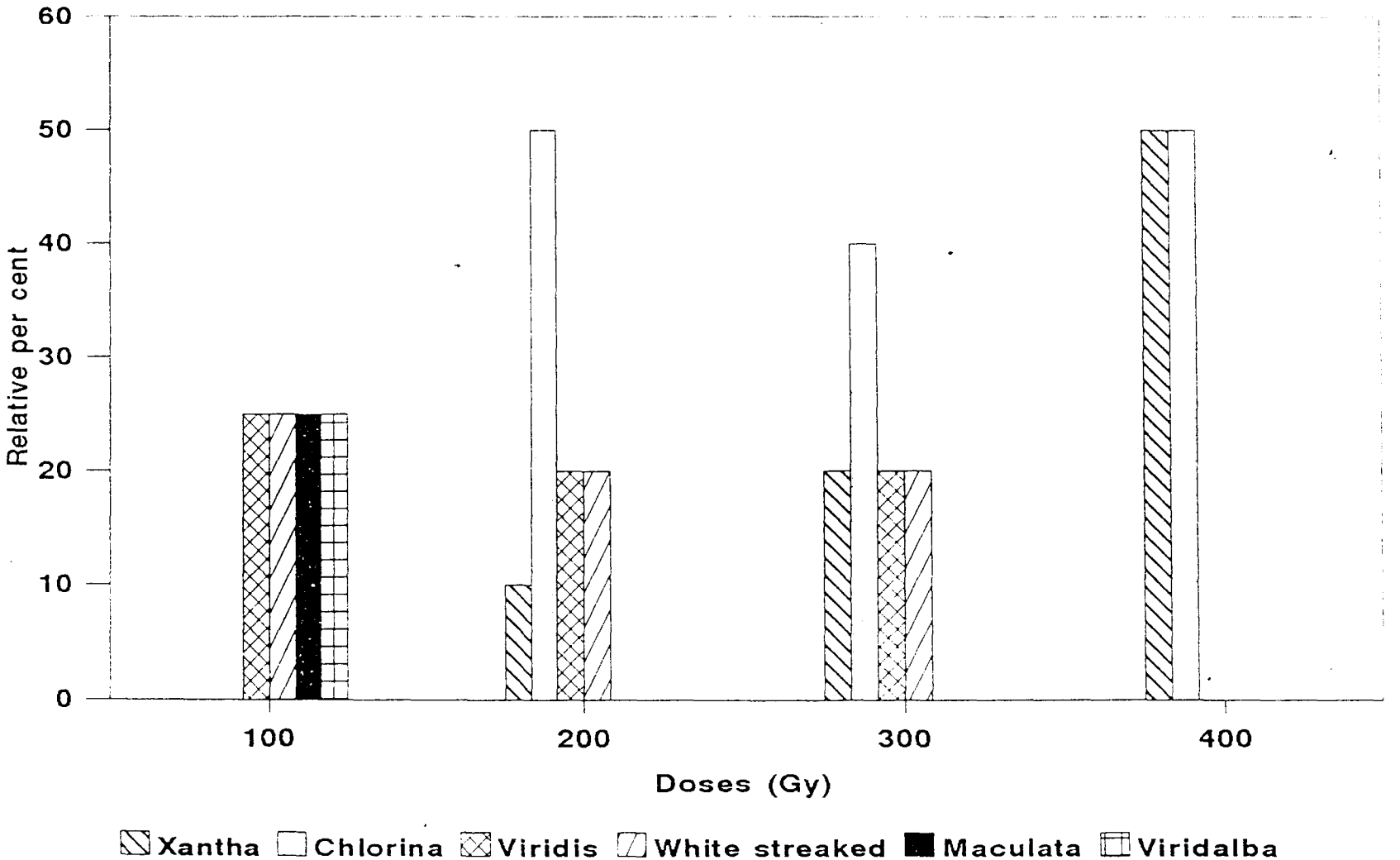


Fig. 3. SPECTRUM OF CHLOROPHYLL MUTATIONS (GAMMA RAYS)

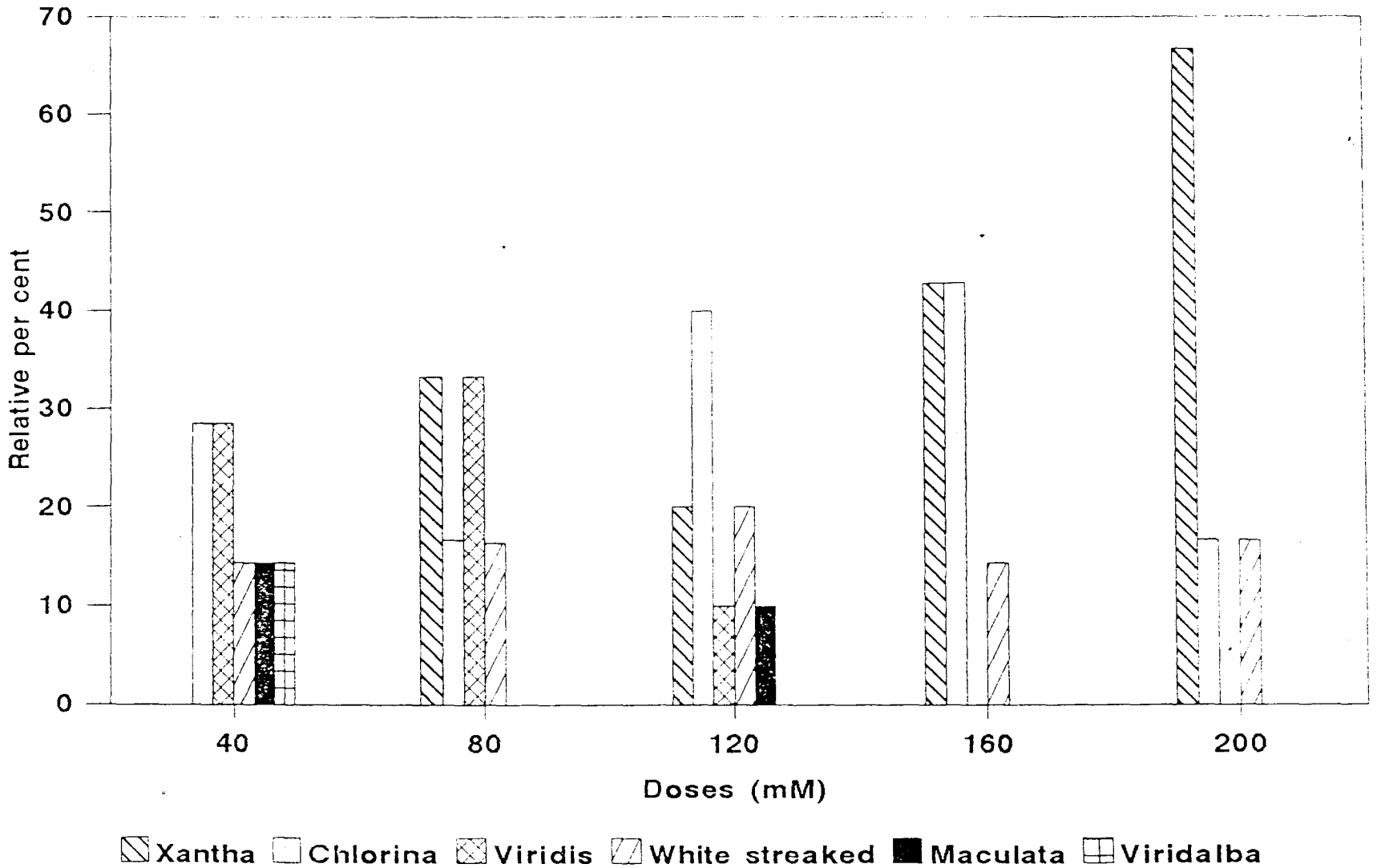


Fig. 4. SPECTRUM OF CHLOROPHYLL MUTATIONS (EMS)

Table 6. Frequency and Relative per cent of M_2 progeny rows segreating for single and multiple chlorophyll mutations (Gamma rays)

Treatments (Millimoles)	Number of chlorophyll mutants		Plant progenies segregating for mutation of			
	Segregating rows	Mutants	Frequency		Relative %	
			1 type	2 types	1 type	2 types
100	7	12	3	4	42.85	57.14
200	8	10	6	2	75.00	25.00
300	4	5	3	1	75.00	25.00
400	1	2	-	1	0.00	100.00

Table 7. Frequency and Relative per cent of M_2 progeny rows segreating for single and multiple chlorophyll mutations (EMS)

Treatments (Millimoles)	Number of chlorophyll mutants		Plant progenies segregating for mutation of			
	Segregating rows	Mutants	Frequency		Relative %	
			1 type	2 types	1 type	2 types
40	4	6	2	2	50.00	50.00
80	5	6	4	1	80.00	20.00
120	6	10	5	1	83.33	16.67
160	5	7	4	1	80.00	20.00
200	4	6	4	0	100.00	0.00

and xantha combination was the most frequent among other types (Fig. 5).

The effect of different concentrations of EMS on single and multiple chlorophyll mutations are presented in Table 7.

The relative per cent of progeny rows for multiple chlorophyll mutations decreased with an increase in dose except at 160 mM at which a slight increase was noticed, whereas frequency of progeny rows for single chlorophyll mutation increased with decrease in multiple chlorophyll frequency.

The composition of multiple chlorophyll mutations was either viridis and chlorina or white streaked and maculata types in 40 mM chlorina and xantha types in 80 mM, white streaked and maculata types in 120 mM and chlorina and white streaked in 160 mM white streaked and maculata type was more frequent in EMS treated population (Fig 6.).

A comparison of the effects of two mutagens showed that higher rate of multiple chlorophyll mutation frequency

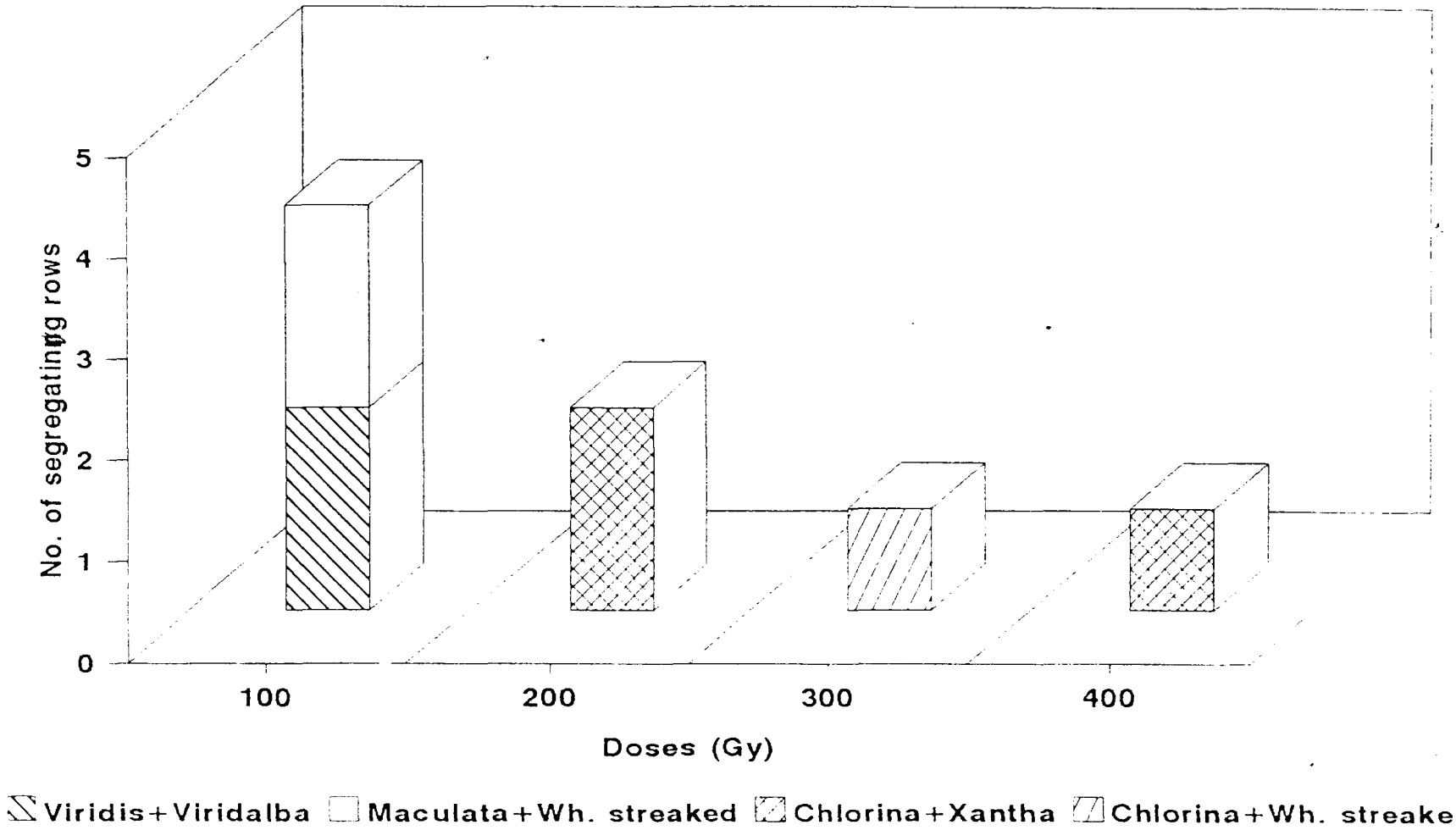


Fig. 5. FREQUENCY AND COMPOSITION OF MULTIPLE CHLOROPHYLL MUTATIONS (GAMMA RAYS)

