BIOLOGICAL EFFECTS OF GAMMA RAYS AND EMS IN THE M_1 GENERATION OF RED GRAM (*Cajanus cajan* L.)

By

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THESIS

submitted in partial fulfilment of
the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "Biological effects of gamma rays and EMS in the M_1 generation of red gram (<u>Cajanus cajan</u> L.)" is a bonafide record of research work done by me during the course of research work and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara,
21-12-1985.

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JAYANTHI, S.

CERTIFICATE

Certified that this thesis entitled "Biological effects of gamma rays and EMS in the M_1 generation of red gram (<u>Cajanus cajan</u> L.)" is a record of research work done independently by Smt. Jayanthi. S., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Introduction

INTRODUCTION

It has long been known that induced mutations could be useful for the solution of specific problems where more conventional methods were insufficient. Recent experience and indeed success in plant breeding have clearly shown that mastering of mutation breeding techniques may become crucial to further success in the breeding of many crop species.

One of the major objectives of plant breeding is maximisation of crop production. It aims at an overall quantitative and qualitative improvement of crop varieties. In this process, breeding behaviour of individual crop with its inherent genetic structure decides the realisation of the breeder's objective. The extent of natural variability, if low, limits the scope of crop improvement by the conventional breeding techniques. In this respect, mutation as a means of artificially creating variability comes to the aid of plant breeders. It can be an alternative, complementary or unique solution in crop improvement. Mutation induction is a real and proven way to create variation within a crop variety and artificial mutagenesis offers a possibility for induction of desired attributes. However, it should not be the end of a plant breeder's efforts. Such artificial induction of variability can serve as the basis for further improvement and evolution of new varieties.

The discovery of enhancing the mutation frequency through mutation treatments in Drosophila melanogaster by Muller (1927) followed by the demonstration of the effectiveness of radiation in crop plants like maize and barley by Stadler (1928) led many plant scientists to adopt mutagenesis in crop improvement efforts. Since then, rapid advancements have resulted in the identification of a wide array of potent chemical mutagens having non-random effect and in the development of effective treatment techniques to make most of the induced mutations to find phenotypic expression. Ionising radiations like X-rays, gamma rays and fast neutrons still remain the most potent tools for inducing variability. Among the chemical mutagens tested widely, ethyl methane sulphorate (EMS) seems to possess many properties favourable to high mutagenic effectiveness as well as high mutagenic efficiency. Both physical and chemical mutagens are found to induce morphological and physiological effects such as reduced germination, lethality, seedling injury and sterility in the M_1 generation.

Pulses as a group are considered one of the most important source of protein to human nutrition. The breeding programmes undertaken in Indian pulses are far less, with the result the genetic variability in these crops has not been fully utilised. One of the main reasons for the stagnation in pulse production in our country is the non-availability of suitable varieties possessing high yield potential both in terms of grain yield and protein content as well as earliness and synchronised flowering. The varieties now cultivated often exhibit lack of one or more of these important attributes. Further, natural variability in the germplasm is found to be a limiting factor to evolve varieties of superior performance. Therefore, induction of mutation often becomes an auxillary source to create more variations that can be utilised in the improvement programme of pulse crops.

Red gram is an important pulse crop of peninsular India and among the various pulse crops, this is one of the most important ones from the point of view of per capita consumption. In India it occupies 2.5 million hectares yielding 1.8 million tonnes of grain and is the second important grain legume. Under the high risk, low management and subsistence semi-arid farming systems, pigeon pea is a widely preferred grain legume intercrop component due to its hardiness and slow early growth. Present yield level, which is very low, could marginally be improved and stabilised through better crop management. Increasing productivity to make the crop suitable for commercial cultivation is the matter of primary importance in pigeon pea breeding. Long period of growth and maturity of some of the existing cultivars limit breeding work to one generation per year. Photosensitivity of the varieties is another aspect which require immediate attention. In order to overcome these problems, the plant must be restructured both morphologically and physiologically. Mutation breeding has the recognised potential to bring about novel changes in plant structure and productivity.

Hence the present work was undertaken to fulfil the following objectives:

1. To investigate the direct effects produced by gamma rays and EMS on red gram.

2. To study the effects of different doses of gamma rays and EMS in the M_1 generation.

Review of Literature

REVIEW OF LITERATURE

In the following review, the mutation studies carried out have been restricted to pulses with special reference to red gram.

Effect of presoaking, concentration and duration of treatment in chemical mutagenesis

Ethyl methane sulphonate (EMS) is one of the most widely used chemical mutagens in crop improvement today. Many variables are involved in the interaction between the chemical and the cells of the embryo. Two important variables that should receive attention are the presoaking period and the duration of post washing after seed treatment. Presoaking of seeds in water permits better control of the rate of penetration of the mutagen while postwashing enables the elution of the remaining mutagen in the seed tissues and the hydraulitic products which may be toxic (Konzak *et al*., 1972).

Sivasubramaniam (1978) in groundnut noticed that presoaking would lead to gradual reduction in germination percentage with the increasing hours of presoaking. The effect of presoaking was more severe in the higher concentration of 60 mM which caused a drastic reduction in survival count compared to the control and other concentrations. Bhatnagar *et al*. (1982) in gram administrated the EMS doses in two durations (6 and 24 hours) of treatment, followed by presoaking for four hours in distilled water. The treatment of 24 hours duration proved lethal to germination. In 6 hours treatment the dose effect was observed where the germination percentage was much higher in lower concentration. Zakri *et al*. (1982) presoaked the soybean seeds for varying periods of 0, 6, 12 and 24 hours, treated with 0.2% EMS for 3 hours. According to them efficiency was enhanced with increasing periods of presoaking. This was evident when plant injury and sterility were taken as the criteria for biological damage. However when lethality was considered, a presoaking period was not necessary for efficiency to be increased.

Effect of mutagens in the M_1 generation

Both physical and chemical mutagens affect the normal biological organisation of an organism and this is expressed in different ways. These effects seen in the M_1 generation can be categorised as effects on (i) germination (ii) survival (iii) growth (iv) fertility

(v) chlorophyll chimeras and (vi) other morphological and developmental abnormalities.

(i) Germination of seeds

Enhancement of mutagen doses resulted in gradual reduction in germination percentage as observed by Willensiek (1965) in peas using gamma rays and EMS; in field beans Shirshov and Shain (1966) following gamma irradiation; by Sidorova et al. in pea using gamma rays; in phaseolus by Bajaj and Saetler (1970) following gamma ray treatment and by AliKhan et al. (1973) in Cajanus cajan with gamma rays and EMS. Dahiya (1973) reported that among the different gamma radiation treatments, 30 and 70 krad decreased germination of the treated mungbean seeds. He also found that the crop was relatively resistant to radiation treatments and a dose as high as 70 krad could be given without drastic effect on the viability of seeds. Louis and Kadambavanasundaram (1973a) could notice complete lethality beyond 60 krad and the LD_{50} was at 40 krad. Studies of Rajasekharan (1973) in dry seeds of black gram (Vigna mungo (L.) Hepper) with gamma rays showed that the LD_{50} for germination was between 110 and 115 krad. Ramaswamy (1973) in Co.1 black gram studied the effects of gamma rays, fast neutrous, EMS, MMS and DES and he noticed that germination was not affected in any of the treatments under controlled conditions, while under field conditions it was drastically reduced at higher doses. Similar results were also noticed under field conditions by Srivastava et al. (1973) in bengal gram using EMS and by Mohanasundaram (1974) in red gram following gamma ray and EMS treatments.

Mujeeb (1974) in bengal gram variety chhola revealed that gamma irradiation with 40 krad and above reduced the germination. Palaniswamy (1975) in cowpea noticed a negative relationship between gamma ray doses and germination percentage and the LD_{50} was found to lie between 40 and 50 krad. In Phaseolus vulgaris, Rubaihayo (1975) reported that when dryseeds were treated with gamma ray doses of 0 - 21 krad, germination percentage was significantly reduced by the 21 krad dose while other treatments indicated no significant difference from the control. In redgram a gradual reduction in germination with increasing doses of gamma rays and EMS was observed by Nadarajan (1976), Sivaswamy (1976) and Srinivasan (1977). Kulkarni and Shivasankar (1978) studied the effects of gamma rays (5 to 25 krad and EMS 0.6 - 1.5%) in horse gram and observed that the effect of employed doses of gamma rays was not drastic. Venkateswaralu et al. (1978) noticed that germination of the M_1 was severely reduced at higher doses (30 and 40 krad) of gamma rays in two red gram varieties.

Studies of Vadivelu (1979) in bengal gram showed that gamma rays to presoaked seeds resulted in higher reduction in germination compared to dry seeds. The LD_{50} was found to lie between 40 and 50 krad for gamma rays and 50 and 60 mM for EMS. When dry seeds of six varieties each of cowpea and pigeonpea were treated with gamma rays (20-80 KR) a reduction in germination was noticed in all cases by Chowdhury and Singh (1980). A progressive decrease in germination with increasing doses was noticed by Subramanian (1980) in Vigna following gamma irradiation; Venkateswaralu et al. (1980) in pigeonpea and by Khan (1981) in mungbean using gamma rays and EMS. Manju (1981) found that in horse gram seeds treated with gamma rays and EMS took more time for germination than the control. She also noticed that none of the seeds germinated following 0.75% and 0.9% EMS treatments. In red gram Premsarkar and Appadurai (1981) obtained the LD_{50} values as 20 and 15 krad when gamma rays was used and 30 and 40 mM when EMS was used. Al Rubeai and Godward (1982) observed that in french bean (Phaseolus vulgaris) seeds irradiated with six different doses from 2.5 krad to 30 krad gamma rays, germination was not affected even by the highest dose. Studies of Balaravi (1982) in red gram with gamma rays revealed that germination was low in all gamma ray treatments and the irradiation effect was drastic at doses above 25 KR. Chaturvedi *et al*. (1982) also reported that in red gram germination was decreased considerably with the enhancement of radiation dose and concentration of EMS. LD_{50} dose was observed as 20-25 krad with gamma rays and 0.3 - 0.4% with EMS.

These mutagens were found to have a stimulatory effect on germination rate in certain crops. Prasad and Das (1973) found that when dormant seeds of six varieties of *Lathyrus sativus* were treated with gamma ray doses 10 to 50 krad, germination was not affected in five varieties while in one variety germination was increased. With gamma irradiation germination in all treatments was earlier and the rate was higher than in the control as recorded by Khan & Hashim (1978) in green gram. Vadivelu (1979) in bengal gram reported that gamma rays of 10, 20, 30 krad to dry seeds, 10 krad to presoaked seeds and 10 mM concentration of EMS induced germination. In horse gram, Manju (1981) observed a higher germination percentage in gamma ray treatments (10 krad to 50 krad) and 0.15% EMS compared to the control.

(ii) Survival of plants

Reduced survival at higher doses of gamma rays was noted in field bean by Shirshov and Shain (1966). Teodoradze

(1966) noticed a decrease in survival with gamma irradiation in French bean and soyabean while 20 krad and above proved lethal for *Phaseolus*. In soybean 12 krad gave a survival of only 0.5 per cent. Jaranowski (1970) reported that when dryseeds of *Pisum arvense* were irradiated with gamma rays there was a sharp fall in survival rate above 25 krad though certain lines tolerated 50 krad. A preliminary study conducted at IARI (1971) showed that in pulses higher doses of gamma rays resulted in sharp decline in survival rate. The results suggested that 30 krad to 50 krad would be the optimum dose for the pulses studied.

Alikhan *et al.* (1973) studied the effect of physical and chemical mutagens in two varieties of red gram and reported a gradual reduction in survival with increasing doses of mutagens. Louis and Kadambavanasundaram (1973b) also reported the same effect in cowpea. However, the reduction was significantly lower only in the 40 and 50 krad treatments and the LD_{50} was found to be around 40 krad. Studies of Rajasekharan (1973) in dryseeds of black gram using gamma rays showed that the LD_{50} for survival ranged between 80 and 85 krad. A gradual reduction in survival rate with increasing doses of gamma rays and EMS was noticed by Ramaswamy (1973) in black gram and Mohanasundaram (1974) in red gram.

In *gymnopsis* 100 and 150 krad gamma ray treatments were found to be lethal by Yadhava and Chowdhury (1974). In cowpea plants, the LD_{50} for survival was found to lie between 40 and 50 krad by Palaniswamy (1975) whereas Constantin *et al.* (1976) noticed that in green house grown populations of soybean survival was unaffected by gamma irradiation doses below 70 krads. However in field it decreased with an increase in dose of both gamma rays and EMS. Similarly proportionate reduction in survival rate with increase in doses of physical and chemical mutagens has been observed by Nadarajan (1976) in red gram, in cowpea by Narasinghani and Kumar (1976); Sivaswamy (1976) in red gram; Krishnaswamy *et al.* (1977) in bengal gram; in red gram by Srinivasan (1977) and in horse gram by Kulkarni and Shiva Sankar (1978). Krishnaswamy *et al.* (1977) also observed the LD_{50} for survival as 100 krad when gamma rays was used.

In pigeonpea Venkateswarlu *et al.* (1978) studied the effects of gamma rays and noticed a drastic reduction (about 60-78%) in survival at 5 krad. Irradiation with 10 krad did not noticably reduce survival, but it was very poor at 30 and 40 krads. Brunner (1979) recommended that doses resulting in 50% survival in the M_1 generation was most effective in mutation breeding. Gamma irradiation to presoaked bengal gram seeds resulted in higher reduction in survival compared to dryseeds (Vadivelu, 1979). A negative relationship between mutagen dose and survival rate was also noticed by Khan (1981) in mungbean and in horse gram by Manju (1981). Premsarkar and Appadurai (1981) in pigeonpea reported that LD_{50} values for survival were attained with 20 and 15 krad when gamma rays was used and 30 and 40 mM concentration when EMS was used. In the combination treatments the half kill dose for survival was reached even at the low dose combination of 5 krad gamma ray + 20 mM EMS. Vinod and Singh (1981) observed when seeds of pigeonpea cultivar T-21 were irradiated with 10, 15 and 20 krad gamma rays, survival of seedlings was adversely affected by gamma irradiation, the same being quite severe in 20 krad gamma rays. Only 59% of the seedlings survived till maturity.

Balaravi (1982) noticed that survival of pigeonpea plants decreased with increasing doses of gamma rays. Chaturvedi et al. (1982) also noticed the same trend in plant survival in pigeonpea and they found that the half kill dose was between 20 and 25 krad with gamma rays and 0.2 to 0.3% with EMS. Krishnaswamy and Rathinam (1982) treated ten varieties of green gram seeds after 12 hours of presoaking with 20, 40, 60 and 80 mM of EMS for 4 hours. After 10 days no determinant to survival was noticed upto 40 mM. At 60 mM, survival was reduced in all the cultivars, the overall decrease being 7.9%. Filippetti and Depace (1983) in *Vicia faba*, used two doses of gamma rays (8 and 12 krad) and observed that both the doses produced survival rate below 50%. The higher dose induced lower survival.

