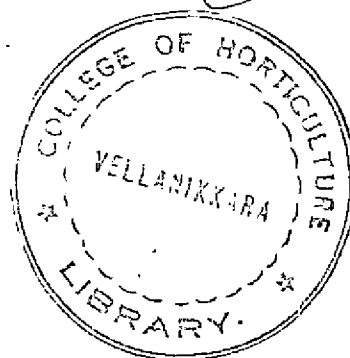


EFFECT OF MUTAGENS ON THE GROWTH RESPONSE
AND MUTATION RATE IN CHILLIES

BY
ASHA M. S.



THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
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COLLEGE OF AGRICULTURE
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DECLARATION

I hereby declare that this thesis entitled "Effect of mutagens on the growth response and mutation rate in chillies" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

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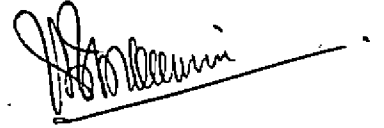
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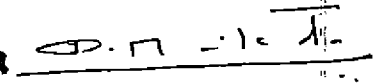
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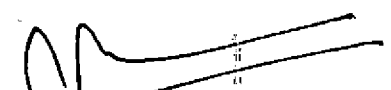


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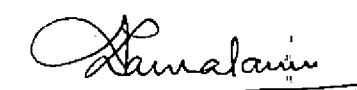
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ASHA, M.S.

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INTRODUCTION

INTRODUCTION

The successful exploitation of atomic radiations and radio mimetic chemicals for inducing alterations in the base sequence of DNA is one of the most potent lines of contemporary breeding research. The success of the green revolution which is to a certain extent a product of induced mutations has already proved that these mutagens can be beneficially utilised for tailoring better varieties of crop plants. Artificially induced variations have been extensively studied and reported in almost all crop plants especially seed propagated ones. The reports made so far show that all morphological and physiological characters within the species boundary and even beyond this can be induced by mutations.

It is well known that a crop plant can be improved in productivity, resistance to various stresses and adaptation to environment, when genetic variability for that particular trait is available in the considered population or species. The process of breeding crop plants has been successful for a long time because genetic variation already present in the population has been used and subsequently further genetic variation made available by crossing plants from

different populations, varieties, species and genera. In some cases however, further progress through the classical methods of breeding becomes more and more difficult. The possibility offered by mutagenic agents in such situations is of considerable interest. Larger genetic variation means the possibility of greater responses to selection and higher chances of improvement. It has been demonstrated and clearly stated by many workers including Gregory (1956) and Pate and Duncan (1963) that radiation is as efficient as hybridisation in supplementing genetic variability for selection. Now it is clear that under certain situations induced mutations are the only solution for the problems faced by the breeders.

Study of mutagen sensitivity is a pre-requisite for initiating practical mutation breeding programmes in any crop plant, as there is a positive correlation between sensitivity and yield of positive variants. The sensitivity of seeds to mutagenic treatment is dependent on various factors including genotype, type of mutagen, the doses employed and many other modifying factors. The response of cells of higher plants to physical and chemical mutagens is influenced to varying degrees by numerous

biological, environmental and chemical factors as reported by Kamra and Brunner (1970). They further added that these factors modify the effectiveness and efficiency of mutagens in higher plants. Though it is not clearly understood why these factors influence mutations and chromosome aberrations, it has been clearly demonstrated that many of these factors must be controlled in mutagen treatment in order to obtain reliable, repeatable and usually optimum results.

Chillies-the attractive red condiment, is now a lucrative commercial crop in India. A native of Latin America, chilli is now cultivated in all parts of the country either as a major crop or in homesteads. Today India is the largest producer and consumer of chillies in the world producing about 5.24 lakh tonnes of dried chillies reaped from nearly 8 lakh hectares. A majority of the types grown in India are of medium pungency. A number of high yielding varieties have been evolved by many research stations in India. Unfortunately almost all the varieties released and recommended for cultivation either for larger scale or for homesteads are highly susceptible to leaf curl complex in all the seasons. The lack of genes responsible for resistance reactions gives a scope

for induced alterations in the existing genotype of this particular crop variety.

The present investigation was taken up as a preliminary trial in the broad area of 'induction of mutations for leaf curl complex resistance in chillies'. The objectives of the present investigation are as follows:

1. to study the effects of gamma rays and EMS in relation to genic status in chillies.
2. to assess the extent of damages created due to the mutagens based on M_1 injury, lethality, sterility and other morphological parameters.
3. to find out the differential response of the varieties to moderate doses of EMS and gamma rays.
4. to find out the medium sensitive varieties under moderate doses of gamma rays and EMS for detailed mutational analysis and
5. to study the general effect of gamma rays on induced variability in various polygenic traits in M_2 generation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The discovery of Muller in 1927 that X-rays could induce genetic changes in *Drosophila* marked the beginning of the era of induced mutagenesis. The utilisation of radiation as a tool for inducing variability in crop plants was first reported by Stadler (1928). After his publication numerous papers appeared in the literature describing the effects of radiation in plants. In recent years radiations like X-rays, gamma rays and neutrons are widely used for induction of mutations. Radiations act at the chromosomal level, causing structural and numerical changes as well as spindle abnormalities or at the molecular level causing changes in the macromolecular structure of DNA.

Soon after the discovery of the mutagenic effects of radiation, search was made for chemicals that would produce cytogenetic changes. Auerbach ^{et al} (1947) was the first to initiate work on chemical mutagens during World War I. With this discovery, workers all over the world started surveying different chemicals for their mutagenic activity. Among the numerous chemicals known, the alkylating agents have been found to be the most efficient in

inducing mutations in a wide range of organisms from bacteria to mammals (Auerbach, 1961). Within this group EMS appears to be more efficient in producing mutations in several organisms including higher plants (Swaminathan et al. 1962). The mutagenic efficiency of EMS was demonstrated by Shrenburg (1960).

The effect of alkylating agents and their mechanism of action in the biological test system have been reviewed by Ross (1962); Loveless (1966); Lawley (1973) and Sun and Singer (1975). High mutagenicity of EMS in barley has been demonstrated by Heslot et al. (1959) and it has been corroborated by Gustafsson (1960) and others. Rao and Natarajan (1965) reported that compared to NMU and MMS, EMS induced higher rates of chlorophyll and viable mutations in M_2 plant basis. The outstanding works of Gustafsson (1963); Yamaguchi and Miah (1964); Kawai and Sato (1965); Konzak et al. (1965); Gaul et al. (1966); Sato and Gaul (1967); Siddiq et al. (1968); Soriano (1968); Kawai (1969) and Mikeelson et al. (1971) gave a clear picture of the research work carried out to study the effectiveness and efficiency of chemical mutagens in various plant species.

Physical and chemical mutagens

Earlier it was hoped to find mutagens which particularly affect specific genes and change them in a desired direction. When it was reported that ionizing radiation acts more or less at random, the hope for gene specificity was directed to chemical mutagens (Micke, 1970). These attempts assumed that particular chemical reactions would take place between a mutagen and a gene which would then result in a particular genetic change only if the right chemical mutagen had been applied. Experimental evidences based on this assumption were reported in micro-organisms and reviewed by Auerbach (1960) and Wegtergaard (1960). However, specific reactions can take place only with the four nuclear bases which are the building bricks of the genes (Micke, 1970). There are also numerous reports to support the fact that the spectrum of induced mutations and of recoverable mutants is not alike if different mutagens are applied (Nilan, 1966; Smith, 1961).

Nilan and Konzak (1961); Ehrenberg et al. (1961) and Gustafsson (1963) reported that the spectrum of chlorophyll deficient mutants may depend on the type of mutagens employed. The reason for such differences

may lie only to a small extent in different mutations induced in the chromosomes. Micke (1970) is of the opinion that many of these differences are the result of a different ratio between gene mutations, small deficiencies and chromosomal aberrations. These reports suggested that it is worthwhile using several mutagens in mutation work as the chances of getting a particular desirable mutant are then increased. As reported by Nilan (1966); Lundquist et al. (1968) and Von Wettstein et al. (1968) the mutation rates of specific loci may also vary depending on the type of mutagens used in addition to other modifying factors.

Kamra and Brunner (1970) reported that in sexually propagated plants, seed treatment using chemical mutagens has yielded very high mutation frequencies and in most cases they are more efficient than ionizing radiations. Studies on rice by Swaminathan (1971) using EMS, gamma rays and fast neutrons have shown that EMS induces a higher frequency of chlorophyll mutants compared to other mutagens. Swaminathan et al. (1962) from their studies on chromosomal aberrations and chlorophyll mutation frequency in barley and wheat, have concluded that in

the evolution of gene placement along chromosomal arms it is likely that linkage groups in which genes without need for recombination are located near the centromeres would have had a selective advantage. The location of genes relating to chlorophyll development in the proximal segments of chromosomes and the high susceptibility of such regions to EMS action may be perhaps the factors involved in the induction of a large number of chlorophyll mutations in EMS treated material." A dose-dependent linear increase in frequency of chlorophyll mutations in both physical and chemical mutagens have also been reported by Siddiq (1967); Siddiq and Swaminathan (1968); Yamaguchi and Miah (1964); Singh (1970) and Nair (1981).

Mutagen dependent effectiveness and efficiency based on chlorophyll segregation in M_2 has also been reported by Kawai and Sato (1965); Sato (1966); Matsuo and Yamaguchi (1967); Ehrenburg et al. (1961); Nair (1981) etc. Swaminathan et al. (1970) showed that the values for effectiveness in neutron treatment were 7-10 times more than that of gamma ray and 2-3 times than for EMS, from a comparison of the effect of different mutagens on rice varieties.

Genic constitution and Radio sensitivity

Much evidence exist that genetic differences even though they are as small as single gene differences, can induce significant changes in radiosensitivity. Gustafsson (1944, 47, 1965); Gustafsson and Tedin (1954); Nilan (1956); Lamprecht (1956 and 1958); Gelin et al. (1958); Smith (1961); Sparrow (1961); Konzak et al. (1961a) and Sparrow et al. (1965) clearly reported that any change in genotypic level can induce significant changes in radiosensitivity which influence not only the total rate but also the spectrum of recoverable mutations. But a clear and specific prediction on the influence of a particular genotype on the mutation spectrum is not available as reported by Mackey (1960a, b). Jagathesan and Swaminathan (1961) and Swaminathan (1965) reported a differential effect of mutagen between species of the same ploidy level and between varieties within the same species in various crops. Enken (1966a and 1966b) concluded that the closer the varieties are in their genotypes, greater is the similarity in their spectra and frequency of mutation. Gregory (1960) stated that "the chief limiting factor in mutation production and mutation

recovery is the genic constitution of the experimental organism and not the type of mutagen used. Thus for the plant breeder, a knowledge of what might be called mutant expectations in his material may be more important than a resolution of the mechanism of mutational change at the submicroscopic level".

Reports on the genotypic level in relation to radiosensitivity are innumerable in cereals but not attempted here. Comparisons among varieties of tomato (Bianchi et al. 1963) barley (Mikaelson and Brunner, 1968) pea (Mukeeb and Siddiqui, 1973) showed variation in respect to radiation among different genotypes indicating the influence of genetic factors, on radiosensitivity. Gamma irradiation of green gram varieties indicated variation in the mutagenic sensitivity in the M_1 generation (Ratnaswamy et al. 1978). Krishnaswami and Rathnam (1982) also reported differential sensitivity to EMS exhibited by ten greengram cultivars. Difference in radiosensitivity was also reported in cucurbits (Vishnoi and Joshi, 1981) Safflower (Mallikarjunaradhya and Channabyregowda, 1981) and Tomato (Georgiov, 1966). In sorghum differential sensitivity to radiations, chemicals and combination treatment was reported by

Sree Ramulu (1970). Mutagen sensitivity has been found to be distinctly different in different races of rice (Fujii, 1962; Kawaii, 1962; Joshua et al. 1965).

As reviewed and reported by Davidson (1960); Konzak (1957); Konzak et al. (1961a and b) and Nilan (1956), the major factors that alter genotypic sensitivity to mutagens include nuclear volume, water content, oxygen pressure, stage of development and hydrogen ion concentration. As these studies are beyond the scope of this investigation, detailed reviews are not included.

Mutagenic effects observable in the first generation

The three main effects of mutagens include physiological damage (primary injury), factor mutations (point mutations or gene mutations) and chromosomal mutations (Chromosomal aberrations). The latter two are transferred to the succeeding generations whereas the primary injury is restricted to the M_1 generation. Plant injury and lethality account for physiological damage and it can be chromosomal or extra-chromosomal in origin. As reported by Gaul (1970) mutagenic treatments with low physiological effects and strong genetic effects are desirable.

Gaul (1959) reported that for a given mutagenic treatment there is correlation between M_1 seedling height and survival on one hand and M_2 mutation frequency on the other hand. Hence a quantitative determination of M_1 injury should be a routine procedure in mutation breeding experiments.

Gaul (1970) listed the following criteria to measure plant injury in the M_1 generation.

1. Seedling height after a particular period of growth.
2. Root length.
3. Emergence under field/laboratory conditions.
4. Survival under field/laboratory conditions.
5. Number of spikes (inflorescence) per plant.
6. Number of seeds per spike.
7. Fruits and/or seeds per plant.

Gaul (1970) reported that with increasing dose the values obtained for each of these biological criteria decrease. As reported by Sparrow (1961) and Gaul (1963, 70) the plant injury may vary depending on the genotype, type of mutagen and doses employed and various other modifying factors. Gaul (1959 and 63) reported a correlation between seedling height and survival and this correlation permits the prediction of the killing rate produced by a definite dose.

Cytological changes are also met with as a result of mutagenic treatments. A general survey of cellular changes due to mutagen treatment has been presented by Sparrow (1961). Catcheside (1945); Darlington and La Cour (1945); Evans (1962); Gustafsson and Von Wettstein (1958); Sparrow (1961) and Swanson (1957) reviewed the types of induced chromosomal mutations; their mitotic and meiotic behaviour and genetic consequences. Using X-rays and thermal neutrons, Caldecott et al. (1954) reported that the frequency of chromosome aberrations is directly proportional to the doses. Gaul (1970) reported that the only type of chromosomal mutation that can be readily recognised in most plants are translocations.

It has been found in barley that the translocation frequency determined by both mitosis and meiosis is the same and increases linearly with radiation dose (Gaul, 1963; Caldecott and Smith, 1952; Caldecott et al. 1954) As reported by Gaul (1970) numerous observations indicate different effects of radiations and chemicals on the production of chromosome mutations. He further concluded that chemical mutagens induce more chromosomal

fragments and fewer chromosome recombinations on comparison with ionizing radiations. Sato and Gaul (1967) reported that EMS produces in barley seeds at least five times more fragments per bridge than X-rays; many fragments and bridges of chromatid type were found after EMS treatment while they were scarcely found after X irradiation.