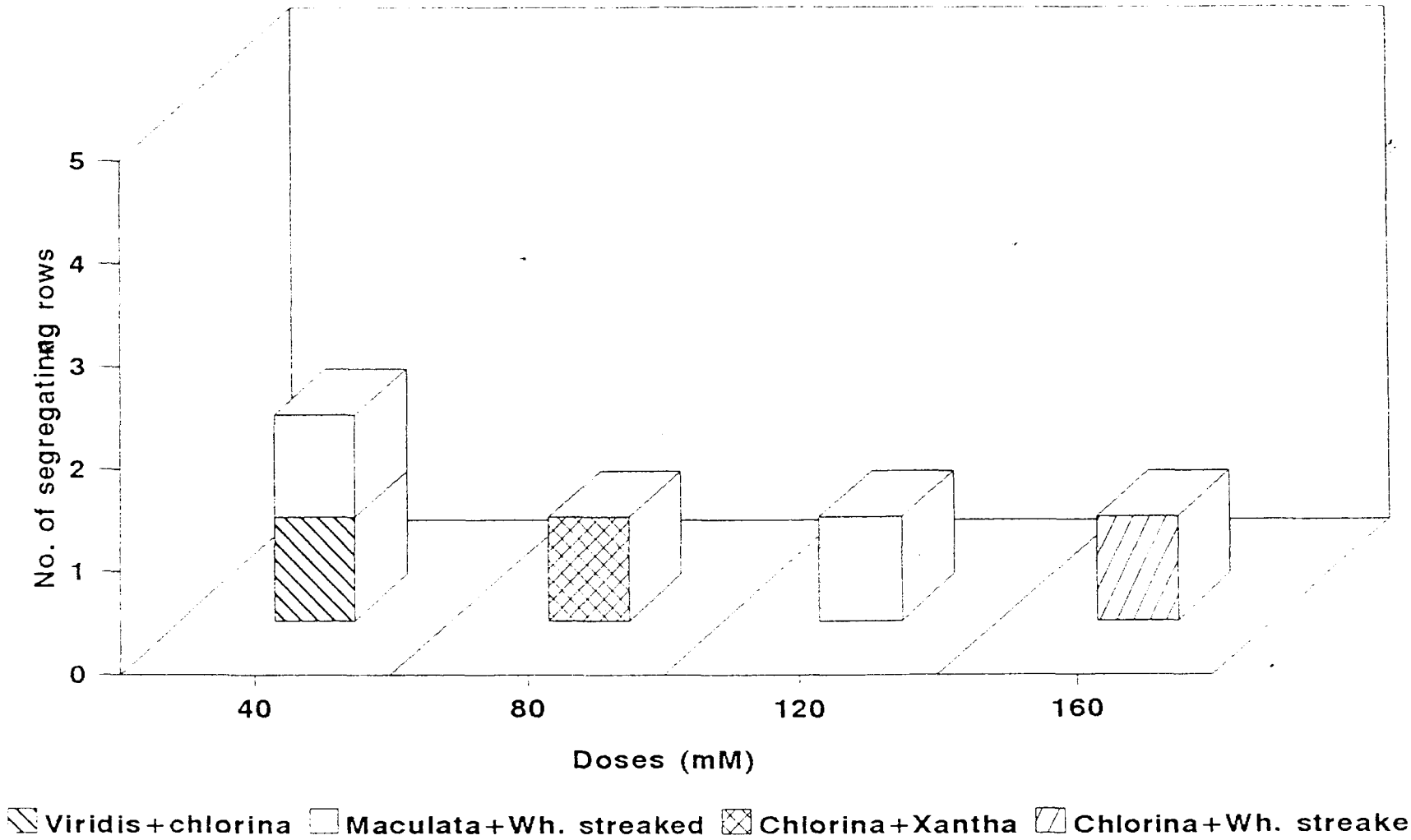


Fig. 6. FREQUENCY AND COMPOSITION OF MULTIPLE CHLOROPHYLL MUTATIONS (EMS)

was at 400 Gray units of gamma rays and 40 mM of EMS but the result obtained in 400 Gray units of gamma rays was not absolute since there was only one segregating row. Avoiding the effect of 400 Gray units the higher rate of multiple chlorophyll mutation was observed at lowest doses of both the mutagens. The composition of multiple chlorophyll mutations differed in two mutagens.

4.1.4 Segregation ratio

The effect of different exposures of gamma rays on segregation ratio are presented in Table 8.

The mean segregation per cent ranged from 6.9 (400 Gray units) to 14.93 (200 Gray units). It was having an initial increase at the lower doses, reached maximum at 200 Gray units and thereafter decreased as the dose increased. The segregation per cent in all the progeny rows was less than 25 per cent and the maximum frequency of M_2 progeny rows with chlorophyll mutations was recorded for those with segregation per cent between 10 and 14.

The effect of different concentrations of EMS on segregation per cent of chlorophyll mutations are given in Table 9.

Table 8. Segregation per cent of chlorophyll mutations in the M_2 generation (gamma rays)

Treatments (Gray units)	Mean Segregation	Relative Frequency of M_2 rows with chlorophyll mutation					
		Segregation percentage of					
	Per cent	< 5	5 - 9	10 - 14	15 - 19	20 - 24	≥ 25
100	12.77	-	2	1	3	1	-
200	14.93	-	-	5	-	3	-
300	11.36	-	1	3	-	-	-
400	6.9	-	1	-	-	-	-

Table 9. Segregation per cent of chlorophyll mutations in the M_2 generation (EMS)

Treatments (Millimoles)	Mean Segregation Ratio	Relative Frequency of M_2 rows with chlorophyll mutation					
		Segregation percentage of					
		< 5	5 - 9	10 - 14	15 - 19	20 - 24	≥ 25
40	10.14	1	1	1	-	1	-
80	12.77	-	1	2	-	1	1
120	8.85	1	2	3	-	-	-
160	13.21	-	-	3	1	1	-
200	9.23	-	2	2	-	-	-

The mean segregation per cent ranged from 8.85 (120 mM) to 13.21 (160 mM) and was not having any direct dose-dependence. The mean segregation per cent in all the progeny rows did not exceed 25 per cent and the maximum frequency of M_2 progeny rows with chlorophyll mutations was recorded for those with segregation per cent betweeen 10 and 14.

4.2 Viable mutations

4.2.1 Macromutations

A total of 1496 M_2 plants from different treatments of gamma rays and EMS were examined through out their life period for any deviation from the normal one.

4.2.1.1 Mutation frequency

The effect of different exposures of gamma rays on the viable mutation frequency are presented in Table 10.

The viable mutation frequency on M_2 progeny row basis ranged from 21.05 to 33.33 per cent. The frequency decreased to minimum in 200 Gray units, there after increased upto 400 Gray units and was maximum (33.33) at 400 Gray units. Hence the frequency did not show any direct-dose dependence

Table 10. Frequency of viable mutants (Gamma rays)

Treatments	Number of M_2 progeny rows scored	Number of M_2 plants scored	Viable mutation frequency			
			M_2 progeny row basis		M_2 plant basis	
			Segregating Rows (No.)	%	Number of mutants	%
Control (0)	9	90	-	-	-	-
100 Gy	20	250	6	30.00	11	4.4
200 Gy	19	145	4	21.05	6	4.13
300 Gy	12	72	3	25.00	3	4.16
400 Gy	3	32	1	33.33	1	3.13

Table 11. Frequency of viable mutants (EMS)

Treatments	Number of M_2 progeny rows scored	Number of M_2 plants scored	Viable mutation frequency			
			M_2 progeny row basis		M_2 plant basis	
			Segregating Rows (No.)	%	Number of mutants	%
Control (0)	9	90	-	-	-	-
40 mM	17	203	3	17.65	5	2.47
80 mM	18	162	4	22.22	4	2.47
120 mM	17	212	3	17.65	5	2.36
160 mM	12	120	4	33.33	4	3.33
200 mM	11	120	4	36.36	4	3.33

The viable mutation frequency estimated on M_2 plant basis had a range from 3.13 to 4.4 per cent. It was not having any direct dose dependence but the maximum frequency at the lowest dose (100 Gray units) and the minimum at the highest dose. (400 Gray units).

The effect of different concentrations of EMS on viable mutation frequency is presented in Table 11.

The viable mutation frequency on M_2 progeny row basis ranged from 17.65 to 36.36 per cent. It showed an increase with dose in all the cases except in 120 mM. The maximum frequency was at 200 mM and minimum at 40 and 120 mM.

The viable mutation frequency on M_2 plant basis ranged from 2.36 to 3.33 per cent. It showed an erratic behaviour with dose.

On comparison of the frequencies of two mutagens, Gamma rays had higher frequencies on M_2 plant basis than EMS.

4.2.1 Spectrum and Segregation ratio

The different types of viable mutations observed included mutations with leaf characters such as size of

leaves (Plate 5), number of leaflets (trifoliate leaf was replaced by increased number of leaflets:plate 6), texture of leaves (thick and soft:plate 7) stem colour mutations (plate 8), pod length mutation (plate 9), seed size mutations (plate 10) and seed colour mutations. (Plate 11).

The spectrum of mutations, segregation ratio and the relative per cent of viable mutations in gamma ray treated population are presented in Table 12.

Leaf mutations

Leaf mutants of two types, viz, small sized and thick-soft textured leaf types in 300 and 100 Gray units respectively were observed in gamma ray irradiated population with a mean segregation per cent of 9.09. Reduction in the normal size of the leaves caused small sized leaf mutants. But this abnormality or mutation appeared only at initial stages. Plant with thick and soft leaves was found as leaf texture mutant in 100 Gray units.

Stem colour mutations

Six stem colour mutations (three in 100 Gray units, two in 200 Gray units and one in 300 Gray units) obtained

Table 12. Spectrum and segregation per cent of different viable mutations (Gamma rays)

Spectrum of mutations	Number of mutants (segregation per cent)					Relative per cent
	Dose (gray units)					
	100	200	300	400	Total	
Leaf						
Small sized	-	-	1 (7.14)	-		
Thick and soft	1 (12.5)	-	-	-		
Total	1 (12.5)	-	1 (7.14)	-	2 (9.09)	7.41
Stem colour						
Brownish green	1 (6.67)[a]	1 (12.5)	-	-		
Light green	1 (6.67)[b]	1 (10.0)[a]	1 (8.33)[a]			
	1 (5.00)[c]					
Total	3 (8.57)	2 (11.11)	1 (8.33)	-	6 (9.23)	22.22
Pod length						
Small	1 (5.88)		-	-		
	1 (5.0)	-	-	-		
			-	-	3 (5.77)	11.11
Large	-	1 (6.67)				
Total	2 (5.41)	1 (6.67)				
Seed size						
Bold	1 (6.67)	1 (6.67)[b]	-	-		
		1 (10.0)				
Small	1 (5.88)	1 (10.0)	1 (7.14)	1 (3.45)		
Long	1 (5.0)	-	-	-		
Total	3 (5.76)	3 (8.57)	1 (7.14)	1 (3.45)	8 (6.15)	29.63
Seed colour						
Dark brown	1(6.67)[a]	1 (6.67)[b]	-	-		
Yellowish brown	1(5.00)[c]	-	-	-		
Greenish brown	1 (7.69)	-	-	-		
	1 (6.67)	-	-	-		
White	1 (6.67)[b]	1 (10.0)[a]	1 (8.33)[a]	-		
Total	5 (7.94)	2 (8.00)	1 (8.33)	-	8 (8.00)	29.65
Associated mutations	3	2	1	-	6	
Total mutants	11 (12.5)	6 (13.95)	3((9.68)	1 (3.45)	21 (10.5)	

a,b,c associated mutations (mutant with change in two characters)

were having highest mean segregation per cent of 9.23. Plant with brownish green and light green coloured stems were observed as mutants, but these variations were associated with seed colour variations also.

