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

BY

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THESIS

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DECLARATION

I hereby declare that this thesis, entitled **Morphological effect of gamma rays and EMS on winged bean [Psophocarpus tetragonolobous (L.)]** is a bonafide record of the research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar titles of any University or Society.

(REEJA S. DHARAN)

College of Agriculture,
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CERTIFICATE

Certified that this thesis, entitled **Morphological effect of gamma rays and EMS on winged bean** [Psophocarpus tetragonolobus (L.)], is a record of research work done independently by Kum. REEJA S. DHARAN, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to

The Eternal Power guiding me

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ABBREVIATIONS

GR	-	Gamma Rays
EMS	-	Ethyl Methane Sulphonate
kR	-	Kilo Rad
Gy	-	Gray
mM	-	Milli Mol
LD_{50}	-	Lethal Dose 50
DAS	-	Days After Sowing
M_1, M_2, M_3	-	First, second and third generations respectively after treatment with a mutagen.
C.R.D.	-	Completely Randomised Design
R.B.D.	-	Randomised Block Design

INTRODUCTION

I. INTRODUCTION

The success of any crop improvement programme depends on the extent of genetic variability available in the breeding population. Induced mutations are considered as an alternative to naturally occuring variation as the source of germplasm for plant improvement programme and as an alternative to hybridization and recombination in plant breeding (Brock, 1971). Mutation breeding has advantages mainly in improvement of a specific character in a well-developed and highly desirable genotype within a short period and in breaking tight linkages, thus helping in obtaining rare combinations and in enlarging variability for quantitative characters. An enlarged spectrum of induced mutations and increased mutation frequency would provide more opportunities in isolating beneficial mutants and the success of an induced mutation breeding programme depends upon the number of useful mutants induced.

The winged bean [*Psophocarpus tetragonolobus* (L.)] is a native of south-east Asia and its maximum diversity occurs in Papua New-Guinea. It was introduced to India in 1799 (Chandel *et al.*, 1979). Winged bean, more commonly known in India as Goa bean is one of the under-exploited tropical legumes. In India its cultivation is limited to north-eastern areas, Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala. It is an exceptional legume; the green pods, leaves and seeds are rich in protein and vitamins, the tuberous roots are uniquely rich in protein and the eds are a source of edible oil. Thus nutritionally the crop is ilar to soybean with its seeds containing 30 to 48 per cent protei d about 15 to 18 per cent oil. Crop also serves as a green manure, cover crop and forage crop besides some medicinal value. Due to such a high potential and its ability to grow in marginal lands, winged bean has become popular in recent years. Though it can become a significant food crop in the humid tropics; it still remains as a backyard crop (Anon., 1975).

However, the crop has some disadvantages (like photosensitivity, indeterminate growth habit and trailing nature) which may cause a setback in its large scale cultivation. Since it is a self-pollinated crop, the amount of natural variability is limited; but there exists a wide scope for inducing variability by mutagenesis and to incorporate such variability in further breeding programmes. The present investigation was therefore undertaken to study the morphological effects produced by two potent mutagens viz., gamma rays and EMS on winged bean in the M_1 generation as the first step in a mutation breeding programme.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

During the last few decades, induced mutagenesis is fruitfully used by plant breeders all over the world for crop improvement. Genetic variability is essential for any crop improvement programme and mutagenesis is extremely useful in creating new variability and in augmenting the existing one.

The possibility that new types of inherited characteristics may appear suddenly, was first suggested by De Vries in 1901. The beginning of the era of induced mutagenesis was marked by the remarkable discovery of Muller in 1927 that X-rays could induce genetic changes in Drosophila. Stadler in 1928 demonstrated the artificial induction of mutations in plants, viz., barley and maize using radiations. With his pioneering work, Gustafsson in 1947, established a very useful and sound basis for mutation breeding.

Apart from ionizing radiations, there are a number of chemical mutagens which also produce mutations in plants when applied singly, combined with other chemicals and in succession or simultaneously with radiation (Ehrenberg et al., 1961 and Konzak et al., 1965).

Though mutagenesis by chemical means was attempted by many workers over a long period (Schiemann, 1912) the active and productive study of chemical mutagens begun only after 1947 when Auerbach and Robson discovered the mutagenicity of sulphur and nitrogen mustards in Drosophila. Freese (1963) classified chemical mutagens as base analogue substitutes, dyes, acids, metals and alkylating agents. Alkylating agents were found to be superior than other chemicals in inducing mutations in a wide range of organisms (Auerbach, 1961). Among the alkylating agents, ethyl methane sulphonate (EMS) appeared to be the outstanding one in inducing mutations in a wide variety of organisms including higher plants (Swaminathan et al., 1962). The relatively low toxicity and high genetic effects of EMS (Gaul, 1961) and its high mutagenic effectiveness and efficiency in higher plants (Konzak et al., 1965) provide scope for its exploitation in crop improvement programmes.

Detailed reviews relating to the induction and recovery of mutations in numerous crops have been presented by many investigators. Here an attempt is made to present an elaborate review of mutation research conducted in leguminous crops.

2.1. Induced mutagenesis in winged bean [*Psophocarpus tetragonolobus* (L.)]

Not much work has been done in winged bean about mutagenesis. Kesavan and Khan (1978) isolated some mutants for earliness and leaf characters after treating two genotypes, viz., UPS-31 and UPS-122 with gamma rays and EMS. The doses ranged from 10 to 80 kR in gamma rays and 0.05 to 2.0 per cent in EMS. Increasing doses of gamma rays caused a very high reduction in seed germination and a drastic reduction of germination was shown by doses over 30 kR. LD_{50} was found to be between 15 and 20 kR. Doses over 25 kR reduced plant height by 50%. In EMS, germination and plant height were found to be reduced at the lower concentrations of 0.05 - 0.2%. 0.05% EMS treatment caused 20% height reduction. One mutant with shortened internodes and dark green miniature leaves at 10 kR dose has been reported in the M_2 generation. Another mutant which flowered on 43rd day from the date of sowing was observed in 25 kR treatment. Mutants for leaf colour, leaf shape and reduced internodes were also reported.

Senanayake (1978) found that good mutants could be obtained by exposing winged bean seeds to 20 - 40 kR gamma rays.

Karikari (1981) reported that by making use of irradiation, a bushy type of plant requiring shorter staking could be developed.

He found significant reduction in vegetative parts, seed yield and tuber yield when the seeds of four genotypes were treated with 15 and 20 kR gamma rays. As the dose increased, a decrease in total dry matter per plant, mean pod length and mean leaf area per plant was observed.

Savithramma (1982) reported a stimulatory effect of lower concentrations of EMS in plant height and days to first flowering in M_1 generation. Higher concentrations of 0.9% EMS showed stimulatory effect in characters like survival, number of pods per plant, number of seeds per pod, hundred seed weight and seed fertility in M_1 generation.

Veeresh (1983) found that seed germination, plant height, pollen fertility and seed fertility were drastically reduced by higher doses of gamma rays in M_1 generation when the seeds of the variety 'Chimbu' were treated with 10,15,20,25,30 and 35 kR of gamma rays. But lower doses showed stimulatory effect in seed germination and survival. He also reported many chlorophyll mutants, dwarf plants, bushy types, early maturing types and leaf mutations in the M_2 generation.

A mutant with a shift in the photoperiod-tuberisation link and/or suppressed flowering have been developed by Klu (1985). Flowerless condition lead to the lack of the seeds which is a vital tool in tuberisation.

Jugran et al. (1986) isolated three dwarf plants in M_3 population of cultivar V-16 from 200 Gy treatment of gamma rays which did not grow beyond 38 cm, while the height of control plants were 241-280 cm. None of these variants showed climbing habit and the reduction in height was mainly due to reduced and dense internodes.

Several early flowering and early maturing mutants were reported (Anon., 1986). Mutants which were semidwarf, requiring no support, having a plant spread of 400 sq. cms and producing more than 35 pods per plant were also identified.

Veeresh and Shivshankar (1989) isolated elevan early mutants, flowering 11-16 days and maturing 27-34 days earlier than the control from gamma rays treated population of the parent variety 'Chimbu'.

Klu et al. (1989) observed a mutant in the M_2 of the accession UPS-122 which did not flower throughout its growth period of 5 months, had few leaves but developed an underground tuber weighing about 100g. Other mutants with variation in seed size and colour were also obtained in the M_3 generation.

2.2. Induced mutagenesis in related pulse crops

2.2.1. Greengram [Vigna radiata (L.) Wilczek]

Using both physical and chemical mutagens, studies on induced mutagenesis have been made by various workers in green gram.

Bhatt et al. (1972) isolated a giant variant from the M_1 generation of 20 kR treatment of gamma rays. This variant manifested gigantism' in almost all the plant characters and bred true in succeeding generations. Dahiya (1973) reported two progenies in M_2 generation of gamma rays treated populations where the size of seeds were twice as that of the control. A stimulative effect on plant growth was observed with moderate exposure of 30 kR treatment of seeds. He also found that the higher dose of radiation of 70 kR induced more significant variation from control than moderate dose of 30 kR. Chlorophyll mutations like albina, xantha, viridis and maculata were also reported. Sree Rangaswamy et al. (1973) observed that the greengram plants treated with gamma rays were shorter than the parents and those treated with 60 kR were the shortest.

Krishnaswamy et al. (1977) identified a linear relationship with regard to increase in dose to reduction in survival and reduction in mean height of X-rays and gamma rays treated plants in the M_1 generation. The chlorophyll mutation frequency which is taken as a reliable index to determine the mutagenic efficiency was found to be maximum at 80 kR of both X-rays and gamma rays. So application of a dose of 80 kR of both the mutagens were recommended for a programme of improvement of the Co.1 strain of green gram. Singh (1981) isolated a mutant from the gamma rays treated population with protruded stigma and increased number of floral parts.

Singh et al. (1988) reported a mutant producing multirecemose inflorescence with more number of pods and higher grain yield. Sinha (1988) isolated a photoinsensitive mutant with increased number of pods per plant and yield than the parent from the M_2 population of 40 kR gamma rays treated population. Satyanarayana et al. (1989) identified a multifoliate leaf mutant where each leaflet was substituted by a trifoliate leaf, giving nine or more leaflets in the M_2 generation of cultivar 'Pant Mung-2' after treatment with 40 kR gamma rays.

Mehetre et al. (1990) reported a reduction in seed germination and plant survival with higher doses of gamma rays treatment. Differential behaviour was observed for characters like plant height, pod length, number of pods per plant and number of branches per plant. He observed more variability at 40 and 50 kR doses, and more harmful effects on all the vegetative as well as reproductive characters at 30 kR dose. Shaikh (1990) selected phenotypically deviant mutants in M_2 generation which were synchronous, early, bushy, erect and disease tolerant.

Seenaiah et al. (1990) isolated an extended stigma flower mutant where the petals were only half developed coupled with male sterility from the M_3 population of 'Pant Mung-2' treated with 40 kR gamma rays. Pod setting was observed as normal with well filled seeds, when crossed with male fertile normal plants. The mutant had an advantage that it can be pollinated without opening the flower.

2.2.2. Red gram [Cajanus cajan (L.) Millsp.]

In red gram considerable work on mutation breeding has been made and many improved lines with higher yield, earliness and dwarfness have been obtained.

Mohamed Ali Khan and Veeraswamy (1974) noticed that the plant height at maturity, number of branches per plant and number of pods per plant in M_1 generation decreased with increase in dose of gamma rays and EMS. Pollen fertility was also found to decrease with increase in dose. They concluded that gamma irradiation is capable of producing more viable mutations than EMS. In the M_2 generation, dwarf mutants with a height of 55-60 cm were obtained as against 105-110 cm in control plants. Early types maturing 35-45 days earlier than the control have also been reported.

Chaturvedi and Sharma (1978a) observed different types of abnormalities in the floral composition of mutant population. They suggested that EMS is more efficient in inducing floral mutations. Six male sterile mutants of tall or dwarf habit have been induced and isolated by treating with 0.1-0.3% EMS (Chaturvedi and Sharma, 1978b).

Venkateswarlu et al. (1978) reported that gamma irradiation reduced germination, seedling height, pollen fertility and survival at maturity; where the reduction was greatly pronounced at 5 kR. Thombre et al. (1981) observed a marked decrease in seed germination and plant survival at high radiation doses. Plant height was observed to be decreasing linearly with increase in doses of the mutagen (Sharma and Chaturvedi, 1982).

Nadarajan et al. (1985) showed that the characters like seed germination, survival of seedlings, pollen fertility and seed fertility decreased gradually with the increase in doses of the mutagens. A slight stimulatory effect was noticed for plant height on 30^{th} day by lower doses of mutagens, while the higher doses reduced the plant height. More height reduction was at earlier stage than at a later stage for the same dose of mutagens. They reported that mutagenic sensitivity not only depends upon the genotype but also on the type of mutagens used.

Arahna (1987), suggested that mutagenic effectiveness was higher for EMS than gamma rays, while efficiency was higher for gamma rays. She observed chlorophyll mutations and mutations with variable leaf shape, and reported that gamma rays induced wider spectrum of chlorophyll mutants. Selvaraj et al. (1989) isolated a mutant from the M_2 population of the variety Co-1 after the treatment of 16 kR gamma irradiation and developed the mutant variety Co-5. It was the shortest duration of the varieties in redgram developed in Tamil Nadu till then. The variety was photoinsensitive, higher yielding and possessed many other superior characters.

2.2.3. Black gram [Vigna mungo (L.) Hepper]

Mutation breeding has resulted in good mutants for seed coat colour, leaf shape and dwarfness in addition *~ other morphological variations in blackgram.

A mutant with densely hairy pods and shiny seeds with yellowish green testa was isolated by Rao and Jana (1974) after exposing the seeds of variety T_9 to a combined treatment of X-rays and EMS. Another mutant isolated was found to have brown testa. After a combined treatment with 40 kR X-rays and 0.2% EMS, Rao and Reddy (1975) have selected a mutant from M_2 generation with reduced size of the petal enclosing the stamens and with protrusion of stigma which they designated as crumpled petal mutants. The mutant set only few pods and seeds though pollen fertility was normal. Different kinds of leaf mutants such as crinkled leaf, waxy leaf, narrow leaf and unifoliate have been isolated by Rao and Jana (1976). The crinkled leaf and waxy leaf mutants had normal fertility whereas the narrow leaf mutant was partially sterile. The unifoliate mutant which was extremely dwarf, found to be completely sterile.

Kundu and Singh (1982) observed albina, chlorina, alboviridis and viridis type of chlorophyll mutations in the M_2 generation of gamma rays treated populations. 50 kR dose was found to give the highest frequency of chlorophyll mutations. They observed a linear relationship between the frequency of chlorophyll mutations and the dose of gamma rays. It was also found that the range towards lateness had widened by gamma rays. An erect, synchronous and determinate type mutant bearing upright pods was selected by Shaikh and Majid (1982) from a M_2 progeny following 50 kR treatment of gamma rays. Also the mutant had an increased number of pods per plant resulting in high harvest index and seed yield per plant.