M_1 sterility is the most easily discernible effect due to mutagen treatment. Sparrow (1961) reported that mutagen induced reduction of reproductive capacity can be due to (1) severe stunting or growth inhibition which prevents flowering (2) flowers are formed, but lack the necessary reproductive structures (3) reproductive structures are present, but pollen is aborted (4) fertilization occurs, but embryos are aborted before maturity or (5) seeds form, but fail to germinate properly or die after germination. Most common is the occurrence of non functional gametes.

Gaul (1970) reported that the mutagen induced sterility may be caused by (1) chromosome mutation (2) factor mutation (3) cytoplasmic mutations and (4) physiological effects. Chromosome mutations are probably the major origin of all mutagen induced

sterility. Muller (1966) concluded that the achievements of increased mutation frequencies is limited by the increased sterility of the M_1 plants and not by the increased M_1 lethality. Gaul and Mittelstenscheid (1960) reported that in certain instances the radiation induced M_1 sterility is transferred into later generations. Most of the radiation induced sterility in M_1 and further generations is probably haplontic according to Muntzing (1930) and EMS induced sterility appears to have a diplontic nature (Sato and Gaul, 1967).

Induced mutation in vegetable crops

In the area of vegetable breeding, most of the work was carried out in crops like Cucumber, Bhindi, Tomato and Chillies. Induced mutation work was carried out by several workers in Cucumber, a commonly cultivated vegetable. Campos and Walderice (1963) studied the effect of ionizing radiations on Cucumis sativus L and Momordica charantia. Roy et al. (1971) conducted irradiation studies in Cucumis sativus L. Whelan (1970) noticed a reduction on seedling emergence in Cucumis sativus following gamma irradiation. Haq and Abidi (1972) studied the effect of gamma irradiation upon

emergence, mortality and survival in M_1 generation of Cucumis and noticed a decreasing trend with increasing dose. Reports on induction of mutations in Bhindi are also available. Patel (1967) isolated dwarf mutants and mutants with altered phyllotaxis in Okra using x-rays. Kuwada (1972) observed plants with increased number of nodes and pods in the X_2 generation and obtained very promising lines in the X_{10} generation. Nandpuri et al. (1970) observed increased variation in plant height, number of days taken for flowering and yield in the R_1 generation, after irradiations in Okra using gamma rays. They isolated bushy type mutants from the above line. Yashivar (1975) obtained some quantitative mutations in Okra after irradiation studies. Rao and Raj (1976) studied the effects of X-rays and ^{60}Co -gamma rays on morphological characteristics in Bhindi. Koshy and Abraham (1978) reported the developmental and morphological abnormalities in Okra following treatment with ^{60}Co -gamma rays. Jehangir and Chandrasekhar (1978) undertook a study to observe comparative mutagenic effects of gamma rays and DES in Bhindi. In the family Solanaceae to which chilli belongs, most of the work was done in tomato and chilli. A good amount of induced mutation work was undertaken by

several workers in tomato. Mac Arthur (1934) obtained mutations in tomato using X-rays. Flower colour change was noticed in a population raised from X-ray treatment of tomato seeds by Young (1940). Lesley and Lesley (1956) isolated many useful mutants in tomato after treatments using X-rays and Yagyu and Morris (1957) studied the cytogenetic effects of X-rays and thermal neutrons on dormant tomato seeds. Verkerk (1959) isolated mutants after neutron irradiation in tomato. Davies (1962) studied the genetic control of radio-sensitivity in tomato using growth measurements and other characters. Nettencourt and Constant (1966) made a comparative study of the effects of chronic gamma irradiation in tomato. Early maturing tomato mutants were isolated by Brock (1966). Jain et al. (1968) analysed the mutations induced in tomato by base specific chemicals. Bose and Maiti (1973) studied the cytogenetic effects of pre and post irradiation treatments with Colchicine and diethyl sulphate in tomato in M_2 generation and isolated very interesting floral, dwarf and sterile mutants. Dhesi and Nandpuri (1964) studied the effects of irradiation on tomato. The comparative mutagenic efficiency of radiations and EMS

in *Lycopersicon* was studied by Majid (1975). Nushikyan (1976) conducted a study on the experimental use of chemical and physical mutagens in tomato breeding. Rao and Rao (1977) studied the phenomenon of gamma ray induced meiotic stickiness in tomato. Kaushik and Kalloo (1979) studied the variability as induced through gamma rays and EMS in some genotypes of tomato. Raghuvanshi et al. (1979) worked on some useful induced mutations in tomato and chilli.

Work on conventional breeding technique in chillies are innumerable. But the more modern and advanced technique of mutation breeding has not received much attention in this crop when compared to crops like cereals and legumes. Studies on the induction of mutations in chillies started even before the middle of the present century. Raghavan and Venkitasubban (1970) studied the cytology of some X-ray derivatives of Capsicum annuum L. Campos and Morgan (1960) studied the genetic control of haploidy in C.frutescens following crosses with X-rayed pollen. Murthy et al. (1968) conducted studies on colchicine induced auto tetraploid in G_3 chilli. A study of the cytological changes in Capsicum

annuum under the influence of chemical mutagens EI, NMU, NEU was conducted by Galukyan (1968). Mutant lines of Capsicum with high yield and good productivity in the first crop were isolated by Videnin and Skripnikova (1970) Iqbal (1969, 1970, 1972) studied the extent of cellular damage and responses of shoot apices subsequent to radiation damage and effect on survival, growth and radio sensitivity of apical meristems of Capsicum annuum. Sahib and Abraham (1970) studied the biological effect of X-rays on K_1 variety of chillies and studied the morphological abnormalities and chromosomal aberrations induced in M_1 . Frequency of chlorophyll mutations in M_2 were also observed by them. Zubrzycki and Pahlen (1973) compared the effects of EMS and X-rays in the induction of mutations in Capsicum annuum. Bensal and Singh (1972) studied a polypetalous mutant of NP 46 A, which breeds true and which was induced by X-rays. Bensal (1973) discovered a mutation in C. annuum Var Np 46 A, in which reproductive parts were transformed to vegetative ones by treatment with EMS and NMU. Subash and Nizam (1973) conducted a preliminary study on the effect of X-ray irradiation on Capsicum annuum and

observed that the period from germination to seed production was reduced from 150 days in the control to 120-130 days in the irradiated material. The effect of X-rays on the mitotic activity and frequency of structural rearrangements in the chromosomes in root cells of the species Capsicum annum was studied by Terzyan et al. (1974). The effectiveness of treating pepper seeds with N-nitroso N-methyl urea was studied by Gukasyan and Akopyan (1974). Subash and Nizam (1974, 75) isolated multicarpellate mutants and aneuploids following treatments with X-rays, r-rays and neutrons in the promising variety of Np-46 A. They discovered that treatment with EI, N-nitroso N-methyl urea and dms, increased the variation in yield in three varieties. Gukasyan and Tamanyan (1976) treated chilli seeds with N-nitroso N-methyl urea and reported that frequency of aberrant cells increased in the year of treatment and several successive generations. Skripnikova (1976) found that forms of interest to the breeders often appeared in treatments with low doses of N-nitroso N-methyl urea. Katiyar (1977, 78) has reported meiotic abnormalities, pollen sterility and desynaptic behaviour in M_1 and M_2 generations in chillies induced by r-rays.

Khan et al. (1979) studied the effect of gamma irradiation on the epidermis of chillies. Rao and Lakshmi (1980) studied the meiotic abnormalities after mutagen treatment which was proportional to the dosage. Ramalingam (76, 77, 80) conducted studies on induced variation in chillies using physical and chemical mutagens. He also isolated two sterile mutants which exhibited desynapsis, after treatments of seeds with N-nitroso N-methyl urea and EMS. He also studied the frequency and spectrum of induced mutation in chillies. Indira and Abraham (1977, 80) conducted induced mutation studies on a purple flowered and purple fruited variety of Capsicum annum L. Subash and Nizam (1977) also studied the meiotic anomalies induced by X-rays in chillies. Khuspe and Ugale (1977) conducted a study on the growth and fruit development in Capsicum annum after treatment with ^{60}Co -gamma rays and EMS. Sonone et al. (1978) conducted cytological studies in natural triploid plant in chilli. Cytogenetical studies in the genus Capsicum has been conducted by Sahrigy and Seehy (1978). Thombre and Mahetre (1979) conducted cytological study in a haploid of red pepper. Murthi and Lakshmi (1980) conducted cytomorphological studies in spontaneous partial

desynaptic mutants in chilli. Patel and Mesharam (1981) studied induced qualitative variation in economic characters by chemical mutagens in red pepper and found that variation in seven characters were increased in the M_2 following treatment with EMS and dms. Sadanandam et al. (1981) isolated a desynaptic mutant in Capsicum induced by the EMS which was phenotypically normal but completely sterile.

Induced mutations and polygenic characters

Almost all economically important characters in plants are known to be governed by polygenes. The expression 'MICRO-MUTATION' is used to mean mutations in polygenes governing quantitative characters leading to small changes in phenotypes. The significance of mutations with small effects in evolution, varietal differentiation and speciation has long been emphasized by students of plant and animal breeding, evolution and genetics. East (1935) has pointed out that the deviations forming the fundamental materials of evolution are the small variations mentioned by Darwin. Baur (1924) in his paper on the means, origin and inheritance of racial differences in Antirrhinum introduced the term "Klein-mutationen" which

Gregory (1969) interpreted as synonymous with micro-mutations. But the first convincing report that physical mutagens like X-rays can induce new genetic variability in quantitative traits was presented by Buzzati Traverso (1955) in Drosophila. The possible role of small mutations in plant breeding became apparent soon.

Gregory's work on Arachis hypogaea (1955, 1956 a,b, 1957, 1961) has clearly shown that "samples of irradiated populations which include only the normal types of intra-varietal variation showed significantly greater multifactorial variability in yield than untreated populations". He found that selection was successful in leading to lines with better production. Experiments of Humphery (1954) and Rawlings et al. (1958) on induced mutations in soyabean clearly showed that the estimates of genetic variations for yield, plant height, maturity time and seed size on the average were five times as large as those of the controls, giving a better chance for selection.

Scossiroli (1966 a) reported that it would be normal to observe some decrease in mean values or quantitative traits measured in the normal looking plants as compared with control since the majority of the small mutations induced would be detrimental. As a general rule, induced

mutations can be successfully used to create any sort of useful variations in quantitatively inherited characters. The classical works of Brock (1957) and Gregory (1968) on improvement on yield and Gustafsson (1965) on adaptability, Brock (1970) on maturity time and sigurbjornsson and Micke (1969) on numerous other traits provide example to this.

The increased variability in mutagen treated population is found to be largely due to increase in genetic components as reported by Brock et al. (1972) and Gaul et al. (1972) and Scossiroli et al. (1966). X-ray and Neutron treatments on soyabeans by Humphrey (1954); and Rawlings et al. (1958) resulted in an increase in genetic variability for yield, plant height, maturity and seed size, oil and protein content. Borojevic and Borojevic (1968) reported that genetic variability for several quantitative characters increased in irradiated population of Triticum aestivum. Improved yield due to selections in irradiated populations have been reported in Barley by Gaul (1961, 65) and in durum wheat by Bogyo et al. (1969).

Reviews on induced micro-mutations by Gaul (1965) and the experiments on selection performed on irradiated populations by Oka et al. (1958); Borojevic (1965); Brock and Latter (1961); Goud (1967) and Scossioli (1965, 1966 a b) gave a clear picture of the role and importance of induced mutations in different crop plants from a plant breeding point of view.

MATERIALS AND METHODS

MATERIALS AND METHODS

Selection of seed material

Twenty varieties of chillies (Capsicum annuum L.) were tested for the present investigation. The details are given in Table 1. The direct effect of the mutagens, ^{60}Co -gamma rays and Ethyl Methane Sulphonate (EMS) on these genotypes was assessed in the M_1 generation with respect to various growth metrics. Genetically pure, well developed seeds obtained from fully ripened fruits of healthy plants, were used for the study. Uniformly dried, healthy seeds having same size and colour, were selected for mutagenic treatment. As the germination percentage for three entries were very poor, data from seventeen varieties were collected and are included in this report. Gamma ray exposed seeds of the varieties, Pant C₁ and Black Suryamukhi along with the control, were carried forward to the second generation to assess the extent of induced variability for various polygenic traits.

Gamma irradiation

Two hundred seeds of each variety were exposed to 20 and 30 kR gamma rays, using a ^{60}Co -gamma shine unit

Table 1. Details of varieties tested for Mutagen sensitivity

<u>No.</u>	<u>Name of variety</u>	<u>Source</u>
1.	CA-52	College of Horticulture Vellanikkara.
2.	CA-99	-do-
3.	Vellanochi	Local
4.	Pant C ₁	College of Agriculture, Vellayani.
5.	K ₂	-do-
6.	G ₄	-do-
7.	CA-47	College of Horticulture, Vellanikkara
8.	CA-48	-do-
9.	Black Suryamukhi	College of Agriculture, Vellayani.
10.	White Kantari	-do-
11.	Blue pendent	-do-
12.	Kantari	-do-
13.	CA-30	College of Horticulture, Vellanikkara.
14.	CA-53	-do-
15.	CA-68	-do-
16.	CA-94	-do-
17.	CA-151	-do-
18.	CA-152	
19.	CA-154	
20.	CA-150	

installed at the Department of Botany, University of Kerala, Kariavottom, Trivandrum. The dose rate employed being 30 kR/hr. The irradiated seeds were sown in pots on the following day of treatment.

EMS treatment

EMS solutions of 0.5 and 1.0 per cent concentrations were prepared in glass distilled water immediately before use and adjusted to neutral pH using phosphate buffer. Two hundred seeds of each variety were used for the treatment. Seeds pre-soaked in distilled water for twelve hours were treated with the above two concentrations of EMS. Before treatment, special care was taken to remove the superficial water on the seeds by gently pressing pre-soaked seeds within the folds of a blotting paper.

The pre-soaked seeds were immersed in the mutagen solution for four hours with intermittent shaking. To facilitate uniform absorption of the mutagen by the seeds, 20 ml of the solution was used, approximately ten times the volume of the seed. The solution was maintained at room temperature throughout the period of treatment.

After treatment, the seeds were washed thoroughly in distilled water and kept in running water for two hours.

