

RELATIVE BIOLOGICAL EFFECTIVENESS OF GAMMA RAYS AND ETHYL METHANE SULPHONATE ON CARDAMOM VARIETIES

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Relative biological effectiveness of gamma rays and ethyl methane sulphonate on cardamom varieties" 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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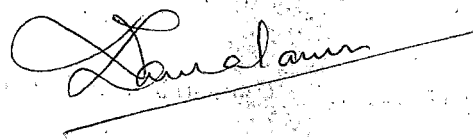
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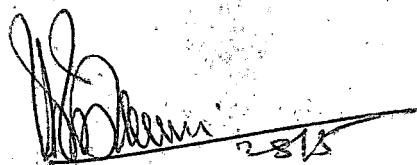
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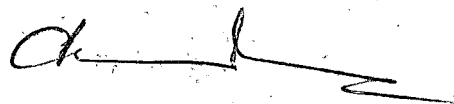


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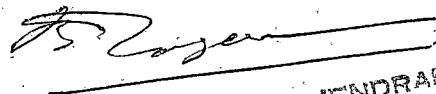
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C O N T E N T S

	Page No.
INTRODUCTION ..	1
REVIEW OF LITERATURE ..	4
MATERIALS AND METHODS ..	29
RESULTS ..	41
DISCUSSION ..	113
SUMMARY ..	131
REFERENCES ..	1 ... 133
ILLUSTRATIONS	

LIST OF TABLES

		Page No.
Table 1	Number of days taken to start germination	42
Table 2	Number of days taken to complete germination from the date of sowing	45
Table 3	Number of days taken to complete germination from the date of first sprout	47
Table 4	Rate of germination (percentage) as on 60th day of sowing under different doses of gamma rays and EMS	50
Table 5	Rate of germination (percentage) as on 90th day of sowing under different doses of gamma rays and EMS	53
Table 6	Rate of germination as on 120th day of sowing under different doses of gamma rays and EMS (percentage)	56
Table 7	Rate of germination (percentage) as on 150th day of sowing under different doses of gamma rays and EMS	59
Table 8	Rate of germination (percentage) as on 180th day of sowing under different doses of gamma rays and EMS	61
Table 9	Rate of germination (percentage) as on 210th day of sowing under different doses of gamma rays and EMS	64
Table 10	Rate of germination (percentage) as on 240th day of sowing under different doses of gamma rays and EMS	67

Table 11	Rate of germination (percentage) as on 270th day of sowing under different doses of gamma rays and EMS	69
Table 12	Germination percentage as influenced by varieties, mutagens and their doses	72
Table 13	Seedling survival on 20th day of sprouting (percentage)	76
Table 14	Survival of plants on 70th day of transplanting (percentage)	78
Table 15-1	Mean plant height(cm) as influenced by varieties, mutagens and their doses at different time intervals	81
Table 15-2	Frequency distribution of plant height variants at different growth phases	85
Table 15-2-A	Statistical analysis of frequency distribution of plant height variants at different growth phases.	86
Table 16-1	Number of leaves per plant at different growth phases ad influenced by varieties, mutagens and their doses	89
Table 16-2	Frequency distribution of leaf number variants at different growth phases	93
Table 16-2-A	Statistical analysis of frequency distribution of leaf number variants at different growth phases	94
Table 17-1	Leaf area (sq.cm.) per plant as influenced by different varieties mutagens and their doses	97

Contd...

Table 17-2	Frequency distribution of leaf area variants as influenced by the different mutagens	99
Table 17-2-A	Statistical analysis of frequency distribution of leaf area variants	100
Table 18-1	Number of tillers per plant as influenced by different varieties mutagens and their doses	101
Table 18-2	Frequency distribution of tiller number variants as influenced by the different mutagens	103
Table 18-2-A	Statistical analysis of frequency distribution of tiller number variants	104
Table 19	Frequency of chlorophyll deficient plants under different treatments (percentage)	106
Table 20	Statistical analysis of frequency of dividing cells under different treatments (percentage)	108
Table 21	Statistical analysis of spectrum of dividing cells under different treatments	110
Table 22	Direction of shift in mean value for plant height, leaf number, leaf area and tiller number per plant.	121

LIST OF FIGURES

- Fig. 1-12 Growth variation as on 45th day of transplanting.
- Fig. 13 Seedling with split lamina.
- Fig. 14 Plant with narrow leaves and deep serration.
- Fig. 15 Chlorophyll variant (striata type)
- Fig. 16 stem dichotomy
- Fig. 17 EMS induced growth retardation.
- Fig. 18-19 Gamma ray induced plant height reduction.

INTRODUCTION

INTRODUCTION

The extent of genetic variability available in the breeding population and the selection technique determine the success of an crop improvement programme. Induced mutation has become an effective method to enlarge genetically conditioned variation considerably within a short time. Numerous work has been done on the induction of mutation in different crops and its utility in furthering the crop prospects has been established beyond doubt. Though it has been now claimed as an excellent tool in the hands of the breeder it took nearly three decades to realize its importance and usefulness for practical plant breeding programmes.