(iii) Plant growth

Seedling growth was reduced by mutagen treatments as observed by Teretchenko (1966); Maslov and Stepanova (1967) in pea using gamma rays and by Narasinghani and Kumar (1969) in cowpea using EMS. In a study conducted at IARI (1971) it was noticed that in bengal gram the higher doses of gamma rays resulted in sharp decline in shoot growth. However, the root growth did not show any dose event relationship when compared to shoot growth. Ojomo and Chheda (1972) also obtained the same results in cowpea plants following gamma irradiation. Similarly a gradual reduction in seedling height with increasing doses of mutagens was noticed by Kwon and Im (1973) in soybean; in cowpea by Louis and Kadambavanasundaram (1973a) following gamma irradiation and in bengal gram by Srivastava *et al*. (1973) using EMS. In green gram gamma ray treated plants were shorter than the parents and those treated with 60 krad were the shortest. (Sreerangaswamy *et al*., 1973)

By increasing mutagen doses seedling height reduced proportionately in red gram (Alikhan and Veeraswamy, 1974 and Mohanasundaram, 1974). Studies of Mujeeb (1974) in bengal gram variety chhola revealed that gamma irradiation with 40 krad and above reduced seedling growth while the lower doses had stimulatory effect. A slight increase in plant growth at lower doses and gradual decrease with higher doses of gamma rays was reported by Rubaihayo (1975) in *Phaseolus vulgaris* and by Nadarajan (1976) in red gram. A decrease in seedling height with increase in dose of gamma rays was reported in black gram by Krishnaswamy *et al.* (1977); in pigeonpea by Venkateswaralu *et al.* (1978) and in *Phaseolus vulgaris* and *Phaseolus limensis* by Subramanian (1979). When dryseeds of mungbean were exposed to varying doses of Co^{60} gamma rays ranging from 10 to 40 krad, it was found that the shoot was more radiosensitive than the root (Khan and Hashim, 1978). Vadivelu (1979) reported that in bengal gram the reduction in seedling height on 30th day was proportionate to the increase in dosage of gamma rays, concentration of EMS and strength of combination. Reduction in height at maturity was dose dependant. Gamma irradiation to presoaked seeds and combination treatments induced greater reduction than gamma irradiation to dryseeds and EMS treatments. Vindhiyavaraman (1979) also obtained the same results in *Vigna marina* with regard to seedling height. But he noticed that the reduction in radicle and plumule length was more pronounced in EMS treatments than in gamma rays.

Venketeswaralu *et al.* (1980) studied the effects of gamma rays (5 to 40 krad) and EMS (0.02 to 0.06 mM) in two streams of pigeonpea and noticed that height was most affected at 30 and 40 krad of gamma rays and 0.04 and 0.06 mM EMS. Khan (1981) in mungbean and Khanna and Maherchandani (1981) in chickpea also confined the result obtained by many earlier scientists i.e. seedling height at maturity decreased linearly with increasing doses of mutagens. Manju (1981) noticed this dose-plant height relationship in all the cases except in 20 krad gamma rays and 0.03% EMS treatment which showed an increase in height. She also noticed a severe plant height reduction with EMS treatment than with gamma rays. In red gram Chaturvedi *et al.* (1982) also obtained the same results and they obtained the LD_{50} dosage with respect to seedling height as 30 krad and 0.2% - 0.3% respectively with gamma ray and EMS treatments.

Krishnaswamy and Rathinam (1982) treated ten green gram varieties with EMS (20, 40, 60 and 80 mM) after 12 hours of persoaking for 4 hours and noticed that all cultivars were tolerant to 20 mM concentration and not recorded any reduction in height. Double that concentration proved repressive to two cultivars out of ten. Rao and Rao (1983) studied the physiological variabilities in black gram due to gamma irradiation (10 to 60 krad) and observed that 20 krad induced increased seedling growth while 60 krad reduced it. Plant height, number of nodes and internodal length were found to be favourably induced in the lower doses while in the higher doses particularly in 50 and 60 krad there was a reduction.

(iv) Fertility

Reduction in fertility in M_1 plants is a reliable parameter for showing the effectiveness of mutagenic treatment as reported by Kivi (1962). Pollen and seed fertility was reduced in proportion to the dose of gamma rays as observed by Bankowska and Rymsza (1970) in *Phaseolus vulgaris* and by Kasprzyk (1970) in broad bean. Louis and Kadambavanasundaram (1973a) also obtained the same result in cowpea and also noticed the LD_{50} for pollen sterility as 25 krad. Rajasekharan (1973) revealed that in black gram variety Pusa-1, pollen fertility was maximum in the gamma irradiated population. In black gram variety Co.1 gamma rays induced high sterility while the chemical mutagens induced low sterility (Ramaswamy, 1973). Vo Hung (1973) found that in pea, pollen fertility was reduced by 15 krad gamma rays. In red gram Mohanasundaram (1974) observed a sudden reduction in pollen and seed fertility due to increasing doses of gamma rays and EMS while Alikhan and Veerasamy (1974), Sivaswamy (1974) and Nadarajan (1976) reported a gradual reduction.

In green gram the decrease in seed fertility was more with gamma rays as compared to EMS (Palaniswamy, 1975; Suresh, 1975).

Krishnaswamy *et al*. (1977) reported that in green gram seed fertility was reduced to 50% in gamma ray treatment. Srinivasan (1977) in his studies with gamma rays, EMS and their combination in red gram, observed the pollen and seed sterility increased markedly with increasing dose/concentration/strength of combination. The combination treatments produced less than additive effect on pollen and seed sterility in M_1 generation. Khan and Hashim (1978) exposed dry seeds of two varieties of mungbean (G-65 and PS-16) to varying doses of Co^{60} gamma rays ranging from 10 to 40 krad. They found that seed fertility in the variety G-65 showed stimulatory effect at 25, 30 and 35 krad. The other variety PS-16 did not show any response. In both the varieties pollen fertility decreased with increasing doses of radiation. Kulkarni and Shivashankar (1978) reported that in horse gram pollen fertility was 50% at 25 krad gamma rays and 73% at 1.5% EMS which has the highest dose employed in that study.

Venkateswaralu *et al*. (1978) reported that in pigeonpea gamma irradiated populations showed lower pollen fertility than the respective controls. Pollen fertility decreased with the increasing doses of gamma rays. Similar results were obtained by Vindhiyavarman in *Vigna marina* (1979) Fatimatesn and Hashim (1980) in mothbean (*Phaseolus acontifolius*); in *Vigna* by Subramanian (1980) and by Khan (1981) in mungbean. The percentage of pollen fertility decreased linearly with increase in doses of gamma rays and EMS except in 0.45% and 0.9% EMS treatments which showed an increase in fertility (Manju, 1981). The reduction was greater with gamma rays than with EMS treatment. Premsarkar and Appadurai (1981) noticed that in red gram higher doses resulted in lower pollen and seed fertility. The reduction in fertility was much enhanced in the combined treatments. Chaturvedi *et al*. (1982) noticed the LD_{50} in respect to pollen fertility as 20-25 krad in gamma ray treatments and 0.2 to 0.3% in EMS treatments. Samolo and Misra (1982) in green gram, Filipoetti and Depace (1983) in *Vicia faba* have also reported a progressive decrease in pollen fertility with increase in dose of these mutagens.

(v) Chlorophyll chimeras

In legumes in the M_1 generation, after mutagenic treatment with EMS, chlorophyll deficient spots were observed by Blixt and Gelin (1965) and they found a close correlation between leaf spotting and mutation rate. They advocated the use of it as a criterion for selecting in M_1 generation for plants which would give higher yield of mutations in the

M_2 generation. Mashanidze and Debelyi (1970) reported that in pea, selection of plants with chlorophyll free spots on the leaves in the M_1 after treatment with chemical mutagens and gamma rays, increased the probability of isolation of mutants in the M_2. Gohal et al. (1970) observed two chlorophyll deficient plants among M_1 plants from the seeds of Cyamopsis tetragonoloba treated with EMS. Kiang and Halloram (1975) observed no chlorophyll mutants in the M_1 generation of soybean with EMS treatment. They were of the opinion that this is to be expected because chlorophyll mutants are normally recessive and would be expressed only through segregation in the M_2 and later generations. Narasinghani and Kumar (1976) also could not spot chlorophyll mutants in the M_1 generation of cowpea plants. Brunner (1979) reported that when seeds of Vicia faba, Pisum sativum and Phaseolus vulgaris were treated with 0.3 - 1% EMS for 1 to 3 hours at 20°C following 8 to 16 hours presoaking followed by 4 to 8 hour post treatment washing, the percentage of chlorophyll and morphological mutation in the M_1 was 52, 49 and 43% respectively. Manju (1981) observed chlorophyll chimeras in the M_1 generation of gamma ray and EMS treated plants. The frequency of such chimeric plants was very low and the frequency was more with gamma rays than with EMS treatments. Chaturvedi et al. (1982) observed that there was a progressive decrease in chlorophyll deficient plants and other morphological abnormalities with the increase in dosage of mutagens and the LD_{50} values were 25 to 30 krad in gamma rays and 0.3 to 0.4% in EMS. They also observed that all the treatments produced less than 20% chlorophyll chimeras.

(vi) Morphological variations

Irradiated plants may show abnormalities in stems, buds, leaves, branches, flowers and fruits. The type of response depends upon the duration of exposure, age and condition of the plant and environment during and after exposure. Plants with vigorous growth were obtained in field bean by Shirshov and Shain (1966) with gamma ray treatments. Srivastava et al. (1973) noticed that by increasing EMS concentration from 0.125 to 0.375% resulted in reduced number of branches in bengal gram. In black gram Rajasekharan (1973) noted a reduction in number of pods in the mutagenic treatments though it did not show any dose relationship. In mungbean as a result of gamma irradiation and EMS treatment, Tikoo and Jain (1974) obtained plants with increased pod number ranging from 100-150 as compared with 25 to 55 in controls. In green gram Suresh (1975) reported that the number of branches per plant increased over the control with the increase in dosage of mutagens. Following a treatment with 0.7% EMS Mohan (1980) in *Phaseolus vulgaris* observed a mutant with dark green rough textured leaves with small epidermal projection, brittle steams and leaf petiols. Subramanian (1980) treated three species of *Vigna* seeds with 10, 20, 30 and 40 krad of gamma rays and he noted that the leaf abnormalities were found to be more in seedlings from the treatment with higher doses of gamma rays. The location of leaflets in *Vigna acontifolia* was very irregular and in certain cases only one or two leaflets were observed in a leaf. A striking morphological variation observed by Manju (1981) in horse gram was the presence of dwarf plants with higher doses of EMS. But dwarf plants were not seen in gamma irradiated M_1 population. Leaf variations such as alteration in the number, size and shape of leaflets were noticed in the first formed, secondary leaflets. These leaves lacked one or two lateral leaflets thereby appearing as bifoliate or unifoliate leaves instead of the normal trifoliate leaf. Filippetti and Pepace (1983) noted that in *Vicia faba* 8 krad dose was most effective in producing broad range of morphological variants although at low frequencies.

Materials and Methods

MATERIALS AND METHODS

The investigations reported herein on the "biological effects of gamma rays and EMS in the M_1 generation of red gram (*Cajanus cajan* L.)" were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the period 1983-'85.

A. Materials

Pure seeds of SA-1 variety of red gram obtained from the Director, School of Genetics, Tamil Nadu Agricultural University, Coimbatore were made use of in the study.

Gamma irradiation was done in the Co^{60} gamma chamber available at the Radiotracer Laboratory attached to the College of Horticulture, Vellanikkara. The source has a dose rate of 288 kR per hour.

The chemical mutagen used was ethyl methane sulphonate (EMS) having a molecular weight of 124.16. The chemical has a specific gravity of 1.18 at 20°C. It was obtained from Sisco Research Laboratories, Bombay.

B. Methods

I. Mutagen treatments

a) Gamma irradiation: Well dried uniform seeds of SA-1 variety of red gram with a moisture content of 10% were sorted out. Five samples of 400 seeds each were irradiated at five different doses of gamma rays viz., 10, 20, 30, 40 and 50 krads.

b) <u>Chemical treatments</u>:- In order to find out the optimum duration of presoaking of seeds, concentration of the chemical and duration of treatment, a preliminary laboratory test was conducted as detailed below.

Two durations of presoaking viz., 2 hours and 4 hours were tried with three concentrations of the chemical viz., 0.5%, 0.75% and 1.0% at two durations of treatment viz., 6 hours and 8 hours. Thus altogether there were twelve treatment combinations as listed below.

1.	2 hours	of presoaking with	0.5% EMS and	6 hours	of treatment.
2.		"	"	8 hours	"
3.		"	0.75% EMS and	6 hours	"
4.		"	"	8 hours	"
5.		"	1% EMS and	6 hours	"
6.		"	"	8 hours	"
7.	4 hours	"	0.5% EMS and	6 hours	"
8.		"	"	8 hours	"
9.		"	0.75% EMS and	6 hours	"
10.		"	"	8 hours	"
11.		"	1% EMS and	6 hours	"
12.		"	"	8 hours	"

Fifty uniform sized air dried seeds were treated as per the schedule given above with two replications. After the treatment, the seeds were washed thoroughly with water for an hour to remove the traces of the chemical from the seeds. Seeds were then kept in petridishes lined with moist filter paper for testing their germinability at room temperature. Germination counts were taken daily for 10 days and the percentages of germination were worked out. These values of germination were then transformed into angular sines and statistically analysed to find out the significance of the difference between the treatments. From the results obtained, the LD_{50} (The dose which gave 50% and above mortality) was found out by employing the method of probit analysis. Based on the results thus obtained, five doses of the chemical at regular intervals with LD_{50} as the highest dose were fixed as 0.3%, 0.4%, 0.5%, 0.6% and 0.7% for further trials.

Uniform sized seeds of SA-1 variety were carefully selected. Six samples of 200 seeds each were counted and presoaked for 2 hours and were treated with five different concentrations of EMS viz., 0.3%, 0.4%, 0.5%, 0.6% and 0.7% with one control treated with water for a duration of six hours. The solution of EMS at different concentrations was prepared with double distilled water without any buffer.

The seeds were treated with the chemical at the room temperature of 25 $\pm$ 1°C for the required duration of six hours. In order to maintain uniform concentration throughout the period of treatment, the solution with the seed was given intermittant shaking. After the period of treatment the seeds were throughly washed in the stream of running water for one hour to remove the traces of the chemical from the seeds.

II. Study of the M_1 generation

a) Laboratory studies

Samples of 20 seeds per dose in both the treatments along with the control were kept in petridishes lined with moist filter paper replicated four times and the following observations were recorded for ten days:-

1. Number of seeds germinated:- Counts of seeds germinated in petridishes were taken every day for ten days to estimate the percentage of germination.

2. Time taken for germination:- Germination counts were taken at intervals of 6 hours. The emergence of radicle was taken as the criterion for germination of seeds.

3. Length of primary root:- This was measured in cm every day.

4. Length of primary shoot: This was measured in cm every day, the measurement being recorded from the point of differenciation of root and shoot upto the base of cotyledon.

From the data obtained on the lengths of primary shoot and root, the shoot-root ratio was worked out utilising the respective values of relative percentages over control.

b) Field studies

Two field experiments were laid out, one with gamma irradiated seeds and another with EMS treated seeds.

(i) Gamma irradiated seeds:- A field experiment was laid out with an untreated control in a 6 x 4 Randomised Block Design. The treated seeds were sown on the same day of treatment. Fifty seeds were sown on the same day of treatment in a row at a spacing of 1 m between rows and 50 cm between plants in a row. The cultural, manurial and plant protection measures were done as per the Package of Practices Recommendations (1982) of the Kerala Agricultural University.

(ii) EMS treated seeds:- Another field experiment was laid out with EMS treated seeds along with a control in a 6 x 4 Randomised Block Design. Fifty seeds per treatment were sown in row adopting a spacing of 1 m between rows and 50 cm between plants in a row. The seeds were sown in the field on the same day after treatment. Cultural, manurial and plant protection measures were adopted as per the Package of Practices Recommendations (1982) of Kerala Agricultural University.

The following observations were recorded.

1. Germination of seeds:- Germination counts were taken in the field on the fifth, tenth and fifteenth day after sowing to estimate the percentage of total seeds germinated.

2. Survival of plants:- Survival counts were taken on the 15th and 30th day after sowing. Percentages of survival were worked out for the different treatments based on the number of seeds sown and the number of seedlings survived.

3. Height of seedlings:- Twenty seedlings selected at random per treatment per replication were marked and the seedling height was measured from the soil surface to the terminal bud. The mean plant height was estimated and expressed as percentage with respect to the control.

4. Pollen fertility:- This was studied based on the stainability with 1:1 glycerine acetocarmine. Study was confined to 20 plants selected at random per treatment per replication. In each case 15 microscopic fields or 500 grains were scored.

5. Chlorophyll chimeras:- The plants in the M_1 generation were examined for chimeric plants exhibiting chlorophyll deficient patches or sectors on their leaves.

6. Seed sterility:- Study was confined to 20 plants selected at random per treatment per replication.

7. Morphological variations:- M_1 plants were examined to locate plants with morphological variations such as dwarf plants, plants with alterations in number, size and shape of leaflets.