Planting technique

The two sets of gamma-ray treated and control seeds soaked for twelve hours, and the EMS treated seeds were sown in pots in 2 replications of 100 seeds each. As far as possible, uniform potting mixture was filled in all the pots. The potting mixture was prepared with cowdung, riversand and soil. Seedlings randomly selected from these pots were transplanted on to the main field on the 30th day of sowing. A spacing of 60 cms between rows and 30 cms between plants was given to the seedlings. Special care was taken to provide uniform field conditions for these plants till harvest. The crop was maintained following Package of practices recommended by the Kerala Agricultural University. Fertilizer applications were done at the rate of 75 kg, 40 kg and 20 kg of NPK per hectare. Half the recommended dose of N and K and full dose of P was given at the time of transplanting. Further fertilizer application with N and K, (37.5 kg N, 10 kg K) was made one month after transplanting. This moderate spacing and fertilizer status was given to check excessive growth. All the field experiments in this study relating to M_1 and M_2 were conducted in the experimental area attached to the Department of Agricultural Botany, College of Agriculture, Vellayani.

Direct effect of the mutagens on the M₁ generation

The effect of gamma rays and EMS on the various genotypes were studied with respect to different growth metrics. It included

1. Germination percentage.
2. Days taken to complete germination.
3. Seedling survival on the 30th day.
4. Number of leaves, shoot and root length on the 30th day.
5. Plant height at 15 days interval.
6. Number of branches per plant, 45, 60 and 75 days after transplanting
7. Days taken to first flower opening.
8. Days taken to harvest from date of sowing.
9. Pollen sterility, and
10. The number of fruits per plant.

Germination

Germination counts in the different treatments were taken from the seventh day of sowing. Total germination percentage was estimated from the values taken on the day after which no further germination was observed. The number of seeds germinated were expressed as percentage values.

Survival of seedlings

Survival of seedlings was determined on the 30th day after sowing. The number of seedlings survived per treatment was counted and expressed as percentage values.

Seedling height

The height of seedlings, 20 days after sowing and at transplanting (30 days after sowing) were measured. Measurements were taken in cms from the soil level to the tip of the shoot. Ten plants selected randomly per replication in each treatment were measured and the average for each dose was calculated.

Number of leaves, root length and shoot length

Observations were taken from a sample of plants randomly selected from each treatment including control. Total number of leaves per seedling were counted and the average taken. The root length and shoot length were measured, as the distance from the demarkating point between the root and shoot to the tip of root and shoot respectively.

Plant height

Plant height was determined at 5 stages of growth at an interval of 15 days namely 30th, 45th, 60th, 75th and 90th days after transplanting.

Number of branches

Number of branches produced per plant was also studied at three stages of plant growth i.e. 45th, 60th and 75th day after transplanting. Primary, secondary and tertiary branches were counted and added together.

Days to flowering

The number of days taken for flowering was calculated from the date of sowing to the date of first flower opening on each plant.

Pollen sterility

Pollen sterility analysis was done using acetocarmine staining technique. Pollen grains collected from flowers at the time of anthesis, stained in acetocarmine-glycerine stain and studied. A minimum of ten microscopic fields were observed from each slide and three slides per treatment per replication was studied. Pollen grains of uniform size and shape and evenly stained were considered as fertile while the pollen which were not stained and of unequal size and shrivelled were counted as sterile. Pollen sterility was calculated as the number of sterile pollen divided by the total number of pollen (sterile and fertile) and expressed in percentage.

Days to maturity

The date at which the first mature fruit was harvested was recorded for each treatment and the duration from date of sowing till date of first harvesting calculated.

Number of fruits per plant

Data on the mean number of fruits produced per plant was studied in the M_1 generation. The total number of fruits produced per plant was taken and the mean calculated.

Observations in M_2 plants

The flowers of ten selected M_1 plants from each treatment were selfed and the seeds extracted from fully ripened fruits. The seeds were uniformly dried and sown plant-wise one month after extraction in pots with four replications. One hundred seeds representing ten plants in each of the treatments including control were sown in each replication. On the 30th day a maximum of fifty seedlings per treatment per replication were transplanted in singles in main field with a spacing of 60 x 30 cms in four replications. The fertilizer dose and mode of applications and crop management were as recommended in package of practices. Special care was taken to provide uniform field conditions for the entire crop till harvest. The following observations were taken in M_2 .

Observations on quantitative characters in M_2 were made following the same technique as for M_1 generation, excluding the border plants and morphologically abnormal ones. In M_2 generation fruit character observations were also taken following the techniques noted below.

Weight of fruits

Fruit weight was determined from fresh ripe fruits in the M_2 generation. Ten randomly selected fruits per treatment were weighed and the mean weight calculated in gms.

Length of fruits

The length of fruits was measured as the distance from the point of attachment to the tip. A sample of ten fruits per treatment was measured and the mean calculated.

No. of seeds per fruit

The seeds were extracted from dry ripe fruits and the number of seeds per fruit counted.

Statistical analysis

Analysis of variance of the data was done following Fischer (1935). Percentage values were transformed by the angular transformation as proposed by Snedecor (1956). There were five treatments, namely two doses each of gamma

1. Chlorophyll mutation frequency:

The chlorophyll deficient mutants were screened out on the 20th day of sowing and their frequency calculated. Due to lack of different types of chlorophyll mutants, the spectrum was not taken into consideration.

2. Viable mutations

Gamma ray treated and control plants were subjected to periodical observations and the visual variants were scored.

3. Quantitative mutations

Detailed observations on quantitative traits like

1. Plant height on 75th and 90th day of transplanting
2. Number of branches/plant " "
3. Number of fruits/plant
4. Weight of fruits
5. Length of fruits
6. Yield per plant
7. No.of seeds/fruit
8. Days taken for flowering and
9. Days taken to harvesting were made and data analysed.

Observations on quantitative characters in M_2 were made following the same technique as for M_1 generation, excluding the border plants and morphologically abnormal ones. In M_2 generation fruit character observations were also taken following the techniques noted below.

Weight of fruits

Fruit weight was determined from fresh ripe fruits in the M_2 generation. Ten randomly selected fruits per treatment were weighed and the mean weight calculated in gms.

Length of fruits

The length of fruits was measured as the distance from the point of attachment to the tip. A sample of ten fruits per treatment was measured and the mean calculated.

No. of seeds per fruit

The seeds were extracted from dry ripe fruits and the number of seeds per fruit counted.

Statistical analysis

Analysis of variance of the data was done following Fischer (1935). Percentage values were transformed by the angular transformation as proposed by Snedecor (1956). There were five treatments, namely two doses each of gamma

rays and EMS and control, seventeen varieties and two replications. The outline of the analysis of variance table showing the source of variations and corresponding degrees of freedom is given below:

Source	degrees of freedom
Total	169
Block	1
Treatment	4
Varieties	16
Interaction	64
Error	84

The M_2 data for various quantitative characters were analysed, using the analysis of variance table. In this case only two varieties and two exposures of gamma rays, along with the control were considered. The outline of the analysis of variance table is as follows:

Source	degrees of freedom
Total	23
Block	3
Variety	1
Treatment	2
Interaction	2
Error	15

RESULTS

RESULTS

GERMINATION PERCENTAGE

The influence of varieties, mutagens and their doses on the germination percentage of seeds is presented in Table 2. The statistical analysis of the data showed significant variation among varieties, mutagens and their interactions. A variety dependent variation in germination was noticed both in control and treated materials. The germination percentage in control ranged from 24 to 72.5 in the varieties V_{13} and V_{14} respectively. EMS showed a drastic reduction in germination compared to gamma ray exposures. In majority of cases higher doses gave a greater reduction in germination compared to lower doses. The maximum reduction in germination was noted in 1 per cent concentration of EMS in almost all the varieties. In the varieties V_{11} and V_{13} exposures of gamma ray had a stimulating effect on germination while the germination was very poor in the untreated seeds. Gamma ray exposed seeds showed a range in value of germination percentage from 13.5 (V_{13}) to 64.5 (V_{14}) under 20 kR and 29.0 (V_{13}) to 64.0 (V_{14}) under 30 kR exposures. The range in value for germination percentage under EMS treat-

Table 2. Germination percentage

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	26.5	29.0	25.0	24.5	18.5	24.7
V ₂	43.5	48.0	34.0	23.5	18.5	33.5
V ₃	39.5	36.0	24.5	27.0	23.0	30.0
V ₄	41.0	56.5	46.5	23.5	30.0	39.5
V ₅	44.5	40.5	41.5	7.5	7.0	28.2
V ₆	33.5	35.0	36.0	11.5	13.0	25.8
V ₇	28.0	29.5	25.0	6.5	9.5	19.7
V ₈	52.0	51.5	59.0	15.5	12.5	38.1
V ₉	68.0	58.0	47.5	26.5	25.0	45.0
V ₁₀	49.0	60.0	44.5	19.5	8.5	36.3
V ₁₁	25.5	37.0	29.5	28.5	25.5	48.67
V ₁₂	83.5	60.5	51.0	19.0	8.0	44.4
V ₁₃	24.0	13.5	29.0	9.0	9.0	16.9
V ₁₄	72.5	64.5	64.0	54.5	39.5	59.0
V ₁₅	62.5	55.5	48.0	26.5	23.0	43.1
V ₁₆	56.5	52.5	57.0	38.0	36.0	47.9
V ₁₇	39.5	31.5	28.5	13.0	14.0	25.2
General Mean	46.38	44.65	40.62	22.0	18.85	

	<u>F values</u>	<u>CD values</u>
Variety	44.29*	4.88
Treatment	194.78*	2.64
Interaction	5.41	10.92

* Significant at 5% level of significance

ments was 6.5 (V_7) to 54.5 (V_{14}) in 0.5 per cent and 7.0 (V_5) to 39.5 (V_{14}) in 1 per cent concentration.

DAYS TAKEN FOR MAXIMUM GERMINATION

Data regarding the effect of gamma rays and EMS on the number of days taken to achieve maximum germination is given in Table 3. Significant delay in germination was seen in both the exposures of gamma rays and EMS compared to the control. Greater delay in germination was shown by EMS compared to gamma rays. Delay in germination was seen to be dose dependent. Higher the exposure, greater was the delay in germination. In majority of the varieties tested, delay in germination due to lower concentration of EMS was even greater than due to the higher dose of gamma rays.

When the number of days taken to complete germination in the control plants ranged from 10 to 16, it was 11 to 20 in 20 kR, 12 to 29 in 30 kR, 11 to 25.5 in 0.5 per cent and 12 to 28.5 in 1 per cent EMS concentration. The minimum number of days taken for germination in both gamma ray and EMS treatments were comparable. It was observed that the maximum delay in germination for both doses of gamma rays was shown by the same variety V_{12} while in the case of EMS, variety V_{10} showed the maximum

Table 3. Days taken for maximum germination

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	11.0	11.5	16.0	11.5	15.5	13.1
V ₂	11.0	12.5	15.0	14.5	17.5	14.1
V ₃	11.0	11.0	15.0	11.0	12.0	12.0
V ₄	12.5	12.0	15.0	14.0	17.5	14.2
V ₅	10.0	11.0	13.5	15.5	17.5	13.5
V ₆	15.5	12.5	13.5	16.0	18.0	15.1
V ₇	12.5	14.0	15.0	16.0	17.5	15.0
V ₈	11.0	11.0	12.0	15.5	17.5	13.4
V ₉	10.5	12.0	13.0	16.0	17.5	13.8
V ₁₀	12.5	13.0	12.0	25.5	29.5	18.3
V ₁₁	16.0	17.5	16.5	16.0	19.0	17.0
V ₁₂	15.5	20.0	29.0	17.0	25.0	21.3
V ₁₃	14.5	19.5	20.0	16.5	18.0	17.7
V ₁₄	10.5	14.5	15.5	17.0	18.0	15.1
V ₁₅	13.0	14.5	15.5	16.5	17.5	15.4
V ₁₆	14.5	15.5	14.5	16.0	23.0	16.7
V ₁₇	13.0	14.0	19.0	16.5	17.5	16.0
General Mean	12.62	13.88	15.88	15.94	18.65	

	<u>F value</u>	<u>CD value</u>
Variety	20.57*	1.42
Treatment	70.65*	0.78
Interaction	4.83*	3.18

* Significant at 5 per cent level of significance.

delay in germination for both the concentrations. A dose-dependent variation was noted for almost all the varieties. As the dose increased, the number of days taken for germination also increased. It was noted that in variety V₆, both doses of gamma rays induced an early germination compared to the control. In the case of V₁₂, 30 kR gave the maximum delay in germination.

SURVIVAL AT SEEDLING STAGE (30 days after sowing)

Effect of mutagens on the survival at seedling stage is given in Table 4. A general reduction in the survival percentage was noticed in the treated population compared to the control. When the general mean in the control showed a survival percentage of 35.53, it was 34.74 and 33.79 in gamma ray exposures, and 18.26 and 16.11 in EMS treatment, which is almost half to that of the control value. The maximum reduction was seen in the case of 1 per cent EMS treatment. The percentage of lethality was more in EMS treatment than in gamma ray exposures.

The survival percentage ranged from 18 to 69.5 in control, 9.5 to 55.5 in 20 kR, 15 to 56.5 in 30 kR, 70 to 40.5 in 0.5 per cent and 7.5 to 38.5 in 1 per cent EMS.

Table 4. Survival at seedling stage (30 days after sowing)

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	18.5	20.0	22.5	20.5	15.5	19.4
V ₂	33.5	36.0	30.5	15.0	12.5	25.5
V ₃	29.0	32.0	21.0	31.0	15.0	25.6
V ₄	17.5	34.0	33.5	14.5	21.0	24.1
V ₅	32.0	21.0	33.0	7.0	7.5	20.1
V ₆	31.5	26.0	30.5	5.5	8.5	20.4
V ₇	18.5	23.0	17.5	5.5	9.0	14.7
V ₈	34.5	27.0	47.5	11.5	11.0	26.3
V ₉	51.0	32.5	45.0	22.5	23.0	38.8
V ₁₀	35.0	48.5	28.0	18.5	8.5	27.7
V ₁₁	25.0	41.0	25.5	25.0	23.5	26.0
V ₁₂	60.0	55.5	51.0	18.5	8.5	38.7
V ₁₃	18.0	9.5	15.0	6.5	7.5	11.3
V ₁₄	69.5	53.5	56.5	40.5	38.5	51.7
V ₁₅	51.0	52.0	41.5	20.0	19.5	36.8
V ₁₆	49.0	41.5	51.5	36.0	31.5	41.9
V ₁₇	30.5	27.5	24.5	12.5	13.5	21.7
General Mean	35.53	34.74	33.79	18.26	16.11	

	<u>F value</u>	<u>CD value</u>
Variety -	39.6*	4.75
Treatment -	111.94*	2.57
Interaction -	3.97*	10.6

* Significant at 5% level of significance

A sharp decline in the percentage of survival was seen in the EMS treatments when compared to control and gamma ray treatments in most of the varieties tested. In varieties V_1 , V_4 and V_{11} gamma ray exposures induced a greater survival percentage than the control.