Pod length mutations

Small (two in 100 Gray units) and large (one in 200 Gray units) sized pod mutants with a mean segregation ratio of 5.77 were observed.

Seed size mutations

A total of eight mutants observed included bold(one in 100 Gray units and two in 200 Gray units), small (one from each dose) and long (one in 100 Gray units) seed types. These seed size mutants showed a mean segregation per cent of 6.15.

Seed colour mutations

Four types recognized were dark brown (one each in 100 and 200 Gray units), yellowish brown (one in 100 Gray units), greenish brown (two in 100 Gray units) and white (one each in 100, 200 and 300 Gray units). A total of eight seed

colour mutations were seen with a mean segregation ratio of 8.0 per cent. Out of this five were associated with stem colour mutations and one with seed size mutation. All the three white seeded types had light green stem and white flower.

A total of twenty one plants were recognised as mutants which exhibited visible variation from the normal ones with respect to morphological characters and among them six had simultaneous change in two characters, especially in stem and seed colour variations. Among the different mutations, seed size and seed colour mutations were more frequent, each with a relative per cent of 29.63. Stem colour, pod length and leaf mutants constituted lower proportions of 22.22, 11.11 and 7.41 per cent respectively.

The spectrum, segregation per cent and relative per cent of viable mutations recorded in EMS treated population were given in Table 13.

Leaf mutations:

Leaf mutations included small sized, tetrafoliate and thick-soft leaves. Tetrafoliate type is characterised by four leaflets instead of three. Four leaf mutations having a

Table 13. Spectrum and segregation per cent of different viable mutation (EMS)

Spectrum of mutations	Number of mutants (segregation per cent) Dose (milli moles)						Relative per cent
	40	80	120	160	200	Total	(%)
Leaf							
Small sized	-	-	1 (4.55)[a]	-	1 (5.56)		
Tetra foliate	1 (4.55)	-	-	-	-		
Thick and soft	1 (4.55)	-	-	-	-		
Total	2 (4.55)	-	1 (4.55)	-	1 (5.56)	4 (4.76)	13.79
Stem colour							
Brownish green	-	-	-	1 (10.0)[a]	-		
Light green	-	-	1 (3.85)[b]	-	1 (5.0)[a]		
Total	-	-	1 (3.85)	1 (10.0)	1 (5.0)	3 (5.36)	10.34
Pod length							
Small	-	-	1 (3.13)	-	-		
Total	-	-	1 (3.13)	-	-	1 (3.13)	3.45
Seed size							
Flat	1 (6.67)[a]	-	-	-	-		
Bold	-	1 (12.5)	-	-	-		
Small	1 (4.55)	-	-	1 (8.33)	-		
	1 (6.67)	1 (6.25)	1 (4.55)[a]	1 (5.56)	1 (7.14)[b]		
	-	-	-	1 (10.0)[a]			
Long	-	-	1 (3.85)	-	1 (7.69)		
Total	3 (8.11)	2 (8.33)	2 (4.16)	3 (7.5)	2 (7.41)	12 (6.25)	41.38
Seed colour							
Dark brown	-	1 (7.14)	-	1 (10.0)[a]	-		
Yellowish brown	-	-	1 (3.13)	1 (8.33)	1 (5.0)[a]		
Greenish brown	-	-	-	-	1 (7.14)[b]		
White	1 (6.67)[a]	1 (8.33)	1 (3.85)[b]	-	-		
Total	1 (6.67)	2 (7.69)	2 (3.45)	2 (9.09)	2 (5.88)	9 (5.81)	31.03
Associated mutations	1	-	2	1*	2		
Total mutants	5 (8.47)	4 (8.00)	5 (6.25)	4 (7.69)	4 (6.15)	22 (7.19)	

a,b - Associated mutations (mutant with change in 2 characters)

* - Mutant with change in three characters

mean segregation per cent of 4.76 were observed in 40, 120 and 200 millimoles of EMS.

Stem colour mutations:

Three stem colour mutations of two types, viz, brownish green and light green were recorded. It was having a mean segregation ratio of 5.36 per cent. All of these mutations were associated with seed colour variations and one among them was having seed size variation also.

Pod length mutations:

Mean segregation per cent of 3.13 was observed for the only one small type of mutation (in 120 mM).

Seedsize mutations:

Four categories identified were flat (one in 40 mM), bold (one in 80 mM), small (two at 40 nM, one in 80), 120 and 200 mM and three in 160 mM and long (one each in 120 and 200 mM) types. Twelve seed size mutations were recorded in all concentrations of EMS with a mean segregation ratio of 6.25 per cent. Among these, four mutations were associated

with other variations also, viz. two with seed colour variations (in 40 and 200 mM), one with leaf mutation (in 120 mM) and one with seed and stem colour mutations (in 160 mM).

Seed colour mutations:

Nine seed colour mutations included dark brown (one each in 80 and 160 mM), yellowish brown (one each in 120, 160 and 200 mM) greenish brown (one in 200 mM) and white (one each in 40, 80 and 120 mM) coloured types. Five of them were associated with other mutations also, of which two with stem colour variation (in 120 and 160 mM), two with seed size variation (in 40 and 200 mM) and one with seed size and stem colour variations (in 160 mM). A mean segregation per cent of 5.81 was recorded for seed colour variations.

Twenty two plants with distinct changes from the normal one were identified as mutants, while five of them exhibited changes in two characters and one, in three characters. All the three stem colour mutants showed variation in seed colour also, but not the same colour change and one such mutant exhibited small seed size also. In two mutants, seed size variation was associated with seed colour variation and in another seed size mutant leaf size was also

reduced. Among different types seed size mutants were observed as the most frequent (41.38%) followed by seed colour mutations (31.03%).

Yield parameters of pod length, sed size mutants and control plants are given in table 14.

Growth habit of podlength and seed size mutants did not change and all other yield characters of them remained unaltered except the number of seeds per pod in pod length mutants and 100 seed weight in seed size mutants.

4.2.2 Micromutations:

Fifteen plants were selected from each line of a particular treatment and the data were recorded. The distribution and mean performance of plants at different levels of gamma rays and EMS are given in tables. 15-21.

Height at 45 days ater sowing (Table 15)

The negative variants showed an increasing trend while positive variants showed a decreasing trend with an increase in doses of gamma rays. But in case of EMS both the variants showed an erratic behaviour with doses, however the

Table 14. Yield parameters of podlength and seedsize mutants

		Gamma rays				EMS				
		PL (cm)	NPF (No.)	NS (No.)	SW (g)		PL (cm)	NPF (No.)	NS (No.)	SW (g)
POD LENGTH mutants										
Small	i	12	8	6	29.4	iii	10	5	5	32.1
	ii	11	6	5	25.9					
Large	i	24	6	12	31.5		-	-	-	-
SEED SIZE mutants										
Flat						i	19	7	9	22.9*
Bold	i	18.2	8	8	39.2	iv	20.3	6	9	42.8
	ii	22.1	7	10	38.5					
	iii	21.5	8	9	44.6					
Small	i	19	7	11	17.6	v	20	7	11	17.9
	ii	16	9	9	15.2	vi	21	7	13	17.3
	iii	22	8	14	12.2	vii	19	6	10	14.7
	iv	21	5	12	12.4	viii	20	5	11	17.3*
						ix	19	4	12	12.9
						x	18	6	10	15.6
						xi	20	7	9	17.0*
						xii	21	5	10	16.4*
Long	i	21	6	9	36.1	ii	21	5	9	33.7
						iii	20	6	9	34.5
CONTROL		18.99	6.23	9.24	30.99		18.99	6.23	9.24	30.99

PL - Pod length (cm)
NPF - Number of pods at first harvest
NS - Number of seeds per pod
SW - 100 seed weight (g)
* - Associated with other changes also

Table 15. Effect on mutagens on height at 45 DAS

Treatments	Range (cm)	Frequency Distribution %			Mean (SE) (cm)	Shift in Mean (cm)	't'
		Negative variants	Control variants	Positive variants			
Control	52-63	-	-	-	59.23 (1.08)	-	-
Gamma rays							
100 Gy	32-65	25.33	62.00	12.67	57.23 (5.22)	-2.00	<1
200 Gy	20-64	63.82	23.80	12.38	48.73 (12.55)	-11.0	<1
300 Gy	21-62	71.11	26.67	2.22	47.96 (11.86)	-11.27	<1
400 Gy	20-59	93.33	6.67	0.00	92.4 (11.4)	-16.83	1.47
EMS							
40 nM	39-64	31.85	52.59	15.56	57.23 (7.05)	-2.00	<1
80 mM	36-64	20.00	56.19	23.81	57.64 (5.17)	-1.54	<1
120 mM	37-63	40.00	45.33	6.67	55.37 (6.49)	-3.86	<1
160 mM	28-64	38.89	50.00	11.11	55.39 (7.06)	-3.84	<1
200 mM	24-63	56.67	36.67	6.67	51.58 (9.57)	-7.65	<1

CONTROL VARIANTS : Mean - S.E ≤ ≥ Mean + S.E

highest frequency of negative variants and lowest of positive variants were at the highest dose (200 mM).

On comparison with control it is seen that the mean shifted in the negative direction with respect to all the doses of gamma rays and EMS. Shift in mean was higher in case of gamma rays than EMS, but none of the treatments either in gamma rays or EMS exhibited significant difference.

ii. **Height at 75 days after sowing (Table 16)**

In gamma ray treated population the negative variants showed an increasing trend and positive variants showed reverse trend with increase indose. In case of EMS, negative and positive variants did not show any linear dose-dependence. Highest frequency of negative variants was at the highest dose in two mutagens.

The mean shifted in the negative direction except in 100 Gray units of gamma rays (0.39 cm) and 80 mM of EMS (0.27 cm). Shift in mean was higher in gamma rays compared to EMS and none of the treatments exhibited significant difference.

Table 16. Effect of mutagens on height at 75 DAS

Treatments	Range (cm)	Frequency Distribution (%)			Mean (SE) (cm)	Shift in mean (cm)	't'
		Negative variants	Control variants	Positive variants			
Control	162-190	-	-	-	171.2 (3.11)	-	-
Gamma rays							
100 Gy	151-192	14.00	67.33	18.67	171.59 (7.71)	+0.39	<1
200 Gy	61-195	50.47	22.86	26.67	147.28 (35.37)	-23.92	<1
300 Gy	72-180	73.33	24.45	2.22	149.2 (32.26)	-22.00	<1
400 Gy	77-162	92.26	7.74	0.00	133.47 (27.28)	-37.47	1.36
EMS							
40 mM	142-188	36.30	39.26	24.44	168.04 (1.035)	-3.16	<1
80 mM	133-188	20.95	50.48	28.57	171.47 (9.95)	+0.27	<1
120 mM	125-184	29.33	57.33	13.33	166.38 (12.61)	-4.82	<1
160 mM	125-187	30.00	55.56	14.44	167.55 (10.35)	-3.65	<1
200 mM	92-182	55.00	36.67	8.33	156.84 (20.62)	-14.36	<1

control variants Mean - S.E $\leq$ $\geq$ Mean + S.E

Days to flower (Table 17)

The negative variants showed decrease with increase in dose and positive variants had no such trend with doses in case of two mutagens. Positive variants exhibited highest freuency than negative variants except at the lowest dose of gamma rays.

The mean shifted in the positive direction viz. towards lateness except in 100 Gray units of gamma rays and 40 mM of EMS. But this positive shift in mean was marginal, with a minimum of 2.1 days and a maximum of 3.68 days in gamma ray exposures and a minimum of 0.91 days and a maximum of 3.56 days in EMS treatment.

Pod length (Table 18)

The negative and positive variants did not exhibit any unidirectional change with dose. Negative variants recorded higher frequencies than positive variants.