Bhamburkar and Bhalla (1985) observed a reduction in germination percentage, increase in pollen sterility and an inverse relationship between dose or concentration with the seedling height and survival percentage of plants in the M_1 generation of mutant population. Ignacimuthu and Babu (1988) reported a reduction in dehydrogenase activity in M_1 and M_2 plants by mutagenesis. A dose dependant decrease was observed in seedling emergence, seedling height, survival and pollen fertility in M_1 and M_2 plants. They observed that the spectrum of chlorophyll mutations was narrow; the spectrum and frequencies of chlorophyll mutations increased with dose of each mutagenic treatment.

Mahna et al. (1989) noted a gradual reduction in germination, emergence percentage and plant height with increasing concentration of the mutagen in the M_1 generation of sodium azide treated seeds. Many abnormalities pertaining to cotyledonary leaf, compound leaf and branching were also observed in the M_1, but they disappeared in further generations. A reduction in plant height and branches per plant, but an increase in the number of pods per plant were reported by Sinha and Bharati (1990).

Singh and Raghuvanshi (1991) isolated a mutant in M_2 population, where the seeds of the mutant were larger and heavier, with a test weight double that of the control which was designated as bold seeded mutant. The mutant showed an increase in total yield per plant and protein content.

2.2.4. Bengal gram (*Cicer arietinum* L.)

Bengal gram received much attention of mutation breeders and information on almost all aspects of mutation breeding is available. Many improved varieties have been released.

Thombre and Phadnawis (1974) irradiated three varieties with gamma rays and isolated mutants for leaf, flower and pod characters. The mutants were found to have an increased crude protein content in their seeds. A mutant with suppressed branching, larger leaves on the main stem, a different flowering pattern, thicker shells and smaller kernals than normal plants was reported by Mouli and Patil (1976) after treating the variety TG-2 with gamma rays. Shakoor et al. (1978) found that irradiation with 10 kR dose of gamma rays caused the development of three mutant plants with upright and compact growth habit.

Khanna and Maherchandani (1980) treated the seeds of three varieties with gamma rays and found that germination in two varieties decreased at higher doses, while in one variety there was no reduction in germination. Seedling height showed a general decrease in the irradiated seeds. A stimulatory effect was noticed on the height of plants at maturity by lower doses of the mutagen, but it reduced as the dose increased.

Shaikh et al. (1980) selected a mutant which is a bit shorter than mother variety, maturing 10 days earlier and having higher number of pods per plant from the 20 kR gamma radiation treated populations of the variety 'Faridpur - 1'.

Vadivelu and Rathinam (1980) made a study on the relative sensitivity of two cultivars of bengal gram and on the frequency of chlorophyll mutations induced by the chemical and physical mutagens and their combinations. Their observations indicated a linear reduction in germination percentage, survival, seedling growth, dry matter production and seed fertility in M_1 and chlorophyll mutations in M_2 with increase in mutagen dose. Chemical mutagen was more potent than physical mutagen and combination treatments showed enhanced effect compared to single treatments.

Subba Rao (1988) observed a linear dependance of germination percentage and seedling height on dosage of gamma rays used. Higher dose was found to cause delayed flowering and reduced number of pods. A very early flowering mutant was identified from 20 kR gamma rays treated progeny of the cultivar C-727 by Haq et al. (1989).

Broad leaved, white flowered, erect, dwarf, bushy, early and chlorophyll deficient chickpea mutants were induced by Shaikh (1990). After exposing the seeds of 'Faridpur-1' variety to gamma irradiation Shamsuzzaman and Shaikh (1991) identified two early maturing mutant lines having a higher hundred seed weight and higher seed yield per plant than the parent variety.

2.2.5. Cowpea [Vigna unguiculata (L.) Walp .]

In cowpea, considerable works on mutation breeding have been made and many improved lines for higher yield and date of flowering have been obtained.

Based on a study of the effect of neutrons and X-rays irradiation in the M_1 generation of two varieties of cowpea, Ojomo and Chheda (1971) reported that various grades of chlorophyll deficient spots were observed only in the first simple leaves and very rarely in the first trifoliate leaf. They also suggested that the morphologically visible changes restricted to alteration in the number and shape of leaflets, in the early trifoliate leaves only.

Louis and Kadambavanasundaram (1973a) studied the effect of gamma rays on germination, survival, plant height and yield attributes in M_1 generation. They (Louis and Kadambavanasundaram, 1973b) observed a retardation and suppression of growth in higher doses and some positively stimulated effects and relatively large increases under lower doses of gamma irradiation in the M_1 generation.

Rao and Reddy (1975) have selected a mutant from the progeny of a M_1 plant obtained after treatment with 0.3% EMS for 12 hours. The mutant was with reduced size of the petal enclosing the stamens and with protrusion of stigma which they designated as crumpled petal mutant. It set only few pods and seeds though pollen fertility was normal. Narsinghani and Kumar (1976) found that survival, number of pods, seed yield per plant and pollen fertility were reduced when treated with 0.25% EMS and 0.025% MMS. Few mutants for larger pod size and leaf shape were also isolated in addition to chlorophyll mutants like albino, xantha, chlorina and striata.

Ojomo and Raji (1978) reported a reduction in germination percentage, seedling height, survival percentage and initial growth. Chowdhury (1983) selected some mutants with increased pod length, pods per plant, seeds per plant and hundred seed weight, and drastically reduced plant height from the gamma irradiated population. Oommen and Gopimony (1984) reported that treatment with both gamma rays and EMS on cowpea resulted in physiological damages in M_1 generation which was evidenced by the reduction in survival of plants, plant growth and pollen fertility. Mutation frequency of gamma rays and EMS estimated on M_1 plant basis was also found to be increasing with increasing doses.

A mutant flowering 28 days earlier than the parent variety and showing a slight reduction in height was isolated from the M_2 generation of 10 kR gamma rays treated material by Gupta et al. (1981).

Kumar et al. (1989) selected a mutant with green leaves, white stems, petiole and rachis, and white veins in stipules and leaflets from EMS treated populations. They reported that stem of the mutant plant was devoid of chlorophyll, but nominal amounts of chlorophyll a & b were found in the leaflets.

2.2.7. Soybean [Glycine max (L.) Merr.]

A number of mutation works have been reported in soybean and many improved varieties have also been developed through mutation breeding.

Kiang and Halloran (1975) reported plants showing abnormal leaflet formation, chlorophyll mutations, abnormal number of cotyledons and abnormal number of primary leaves from the EMS treated material. Choudhary and Fazlul Haque (1976) isolated some mutants for earliness, which matured 15 days earlier to the control with 20 kR of gamma irradiation. The hundred seed weight in the M_2 generation was found to be higher than the control for the 10, 20 and 30 kR treatments.

During 1976, Constantin et al. reported in soybean that seedling height decreased as the dose of neutrons and gamma rays increased. Reduction in germination percentage and delayed flowering was noticed by Patil and Bhalla (1985) in the higher dose-gamma rays treated material. Variation in number, size and shape of leaves were also reported. Besides greater variation in plant height, node number and fertility, FU Laiqing (1986) reported mutants with opposite trifoliate leaves and multi-leaflet leaves from the 15 kR of gamma rays treated material.

From the combined treatment of 25 kR gamma rays and UV radiations a mutant was isolated by Bhatnagar et al. (1989) which was found to possess better germinability, earliness, high yield, smaller seedsize and changed colour of the hilum in comparison to the parent variety. Rajput and Sarwar (1989) reported that they could develop a high yielding, short stature, early mutant in soybean by seed treatment with gamma rays and fast neutrons. Skorupska and Palmer (1989) developed an apetally mutant lacking standard, wing and keel petals and with abnormal style in soybean.

Bhatnagar et al. (1992) performed induced mutagenesis in three varieties of soybean and reported that they could disrupt the negative association between oil and protein content in the parent varieties.

2.2.8. French bean (*Phaseolus vulgaris* L.)

Swarup and Gill (1968) observed poor germination, growth and vigour at a higher dose of X-rays treatment in the M_1 generation. Pande and Seth (1975) isolated several early flowering mutants with fleshy pods by treating with EMS and MMS. Maximum frequency of mutations was observed in 0.3% EMS treatment which also changed some yield components.

A plant with dark green, rough textured leaves with small epidermal projections and high pollen sterility was identified in the M_1 generation by Tara Mohan (1980) after treating 'Carioca' variety with 0.7% EMS solution. Tulmann Neto and Alberini (1989) subjected the seeds of the cultivar 'Carioca' to 32 kR of gamma radiation and selected a bushy mutant in the M_2. Mutant was found to be maturing 5-14 days earlier than 'Carioca' and giving yields higher than 'Carioca' and other cultivars under favourable conditions. Ignacimuthu and Babu (1990) noticed variation in the yield of wild and cultivated beans following seed irradiation with gamma rays and EMS.

2.2.9. Lentil (*Lens culinaris* Medic.)

Sharma and Sharma (1979) reported two types of induced leaf mutations. One was a boat-leaf mutant having 3 to 4 boat shaped leaflets per leaf developing from the same place due to a reduction in the length of leaf rachis and another was a crinkled leaf mutant having twisting, folding, shrinking and irregular serration on the margin of the leaflets. Tyagi and Sharma (1981) identified great reduction in shoot length and root length at higher doses of gamma irradiation of seeds.

A dwarf 'shy' mutant with narrow leaflets showing longitudinal bending and few flowers and fruits was isolated from the M_1 population after seed irradiation with gamma rays by Sinha (1989). Shaikh (1990) noted that maximum frequency of variants were occured in 0.5 mM treatment of sodium azide and he selected those mutants with higher number of pods. A very late flowering dwarf mutant was reported by Sinha and Chowdhury (1991). Leaf length, leaflet length, number of pods per plant and seeds per plant were showed a reduction in the mutant.

2.2.10. Horse gram [Macrotyloma uniflorum (Lam.) Verdic.]

Kulkarni and Shivashankar (1978) obtained some mutants for dwarfness and testa colour by treating with EMS and gamma rays. The frequency of chlorophyll mutations increased with increasing EMS concentrations upto 1.2% and then decreased, where as in gamma rays the increase was upto 15 kR and then decreased. But the frequency of macromutations decreased with the increase in EMS concentration. The spectrum and frequency of macromutations was much higher in EMS than in gamma rays.

Manju (1981) treated hórsegram seeds with gamma rays and EMS and reported that, gamma rays have a stimulatory effect on germination, while the increased doses of EMS caused a reduction in germination. Both the mutagens reduced survival percentage, plant height and pollen fertility. EMS caused wider spectrum of chlorophyll mutation and high segregation ratio of chlorophyll mutants. Morphological variants produced were with respect to growth habit, leaf size and shape and time of flowering.

2.2.11. Khesari (_Lathyrus sativus_ L.)

Nerkar (1976) treated the seeds with different mutagens and observed a large number of viable mutations for plant height, maturity, leaf shape and size, flower colour, pod size and shape. EMS and NMU (N-nitroso N - methyl urea) were found to be more efficient for the production of mutants than gamma rays.

2.2.12. Field bean [_Lablab purpureus_ (L.) Sweet.]

A macromutant was isolated by Sivasubramanian _et al._ (1989) after treating the variety Co-6 with 24 kR of gamma radiation. Mutant showed variation in growth habit, flower colour and arrangement, pod colour, pod shape, cooking quality, bean size, shape and bean colour. The high yielding mutant was later released as a variety with name Co-10.

2.2.13. Cluster bean [Cyamopsis tetragonoloba (L.) Taubert]

Chowdhury et al. (1975) observed greater variation for six quantitative characters when subjected to 10-250 kR of gamma irradiation. Plant height and peduncle length were found to be increased, but number of branches per plant, bunches per plant and yield per plant were less than the control in the M_2 generation.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

The present investigation to assess the morphological effect of gamma rays and Ethyl Methane Sulphonate (EMS) on winged bean was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 1991-1993.

3.1. MATERIALS

3.1.1. Biological Material

The crop selected for the present study was PT-62 variety of winged bean [*Psophocarpus tetragonolobus* (L.)] which is an excellent type recommended by the K.A.U. Seeds of the cultivar were obtained from the Instructional Farm, College of Agriculture, Vellayani.

3.1.2. Mutagens

Two types of mutagens, viz., physical and chemical were used for the study. The physical mutagen employed was gamma rays. The gamma irradiation facilities available at the Radio-Tracer Laboratory, College of Horticulture, Kerala Agricultural University, Vellanikkara were utilised. $Cobalt_{60}$ gamma cell unit was employed for irradiation. The source was operating at an intensity of 936 Gray per hour.

The chemical used was EMS : CH_3SO_2-O-C_2H_5, having a molecular weight of 124.16. The chemical has a specific gravity of 1.204 at 20°C.

3.2. METHODS

3.2.1. Mutagen treatments

3.2.1.1. Gamma irradiation

Seeds of uniform size were sorted out. The moisture content of the seeds was approximately 12 per cent. Five samples of 160 seeds each were irradiated at five doses of gamma rays, viz., 100,200, 300, 400 and 500 Gy.

3.2.1.2. EMS treatment

Five samples of 90 seeds each were selected and presoaked in distilled water for four hours. This was followed by treatment with EMS at concentrations of 40,80,120,160 and 200 mM prepared in double distilled water. The duration of treatment was six hours. The volume of the mutagen solution was approximately seven times the volume of dry seeds (200 ml per 90 seeds). The treatment was conducted at the room temperature of 27±1°C and intermittent shaking was given to maintain uniform concentration. After treatment, the seeds were washed in double distilled water three to four times, followed by running water for about an hour to remove the traces of the chemical from the seeds.

3.2.2. Study of the M_1 generation

3.2.2.1. Laboratory studies

Samples of 20 seeds per dose of gamma rays treatment and 10 seeds per dose of EMS treatment were sown in petridishes, replicated three times and the following observations were recorded.

a. Germination of seeds

Number of seeds germinating on each day was counted to estimate the percentage of germination. The appearance of radicle upto 5 mm length was taken as the criterian for germination.

b. Days taken to complete germination

Days were counted till the completion of germination of seeds in each of the sample upto two weeks.

c. Root length, shoot length and root-shoot ratio

The seedlings raised in petridishes were carefully taken out on the fifteenth day of sowing and the length of primary root and shoot of each seedling was measured. The mean root length and shoot length for each treatment were estimated. The root-shoot ratio was worked out for each seedling from the data on length of primary root and shoot.

3.2.2.2. Field studies

The gamma irradiated seeds were sown in the field on the next day of treatment along with the unirradiated control. The experiment was laid out in Randomised Block Design with six treatments and three replications. Thirty seeds were sown per treatment at a spacing of 60x30 cm.