HEIGHT OF SEEDLINGS (20 days after sowing)

Data regarding the height of seedlings 20 days after sowing, is represented in Table 5. The statistical analysis showed a significant difference among varieties, treatments and their interactions. The reduction in height was more in the case of gamma ray treatments than in EMS treatments, though both treatments were significantly different from the control. The effect of both the doses of EMS on the seedling height was comparable. A dose dependence could be noticed in that higher the dose less was the height. The higher dose of gamma rays induced a reduction almost half to that of the control plants. Differences in height were also significant among the various varieties tested. Variety V_{15} showed the maximum height at 20 days after sowing (4.71 cm) followed closely by variety V_1 (4.54 cm).

In control plants the height (in cm) ranged from 2.25 to 7.0 while in 20 kR it was 1.75 to 6.3 and 1.35 to 5.4 in 30 kR. In the case of EMS, it ranged from 2.1 to 5.1

Table 5. Height of seedlings (cm) (20 days after sowing)

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	4.75	3.5	3.0	5.0	6.45	4.54
V ₂	4.50	3.25	2.5	4.25	6.05	4.21
V ₃	4.25	2.75	2.0	4.20	5.4	3.82
V ₄	4.0	2.75	2.0	2.75	2.5	2.80
V ₅	5.50	3.5	2.75	3.45	3.5	3.74
V ₆	5.75	2.5	2.0	5.1	2.55	3.58
V ₇	3.25	2.25	1.75	2.9	2.35	2.50
V ₈	4.25	2.50	2.0	2.8	2.75	2.96
V ₉	2.50	1.75	1.75	3.45	2.40	2.37
V ₁₀	2.50	1.75	1.75	4.6	2.20	2.56
V ₁₁	2.25	2.0	1.75	3.9	4.45	2.87
V ₁₂	4.25	2.8	1.35	3.25	2.55	2.84
V ₁₃	4.85	4.3	2.15	2.1	2.20	3.12
V ₁₄	6.85	5.1	4.3	2.55	3.20	4.40
V ₁₅	6.85	6.3	4.55	3.9	1.95	4.71
V ₁₆	7.0	3.5	5.40	3.2	2.50	4.32
V ₁₇	6.3	5.3	4.25	2.15	3.25	4.25
General Mean	4.74	3.28	2.66	3.53	3.31	

		<u>F value</u>	<u>CD value</u>
Variety	-	25.44*	0.45
Treatment	-	79.16*	0.24
Interaction	-	10.44*	1.0

* Significant at 5% level of significance

in 0.5 per cent and 1.95 to 6.45 in 1 per cent concentrations. The minimum height was noticed in 30 kR treatment in variety V_{12} . In varieties V_1 , V_2 , V_3 and V_{11} it was observed that EMS treatments increased the plant height.

HEIGHT OF SEEDLINGS (30 days after sowing)

Table 6 represents the height of seedlings on the 30th day of sowing. There was significant difference among varieties, treatments and in their interactions. The height was significantly reduced in the treatments compared to control. The reduction in height was found to be more in the case of gamma ray exposures than EMS. The lower doses of gamma rays induced a height comparable to both the EMS treatments. A dose dependence could be noticed in the case of gamma ray exposures; higher dose of gamma rays reduced the height in almost all the varieties.

The height in the control plants ranged from 3 to 14 cm, while in 20 kR it was 2.25 to 11.95 cm and in 30 kR 1.75 to 11.6 cm. In the case of EMS treatments it ranged from 2.8 to 9.35 cm in 0.5 per cent and 1.95 to 15.7 cm in 1 per cent concentrations. The minimum height was comparable in all the treatments. The minimum height in the gamma ray treatments was observed in a variety which had a

Table 6. Height of seedlings (cm) (30 days after sowing)

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	4.5	3.85	2.75	9.35	8.3	5.75
V ₂	7.0	3.75	3.50	8.1	11.35	6.74
V ₃	6.35	4.0	3.25	6.5	10.1	6.04
V ₄	3.75	3.0	2.75	4.0	15.7	5.84
V ₅	9.25	3.5	3.25	4.9	4.4	5.06
V ₆	9.75	3.0	3.0	7.8	2.45	5.20
V ₇	4.75	2.75	2.0	3.75	1.95	3.04
V ₈	5.75	2.75	4.0	2.95	2.50	3.59
V ₉	6.25	2.5	2.75	6.0	3.95	4.29
V ₁₀	3.0	2.25	1.75	5.5	2.95	3.09
V ₁₁	3.0	2.5	2.25	8.7	12.35	5.76
V ₁₂	6.5	4.55	2.05	2.8	4.1	4.00
V ₁₃	8.2	6.7	4.2	3.15	2.45	4.94
V ₁₄	13.8	10.9	9.05	3.45	4.05	8.25
V ₁₅	12.9	11.95	9.1	7.7	3.9	9.11
V ₁₆	14.0	10.9	11.6	5.0	4.15	9.13
V ₁₇	13.35	11.05	9.2	4.35	4.8	8.65
General Mean	7.8	5.29	4.5	5.53	5.85	

	F value	CD value
Variety	- 21.43*	1.22
Treatment	- 27.43*	0.66
Interaction	- 10.32*	2.74

* Significant at 5% level of significance

very low height in control plants (3 cm) whereas in the case of EMS treatments, the minimum height was observed in plants with medium height. In most of the varieties gamma rays induced more reduction in height than EMS treatments. In some cases, it was seen that 1 per cent concentration of EMS induced a height much greater than the control plants. The maximum height of seedlings (15.7 cm) was noted in 1 per cent EMS treatment in variety V_4 which had a very low height in the control (3.75 cm).

NUMBER OF LEAVES AT TRANSPLANTING (30 days after sowing)

Table 7 represents the data regarding number of leaves per seedling at the time of transplanting, as affected by gamma ray and EMS treatments. The treatments differed significantly from the control. The varieties showed a significant difference, as also the interaction between varieties and treatments. The maximum reduction in the number of leaves was noticed in the higher dose of EMS, where the number of leaves was almost half of the control plants. The effect of the lower dose of EMS was comparable to the effect of both the doses of gamma rays. Variety V_3 showed the maximum number of leaves among the varieties tested while the minimum number of leaves was noticed in the variety V_{12} .

Table 7. No. of leaves at transplanting (30 days after sowing)

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	9.5	9.0	8.5	5.5	4.5	7.4
V ₂	11.5	7.0	7.0	10.5	9.0	9.0
V ₃	20.0	9.0	7.5	7.5	7.0	10.2
V ₄	7.5	6.0	6.0	8.0	7.0	6.9
V ₅	10.5	6.5	7.0	7.5	6.5	7.6
V ₆	15.5	7.0	6.5	7.5	4.5	8.2
V ₇	9.5	8.0	7.0	7.5	4.5	7.3
V ₈	11.5	10.0	8.5	7.5	5.5	8.6
V ₉	10.0	6.0	8.0	9.5	8.0	8.3
V ₁₀	7.5	5.5	5.0	7.5	4.0	5.9
V ₁₁	9.5	6.5	6.5	7.5	8.5	7.7
V ₁₂	8.0	5.5	4.5	6.5	4.0	5.7
V ₁₃	14.5	11.5	9.5	5.5	4.5	9.1
V ₁₄	11.5	9.5	8.5	5.5	5.5	3.1
V ₁₅	9.5	9.0	7.5	7.0	7.0	8.0
V ₁₆	10.5	10.5	9.0	7.0	5.0	8.4
V ₁₇	10.5	8.5	7.5	7.0	6.0	7.9
General Mean	11.0	7.94	7.29	7.32	5.94	

		<u>F value</u>	<u>CD value</u>
Variety	-	5.1*	1.38
Treatment	-	50.11*	0.74
Interaction	-	2.76*	3.1

* Significant at 5% level of significance

The number of leaves in the control population ranged from 7.5 to 20 whereas it was 5.5 to 11.5 in 20 kR, 4.5 to 9.5 in 30 kR, 5.5 to 10.5 in 0.5 per cent and 4 to 9.0 in 1 per cent EMS concentration. In all the varieties 1 per cent EMS treatment showed the minimum number of leaves. A dose dependent reduction in number of leaves was seen for both the mutagens. As the mutagen dose increased, the number of leaves were reduced.

ROOT LENGTH AT TRANSPLANTING (30 days after sowing)

Length of roots at the time of transplanting is represented in Table 8. Statistical analysis of the data showed significant variation among different varieties, mutagens, and in their interactions. The reduction in root length was more under EMS treatments than the gamma ray exposures. When EMS induced about 50 per cent reduction in root length compared to control, gamma rays gave only about twenty five per cent reduction. Reduction in length was dependent on the dose of the mutagens. Higher the dose, greater was the reduction in root length. The maximum reduction in length was noted under 1 per cent concentration of EMS in all the varieties tested.

Table 8. Root length of seedlings at transplanting
(30 days after sowing)

Variety	Control	Gamma rays		EMS		General Mean
		20 k R	30 kR	0.5%	1.0%	
V ₁	12.70	7.75	6.70	4.35	4.10	7.12
V ₂	9.65	6.40	7.15	4.45	4.80	6.49
V ₃	11.25	8.25	7.75	3.65	3.30	6.84
V ₄	7.50	5.75	6.50	5.15	4.20	5.82
V ₅	9.50	5.75	7.00	5.90	4.25	6.48
V ₆	12.25	5.70	3.55	4.40	3.30	5.84
V ₇	12.50	6.40	6.00	3.60	3.30	6.36
V ₈	12.25	6.10	6.60	4.40	3.20	6.51
V ₉	9.25	5.60	5.00	5.45	5.60	6.18
V ₁₀	8.35	5.25	4.70	11.70	4.95	6.99
V ₁₁	6.00	6.20	5.20	4.85	3.05	5.06
V ₁₂	9.90	10.50	9.70	10.90	3.95	8.99
V ₁₃	15.40	12.15	11.45	6.30	4.55	9.97
V ₁₄	12.15	9.90	9.25	5.5	4.75	8.81
V ₁₅	13.25	10.45	9.55	4.15	4.30	8.34
V ₁₆	12.30	10.85	10.50	5.00	4.25	8.58
V ₁₇	10.05	8.20	8.15	5.20	3.85	7.09
General Mean	10.84	7.72	7.34	5.59	4.10	

	<u>F value</u>	<u>CD value</u>
Variety -	7.62*	1.32
Treatment -	98.87*	0.72
Interaction -	2.86*	2.98

* Significant at 5% level of significance

The length of roots in control population ranged from 6 to 13.25 cm while it was 5.25 to 12.15 cm under 20 kR, 3.5 to 11.45 cm under 30 kR, 3.6 to 11.7 cm in 0.5 per cent and 3.05 to 5.6 cm in one per cent EMS. A dose dependent variation on varieties was noted in general. The maximum reduction in root length was shown by the higher dose of EMS for the variety V_{11} , which also had the shortest root length in the untreated condition. The minimum values noted under 20 kR, 30 kR and 0.5 per cent EMS were in varieties having a higher root length in control plants. In the case of both exposures of gamma rays, the minimum reduction was noted in the same variety. The maximum reduction in root length was noted under 1 per cent EMS (3.05 cm) in V_{11} , followed by 0.5 per cent EMS (3.6 cm) in V_7 . The maximum reduction in value under 30 kR gamma rays was comparable to that of the lower concentration of EMS. It was interesting to note that variety V_{12} gave a higher length of roots in lower doses of both the mutagens when compared with the control.

SHOOT LENGTH AT TRANSPLANTING (30 days after sowing)

Data regarding the shoot length of seedlings at the time of transplanting is given in Table 9. Statistical analysis of the data showed significant variation among

Table 9. Shoot length at transplanting
(30 days after sowing)

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	8.15	5.65	5.4	5.15	4.95	5.86
V ₂	9.7	4.0	4.8	9.0	7.9	7.08
V ₃	12.75	6.5	6.4	5.9	4.35	7.18
V ₄	6.55	4.7	4.35	7.6	7.05	6.05
V ₅	10.65	5.1	5.05	6.0	5.15	6.39
V ₆	10.1	5.05	4.35	6.9	5.0	6.28
V ₇	6.95	6.4	3.8	5.3	3.2	5.13
V ₈	10.9	6.15	5.35	4.95	4.2	6.31
V ₉	11.9	3.35	5.5	6.35	6.0	6.62
V ₁₀	4.5	2.5	2.9	8.0	3.05	4.19
V ₁₁	5.5	3.9	4.25	6.85	6.3	5.36
V ₁₂	9.25	5.6	2.55	5.85	3.9	5.43
V ₁₃	14.5	10.55	6.0	5.7	4.9	8.33
V ₁₄	12.95	10.8	8.75	6.7	5.5	8.94
V ₁₅	14.55	13.85	9.9	4.95	5.45	9.74
V ₁₆	15.9	14.35	13.85	6.9	6.5	11.5
V ₁₇	12.1	10.5	7.9	5.5	6.85	8.57
General Mean	10.41	7.00	5.95	6.33	5.31	

	<u>F value</u>	<u>CD value</u>
Variety -	19.04*	1.22
Treatment -	74.00*	0.66
Interaction-	4.76*	2.72

* Significant at 5% level of significance

different varieties, mutagens and interaction among varieties and treatments. The maximum reduction was noted in 1 per cent EMS concentration followed by 30 kR gamma rays. The lower doses of both the mutagens induced a comparable reduction in shoot length. In the higher doses of mutagens the length of shoot was almost half of the control, whereas in the lower doses it was reduced to about twenty five per cent of the control. Higher the dose of mutagen, less was the shoot length.

The shoot length (in cm) ranged from 4.5 to 15.9 in control, 2.5 to 14.35 in 20 kR, 2.9 to 13.85 in 30 kR of gamma rays, 4.95 to 9.0 in 0.5 per cent, and 3.2 to 7.9 in 1 per cent EMS concentration. The minimum shoot length for gamma ray treatments (2.5, 2.9) was shown by variety V_{10} . The maximum shoot length in the treated condition was also noted in the variety with the maximum shoot length (V_{16}). In the case of EMS treatments, the minimum shoot length was more than in case of gamma ray treatment, but the minimum reduction in shoot length was less than gamma ray exposures. The minimum shoot length in EMS treated population was also noticed in a variety with comparatively short roots (6.95), as its control. In two varieties V_4 and V_{11} , EMS treatments increased the length of shoot compared to the control.