III. Statistical analysis

In the M_1 generation, the data were statistically analysed to find out the significance of difference between the treatment groups and control, employing the method of analysis of variance. In the case of germination, survival, pollen and seed fertility, the percentage values were transformed into angular sines and subjected to statistical analysis. The data on germination under laboratory conditions were tested for their significance by χ^2 method.

Results

RESULTS

Results of observations on the "Biological effects of gamma rays and EMS on M_1 generation of red gram", based on the study conducted during 1983-'85 are presented in this chapter. The data collected from the M_1 generation were subjected to suitable analyses and the mean values are presented in Tables 1 to 14. Values on the corresponding analyses of variance are presented in Appendices I to VII. In the case of germination, survival, pollen and seed fertility, the percentage values were transformed into angular sines and subjected to statistical analyses. In those cases, the original mean values are given in brackets in the respective tables.

Preliminary laboratory test

The data on the effect of presoaking of seeds, concentration of EMS and duration of treatment on germination of seeds obtained in the preliminary laboratory test are presented in Table 1 and the corresponding Anova in Table 2.

(TABLES 1 & 2)

The results presented in the above tables have indicated the following: The two durations of presoaking viz., 2 hours and 4 hours tried in the present case did not

Table 1. Effect of presoaking of seeds, concentration of EMS and duration of treatment on germination of seeds (Preliminary laboratory test)

	Treatments					Germination (in %)
1.	2 hr presoaking	+ 0.5%	EMS	+ 6 hr	treatment	89.0
2.	" "	+ "	"	+ 8 hr	"	93.0
3.	" "	+ 0.75%	"	+ 6 hr	"	85.0
4.	" "	+ "	"	+ 8 hr	"	57.0
5.	" "	+ 1%	"	+ 6 hr	"	6.0
6.	" "	+ "	"	+ 8 hr	"	2.0
7.	4 hr "	+ 0.5%	"	+ 6 hr	"	84.0
8.	" "	+ "	"	+ 8 hr	"	93.0
9.	" "	+ 0.75%	"	+ 6 hr	"	67.0
10.	" "	+ "	"	+ 8 hr	"	25.0
11.	" "	+ 1%	"	+ 6 hr	"	2.0
12.	" "	+ "	"	+ 8 hr	"	2.0

Table 2. ANOVA table of preliminary laboratory test of 2 x 3 x 2 CRD (Factorial design)

Source	Degrees of freedom	Sum of squares	Mean squares	F value
Presoaking hours	1	237.38	237.38	3.66
Concentration of EMS	2	16924.34	8462.17	64.77*
Presoaking hours x concentration of EMS	2	344.02	172.01	2.66
Duration of treatment	1	266.39	266.39	4.11
Presoaking hour x duration of treatment	1	1.41	1.41	2.12
Concentration of EMS x duration of treatments	2	841.57	420.79	6.49*
Presoaking hour x concentration of EMS x duration of treatment	2	35.67	17.83	0.28
Error	12	777.25	64.77	
Total	23	19428.03		

* Significant at 1% level

differ significantly. Same was the case with reference to the two durations of treatments of the chemical viz., six hours and eight hours. However, the three concentrations of the chemical tried in the present study viz., 0.5%, 0.75% and 1% produced significant differences on the germination of seeds. As is seen from the results, an increase in the concentration of the chemical is bringing about a corresponding reduction. When the treatment involving a concentration of 0.5% of the chemical gives 91% germination of seeds, the corresponding values for 0.75% and 1% are seen to be 58.5% and 3% respectively. Among the different interactions, only concentration of EMS x duration of treatment is seen to have significant influence on seed germination.

Based on the results of preliminary laboratory test, field trials were conducted. In all these field trials seeds were persoaked for two hours and were treated with the chemical for six hours since there was no significant difference between 2 hour presoaking and 4 hour presoaking and also between 6 hour treatment and 8 hour treatment. However, the three concentrations of the chemical tried in the preliminary laboratory studies viz., 0.5%, 0.75% and 1% differed significantly on their effects on seed germination. On probit analysis of the data on germination, the LD_{50} (the dose at which there was 50% and above mortality) was calculated as 0.7%. Five concentrations at regular intervals with LD_{50} as the highest viz., 0.7%, 0.6%, 0.5%, 0.4% and 0.3% were tried in the field trials.

As programmed the seeds kept for germination in the preliminary laboratory test could not be observed for 30 days for want of asceptic conditions.

<u>Effect of mutagens on the M_1 generation</u>

1. <u>Germination of seeds</u>

Observations on the effects of mutagens on the germination of seeds and the mean time taken for germination are given in Table 3.

(TABLE 3)

From the results presented in the above table, it is seen that the treatment differences are not significant either for germination of seeds or for mean period of germination. This is the case for both gamma rays and EMS.

In the case of gamma irradiation, the germination percentage of seeds does not seem to have any regularity with dose. Among the five doses tried the germination values are found to vary from 96.3% in 20 krad to 87.5% in 30 and 40 krads. The lower doses of gamma irradiation viz., 10 and 20 krads are seen to have a stimulatory effect

Table 3. Effect of mutagens on the total seed germination (Laboratory conditions)

Treatments	Germination percentage	Relative percentage over control	Period of germination	
			Mean period in hours	Relative percentage over control
Gamma irradiation dose				
Control	87.5	100.0	49.88	100.0
10 krad	92.5	105.7	49.54	99.3
20 krad	96.3	110.0	49.24	98.7
30 krad	87.5	100.0	51.08	102.4
40 krad	87.5	100.0	51.77	103.8
50 krad	92.5	105.7	53.18	106.6
Significance	NS		NS	
S.E.			0.71	
EMS				
Control	91.3	100.0	15.24	100.0
0.3%	96.3	105.5	10.83	71.1
0.4%	92.5	101.4	14.35	94.2
0.5%	88.8	97.3	16.14	105.9
0.6%	81.3	89.0	17.81	116.9
0.7%	77.5	84.9	24.00	157.5
Significance	NS		NS	
S.E.			1.14	

on seed germination. The mean period taken for germination of seeds is seen to vary from 49.24 hours in the treatment with 20 krads to 53.18 hours in the treatment with 50 krads. With reference to time taken for germination, it can be seen that it is increasing along with the increase in doses of gamma irradiation.

In the case of seeds treated with EMS, the percentage of germination is seen to vary from 96.3 in the treatment with 0.3% solution to 77.5 in the treatment with 0.7% solution. It is also seen that the decrease in the percentage of germination is proportional to the increase in the concentration of the chemical. The time taken by the seeds for germination is found to vary from 10.83 hours in the treatment with 0.3% solution to 24 hours in the treatment with 0.7% solution and the increase in the time taken for germination is seen to be proportional to the increase in the concentration of the chemical. It is also specifically noticeable that lower doses of EMS are seem to have a stimulatory effect on germination of seeds.

A comparison of the effects of different doses of gamma irradiation and EMS tried in this case, on total seed germination has indicated that both the mutagens have almost the same effects in reducing the percentage of germination. However, seeds treated with gamma rays are observed to take a longer period for germination as compared to those treated with EMS.

The data on the effects of mutagens on the percentage of total seed germination under field conditions are presented in Table 4.

(TABLE 4)

The results presented in the above table have indicated that in the case of gamma irradiation, the percentage of germination does not appear to have any relationship with the dose, either on the 5th day or on the 10th day or on the 15th day. On the 5th day percentage of germination is found to be decreasing from 10 krads to 30 krads after which it is increasing in the 40 krad dose following a rapid reduction in the 50 krad dose. The same is the case on the 10th and 15th days. It is also seen that commencing from the 5th day, germination is found to reach its maximum on the 10th day after which no further increase is seen.

In the case of EMS the percentage of germination is seen to be inversely proportional to the concentration of the chemical. Commencing from the 5th day, germination is seen to be increasing on the 10th day and from there again increasing on the 15th day.

Table 4. Effect of mutagens on the percentage of total seed germination
(Field conditions)

Period	Control	Gamma irradiation dose in krad					Control	EMS - dose in %				
		10	20	30	40	50		0.3	0.4	0.5	0.6	0.7
5th day	27.0	35.5	29.5	27.5	43.5	10.5	65.5	50.5	55.0	51.5	46.5	23.5
10th day	75.0	80.0	60.5	69.5	56.0	44.0	80.5	62.5	61.0	51.5	46.5	29.0
15th day	75.0	80.0	60.5	69.5	56.0	44.0	82.0	69.5	65.5	55.0	52.0	33.0

A comparison of the two mutagens based on their effects on the percentage of total seed germination under field condition has shown the following. On the 5th day, seeds treated with gamma rays have exhibited a lower percentage of germination as compared to those treated with EMS. However, this situation ~~is~~ seems to be reversed, on the 10th and 15th days. Again gamma ray treated seeds are seen to attain maximum germination on the 10th day while those treated with EMS reach the maximum only on the 15th day.

Observations on the germination percentages of seeds on the 15th day after sowing under field conditions are transformed into angular sines. They are then expressed as relative percentage over control. These values along with the mean germination percentage in brackets are presented in Table 5.

(TABLE 5)

As is seen from the above table, the treatment differences are significant both for gamma irradiation and EMS.

With regard to gamma irradiation, the seeds subjected to 10 krad dose have exhibited highest germination which is on par with that in the control. Germination value in the control is also observed to be on par with that of seeds

Table 5. Effect of mutagens on germination percentage with respect to the control on the 15th day (Field conditions)

Treatments	Germination percentage		Relative percentage over control
<u>Gamma irradiation dose</u>			
Control	60.00	(75.0)	100.0
10 krad	63.43	(80.0)	106.7
20 krad	51.35	(60.5)	80.7
30 krad	56.17	(69.5)	92.7
40 krad	48.44	(56.0)	74.7
50 krad	41.55	(44.0)	58.7
Significance	Significant at 1% level		
SE	1.71		
CD	5.15		
<u>EMS</u>			
Control	64.89	(82.0)	100.0
0.3%	56.48	(69.5)	84.8
0.4%	54.02	(65.5)	80.0
0.5%	47.87	(55.0)	67.1
0.6%	46.15	(52.0)	63.4
0.7%	35.06	(33.0)	40.2
Significance	Significant at 1% level		
SE	2.56		
CD	7.73		

in the 40 krad dose. Seeds which have received 50 krad dose have recorded the lowest germination value. Thus there seems to be no direct relationship between dose and germination value.

With regard to EMS, maximum germination is noticable in the control which is significantly higher to all other treatments. This is followed by the dose 0.3% which has exhibited higher germination over the rest. However, it is on par with its nearest higher dose, 0.4% which is also seen to be on par with its immediate higher dose 0.5% which again is observed to be on par with its immediate higher dose 0.6%. The dose 0.7% has recorded the lowest germination value among the different concentrations of EMS tried. It is specially noticed that in the case of EMS the germination values are inversely proportional to its concentration.

2. Survival of plants

Data on the effect of mutagens on the number of seedlings survived under laboratory conditions for a period of 10 days are presented in Table 6.

(TABLE 6)

Results presented in the above table have revealed that in the case of gamma irradiation maximum germination is

Table 6. Effect of mutagens on number of seedlings survived (Laboratory conditions)

Treatments	No.of seeds kept for germination	Growth periods (in days)									
		1	2	3	4	5	6	7	8	9	10
Gamma irradiation dose											
Control	80	1	70	70	70	70	64	64	60	60	-
10 krad	80	7	74	74	72	70	68	58	58	58	-
20 krad	80	6	77	77	77	74	63	60	60	57	-
30 krad	80	-	70	70	70	68	64	58	58	57	-
40 krad	80	-	70	70	68	65	61	57	56	55	-
50 krad	80	-	74	74	73	70	66	59	56	54	-
EMS											
Control	80	4	67	73	73	72	68	67	65	65	-
0.3%	80	21	77	77	77	71	66	64	63	60	-
0.4%	80	7	71	74	74	72	68	62	60	55	-
0.5%	80	4	70	71	71	66	59	57	57	55	-
0.6%	80	5	62	65	65	63	62	55	51	-	-
0.7%	80	1	56	62	62	61	60	55	45	-	-

seen to have achieved on the 2nd day after keeping the seeds for germination. This is the case with respect to control also. From the second day to the third day no reduction in the survival of the seedlings is seen in any of the treatments including the control. A slight reduction in the survival of seedlings is seen thereafter in the treatments with 10 krads, 40 krads and 50 krads as indicated by lower values on the fourth day. This reduction in the survival of seedlings is uniformly seen not only in the treated seeds but also in the control from the 6th day till the 9th day.

In the case of EMS, maximum germination is seen to have achieved on the 3rd day after keeping the seeds for germination. No reduction in the survival of seedlings is observed from the 3rd day to the 4th day. However, from the 5th day onwards reduction in the survival of seedlings is observed not only in the treated seeds but also in the untreated control till the 9th day.

The data pertaining to the percentage of plants survived on the 9th day and also the relative percentage over control are given in Table 7.

(TABLE 7)

As is seen from the results presented above, the different doses of the mutagens tried have not produced any

Table 7. Effect of mutagens on the survival of plants (in %) on the last day. (Laboratory conditions)

Treatments	Survival percentage	Relative percentage over control
Gamma irradiation dose		
Control	75.0	100.0
10 krad	72.5	96.6
20 krad	71.3	95.0
30 krad	71.3	95.0
40 krad	68.8	91.7
50 krad	67.5	90.0
Significance	NS	
S.E.	2.74	
EMS		
Control	81.3	100.0
0.3%	75.0	92.3
0.4%	68.8	84.6
0.5%	68.8	84.6
0.6%	63.8	78.5
0.7%	56.3	69.2
Significance	NS	
S.E.	2.29	

significant effect on the survival of plants expressed as percentage. This is true not only with reference to gamma irradiation but also to EMS. Both the mutagens have reduced the percentage of survival of plants as compared to the control. In both the cases the reduction is seen to be directly proportional to the dose. When the range in the survival percentage of the seedlings among the various gamma ray treatments is from 72.5 to 67.5, the same for the various concentrations of EMS is observed to be from 75.0 to 56.3.

Observations on the percentage of survival of plants on the 30th day under field conditions were transformed into angular sines. The relative percentages over the control of survival and lethality were then worked out. These values along with the actual survival percentages in brackets are presented in Table 8.

(TABLE 8)

As indicated by the figures presented in the above table, the treatment effects have produced significant differences in the survival percentages both in case of gamma irradiation and EMS. Both mutagens are also seem to be capable of reducing the survival percentages as indicated by the highest survival values in the untreated controls.

Table 8. Effect of mutagens on the survival of plants on the 30th day (Field conditions)

Treatments	Survival percentage		Relative percentage over control	
			Survival	Lethality
Gamma irradiation dose				
Control	61.34	(77.0)	100.0	0.0
10 krad	57.10	(70.5)	91.6	8.4
20 krad	48.16	(55.5)	72.1	27.9
30 krad	51.94	(62.0)	80.5	19.5
40 krad	50.18	(59.0)	76.6	23.4
50 krad	38.94	(39.5)	51.3	48.7
Significance	Significant at 1% level			
SE	1.68			
CD	5.05			
EMS				
Control	64.89	(82.0)	100.0	0.0
0.3%	54.94	(67.0)	81.7	18.3
0.4%	51.06	(60.5)	73.8	26.2
0.5%	46.15	(52.0)	63.4	36.6
0.6%	42.99	(46.5)	56.7	43.3
0.7%	33.52	(30.5)	37.2	62.8
Significance	Significant at 1% level			
SE	2.89			
CD	8.73			

In the case of gamma irradiation, the control and the lowest dose 10 krad are observed to be on par with each other. This is followed by the doses 30 krads, 40 krads and 20 krads in that order which are also seen to be on par with one another and significantly inferior to the dose 10 krad but significantly superior to the dose of 50 krad which has recorded the minimum survival value.

In the case of EMS, the survival values are seen to be inversely proportional to the dose. The doses 0.3% and 0.4% are observed to be on par which is significantly inferior to control but superior to the dose 0.5% which is also on par with the dose 0.6%. The highest dose 0.7% is seen to be significantly inferior to all the rest in terms of survival values.

3. <u>Plant growth</u>

Observations relating to the effect of mutagens on root elongation under laboratory conditions are given in Table 9.