Comparison of the mean performance of plants showed that the mean changed in the negative direction in different treatments of gamma rays and EMS. The shift in mean was

Table 17. Effect of mutagens on days to flower

Treatments	Range (cm)	Frequency Distribution (%)			Mean (SE) days	Shift in mean days	't
		Negative variants	Control variants	Positive variants			
Control	65-82	-	-	-	77.28 (1.6189)		
Gamma rays							
100 Gy	63-90	7.33	90.67	2.00	76.29 (5.21)	-0.96	<
200 Gy	64-92	5.71	70.48	23.51	79.35 (6.5)	2.10	<
300 Gy	66-89	2.22	75.56	22.22	79.4 (5.42)	2.15	<
400 Gy	72.94	0.00	80.0	20.0	80.93 (4.99)	3.68	<
EMS							
40 mM	66.90	3.70	91.11	5.19	76.21 (3.56)	-1.04	<
80 mM	66.88	0.00	93.33	6.67	78.16 (4.24)	0.91	<
120 mM	67.90	0.00	89.33	10.67	79.67 (4.07)	2.42	<
160 mM	68.98	0.00	93.33	6.67	78.91 (4.12)	1.66	<
200 mM	71.91	0.00	83.33	16.67	83.81 (3.69)	3.56	<

Table 18. Effect of mutagens on pod length

Treatments	Range (cm)	Frequency distribution % Negative variants	Control group	Positive variants	Mean (SE) (cm)	Shift in Mean (cm)	't'
Control	16-22	-	-	-	18.99 (0.62)	-	-
Gammarays							
100 Gy	11-24	36.64	46.36	17.33	18.07 (2.43)	-0.91	<1
200 Gy	12-22	32.00	60.00	8.00	18.89 (1.99)	-0.10	<1
300 Gy	14-22	40.00	46.67	13.33	17.40 (2.24)	-1.59	<1
400 Gy	15-21	42.22	48.88	8.88	18.00 (1.73)	-0.99	<1
EMS							
40 mM	14-22	20.00	56.19	23.81	18.68 (2.16)	-0.31	<1
80 mM	14-21	20.95	50.48	28.57	18.82 (1.85)	-0.17	<1
120 mM	10-21	36.30	39.26	24.44	17.89 (1.92)	-1.10	<1
160 mM	14-22	40.00	45.33	14.67	18.36 (2.75)	-0.63	<1
200 mM	14-22	50.47	22.86	26.67	18.45 (1.85)	-0.54	<1

maximum (1.59 cm) at 300 Gray units of gamma rays and 120 mM of EMS (1.11 cm). None of the treatments exhibited significant change.

Number of pods per plant (Table 19)

Both positive and negative variants did not show continuous increase or decrease with increase in dose of gamma rays and EMS. Negative variants had higher frequency than positive variants in all the treatments of the two mutagens.

The mean shifted in the negative direction in all the treatments of both the mutagens. The maximum negative shifts of 2.43 and 2.23 were recorded at 400 Gray units of gamma rays and 120 mM of EMS. Shift in mean did not vary too much in both cases, but a significant reduction was noticed in 400 Gray units of gamma rays.

Number of seeds per pod (Table 20)

Negative variants did not exhibit any regular change in gamma ray and EMS treatments. Positive variants showed a similar behaviour in case of gamma rays, but a decreasing trend with increase in dose of EMS. Negative

Table 19. Effect of mutagens on number of pods per plant (first harvest)

Treatments	Frequency of distribution (%)					Shift in Mean (no.)	't'
	Range (no.)	Negative variants	Control group	Positive variants	Mean (SE) (no.)		
Control	5-10	-	-	-	6.23 (0.74)	-	-
Gammarays							
100 Gy	2-10	36.64	46.00	17.33	5.74 (1.71)	-0.49	<1
200 Gy	1-12	23.80	55.24	20.96	5.42 (2.39)	-0.81	<1
300 Gy	2-11	42.22	48.88	8.88	4.78 (1.45)	-1.45	<1
400 Gy	2-6	40.00	46.67	13.33	3.8 (0.55)	-2.43	2.02
EMS							
40 mM	2-10	26.67	52.59	20.74	5.66 (1.61)	-0.57	<1
80 mM	2-10	23.81	59.05	17.14	5.01 (1.54)	-1.22	<1
120 mM	2-7	32.00	60.00	8.00	4.0 (1.24)	-2.23	1.54
160 mM	1-8	23.33	64.45	12.22	4.3 (1.04)	-1.92	1.47
200 mM	2-7	23.33	61.67	15.00	4.2 (0.87)	-2.03	1.78

control group

Table 20. Effect on mutagens on number of seeds per pod

Treatments	Range (no.)	Frequency distribution (%) Negative variant	Control group	Positive variants	Mean (SE) (no.)	Shift in Mean (no.)	't'
Control	6-12	-	-	-	9.24 (0.419)	-	-
Gammarays							
100 Gy	6-12	30.66	49.33	20.00	8.85 (0.74)	-0.39	<1
200 Gy	5-12	47.62	30.47	21.91	8.51 (1.02)	-0.73	<1
300 Gy	5-14	46.67	48.89	4.41	8.33 (1.42)	-0.91	<1
400 Gy	7-12	73.33	26.67	0.00	9 (0.577)	-0.24	<1
EMS							
40 mM	6-13	31.85	48.15	20.00	8.99 (0.69)	-0.25	<1
80 mM	5-12	46.66	41.90	11.43	9.11 (1.62)	-0.11	<1
120 mM	5-11	70.66	26.67	2.67	8.64 (1.00)	-0.60	<1
160 mM	5-12	58.89	40,00	1.11	8.57 (0.94)	-0.67	<1
200 mM	5-12	53.33	46.67	0.00	8.75 (0.99)	-0.49	<1

control group Mean - S.E ≤ > Mean + S.E

variants occured at a higher frequency compared to positive variants. Highest frequency of negative variants was at the highest dose in case of gamma rays.

The mean shifted in the negative direction in all the treatments of gamma rays and EMS. The maximum negative shift was 0.91 and 0.67 at 300 Gray units of gamma rays and 160 mM of EMS respectively. The treatments were not significantly different from the control.

100 seed weight (Table 21)

Negative and positive variants in two mutagens did not have any direct dose-dependence. Negative variants recorded higher frequency compared to positive variants

The mean shifted in the negative direction by a maximum of 3.18 and 2.98 gm in 400 Gray units of gamma rays and 200 mM of EMS. The treatments with significant differences were not recorded.

4.3 Mutagenic effectiveness and mutagenic efficiency

The mutagenic effectiveness and efficiency of different doses of gamma rays in inducing chlorophyll mutations are given in Table 22.

Table 21. Effect of mutagens on 100 Seed weight

Treatments	Frequency distribution (%) Range (gm)	Negative variants	Control group	Positive variants	Mean (SE) (gm)	Shift in Mean (gm)	t
Control	25.4-34.5	-	-	-	30.99 (1.026)	-	-
Gammarays							
100 Gy	17.6-39.2	39.33	54.0	6.67	30.31 (3.14)	-0.68	<1
200 Gy	15.2-44.6	50.47	43.8	5.71	30.36 (3.1)	-0.63	<1
300 Gy	12.2-33.3	48.89	46.67	4.44	28.49 (3.16)	-2.5	<1
400 Gy	12.4-33.3	33.33	60.00	6.67	27.81 (4.83)	-3.18	<1
EMS							
40 mM	17.3-36.6	32.59	62.22	5.19	29.71 (2.93)	-3.18	<1
80 mM	14.7-42.8	25.71	65.71	8.57	29.36 (3.10)	-1.63	<1
120 mM	17.3-36	34.66	62.67	2.67	29.65 (3.03)	-1.34	<1
160 mM	12.9-34	37.78	58.89	3.33	28.38 (3.13)	-2.61	<1
200 mM	16.4-34.8	35.00	58.33	6.67	28.01 (2.87)	-2.98	1.03

control group - Mean - S.E. ≤ > Mean + S.E.

Table 22. MUTAGENIC EFFECTIVENESS AND MUTAGENIC EFFICIENCY (Gamma rays)

Treatments (Gray units)	Chlorophyll mutation frequency %	Relative percentage			Mutagenic effectiveness %	Mutagenic efficiency		
		Lethality %	Injury %	Sterility %		Lethality %	Injury %	Sterility %
100	4.29	10.71	3.38	13.33	42.9	40.03	126.92	32.18
200	6.71	2.68	18.57	13.33	33.55	249.94	36.13	50.33
300	6.33	8.86	19.03	23.33	21.10	71.43	33.26	27.13
400	5.88	5.88	28.41	26.67	14.70	99.96	20.69	22.04

Table 23. MUTAGENIC EFFECTIVENESS AND MUTAGENIC EFFICIENCY (EMS)

Treatments (Millimoles)	Chlorophyll mutation frequency %	Relative percentage			Mutagenic effectiveness %	Mutagenic efficiency		
		Lethality %	Injury %	Sterility %		Lethality %	Injury %	Sterility %
40	3.37	2.40	3.38	16.67	112.33	148.24	99.78	20.51
80	3.55	4.14	2.6	16.67	59.16	85.70	136.53	21.29
120	4.52	4.87	6.5	23.37	50.22	110.99	69.54	69.53
160	5.65	3.33	6.48	20.00	47.08	169.66	87.19	28.25
200	4.62	7.69	12.92	20.00	38.80	60.06	35.76	23.10

It is seen that the percentage of injury and steriltiy had a linear relationship with dose, but percentage of lethality was inconsistant with dose. At first, lethality decreased from 10.71 (100 Gray units) to 2.68 (200 Gray units), from where it showed an increase at 300 Gray units and thereafter again it decreased.

Mutagenic effectivenes showed a decreasing trend with increase in dose. The minimum (14.7) was at 400 Gray units and maximum (42.9) at 100 Gray units.

Mutagenic efficiencies on lethality and sterility basis first increased and become maximum at 200 Gray units and thereafter it decreased viz, mutagenic efficiency on lethality or sterility basis did not have any direct dose-dependence. But efficiency on the basis of injury showed an inverse dose relationship.

The data pertaining to the mutagenic effectiveness and efficiency of different concentrations of EMS in inducing chlorophyll mutations are given in Table 23.

The lethality and injury percentages were minimum (2.403, 2.6) at 40 and 80 mM and maximum (7.69 , 12.92) at 200 mM. The sterility percentage was minimum (16.67) at 40

and 80 mM and maximum (23.37) at 120 mM. These three did not have any direct dose-dependence, and showed an erratic behaviour with dose.

Mutagenic effectiveness was found to be high (112.33) at the lowest dose of EMS (40 mM) and it showed an inverse relationship with dose.

Mutagenic efficieny of EMS was seen to be high at 160, 80 and 120 mM respectively on the bais of lethality injury and sterility. Mutagenic efficieny on the basis of lethality first decreased, thereafter increased with increase in dose except at 200 mM. Mutagenic efficiency on injury basis did not exhibit any stable response with dose. The efficiency on the basis of sterility increased with increase in doses upto 120 mM and above which it showed a decreasing trend.

Plate 1 - Spectrum of chlorophyll mutations

Plate 2 - Spectrum of chlorophyll mutations

Plate -1

Plate-2

Plate 3 - Xantha seedling

Plate 4 - Variation in chlorophyll content of chlorophyll mutant and normal plant

Plate-3

Plate-4

Plate 5 - Variation in the size of leaf

1. MUTANT 2. NORMAL

Plate 6 - Variation in the number of leaflets

Plate - 5

Plate - 6

Plate 7 - Variation in the texture of leaves

Plate 8 - Variation in the colour of vines

1. LIGHT GREEN 2. DARK GREEN 3. NORMAL

Plate-7

Plate-8

Plate 9 - Variation in the length of pod

1. SMALL 2. LONG 3. NORMAL

Plate 10 - Variation in the size of the seeds

1. NORMAL 2. SMALL 3. FLAT 4. LONG 5. BOLD.

Plate-9

Plate-10

Plate 11 - Variation in the colour of the seeds

1 NORMAL 2. BLACK 3. YELLOWISH BROWN

4. GREENISH BROWN 5. WHITE

Plate-11

DISCUSSION

V. DISCUSSION

Among legumes, mutation technique has more profitably been exploited as an effective supplementary tool in genetic improvement in major pulse crops. In winged bean, nutritionally very important but an under-exploited pulse crop, mutation induction can create variability and thus allows to exploit the high potentials of this crop. Various types of mutagens are now being used to create variability by inducing useful mutations in crop plants. Different doses of gamma exposures and different concentrations of EMS were tried and reported to be effective in various pulse crops by several workers. In the present study also, mutation induction was done with well established and most commonly used mutagens, viz., gamma rays and EMS (Micke *et al.*, 1987). Although the frequency of valuable mutations are less and most of the mutations are deleterious in nature, one can isolate the mutants suitable for modern agricultural systems by applying appropriate selection techniques. The segregation and visual selection of easily identifiable characters can be made efficiently which often is the prime basis for selecting macro mutants. Hence to obtain maximum output of beneficial mutations it is necessary to assess the

mutation rate. The present study as a continuation of a departmental project initiated with the objective of finding out the mutagenicity of gamma rays and EMS in the M_2 generation of winged bean.