HEIGHT OF PLANTS (30 days after transplanting)

The height of plants at 30 days after transplanting as affected by both the mutagens is given in Table 10. There was a significant variation among varieties, treatments and in their interactions. The gamma ray treatments induced greater reduction in height compared to the control plants. The least height was seen in the case of higher dose of gamma rays.

In control plants, the height ranged from 8 to 31.25 cm, while in 20 kR it was 5.8 to 22.5 cm and 4.7 to 20.5 cm in 30 kR of gamma rays. In EMS treatments, it was 7.1 to 19.3 cm in 0.5 per cent and 6.95 to 17.75 cm in 1 per cent concentrations. In the case of both EMS treatments, the maximum values in height have exceeded the values of the respective control plants. In all four treatments the minimum values were noticed in the variety V_{10} , which had a height of 11.5 cm in untreated condition. Both gamma ray and EMS treatments of the variety V_1 induced a height more than the control plants.

HEIGHT OF PLANTS (45 days after transplanting)

The height of plants as on 45 days after transplanting is given in Table 11. There was significant difference

Table 10. Height of plants at 30 days after transplanting

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	7.4	8.75	9.45	8.35	7.4	8.37
V ₂	9.95	8.0	6.7	7.05	16.35	9.61
V ₃	13.4	7.8	8.45	11.3	10.25	10.24
V ₄	9.15	9.0	7.25	9.95	9.75	9.02
V ₅	13.65	6.85	6.95	19.3	7.9	10.93
V ₆	8.0	13.4	5.3	10.9	14.5	10.42
V ₇	13.4	5.8	6.45	15.75	13.75	11.03
V ₈	12.8	7.1	7.0	8.55	17.75	10.64
V ₉	8.55	7.6	11.1	17.3	14.5	11.81
V ₁₀	11.5	5.8	4.7	7.1	6.95	7.21
V ₁₁	12.0	6.4	8.55	16.0	9.75	10.54
V ₁₂	12.45	9.3	5.3	11.55	9.75	9.67
V ₁₃	19.45	16.5	16.3	11.4	10.4	14.81
V ₁₄	16.8	18.6	16.8	8.75	9.75	14.14
V ₁₅	22.65	15.65	19.8	12.9	11.9	16.58
V ₁₆	31.25	19.3	19.65	11.75	8.9	18.17
V ₁₇	25.3	22.5	20.5	13.7	12.55	18.91
General Mean	14.57	11.0	10.6	11.89	11.3	

	<u>F value</u>	<u>CD value</u>
Variety	- 238.38*	0.626
Treatment	- 170.82*	0.339
Interaction	- 69.92*	1.4

* Significant at 5% level of significance

Table 11. Height of plants at 45 days after transplanting

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	15.9	16.15	17.15	18.2	12.9	16.06
V ₂	26.4	13.3	17.75	15.49	17.8	18.15
V ₃	22.25	15.05	18.65	17.15	15.75	17.77
V ₄	24.50	21.5	14.9	11.9	10.05	16.57
V ₅	23.65	15.05	18.75	22.8	12.4	18.53
V ₆	21.1	18.7	16.0	17.8	16.0	17.92
V ₇	21.4	13.9	16.2	13.7	10.9	15.22
V ₈	24.35	18.35	16.4	12.7	21.55	18.67
V ₉	19.6	21.55	23.4	22.55	17.95	21.01
V ₁₀	18.4	13.0	10.25	13.1	13.25	13.6
V ₁₁	19.65	17.0	19.0	22.25	14.9	18.56
V ₁₂	13.4	12.0	9.0	19.85	13.25	13.68
V ₁₃	28.55	22.35	22.0	19.3	18.9	20.82
V ₁₄	24.0	19.9	21.35	15.4	16.8	19.49
V ₁₅	23.75	18.8	20.95	16.4	14.95	18.97
V ₁₆	37.0	21.15	25.9	11.7	9.9	21.14
V ₁₇	29.3	25.65	25.35	10.35	15.05	21.14
Mean	22.72	17.85	18.46	16.51	14.85	

		<u>F value</u>	<u>CD value</u>
Variety	-	4.22*	3.31
Treatment	-	21.55*	1.79
Interaction	-	2.20*	7.40

* Significant at 0.5% level of significance

among varieties, treatments and interaction between varieties and treatments. All the treatments showed a reduction in height compared to control. The EMS treatments induced more reduction in height than gamma rays. The maximum reduction in height was seen in 1 per cent EMS treatment (14.85 cm).

In the control population the height ranged from 13.4 to 37.9 cm, whereas it was 13 to 25.7 cm in 20 kR and in 30 kR it was 9.0 to 25.9 cm. In EMS treatment, it ranged from 11.7 to 22.8 cm in 0.5 per cent and 9.9 to 21.55 cm in 1 per cent. Variety V_{16} which showed the maximum height for control and gamma ray treatments, showed the minimum height for EMS treatments. The minimum height in gamma ray exposures was observed in varieties V_{10} and V_{12} with a minimum height in control. In variety V_9 , all the treatments gave a higher value than the control.

HEIGHT OF PLANTS, (60 days after transplanting)

Data regarding the height of plants, 60 days after transplanting is presented in Table 12. Statistical analysis showed significant difference among varieties and treatments. A reduction in height was noticed as a result of mutagen treatment. EMS showed a more reduction in height.

Table 12. Height of plants at 60 days after transplanting

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	37.65	19.4	27.35	17.8	20.5	24.5
V ₂	21.4	23.0	23.15	23.4	19.05	22.0
V ₃	28.75	25.3	24.3	18.65	19.0	23.2
V ₄	30.5	30.25	26.0	17.2	14.65	23.72
V ₅	35.25	21.9	31.5	28.8	17.15	26.92
V ₆	35.1	22.6	32.5	22.25	19.25	27.34
V ₇	32.15	27.3	28.1	15.2	24.9	25.53
V ₈	34.15	33.15	34.25	21.7	24.45	29.54
V ₉	31.0	29.5	29.85	24.1	21.75	27.24
V ₁₀	22.65	21.75	19.55	23.7	20.3	22.59
V ₁₁	32.6	31.0	31.0	24.85	20.25	27.94
V ₁₂	16.15	14.65	13.55	18.3	16.4	15.31
V ₁₃	22.0	26.5	24.65	26.25	21.8	24.24
V ₁₄	29.1	23.5	26.95	21.7	17.3	23.71
V ₁₅	24.95	23.1	25.45	17.9	16.6	21.6
V ₁₆	40.75	22.8	29.0	17.7	10.4	24.13
V ₁₇	33.8	27.1	27.8	10.05	16.75	23.1
General Mean	30.17	25.16	26.76	20.56	18.85	

	<u>F value</u>	<u>CD value</u>
Variety -	3.92*	4.49
Treatment -	28.69*	2.43
Interaction-	1.40	10.03

* Significant at 5% level of significance

As the dose increased, the reduction in height was more.

The mean height of plants ranged from 16.15 to 40.75 cm in the control, 14.65 to 33.15 cm in 20 kR, 13.55 to 34.25 in 30 kR, 10.1 to 28.8 cm in 0.5 per cent and 10.4 to 24.45 cm in 1.0 per cent EMS treatment. Variety V₁₆ which showed the maximum height in control gave the minimum height in the 1 per cent EMS treatment.

HEIGHT OF PLANTS (75 days after transplanting)

The height of plants 75 days after transplanting as affected by both the mutagens is presented in Table 13. The statistical analysis of the data showed significant differences among varieties, treatments and their interactions. In general, a reduction in height was noted due to mutagen treatment compared to the control. This reduction was more marked in EMS treatment than in gamma rays. The maximum reduction was seen in the case of the higher concentration of EMS. The mean height of plants ranged from 28.3 to 48.5 cm in control, 16.3 to 40.5 cm in 20 kR, 26.2 to 40.1 cm in 30 kR, 18.4 to 32 cm in 0.5 per cent and 15.1 to 32.2 cm in 1 per cent EMS treatment. The maximum reduction in height in the various treatments (15.1 cm) was seen in the higher dose

Table 13. Height of plants at 75 days after transplanting

Variety Control	Gamma rays		EMS		General Mean	
	20 k R	30 kR	0.5%	1.0%		
V ₁	40.5	40.5	34.0	27.85	21.2	32.81
V ₂	44.25	28.85	29.75	19.05	22.1	28.8
V ₃	32.25	25.85	32.15	21.8	21.8	26.78
V ₄	33.9	35.5	29.9	20.35	19.6	27.85
V ₅	48.5	29.75	28.6	32.0	20.75	31.92
V ₆	46.5	33.25	40.1	29.0	21.25	34.02
V ₇	34.9	35.15	35.15	20.8	30.25	31.25
V ₈	44.25	38.2	35.5	18.35	26.1	32.48
V ₉	41.25	36.4	36.0	31.2	24.55	33.88
V ₁₀	31.75	25.25	27.0	21.85	23.5	25.87
V ₁₁	38.25	34.0	37.6	22.25	23.2	31.06
V ₁₂	24.25	27.1	26.55	31.75	32.15	28.36
V ₁₄	28.25	27.1	26.6	25.9	21.95	25.96
V ₁₅	31.10	16.25	26.15	23.25	20.15	25.38
V ₁₆	40.5	23.35	28.15	22.1	15.05	25.83
V ₁₇	35.5	28.0	28.85	19.0	22.75	26.82
General Mean	37.24	30.91	31.38	24.16	22.9	

		<u>F value</u>	<u>CD value</u>
Variety	-	4.33*	4.23
Treatment	-	49.12*	2.37
Interaction	-	1.9*	9.46

* Significant at 5% level of significance

of EMS in the variety V₁₆.

HEIGHT OF PLANTS, (90 days after transplanting)

Table 14 represents the data regarding height of plants, 90 days after transplanting. Statistical analysis of the data showed significant difference among treatments, varieties and in their interactions. In general, a reduction in height was seen in all the treatments compared to control. This reduction was more in EMS treatments. The effect of both doses of gamma rays was on par, as also the effect of both concentrations of EMS; but both mutagen treatments significantly differed from the control and from each other. The maximum reduction in height was noticed in 1 per cent EMS treatment in general. The height ranged from 25 to 51.4 cm in control, 23.6 to 42.3 cm in 20 kR, 26.4 to 42.9 cm in 30 kR, 21.8 to 39.1 cm in 0.5 per cent and 18.8 to 33.4 cm in 1 per cent EMS concentrations. The variety V₁₃ which had 25 cm as its control height, showed an increased height in all the treatments; the EMS treatments giving the maximum height.

NUMBER OF BRANCHES PER PLANT (45 days after transplanting)

The mean number of branches per plant on the 45th day of transplanting is given in Table 15. There was a

Table 14. Height of plants at 90 days after transplanting

Variety Control	Gamma rays		EMS		General Mean	
	20 KR	30 KR	0.5%	1.0%		
V ₁	42.75	43.0	39.0	28.85	22.9	35.3
V ₂	48.0	31.0	32.3	22.9	22.7	31.38
V ₃	34.5	28.0	38.9	24.85	23.95	30.16
V ₄	38.5	36.0	36.85	23.45	24.9	31.94
V ₅	50.85	33.0	37.25	36.75	22.7	36.11
V ₆	51.4	38.4	42.9	32.05	24.9	37.93
V ₇	36.6	39.65	38.95	23.75	29.3	33.65
V ₈	46.95	42.65	38.5	21.75	33.25	36.62
V ₉	44.75	39.15	40.0	39.1	26.95	37.99
V ₁₀	35.1	30.25	30.9	24.8	25.55	29.32
V ₁₁	44.6	37.15	40.6	25.05	28.1	35.10
V ₁₂	25.0	30.1	28.25	32.9	33.35	29.92
V ₁₄	29.0	29.0	29.65	28.75	23.9	28.06
V ₁₅	32.5	27.4	26.35	25.55	21.3	26.62
V ₁₆	40.75	23.6	29.15	27.6	18.75	27.97
V ₁₇	37.15	29.3	29.0	21.9	19.3	27.33
General Mean	39.9	33.64	34.91	27.5	25.11	

		<u>F value</u>	<u>CD value</u>
Variety	-	6.49*	4.35
Treatment	-	47.81*	2.43
Interaction	-	1.94*	99.72

* Significant at 5% level of significance

Table 15. Number of branches per plant at 45th day after transplanting

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	4.5	4.15	3.0	4.5	3.4	3.91
V ₂	7.95	5.35	2.40	6.15	9.3	6.23
V ₃	3.9	2.25	2.85	5.80	3.05	3.57
V ₄	5.5	4.25	1.9	2.70	2.75	3.42
V ₅	4.5	2.5	2.0	5.90	2.45	3.47
V ₆	2.85	3.15	3.85	3.30	3.25	3.28
V ₇	5.35	3.25	2.45	1.15	3.4	3.12
V ₈	4.95	2.5	2.0	1.25	3.9	2.92
V ₉	3.00	2.15	3.3	3.20	4.25	3.18
V ₁₁	3.15	5.8	5.75	6.25	4.35	5.06
V ₁₂	3.90	4.15	3.0	4.5	1.7	3.45
V ₁₃	4.85	5.5	4.75	3.75	4.55	4.68
V ₁₄	6.00	5.65	5.45	3.9	2.05	4.61
V ₁₅	7.15	5.5	5.6	4.2	5.1	5.51
V ₁₆	12.25	5.2	6.85	3.05	2.6	5.99
V ₁₇	7.50	7.85	7.65	2.75	4.0	5.85
General Mean	5.46	4.29	3.93	3.90	3.76	
			<u>F value</u>		<u>CD value</u>	
	Variety		8.27*		1.149	
	Treatment		9.36*		0.642	
	Interaction		3.18*		2.569	

* Significant at 5% level of significance.

significant difference among varieties, treatments and interaction between varieties and treatments. A dose dependent reduction in the number of branches per plant was noted in all the treatments compared to control. As the dose level increased the number of branches was reduced in most of the varieties. The maximum reduction in number of branches was seen in 1 per cent EMS treatment.

The number of branches ranged from 2.85 to 12.25 in control, 2.15 to 7.35 in 20 kR, 1.9 to 7.65 in 30 kR, 1.15 to 6.25 in 0.5 per cent and 1.7 to 9.3 in 1 per cent EMS. A reduction in number of branches with increase in dose was seen for both mutagens in most of the varieties. Gamma ray treatment of variety V₁₇ did not reduce the number of branches, but EMS concentrations reduced the number of branches to more than fifty per cent of control. In the case of variety V₁₆, the control showed a maximum value of 12.25 whereas the mutagen treatments significantly reduced the number of branches. Varieties V₆ and V₁₁ showed a higher number of branches per plant in all the four treatments compared to the control.