(TABLE 9)

From the results presented above, it is seen that root growth commences within one day after the seeds are kept for germination and is continuously increasing till the 8th day i.e. the entire period during which the materials

Table 9. Effect of mutagens on root elongation in cm (Laboratory conditions).

Treatments	Growth period (in days)							
	1	2	3	4	5	6	7	8
<u>Gamma irradiation dose</u>								
Control	0.3	1.7	2.8	4.7	7.1	9.1	9.7	10.0
10 krad	0.2	1.1	2.7	4.4	6.0	7.3	8.8	8.9
20 krad	0.2	1.3	2.9	3.9	5.0	5.6	5.9	6.3
30 krad	0.0	1.0	2.6	3.8	5.3	6.5	6.9	7.0
40 krad	0.0	0.9	2.7	2.9	4.1	4.1	4.8	5.2
50 krad	0.0	1.0	2.4	3.1	3.4	3.7	3.9	4.0
<u>EMS</u>								
Control	1.5	2.9	5.5	6.5	9.5	9.6	10.1	10.2
0.3%	0.8	2.3	3.3	4.7	6.2	7.1	7.5	7.5
0.4%	0.9	1.8	3.5	3.9	5.7	6.8	7.2	7.2
0.5%	0.8	1.6	2.9	3.4	5.2	5.9	6.5	6.7
0.6%	0.8	1.5	2.6	3.3	4.9	5.2	6.0	6.2
0.7%	0.7	1.2	2.1	2.5	3.5	4.8	5.6	5.7

have been kept under observation. It is also seen that both gamma irradiation and EMS have an inhibitory effect on root elongation since the untreated controls have exhibited greater elongation. In general it can be said that the rate of suppression of root elongation is directly proportional to the increasing dose of the mutagen. This is the case for not only gamma irradiation but also for EMS. Among the various doses of gamma irradiation tried, the root elongation is found to vary from 8.9 cm to 4.0 cm. The corresponding range for the different doses of EMS is observed to be from 7.5 cm to 5.7 cm. Both gamma rays and EMS are seen to have approximately the same effect in suppressing root elongation.

Results of observations on the effect of mutagens on the shoot elongation under laboratory conditions are presented in Table 10.

(TABLE 10)

As is seen from the above table, shoot growth commences only on the third day after the seeds are kept for germination. This constantly increases till the 8th day after which the materials could not be further observed for want of aseptic conditions. In the case of both the mutagens tried, the untreated controls have registered

Table 10. Effect of mutagens on shoot elongation in cm (Laboratory conditions)

Treatments	Growth period (in days)							
	1	2	3	4	5	6	7	8
Gamma irradiation dose								
Control	-	-	0.5	1.5	2.1	5.8	6.0	6.7
10 krad	-	-	0.5	1.5	2.0	3.8	4.2	5.0
20 krad	-	-	0.4	1.5	2.2	3.6	4.1	4.5
30 krad	-	-	0.5	1.1	2.6	3.5	4.0	4.0
40 krad	-	-	0.8	1.4	2.1	2.5	3.0	3.4
50 krad	-	-	-	0.2	1.9	2.3	2.8	3.2
EMS								
Control	-	-	1.0	1.6	3.1	4.8	5.9	7.2
0.3%	-	-	0.5	0.7	2.4	3.5	4.3	5.4
0.4%	-	-	0.9	1.0	2.6	3.9	4.0	5.1
0.5%	-	-	0.6	1.2	2.8	3.0	3.7	4.4
0.6%	-	-	0.7	1.1	2.2	2.7	3.5	4.2
0.7%	-	-	0.5	0.7	2.0	2.8	3.2	4.1

maximum amount of shoot elongation thereby indicating the suppressing effect of both the mutagens in shoot elongation. Among the various doses of gamma irradiation and EMS tried, the rate of suppression of shoot elongation is observed to be directly proportional to the increase in dose of the mutagen. When the range of shoot growth on the 8th day in the various doses of gamma irradiation tried, is from 5.0 cm to 3.2 cm, the same in the case of EMS is from 5.4 cm to 4.1 cm. Thus it can be said that both the mutagens have approximately equal effect in suppressing shoot growth.

Utilising the values of root and shoot elongation of 8th day, shoot/root ratio was computed. These values along with the relative percentages of root and shoot lengths over the respective controls are presented in Table 11.

(TABLE 11)

The results presented in the above table have indicated the following: The treatment effects have produced significant differences not only in the case of root length but also in the case of shoot length. This is found to be the same in the case of gamma irradiation and also EMS. Each dose tried is observed to be significantly different from every other dose tried of both the mutagens in respect of shoot as well as root elongation except in the case of the dose 0.6% EMS which is seen to be on par with the dose 0.7% of EMS in the case of shoot elongation.

Among the five doses of gamma irradiation tried, the shoot/root ratio is found to vary from 1.19 to 0.83. The doses 20 krad and 50 krad as compared to control inhibit root growth more as compared to shoot growth since these treatments have recorded shoot/root ratio of over one. In the other doses, suppression of shoot growth is seen to more than the root growth, as compared to the control, since these treatments have produced shoot/root ratio of less than one.

Among the five doses of EMS tried the shoot/root ratio is found to vary from 1.02 to 0.93. The doses was 0.3 and 0.7 per cent as compared to control inhibit root growth more as compared to shoot growth since these treatments have recorded shoot/root ratio of over one. In the other doses, suppression of shoot growth is seen to more than the root growth, as compared to the control, since these treatments have produced shoot/root ratio of less than one.

Observations on plant height measured on 15th and 30th day after sowing of seeds treated with different doses

Table 11. Effect of mutagens on plant growth under laboratory conditions.

Treatments	Root length in cm	Relative percentage over control	Shoot length in cm	Relative percentage over control	Shoot/ root ratio
<u>Gamma irradiation dose</u>					
Control	10.0	100.0	6.7	100.0	1.00
10 krad	8.9	89.6	5.0	74.6	0.83
20 krad	6.3	62.4	4.5	67.2	1.08
30 krad	7.0	69.9	4.0	59.7	0.85
40 krad	5.2	52.1	3.4	50.7	0.97
50 krad	4.0	40.3	3.2	47.8	1.19
Significance	Significant at 1% level		Significant at 1% level		
SE	0.21		0.11		
CD	0.62		0.16		
<u>EMS</u>					
Control	10.2	100.0	7.2	100.0	1.00
0.3%	7.5	73.7	5.4	75.2	1.02
0.4%	7.2	71.3	5.1	70.8	0.99
0.5%	6.7	65.8	4.4	61.2	0.93
0.6%	6.2	60.7	4.2	58.0	0.96
0.7%	5.7	55.7	4.1	56.4	1.01
Significance	Significant at 1% level		Significant at 1% level		
SE	0.36		0.32		
CD	0.12		0.11		

of gamma rays and EMS are presented in Table 12 along with the relative percentages over control.

(TABLE 12)

The results presented in the above table reveal that both the mutagens are found to reduce plant height as indicated by the higher values in the respective untreated controls. The reduction in plant height is observed to be directly proportional to the increase in dose of the mutagen. This is true not only with reference to gamma irradiation but also with EMS.

Plant height on the 15th day is found to range from 15.6 in 10 krad to 12.3 in 50 krad of gamma irradiation. The corresponding figures for EMS are seen to be from 12.7 for the dose 0.3% to 4.8 for the dose 0.7%.

The results have also indicated significant differences in plant height on the 30th day among the various doses of the mutagens tried. Among the doses of the two mutagens tried, the reduction in plant height on the 30th day is seen to be directly proportional to the increase in dose.

In the case of gamma irradiation, the range in plant height on the 30th day is observed to be 58.8 for the dose 10 krad to 45.3 for the dose 50 krad, which

Table 12. Effect of mutagens on plant growth under field conditions

Treatments	Plant height in cm on				Height reduction on 30th day (injury %)
	15th day	Relative percentage over control	30th day	Relative percentage over control	
Gamma irradiation dose					
Control	16.1	100.0	61.9	100.0	0.0
10 krad	15.6	96.9	58.8	94.9	5.1
20 krad	14.8	91.9	54.0	87.2	12.8
30 krad	13.3	82.9	50.6	81.6	18.4
40 krad	12.6	78.3	46.7	75.4	24.5
50 krad	12.3	76.5	45.3	73.1	26.9
Significance	-		Significant at 1% level		
SE	-		0.79		
CD	-		2.38		
EMS					
Control	15.1	100.0	54.0	100.0	0.0
0.3%	12.7	83.7	42.3	78.2	21.8
0.4%	11.0	72.8	36.3	67.2	32.8
0.5%	9.0	59.7	33.7	62.3	37.7
0.6%	7.5	49.6	31.7	58.6	41.4
0.7%	4.8	31.8	29.5	54.5	45.5
Significance	-		Significant at 1% level		
SE	-		1.51		
CD	-		4.55		

is seen to be on par with the dose 40 krad. All the rest are seen to be significantly different from one another.

In the case of EMS variation in plant height on the 30th day is seen to be 32.3 for the dose 0.3% to 29.5 for the dose 0.7%. The doses 0.4% and 0.5% are observed to be on par while doses 0.5%, 0.6% and 0.7% are also seen to be on par.

4. Pollen fertility

Percentages of pollen fertility in the plants of the different treatments of both the mutagens were transformed into angular sines. The relative percentages over the control were then computed. The above values along with the pollen fertility percentages in brackets are presented in Table 13.

(TABLE 13)

As is seen from the table above, the treatment effects have produced significant differences in pollen fertility percentages in the case of gamma irradiation and EMS. The values presented in the above table have clearly indicated the effect of mutagens in reducing pollen fertility as against both the mutagens. The untreated controls have registered the highest percentage of pollen fertility. The reduction in fertility is also seen to be

Table 13. Effect of mutagens on pollen fertility

Treatments	Pollen fertility percentage		Relative percentage over control of	
			Fertility	Sterility
<u>Gamma irradiation dose</u>				
Control	72.05	(90.5)	100.0	0.0
10 krad	67.94	(85.9)	94.9	5.1
20 krad	64.59	(81.6)	90.1	9.9
30 krad	61.21	(76.8)	84.8	15.2
40 krad	58.37	(72.5)	80.1	19.1
50 krad	55.79	(68.4)	75.5	24.5
Significance	Significant at 1% level			
SE	1.16			
CD	3.48			
<u>EMS</u>				
Control	72.05	(90.5)	100.0	0.0
0.3%	61.96	(77.9)	86.1	13.9
0.4%	58.56	(72.8)	80.4	19.6
0.5%	55.37	(67.7)	74.8	25.2
0.6%	50.24	(59.1)	65.3	34.7
0.7%	47.69	(54.7)	60.4	39.6
Significance	Significant at 1% level			
SE	0.62			
CD	1.88			

directly proportional to the increase in dose not only for gamma rays, but also for EMS. When the range in pollen fertility percentages is from 67.94 to 55.79 among the different doses of gamma rays, the corresponding figures in EMS is from 61.96 to 47.69. In the case of gamma rays, the dose 30 krad is seen to be on par with the dose 40 krad which in turn again is found to be on par with the dose 50 krad. In the case of EMS, all the concentrations tried are found to be significantly different from one another.

5. Seed fertility

Percentages of seed fertility in the plants belonging to different doses of gamma irradiation and EMS were converted into angular sines. The relative percentages of fertility and sterility were computed. Data pertaining to the above along with seed fertility percentage in bracket are furnished in Table 14.

(TABLE 14)

As is seen in the table given above, the treatment effects have produced significant differences in seed fertility percentages in respect to both the mutagens tried. The respective untreated controls have registered the highest seed fertility values as compared to the treated

Table 14. Effect of mutagens on seed fertility

Treatments	Seed fertility percentage		Relative percentage over control of	
			Fertility	Sterility
<u>Gamma irradiation dose</u>				
Control	69.21	(87.4)	100.0	0.0
10 krad	64.01	(80.8)	92.6	7.4
20 krad	60.67	(76.0)	87.1	12.9
30 krad	59.21	(73.8)	84.5	15.5
40 krad	57.42	(71.0)	81.4	18.6
50 krad	53.91	(65.3)	74.8	25.2
Significance	Significant at 1% level			
SE	0.75			
CD	2.26			
<u>EMS</u>				
Control	66.66	(84.3)	100.0	0.0
0.3%	53.01	(63.8)	75.7	24.3
0.4%	50.94	(60.3)	71.5	28.5
0.5%	47.18	(53.8)	63.8	36.2
0.6%	42.88	(46.3)	54.9	45.1
0.7%	37.76	(37.5)	44.5	55.5
Significance	Significant at 1% level			
SE	0.81			
CD	2.43			

plants thereby indicating the direct effect of both the mutagens in reducing the seed fertility percentages. The decrease in seed fertility is also seen to be directly proportional to the increase in dose of the mutagen. This is seen to be the case for both the mutagens.

Among the various doses of gamma irradiation tried, the seed fertility percentage is found to vary from 64.01 to 53.91. It is also seen that the dose 20 krad is on par with the dose 30 krad which in turn is on par with the dose 40 krad.

In the case of EMS seed fertility is found to vary from 53.01 to 37.76 among the five doses tried. The dose 0.3% is observed to be on par with the dose 0.4%.

6. Chlorophyll chimeras

The chlorophyll chimeras were observed only among EMS treated plants and their frequency was observed to be very low in M_1 generation. Chlorophyll deficient patches were found among EMS treated plants specifically in the treatments involving concentrations of 0.3%, 0.4% and 0.5%. These patches were found on the leaves of seven plants in 0.3%, four plants in 0.4% and two plants in 0.5% EMS treatment. Variations were also observed in the nature and extent of patches (Plates Ia, Ib and Ic). In some cases, the chlorophyll deficient patches appeared in the early stages and later disappeared. One plant in 0.3% treatment showed one chimeric branch whereas other branches were normal.

7. Morphological abnormalities

The mutagenic treatments induced a few morphological variations in the M_1 population. Some of the morphological abnormalities noticed during the investigations were the following:

Morphological variations were observed only among EMS treated plants. A striking morphological variation observed was the presence of dwarf plants in the higher doses of EMS treatment viz., 0.6% and 0.7% (Plates IIa, IIb and IIc). The highest dose (0.7%) produced dwarf plants with few branches (Plate IIb). However, such dwarf plants were not observed in the gamma irradiation treatments in the M_1 generation.

Leaf variations such as alternation in size and shape of leaflets were noticed among the treated plants. With regard to gamma irradiation, higher doses (40 and 50 krads) exhibited crinkling of leaflets in the early stages of growth period. However, these plants recovered and

I. Chlorophyll chimeras (EMS treated plants)

Plate Ia. Variations in chlorophyll chimeras.

1. Control	
2, 3, 4 and 5	- 0.3%
6, 7 and 8	- 0.4%
9 and 10	- 0.5%

Plate Ib. Chimeric twig - 0.3%

Plate Ic. Chimeric plant - 0.6%

Plate I a.

Plate I b.

Plate I c.

II. Dwarf plants

Plate IIa. Dwarf plant - 0.6%

Plate IIb. Dwarf plant with few leaves - 0.7%

Plate II a.
(Size reduced to 1/9th)

Plate II b.
(Size reduced to 1/8th)

Plate IIc. Dwarf plant - 0.7%

Plate IId. Control

Plate IIc.
(Size reduced to 1/5th)

Plate IId.
(Size reduced to 1/15th)

produced normal leaves afterwards. Gamma irradiation did not show any morphological variations in the later stages.

Crinkled appearance of the leaflets was a striking morphological variation among EMS treated plants. This was observed in six plants in 0.3%, two plants in 0.4%, one plant in 0.5%, one plant in 0.6% and two plants in 0.7%. There was variation in the pattern of crinkling also (Plates IIIa and IIIc). Clustering of leaflets was seen in one plant in 0.3% EMS treatment (Plate IIIb).

Small and narrow leaflets with round apex were observed in one plant in 0.3% EMS and in one plant in 0.6% (Plates IVa and IVb). Small leaved plants were also noticed at the rate of two plants in 0.4%. Number of branches and leaves were low in higher concentrations of EMS viz., 0.6% and 0.7%.