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

The following observations were recorded

a) Germination percentage.

b) Days taken to complete germination.

c) Survival counts.

d) Plant height.

e) Chlorophyll and other morphological variants.

f) Flowering.

g) Number of pods per plant.

h) Length of pod.

i) Weight of pod.

j) Fruit yield per plant.

k) Number of seeds per pod.

l) Hundred seed weight.

m) Pollen sterility.

n) Seed sterility.

a. Germination percentage

Counts of germinated seeds for each treatment were made everyday after sowing and the percentage of germination was estimated from the value taken on the day after which no germination was observed (28 days). The emergence of plumule from the soil was taken as the criterian for germination.

b. Days taken to complete germination

The duration from the date of sowing till the date of last sprout was calculated for each treatment for obtaining the number of days taken to complete germination.

c. Survival counts

Total number of plants surviving in each treatment was counted on the 30^{th}, 60^{th}, 90^{th} and 120^{th} day after sowing and the survival data estimated on the basis of the number of seeds sown was expressed in percentage values.

d. Plant height

Plant height was measured on the 30th, 60th, 90th and 120th day after sowing from the soil surface to the terminal bud. The mean height for each treatment was calculated.

e. Chlorophyll and other morphological variants

i) Chlorophyll chimeras

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

ii) Morphological variants

The population was examined at regular intervals for the presence of morphological variations, due to the direct effect of the mutagen, such as dwarf plants and the plants with alterations in number, size and shape of leaflets in the early formed secondary leaves.

f. Flowering

i) Days to first flowering

Number of days taken for flowering to commence in individual plants of each treatment was noted and the mean value for each treatment calculated.

ii) Days taken upto last flowering

The duration from the date of sowing till the date of last flowering was noted for each treatment and the mean value worked out.

g. Number of pods per plant

Number of pods were counted in individual plants of each treatment and the mean value worked out.

h. Length of pod

Five pods were selected at random from each plant of each treatment. The length of pod measured and the mean pod length per treatment estimated.

i. Weight of pod

Five pods were selected at random from each plant, their fresh weight determined and the mean weight of pod per treatment worked out.

j. Fruit yield per plant

Fresh fruit yield was estimated for each plant of each treatment and the mean value for each treatment estimated.

There was the necessity of collecting M_1 seeds for further studies. So only dry pods were collected and weighed to determine the weight of pod and fruit yield per plant. Some fresh green pods were collected from the check plants, weighed, dried and again weighed to find out the loss of weight on drying. Then the weight of dry pods was multiplied by this factor to convert it as the weight of fresh pods.

k. Number of seeds per pod

The five pods selected at random from each plant of each treatment was split open and the number of well developed seeds counted. The mean value was worked out for each treatment.

l. Hundred seed weight

Seeds were taken from the pods collected from each plant of each treatment, hundred seed weight determined and for each treatment the mean weight worked out.

m. Pollen sterility

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

n. Seed sterility

Samples of ten seeds collected randomly from each plant of each treatment were used to determine seed sterility by germination test. Germinated seeds were considered as fertile and the ungerminated seeds as sterile. Sterility of each plant was estimated as the percentage of the number of sterile seed to the total number of seeds put for germination test. The mean seeds sterility of each treatment was estimated and expressed in percentage values.

3.2.2.3 Statistical analysis

The data were subjected to analysis of variance (ANOVA). Transformations were used wherever necessary. Since the number of seeds or plants was different for gamma rays and EMS treatments, the ANOVA was done assigning weights to the observations, where the weights were the number of plants per plot. The ANOVA was done as follows:

ANOVA

Source	Design of Experiment	
	C.R.D Laboratory study D.F.	R.B.D. Field study D.F.
Replication	-	2
Treatments	11	10
a. Between levels of gamma rays	5	4
b. Between levels of EMS	5	5
c. Gamma rays Vs EMS	1	1
Error	24	20
TOTAL	35	32

Note : Highest dose of gamma rays, viz., 500Gy showed no germination in the field. So ANOVA for all observations in the field, except germination percentage was done eliminating that level. Thus the degrees of freedom (D.F.) between levels of gamma rays became four and that of the treatments in total became ten.

RESULTS

IV. RESULTS

The morphological effect of gamma irradiation and EMS treatment on winged bean in the M_1 generation have been studied and the results are presented below.

4.1. Preliminary laboratory study

4.1.1. Germination of seeds

The data on germination percentage of seeds in the petridish study are given in Table 4.1.1 and Fig. 4.1.

Percentage of germination was significantly high in gamma rays treated seeds than EMS treated seeds, ie., 36% increase in gamma rays in comparison with EMS. Significant variation was observed at various levels of gamma rays, while the difference was not significant in respect of EMS. The germination percentage showed an increasing trend upto the levels of 300 Gy and then a decreasing trend at higher doses. Maximum germination percentage among gamma rays treatments was observed at 300 Gy. In the case of EMS treatment an irregular trend was noticed, but all the treatments were inferior to the control.

According to the regression equation of $Y = bo + b_1x + b_2x^2$, the germination percentage in gamma rays treatment was found to decrease at the level of $\geq$ 264 Gy, though the highest value for germination percentage was observed at 300 Gy. This equation explains 94% of the variation in germination percentage due to various levels of gamma rays. In the case of EMS treatment, a linear regression relationship of the form, $Y = 57.18 - 2.87x$, ie. $Y = a + bx$, was found to explain 53.6% of the variation in germination percentage attributed to various levels of EMS.

4.1.2. Days taken to complete germination

The mean number of days taken to complete germination is given in Table 4.1.1. When gamma rays was applied 16 days were taken for completion of germination, at the same time it took only 12 days to complete germination in EMS application. Significant differences in the number of days taken for complete germination were observed at different levels of gamma rays. When the dose of gamma rays is increased, the days taken to complete germination decreased with an exception at the dose of 400 Gy. But the EMS treatment showed no significant difference at the various doses, however 120 mM treatment showed the least number of days, ie. 10 days, for completion of germination.

4.1.3. Root length

The effect of the mutagens on root length on fifteenth day after sowing are presented in Table 4.1.2 and Fig.4.2. Root length

Table 4.1.1. Effect of mutagens on the total seed germination (Laboratory conditions)

Treatments	Germination percentage	Days taken to complete germination
Gamma rays		
Control	56.74 (48.86)	18
100 Gy	66.83 (54.81)	17
200 Gy	73.56 (59.03)	17
300 Gy	80.16 (63.52)	15
400 Gy	72.32 (58.23)	17
500 Gy	58.49 (49.86)	13
Mean	68.02	16
$F_{5,24}$	4.56**	2.65*
C.D.	7.74	3.13
SEd.	3.75	1.52
EMS		
Control	63.40 (52.75)	13
40 mM	46.65 (43.06)	12
80 mM	53.35 (46.90)	13
120 mM	46.65 (43.06)	10
160 mM	43.31 (41.14)	13
200 mM	46.65 (43.06)	13
Mean	50.0	12
$F_{5,24}$	1.28^{NS}	0.91^{NS}
SEd.	0.62	0.44
$F_{1,24}$[GR Vs EMS]	32.73**	25.49**

NS - Not significant; * - Significant at 5% level
** - Significant at 1% level.
Figures in parenthesis are the transformed percentages in angles

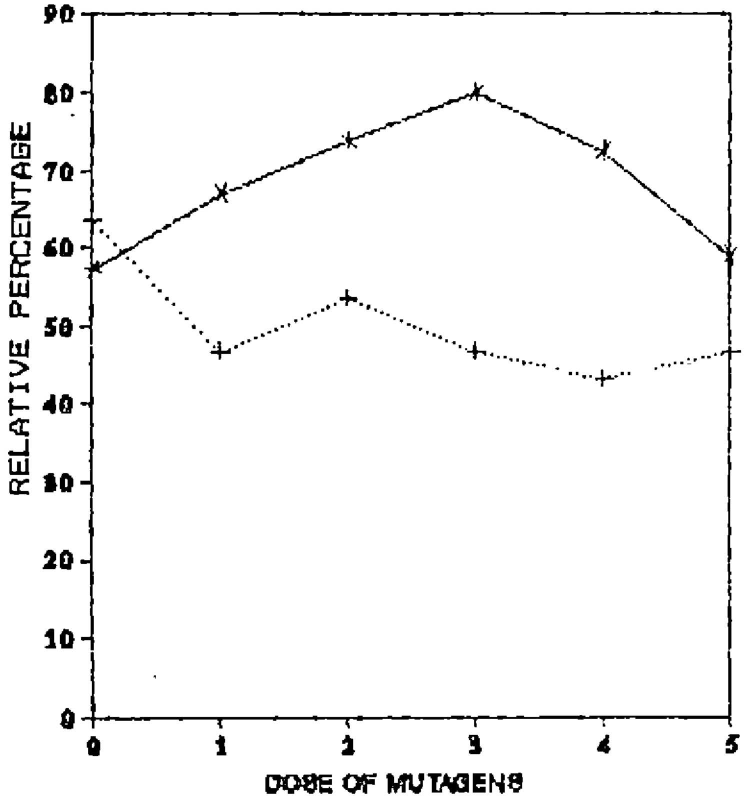
GERMINATION PERCENTAGE

GAMMA RAYS : $Y = 55.16 + 16.79X - 3.18X^2$, ($R^2 = 0.94$) ; where y = Germination percentage
x = dose/100

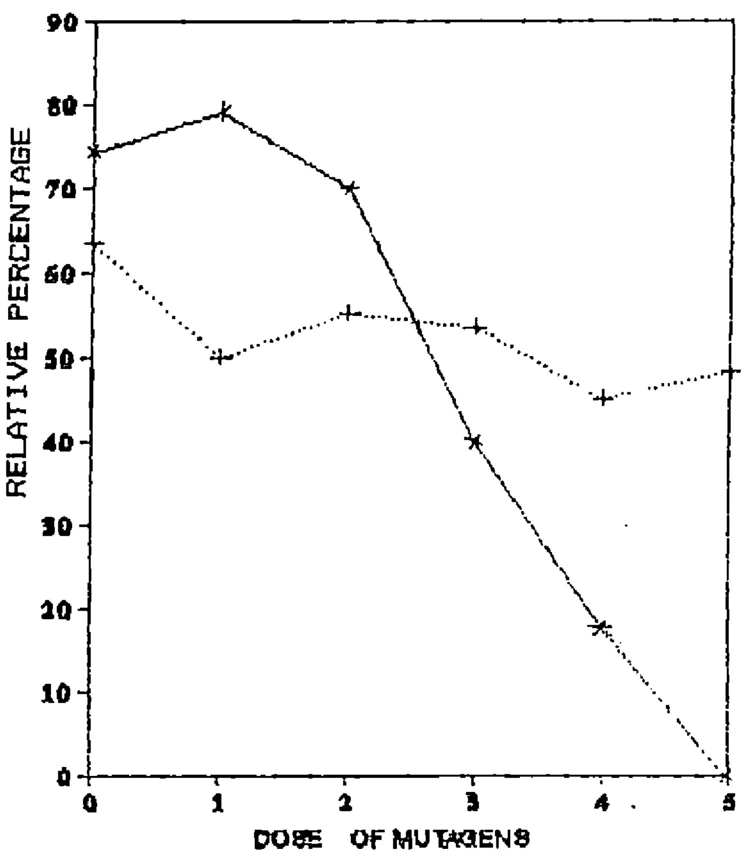
EMS : $Y = 57.18 - 2.87X$, ($R^2 = 0.536$) ; where y = Germination percentage ; x = dose

FIG.4.1 EFFECT OF MUTAGENS ON SEED GERMINATION

LABORATORY STUDY



FIELD STUDY



GAMMA RAYS ——— EMS ....

DOSES	GAMMA RAYS	EMS
0	CONTROL	CONTROL
1	100 Gy	40 mM
2	200 Gy	80 mM
3	300 Gy	120 mM
4	400 Gy	160 mM
5	500 Gy	200 mM

in plants of EMS treatment was more than double that of the gamma rays treatment. Significant differences were observed at various levels of gamma rays as welll as EMS. A gradual reduction with increase in the dose of the mutagen, was showed by gamma rays treatment. The reduction in root length was from 22% (100 Gy) to 72% (500 Gy) of control. In the case of EMS, 40 mM and 120 mM treatments gave a significantly high value for root length than control, while 80 mM and 200 mM treatments caused a significant reduction.

The changes in root length at higher doses of gamma rays were explained by the modified exponential model, $Y = 7.667 - 2.587 \times 1.21^{X}$. The chi-square ($X^2$) test of goodness of fit form the value of 0.27 which is not significant at 5% level, indicating that the fitted model explains the relationship between the applied doses and retardation of root growth. However, when the plants were treated with EMS such a trend was not observed.

4.1.4. Shoot length

The effect of treatments on shoot length on the fifteenth day after sowing are presented in Table. 4.1.2 and Fig. 4.3. Mean shoot length of EMS treatment was more than three times that of the gamma rays treatment. Significant variation was observed at various levels of gamma rays and EMS. In the case of gamma rays the untreated control registered maximum amount of shoot elongation, thereby indicating the suppressing effect of the mutagen in shoot elongation. Among the various doses of gamma irradiation tried, the rate of suppression of shoot elongation was directly proportional to the increase in dose of the mutagen. Suppressi(from 37% at 100 Gy (3.56 cm) to 80% at 500 Gy (1.16 cm). Effect of EMS was found insignificant at lower doses, but a significant increase in shoot length was showed by higher doses of 120 mM and 160 mM. The highest dose of 200 mM gave a highly significant suppressive effect on shoot length.

The modified exponential model, $Y = 0.597 + 5.073 \times 0.663^{x}$ explains the changes in shoot length at higher doses of gamma rays The chi-square (X^2) test of goodness of fit form the value of 0.07 which is not significant at 5% level indicating that the relationship between the applied doses and the retardation of shoot growth is explained by the fitted model. However, such a trend was not observed when the plants were treated with EMS.

4.1.5. Root-shoot ratio

The effect of mutagens on seedling growth are presented in plates 1 & 2 and the variation in root to shoot ratio on the fifteenth

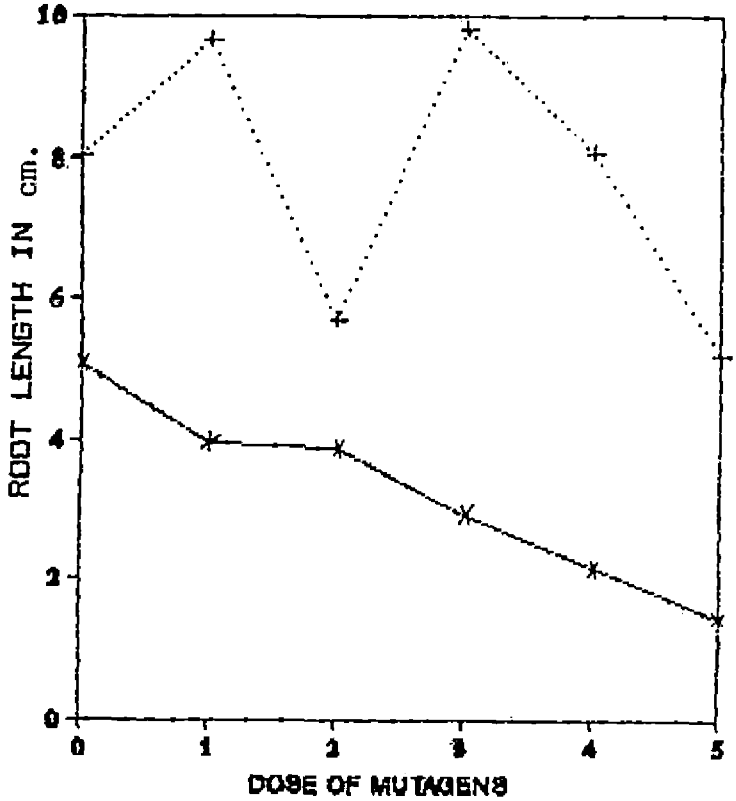
(Laboratory conditions)

Treatments	Root length (cm)	Shoot length (cm)	[a] Root-shoot ratio
Gamma rays			
Control	5.08	5.67	1.17
100 Gy	3.97	3.56	1.21
200 Gy	3.89	2.83	1.48
300 Gy	2.92	2.30	1.22
400 Gy	2.15	1.58	1.45
500 Gy	1.41	1.16	1.27
Mean	3.24	2.85	1.29
$F_{5,24}$	15.33**	6.08**	1.81^{NS}
C.D.	0.99	1.93	-
SEd.	0.48	0.94	0.14
EMS			
Control	8.02	10.33	1.02
40 mM	9.68	10.58	0.99
80 mM	5.70	10.40	0.73
120 mM	9.83	13.79	0.79
160 mM	8.09	14.05	0.73
200 mM	5.21	5.27	1.14
Mean	7.75	10.74	0.94
$F_{5,24}$	16.12**	11.61**	1.19^{NS}
C.D.	1.41	2.72	-
SEd.	0.68	1.32	0.19
$F_{1,24}$[GRVsEMS]	348.93**	286.10**	26.94**

NS - Not significant, ** - Significant at 1% level

a - Root-shoot ratio calculated based on original values and not based on mean values.