NUMBER OF BRANCHES PER PLANT (60 days after transplanting)

Table 16 represents the data regarding the number of branches per plant, 60 days after transplanting, as affected by both the mutagens. Statistical analysis showed significant differences among varieties, treatments and interaction between them. There was a reduction in the number of branches per plant due to the effect of different treatments. EMS concentrations showed a greater reduction in the number of branches than the gamma ray exposures.

The values ranged from 3.8 to 15.3 in the control, 3.8 to 11.5 in the 20 kR, 4.5 to 10.4 in the 30 kR, 1.9 to 7 in 0.5 per cent and 2.05 to 8.55 in 1 per cent EMS treatments. Variety V_{17} gave a higher value in the gamma ray treatments compared to the control, whereas in the EMS concentrations it was reduced to half of that of the control. Varieties V_9 , V_{10} and V_{13} showed more number of branches in the treatments compared to the control.

NUMBER OF BRANCHES PER PLANT (75 days after transplanting)

Data regarding the number of branches per plant, 75 days after transplanting, is given in Table 17. There was a significant difference among varieties,

Table 16. Number of branches per plant at 60th day after transplanting

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	11.15	5.35	4.5	6.15	4.30	6.29
V ₂	12.8	7.55	9.1	4.15	8.00	8.32
V ₃	6.6	6.15	6.5	4.95	5.45	5.93
V ₄	7.25	6.25	4.7	3.95	5.25	5.48
V ₅	8.25	3.75	5.0	5.40	2.80	5.04
V ₆	10.05	4.45	4.9	7.10	8.55	7.01
V ₇	6.65	7.00	6.3	2.90	4.05	5.33
V ₈	7.15	6.60	5.4	1.90	3.4	4.89
V ₉	3.75	4.75	5.25	4.45	7.25	5.09
V ₁₀	4.05	6.25	6.25	5.00	5.25	5.36
V ₁₁	9.85	7.35	10.4	7.75	5.90	8.25
V ₁₂	5.20	5.65	5.35	8.70	2.05	5.39
V ₁₃	6.65	9.50	7.9	8.55	8.4	8.2
V ₁₄	7.20	5.85	8.65	3.75	3.05	5.7
V ₁₅	11.30	6.00	10.35	6.25	5.80	7.94
V ₁₆	15.25	6.65	7.00	4.45	4.25	7.52
V ₁₇	9.35	11.5	10.15	4.05	4.25	7.86
General Mean	8.38	6.51	6.92	5.26	5.18	

	<u>F value</u>	<u>CD value</u>
Variety	3.21*	2.05
Treatment	11.33*	1.11
Interaction	1.56*	4.58

* Significant at 5% level of significance

Table 17. Number of branches per plant, 75 days after transplanting

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	11.4	9.0	5.5	8.25	6.15	8.06
V ₂	14.6	9.25	9.15	5.9	9.10	9.60
V ₃	8.9	5.9	9.4	8.45	8.30	8.19
V ₄	7.25	7.25	5.8	6.1	6.05	6.49
V ₅	8.85	5.0	3.9	9.9	3.95	6.32
V ₆	11.5	5.9	6.85	9.1	7.05	8.03
V ₇	6.5	7.0	8.85	4.4	5.05	6.36
V ₈	9.0	8.3	6.6	4.05	4.2	6.43
V ₉	5.75	5.4	6.4	5.05	7.9	6.10
V ₁₀	5.5	6.75	6.0	6.9	7.25	6.48
V ₁₁	10.15	6.0	11.6	8.4	8.65	8.96
V ₁₃	9.5	10.0	8.4	10.5	9.9	9.66
V ₁₄	7.65	6.25	7.9	4.7	3.25	5.95
V ₁₅	11.7	7.15	11.25	7.0	6.25	8.67
V ₁₆	14.25	7.6	6.5	6.25	3.55	7.63
V ₁₇	9.7	11.8	11.3	5.1	5.25	8.63
General Mean	9.51	7.41	7.84	6.88	6.37	

	<u>F. value</u>	<u>CD value</u>
Variety	2.82*	1.897
Treatment	10.23*	1.06
Interaction	2.00*	4.24

* Significant at 5% level of significance

treatments and interaction between varieties and treatments. All the treatments showed a marked reduction in the number of branches per plant, compared to the control. The gamma ray exposures were comparable with each other as also the EMS treatments, but they significantly differed from the control. The maximum reduction in the number of branches was seen in 1 per cent EMS treatment (3.25).

The number of branches per plant ranged from 5.5 to 14.3 in the control, 5 to 11.8 in 20 kR, 3.9 to 11.6 in 30 kR, 4.1 to 9.9 in 0.5 per cent and 3.3 to 9.1 in 1 per cent EMS concentration. The variety V_1 and V_7 showed a higher value in the gamma ray treatments, which was almost reduced to half in the EMS treatments. The minimum number of branches was seen in the 1 per cent EMS concentration in the variety V_{14} (3.25) which had a few number of branches in the control. Variety V_{10} showed an increase in the number of branches in all the four treatments compared to the control.

NUMBER OF DAYS TAKEN FOR FLOWERING

Data regarding the number of days required for flowering as affected by the different treatments is represented in Table 18. An increase in the mean number

Table 18. Number of days taken for flowering

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	80.25	84.50	87.9	61.25	73.9	77.56
V ₂	75.60	76.00	82.75	73.05	62.25	73.95
V ₃	82.0	85.60	80.55	65.35	71.75	77.05
V ₄	76.85	83.15	86.35	65.05	70.9	76.46
V ₅	84.20	88.0	89.25	69.3	70.5	80.25
V ₆	84.50	84.7	87.15	72.9	93.55	80.56
V ₇	81.20	88.05	89.80	73.05	73.8	81.18
V ₈	80.20	87.55	88.70	83.6	81.9	84.39
V ₉	76.90	78.15	81.85	62.9	66.5	67.26
V ₁₁	81.20	91.90	83.45	65.6	67.75	77.98
V ₁₂	64.35	70.6	70.6	72.35	71.7	69.92
V ₁₄	64.20	72.3	75.0	79.0	81.35	94.37
V ₁₅	62.50	68.5	69.45	82.0	83.85	73.26
V ₁₆	67.15	71.0	70.3	74.9	78.1	72.29
V ₁₇	63.85	68.25	69.9	82.7	83.65	73.67
General Mean	74.99	79.88	80.87	72.2	74.1	

	<u>F value</u>	<u>CD value</u>
Variety -	9.91*	6.33
Treatment -	27.04*	2.11
Interaction -	6.66*	7.95

* Significant at 5% level of significance

of days taken for flowering was seen in both doses of gamma rays. The EMS treatments did not show any significant difference from the control. Among the gamma ray treatments, 30 kR induced the maximum number of days to flowering followed by 20 kR treatment.

In the control, the number of days to flowering ranged from 62.5 to 84.5. In the gamma ray treatments it ranged from 68.3 to 91.9 in 20 kR and 69.5 to 89.8 in 30 kR. In the EMS treatments, it ranged from 61.3 to 81.6 in 0.5 per cent and 62.3 to 83.9 in 1 per cent concentration. Generally in most varieties it was seen that gamma ray treatment increased the days to flowering while the EMS treatment reduced it. The maximum number of days to flowering was induced by 20 kR treatment in the variety V_{11} and the minimum number of days by the 0.5 per cent treatment in the variety V_1 .

NUMBER OF DAYS TAKEN TO MATURITY

Total number of days taken to maturity from the date of sowing is given in Table 19. The varieties, treatments and the interactions showed significant difference. The number of days to maturity significantly increased in different treatments compared to control. An increase

Table 19. Number of days taken to maturity

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	115.5	122.5	135.5	133.5	134.8	128.36
V ₂	118.65	125.85	130.5	134.4	134.72	128.83
V ₃	122.15	128.3	123.15	126.2	126.1	125.18
V ₄	117.0	122.5	128.25	125.65	127.25	124.13
V ₅	124.0	128.75	133.5	114.55	123.05	124.77
V ₆	134.0	131.3	130.05	106.85	109.81	122.4
V ₇	121.85	123.0	123.4	108.85	110.5	117.52
V ₈	119.15	123.0	126.75	121.0	128.15	123.61
V ₉	116.5	119.25	122.4	97.0	100.85	111.2
V ₁₁	118.0	119.5	129.25	135.1	140.0	128.35
V ₁₃	107.15	114.75	113.85	108.15	106.95	110.17
V ₁₄	114.75	112.85	116.5	127.05	133.3	120.89
V ₁₅	107.0	104.65	112.15	137.75	137.7	119.85
V ₁₆	105.65	115.25	110.75	133.35	134.7	119.94
V ₁₇	115.0	118.25	137.35	141.05	141.8	130.69
General Mean	117.09	120.65	124.38	123.36	125.98	

	<u>F value</u>	<u>CD value</u>
Variety -	65.36*	2.12
Treatment -	68.55*	1.22
Interaction -	22.79*	4.73

* Significant at 5% level of significance

in the number of days to maturity was seen with increase in dose of both the mutagens.

In the untreated varieties the number of days to maturity ranged from 105.7 to 134 days and in the various treatments, the range was from 104.7 to 131.3 in 20 kR, 110.8 to 137.4 in 30 kR, 97 to 141.05 in 0.5 per cent and 100.9 to 141.8 in 1 per cent EMS concentration. The maximum number of days to maturity in 30 kR gamma rays and the EMS treatments were exhibited by the same variety (V_{17}) which took 115 days to mature in the control population. In 20 kR treatment, the maximum value was shown by the variety V_6 , which also had a very high value in its control. EMS treatments of certain varieties gave a lower value than the control in the number of days to maturity, though generally an increase in the number of days to maturity was noted for most of the varieties with increase in doses.

POLLEN STERILITY PERCENTAGE

Induced pollen sterility due to the various treatments is given in Table 20. Statistical analysis of the data showed significant differences among varieties, treatments and between the interactions of treatments and varieties. The pollen sterility increased with

Table 20. Pollen sterility percentage

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 kR	0.5%	1.0%	
V ₁	13.905	29.06	30.37	26.675	30.805	25.763
V ₂	13.685	23.015	42.115	20.025	20.155	23.599
V ₃	16.795	23.945	23.075	16.165	21.51	20.298
V ₄	19.775	19.605	42.13	14.535	18.515	22.912
V ₅	24.605	29.465	15.815	20.905	31.015	24.361
V ₆	19.685	27.9	31.425	13.575	29.96	24.509
V ₇	13.545	27.125	51.56	15.575	26.24	26.809
V ₈	17.345	15.365	21.32	26.38	32.47	22.576
V ₉	20.495	18.425	87.70	23.225	40.095	27.988
V ₁₁	18.7	20.555	50.92	21.09	15.66	25.385
V ₁₃	15.1	22.0	37.87	28.795	36.35	28.023
V ₁₄	9.645	18.93	20.585	15.37	20.545	17.015
V ₁₅	21.045	26.26	25.895	35.81	34.295	28.661
V ₁₆	15.08	21.53	33.875	37.155	23.275	26.193
V ₁₇	14.405	23.025	43.02	42.705	28.36	30.303
General Mean	16.921	22.9	33.845	23.866	27.283	

		<u>F value</u>	<u>CD value</u>
Variety	-	2.88*	5.692
Treatment	-	28.56*	3.28
Interaction	-	2.81*	12.728

* Significant at 5% level of significance

increase in doses. The sterility was maximum at 30 kR gamma treatment followed by 1 per cent EMS treatment. The lower doses of both the mutagens had a comparable effect.

In the control population sterility showed a maximum of 24.61 per cent and a minimum of 9.65 per cent whereas in the various treatments it ranged from 15.31 to 29.45 per cent in 20 kR and 15.82 to 51.56 per cent in 30 kR gamma rays, 13.58 to 42.71 per cent in 0.5 per cent EMS and 15.66 to 40.10 per cent in 1 per cent EMS. The maximum pollen sterility was seen in 30 kR gamma ray treatment of the variety V_7 , which had a minimum percentage of sterility in the control condition. The variety V_5 which showed the minimum pollen sterility in the 30 kR treatment, showed the maximum pollen sterility in the 20 kR and control treatment.

NUMBER OF FRUITS PER PLANT

Table 21 represents the data regarding the mean number of fruits per plant in the M_1 generation. Statistical analysis showed significant differences among varieties and treatments. The number of fruits showed significant reduction over control. Among the different treatments, the maximum reduction was seen in 1 per cent

Table 21. Number of fruits per plant

Variety	Control	Gamma rays		EMS		General Mean
		20 kR	30 KR	0.5%	1.0%	
V ₁	47.05	42.95	30.5	11.0	10.75	28.45
V ₂	89.35	65.1	20.5	12.0	11.25	40.28
V ₃	36.35	37.1	47.4	15.2	5	29.57
V ₄	25.5	39.0	33.5	21.9	20.5	28.08
V ₅	29.9	26.2	21.5	22.0	21.1	24.14
V ₆	64.65	63.0	56.5	16.5	15.75	43.28
V ₇	63.25	61.0	58.3	20.1	21.15	44.76
V ₈	59.5	44.25	33.65	16.1	17.35	34.17
V ₉	47.75	24.1	34.75	18.6	17.75	28.59
V ₁₁	89.25	77.0	64.0	22.25	20.3	54.56
V ₁₃	13.65	12.0	12.75	16.4	19.55	14.87
V ₁₄	21.65	12.0	13.65	15.0	11.55	14.77
V ₁₅	27.0	15.5	14.2	13.0	9.5	15.84
V ₁₆	18.4	18.0	19.15	7.3	6.75	13.92
V ₁₇	20.2	6.0	3.0	11.3	12.0	10.5
General Mean	43.56	36.21	30.89	15.9	14.68	

		<u>F value</u>	<u>CD value</u>
Variety	-	7.25*	13.82
Treatment	-	20.14*	7.98
Interaction	-	1.37*	30.91

* Significant at 5% level of significance

EMS concentration and the minimum reduction in the lower dose of gamma rays. The EMS treatments showed more reduction in number of fruits than in gamma ray exposures. The number of fruits in the EMS treatments was reduced to half of the gamma ray treatment. In both EMS and gamma ray treatments a gradual decrease in number of fruits per plant with increase in dose was observed. When the number of fruits per plant in the control ranged from 13.65 to 89.35, it was 6 to 77 in 20 kR, 3 to 64 in 30 kR gamma rays, 7.3 to 22.25 in 0.5 per cent and 5 to 21.1 in 1 per cent EMS concentration. An increase in number of fruits was seen with increased dose of gamma rays in variety V_3 and V_4 , but it can be seen that EMS concentrations gave a significant reduction compared to control.