Another observation in EMS treated plants with 0.3% solution was early flowering by 25 days compared to that of control. Higher doses viz., 0.6% and 0.7% showed a delay in flowering by 30-40 days. Number of flowers and pods per plant was very low in these concentrations compared to control and other treatments.

III. Variations in the morphology of leaves (EMS treated plants)

Plate IIIa. Variations in leaf crinkling.

1. Control	
2, 3 and 4	- 0.3%
5 and 6	- 0.4%
7	- 0.5%
8	- 0.6%
9 and 10	- 0.7%

Plate IIIb. Plant with clustered leaves - 0.3%

Plate IIIc. Plant with crinkled leaves - 0.6%

Plate IIIa.

Plate IIIb.

Plate IIIc.

IV. Plants with round apexed leaves.

Plate IVa. 0.3%

Plate IVb. 0.6%

Plate IVa

Plate IV b.

Discussion

DISCUSSION

Induced mutagenesis has become one of the important tools in recent years in the hands of plant breeders to improve the crop varieties according to the needs and requirements of the farmers and farming systems. The term mutation was introduced into biology by Hugo De Vries in 1900. He suggested the concept of inducing artificial mutations and utilizing them in the breeding programme. Artificial induction of mutations as an approach in the breeding programme was recognised by many biologists in the early years of this century. However, it is only after the classical work of Muller (1927) in Drosophila on the mutagenic property of X-rays that induction of mutations has been widely practised in the crop improvement programme. Subsequently, Stadler (1928) artificially induced mutations in barley and maize using radiations. These discoveries of Muller and Stadler provided a firm footing and paved the way for further mutation breeding research. With his pioneering work in some agricultural crops like barley, wheat, oats, rye, pea etc. Gustafsson (1947) recognised the practical utilisation of radiation to induce useful mutations.

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

Red gram is an important pulse crop of peninsular India. It is perhaps the most important pulse crop from the point of view of percapita consumption. Because of its high protein content of 22.3%, it is an excellent source of protein especially in the vegetarian kitchen. Eventhough it is widely cultivated in the peninsular India because of its adaptability to low management levels, its importance as a rich source of protein and vitamins has not been fully recognized. In view of the limited amount of variability which is presently available in pulses in general and in red gram in particular, probably because of its essentially self-fertilized nature, 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. It also assumes great significance and high practical value.

The present study was taken up with the objective of finding out the direct effects produced by gamma irradiation and EMS on red gram and also the effects of different doses of the mutagens in the M_1 generation. In studies like this, the optimum dose range of the mutagens is very important since higher doses will bring about lethality. Information available from similar studies of allied crops is of great value in deciding the doses to be tried of the mutagens.

Gamma irradiation forms one of the widely used physical mutagens of the day. Different doses of gamma irradiation are reported to be effective in various materials. In the case of the different pulse crops different dose ranges have been reported to be effective by various authors as detailed below.

Author/Authors	Year	Crop	Dose range in krads
1. Dahiya	1973	Mungbean	30-70
2. Prasad and Das	1973	*Lathyrus Sativus*	10-50
3. Ramakanth and Seetharaman	1979	*Dolichos lablab*	10-50
4. Vindhiyavarman	1979	*Vigna marina*	10-100
5. Manju	1981	Horse gram	10-50
6. Kundu and Singh	1982	Black gram	10-50
7. Na Lampang *et al*.	1982	Black gram	0-90
8. Rao and Rao	1983	Black gram	10-60

As such inclusion of gamma rays as a mutagen in a dose range from 50 krad to 10 krad is amply justified.

Ethyl methane sulphonate, popularly known as EMS, is the chemical mutagen included in the present study. This has been recognized as one of the most efficient and effective chemical mutagen throughout the world. Informations available in literature on the effective dose range of EMS on pulses in general and red gram in particular are conflicting. Effective dose range of any chemical mutagen as a matter of fact is conditioned by the moisture content of material to be treated, the concentration of the chemical and also the duration of the treatment. A change in any one of the three factors will be reflected in the effectiveness of the chemical as a mutagen. As such, in the case of chemical mutagen it is always desirable to fix the dose range based on some preliminary observations with the material under the conditions of experimentation. It is in this context that a preliminary laboratory trial formed the basis for fixing the doses of EMS in the present investigation.

Results of the preliminary laboratory test involving two durations of presoaking viz., 2 hours and 4 hours with three concentrations of the chemical viz., 0.5%, 0.75% and 1% at two durations of treatments i.e. 6 hours and 8 hours have yielded interesting and valid conclusions. As indicated by the results, there has been no significant difference between 2 hours of presoaking and 4 hours of presoaking and also between 6 hours of treatment and 8 hours of treatment. In other words 2 hours of presoaking with 6 hours of treatment is seen to be as effective as 4 hours of presoaking and 8 hours of treatment. The only significant difference observed is between the three concentrations of the chemical mutagen viz., 0.5%, 0.75% and 1%. The percentage of germination of seeds treated with the mutagen in the above concentrations is found to vary from 91 for 0.5% to 3 for 1%. From this the LD_{50} (i.e. the dose at which there is 50% and above mortality) has been estimated to be 0.7% which has been fixed as the highest concentration of the chemical mutagen in all further studies. Five concentrations at regular intervals with LD_{50} as the highest dose viz., 0.3%, 0.4%, 0.5%, 0.6% and 0.7% with 6 hour treatment of seeds presoaked for 2 hours tried in all subsequent experiments, are therefore based on actual experimental results of the preliminary laboratory test.

The present study has been undertaken with a view to finding out the biological effects of gamma rays and EMS in the M_1 generation of red gram. In other words it is to explain the possible changes which the mutagens can bring about in the various life processes of red gram that the study has been carried out. In all sexually propagated flowering plants, seed is the initial starting point of the life cycle, the visible activity of which begin with germination of seed. A seed after germination yields a seedling which after growth and development produces flowers in which the essential reproductive organs, the androecium and gynoecium are located. After pollination and fertilization, fresh fruit containing seeds are produced, these seeds again forming the basis for the beginning of next cycle. Hence in any study pertaining to the effects of an agent on the life activities of any plant, it is essential that the study must be initiated from the beginning of the life cycle and completed with the end of the same. In the light of these facts, studies undertaken in the present case on the effects of the mutagens on seed germination, seedling survival, plant growth, pollen and seed fertility etc. are fully justifiable.

Results of studies on the effects of mutagens on seed germination both under laboratory and field conditions have yielded valuable informations. In the laboratory trials, no significant effect is seen to have been produced by the different doses of gamma irradiation or those of EMS either on the germination percentage of seeds or in the time taken for germination. The lower doses of both the mutagens viz., 10 krad and 20 krad of gamma irradiation and 0.3% and 0.4% of EMS are observed to have some stimulatory effect on the percentage of germination by these treatments over their respective controls (vide Fig.1). Similar stimulatory effect of gamma irradiation and EMS on germination have earlier been reported by Vadivelu (1979) in Bengal gram and Manju (1981) in horse gram. In the case of gamma irradiation, no striking relationship is observable in the percentages of germination and in the doses. This is not true with reference to EMS wherein the percentage of germination is seen to be proportionately decreasing with the increasing concentrations of the chemical. This is further substantiated by the mean time taken for germination by the seeds in the respective treatments. As in the case of percentages of germination, the mean time taken by seeds for germination in the various treatments of gamma rays does not appear any bearing with dose. However, EMS gives a different picture in this regard. The time taken by seeds for germination is found to be proportionately increasing with increasing doses of the mutagen (vide Fig.1). In other words the lowest concentration of EMS viz., 0.3% is seen to have registered a germination percentage of 96.3 with the mean period for germination being 10.83 hours as compared to the highest concentration of EMS of 0.7% with a germination percentage of 77.5 and a mean period of germination of 24 hours. Seeds treated with gamma rays are 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 in which case, the seeds have been soaked in various concentrations of the acquous solution of the chemical.

Results of field trial on the effects of mutagens on germination of seeds are in agreement of those conducted in the laboratory, except in the fact that the treatment effects both under gamma irradiation and EMS are seen to be significant with reference to the germination percentages. Absence of any linearity in the germination values registered in the various treatments with reference to doses of gamma irradiation, slight increase over the control in the germination value of the lower doses of gamma irradiation, a proportionate reduction in the germination percentages obtained in the various treatments of EMS along with the increasing concentration of the chemical etc. obtained in the field experiments of the present investigation support and strengthen the trends obtained in the laboratory studies. However, the EMS treated seeds are found to take a longer time interval as compared to the gamma ray treated seeds for attaining maximum germination in the field. This observation is contrary to the results of the laboratory studies. A reasonable explanation of the same demands further detailed investigation. It also observed that seeds treated with gamma rays and EMS have

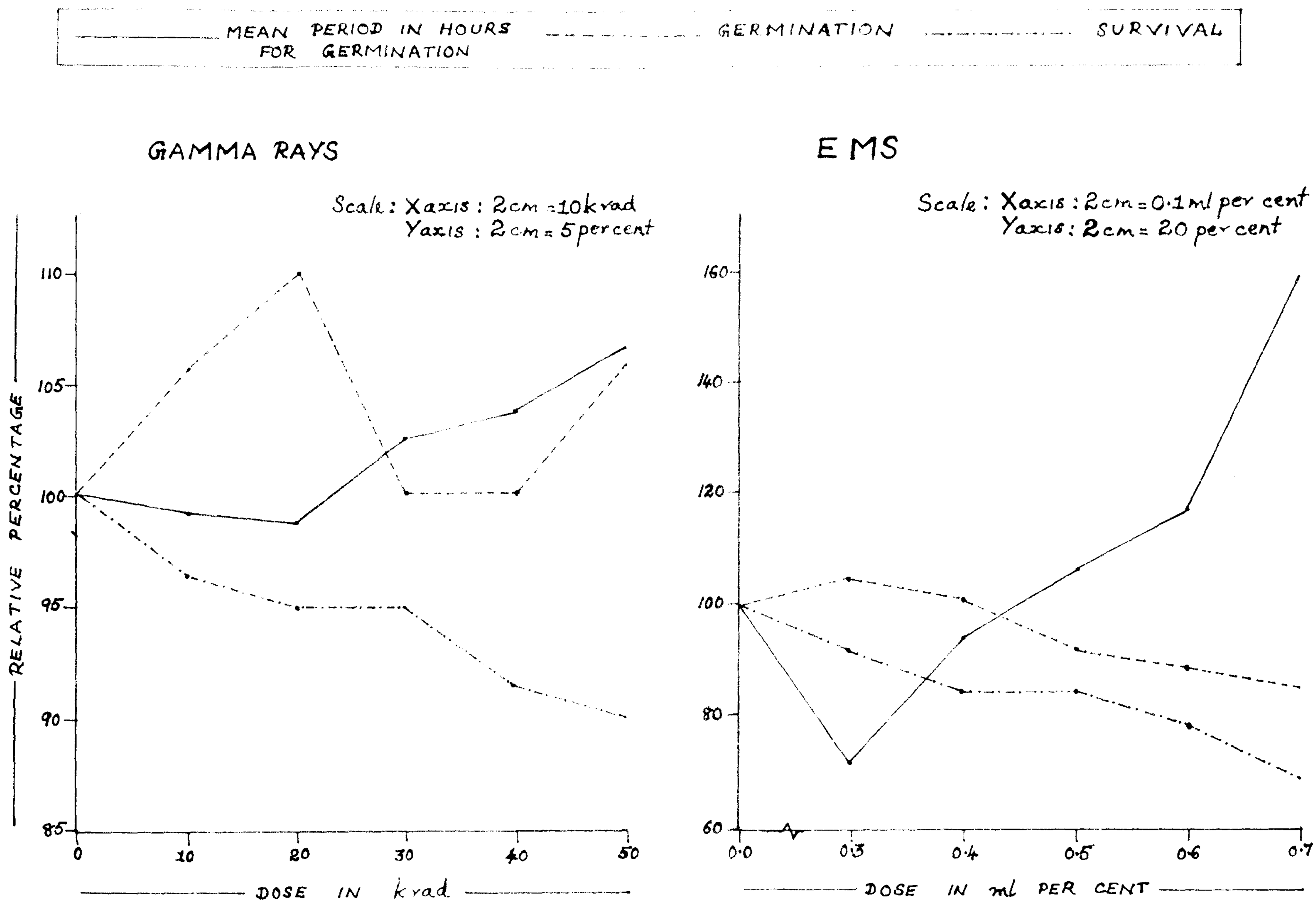


FIG-1. EFFECT OF MUTAGENS ON THE GERMINATION, PERIOD FOR GERMINATION AND SURVIVAL.

registered a lower germination value in the field when compared to the same in the laboratory. This is perhaps because of the ideal conditions for germination in the laboratory in comparison with the same in the field.

Next to germination, the mutagens are likely to have some favourable or unfavourable effects on the seedlings. Effects of the mutagens, gamma rays and EMS, on the seedlings have been studied in the present case both under laboratory and field conditions. In both the cases the results in all essential features agree with each other. It is true that the treatment effects did not produce significant differences on the survival percentage of seedlings in the trial conducted under laboratory situation. However, the trend of the results has given strong indications that both the mutagens reduce survival percentages of seedlings (vide Fig.1), the reduction in survival values being proportional to the increase in the dose of the mutagen. These findings have been further supported by the results of trial conducted in the field in which significant differences could be noticed in the survival percentages registered by treatments involving different doses of gamma irradiation and EMS. Both the mutagens have reduced the survival percentages, the reduction being within the limits of acceptable errors, proportional to the increase in the dose of the mutagen (vide Fig.2). These findings are in

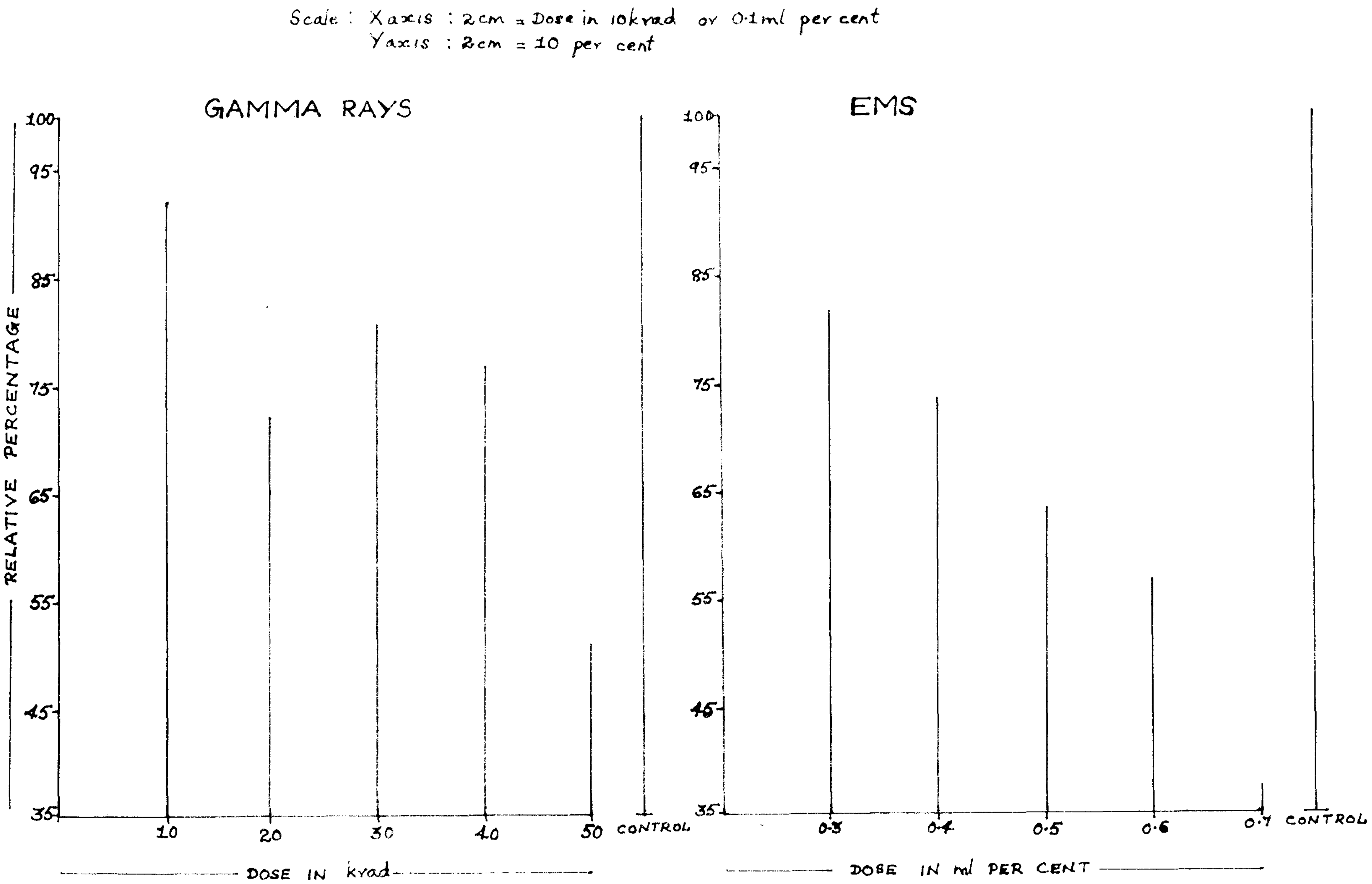


FIG-2. EFFECT OF MUTAGENS ON SURVIVAL OF PLANTS ON THE 30th DAY.

agreement with the earlier reports of Shirshov and Shain (1966) in French bean and soybean; Jaranowski (1970) in *Pisum arvense*; Louis and Kadambavanasundaram (1973b) in cowpea, Ramaswamy (1973) in black gram, Narasinghani and Kumar (1976) in cowpea; Krishnaswamy *et al*. (1976) in bengal gram; Kulkarni and Shivasankar (1978) in horse gram Khan (1981) in mungbean and Manju (1981) in horse gram.