FIG.4.2 EFFECT OF MUTAGENS ON ROOT LENGTH



GAMMA RAYS ——— EMS ········

DOSES	GAMMA RAYS	EMS
0	CONTROL	CONTROL
1	100 Gy	40 mM
2	200 Gy	80 mM
3	300 Gy	120 mM
4	400 Gy	160 mM
5	500 Gy	200 mM

FIG.4.3 EFFECT OF MUTAGENS ON SHOOT LENGTH

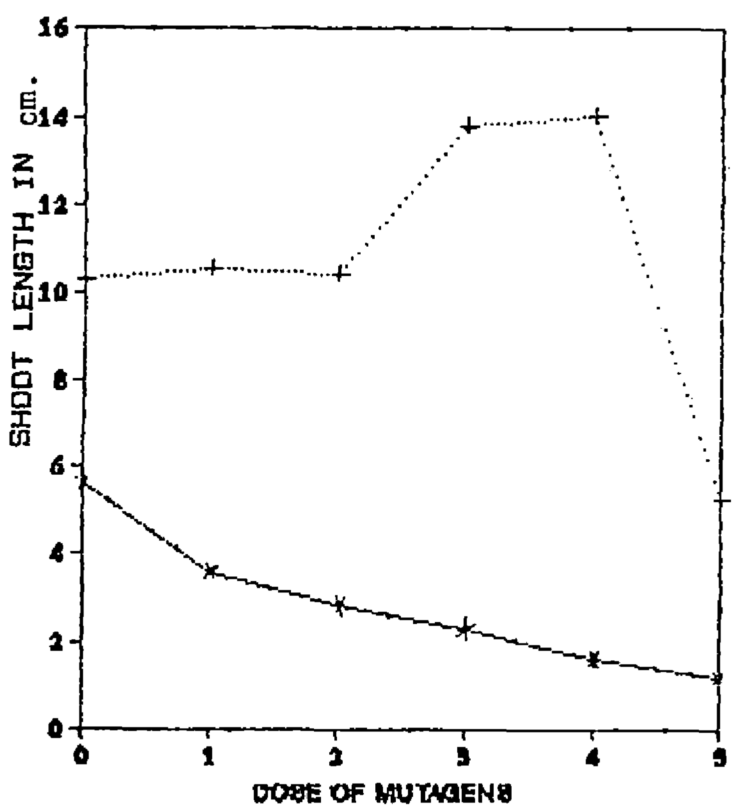


Plate 1. Variation in seedling growth induced by gamma rays.
[Treatment No. 1-control 2-100 Gy 3-200 Gy
4-300 Gy 5-400 Gy 6-500 Gy]

Plate 2. Variation in seedling growth induced by EMS.
[Treatment No. 7-control 8-40mM 9-80mM
10-120mM 11-160mM 12-200mM]

Plate 1

Plate 2

day after sowing due to treatments are presented in Table 4.1.2. Root-shoot ratio was significantly high in gamma rays treated populations than the EMS treated populations. No significant variation was observed at various levels of gamma rays as well as EMS. However, the values for ratio were slightly higher than that of the control in gamma rays treatments indicating that the growth of the shoot was more affected than that of the root. But the ratios were slightly less than that of the control except at 200 mM dose in the case of EMS, which indicated a higher growth inhibition for the root than for the shoot.

4.2. Field study

4.2.1. Germination of seeds

The effects of mutagens on the germination percentage under field conditions are presented in Table 4.2.1 and graphically represented in Fig. 4.1. Gamma rays and EMS treated seeds showed significant variation in germination, ie. 12% reduction in gamma rays in comparison with EMS. Significant variation was observed at various levels of gamma rays, while the difference was not significant in respect of EMS. Gamma rays showed a slight increase over control at 100 Gy for germination, and thereafter it decreased gradually with increase in dose. Highest dose of gamma rays, viz., 500 Gy registered no germination at all. Though all the treatments of EMS were inferior to control in affecting germination, greater suppression was expressed by highest doses of 160 mM and 200 mM.

Though highest value for germination percentage was observed at 100 Gy, when the data were subjected to regression analysis using the regression equation of $Y = bo + b_1\sqrt{x} + b_2x$, the germination percentage was found to decrease at the level of $\geq$36 Gy. This equation explains 98.7% of the variation due to the various levels of gamma rays. A linear regression relationship of the form, Y = a + bx (y = 59.06 - 2.63x) was found to explain 58% of the variation in germination percentage attributed to the various levels of EMS.

4.2.2. Days taken to complete germination

The number of days taken to complete germination are seen in Table 4.2.1. When gamma rays treated seeds took 25 days to complete germination, the EMS treated seeds completed germination in about 16 days. Different levels of gamma rays as well as EMS showed no significant differences in the number of days taken for completion of germination. However, a slight increase in the number of days to complete germination was observed at 120 mM dose of EMS in comparison to the control.

Table 4.2.1. Effect of mutagens on the total seed germination (Field conditions)

Treatments	Germination percentage	Days taken to complete germination
Gamma rays		
Control	74.02 (59.33)	26
100 Gy	78.98 (62.69)	24
200 Gy	70.04 (56.79)	26
300 Gy	40.0 (39.22)	24
400 Gy	17.59 (24.79)	27
500 Gy	0.0 (0)	-
Mean	46.77	25
$F_{5,24}$	57.70**	(a) 0.25^{NS}
C.D.	9.46	-
SEd.	4.56	2.20
EMS		
Control	63.44 (52.77)	15
40 mM	49.80 (44.87)	13
80 mM	55.07 (47.89)	14
120 mM	53.34 (46.89)	18
160 mM	44.93 (42.07)	16
200 mM	48.28 (43.99)	17
Mean	52.48	16
$F_{5,22}$	0.89^{NS}	(b) 1.06^{NS}
SEd.	5.59	2.69
$F_{1,22}$ [GR Vs EMS]	8.16**	(c) 82.60**

NS - Not significant; ** - Significant at 1% level
Figures in parenthesis are the transformed percentages in angles.
a- $F_{4,20}$ b- $F_{5,20}$ c- $F_{1,20}$

GERMINATION PERCENTAGE

GAMMA RAYS : $Y = 74.06 + 39.98\sqrt{x} - 33.26x$, ($R^2 = 0.987$) ; where y = Germination percentage, $x = \sqrt{dose/100}$

EMS : $Y = 59.06 - 2.63X$, ($R^2 = 0.58$) ; where y = Germination percentage, x = dose.

4.2.3. Survival of plants

The results on the survival percentage of plants on the thirtieth , sixtieth, ninetieth and one hundred and twentieth day after sowing are given in Table 4.2.2 and in Fig. 4.4. As indicated by the table, there were no significant differences between gamma rays and EMS treatments in the survival percentages of plants at any day after sowing. At all the periods of plant growth, survival percentage was high for gamma rays than EMS. Various doses of gamma rays showed significant variation in survival. Lowest dose of 100 Gy lead to a slight increase in the value of survival percentage in comparison with the control, and thereafter a gradual reduction was noticed with increase in dose. The same trend was noticed at all the periods of plant growth of gamma rays treatments. As growth period advanced, survival percentage decreased by all the doses of gamma rays, while the reverse was the case in control.

No significant influence was noticed in survival percentage at different periods of plant growth by different doses of EMS. However, all the levels of EMS were found inferior to the control, and among the treatments 160mM concentration was most inferior in affecting survival. The same trend was shown at all the different growth periods of plants.

Table 4.2.2. Effect of mutagens on the percentage of plants survived under field conditions

Treatments	At 30DAS	At 60DAS	At 90DAS	At 120DAS
amma rays				
Control	62.51 (52.22)	69.21 (56.27)	70.55 (57.11)	70.91 (57.34)
100 Gy	78.98 (62.69)	76.72 (61.13)	76.85 (61.21)	74.56(59.69)
200 Gy	66.74 (54.76)	67.85 (55.44)	66.69 (54.73)	65.68 (54.11)
300 Gy	40.0 (39.22)	38.88 (38.56)	38.88 (38.56)	38.88 (38.56)
400 Gy	16.29 (23.79)	13.21 (21.31)	12.17 (20.41)	12.17 (20.41)
Mean	52.9	53.17	53.03	52.44
$F_{4,20}$	18.34**	21.53**	25.53**	20.52**
C.D.	10.51	10.48	9.86	10.76
SEd.	5.04	5.02	4.73	5.16
EMS				
Control	60.04 (50.77)	63.44 (52.77)	60.04 (50.76)	58.38 (49.8)
40 mM	46.03 (42.70)	46.14 (42.77)	48.13 (43.91)	48.13 (43.91)
80 mM	55.07 (47.89)	53.39 (46.92)	53.35 (46.90)	51.63 (45.92)
120 mM	50.0 (44.98)	53.34 (46.89)	53.34 (46.89)	51.67 (45.94)
160 mM	41.32 (39.98)	43.22 (41.09)	39.80 (39.10)	39.80 (39.10)
200 mM	43.26 (41.11)	42.98 (40.95)	46.50 (42.98)	46.5 (42.98)
Mean	49.29	50.42	50.19	49.35
$F_{5,20}$	0.90^{NS}	1.09^{NS}	0.96^{NS}	0.64^{NS}
SEd.	6.17	6.16	5.79	6.32
$F_{1,20}$ [GR Vs EMS]	0.67^{NS}	0.30^{NS}	0.34^{NS}	0.33^{NS}

NS - Not significant ** - Significant at 1% level

Figures in parenthesis are the transformed percentages in angles.

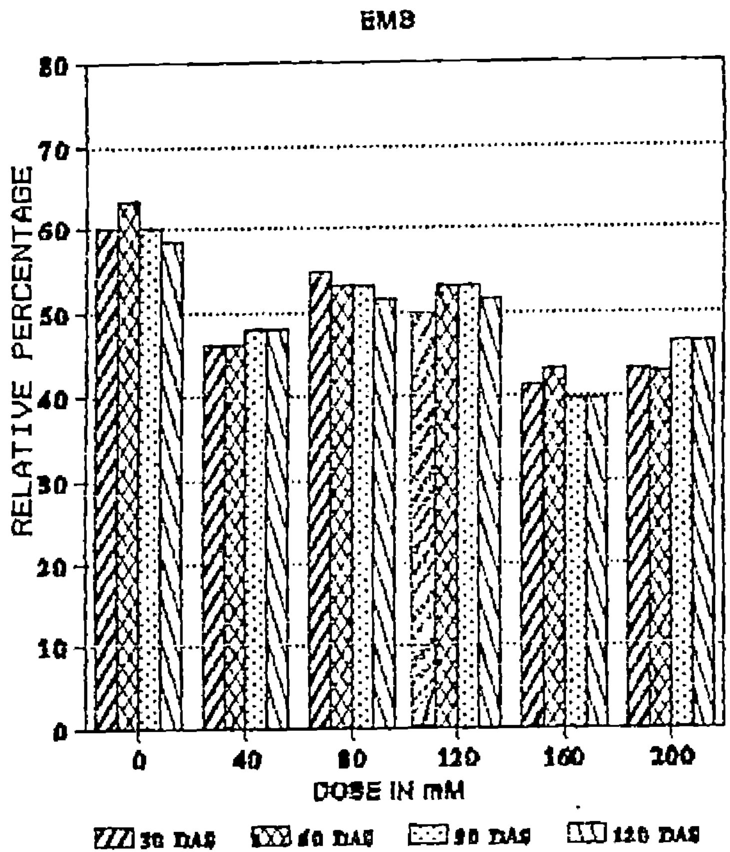
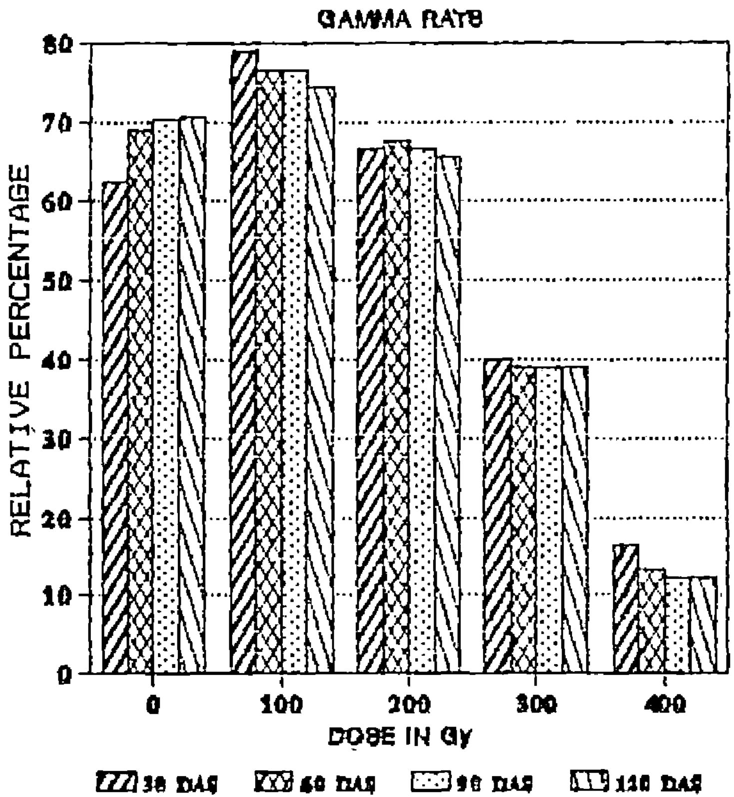

EMS
RELATIVE PERCENTAGE
0
10
20
30
40
50
60
70
80
0
40
80
120
160
200
DOSE IN mM
30 DAS
60 DAS
90 DAS
120 DAS

FIG.4.4 EFFECT OF MUTAGENS ON THE SURVIVAL OF PLANTS



4.2.4. Height of plants

The height of plants on 30, 60, 90 and 120 days after sowing with respect to treatments are given in Table 4.2.3 and in Fig. 4.5. Significant differences were observed between gamma rays and EMS treatments only in the later stages of plant growth, ie. at the 90^{th} and 120^{th} day after sowing. The 28% reduction in height observed in the gamma rays treated population in comparison to the height of EMS treated plants at the ninetieth day after sowing, was increased to 22% reduction at the 120^{th} day after sowing. Various doses of gamma rays caused significant variation in plant height at all the different periods of plant growth. A slight increase of height was noticed at 100 Gy dose in comparison to control and thereafter an inverse relationship was noticed between the increase in dose and the plant height. The trend was the same for all the different periods of plant growth. 400 Gy dose recorded as high as 53% reduction in height in comparison to control at the last stage of 120 days after sowing. At any stage, there was no significant variation in plant height of EMS treated plants.