The handling of mutagen treated material is a very important aspect in mutation breeding research. The after effects of mutation in the M_2 generation are determined by the mode of action of mutagens and interactions of many factors in an organism. The proportion of mutated plants to normal ones namely, the mutation rate can be computed on the basis of number of mutations per 100 M_1 plants (Gustafsson, 1940) or spikes (Stadler, 1928) and the number of mutants per 100 M_2 plants (Gaul, 1960). Gaul (1960) concluded that the mutation rate per 100 M_2 plants provides the best index as it is proportional to the initial mutation rate. The output of mutations are quantitatively expressed by the rate of mutations and qualitatively by the spectrum of mutations. According to Gustafsson (1947) chlorophyll mutations are more frequent and can easily be recognised. Gaul (1964), Nilan et al. (1965) and Kawai (1969) suggested that a cholorophyll test in the M_2 would provide a good guide line for assessing the effective mutagen treatments. But the success of a

mutagen treatment depends upon its ability to create diverse, but useful mutants capable of producing viable seeds. So an estimate of viable mutations is essential for all practical mutation studies. Induced mutations in polygenes occur towards positive or negative direction or towards both of the two, Konzak et al. (1965) proposed the the mutagenic efficiency and mutagenic effectiveness in relation to chlorophyll mutation frequency which determine the usefulness of any mutation breeding programme. Hence the parameters, frequency and spectrum of chlorophyll and viable mutations including both positive and negative mutations, mutagenic effectiveness and mutagenic efficiency are capable of giving satisfactory answers to the problems contained in the objective of the study. The results obtained are discussed under the following sections.

5.1. Cholorophyll mutations

5.1.1. Frequency

In the present study chlorophyll mutations were classified on M_2 progeny row and M_2 seedling basis. The chlorophyll mutation frequency estimated on M_2 progeny row

and M_2 seedling basis showed a similar varying pattern with dose. The chlorophyll mutation frequency increased at the lower doses and decreased at higher doses. This pattern of change in frequency in relation to dose was same not only for gamma rays but also for EMS. Similar results have been reported by Khan (1981) in greengram, Rangaswamy (1989) in cowpea and Vanniarajan et al. (1993) in blackgram. This indicates the elimination of mutations at the higher doses either for gamma ray irradiation or EMS treatments as reported by Singh et al. (1989) in lentil. Saturation or decrease in induced mutation frequency due to more lethal mutations beyond a particular dose was reported by Swaminathan (1961).

An insight into the chlorophyll mutation frequency revealed that the lower doses of gamma rays and higher doses of EMS are equally effective when the frequency is considered on M_2 progeny row basis. But based on M_2 seedling basis which is more important, it was found that the frequency of chlorophyll mutations was more for gamma ray exposures. In general, the frequency and its range were high for gamma rays. This was in agreement with the results of Venkateswaralu et al. (1980) and Brenda (1987) in pigeon pea.

5.1.2. Spectrum

In the present investigation a fairly wide spectrum of chlorophyll mutations was identified including xantha, chlorina, viridis, viridalba, white streaked and maculata types as reported earlier by previous investigators in legumes. [Jana (1964), Ramaswamy (1973), Kundu and Singh (1982a) and Vanniarajan et al. (1993) in blackgram; Louis and Kadambavana Sundaram (1973), Narasinhan and Kumar (1976) and Thirugunakumar (1986) in cowpea; Ratnaswamy et al. (1978), Grover and Virk (1984) and Kamini et al. (1988) in greengram; Chaturvedi et al. (1982), Nadarajan et al. (1982) and Brenda (1987) in pigeon pea; Manju (1981) in horsegram and Singh et al. (1989) in lentil]. Similar types were also observed in winged bean by Veeresh and Sivasankar (1986) after gamma ray exposure and Savithramma (1987) after EMS treatment. Leaf streaking was also reported in winged bean by Armachevilo and Bernardo (1981) after gamma rays and EMS treatments.

In the present study none of the treatments either in gamma rays or EMS exhibited all the six cholorophyll mutations. Chlorina type was maximum followed by viridis and white streaked in gamma ray exposures where as chlorina and

xantha types were maximum in EMS treatments.. Considering the effects of two mutagens in general it was seen that chlorina appeared as the most frequent one. Higher proportion of chlorina was earlier reported by Veeresh and Sivasankar(1986) after gamma ray exposures in winged bean and Vanniarajan et al. (1993) after gamma rays and EMS treatments in blackgram. The reason for the appearance of greater number of chlorina might be attributed to the involvement of polygenes in chlorophyll mutations according to Gaul (1964).

Different chlorophyll mutations except xantha showed inconsistant behaviour with doses of both the mutagens and occurred randomly. Such an erratic behaviour of different chlorophyll mutations in relation to dose was also reported by Brenda (1987) in redgram after gamma ray and EMS treatments. Xantha type occurred with maximum frequency at the highest dose. This was in agreement with the results of Packairaj (1988) in cowpea. Nadarajan et al. (1985) reported that sensitivity depended upon its genetic architecture and the mutagens employed as suggested earlier by Blixt (1968).

5.1.3. Single and multiple chlorophyll mutation frequency

Isolation of two or more chlorophyll mutations from a segregating M_2 progeny row has been referred as multiple

mutations. In the case of highest dose of gamma rays only one progeny row was found segregating for chlorophyll mutations and hence the effect of that particular dose could not be considered absolute inspite of the occurrence of high multiple mutation frequency. Hence the effect avoiding the highest dose (400 Gray units of gamma rays) revealed, in general, that the higher proportion of multiple chlorophyll mutations was at the lower doses and lower proportion at the higher doses of both the mutagens. Decrease in multiple chlorophyll mutation frequency with increase in dose was followed by a progressive increase in single mutation frequency. This indicates that mutagenic action tended to become more specific at higher doses as reported by Ramaswamy (1973) in blackgram. Similar trend in multiple/single chlorophyll mutation frequency was reported by Packairaj (1988) and Rangaswamy (1989) in cowpea. The low survival or germination rate at higher doses in the previous generation study might be due to this specific action leading to more frequent lethal mutations at higher doses as reported by Swaminathan (1964). The avoidance of mutations as lethal ones might be one of the reasons for low chlorophyll mutation frequency at higher doses in M_2.

5.1.4. Segregation ratio

In the present investigation, segregation ratios of chlorophyll mutants did not show any dose-dependence either for gamma irradiation or EMS treatment. Such a behaviour was reported by Brenda (1987) in pigeonpea and Packairaj (1988) and Rangaswamy (1989) in cowpea after gamma ray and EMS treatments. It was seen that most of the mutants recorded segregation per cent in between 10 and 14 and always less than 25 except in 80 mM of EMS (25%). The lower range of segregation ratio was noticed for chlorophyll mutations by Rangaswamy (1989) in cowpea following gamma ray exposures and EMS treatments. Moh and Alan (1964) from their study on Phaseolus vulgaris concluded that induced genetic changes with low transmission frequency were probably associated with minute chromosomal aberrations like deletions which affected gene transmission. This might be one of the reasons for not obtaining the excepted mendelian segregation ratio of 0.25. Other reasons as explained by Swaminathan (1961) might be smallness of mutated sector involving more number of initial cells due to diplontic selection.

5.2. VIABLE MUTATIONS

5.2.1. Macromutations

5.2.1.1. Frequency

Mutation frequencies on m_2 progeny row or plant basis did not show any direct dose dependence either for gamma rays or EMS, but the frequency on M_2 plant basis decreased at the higher doses of gamma rays. The mutagenic effect at higher doses might become drastic in nature leading to more lethal mutations due to the dose dependent influence on metabolism, genetic changes, cytological disturbances and growth (Khanna, 1991). This might be one of the reasons for reducing frequency at higher doses. Similar results were also reported by Veeresh and Sivasankar (1987) after gamma ray irradiation and Savithramma (1987) after EMS treatments in winged bean. An inconsistent behaviour of viable mutations in relation to dose was reported by Brenda (1987) after gamma ray and EMS treatments in red gram. In case of EMS, the decrease in frequency at higher doses was not pronounced. Among the two mutagens, gamma rays showed higher values at three lower doses than any of the concentrations of EMS tried. This was in agreement with the results of previous generation study by Reeja (1993) that the mutagenic effects on several characters were less pronounced for EMS especially in lower doses.

5.2.1.2. Spectrum

In the present study many morphological mutants were produced by gamma ray exposures and EMS treatments with respect to leaf, stem, pod and seed characters. However their genetic control and inheritance was not known. Two types of leaf mutants viz., small sized and thick-soft textured from both gamma ray and EMS treated population and another type with a few tetra foliate leaves from EMS treated population were isolated. Such leaf mutants with modification in the size, texture and number were reported by Kesavan and Khan (1978) and Veeresh and Sivasankar (1986) in winged bean; Borikar et al. (1983) in cowpea, Pulivarthy and Mary (1987) in blackgram, Sathyanarayana et al. (1989) in blackgram and greengram and Singh and Yadav (1991) in greengram. Stem colour mutations noticed included light green, and dark brownish green coloured types. Most of them were associated with seed colour mutations also. Variations in stem colour were reported by Rangaswamy (1989) in cowpea after gamma ray and EMS treatments. Mutations affecting pigmentation might cause associated colour changes in stem and seed. Such mutations affecting anthocyanin pigmentation were reported in stem, petiole and seed and an

anthocyaninless mutant was isolated by Jana and Rao (1974) in blackgram after X-ray irradiation. Pod length mutants (small types), seed size mutants including bold, small and long types and seed colour mutants like dark brown, yellowish brown, green brown and white coloured types were noticed in both mutagen treated plants. In addition to these mutants, pod length mutant with long pods from gamma ray treated plants and seed size mutant with flat seeds from EMS treated plants were also isolated. Distinct changes in pod length and seed size were reported by Bhadra and Jain (1986) after gamma ray and EMS treatments, Bhalla and Bhamburkar (1987) after gamma ray and Hydrazine treatments in blackgram, Singh and Agarwal (1986) after gamma ray, NMU and EMS treatments in cluster bean and Sumanggono (1987) and Singh and Yadav (1991) gamma ray treatment in greengram. Seed coat colour mutations following mutagenic treatment with gamma rays were reported by Thirugunakumar (1986) in cowpea, Bhamburkar and Bhalla (1987) in blackgram and Singh and Yadav (1991) in green gram. According to Gunkel and Sparrow (1961) some of these morphological variations might be attributed to chromosome breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism and accumulation of free aminoacids.

5.2.2. Micro mutations

Genetic variability created using physical and chemical mutagens has become the most potent line of approach. Khan (1988c) reported that practical role of induced mutations in crop improvement of pulse crops can best be assessed on the basis of quantitative characters. Gregory (1965) hypothesised that normal looking plants of mutagen treated population may be variously mutated with large number of small changes and these mutations there by increase the scope of selection of economically important characters.

In the present study almost all the treatments of two mutagens showed both positive and negative variants for characters like height at 45 and 75 days after sowing, pod length, number of pods at first harvest, number of seeds per pod and 100 seed weight. But in case of character, days to flower all the treatments except the lowest doses produced only positive variants viz, the character changed towards lateness. A general conclusion drawn from earlier studies clearly demonstrates that mutation can create genetic variation in any direction or either of the two. Lower frequency of negative variants was observed at two lower

doses for all characters except 100 seed weight (gamma rays), pod length (EMS) and days to flower. Similarly high frequencies of positive variants were also recorded at two lower doses for all characters except 100 seed weight (EMS) and days to flower (EMS) indicating less injury in lower doses.