Other than reducing the survival percentage of seedling, it is reasonable to assume that the mutagens may exert their influence on other growth parameters of the seedlings. A plant is composed of the roots as well as the shoots, the increase in length of which especially in the early growth period can very well be considered as true indices of plant growth. It is on this background that the effects of the different doses of the mutagens on root and shoot elongations have been studied under laboratory conditions in the present investigations. The results point to clear indications that both the mutagens, the gamma rays and EMS, suppress root as well as shoot elongation in red gram with almost identical effects (vide Fig. 3, 4, 5, 6 and 7). It is also further seen that the rate of suppression in case of both root and shoot is directly proportional to the increase in dose not only with reference to gamma irradiation but also with EMS. A similar reduction in seedling growth by gamma irradiation and EMS has previously

FIG-3. EFFECT OF GAMMA RAYS ON THE RATE OF ROOT GROWTH.

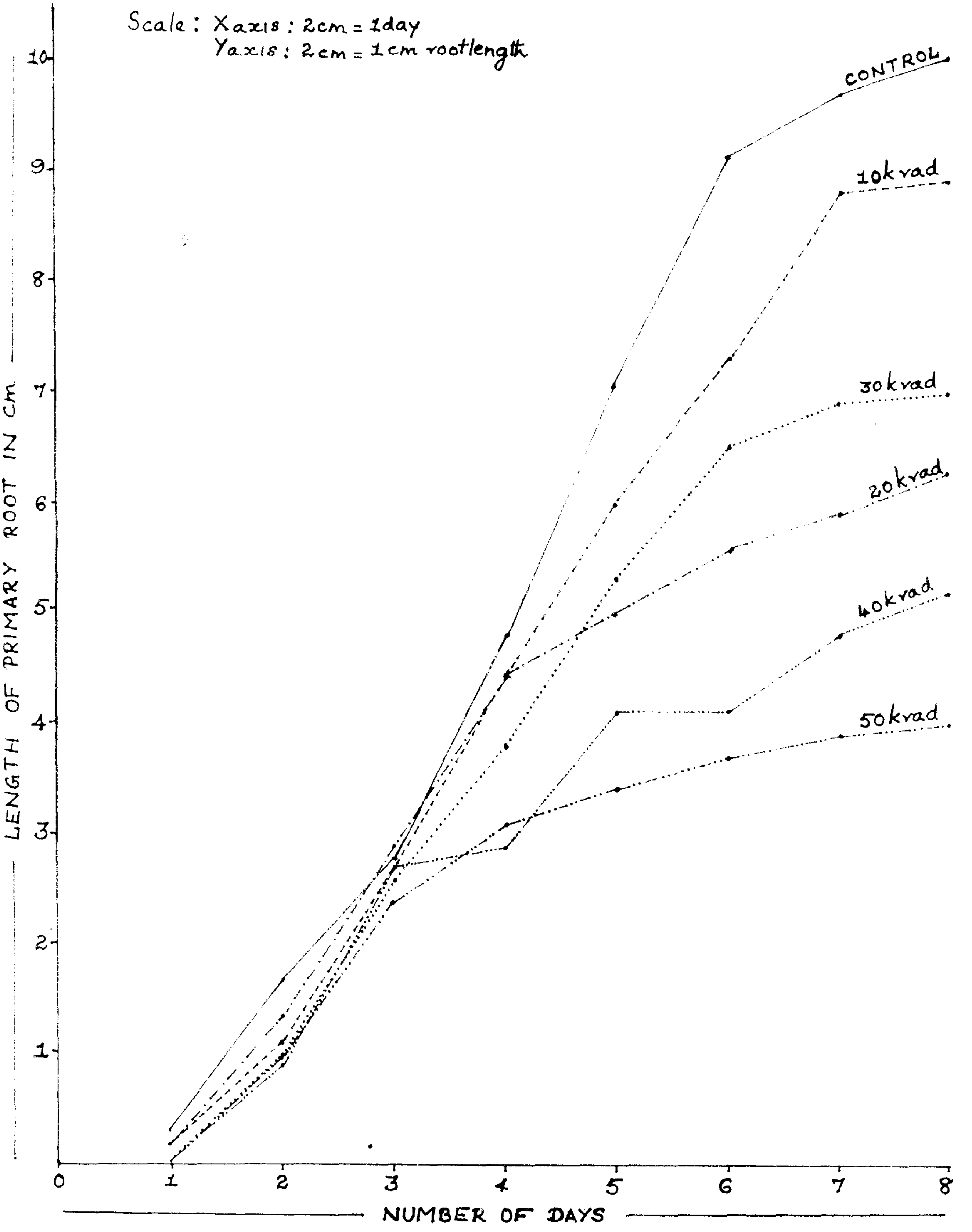


FIG-4. EFFECT OF EMS ON THE RATE OF ROOT GROWTH.

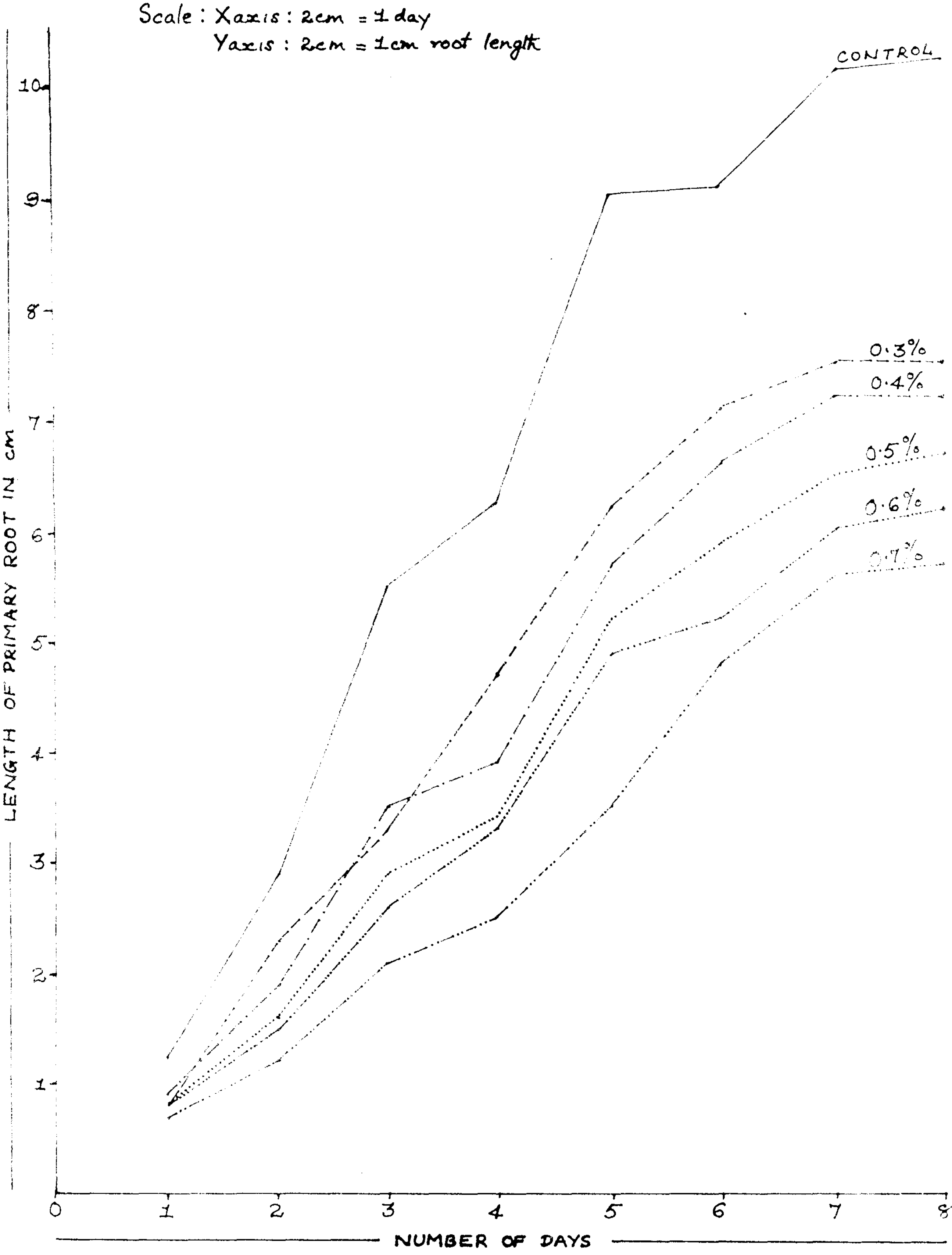


FIG.5. EFFECT OF GAMMA RAYS ON THE RATE OF SHOOT GROWTH

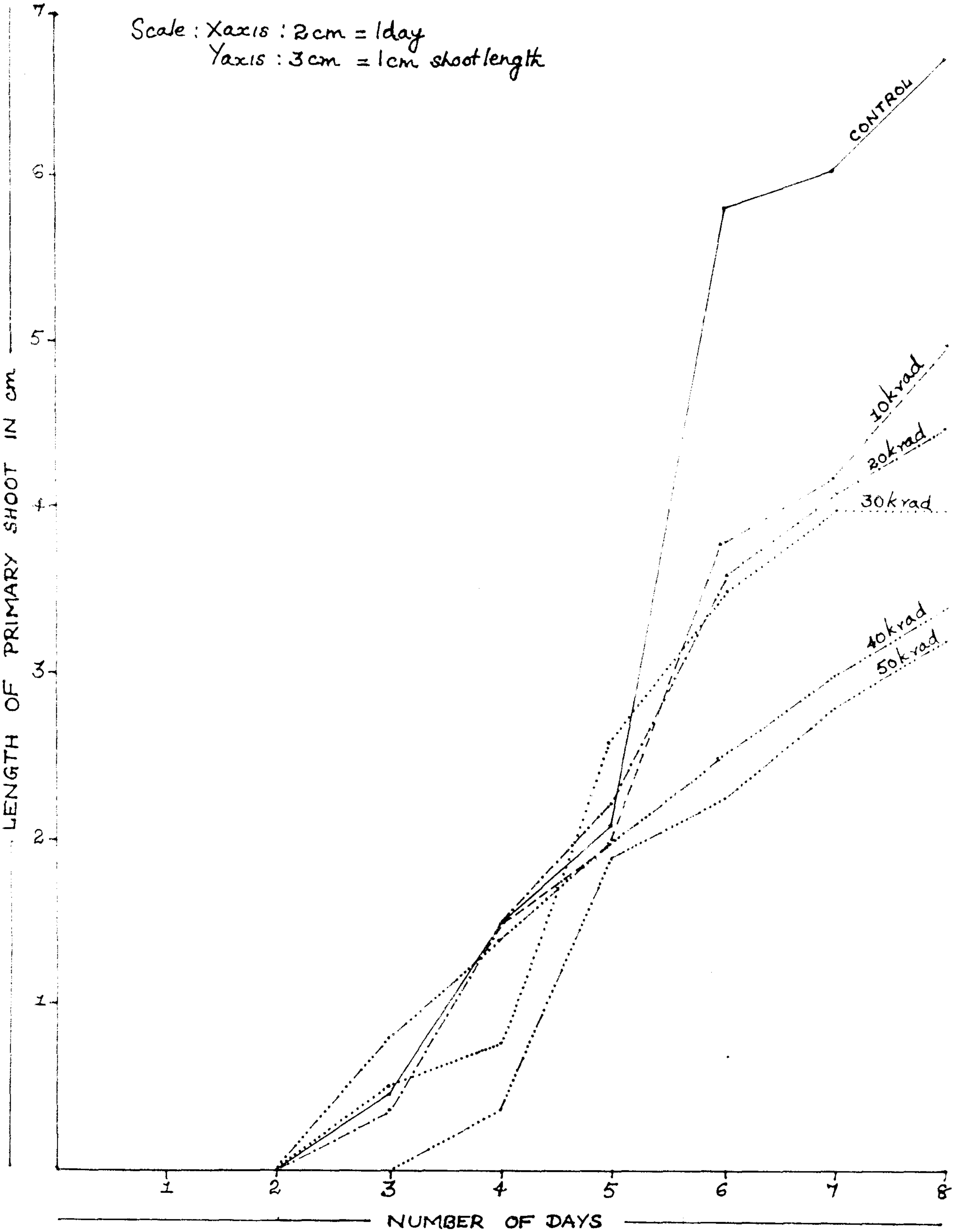
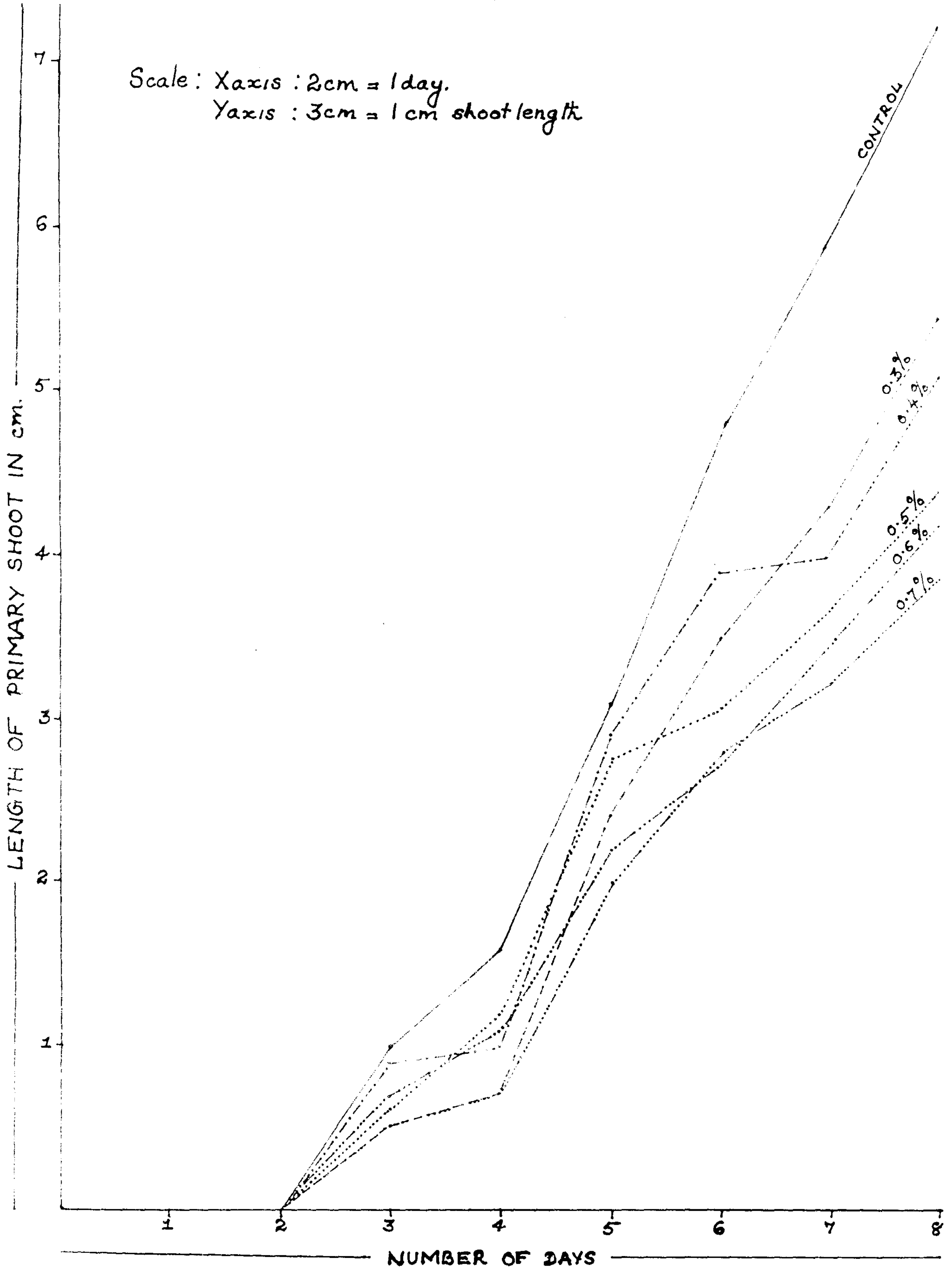