According to the regression equation of $Y = bo + b_1\sqrt{x} + b_2x$, the plant height at 120 days after sowing in gamma rays treatment was found to decrease at a level of ≥ 33.6 Gy, though the highest value for plant height was observed at 100 Gy. The

Table 4.2.3. Effect of mutagens on plant height under field conditions on different days after sowing (m)

Treatments	30DAS	60DAS	90DAS	120DAS
Gamma rays				
Control	0.22	2.39	3.85	6.38
100 Gy	0.26	2.47	4.53	7.28
200 Gy	0.31	2.15	3.57	5.39
300 Gy	0.13	1.04	1.86	3.62
400 Gy	0.08	0.59	1.32	2.99
Mean	0.20	1.73	3.03	5.13
$F_{4,20}$	11.34**	25.15**	23.01**	18.82**
C.D.	0.08	0.50	0.84	1.23
SEd.	0.039	0.24	0.40	0.59
EMS				
Control	0.17	1.37	3.60	6.54
40 mM	0.19	1.58	4.62	7.07
80 mM	0.24	1.82	4.45	6.42
120 mM	0.19	1.79	4.70	6.78
160 mM	0.17	1.09	3.69	6.32
200 mM	0.19	1.30	3.99	6.17
Mean	0.19	1.49	4.18	6.55
$F_{5,20}$	0.53^{NS}	1.90^{NS}	1.87^{NS}	0.41^{NS}
SEd.	0.048	0.296	0.494	0.72
$F_{1,20}$ [GR Vs EMS]	0.09^{NS}	4.17^{NS}	35.98**	25.64**

NS - Not significant ** - Significant at 1% level

PLANT HEIGHT AT 120DAS

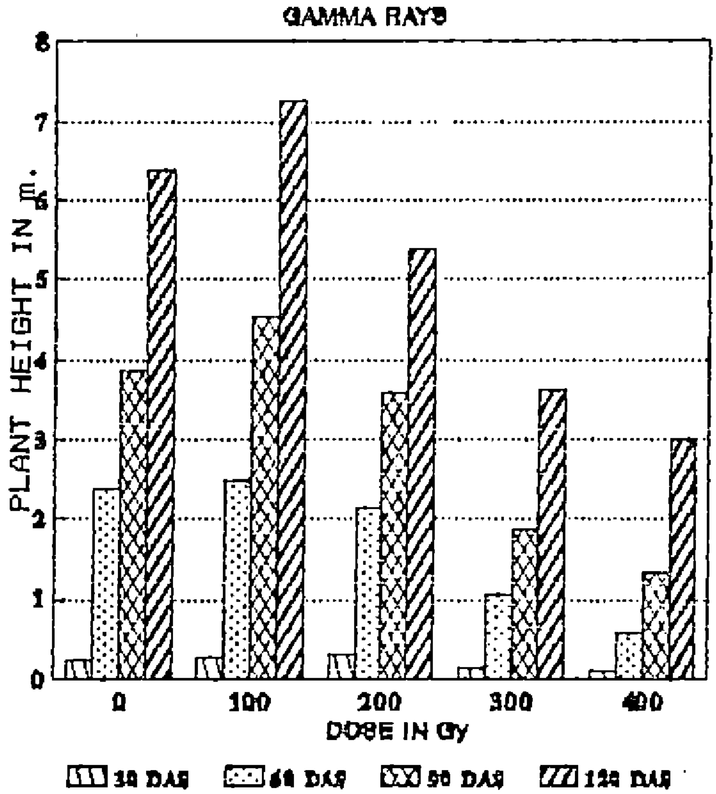
GAMMA RAYS ; $Y = 6.46 + 2.68\sqrt{x} - 2.31x$, $(R^2 = 0.944)$

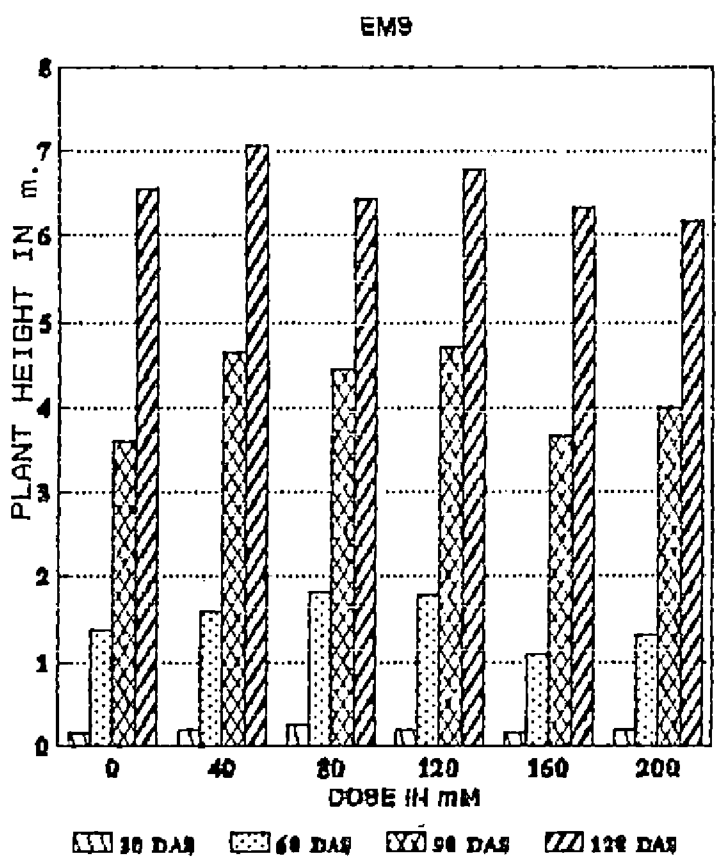
EMS : $Y = 6.57 + 0.696\sqrt{x} - 0.395x$, $(R^2 = 0.63)$; where

y = plant height at 120 DAS

$x = \sqrt{(dose/100)}$

FIG.4.5 EFFECT OF MUTAGENS ON THE HEIGHT OF PLANTS





equation was found to explain 94.4% of the variation caused by the various levels of gamma rays. Using the same equation, the height was found to decrease at the level of $\geq$31 mM in EMS treatment at 120 days after sowing even when the highest value for plant height was observed at 40 mM. 63% of the variation in plant height due to various levels of EMS was explained by the equation.

4.2.5. Chlorophyll chimeras

Chlorophyll chimeras on the leaves were observed among the gamma rays treated plants, while such chimeric plants were completely absent in the M_1 generation in all the EMS treatments. Chlorophyll deficient patches were found in the plants of all the different doses of gamma rays treatment, but variations were there in the nature and extent of patches (Plates : 3, 4, 5, 6). In most cases, chlorophyll deficient patches appeared in the early stages and later disappeared.

4.2.6. Morphological variations

Morphological abnormalities in the M_1 generation were seen in some of the gamma rays treated plants in the present investigation. These included alterations in number, size and shape of leaflets in the first formed secondary leaves. In some cases, these leaves lacked one or two lateral leaflets, thereby appearing as a bifoliate

Plate 3. Control plant.

Plate 4. Variation in chlorophyll content of leaves induced by 200 Gy treatment of gamma rays.

Plate 3


Plate 4

Plate 5. Variation in chlorophyll content of earlier leaves induced by 300 Gy treatment of gamma rays.

Plate 6. Variation in chlorophyll content in the first formed secondary leaves induced by 100 Gy treatment of gamma rays.

Plate 5

Plate 6

Plate 7. Seedling with unifoliate leaf having chlorophyll deficient patch (Treatment No.4) induced by 300 Gy treatment of gamma rays.

Plate 8. Variation in the size of leaf induced by treatment with 400 Gy of gamma rays.
[Increased size of one among the three leaflets]

Plate 7

Plate 8

Plate 9. Variation in the shape of leaf induced by 300 Gy treatment of gamma rays.

[Crinkling of the first formed secondary leaves]

Plate 10. Variation in the shape of leaves induced by 200 Gy treatment of gamma rays.

[Leaflets with round apex]

Plate 9

Plate 10

Plate 11. Variation in the colour of seeds induced by gamma rays.

Plate 12. Variation in the size of seeds induced by gamma rays.

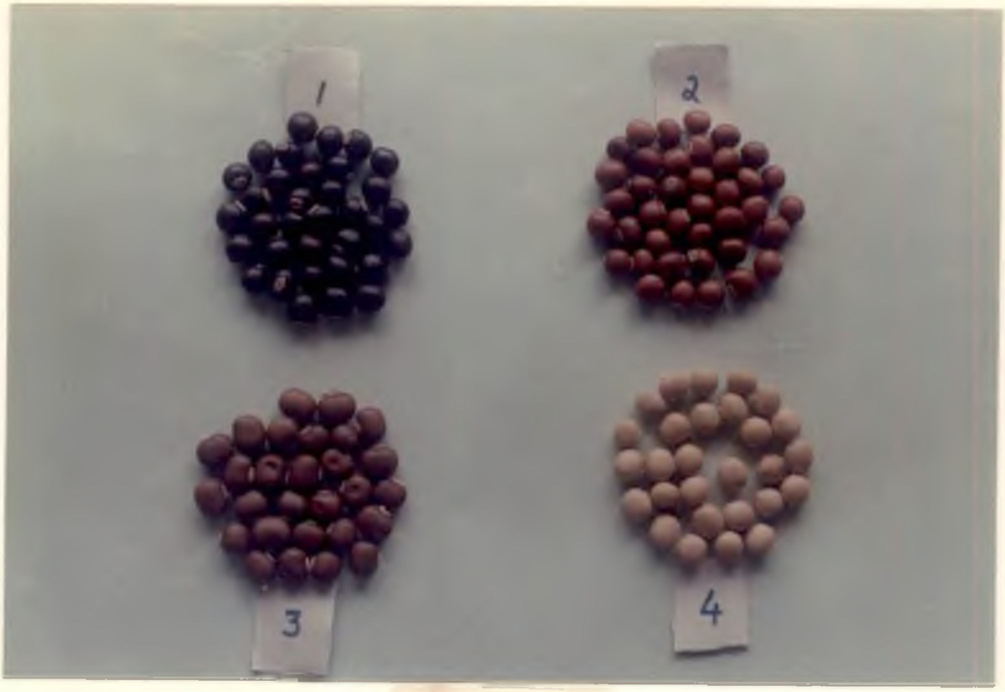


Plate 11

Plate 12

Plate 13. Variation in the shape of seeds induced by gamma rays.

Plate 14. Presence of cracks on the seedcoat surface induced by gamma rays.

Plate 13

Plate 14

or unifoliate leaf instead of normal trifoliate leaf. Certain plants showed an increase in size of one of the three leaflets in a leaf and certain others exhibited crinkling of leaflets in the early stages of growth period (plates : 7, 8, 9, 10). However, these plants recovered and produced normal leaflets afterwards. Morphological variations also included changes in size, colour and the presence or absence of cracks or crinkles on the surfaces of seedcoats (Plates : 11, 12, 13, 14) of the seeds. In addition some non-flowering and non-fruiting plants were also observed at higher doses of gamma rays and at all doses of EMS.

4.2.7. Days to first-flowering

The results of treatments on the number of days to first flowering are given in Table 4.2.4. Effect of gamma rays and EMS on the number of days to first flowering was similar. But significant variation was observed at various levels of gamma rays. An insignificant reduction was noticed at lower levels of 100 Gy and 200 Gy in the number of days to first flowering and a significant increase at 300 Gy and 400 Gy in comparison to control. The range for number of days taken for first flowering was from 75 days (85.32 per cent of control) to 107 days (121.1 per cent of control). Various concentrations of EMS caused no significant variation in the number of days to flowering, but plants in all the treatments flowered earlier than the control.

Table 4.2.4. Effect of mutagens on flowering

Treatments	Days to first flowering	Days taken upto the last flowering
Gamma rays		
Control	88	144
100 Gy	75	148
200 Gy	84	151
300 Gy	105	156
400 Gy	107	139
Mean	92	148
$F_{4,20}$	8.87**	1.54^{NS}
C.D.	13.56	-
SEd.	6.50	7.29
EMS		
Control	92	133
40 mM	84	131
80 mM	83	127
120 mM	86	126
160 mM	91	136
200 mM	89	128
Mean	88	130
$F_{5,20}$	0.38^{NS}	0.39^{NS}
SEd.	7.96	8.94
$F_{1,20}$[GR Vs EMS]	1.90^{NS}	26.0**

NS - Not significant; ** - Significant at 1% level

4.2.8. Days taken upto the last flowering

Maximum number of days taken for last flowering was 148 days in case of gamma rays and 130 days in case of EMS (Table 4.2.4). The effect of various levels of gamma rays as well as EMS were found insignificant. However, all the doses of gamma rays except 400 Gy took more number of days and all the doses of EMS except 160 mM took lesser number of days compared with the respective controls to the last flowering.

4.2.9. Number of pods per plant

The data on the number of pods per plant are given in Table 4.2.5. Number of pods per plant in gamma rays treatment was approximately two times than that of EMS treatment. There was significant variation at different doses of gamma rays, but the variation was not significant in the case of EMS. Lower doses of both mutagens caused an increase in the number of pods per plant, which later decreased gradually with increase in dose.

4.2.10. Weight of pod

Table 4.2.5 shows the results of weight of pod of the various treatments. EMS treated populations showed significant increase in the weight of pods than the gamma rays treated populations. Various levels of gamma rays showed significant variation and all the treatments were inferior to control, ie. the weight of pod was found inversily related to the dose of mutagen. No significant difference was noticed at various levels of EMS, but all treatments were superior to control. A stimulatory effect was noticed at the lowest dose of 40 mM, and thereafter the weight of pod decreased gradually with increase in dose of EMS.

4.2.11. Length of pod

The effect of mutagens on pod length are presented in Table 4.2.5. Length of pod was found to be significantly high in EMS treated populations than in gamma rays treated populations. All the treatments gave values lesser than control for pod length in case of gamma rays and the various levels showed significant variation. Linear reduction was noticed in the length of pod with the increase of dosage from control. Variation between different levels of EMS was insignificant. However, a stimulatory effect was observed at lower doses of 40 mM and 80 mM and then pod length gradually decreased with dose, but all the values were superior to control.