A comparison of treatments with control showed that the mean performance of plants in different treatments shifted for the seven characters studied. A negative shift was exhibited for the characters like height at 45 and 75 days after sowing, pod length, pod number, seed number and 100 seed weight. However the range of variants changed bidirectionally for all these characters. Even though higher frequency of positive variants was at the lower doses a negative shift was noticed because the negative variants exceeded the positive variants even in the lower doses. A reduction in plant height as a result of mutagenic treatments has been reported by several workers. Kundu and Singh (1982b), after gamma irradiation reported reduction in plant height in blackgram. Sumabai (1989) observed that reduction of vine length was maximum for higher exposures of gamma rays in sweet potato. Negative shift was noticed by Palaniswamy

(1975) for mean values of characters like number of pods per plant, pod length, number of seeds per pod and 100 seed weight in cowpea after gamma rays and EMS treatments. All the treatments changed the days to flower towards lateness and positive shift in mean was recorded in two lower doses for height at 75 days after sowing. Khan (1983), after gamma ray and hydrazine hydrate treatments, reported that the mean values of the treated population shifted in positive direction for days to flower in greengram. Increased plant height after mutagenic treatment was reported by Kamannavan (1985) in Chilli.

In the present study, the mean performance was found minimum for higher exposures or treatments and maximum for the control and lower doses. Nadarajan (1983) reported that the mean values of all characters were lower than that in control except for days to flower in which an increase in mean was observed. Maximum shift at higher doses was also reported by Khan (1988b) in greengram and Sumabai (1989) in Sweet potato. Reduction in growth and yield characters at higher doses of mutagen treated material was attributed to abnormal cytological behaviour due to chromosomal damage and miotic inhibition (Sparrow et al.

(1952). Delayed flowering and reduced number of pods at first harvest at higher doses might be due to lower metabolic activities in the vegetative phase. Positive shift for height at 75 days after sowing might be resulting from the stimulatory effect of the mutagens or less inhibition at lower doses and also due to the recovery of mutants as cited by Khanna (1991).

5.3. Mutagenic effectiveness and mutagenic efficiency

By estimating the effectiveness and efficiency the potency of a mutagen can well be assessed. Konzak et al. (1965) proposed mutagenic effectiveness which means the rate of mutation induction as dependent upon the mutagenic dose and mutagenic efficiency which refers to mutagenic rate in relation to various biological effects, usually a measure of damage. Since the chlorophyll mutations were parallel to other mutations chlorophyll mutation frequency was taken into consideration for estimating effectiveness and efficiency as suggested by Kawai (1969).

In the present study in both the mutagens the treatment with lowest dose was more effective in inducing chlorophyll mutations. Similar results were reported by

Brenda (1987) after mutagenic treatment with gamma rays and EMS in redgram and Veeresh and Sivasankar (1986) after gamma ray treatment in winged bean. The maximum mutagenic effectiveness at the lower doses might be due to the absence of corresponding increase in the mutation rate with dose. Ehrenberg et al. (1962) emphasised that at higher doses, mutation rate is lower than expected and the dose-rate of mutation curves may deviate from linearity.

According to Gaul et al. (1972) the effectiveness of a mutagen was more of theoretical importance than of any immediate practical implications. Efficiency was estimated based on the biological damages such as % lethality, % injury and % sterility.

In the present study lethality percentrage with respect to both the mutagens did not have any linear relationship with doses. Injury and sterility percentages showed direct dose relationship with doses of gamma rays, where as in EMS no such an increasing trend was observed. The mutagenic efficiency of gamma rays on the basis of lethality and sterility did not exhibit any direct dose dependence, but on the basis of injury, inverse relationship

with dose was observed. In case of EMS the mutagenic efficiency on the basis of lethality, injury and sterility percentages did not have any stable response with dose. Considering the efficiency of gamma rays and EMS, it was found that among various exposures of gamma rays, 200 Gray units was the most efficient one on the basis of lethality and sterility and 100 Gray units on the basis of injury. Among different concentrations of EMS the maximum values for efficiency on the basis of lethality, injury and sterility percentages were recorded at 160, 80 and 120 mM respectively. Higher efficiency was observed at lower doses of gamma rays and middle doses of EMS. High efficiency at lower doses were reported by Veeresh and Sivasankar (1986) in winged bean and Brenda (1987) in redgram. The greater efficiency at the lower doses might be due to reduced lethality, injury and sterility. To obtain high efficiency at higher doses, mutagenic effect may greatly surpass other damage effects in the cells and toxic effects as suggested by Gaul et al (1972).

In the present investigation among the doses tried for gammarays, lower doses tried were more capable of inducing chlorophyll and viable mutations and also were found

as the most effective and efficient ones, where as in EMS mutagenic effects as chlorophyll and viable mutations were more frequent only in higher doses and middle or higher doses were appeared as the most efficient ones. Since lower doses viz., 100 and 200 gray units were found as the the most effective and efficient ones the dose range around this can be tried in future mutation breeding studies in this crop and recurrent irradiation techniques has to be tried to overcome lethal effects. The desirable variations isolated, if heritable, can be selected and utilized to evolve new varieties with added advantages. Long poded and bold seeded mutants isolated mostly from the lower doses with less ill effects needed to be tested for other yield characters in the subsequent generations. Eventhough no bushy or dwarf types could be spotted out, the mutants with small seed size, low 100 seed weight and more number of seeds provide scope for further selection of new plant types. Shift in mean values for growth and yield characters indicate the micromutations and emphasis the possibility of micromutational selections in further generations.

SUMMARY

VI. SUMMARY

A research programme on the mutagenicity of gammarays and EMS in the M_2 generation of winged bean [*Psophocarpus tetragonolobus* (L.)] was carried out at the Department of plant breeding, College of Agriculture, Vellayani. Seeds collected individually from M_1 plants which were initially subjected to four doses of gamma rays (100, 200, 300 and 400 Gray units) and five doses of EMS (40, 80, 120, 160 and 200 millimoles) were sown in non-replicated progeny row trial to raise the M_2 generation. The mutagenicity of gamma rays and EMS was studied based on chlorophyll and viable mutations. All the seedlings which exhibited deviations from the normal ones by colour differences or by the presence of white patches on the leaves were considered as chlorophyll mutants and the plants which exhibited deviations from the normal ones for morphological characters were isolated as viable mutants. Observations were recorded on height at 45 and 75 days after sowing, days to flower, podlength, number of pods per plant at first harvest, number of seeds per pod and 100 seed weight. The chlorophyll and viable mutants were classified on M_2 progeny row and M_2 seedling basis.

The chlorophyll and viable mutants were expressed quantitatively by frequency and qualitatively by spectrum. The frequency distribution, range and mean performance of plants in each treatment were estimated. The results obtained are summarised as below :-

Quantitative analysis of chlorophyll mutations showed that mutation frequencies estimated on M_2 progeny row and M_2 seedling basis recorded an increase at the lower dose and a decrease at the higher doses of gamma rays. In case of EMS, the frequencies increased upto 160mM and above which it showed a decrease. The frequencies were maximum at a particular dose of both mutagens, eventhough chlorophyll mutations were classified on M_2 progeny row and M_2 seedling basis. Among the different doses tried maximum frequency was at 200 Gray units of gamma rays and 160mM of EMS. In general, it was found that, the frequency and its range of chlorophyll mutations was high for gamma rays.

When chlorophyll mutations were qualitatively analysed, a fairly large spectrum including xantha, chlorina, viridis, white streaked, maculata and viridalba was identified

for gamma rays and EMS, however none of treatments of two mutagens produced six types together. Among the six chlorophyll mutations, chlorina appeared as the most frequent in both the mutagens and xantha showed a direct relationship with dose.

In case of EMS, a clear evidence for the decrease in multiple mutation frequency in relation to increase dose was observed.

Most of the chlorophyll mutants segregated at a ratio in between 10 and 14 per cent or less than 25 per cent.

Quantitative analysis of viable mutations showed that in both the mutagens the maximum frequency on M_2 plant basis was at the higher doses, however the frequency didnot show any direct dose-dependence.

Twenty one viable mutants with respect to various morphological characters of leaf, stem, pod and seed were isolated from gamma ray treated population and twenty two from EMS treated population.

A negative shift was noticed for the characters like height at 45 and 75 days after sowing, pod length, pod number, seed number and 100 seed weight and a positive shift for days to flower. Even in the lower doses, negative variants exceeded positive variants and hence a negative shift was noticed for the characters. However the range of characters widened in both direction in lower doses.

The reduction in mean performance was found to be minimum for higher doses and maximum for control and lower doses.

Among the various doses, 100 Gray units of gamma rays and 40mM of EMS were identified as the most effective doses.

In case of gamma rays, 100 Gray units was identified as the most efficient dose when mutagenic efficiency is estimated on the basis of injury, but 200 Gray units appeared as the most efficient one on the basis of lethality or sterility. The lower two doses can be considered as the most efficient ones. But in EMS mutagenic efficiency was maximum at 120,160 and 80 mM respectively on the basis of sterility, lethality and injury basis.

REFERENCES

REFERENCES

* Agarakova, S.N., Sobelev, N.A and Yakovlev, A.G. (1976). Effect of ethylene imine and dimethyl sulphate vapour on the variability of characters in pea Kulthur, 5 : 37-43.

* Ahnstroem, G (1977). Radiobiology, Manual on Mut. Breed Tech. Rep. Ser., IAEA, 199 : 21-28.

Armachevilo, J.G. and Bernardo, F.A (1981). Effects of EMS and Co^{60} gamma irradiation in winged bean Ann Trop Research, 3 : 241-249.

Athwal, D.S (1963). Some X-ray induced mutations in Cicer aeritinum. Indian J. Genet, 23 (1) : 50-57.

Aurebache, C. and Robson, J.M. (1946). Chemical production of mutations. Nature, 157 : 302.

* Bacq, C. M. and Alexander, P. (1961). Fundamentals of Radiobiology, 2nd ed, Pergamon Press, Oxford.

Badani, P.S. and Bhalla, J.K. (1992). Mutagenic fields and sodium azide in clusterbean. Advances in plant Sci., 5 (2) : 534-541.

Bahl, J. R. and Gupta, P.K. (1982). Chlorophyll mutations in mungbean. (V. radiata). Theor. Appl. Genet, 63 : 23-26.

Bandopadhyay, B. and Bose, S. (1980). Chemically induced variants in blackgram (Phaseolus mungo. L). Current Science, 49 : 106-107.

Bhadra, S.K. and Jain. H.K. (1986). Genetics of some induced plant type mutations in blackgram. (Vigna mungo). Bangladesh. J. Bot, 15 : 27-32.

Bhagat, S.C., Bhatia, C.R., Gopikrishna, D.C., Mitra, R.K., Marahari, P., Pawar, S.E. and Thakare, R.G (1979). Seed protein improvement in cereals and grains legumes, IAEA pp, 225

Bhalla, J.K. (1989). Mutation research in legumes. In recent advances in Genetics and cytogenetics (eds: S.A. Farook and Irfan A.Khan), pp 163-178.

Bhamburkar, S. and Bhalla, J.K. (1987). Morphological and biochemical characterisation of induced mutations in blackgram. (Vigna mungo L. Hepper) Natl. Symp on "Genome Manipulation". Dec 26-27 1987, Patna.

Bhatia, C.R., Thakare, R.G., Pawar S.E. and Kale, D.M. (1991). Induced mutation for yield and yield components showing altered partitioning of dry matter. In plants mutatin breeding for crop improvement. Proc. Intl. Symp. on the contribution of plant mutation breeding to crop improvement IAEA and IAI of United Nations. IAEA 43-53 ISBN 92-01-01 0191-7.

*

Blixt, S. (1961). Quantitative studies of induced mutations in peas. Agric Hort. Gen ,18 : 219-227.

*

Blixt, S. (1966). Studies of induced mutations in peas. XIII. Segregation of an albina mutant. Agric. Hort. Genet , 24 : 48-55.

Blixt, S. (1968). Studies of induced mutations in peas. XXIV. Genetically conditioned differences in radiation sensitivity 2. Hereditas , 59, 303-326.

*

Blixt, S., Ehrenberg, L and Gelin, O. (1963). Studies of induced mutations in peas. VII. Mutation spectrum and mutation rate of different mutagenic agents. Agric. Hort. Grenet, Landskrona, 21 : 178-216.

Borikar, S.T. Heidge, V.S. and Salunke, M.R. (1983). Induced leaf mutations in cowpea. Trop. Grain Legume Bull, 3 (28) : 8-10.

Brenda V. A (1987). Biological effects of gamma rays and EMS in the M_2 generation of Redgram. (Cajanus Cajan. L.). MSc (Ag) Thesis. Fac Agri., Kerala Agric. Univ.