FIG.6. EFFECT OF EMS ON THE RATE OF SHOOT GROWTH



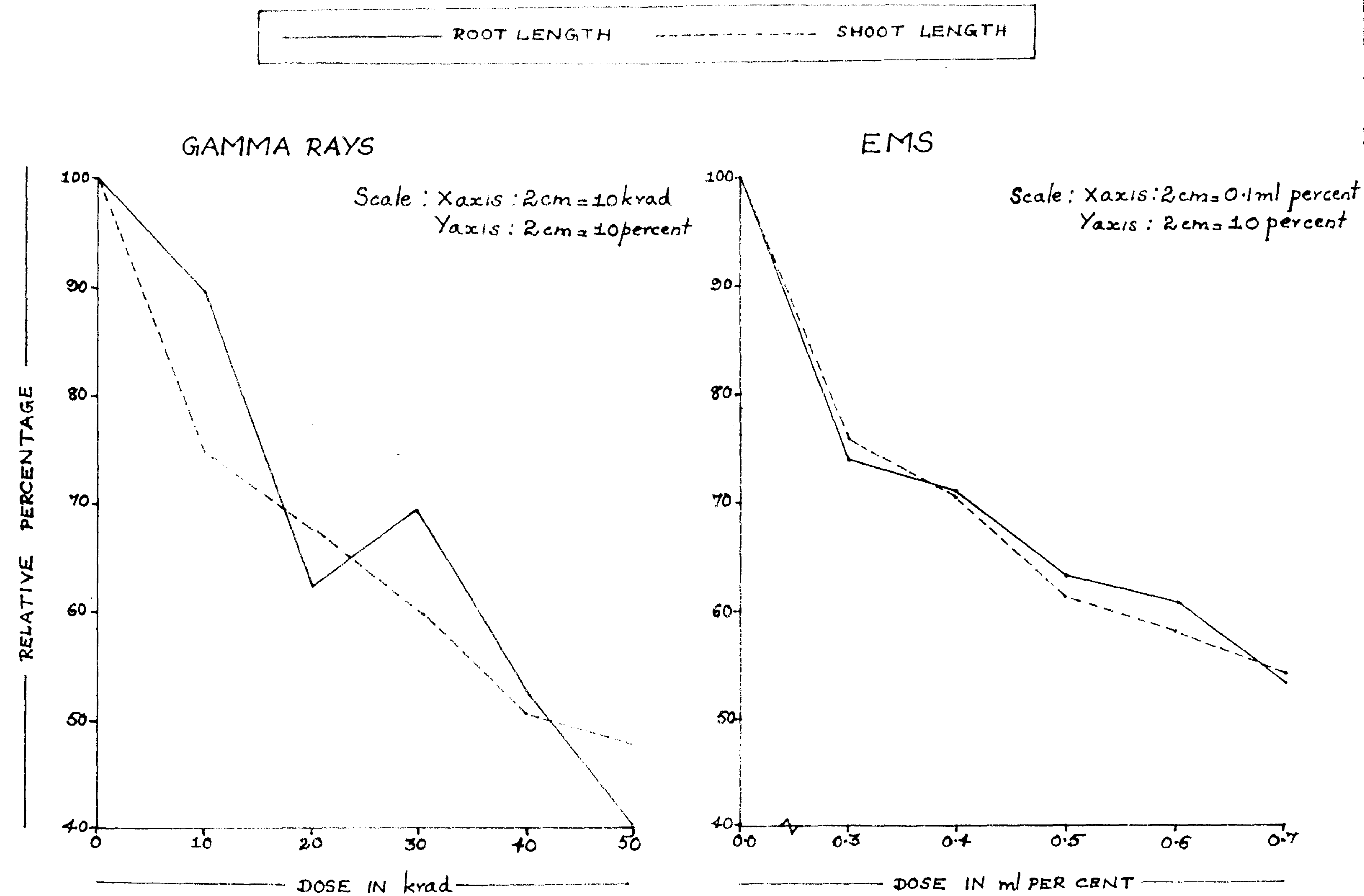


FIG.7 EFFECT OF MUTAGENS ON THE ROOT AND SHOOT LENGTHS.

been reported by Teretchenko (1966); Maslov and Stepanova (1967) in pea using gamma rays and by Naresinghani and Kumar (1969) in cowpea.

The shoot-root ratio calculated on the basis of relative percentages of root as well as shoot elongation over their respective controls has shown that the doses of 20 krad and 50 krad of gamma rays and 0.3% and 0.7% EMS have suppressed root elongation more than shoot elongation as is indicated by the magnitude of shoot/root ratios over one in the above treatments. Again the doses 10, 30 and 40 krads of gamma irradiation and 0.4%, 0.5% and 0.6% of EMS have shown their preference in the suppression of shoot elongation rather than root elongation, since the shoot/root ratios registered against these treatments have been found to be less than one. However, these findings will have to be supported by further detailed investigations.

Results of observations on the effect of mutagens on plant growth under field conditions, measured in terms of plant height, are again in support of those obtained in the laboratory test. The treatment effects in respect of both the mutagens have brought out significant differences in plant growth expressed in terms of height of plant on the 30th day. Both gamma irradiation and EMS are found to reduce plant height, the rate of reduction being directly proportional to the increase in dose of the mutagen (vide Fig.8). This is in agreement with the results from IARI (1971) with reference to gamma irradiation in case of bengal gram. It is also observed that among the two mutagens included in the present investigation and also within the doses tried, EMS is seen to be more effective in reducing plant height under field conditions. However, this needs further confirmations by conducting detailed experiments.

A plant after its growth phase enters into its productive phase when structures directly or indirectly connected with reproduction take their origin on the plant. Among the various structures concerned with reproduction in plants, androecium and gynoecium are considered as essential structures. It might be possible that the mutagens may have some action on these entities either directly or indirectly resulting on a change in the normal fertility status of the plant. In this context studies undertaken on the effect of mutagens on pollen as well as seed fertilities in the present investigation become meaningful.

Results of studies on the effect of mutagens on pollen fertility have indicated significant treatment effects in respect of pollen fertility percentages with reference to both gamma rays and EMS. Both the mutagens are observed to be capable of reducing pollen fertility, the rate of reduction

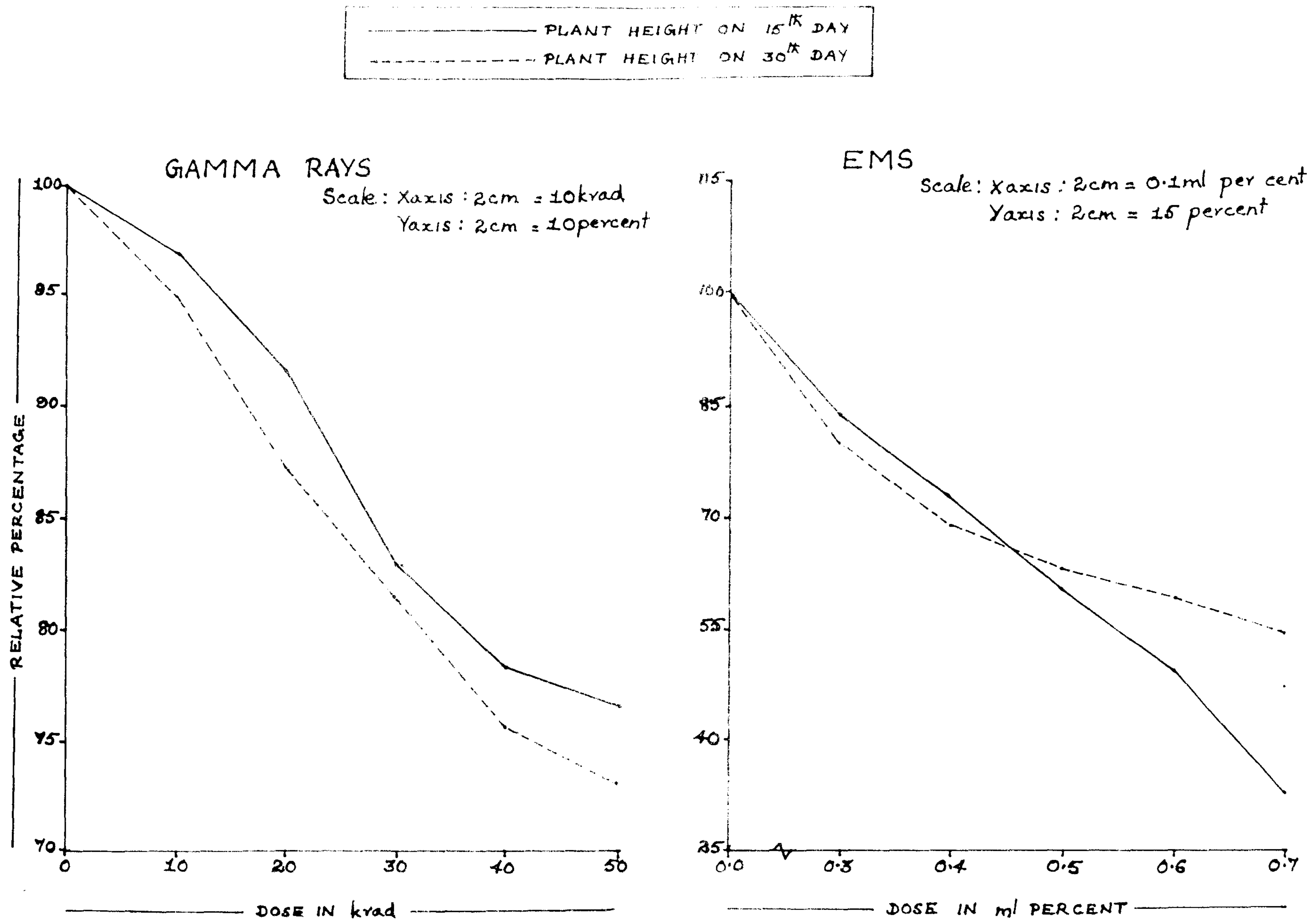


FIG.8. EFFCT OF MUTAGENS ON THE RATE OF GROWTH OF PLANTS.

being directly proportional to the increase in doses of the mutagens (vide Fig.9). This is observed to be the case uniformly for both gamma rays and EMS. Moreover it is also observed that within the limits of acceptable error, EMS is seen to be more effective in reducing pollen fertility in comparison with gamma rays. This inference is drawn from the fact that within the dose range of gamma rays and EMS included in the present study, reduction in fertility percentage of pollen grains is found to vary from 67.94 to 55.79 in case of gamma irradiation and 61.96 to 47.69 in case of EMS.

Results of studies on seed fertility obtained in the present case clearly support those relating to pollen fertility. Both gamma rays and EMS have registered significant treatment effects on seed fertility. Both the mutagens are also found to be reducing seed fertility, the rate of reduction being directly proportional to increase in the dose of the mutagens (vide Fig.10). The higher efficiency of EMS over gamma irradiation in reducing pollen fertility is seen to be more clearly pronounced in case of seed fertility.

Thus it is clearly seen that the mutagens have similar effects in changing the fertility of both the pollen and the seed. This is easily understandable and is in agreement with simple reasoning that pollen is one of the two essential entities involved in the formation of seed. As such

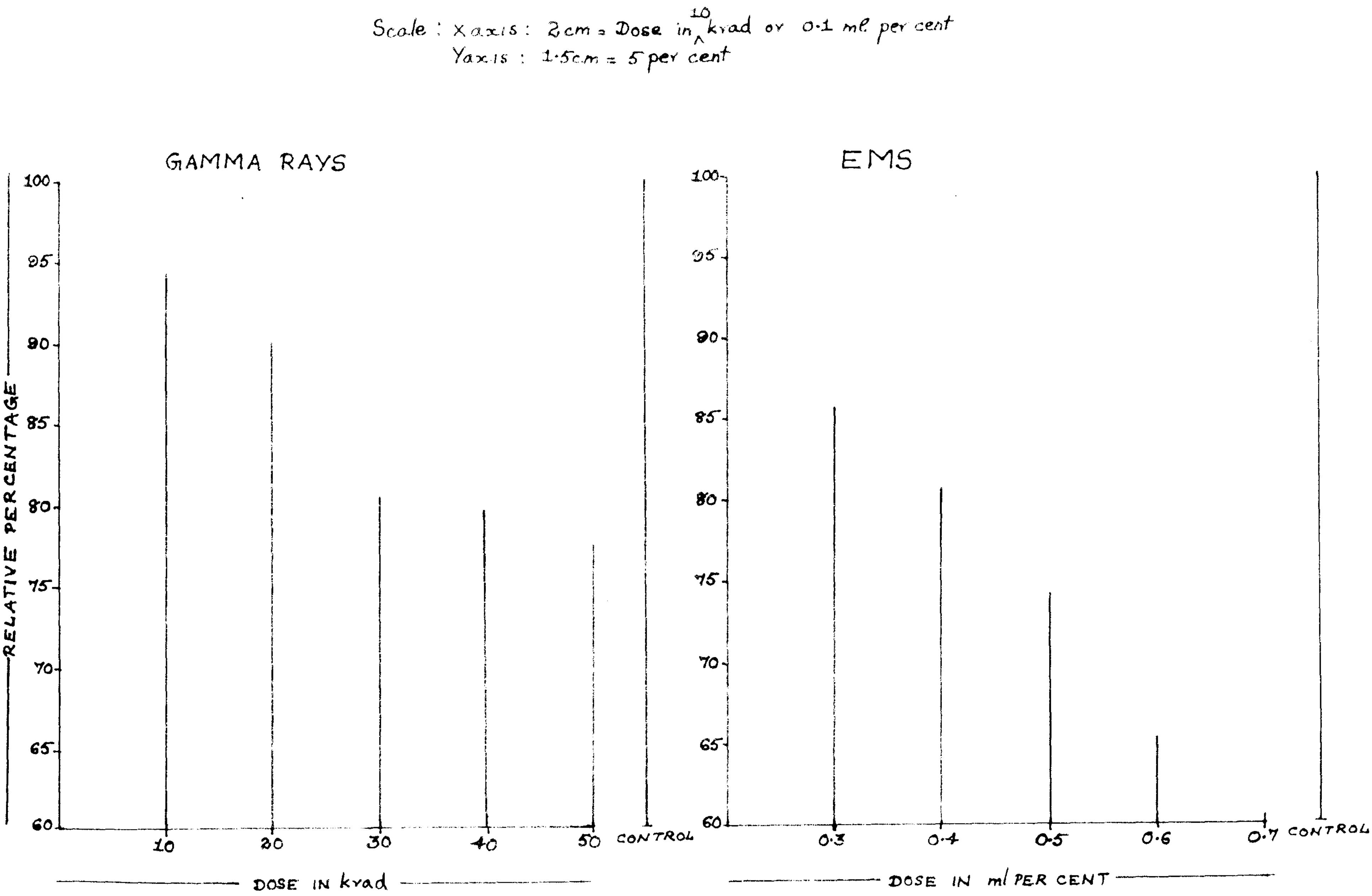


FIG.9. EFFECT OF MUTAGENS ON POLLEN FERTILITY.