4.2.12. Fruit yield per plant

The results of treatments on the fruit yield per plant are given in Table 4.2.5 and Fig. 4.6. A significantly high, ie. about 60%, increase was registered for the yield of fruit per plant in

Effect of mutagens on number of pods per plant, weight of pod, length of pod and fruit yield per plant

Treatments	No. of pods/ plant	Weight of pod (g)	Length of pod (cm)	Fruit yield/ plant (g)
Gamma rays				
Control	7.44	19.63	20.69	147.27
100 Gy	9.50	16.77	19.58	154.68
200 Gy	6.18	12.08	16.66	77.29
300 Gy	4.74	9.52	15.46	53.28
400 Gy	2.89	7.76	13.63	36.41
Mean	6.15	13.15	17.20	93.79
$F_{4,20}$	3.99**	14.21**	9.02**	4.92**
C.D.	3.73	3.88	2.86	72.19
SEd.	1.79	1.86	1.37	34.61
EMS				
Control	3.47	15.31	17.98	57.53
40 mM	4.08	19.86	20.16	75.40
80 mM	4.00	17.44	20.02	71.95
120 mM	3.41	17.23	18.95	60.59
160 mM	3.20	16.48	18.23	37.77
200 mM	3.13	15.95	17.15	48.72
Mean	3.55	17.04	18.75	58.66
$F_{5,20}$	0.07^{NS}	0.98^{NS}	1.0^{NS}	0.22^{NS}
SEd.	2.19	2.28	1.68	42.38
$F_{1,20}$ [GR Vs EMS]	9.42**	19.56**	5.61*	4.58*

NS - Not significant   * - Significant at 5% level

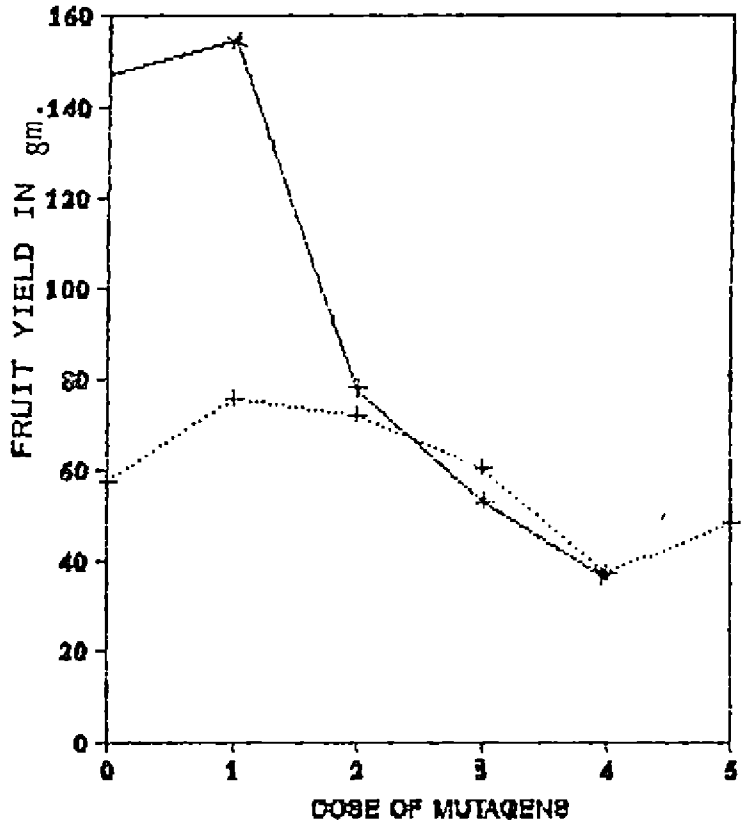
** - Significant at 1% level

FRUIT YIELD PER PLANT

GAMMA RAYS : $Y = 150.76 + 27.05\sqrt{x} - 45.11x$, $(R^2 = 0.899)$

EMS : $Y = 58.41 + 33.95\sqrt{x} - 18.87x$, $(R^2 = 0.737)$; where y = yield, $x = \sqrt{(dose/100)}$

FIG.4.6 EFFECT OF MUTAGENS ON FRUIT YIELD PER PLANT



GAMMA RAYS ——— EMS ········

DOSES	GAMMA RAYS	EMS
0	CONTROL	CONTROL
1	100 Gy	40 mM
2	200 Gy	80 mM
3	300 Gy	120 mM
4	400 Gy	160 mM
5	——	200 mM

gamma rays in comparison to EMS. Different doses showed significant variation in gamma rays while the difference was not significant in respect of EMS treated populations. Lowest doses of both the mutagens caused a slight stimulation, but thereafter fruit yield decreased linearly with increase in dose of mutagen. The range for fruit yield per plant was higher in gamma rays treated population which ranged from 24% at 400 Gy to 105% at 100 Gy in comparison to the control.

A regression equation of the form, $Y = bo + b_1\sqrt{x} + b_2x$ was found to fit to the values of fruit yield per plant both in gamma rays and EMS treatments. In gamma rays treatment, though highest value for yield was observed at 100 Gy, the yield was found to decrease at the level of ≥ 9 Gy as per the equation; and the equation explains 90% of the variation in yield due to various levels of gamma rays. Though highest value for yield in case of EMS treatment was observed at 40 mM, according to the equation, the yield was found to decrease at the level of ≥ 32.4 mM. This equation explains 74% of the variation in yield due to various levels of EMS.

4.2.13. <u>Number of seeds per pod</u>

Observations on the number of seeds per pod indicated a significant increase in the EMS treatment than gamma rays treatment

Table 4.2.6. Effect of mutagens on the number of seeds per pod and hundred seed weight

Treatments	No. of seeds/ pod	Hundred seed weight (g)
Gamma rays		
Control	10.38	33.08
100 Gy	8.81	31.36
200 Gy	6.95	32.13
300 Gy	6.05	28.40
400 Gy	3.36	28.17
Mean	7.11	30.63
$F_{4,20}$	18.31**	2.18^{NS}
C.D.	1.85	-
SEd.	0.89	2.14
EMS		
Control	9.42	28.91
40 mM	11.16	32.04
80 mM	10.81	29.46
120 mM	8.98	31.37
160 mM	9.37	29.22
200 mM	8.71	27.52
Mean	9.74	29.75
$F_{5,20}$	1.70^{NS}	0.81^{NS}
SEd.	1.09	2.62
$F_{1,20}$ [GR Vs EMS]	39.14**	0.75^{NS}

NS - Not significant; ** - Significant at 1% level

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Treatments	No. of seeds/ pod	Hundred seed weight (g)
Gamma rays		
Control	10.38	33.08
100 Gy	8.81	31.36
200 Gy	6.95	32.13
300 Gy	6.05	28.40
400 Gy	3.36	28.17
Mean	7.11	30.63
$F_{4,20}$	18.31**	2.18^{NS}
C.D.	1.85	-
SEd.	0.89	2.14
EMS		
Control	9.42	28.91
40 mM	11.16	32.04
80 mM	10.81	29.46
120 mM	8.98	31.37
160 mM	9.37	29.22
200 mM	8.71	27.52
Mean	9.74	29.75
$F_{5,20}$	1.70^{NS}	0.81^{NS}
SEd.	1.09	2.62
$F_{1,20}$ [GR Vs EMS]	39.14**	0.75^{NS}

NS - Not significant; ** - Significant at 1% level

(Table 4.2.6). Significant variation was shown by different levels of gamma rays and all the treatments were inferior to the control. Reduction in the number of seeds per pod was inversily proportional to the doses and the reduction was as high as 68% of that of the control at the highest dose of gamma rays among the survived doses. Effects of various doses of EMS were found to be insignificant, but a slight stimulatory effect was noticed at the lower doses.

4.2.14. Hundred seed weight

The results of treatments on 100-seed weight are presented in Table 4.2.6. Gamma rays as well as EMS treatment registered more or less similar values for hundred seed weight. Same was the case when various levels of gamma rays were compared with each other; however, a slight decrease in 100-seed weight was shown by higher doses. Various levels of EMS treatment was also found to have no effect on 100-seed weight, but a slight increase was observed at the lowest dose of EMS employed.

4.2.15. Pollen sterility

The pollen sterility percentage as influenced by treatments are presented in Table 4.2.7 and are graphically represented in Fig. 4.7. Gamma rays treated populations showed higher pollen sterility which was approximately seven times higher than that of EMS treated

Table 4.2.7. Effect of mutagens on pollen sterility and seed sterility

Treatments	Pollen sterility (%)	Seed sterility (%)
Gamma rays		
Control	5.86 (14.01)	10.76 (19.14)
100 Gy	6.59 (14.87)	18.59 (25.53)
200 Gy	52.78 (46.57)	19.73 (26.36)
300 Gy	53.12 (46.77)	24.18 (29.44)
400 Gy	48.56 (44.16)	23.82 (29.20)
Mean	33.38	19.42
$F_{4,20}$	11.13**	2.87^{NS}
EMS		
Control	5.11 (13.06)	11.71 (20.0)
40 mM	2.58 (9.23)	12.80 (20.96)
80 mM	4.15 (11.75)	10.63 (19.02)
120 mM	3.84 (11.30)	18.39 (25.39)
160 mM	5.16 (13.12)	17.49 (24.71)
200 mM	6.89 (15.21)	19.62 (26.28)
Mean	4.62	15.11
$F_{5,20}$	0.14^{NS}	1.56^{NS}
$F_{1,20}$ [GR Vs EMS]	49.64**	2.09^{NS}

NS - Not significant; ** - Significant at 1% level

Figures in parenthesis are the transformed percentages in angles.

Note:- Separate C.D. table for pollen sterility and seed sterility (Table 4.2.7.1) attached.

Table 4.2.7.1. C.D. Values

Seed Sterility

	1	2	3	4	5	6	7	8	9	10	11	
2	15.68	6.25	6.26	7.23	11.55	7.04	6.67	6.58	6.52	7.23	6.99	1
3	15.5	15.27	6.30	7.27	11.58	7.08	6.71	6.62	6.56	7.27	7.03	2
4.	15.03	14.8	14.61	7.28	11.58	7.09	6.72	6.63	6.57	7.28	7.04	3
5.	15.19	14.97	14.77	14.28	12.14	7.96	7.64	7.55	7.51	8.13	7.91	4
6.	15.75	15.53	15.34	14.87	15.03	12.03	11.81	11.76	11.73	12.14	11.99	5
7.	15.86	15.64	15.46	14.99	15.15	15.71	7.46	7.38	7.33	7.96	7.75	6
8.	15.75	15.53	15.34	14.87	15.04	15.59	15.71	7.02	6.97	7.64	7.41	7
9.	15.85	15.63	15.44	14.98	15.14	15.69	15.81	15.70	6.88	7.55	7.32	8
10.	15.64	15.42	15.23	14.76	14.92	15.49	15.60	15.49	15.59	7.51	7.28	9
11	15.93	15.71	15.52	15.06	15.22	15.77	15.89	15.78	15.87	15.67	7.91	10

Pollen Sterility

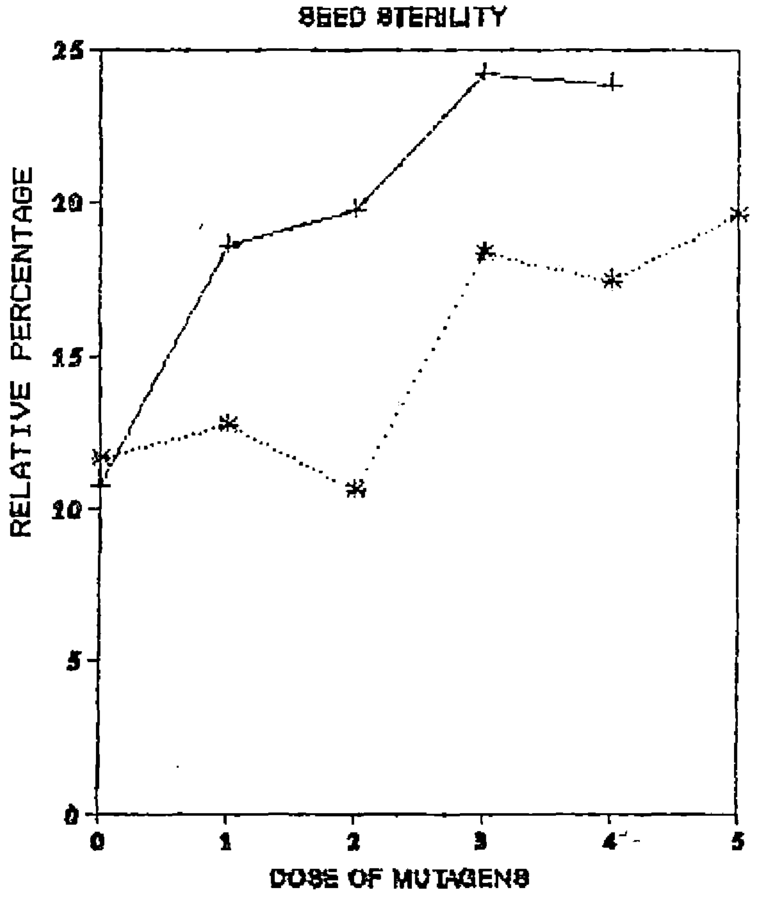
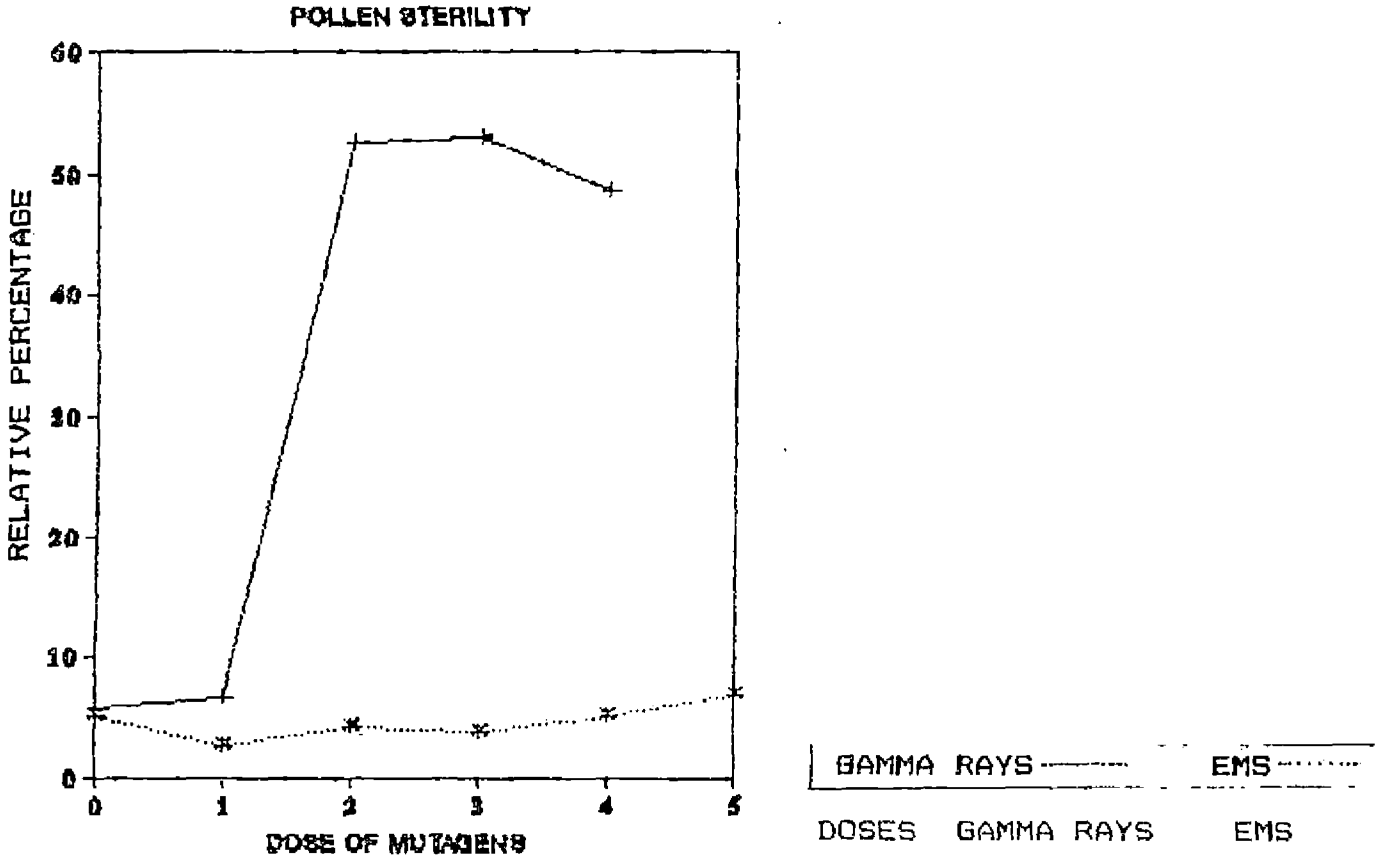

SEED STERILITY
RELATIVE PERCENTAGE
0
5
10
15
20
25
0
1
2
3
4
5
DOSE OF MUTAGENS
0 CONTROL CONTROL
1 100 Gy 40 mM
2 200 Gy 80 mM
3 300 Gy 120 mM
4 400 Gy 160 mM
5 —— 200 mM

FIG.4.7 EFFECT OF MUTAGENS ON POLLEN STERILITY AND SEED STERILITY



populations. Pollen sterility of various levels of gamma rays differed significantly and except at 100 Gy dose, the estimates were around 50%. Effects of different levels of EMS on pollen sterility was found to be similar to each other and to the control. But at 40 mM treatment of EMS, pollen sterility was nearly half of that in control.