M_2 Generation

Mean height of plants

The mean height of plants in M_2 at 75 and 90 days after transplanting is given in Table 22. Significant difference in the mean height was noted between the two varieties, but there was no significant difference between

treatments. The maximum height was seen in 30 kR dose of gamma rays in the two varieties, both at 75 days and 90 days of transplanting. A reduction in height in 20 kR exposure could be noted compared to the control. Variety V_2 showed the maximum height at 75 days in the control plants followed by 30 kR and 20 kR. At 90 days of transplanting, the second variety showed an insignificant increase in value with increase in exposures.

Mean number of branches per plant

The mean number of branches per plant in M_2 generation is given in Table 23. No significant differences in varieties or treatments could be seen at 75 days after transplanting. At 90 days after transplanting, significant differences were seen between varieties, treatments and their interactions. At 75 days of transplanting in both the varieties, 20 kR gave the minimum values compared to other treatments. At 90 days of transplanting, the second variety showed a higher number of branches per plant than the first variety. Comparison between treatments showed that 30 kR treatment significantly increased the number of branches per plant at 90 days after transplanting. This could be noticed in the case of both varieties.

Table 22. Mean height of plants in M_2 generation

	75 days after planting			90 days after planting		
	Control	20 kR	30 kR	Control	20 kR	30 kR
V_1	25.85	25.1	30.75	29.83	28.55	31.45
V_2	35.85	29.55	31.2	33.48	35.20	35.48
		F value	CD value	F value	CD value	
Variety		14.41*	2.77	13.53*	2.75	
Treatment		3.34	3.396	0.771	3.37	
Interaction		4.479*	4.804	0.528	4.77	

Table 23. Mean number of branches per plant in M_2 generation

	75 days after planting			90 days after planting		
	Control	20 kR	30 kR	Control	20 kR	30 kR
V_1	12.25	9.8	13.15	16.2	13.0	22.8
V_2	12.20	11.05	11.1	18.9	20.8	22.3
		F value	CD value	F value	CD value	
Variety		0.08	2.69	10.35*	2.20	
Treatment		1.43	2.535	11.72*	2.696	
Interaction		1.05	3.585	5.36*	3.813	

* Significant at 5 per cent level

Number of days to flowering and harvest

The number of days to flowering and harvest in the M_2 generation is given in Table 24. Significant differences between treatments were noticed. The varieties did not differ significantly. Comparison between treatments showed that the duration to flowering and harvest were reduced in the gamma ray treatments compared to control. The maximum reduction in flowering and harvest was seen in the 30 kR treatment, in case of both varieties. A dose dependent decrease in number of days to flowering and the number of days to harvest were noticed in Variety V_2 and V_1 respectively.

Mean number and length of fruits

The number and length of fruits per plant in M_2 generation is given in Table 25. Analysis of data showed a significant difference between varieties and interaction between varieties and treatments in both the number of fruits per plant and length of fruits. The number of fruits per plant was greater in the first variety, which had shorter fruits than the second variety. 30 kR treatment of the first variety gave the maximum number of fruits while in case of second variety, 20 kR induced the maximum number of fruits, which was reduced

Table 24. Mean number of days to flowering and harvest in M_2 generation

	Days to flowering			Days to harvest		
	Control	20 kR	30 kR	Control	20 kR	30 kR
V_1	69.63	61.15	63.85	106.85	104.15	103.73
V_2	68.20	64.0	63.48	109.03	103.23	104.55

	F value	CD value	F value	CD value
Variety	0.262	1.453	0.910	
Treatment	32.58*	1.780	13.89*	
Interaction	3.521	2.517	1.538	

Table 25. Number and Length of fruits per plant in M_2 generation

	Mean number of fruits/plant			Mean length of fruits		
	Control	20 kR	30 kR	Control	20 kR	30 kR
V_1	39.13	40.5	59.82	3.8	5.0	4.9
V_2	33.05	40.53	24.0	6.5	6.3	5.7

	F value	CD value	F value	CD value
Variety	11.23*	8.829	82.52*	0.377
Treatment	0.71*	10.813	2.95*	0.462
Interaction	9.47*	15.29	10.84*	0.653

* Significant at 5% level

significantly in the 30 kR. The length of fruit was maximum in the 20 kR of the first variety while in the second variety gamma ray exposures reduced the length of fruits.

Mean fruit weight and number of seeds per fruit

The mean fruit weight and number of seeds per fruit in M_2 generation is given in Table 26. Significant differences in the fruit weight was noted between varieties. The second variety had heavier fruits than the first variety. In the case of first variety, the weight increased with increase in dose of gamma rays, the maximum weight being at 30 kR. In case of the second variety a reduction in weight was noted in both treatments, the minimum value being at 30 kR. The varieties, treatments and their interaction showed significant differences in the number of seeds per fruit. Variety V_2 had more number of seeds per fruit than variety V_1 . An increase in number of seeds per fruit was seen in case of both the varieties with increase in dose. The maximum number of seeds per fruit was seen in 30 kR treatment of both the varieties.

Table 26. Fruit weight and number of seeds/
fruit in the M_2 generation

	Mean weight of fruits			No. of seeds/fruit		
	Control	20 kR	30 kR	Control	20 kR	30 kR
V_1	0.426	0.645	0.777	25.0	25.9	29.2
V_2	0.917	0.899	0.686	31.3	35.6	44.18
	<u>F value</u>	<u>CD value</u>		<u>F value</u>	<u>CD value</u>	
Variety	22.83*	0.046		212.98*	4.764	
Treatment	2.36	0.119		52.69*	5.835	
Interaction	13.38*	0.168		14.84*	8.253	

* Significant at 5% level

DISCUSSION

DISCUSSION

Germination:

During the present investigation it has been found in almost all the varieties that the two exposures of gamma rays and two concentrations of EMS significantly reduced the percentage germination of seeds. Reduction in germination as a result of mutagen treatment, as was noted in this particular crop variety was reported by many scientists in various crops including Gustafsson and Gadd (1965) in Poa pratensis, Rangaswamy (1969) in sorghum, Roy et al. (1971) in Cucumis sativus, Bohera and Patnaik (1979) in Amaranthus, Majid (1975) in Lycopersicon etc. In both the mutagens a progressive reduction in percentage of germination was noticed with increase in dose level. Fujii and Matsumura (1958) observed decreasing germination with increasing dosages of radiations in several crop plants. Bhaskaran (1959) found that the germination percentage decreased with increase in the dose of X-ray in all the three species of wheat studied by him. A proportionate reduction in germination as was noticed in the present investigation was reported by Sree Ramulu (1970) with increase in dose

of both physical and chemical mutagens in Sorghum. Goud et al. (1970) also reported a decreasing trend with increase in dosage. Gregory (1955, 1968), Bilques and Martin (1961) and Giles and De Winck (1969) also reported the same trend in PeaChut with radiation doses.

A drastic reduction in germination in EMS treatments compared to gamma rays was noted in the present investigation. This is in line with the reports made by Rao and Ayengar (1974), Ganeshan (1970), Siddiq and Swaminathan (1968), Yamogata et al. (1965) in rice that chemical mutagens such as DES, EMS and NMU will drastically reduce germination of seeds. In Bajra, Singh et al. (1978) reported that gamma rays had little or no effect on germination whereas EMS resulted in drastic reduction.

A genotypic variation in the response of the mutagen as regards the percentage germination was noted both in the case of gamma rays and EMS in the present investigation. This to a certain extent, demonstrates that the germination test can be adopted to assess the sensitivity of different varieties of chillies to various mutagens. It has already been reported in various crops that

genetic differences, even though they are as small as single gene differences, can induce significant changes in radio sensitivity, which influence not only the total rate but also the spectrum of recoverable mutations (Gustafsson, 1944, 47 and 65; Gustafsson and Tedin, 1954; Nilan, 1956, Lamprecht, 1956 and 1958, Gelin et al. 1958, Smith, 1961, Sparrow, 1961, Konzak et al. 1961a and Sparrow et al. 1965). Mackey (1960 a,b) clearly demonstrated that although nobody is able to predict the influence of a particular genotype on the mutation spectrum, the choice of the parent material is certainly a most decisive part of any programme in mutation breeding.

The influence of mutagens in germination was attributed by Skoog (1935) and Smith and Kerstern (1942) to the destruction of auxins, while Gordon and Webber (1955) and Gordon (1957) suggested that it would be due to inhibition of synthesis of auxins. It is well recognised that factors like temperature, water content, oxygen tension, protecting substances in the seed etc. may affect seed germination and growth. Sydorenko (1962) based on his studies on the germination of irradiated corn seeds at higher doses of ionizing and UV radiation suggested that the activities of catalase, peroxidase and isocitric

dehydrogenase decreased in the irradiated material. Brock (1965 b) after studying the response of Trifolium subterraneum to X-rays and thermal neutrons attributed reduction in germination to radiation induced gross chromosomal breakage. Sinha and Godward (1972) observed reduction in germination in Lens culinaris following gamma ray treatment and attributed the reduction to disturbances caused at physiochemical level of the cells or acute chromosomal damage or both. Venkateswarlu et al. (1978) noticed reduced germination in Pigeon pea, following irradiation and suggested it to be due to threshold physiological effect of X-rays in the species. The physiological effect of mutagens in inhibiting germination was also reported by Chauhan and Singh (1970) that gamma rays cause disruption and disorganise the tunical layer and results in poor germination of exposed seeds. A most striking effect is the impairment of mitosis and virtual elimination of cell division in meristematic zone during germination of seeds as reported by Cherry and Hageman (1961).

Chemical mutagens are also known to cause reduction in germination of seeds (Siddiq and Swaminathan, 1968; Chandrasekhar and Reddi, 1971; Rao and Ayengar, 1964;

Sree Ramulu, 1970). Several explanations are suggested for the action of chemicals on seeds. Alkylating agents are known to react with the genetic material DNA by alkylating phosphate groups (Alexander and Stacey, 1957). Inhibition of germination with EMS treatment was attributed by Freeze - Gertzen et al. (1963) to the formation of acids upon hydrolysis which in turn reduce the pH of the medium, making it toxic.

Rate of germination of seeds:

The present study has shown that both gamma rays and EMS induced delay in germination based on the genotypes tested. The maximum delay in germination was shown by EMS treatment. Delay in germination as was noted in the present investigation have been reported by many workers including Gregory (1955, 1968) in Pisum and Favret (1963) and Gaul (1967) in barley. The effect of EMS in delaying germination of seeds has been clearly demonstrated by Van der Veen and Hilderling (1965) in tomato, Osone (1966) and Sree Ramulu (1970) in rice and Chandrasekhar and Reddi (1971) in Sorghum. This kind of delay in germination is seen to be attributed to physiological damages due to both chromosomal and extra chromosomal

origin. Scarascia (1956) after studying the effects of radiation in four cultivars of N. tabacum attributed the delay in germination to chromosome aberrations, whereas Gaul et al. (1966) after studying the effect of EMS in barley attributed the delay to be due to the physiological damages caused by alkylating agents and their hydrolytic products. Bianchi et al. (1963) observed proportionate delay in seedling sprouting in tomato varieties and suggested this as the first identification of physiological damage.

Survival:

It has been noted in the present investigation that in general, both the mutagens reduced the survival of seedlings depending on the exposures tested. A variety dependent variation has also been noted within the same exposure of a particular mutagen. Reduction in survival was more in EMS than in gamma rays. Rate of survival is taken as a quantitative measure to assess the effect of different mutagens and their doses. A mutagen dependent variation in survival percentage as was noted in the present investigation has been reported by D' Amato et al. (1962) in wheat. Ionizing radiations decrease the survival percentage much as reported by

Tomohira et al. (1964) in Capsicum and Datura, Matsumura (1966) and Chaudhary (1978) in wheat, Sahib and Abraham (1972) in Capsicum, Venkateswaralu et al. (1978) in Pigeon pea etc. Konzak et al. (1965) attributed the decrease in survival percentage to the reduced cell growth resulting from cytological abnormalities and also due to the decrease in the synthesis of auxins and other physiological changes.

Seedling characters:

A quick and simple method to determine the effect of a mutagenic seed treatment is the study of seedling characters, especially the measurement of seedling height both root and shoot. In the present investigation, length of root, shoot and the number of leaves of seedlings were observed to be reduced in the treated population. This is in line with the reports made by Vishnoi and Joshi (1981) in Cucurbits after treating the seeds with K-rays. Sinha and Godward (1972) after studying the effect of different doses of gamma irradiation in two varieties of lentil reported that the root appeared to be more radiosensitive than the shoot. The same trend was noted in the present investigation also. Revell (1953),

Swaminathan et al. (1962) and Bhandari and Natarajan (1966) attributed the differential response of root and shoot to their anatomical and physiological differences, and the differences between their growth mechanisms. They further stated that the shoot growth is mostly due to cell elongation, whereas the root growth is more dependent on cell division. The differences between radiosensitivity of root and shoot has also been reported by Dumanovic and Ehrenberg (1965) and Avanzi et al. (1966). Johnstone and Klepinger (1967) observed in Yuca brevifolia after seed treatment that roots are more radiosensitive than shoots and that the excessive growth inhibition of root would limit the survival of the species.

Rate of growth in plants:

In the present investigation rate of growth was determined by observation on plant height and branching ability at fortnightly intervals. The observations made from 30th day of transplanting to crop maturity clearly showed that the rate of growth was markedly reduced by both the mutagens. A variety dependent effect was also noted in both the mutagens. Growth reduction was more marked in EMS treatments compared to gamma rays.

Numerous reports made clearly supports the results obtained in the present investigation. Caldecott et al. (1952) observed a reduction in growth of barley seedlings following X-irradiation of seeds. Konzak et al. (1961a,b) also observed reduction in growth of seedlings in wheat with an increase in dose of fast neutrons, thermal neutrons and X-rays. Woodstock and Justice (1967) after studies in Zea mays, wheat, sorghum and radish reported a proportional decrease in growth rate depending on the increase in exposure level of gamma rays. The same results were reported by Roy et al. (1971) in Cucumis sativus with X-irradiation and Venkateswarlu et al. (1978) in Pigeon pea after gamma ray treatment. Reduction in growth rate as affected by the different doses of chemical mutagens were reported by Scarascia et al. (1961), Konzak et al. (1961a, b) and Rehman and Soriano (1973).