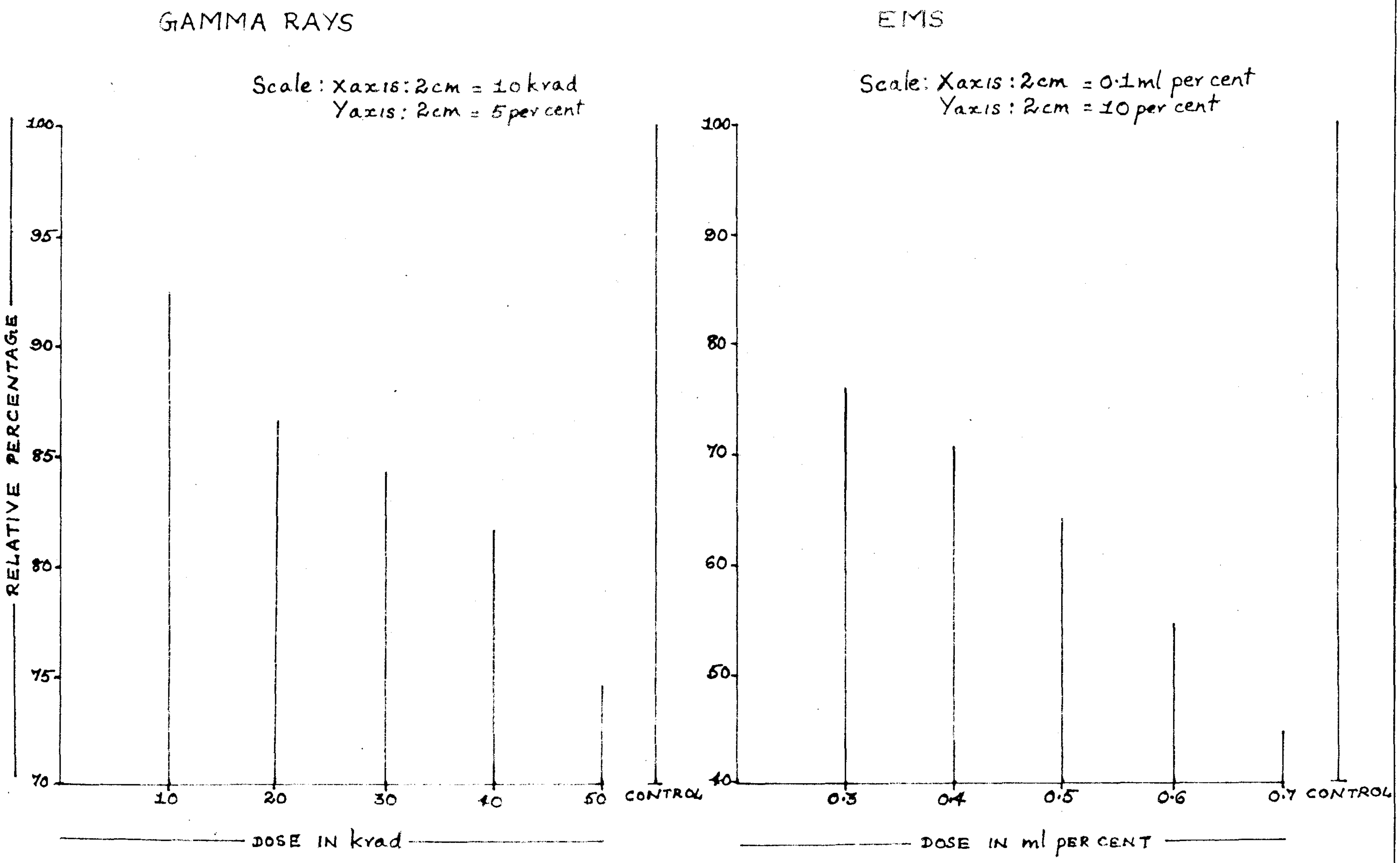


FIG. 10. EFFECT OF MUTAGENS ON SEED FERTILITY.

any change affecting pollen is likely to be reflected in the seed as well.

The frequency of chlorophyll chimeras has been observed to be very low and has been spotted only in EMS treated population. No chimeric plant has been registered in the gamma ray treated population. This is not in agreement with the report of Manju (1981) in horse gram. This difference may perhaps be due to the limited size of the population or to the nature of the material subjected for the study in the present case. However, this needs further confirmation by subsequent studies. Even in the EMS treated population chlorophyll chimeras have been observed to confine only to the lower doses of 0.3%, 0.4% and 0.5%. None has been realised in the higher doses of 0.6% and 0.7%. Again the frequency of chimeric plants is found to be decreasing along with the increase in the concentration of EMS. As for example when seven chimeric plants have been spotted in 0.3% concentration of EMS, only four and two have been seen in 0.4% and 0.5% concentrations respectively. This is indicative of the fact that under conditions of experimentation in the present case lower doses of EMS are ideal for bringing a change or destruction to the chlorophyll in the desired level. It has also been seen that there are variations in the nature and extent of patches, these variations being such as to have no dose dependance. A unique plant observed in the treatment involving 0.3% solution of EMS needs special mention. One branch of this plant produced chlorophyll chimeras whereas other branches were normal. This may perhaps be due to the fact that only part of the embryo has been affected by the mutagen, the rest remaining normal. Similar incidence have been reported by Swarup and Gill (1960) in French bean; Beshanidse and Debelyi (1970) and Shifrin (1972) in pea; Gohal et al. (1970) in cluster beans and Manju (1981) in horse gram.

Different types of morphological abnormalities have been caused by mutagens. In the present case, such morphological variations are seen to be confined to only EMS treated population. The chief among the different types of morphological variations observed in the present study are dwarf plants, plants with crinkled leaves, plants with small narrow leaflets with round apex, plants with reduced canopy size, plants with prolonged as well as reduced flowering duration and plants with lesser number of flowers and pods. Failure in the recovery of such morphologically different types in the gamma ray treated population may probably be due to the limited size of the population and also due to the nature of the material investigated. In the EMS treated population no dose dependance is seen in the realization of mutants with the above type of morphological types of variation. However, there has been a predominance of such types appearing in the higher doses of EMS viz., 0.7% and 0.6%. Appearance of such morphological variations in the M_1 generation has been reported by Sjodin (1962) in Vicia; Ashri and Goldin (1965) in Ground nut; Shirshov and Shain (1966) in pea and Manju (1981) in horse gram.

From the foregoing discussions it becomes clear that the two mutagens, gamma rays and EMS within the dose range tried in the present case, are capable of altering seed germination, seedling survival, shoot as well as root elongation, fertility of pollen and seed, chlorophyll as well as morphological abnormalities resulting on a wider spectrum of heritable variation in the treated material. The variability thus induced will offer better scope for the breeder to select the genotype with the desired combination of characteristics.

Summary

SUMMARY

Investigations on the "Biological effects of gamma rays and EMS on the M_1 generation of red gram (*Cajanus cajan* L.)" were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the period 1983-'85. Pure seeds of SA.1 variety of red gram were subjected to five different doses of gamma irradiation (10 to 50 krad) and five doses of EMS (0.3% to 0.7%) and the M_1 generation was raised and studied. Observations on seed germination, seedling survival, shoot as well as root elongations, pollen and seed fertilities, chlorophyll chimeras, morphological variations etc. were recorded from the M_1 generation plant. The data so collected were subjected to suitable statistical analysis. The important findings are summarised below.

1. In the preliminary laboratory test it is seen that (a) 2 hour presoaking of seeds is as effective as 4 hour presoaking, (b) 6 hour treatment of EMS does not differ significantly from 8 hour treatment (c) seed germination is drastically reduced from 91% to 3% when concentration of EMS increases from 0.5% to 1% and (d) the LD_{50} for EMS is 0.7%.

2. Studies on the effect of mutagens on germination of seeds have shown that (a) the treatment effects are not significant either on the germination percentage of seed or in the time taken for germination (b) Lower doses of both the mutagens stimulate, germination (c) no dose dependance on the percentage of germination is observed in the case of gamma irradiation while in the case of EMS the percentage of germination proportionately decreases with increasing concentrations of the chemical. Seeds treated with gamma rays take a longer time for germination as compared to those treated with EMS. No dose dependance is observed in the case of seeds treated with gamma rays in the time taken for germination, whereas in the case of EMS the time taken by seeds for germination proportionately increases with increasing doses of the mutagen. Results of field trial on the effects of mutagen on seed germination have registered significant treatment effects on germination. These results perfectly agree with those obtained in the laboratory trials.

3. Studies on the effect of mutagens on survival of seedlings conducted in both laboratory and field conditions have registered significant treatment effects only in the case of field trials. In both the cases both gamma rays and EMS have reduced the survival percentage of seedlings, the rate of reduction being proportional to increasing doses of

the mutagen in both the cases under both the situations.

4. Results of laboratory studies conducted on the effect of mutagens on root and shoot elongations have shown that (a) both the mutagens suppress root and shoot elongations with identical effects and (b) rate of suppression is directly proportional to increase in doses of both the mutagens.

5. Results of field studies conducted on the effect of mutagens on plant growth expressed in terms of plant height on the 30th day have produced significant treatment effects. Both gamma rays and EMS have reduced plant height, the rate of reduction being directly proportional to increase in doses.

6. Results of studies on the effect of mutagens on pollen as well as seed fertility have indicated significant treatment effects in both. Both gamma rays and EMS have reduced pollen and seed fertilities. The rate of reduction in the fertility of pollen and seed has been directly proportional to the increase in doses of both the mutagens. Among the two mutagens EMS is more effective in reducing pollen and seed fertilities.

7. Chlorophyll chimeras in very low frequencies have been observed only in the EMS treated population.

8. Striking morphological variations observed in the M_1 generation include dwarf plants, plants with crinkled leaves, those with small narrow leaflets with round apex, those with reduced canopy size, those with prolonged as well as reduced flowering durations and those with lesser number of flowers and fruits. These morphological variations are restricted to EMS treated population.

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* Originals not seen

Appendices

Appendix I

Analysis of variance table for the effect of mutagens on the germination of plants on the 15th day
(Field conditions)

Source	Degrees of freedom	Sum of squares	Mean square	F value
<u>Gamma rays</u>				
Block	3	289.41	96.47	8.25**
Treatment	5	1235.91	247.18	21.15**
Error	15	175.30	11.69	
Total	23	1700.62		
<u>EMS</u>				
Block	3	74.25	24.75	0.94**
Treatment	5	2121.87	424.37	16.14**
Error	15	394.61	26.30	
Total	23	259.06		

** Significant at 1% level

Appendix II

Analysis of variance table for the effect of mutagens on the survival of plants on the 30th day
(Field conditions)

Source	Degrees of freedom	Sum of squares	Mean square	F value
Gamma rays				
Block	3	185.11	61.70	5.49**
Treatment	5	1230.24	246.05	21.91**
Error	15	168.46	11.23	
Total	23	1583.84		
EMS				
Block	3	192.68	64.23	1.91
Treatment	5	2712.61	54.25	16.16**
Error	15	503.45	33.56	
Total	23	3408.74		

** Significant at 1% level

Appendix III

Analysis of variance table for the effect of mutagens on root growth (Laboratory conditions)

Source	Degrees of freedom	Sum of squares	Mean square	F value
Gamma rays				
Block	3	0.14	4.68	0.28
Treatment	5	102.24	20.45	120.36**
Error	15	2.55	0.17	
Total	23	104.93		
EMS				
Block	3	0.49	0.17	2.9
Treatment	5	51.05	10.21	176.43**
Error	15	0.87	5.8	
Total	23	52.41		

** Significant at 1% level

Appendix IV

Analysis of variance table for the effect of mutagens on shoot growth (Laboratory conditions)

Source	Degrees of freedom	Sum of squares	Mean square	F value
<u>Gamma rays</u>				
Block	3	0.64	0.21	4.20
Treatments	5	31.32	6.26	125.28**
Error	15	0.73	0.05	
Total	23	32.69		
<u>EMS</u>				
Block	3	0.14	4.59	1.02
Treatments	5	29.95	5.99	132.69**
Error	15	0.68	4.51	
Total	23	30.77		

** Significant at 1% level

Appendix V

Analysis of variance table for the effect of mutagens on plant growth under field conditions

Source	Degrees of freedom	Sum of squares	Mean square	F value
<u>Gamma rays</u>				
Block	3	37.52	12.51	5.01
Treatment	5	859.42	171.88	68.86**
Error	15	37.44	2.49	
Total	23	934.39		
<u>EMS</u>				
Block	3	28.49	9.49	1.04
Treatment	5	1635.78	327.16	35.92**
Error	15	136.62	9.11	
Total	23	1800.89		

** Significant at 1% level

Appendix VI

Analysis of variance table for the effect of mutagens on pollen fertility

Source	Degrees of freedom	Sum of squares	Mean squares	F value
Gamma rays				
Block	3	22.39	7.46	1.39
Treatment	5	755.05	151.01	28.25**
Error	15	80.17	5.34	
Total	23	857.61		
EMS				
Block	3	5.58	1.86	1.19
Treatment	5	1445.59	289.12	186.29
Error	15	23.28	1.55	
Total	23	1474.44		

** Significant at 1% level

Appendix VII

Analysis of variance table for the effect of mutagens on seed fertility

Source	Degrees of freedom	Sum of squares	Mean squares	F.value
Gamma rays				
Block	3	5.73	1.91	0.85
Treatment	5	565.62	113.12	50.46**
Error	15	33.63	2.24	
Total	23	604.98		
EMS				
Block	3	20.85	6.95	2.66
Treatment	5	1994.44	398.89	152.89**
Error	15	39.13	2.61	
Total	23	2054.42		

** Significant at 1% level

BIOLOGICAL EFFECTS OF GAMMA RAYS AND EMS IN THE M_1 GENERATION OF RED GRAM (*Cajanus cajan* L.)

By

JAYANTHI, S.

ABSTRACT OF THE THESIS

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Department of Agricultural Botany
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ABSTRACT

The studies reported herein were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the period 1983-'85.

Seeds of SA-1 variety of red gram (Cajanus cajan L.) were subjected to induced mutagenesis using five doses of gamma rays (10, 20, 30, 40 and 50 krad) and five doses of EMS (0.3%, 0.4%, 0.5%, 0.6% and 0.7%) and their biological effects on the M_1 generation were studied.

In the preliminary laboratory test it was found that the two presoaking times viz., 2 hours and 4 hours did not differ significantly. Same was the case with the two durations of chemical treatments viz., 6 hours and 8 hours. The three concentrations of the chemical viz., 0.5%, 0.75% and 1% tried did differ significantly. The LD_{50} value was obtained as 0.7%.

Lower doses of both gamma rays and EMS stimulated seed germination. No dose dependance on the percentage of germination was noticed in the case of gamma irradiation while in the case of EMS, germination percentage proportionately decreased with increasing concentrations of the chemical. Gamma ray treated seeds took longer time for germination compared to those treated with EMS.

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

Root length, shoot length and plant height were reduced by gamma rays and EMS, the rate of reduction being directly proportional to the increase in doses.

Pollen and seed fertilities decreased linearly with increase in doses of both gamma rays and EMS. Among the two mutagens, EMS was more effective in reducing pollen and seed fertilities.

Chlorophyll chimeras, in very low frequencies were observed only in the EMS treated population. Morphological variations noticed included dwarf plants, plants with crinkled leaves, those with reduced canopy size, those with prolonged as well as reduced flowering durations, those with lesser number of flowers and fruits. These morphological variations were restricted to EMS treated populations.