4.2.16. Seed sterility

Table 4.2.7 and Fig.4.7 shows the results of seed sterility percentage. Effect of gamma rays and EMS on seed sterility was not significantly different and gave similar values. Various levels of gamma rays showed no significant difference; however, slightly increasing trend was noticed for seed sterility with the increase in the dose of gamma rays in comparison to the control. EMS treated populations also recorded no significant effe at its various levels, though slightly higher values for seed stelity were observed at the higher concentrations in comparison to th control.

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DISCUSSION

V. DISCUSSION

New concepts suggest that mutations are neither rare events nor largely deleterious in effects. Undesirable changes produced by the action of mutagens are manifested as M_1 damages and for the economic use of mutations in plant breeding, efficient treatments producing greater proportion of mutations to damages are essential. A remarkable amount of work has been done in the recent years by employing physical and chemical mutagens for crop improvement.

Winged bean is one of the most underexploited tropical legumes. It is an excellent source of dietary proteins and oils, and all parts of this plant are consumed as food. In view of the limited amount of variability which is presently available in legumes in general and in winged bean in particular, probably because of its essentially self-fertilized nature (Anon., 1975), it is considered essential to undertake methods of inducing genetic variability through induced mutagenesis.

In the light of the factors mentioned above, the choice of the problem is fully justifiable. The present investigation was undertaken to study the direct effect of 6 doses of gamma rays (100 to 500 Gy) and 6 concentrations of EMS (40 to 200 mM) on winged bean and also the effects of different doses of the mutagens in the M_1 generation. A brief discussion on the varied responses of winged bean to mutagenic treatments in the M_1 generation is given in the following sections.

5.1. Germination percentage

Generally mutagens have harmful effects on organisms, and the mutagen treatments reduce germination. In the present investigation, germinability of gamma irradiated seeds was better in all the treatments compared to the control in the laboratory trials. In field study also a slight stimulatory effect was noticed at lower doses of gamma rays. Similar observations were made by Swarup and Gill (1968) and Rukmanski (1973) in french bean, Mujeeb (1974) in Cicer, Khan and Heshim (1978) in greengram, Manju (1981) in horsegram and Patil and Bhalla (1985) in soybean. Stimulatory effect on germination may be due to higher activity of certain enzymes involved in the synthesis of auxins (Casarett, 1968). In field trials, greater reduction in germination was noticed at higher doses compared to lower doses of gamma rays and no germination was observed at the highest dose of 500 Gy. This is in agreement with the results obtained by Louis and Kadambavanasundaram (1973b) in cowpea, Venkateswarlu et al. (1978) in pigeonpea, Kesavan and Khan (1978) in winged bean, Vadivelu and Rathinam (1980) in bengal gram, Veeresh (1983) in winged bean, Nadarajan _et al_. (1985) in _Cajanus cajan_, Subba Rao (1988) in _Cicer_ and Mehetre _et al_. (1990) in mungbean. In field trials mean germination percentage was higher in treatments with EMS than that with gamma rays which was in agreement with the results of Siddiq and Swaminathan (1968) in rice and Manju (1981) in horsegram. Higher reduction in germination with gamma rays treatment may be due to gross chromosomal breakage caused by it.

With regard to EMS treatment, no significant effect was seen to have been produced by the different doses either in the laboratory or in the field studies, which might be due to the sublethal nature of doses used. Jayanthi (1986) observed this effect in redgram after EMS treatment in the laboratory trials. Here no striking relationship is observable between the percentages of germination and the dose. However, all the treatments were inferior to control in studies conducted in the laboratory as well as in the field. A dose dependent decrease in germination with chemical mutagens was reported by Oommen (1980) in cowpea, Manju (1981) in horsegram and Mahna _et al_. (1989) in blackgram. In laboratory studies, mean germination percentage was higher in gamma rays treatment than the EMS treatment. Similar report was given by Mohamed Ali Khan _et al_. (1973) in redgram. The reduction in germination after EMS treatment may be due to the effects of toxic acids produced by hydrolysis of the chemical on the seeds.

5.2. Days taken to complete germination

Seeds treated with gamma rays were observed to take a much higher time interval for germination as compared to those treated with various concentrations of EMS. This is because of the dry nature of seeds treated with gamma rays unlike in the case of EMS, where the seeds have been scaked in distilled water followed by various concentrations of the aqueous solution of the chemical. An early germination was noticed at all the doses of gamma rays in comparison to the control in the laboratory experiment. Mujeeb (1974) reported earlier germination in gram at lower doses of gamma rays. This may be due to the influence of gamma rays on plant hormones and plant growth regulators which causes a reduction in the period taken for completion of germination.

However, the mean number of days taken by the seeds for completion of germination by various treatments of EMS and by various treatments of gamma rays in the field does not appear any bearing with dose. Various investigators have been reported that the duration of seed germination is not affected by low doses of mutagens (Sjodin, 1962; Wellensiek, 1965 and Ojomo and Chheda, 1971). Sjodin (1962) proposed that the first phase of germination is the swelling of cells by hydration followed by enzymatic action and metabolism, the energy for which will be already available in the seeds. This stage is unaffected by mutagens, leading to a lack of effectiveness of mutagens on the period of germination.

5.3. Seedling growth

Measurement of root length and shoot length especially in the early growth period can be considered as a true index of the effectiveness of mutagenic treatments in many plants. The presence of an electrostatic field or a toxin chemical has been reported to influence plant growth (Ehrenberg, 1960). Root length and shoot length at all the doses of gamma rays tried were reduced gradually with increase in dose, with a drastic reduction at higher doses compared with the control. From the root-shoot ratio it is clear that growth of shoot was more affected than root growth by gamma rays treatments. The results are in agreement with studies conducted by Kulkarni and Shivashankar (1978) in horsegram, Krishnaswami and Rathinam (1980) in greengram, Oommen (1980) in cowpea, Gomathinayagam and Rajasekharan (1981) in sorghum, Veeresh (1983) in winged bean and Mini (1989) in cowpea.

With the application of EMS, both root length and shoot length were reduced drastically at the highest dose. This is in agreement with the result of Vindhiya Varman *et al.* (1981) in *Vigna marina*. In other doses, a definite trend was not observed for any of the charaters. A significant increase in root length was noticed at 40 mM and 120 mM, and in shoot length at 120 mM and 160 mM. But 80 mM showed much reduction in root length in comparison to control. The root-shoot ratio shows that in general, growth of the root was more affected than that of the shoot in EMS treatments; however, at the highest dose of 200 mM, shoot growth was drastically affected than root growth in comparison to control. Higher growth inhibition for the root was reported by Oommen (1980) in cowpea, Manju (1981) in horsegram, Tyagi and Sharma (1981) in lentil, Bhamburkar and Bhalla (1985) in blackgram and Mini (1989) in cowpea. Gamma rays caused drastic reduction in both root length and shoot length. Control of EMS treatment showed higher values for both the characters in comparison to the control in gamma rays treatment. Presoaking of seeds in EMS treatment caused better initial growth in the laboratory than the dry seeds used for gamma rays treatment and this may be the reason for higher values for root length and shoot length in EMS treatment.

Reduction in seedling growth by the mutagens in M_1 generation has been attributed to physiological and biochemical disturbances in the seed and seedlings (Gordon, 1957) and inhibition of DNA synthesis (Mikaelsen, 1968). These effects were greatly pronounced in gamma rays treatment than that of EMS in the present study.

5.4. Survival of plants

Effectiveness of dose levels for a particular mutagen can be estimated based on survival of seedlings in M_1 generation. Gamma rays and EMS treatments showed not much variation in their mean values for survival percentage. At any time of growth of the plants, the survival percentage was found to decrease with increase in dose of gamma rays, except at 100 Gy treatment where an increase in survival was noticed. The enhanced survival was due to increased germination. Similar results have been obtained following seed irradiation by Louis and Kadambavanasundaram (1973b) in cowpea, Mohamed Ali Khan et al. (1973) in redgram, Choudhary and Fazlul Haque (1976) in soybean, Krishnaswami and Rathinam (1980) in greengram, Manju (1981) in horsegram, Thombre et al. (1981) in redgram and Veeresh (1983) in winged bean. Reduction in survival at higher doses may be due to radiation-caused physiological and cytological disturbances including chromosomal and extrachromosomal damage of cells (Sato and Gaul, 1967). It will lead to a failure of shoot elongation after initial growth. Gradual reduction in survival with the advancement of growth may be due to the prolonged lethal action of radiation leading to post-germination mortality.

Survival percentages at various levels of EMS showed values lower than that of the control at any growth period of plants. However, these values were neither in any definite trend nor with significant reduction from control. Reduction in survival following EMS treatment were obtained in barley and wheat (Swaminathan et al., 1962), pea (Heringa, 1964), cowpea (Narsinghani and Kumar, 1976), bengal gram (Vadivelu and Rathinam, 1980), winged bean (Savithramma, 1982) and redgram (Nadarajan et al., 1985). A report on M_1 plant survival was given by Zannone (1965) in *Vicia sativa* that survival was unaffected by various doses of EMS except the 0.2 per cent. Survival percentage was almost the same at all the different periods for each particular dose of EMS indicating that chemically induced lethality was mostly expressed through inhibition of germination and not by post-germination lethality.

5.5. Plant height

Height measured at all the different periods of plant growth showed a reduction with increase in dose of gamma rays except at 100 Gy where a slight stimulation of growth was noticed. This is in agreement with the results obtained by Nair (1971) in rice, Mohamed Ali Khan and Veeraswamy (1974) in redgram, Venkateswarlu et al. (1978) in pigeonpea, Vadivelu and Rathinam (1980) in bengalgram, Manju (1981) in horsegram, Nadarajan et al. (1985) in redgram and Subba Rao (1988) in Cicer. Height reduction was highest at 60^{th} day after sowing than later stages in case of gamma rays treatment. This indicates a recovery of plants from injury at later stages of growth. The recovery may be due to the elimination of damaged zones by inhibition of cell division and by development of uninjured meristems which replaced the injured ones as growth proceeded (Nair, 1971). Reduced growth in mutagen treated materials at higher doses was attributed to abnormal cytological behaviour due to chromosomal damage and mitotic inhibition (Sparrow et al., 1952). Gordon (1957) opined that reduction which induce physiological changes may involve a number of inter-related non-specific factors such as inhibition of DNA synthesis and variation in auxin level leading to a reduced growth of the treated plants. Mikaelsen (1968) reported inhibition of DNA synthesis as the cause of growth inhibition.

Treatment with EMS caused not much variation in plant height at any stage of plant growth. Lower doses showed a very slight increase in height. Increase in plant height following lower concentrations of EMS was observed in redgram by Chaturvedi and Sharma (1978b) and in winged bean by Savithramma (1982). Singh and Reghuvanshi (1991) reported mutants with increased plant height after a combined treatment of gamma rays and EMS in blackgram. Mutagens at lower concentrations may not be harmful and have some beneficial effects on plant growth leading to a stimulation of growth at their lower levels. Savithramma (1982) observed a non-significant effect of EMS at its higher concentrations on plant height of winged bean. The innate capacity of tissues for growth differs greatly. Lack of significant variation in height of EMS treated plants may be due to the resistance of the tissues to accept any change in the chemical composition at the time of treatment because of a successful competition of the biochemical reactions to the mutation yielding reactions inside the seeds. However, this needs further confirmation by subsequent studies. Among the two mutagens, gamma rays was found more effective in reducing plant height in the present study, which may be due to the more physiological and cytological damage caused by gamma rays in comparison to EMS.

5.6. Chlorophyll chimeras

Chlorophyll chimeras has been spotted in the leaves in all the different doses of gamma rays. This is in agreement with the report of Ojomo and Chheda (1971) and Louis and Kadambavanasundaram (1973a) in cowpea, Krishnaswamy et al. (1977) in greengram, Oommen (1980) in cowpea, Manju (1981) in horsegram and Mini (1989) in cowpea. Destruction of chlorophyll may be the reason for the occurance of chlorophyll deficient patches. There was variation in the nature and extent of patches, but these variations have no dose dependance.

In none of the EMS treated plants, chlorophyll chimeras were found. Similar reports were given in soybean (Kiang and Halloran, 1975) and in cowpea (Narsinghani and Kumar, 1976 and Mini, 1989). The difference in the appearance of chlorophyll chimeras in the two mutagen treatments may be due to the nature of the material subjected for the present study or to the limited population size. But this should be confirmed by subsequent studies.

5.7. Morphological variation

In the present investigation morphological variations are seen to be mostly confined to gamma rays treated population. Dwarf plants, plants with bifoliate and unifoliate leaves, plants with crinkled leaves or with enlarged size of one among the three leaflets of a leaf and leaves with round apex were present more commonly with the higher doses of gamma rays treatments. In both gamma rays and EMS treatments non-flowering and non-fruiting variants were also identified. Morphological variation including changed size, colour and the presence or absence of cracks and crinkles on the seedcoat surfaces of the seeds were also observed from various gamma rays treatments. No dose dependance of the mutagen is seen in the realization of plants showing these types of variations. Similar morphological variants were reported in the M_1 generation by Bhatt et al. (1972) in greengram, Louis and Kadambavanasundaram (1973a) and Oommen (1980) in cowpea, Patil and Bhalla (1985) in soybean, Fu Laiqing (1986) in soybean and Mini (1989) in cowpea after gamma irradiation. Klu et al. (1989) reported a non-flowering mutant in M_2 and some mutants with variations in seed size and seed coat colour in M_3 following seed irradiation of winged bean with gamma rays. Except some non-flowering and non-fruiting variants, no other morphological variations were observed with EMS treatment. These differences between gamma rays and EMS treatments may be due to the limited size of the population or to the nature of material investigated. These morphological variations may be attributed to chromosome breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism and accumulation of free aminoacids, as cited by Gunckel and Sparrow (1961). Dwarf plants may be produced as a result of the inactivation of respiratory enzymes by the higher doses of the mutagen.