The explanations offered for the delay and reduction in growth rate are many. Smith and Kersten (1942) attributed the decrease in growth of seedlings following X-ray treatment to the destruction of auxins caused by ionizing radiations. Sparrow et al. (1952) suggested

that the abnormal cytological behaviour due to chromosomal damage and mitotic inhibition can be attributed to reduced growth in mutagen treated materials. Pele and Howard (1955) based on their studies on X-rayed seeds suggested that the possible interference of irradiation with the synthesis of new DNA may lead to inhibition of growth. Gordon (1957) opined that radiation which induce physiological changes may involve a number of interrelated non-specific factors such as inhibition of DNA synthesis and variation in auxin level which may ultimately lead to delay and suppression of growth in the exposed materials. Evans and Sparrow (1961) believed that the influence of ionizing radiations on growth can be attributed basically to the genic loss due to chromosomal aberrations. Evans (1965) after having detailed analysis on growth rate in X-rayed Vicia faba stated that the effect may either be due to chromosomal aberrations or due to mitotic delay. The phenomenon of mitotic delay due to irradiation has been reported as the major cause of growth retardation by Evans et al. (1957) and Evans and Scott (1964). Ananthaswamy et al. (1971) observed inhibition of seedling

growth in gamma irradiated wheat seeds and suggested that the adverse effect of seedlings might be due to specific effect on certain respiratory systems operating during crop growth. Sinha and Godward (1972) pointed out that growth inhibition at higher doses may be due to gross cellular injury either to genic controlled biochemical and physiological processes or due to chromosomal aberration or both. From a detailed study on the effect of ionizing radiation and post treatments with growth substances on rice, El-Aishy (1976) concluded that marked decrease in length of coleoptile and first leaf might be due to an increase in the production of active radicals that are responsible for seed lethality or to the increase of radiation induced gross chromosomal alterations which may result in lethality or suppressed growth of seedlings.

Crop maturity:

Delay in crop maturity, both for first flower opening and harvesting of fruits was experienced in the treated population, compared to the untreated controls. As in the case of all other criteria discussed above, the influence of varieties, types of mutagens and their doses

were found to influence crop maturity. The present findings support the reports made by Tadin (1954) and Down and Anderson (1956) that flowering duration is a character which can be affected by mutagen treatment. Bianchi et al. (1963) observed delay in flowering following irradiation in tomato and attributed it to the delay in the beginning of germination and growth. Iqbal (1972) exposed the seedlings of Capsicum annuum to gamma rays at different stages of development and reported that flowering was delayed due to retarded growth of seedlings. A progressive delay with increase in the exposure level as was seen in the present investigation has been reported by Gunckel (1965) after chronic gamma irradiations.

In the present investigation, data related to days taken to complete germination and rate of growth of plants clearly demonstrated that higher the exposures of both the mutagens, greater was time taken for complete germination and rate of growth of plants was also very slow. The delay in flowering and fruit maturity clearly shows a close association with the above two factors. The delay in germination and slow growth rate may lead

to prolonged delay in complete physiological crop maturity and may be the reason for lateness in flowering and fruit maturity in the treated population.

Fruit yield per plant:

During the course of the present study it was made clear that the mutagens adversely affects the fruit yield per plant. Number of fruits per plant was reduced significantly by both the mutagens; higher the exposure level greater was the reduction in number of fruits per plant. Reduction in yield due to mutagen treatment has been reported in various crops including leguminous crops by Tedin (1954), Zacharias (1956), Gottschalk (1965), Bora et al. (1961), Jana (1962) etc. Reduction in yield due to mutagen treatment may be due to their adverse effect on growth and growth rate and/or due to induced pollen sterility, as a result of chromosome structural alteration. Caldecolt et al. (1954) reported that the reduction in yield in M_1 generation can be due to radiation induced structural changes in chromosomes involving translocations, inversions and deletions. Sree Ramulu (1970) based on his studies in sorghum using X-rays reported that the cause of reduction in yield can be attributed to reduced pollen fertility.

Pollen sterility

Sterility is one of the most important M_1 damages included in plants by mutagen. treatment. The intensity of sterility is known to vary depending on the type and dose of the mutagen employed. The results of the present investigation on pollen sterility revealed a linear increase in sterility with increase in dose of the mutagens. For moderate doses as studied in the present investigation, the same trend has been reported in rice varieties treated with different mutagens by Beachell (1957), Chang and Hsieh (1957), Siddiq (1967) and Singh (1970). It has also been noted in the present study that a higher percentage of sterility was induced by gamma rays compared to EMS. Rao and Ayengar (1964) and Siddiq and Swaminathan (1968) have also reported the same trend.

A genetic status in relation to induced pollen sterility was also noted in the present investigation. This supports the views expressed by Gustafsson (1947) and Sahai and Dalal (1973) that the induced pollen sterility by the different mutagens may vary depending on the genotype of the materials under study.

Gaul (1970) stated that mutagen-induced sterility may be caused by (1) Chromosome mutations, (2) Factor mutations (3) Cytoplasmatic mutations, and (4) Physiological effect. Of these, chromosome mutations are probably the major origin of all mutagen-induced sterility. Katayama (1963) found a direct correlation between M_1 sterility and the frequency of translocation in rice. Singh (1970) observed that gamma rays induced a high frequency of translocation in rice and this might be correlated with pollen sterility as was noticed in the present investigation. Singh (1970) also reported that chemical mutagens induced a marked reduction in pollen and seed sterility, even though the extent of chromosomal aberrations was negligible. According to Bender and Gaul (1966,1967) and Sato and Gaul (1967) radiation induced M_1 sterility might be due to detectable chromosome aberrations and cryptic deficiencies and the sterility induced by EMS and other chemical mutagens might be due to cryptic deficiencies and specific gene mutations. Gaul et al. (1966) and Sato and Gaul (1967) reported that radiation induced sterility is mostly haplontic and EMS induced sterility is diplontic in

nature. According to Gaul (1970) "the actual origin of EMS induced sterility may be gene mutations, or more probably 'invisible deficiencies' the frequency of which may be relative higher than that after ionizing radiations". Though the treated plants were not examined cytologically, the dose dependent increase in frequency of high sterility in both the mutagens noted in the present study may be considered to provide support to the observation of Bender and Gaul (1966, 1967) and Sato and Gaul (1967), that the M_1 sterility depends on the type of mutagens and the doses in inducing higher percentages of chromosomal aberrations or gene mutations.

Mutagen induced sterility has also been reported by many workers in various crops including Venkateswarlu et al. (1978), Rai and Das (1978), Sahai and Dalal (1973) Freeze-Gertzen et al. (1968), Sato and Gaul (1967), Gustafsson (1947). A higher frequency of chromosome stickiness and pollen sterility induced by gamma rays was reported by Rao and Rao (1977). Katiyar (1978) in detail analysed gamma ray induced chromosome aberration and pollen steri-

lity in Capsicum annuum . Rao and Lakshmi (1980) suggested pollen sterility to be the result of cumulative effects of aberrant meiotic stages and physiological and genetic damage caused by chromosome breakage following formation of antimetabolic agents in the cell.

Induced polygenic traits in the M_2 generation.

Most of the economically important traits in plants are governed by polygenes. In the present investigation, the effects of gamma rays on polygenic traits like plant height, days to flowering and maturity, number of fruits per plant, weight and length of fruits and number of seeds per fruit were analysed. Among the above characters analysed, number of days taken to first flower opening and fruit maturity showed significant reduction in mean values in M_2 generation. As regards plant height and rate of growth of plants in general, 20 kR showed a significant reduction compared to control and 30 kR exposure. In the case of fruit characters 30 kR exposures showed a significant reduction compared to 20 kR and control population. A reduction in mean values in M_2

generation for the polygenic traits have been reported in various crop plants and it is stated that the shift in mean value in the segregating generation will depend on the frequency of both negative and positive mutants induced.

A reduction in mean value as was noted in the present investigation has been reported by Brock (1965a), Ehrenburg et al. (1965), Gaul (1967) and Scossiroli (1964) in wheat. In extensive studies performed by Scossiroli (1966 a,b) and Scossiroli et al. (1966) on wheat, this effect was shown in the same population for a large number of characters. Gregory (1965) found that yield of dry peanut pods on the average decreased by irradiation. Oka et al. (1958), Matsuo and Onozawa (1965), Ota et al. (1962), Kawai (1962) Yamaguchi (1964) Miah and Bhatti (1968) and Sharma and Saini (1970) in rice, Gupta (1970) in barley, Bhatt et al. (1968) in wheat and Daly (1960) and Bhatia and Van der Veen (1965) in Arabidopsis have however reported that there is no significant reduction in mean values in irradiated population. Gaul (1970) has pointed out that in most instances, the mean values of mutagen treated populations

are lower than in untreated populations. Rajendran (1975) has reported a significant reduction in mean value for number of days to flowering in safflower under gamma ray treatment but for other characters, occasionally shifts in positive and negative directions were significant. Shift in mean values in the treated population compared to untreated control met with in the present investigation clearly shows a better scope for selection of desirable mutants both in negative and positive directions from the segregating population of chillies induced by gamma rays.

SUMMARY

SUMMARY

The present investigation was carried out in the premises of the Department of Agricultural Botany, College of Agriculture, Vellayani. This was taken up as a preliminary trial on the broad area of 'Induction of mutations for leaf curl complex resistance in chillies'. The direct effect of mutagens, ^{60}Co -gamma rays and Ethyl Methane sulphonate (EMS) on twenty varieties of chillies was assessed in the M_1 generation with respect to various growth metrics. Data were collected on 1) Germination percentage and days taken to complete germination; 2) Seedling survival at transplanting 3) number of leaves, root length and shoot length at transplanting 4) plant height at 15 days interval 5) number of branches per plant at 45, 60 and 75 days after transplanting 6) days taken to first flower opening 7) days taken to harvest 8) pollen sterility percentage and 9) number of fruits per plant. Gamma ray exposed seeds of two varieties Pant C_1 and Black Suryamukhi along with control were carried forward to the M_2 generation to assess the extent of induced variability for various polygenic traits like plant height and number of branches per plant, fruit characteristics and days taken to flowering and harvest.

The tabulated data were analysed statistically following Fischer (1935). From the ANOVA, it was found that in almost all the varieties the two mutagens significantly reduced the germination and survival percentages, root and shoot length at transplanting, the plant height and number of branches and the number of fruits per plant while the number of days to germination and number of days to flowering and harvest were significantly delayed and pollen sterility increased. A dose dependent increase or decrease could be noted in almost all the varieties.

EMS induced a more drastic reduction in germination percentage and greater delay in germination compared to gamma ray exposures. The maximum reduction in germination was noted in 1 per cent concentration of EMS in almost all the varieties. A sharp decline in survival percentage could also be noted in the EMS treatments compared to the gamma ray exposures. A similar trend was also seen in the case of the number of leaves, root length and shoot length at transplanting. The maximum reduction in number of leaves was seen in the higher dose of EMS where the number of leaves was almost half of the control. When EMS induced about fifty per cent reduction in root length compared to control, gamma rays gave only about twenty five per cent reduction. The higher doses of both the

mutagens induced a more reduction in shoot length.

The height of seedlings both at 20 days and 30 days after transplanting were significantly reduced by mutagen treatments. The reduction in height was more in the case of gamma ray treatments than EMS. The higher dose of gamma rays induced a reduction almost half to that of the control plants. The height of plants taken at 15 days interval from the 30th day of transplanting also showed a reduction in the mutagen treated population. The reduction in height was more in the case of EMS treatment at all stages of plant growth except at 30 days after transplanting. EMS concentrations induced a greater reduction in number of branches than the gamma ray exposures at all stages of plant growth.

An increase in the mean number of days to flowering was seen in both doses of gamma rays, whereas EMS treatments did not show any significant difference from the control. An increase in the number of days to maturity was seen with increase in dose of both the mutagens. Pollen sterility percentage also increased with increase in dose of mutagens. The sterility was maximum at 30 kR gamma ray treatment followed by 1 per cent EMS treatment. The lower dose of both the mutagens had a comparable effect. The number of

fruits also showed a significant reduction in mutagen treatment over control. Among the different treatments, the maximum reduction was seen in 1 per cent EMS concentration and the minimum reduction in the lower dose of gamma rays. EMS treatments showed more reduction in number of fruits than in gamma ray exposures.

In the M_2 generation the effects of gamma rays on polygenic traits like plant height, days to flowering and maturity and fruit characteristics were analysed. The number of days to first flower opening and maturity showed a significant reduction in mean values in M_2 generation. In case of plant height and rate of growth of plants in general, 20 kR gamma rays showed a significant reduction and as regards the fruit characters 30 kR exposures showed a significant reduction in mean values. This shift in mean values for various polygenic traits in the segregating generation due to gamma rays projects scope for a positive response to selection and further improvement in this particular crop variety for increasing unit area production. Detailed analysis on the extent of variability created by the mutagens and the selection of the desirable types based on the present day need are suggested as the future line of work in this particular crop variety.

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EFFECT OF MUTAGENS ON THE GROWTH RESPONSE AND MUTATION RATE IN CHILLIES

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ABSTRACT OF THE THESIS
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ABSTRACT

Varietal sensitivity of chillies (Capsicum annuum L.) to the most potent mutagens ^{60}Co -gamma rays and Ethyl Methane sulphonate was assessed by using twenty available genotypes. The experiments were conducted at the Department of Agricultural Botany, College of Agriculture, Vellayani, during the summer seasons of 1982 and 1983. The sensitivity of the varieties was assessed based on the direct effect of the mutagens in the M_1 generation. The parameters chosen include germination percentage days taken to complete germination, growth rate based on plant height and branching ability, days taken to flowering and maturity and various fruit characters. To assess the induced variability due to gamma rays, seeds of M_1 population from the two most popular varieties, Pant C_1 and Black Suryamukhi along with the control were carried forward to the M_2 generation and the general effect studied based on various growth metrics including plant height and number of branches and other yield attributing characters.

The statistical analysis of the data collected from the first generation clearly demonstrated that there exists a wide variation for sensitivity among the different genotypes of chillies to both the mutagens. In majority of

the cases, the morphological and yield attributing characters showed a significant reduction in treated population compared to the control. While comparing the effect of the two mutagens, it is seen that EMS is more effective in reducing the mean values in growth metrics compared to gamma rays. Delay in germination and growth rate were induced by the mutagens. Lethality and sterility were induced by both the mutagens in almost all the varieties. Based on lethality and sterility, it was possible to classify the varieties as least sensitive, moderately sensitive and sensitive varieties.

Analysis of induced variability in M_2 generation showed a significant shift in mean value either in positive or negative direction, based on the character under study. This clearly demonstrated that a positive response to selection can be created by gamma rays in chillies.