Casarett, A.P. (1968). Radiation Biology. Prentice-Hall Englowood Cliffs, New Jersey.

Chary, S.N. and Bhalla, J.K. (1988). Induced viable mutations in Pigeon pea. (Cajanus cajan. L. Millsp.) Indian j.Bot., 11 : 169-176.

Chaturvedi, S.N. and Sharma, R.P. (1978a). Induced mutations in redgram with special reference to floral composition. Current science, 47 (10) : 349-352.

Chaturvedi, S.N. and Sharma, R.P. (1978b) NMu induced polycarpellary mutant in redgram, Current Science, 47 (20) : 960-961.

Chaturvedi, S.N, Sharma, R.R., Singh Mendra and Palliwal (1982). Note on frequency and spectrum of chlorophyll mutations in Pigeon pea with special reference to biological damage. Indian J. Agric. Sci., 51 : 248-250.

Chomchalow, N., Haq, N. and Pongpangan, L. (1982). Genetic resources and breeding of the winged bean. In: Genetic resources and the plant breeder (Ed. Singh, R.B. and Chomchalow, N) IBPGR, ISBN. No. 92-9043-102-4.

Chowdhari, R.K. (1983). A bold seeded dwarf mutant of cowpea. Trop. Grain. legume Bull., 27 : 10-12.

Dahiya, B.S. (1973). Improvement of mungbean through induced mutation. Indian J. Genet., 33 (3) : 460-468.

Donini, B.S. (1977). Breeding methods and applied mutagenesis in fruit plants. In The Use of Ionization Radiation in Agriculture (Proceedings of workshop, Wageninger 1976). Biological Science EuR - 5815EN, pp 453-486.

Donini, B., Kawai, T. and Micke, A. (1984). Spectrum of mutant characters utilized in developing improved cultivars. In Selection in Mutation breeding, Vienna IAEA pp. 243-282.

Ehrenberg, L., Gustafsson, A. and Landquist, U. (1962). The mutagenic effect of ionizing radiations and reactive ethylene derivatives in barley Hereditas, 47 : 243-282

*

Freeze, E. (1971). Chemical mutagens Principles and Methods for their Detection (Eds. Hollaendes et al.) Vol I. Plenum Press New york 1-56 pp.

*

Freisleben, R and Lein, A. (1942). Uber die Auffinding einer Mehitauresistenten Mutante nach. Rontgen bestra blung einer anfalligen Linie van Sommer gerste Naturusissen Schaffen, 30: 608

Ganguli, P.K (1993). Induced mutatins as a method in crop improvement In : Advances in plant breeding, pp 228-252.

*

Gaul, H. (1960). Critical analyssis of the methods for determining with mutagens. Genet Agr. Paiva, 12 : 247-318.

Gaul, H. (1961). Use of induced mutants in seed propagated species. In: Mutation and Plant breeding NAS-NRC, 891 : 206-251.

Gaul, H. (1964). Mutations in plant breeding. Rad. Bot., 4 : 155-232.

Gaul, H. (1965). The concept of Macro an dmicro mutations and results on induced micromutations in barley. In : The use of Induced Mutations in Plant breeding. Pergamon Press, Oxford; 408-426 pp.

*

Gaul, H., Frimmel, G, Gichner, T and Ulonska, E (1972). Efficiency of mutagenesis: Induced mutations and plant improvement (Proc. Study group meeting, Buenos Aires, 1970) IAEA, Vienna.

Gottschalk, W and Wolff, G. (1983). Induced mutations in plantbreeding. Monograph of Theroretical and applied Genetics., 7 : 238.

Gregory, W.C. (1965). Mutation frequency, magnitude of change and probability of improvement in adaptation. The use of induced mutatins in plant breeding. Rad. Bot. (Suppl.), 5 : 432-441.

*

Grover, I.S and Tejpal, S.K (1980) Induced mutation in greengram. (P. aureus). Genet. Pol., 20 (4) : 529-540.

*

Grover, I.S and Virk, G.S (1984) Induced mutation in mung bean (Vigna. radiata L. Wilezek). Genet. Agr; 38 : 237-248.

*

Gunkel, J.H. and Sparrow, A.H. (1961). Ionizing radiations, biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia of Plant physiology. (W. Rubland Ed.) Berlin PP: 555-611.

*

Gustafsson, A. (1940). The mutation system of chlorophyll apparatus. Lundus Univ. Arssku 36 : 1-40.

Gustafsson, A. (1947). Mutations in agricultural plants. Hereditas, 33 : 1-100.

Gustafsson, A (1963). Productive mutations induced in barley by ionizing radiations and chemical mutagens. Hereditàs, 50 : 111-163.

*

Haq, M.A. (1982). Germplasm resources, breeding and genetics of the winged bean. Z. Pf lazenzuehtq, 88: 1-12.

Haq, M. A., Siddiq, M. and Hassan, M. (1989). A very early flowering and photoperiod insensitive induced mutant in chickpea. (Cicer arietinum L.). Mut breed Newsl., 34 (19).

Hassan, S. and Khan, A (1991) NIFA-88 a high yielding mutant chickpea variety. Mut. breed. Newsl., 37 (3).

*

Heslot, H (1977). Review of main mutagenic compounds. Manual on Mut Breed. Tecs Rep ser 119, IAEA; 51-59.

Ignacinuthu, S. and Babu, C.R (1988). Radiosensitivity of the wild and cultivated urd beans, J. Nuclear Agric. Biol., 19 (2) : 119-123.

Jana, M. K. (1963). X ray induced tall mutant of blackgram, Current Science, 32 : 469-470.

*

Jana, M. K. (1964). Effects of X-ray and Neutron irradiation of seeds of Phaseolus mungo (L). Genet. Agr ,8 : 617-628.

Jana, M. K. and Rao S.A. (1974). Inheritance of pigmentation in blackgram. Indian J. Genet. pl. Breed, 34 : 36-40.

Jugran,H. M., Nath, P., Banerji, B.K. and Datta, S.K. (1986). Gamma rays Induced Dwarf mutant of winged bean (Psophocarpus tetragonolobus L. D.C. J. Nuclear Agric Biol., 15 (3) : 175-178.

Kamannavan. P.Y. (1985). Studies on mutagenesis in chilli (Capsicum annum. L.). Thesis abstracts, Directorate of Publications , Haryana Agric. Univ., 9 (1) : 50-51.

Kamini, K., Akhaury, S.B. and Kumar, J. (1988). Mutagenic effect of gamma rays in two varieties of Phaseolus aureus. (L). Proc. Cytol Genet, 1 : 188-191.

Karikari, S.K. (1981). The effect of seed irradiation on plant characteristics and yield of winged bean. (Phosphocarpus tetragonolobus. L.DC). In 2nd International Seminar on Winged bean, Colombo, Srilanka, 1981.

Kawai, T. (1969). Relative Effectiveness of physical and chemical mutagens. Induced Mutations in plants. (Proc. Symp., Pulmann 1969) IAEA, Vienna : pp 137-152.

Kesavan, V. and Khan, T.N.(1978). Induced mutation in winged bean In: Seed Protein Improvement in Grain legumes., IAEA, Vienna : pp 276.

Khan, I. A. (1981), Mutation studies in mungbean (Phaseolus aureus Roxb.) Bot. Bull Academia Sinica, 22 : 113-121.

Khan, I. A. (1983), Mutation studies in mungbean (P. aureus). VI. Estimates of genetic variability Bot. Bull. Acad Sci Taipci, 24 (2) : 121-128.

Khan, I. A. (1986). Evaluation of quantitative characters in the single and combination treatment of EMS and gamma rays in mungbean (Vigna radiata (L) Wilezek). In Perpectives in Cytology and Genetics., 5 : 297-301.

Khan, M. I.A. (1987a). Azide mutagenesis in blackgram Natl. Symp on "Genome Manipulation," Dec. 26-27, 1987 Patna Univ.

Khan, I. A. (1987b). Effect of selection for improvement of quantitative characters in irradiated population of mungbean. (Vigna radiata (L.) Wilzek) J. Nuclear Agric Biol., 1 : 5-8.

Khan, I. A. (1988a). Sodium azide induced genetic variability in blackgram. (Vigna mungo. (L) Hepper) Genome, 30 (suppl) : 145.

Khan, I. A. (1988b). Mutation studies in mungbean (Vigna radiata. (L) Wilzek) IX Estimate of genetic variability Legume Research, 11 (2) : 89-93.

Khan, I. A. (1988c). Induced mutation and their role in improvement of quantitative characters in mungbean. Proc. conf. on Cytol. Genet, 1 : 211-215.

Khan, I. A. (1989). Studies on pattern of induced mutability in mungbean. Bengladesh. J. Agric. Research., 14 (1) : 15-18.

Khan, I.A. and Hashim. M. (1978). Radiation induced variability in quantitative traits of mungbean. J. Cytol. Genet. 13 : 12-15.

Khanna, V.K. (1991). Effect of gamma irradiation of seeds on deoxy ribonucleic acid content in chickpea. Indian J. Pulses Res, 4 (1): 1-3.

Kharkwal, M.C. (1983). Mutation breeding for chickpea improvement. Intl. Chickpea Newsl., 9 ; 45.

Klu, G. Y.P. (1985). An induced flowerless mutnt in winged bean. (Psophocarpus tetragonolobus. (L) D.C.). Trop Grain Leg. Bull., 30 : 37.

Konzak, C.F., Nilan, R.A., Wagner, J. and Foster, R.J (1965). Efficient chemical mutagenesis. In : The use of Induced Mutations in Plant Breeding (Rep. RAO/IAEA. Tech meeting Rome, 1964) Peryamon Press, pp. 49-70.

*

Kozera, W. and Roszho, A. (1986). The influence of fast neutrons on the size and variation of morphological characters in M_2 plants of 2 varieties of dwarf bean (Phaseoulus vulgarus. L.) Genetica polonica, 26 (3) : 367-373.

Kulkarni, U.G., Patil, S.S. and Goud, J.V (1990). Induced mutagens and selective response of yield in greengram. J. Maharashtra Agrl. Univ., 15 (2) : 220-222.

Kundu, S.K. and Singh, D.P (1982a). Note on gamma rays induced variability for flowering and chlorophyll mutations in blackgram. Indian J Agric. Sci. 52 : 190-191.

Kundu, S. K. and Singh, D.P (1982b). EMS induced variability for quantitative characters in blackgram Madras Agric. J., 69 : 644-646.

Louis, H.I. and Kadambavanasundaram, M. (1973). Stimulatory effect of gammarays on the growth of cowpea, Madrass Agric. J. 60 : 1846-1852.

*

Loveless, (1966). Genetic and Applied Effects of Alkylating agents, Butterworths, London.

Manju. P. (1981). Mutation breeding in horsegram MSc. (Ag) Thesis, Fac. Agric., Kerala Agri Univ.

*

Mekhandzchiev, A. and Vassileva, M. (1975). Mutagenic effect of gamma rays and fast neutrons on Pisum sativum. Comptes Rendus de l'. Academic Agricole Georgi Dimitrov, 8 (4) : 27-33.

*

Meono, M. E. (1975). Cholorophyll mutations induced by gamma irradiation in Phaseolus vulgaris (L) Revista de Biologia Tropical, 23 (1) : 125-132.

Micke, A. (1984). Mutation breeding of grain legumes, Plant Soil, 82 : 337-358.

Micke, A., Domini, B. and Maluszynski, M (1987). Induced mutations for cro improvement a review. Trop. Agric. (Trinidad): 64 (4) : 259-278.

*

Moh, C. C and Alan, J.J. (1964). Bean mutant induced by ionizing radiations Turrialba, 14 : 82-84.

*

Muller, H.J. (1927). Artificial mutation of the gene. Science, 66 : 84-87.

Nadarajan, N and Ramalingam R. Sethupathy (1982). Mutagenic effectiveness and efficiency in Cajannus cajan. (L) Mill sp. Madrass Agric. J., 69 (2) : 71-75.

Nadarajan, N., Ramalingam R., Sthupathi and Sivaswamy, N. (1983). Induced variations in quantitative characters in Red gram. Madrass Agric. J., 70 : 219-222.

Nadarajan, N., Ramalingam R. Sethupathy and Sivaswamy. N. (1985). Biological effects of mutagenic treatments in Cajannus cajan. (L) Mill sp. Madras Agric J., 72 (6) : 301-305

Narasinghan, V.G, and Kumar, S. (1976). Mutations studies in cowpea. Indian. J. Agric. Sci., 46 (2) : 61-64.