5.8. Flowering

Days to first flowering in M_1 generation was delayed at higher doses of gamma rays and it was slightly earlier at lower doses. This is in agreement with the result of Veeresh (1983) in winged bean, Patil and Bhalla (1985) in soybean, Subba Rao (1988) in Cicer and Kothekar (1989) in moth bean in the M_1 generation. Higher doses of gamma rays may be lowering the rate of metabolic activities in the plants in their vegetative stage and thus leading to a prolonged vegetative phase and late flowering, and the reverse will be the case at lower doses. There was no appreciable change in the number of days to flowering in the EMS treatments. However in all the treatments, plants flowered slightly earlier than the control. Such variation may be owing to an absence of profuse and persistent vegetative growth and mitotic arrest in the flower primordia (Rajput, 1974) of treated plants in comparison to control. Similar report was given by Narsinghani and Kumar (1976) in cowpea. Mean days to flowering was almost the same in both gamma rays and EMS treatments.

Regarding the number of days till the last flowering, when gamma rays treated plants took a mean number of 148 days to complete flowering, EMS treated plants gave a mean value of 130 days from sowing to the last flowering. The exact reason for this is not known, but the higher beneficial effect of gamma rays in general, than EMS in plants can be considered as the reason for it. There was not much variation within the different doses of EMS.

Except the 400 Gy, all the other doses of gamma rays caused a slight later completion of flowering than control. This is in agreement with the observations of Nadarajan et al. (1983) in redgram, Kundu and Singh (1982) in blackgram and Sinha and Chowdhury (1991) in lentil. Except 160 mM all the other doses of EMS caused an earlier completion of flowering in comparison with control. In general, gamma rays treated plants showed a longer reproductive phase than the EMS treated ones. All such variations may be owing to changed metabolic rates in the plants due to the action of mutagens. However, this must be confirmed by further investigations.

5.9. Yield contributing characters

5.9.1. Number of pods per plant and fruit yield per plant

Gamma rays treatment caused a gradual reduction with increase in dose for both t characters. But 100 Gy dose showed a stimulatory effect for both the characters. Such stimulation at lower doses followed by gradual reduction with increase in dose at higher doses of ionizing radiations was reported by Louis and Kadambavanasundaram (1973a) in cowpea, Krishnaswamy et al. (1977) in greengram and Subba Rao (1988) in *Cicer*.

EMS showed no significant variation at any of its doses for both the characters. However at the highest two doses of 160 mM and 200 mM a decreasing trend was observed for both the characters compared to the control. This is in agreement with the results obtained by Heringa (1964) in pea, Ehrenberg et al. (1966) in barley, Mohamed Ali Khan and Veeraswamy (1974) in redgram, Rao and Reddy (1975) in cowpea, Narsinghani and Kumar (1976) in cowpea and Nadarajan et al. (1983) in redgram. Mean number of pods as well as mean fruit yield per plant in gamma rays treatment was nearly double than those in the EMS treatment. Comparatively lesser vegetative growth and longer reproductive phase may be the reason for these.

5.9.2. Length of pod and weight of pod

Both the characters showed a decreasing trend with increase in dose of gamma rays. Similar reports have been made by Louis and Kadambavanasundaram (1973b) in cowpea, Veeresh (1983) and Jugran et al. (1986) in winged bean and Mini (1989) in cowpea. A contradictory report was given by Shaikh and Majid (1982) in blackgram and Klu et al. (1989) in winged bean, where they could get larger pods from mutant plants than those in control.

By EMS treatment both length and weight of pods were increased in comparison to the control, but this increase was not statistically significant. Such increase was reported following EMS treatment in cowpea (Rao and Reddy, 1975 and Narsinghani and Kumar, 1976) and in winged bean (Savithramma, 1982). Mean length and mean weight of pod were higher in EMS treatment in comparison to gamma rays. Such increases in EMS treatment may be due to the lesser number of pods produced in them.

5.9.3. Number of seeds per pod and hundred seed weight

In general, both the characters reduced with increase in dosage of gamma rays. Reduction was very high at higher doses for the number of seeds per pod, where as for hundred seed weight, the reduction was not significant. This is in agreement with the observations of Swarup and Gill (1968) in french bean, Louis and Kadambavanasundaram (1973a) in cowpea, Choudhary and Fazlul Haque (1976) in soybean, Veeresh (1983) in winged bean and Mini (1989) in cowpea.

Number of seeds per pod and hundred seed weight were not affected by treatment with any of the doses of EMS. However at the highest dose of 200 mM a slight reduction was observed for both the characters. Such decrease with EMS treatment was observed by Narsinghani and Kumar (1976) and Mini (1989) in cowpea. But at lower doses a stimulatory effect was noticed for both the characters, which is in agreement with the result of Khan (1988) in mungbean. Savithramma (1982) reported variation both in positive and negative directions for number of seeds per pod and hundred seed weight after EMS treatment. Number of seeds per pod was significantly higher in EMS treatment compared to gamma rays treatment which may be due to the lesser number of pods produced by the plants of EMS treatment. But in hundred seed weight the treatment with neither gamma rays nor EMS showed significant variation.

Some of these yield contributing characters showed stimulatory effects at lower doses of gamma rays, the reason for which will be that ionizing radiations in small and appropriate doses may be useful in increasing crop yields in winged bean. But the higher doses of gamma rays cause a gradual decrease in many yield contributing factors with increase of dose, due to the delay in initiation of flowering, growth inhibition and reduced fertility.

Increase in vegetative growth during the later part of plant growth in the EMS treatment with higher doses may be causing a reduction in the values of the yield contributing factors. Stimulatory effect at lower doses of EMS is due to the beneficial effect of the mutagen on physiological and biochemical processes of the plant.

5.10. Pollen sterility and seed sterility

The effect on pollen sterility and seed sterility in M_1 generation has been considered as one of the prime factors to estimate the efficiency of mutagens. In general, pollen sterility and seed sterility were observed to increase with increasing dose of gamma rays. Higher doses showed very high sterility, but at the highest surviving dose of 400 Gy a slight decrease was noted in these values compared to 300 Gy treatment. Increase in pollen sterility and seed sterility with increase in dose of gamma rays was noted by Nerkar (1977) in *Lathyrus*, Gomathinayagam and Rajasekharan (1981) in sorghum, Veeresh (1983) in winged bean, Nadarajan *et al*. (1985) and Jayanthi (1986) in redgram and Mini (1989) in cowpea. Gaul *et al*. (1966) suggested that cryptic structural differences in chromosomes and chromosomal aberrations are the cause for M_1 sterility with radiations. Sparrow (1961) opined that abnormalities due to chromosomal aberrations, gene mutations, cytoplasmic mutations and physiological effects may lead to reduction in reproductive capacity, which may be expressed by severe stunting or inhibition of growth, formation of flowers lacking reproductive structures, aborted pollen grains or embryo, and reduced accumulation of food in developing seeds.

EMS also caused increase in pollen sterility and seed sterility with increase of dosage, but neither of the characters were statistically significant. Higher doses caused high sterility of the seeds. Observations in Lathyrus (Nerkar, 1977), Vigna marina (Vindhiya Varman et al., 1981), winged bean (Savithramma, 1982), redgram (Nadarajan et al., 1985 and Jayanthi, 1986) were also suggested an increase of pollen sterility and seed sterility values with increase in dosage of the mutagen. Several workers (Ehrenberg et al., 1961, 1966; Gaul et al., 1966; Sato and Gaul, 1967) reported that high sterility cannot be attributed to chromosomal aberrations but to other reasons including specific gene alteration and cryptic deficiencies.

Gamma rays is seen to be more effective in increasing pollen sterility in comparison to EMS. This inference is drawn from the fact that within the dose range of gamma rays and EMS included in the present study, increase in pollen sterility percentage is found to vary from 2.58 to 6.89 in the case of EMS and 5.86 to 53.12 in the case of gamma rays. Increased fertility of EMS treatment may be due to the fact that the plants which survived were those that had resisted the higher lethal effects of the chemical. Chemicals and gamma rays were equal in their capacity to induce chromosomal aberrations, but the aberrations caused by chemicals were largely eliminated during ontogeny (Akhun-Zade, 1977). This may be the reason for reduced sterility observed with EMS treatment than with gamma rays in the present investigation. However seed sterility percentage is not showing significant difference between that of gamma rays and EMS. Sterility induced by radiation is reported to be mostly haplontic, but a larger part of the EMS induced sterility is diplontic in nature (Gaul et al., 1966 : Sato and Gaul, 1967). This may be the reason for the appearance of more pollen sterility in gamma rays treatment, and comparatively high seed sterility in EMS treatment eventhough its pollen sterility is much less.

In the present investigation, it became clear that the dose range tried for gamma rays is capable of causing variability in seed germination, growth, survival, flowering, yield contributing factors, pollen sterility, chlorophyll variations and morphological abnormalities. But EMS was found to be not much effective at any of its doses tried; however in seedling growth it brought about a significant variation. Lesser variability caused by EMS treatment indicated a more uniform effect of EMS on the biological material resulting from the relative insensitivity of the action of modifying factors and absence of secondary physiological effects. Stimulatory effect was noticed at lower doses of gamma rays and EMS, which may be due to the reason that, under certain conditions the mutagens may even result in an increase in the physiologically active growth promoting substances, perhaps by the destruction of inhibitory substances (Casarett, 1968). The reasons for the variations caused by the mutagens used should be confirmed by further investigations. Many of the variations in the treated material may be heritable and so desirable genotypes can be selected by plant breeders to evolve new varieties, with added advantages along with the desirable characters of the parent variety.

SUMMARY

VI. SUMMARY

The present investigation was undertaken as the first step to obtain information on the extent of genetic variability that could be induced by gamma rays (100 to 500 Gy) and EMS (40 to 200 mM) in winged bean [Psophocarpus tetragonolobus (L.)] variety PT-62. The direct effects of different dc [illegible] MS were estimated with respect to the various growth and yield metrices in the M_1 generation.

The experiment was laid out during Rabi, 1992 in R.B.D. with three replications. Data were collected on seed germination, seedling growth, survival, plant height, chlorophyll and morphological variations, flowering, yield contributing factors, pollen sterility and seed sterility. The tabulated data were analysed statistically.

The germination percentage of seeds was reduced by gamma rays and EMS. But gamma rays caused stimulation of germination at all the doses in the laboratory trials and at the lowest dose in the field. In general, the effect was more severe with gamma rays than with EMS in the field study. Though the number of days taken to complete germination was not much affected by either of the mutagens in the field, gamma rays caused a slightly earlier germination of seeds in the laboratory study.

Seedling growth, ie. the growth of root and shoot drastically decreased by gamma rays. There was a linear reduction of growth with increase in dose of gamma rays. EMS reduced seedling growth drastically at the highest dose. Root-shoot ratios indicated that gamma rays causea more inhibition of shoot growth, where as EMS caused more inhibition of root growth.

Both the mutagens reduced the survival of plants, but EMS did not produce drastic effects. Gamma rays caused a decrease in plant survival with increase in dosage with a stimulatory effect at the lowest dose.

An inverse relationship was observed between plant height and dose of gamma rays with maximum reduction at the highest doses and a beneficial effect at the lowest dose. EMS brought about not much variation in plant height.

Chlorophyll chimeras and morphological variants were observed mostly in gamma rays treated populations than in EMS treated ones, however there was no dose dependance. These plants recovered to normalcy afterwards. Morphological variations mostly included alteration in number, size and shape of leaves, and size and colour of seeds.

Though days to first flowering was reduced by both the mutagens, the effect of EMS was not significant. Higher doses of gamma rays showed much delay in flowering. Days for completion of flowering was not much affected by both the mutagens. However, the gamma rays treated populations were found to be in the reproductive phase for longer period than EMS treated populations.

Yield contributing factors like the number of pods per plant and fruit yield per plant decreased gradually with increase in dose of gamma rays, though EMS did not cause much variation in these characters. Lower doses of both mutagens however, produced stimulatory effects. Weight and length of pod slightly increased in EMS treatment but decreased linearly with increase in dose of gamma rays treatment. Number of seeds per pod showed an inverse relationship with dose of gamma rays, while there was no significant effect for EMS. Both the mutagens exhibited more or less the same effect of control for hundred seed weight.

Greater pollen sterility was induced by gamma rays. Except the lowest dose of 100 Gy, all doses caused very high sterility. Effect of EMS on pollen sterility was not much pronounced. Seed sterility percentage was found to increase with the treatment of both the mutagens, however, the effects were not significant.

In general, gamma rays produced greater variability for morphological characters in winged bean in the M_1 generation than EMS. From the point of practical plant breeding these variations are only indicative of effective mutagenic action. The real scope of mutation breeding can be assessed only after the study of mutations in the M_2 generation.

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* Originals not seen.

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

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ABSTRACT

Seeds of winged bean [Psophocarpus tetragonolobus (L.)] variety PT-62 were subjected to induced mutagenesis using six doses of gamma rays (100 to 500 Gy) and six doses of EMS (40 to 200 mM) to obtain information on the extent of genetic variability that can be induced in the M_1 generation.

The germination percentage was observed to be decreased by both the mutagens, however the effect was more severe with gamma rays in the field study. But gamma rays caused a stimulation of germination and an earlier germination, at all the doses in the laboratory trials. Number of days taken to complete germination was not much affected by either of the mutagens in the field.

The growth of root and shoot were reduced by gamma rays linearly with increase of dose, and by EMS drastically at its highest dose, however gamma rays showed greater inhibition of shoot growth and EMS caused greater inhibition of root growth.

Survival percentage was observed to be reduced by both mutagens, but EMS was of not much effect. Gamma rays caused a stimulatory effect at its lowest dose.

Plant height was beneficially affected by gamma rays at the lowest dose and drastically reduced at higher doses. EMS caused not much variation in plant height.

Chlorophyll chimeras and morphological variations were mostly observed in gamma rays treated populations. Morphological variations mostly included alteration in number, size and shape of leaves and size and colour of seeds.

Earlier flowering was observed in lower doses of gamma rays, but higher doses caused delayed flowering. Days taken to first flowering was not affected by EMS treatments. Both mutagens exhibited not much effect on the number of days to last flowering. However gamma rays treated populations were in reproductive phase for longer period than EMS treated populations.

Number of pods per plant and fruit yield per plant were not affected by EMS treatment. But lower dose of gamma rays caused a stimulatory effect, and thereafter a gradual reduction with increase in dose. Weight and length of pod were slightly increased by EMS treatment, and decreased linearly with increase in dose of gamma rays treatment. EMS caused no significant effect on number of seeds per pod, whereas gamma rays showed an inverse relationship with dose. Both the mutagens showed not much variation in hundred seed weight.

Greater pollen sterility was induced by gamma rays treatments, but the effect of EMS was not pronounced. An insignificant increase in seed sterility was exhibited by both the mutagens.

In the present investigation greater variability for morphological characters was produced by gamma rays than EMS in the M_1 generation of winged bean.