NAS (National Academy of Sciences) (1975). The winged bean a high protein crop for the tropics, NAS Washington, DC. 42 pp.

Nilan, R.A., Konzak, C.F., Wagner, J. and Gegault, R.R (1965). Effectiveness and efficiency of radiations for inducing genetic and cytogenetic changes. Rad. Bot, 5 : 71-89.

Packairaj, D. (1988). Studies on induced mutagenesis of parents and hybrids in cowpea (V. unguiculata L. Walp). MSc (Ag) Thesis, TNAU, Coimbatore.

Palaniswamy, G.A. (1975). Investigations on the induction of mutations in cowpea (V. sinensis L. Savi) MSc. Ag. Thesis, TNAU

Palaniswamy, G.A., Nagarajan, R. and Loganathan, N.S (1978). A note on chlorophyll mutation in gamma irradiated cowpea. (Vigna sinensis L. Savi) C.152 Madrass Agric J., 65 : 262-263.

Pawar, S.E., Thakare, R.G, Mishra, R.,Krishna, T.G. and Bhatia, C.R (1984). Induced mutations for the crop improvement of pulse crops. Pulse production, 40 : 361-367.

Pulivarthy, H.R. and Mary, T.N. (1987). Induced high yielding mutant in greengram (Vigna radiata L. Wilczek), Mutation Breed News, 30 : 12

Rajput, M.A. (1974). Increased variability in M_2 of gamma irradiated mung beans (Phaseolus aureus Roxb.). Rad. Prot., 14 : 85-89.

Ramaswamy, N.M. (1973). Investigations on induced mutagenesis in blackgram (Phaseolus mungo. L.) Dessertation, TNAU.

Rangaswamy, K. (1989). Studies on induced mutagenesis in homozygous and heterozygous genotypes of cowpea. (Vigna unguiculata. L. Walp.) MSc (Ag) Thesis TNAU., Coimbatore.

Rao, D. M. (1984). Induction of mutations in pigeonpea. Mut. Breed. Newsl, 24 : 8.

Rao, S. A. and Jana M.K. (1976). Leaf mutations induced in blackgram by x-rays and EMS. Environ. Expt. Bot., 16; 151-154.

Rao, S. A., Rao, S.P. and Jana, M.K. (1975a). New plant type in blackgram. Curr. Sci., 44 : 679-680.

Rao, C. H., Tickoo, J.L., Hayat Ram and Jain, H.K (1975b). Improvement of pulse crops through induced mutations. In : Breeding for seed protein Improvement using Nuclear Techniques. IAEA, Vienna. pp 125-131.

Rathnaswamy, R., Krishnaswamy, S. and Narappan, P.V. (1978). Radiosensitivity studies in greengram. (Vigna radiata L. Wilczek). Madras. Agric J., 65 : 351-356.

Reeja, S. Dharan (1993). Morphological effect of gamma rays and EMS on winged bean (Phsophocarpus tetragonolobus L D.C). MSc (Ag) Thesis, Fac. Agric, Kerala Agric. Univ.

*

Rukmanski, G. (1972). Chlorophyll mutations in French bean and their possible use of assessing mutability of varieties. Genetika i selektsuya, 5 (4) : 307-314.

Santos, I.S. (1969). Induction of mutation in mungbean. (Phaseolus aureus Roxb.) In : Induced mutations in plants. (Proc. Symp. Pullman, 1969), IAEA, Vienna, pp : 169-179 .

Sathyanarajana, A., Rao, Y.K. Seenaiah, P and Kodandaramaiah.P (1989). Multifoliate leaf mutants of mung bean and urdbean. Mut. Breed Newsl., 33 : 33-17.

Savithramma, D.L. (1987). Studies on induced mutagenesis in winged bean through EMS. Mysore J. Agric, Sci. 21 (1) : 93.

*

Scholz, F and Lehmann, C.O (1958, 1959, 1961, 1962). Die Gaterslebener Mutation der Satgerste in Beziehung Zur Formenmanning flatigkeit der Art Hordeum Vulgare L. 1,11,111,IV. Kultur Ptanze, 6 : 123-314.

Seth, S., Chandra, S and Chaudhari, B.D. (1983). Effect of irradiation on earliness in mungbean. Indian J. Heridity, 15 : (1/4) : 31-36.

Shik, M. A.Q. (1991). Pulses improvement through nuclear and conventional techniques. In Advances in pulses research in Bengladesh, Proc of Second national workshop on pulses 6-8 June 1989, ICRISAT. 35-41 ISBN 92-9066-199-1.

Singh, V. P. and Chaturvedi, S.N (1981). The productivity of some mutants of mungbean. (V. radiata) : 2. Variation in size and number of pods. Genet Agrar, 35 (3/4) : 295-300.

Singh, V. P. and Suman Agarwal (1986). Induced high yielding mutants in cluster bean, Indian J. of Agric Science, 56 (10) : 695-700.

Singh, V.P. and yadav, R.D.S (1991). Induced mutations for qualitative and quantitative traits in greengram. (Vigna radiata L. Wilezek. cv. T44). J. Genet Breed, 45 (1) : 1-5.

Singh, V.P., Yadav, R.D.S. and Singh, R.M. (1988). A multiracemose inflorescence mutant of greengram induced by gammarays. Indian J. Genet , 48 (1) : 111-112.

Singh, D., Singh, R.M. and Singh, J (1989). Effect of gammarays, EMS, Hydroxilamine on type and frequency of chlorophyll mutations in lentil, Lens, 16 (2) : 3-5.

Sinha, R.P (1987). Yellow testa small seeded mutant of mungbean. (Vigna radiata. L. Wilezek). J. Nuclear Agric. Biol., 16 (3) : 158-159.

Sinha, R.P (1988). Early maturing dwarf mutant of urdbean. (Vigna mungo L. Hepper). J. Nuclear Agric Biol., 17 (1) : 61-62.

Sinha, S.S.N and Himanshu, R.S (1984). Effect of gamma irradiation on chlorophyll metabolism in Vigna and Phaseolus species. Cytol., 49 : 279-287.

Song, H. S., Kim, J.R., Oh, J.H. and Kwon, S.H. (1988). Radiosensitivity and improvement of yield and disease resistance by induced mutations in mungbean. (Vigna radiato L. Wilzek.) In: Improvement of grain legume production using induced mutations (Proc workshop., Pullman., USA, 1-5 July 1986), IAEA : 89-109

Sparrow, A.H., Moses, M.J. and Dubow., R.J. (1952). Relationship between inonizing radiations, chromosome breakage and certain other nuclear disturbances Exp. Cell. Res. (suppl.), 2 : 245-267.

Stadler, L.J. (1928). Mutations in barley induced by X-ray and radium Science, 68 : 186-187.

Sumabai, D.I (1989). Genic manipulation in sweet potato adopting induced mutations. PhD Thesis. Fac. Agri., Kerala Agric Univ.

Sumanggono, R. (1987). Mutants derived from irradiating mungbean. cv. manyar, TVIS News, Taiwan, 2 (2) : 6.

Sunny. K. Oommen and Gopimony, R. (1984). Efficient mutagenesis in cowpea. Agric. Res. J. kerala., 12 (1) : 57-62.

Swaminathan, M.S. (1961). Effect of diplontic selection on the frequency and spectrum of mutations induced in polypoids following seed irradiation, Symp. on the effects of ionizing radiations on seeds. IAEA, pp. 279-288.

Swaminathan, M.S, (1964). The use of induced mutations in plant breeding. J.Sci. Indian Res., 23 : 455-458.

Thirugunakumar, S. (1986). Studies on induced mutagenesis in cowpea. (V. unguiculata. L) Walp). MSc (Ag) Thesis, TNAU, Coimbatore.

Tulmann Neto, A. (1990). Genetic improvement of beans through mutation induction. In : Genetic improvement of pulse crops. Vol. I. Premier Publishing House, Hyderabad, pp 29-328.

*

Turishcheva, M.S, Taran, S.L. Beletskil Yuo, Belkina, G.G. and Odintsova, M.S (1987). Structure and Function of the chloroplasts in viable plastom mutants of sunflower. Fiziologiya Rastenic, 34 (6) : 1097-1102.

Vanniarajan, C., Vivekanandan, P and Ramalingam, T. (1993). Spectrum and frequency of chlorophyll and viable mutations in M_2 generation of blackgram. Crop. Improv : 2 (2) : 215-218.

*

Varadanyan, K.H. (1976). Study of chlorophyll mutations in French beans after treatment with chemical mutagens. Biol. Zh. Arm., 29 (7) : 78-82.

Veeresh, L.C. and Sivasankar, G. (1986). Radiosensitivity and frequency of chlorophyll and viable mutations in winged bean. Trop. grain Legume Bull, 33 : 37-41.

Veeresh, L.C. and Sivasankar, G. (1987). Early mutants in winged bean. Indian J. Genet. Plant breed., 47 (3): 353.

Venkateswaralu, S., Singh, R.M., Singh,R.B. and Singh, B.B. (1976) EMS induced multicarpellate condition in Cajanus cajan. Current Science., 45 : 773-774.

Venkateswaralu, S., Singh, R.M., Singh, R.B. and Singh, B.D. (1978). Radiosensitivity and frequency of chlorophyll mutations in pigeonpea. Indian J. Genet., 38 : 90-94.

Venkateswaralu, S. Singh, R.M., and Reddy, L.J. (1980). Induced mutagenesis in pigeonpea with gamma rays EMS and hydeoxylamine. Proc. Intl. Workshop on Pigeon peas. ICRISAT, 2 : 67-73.

*

Vishnoi, A.K. and Gupta, P.K 1980. Induced mutagen4esis in Vicia faba L.I Chlorophyll mutations induced by gamma ryas, EMS and hydrazine. Cytobios, 27 : 81-87.

*

Yankulov, M.T., Issai, E. and Abreu, S. (1979). Certain aspects of the sensitivity and mutability of two varieties of beans under the influence irradiation with gamma rays and EMS. Comptes Randus des Sciences 32 (2) : 205-208.

* - Originals not seen

MUTAGENICITY OF GAMMA RAYS AND EMS ON WINGED BEAN [*Psophocarpus tetragonolobus* (L.)]

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ABSTRACT OF THE THESIS

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ABSTRACT

A study on mutagenicity of gamma rays and EMS in the M_2 generation of winged bean [*Phophocarpus tetragonolobus* (L.)] was carried out in non-replicated progeny row trial with seeds collected individualy from M_1 plants which were initially treated with four doses of gammarays (100, 200, 300 and 400 Gy units) and five doses of EMS (40, 80, 120, 160 and 200 mM). Observations on chlorophyll and viable mutations and other characters like height at 45 DAS and 75 DAS, days to flower, pod length, pod number, seed number and 100 seed weight were recorded. The chlorophyll and viable mutations were quantitatively expressed by frequency and qualitatively by spectrum. The frequency distribution, range and mean performance of plants in each treatment were estimated.

Quantitative analysis of chlorophyll mutations showed that mutation frequencies recorded an increase at the lower doses and a decrease at the higher doses. The frequencies were maximum at the same dose for both the mutagens viz., at 200 Gy units of gamma rays and 160 mM of EMS. Among the two mutagens, high frequencies were noticed for gamma rays.

Quantitative analyais of chlorophyll mutations identified six types for both the mutagens viz., xantha, chlorina, viridis, maculata, viridalba and white streaked types. But none of the treatments produced six types together. Among the different types, chlorina appeared as the most frequent one. In case of EMS, the decrease in multiple mutation frequency with increase in dose was clearly observed. The segregation per cent of most of the chlorophyll mutants was in between 10 and 14.

Quantitative analysis of viable mutations revealed that maximum frequency on M_2 plant basis was not at the higher doses for both the mutagens. Twenty one viable mutants with change in characters of leaf, stem, pod and seed were isolated from gamma ray treated population and twenty two from EMS treated population.

A negative shift in mean was noticed for all the characters except days to flower due to more negative variants than positive variants even in the lower doses. The mean performance of plants was found to be minimum for higher doses and maximum for control and lower doses.

Mutagenic effectiveness was found to be high in lower doses viz., 100 Gy units of gamma rays and 40mM of EMS. The efficient doses of gamma rays identified on the basis of injury and lethality or sterility were 100 Gy units and 200 Gy units respectively.. In EMS the efficiency on the basis of sterility, lethality and injury was maximum at 120, 160 and 80 mM respectively.