Various mutagens are available which can be beneficially utilized for tailoring better varieties of crop plants. The utilisation of radiations and chemical mutagens as potent tools for inducing alterations in the genetic make up of organisms is one of the most important lines of contemporary breeding research. Among physical mutagens gamma rays have been observed to be the most potent agent and used widely for irradiating seeds and seed material. Ethyl methane sulphonate is also one of the most potent chemical mutagen that can be utilized for economic mutation breeding.

Efficient treatments producing greater proportion of mutations to damages are essential for an economic mutation breeding programme. A quantitative determination of M_1 injury helps in fixing doses of the mutagen which results in low plant injury with

high genetic effects. The spectrum of induced mutations and recoverable mutations vary depending on the mutagens used (Nilan, 1966 and Smith 1961). Ehrenberg et al. (1959) and Sharma and Benseal (1970) reported that EMS is much superior to gamma rays and at the same time caused much less reduction in fertility.

Cardamom, the queen of spices is one of the most important dollar yielding crops of India. In Kerala, it is extensively grown in the high ranges of Idukki and Wynad districts. In South India from time immemorial, it is used in curries, cakes, sweetmeats and pickles as well as in medicine. It can be propagated vegetatively and also through seeds and is generally grown in a variety of environmental conditions. Indian cardamom industry can have a sound footing in the world market with increased productivity and improved quality which is possible only through concentrated efforts of research and development in various aspects of crop improvement.

There exists a wide variability for yield and other yield attributes in cardamom. But so far no systematic approach has been made to assess the extent of variability available within or between cardamom varieties. This to a large extent is due to their mode of cultivation in different pockets under high range conditions. This appears to be a limitation for detailed analysis in the natural variability in this particular crop variety. But

induced mutagenesis the extent of variability created can be assessed within a restricted size of population under controlled condition.

The present investigation was taken up as a preliminary trial in the broad area of 'Induced mutations in cardamom'. The objectives of the investigation were,

1. To find out the relative efficacy of ^{60}Co gamma rays and ethylmethane sulphonate in inducing morphological and cytological alterations in cardamom.
2. To analyse the factors concerned with mutagenic effect.
3. To determine the direct effect of the mutagen on this particular crop variety.
4. To assess the varietal reactions to both the potent mutagens widely used at present.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Induced mutations are being used to a larger extent in genetic researches. The birth of mutation breeding technique was actually a boon to plant breeders, since it facilitated the enrichment of crop improvement programmes to a greater extent. A good amount of genetic diversity in the parent population is the cornerstone of any successful crop improvement programme. Induced mutation enables crop improvement through enhanced genetic variability (Konzak, 1984). The enhanced genetic variability through induced mutation can serve as the basis for widening the desirable attributes of crop plants and building up exotic varieties.

The credit for focussing the attention of geneticists on to the phenomenon of induced mutation and their utility in breeding goes to Hugo De Vries in 1901. The outstanding work of De Vries stimulated other investigators to create mutation artificially in different organisms by various physical and chemical agents. X-rays and other radiations were applied on to living cells of plants by Koernicke (1905) and Gagner (1908). But it was the remarkable discovery of Muller in 1927 that X-rays could induce genetic changes in *Drosophila*, that marked the beginning of the era of induced mutagenesis. This was closely followed by the successful experiments of Stadler (1928 a, b) in inducing mutation in barley and maize using ionising radiations.

Similar findings by Gagner and Blakeslee (1927) and Good Speed (1929) in Datura stramonium and Nicotiana confirmed this. Different types of physical mutagens were attempted thereafter by many scientists to put it in practice (Gustafsson, 1947; Delaunay, 1931; Sapehin, 1936; Freisleben and Lein, 1944). This in turn led to many valuable achievements (Dubinin, 1964 and Gaul, 1964).

Sparrow et al. (1958) reviewed the investigations relating to the effects of radiations during 1896 to 1955. A clear picture of the attempts made with ionising radiations have been published by many scientists including Gregory (1956 a, b, 1968), Gaul (1959), 1961, 1964), Sparrow (1961), Evans and Sparrow (1961), Sparrow et al. (1965, 1968), Sparrow and Pond (1956), Gustafsson (1963), Yamaguchi (1964), Brock (1965 a, b), Milan et al. (1965), Gottschalk (1965, 1969), Gottschalk and Baquar (1973), Iqbal, (1969, 1970, 1972), Tanaka (1968), Mikaelson (1968), Mikaelson and Brunner (1968), Broertjes (1969, 1972) and Harten et al. (1972, 1973).

Recently the mutagenic property of chemicals was brought to light by Auerbach et al. (1947). After this discovery scientists all over the world tried various chemicals for their mutagenic property. Rapoport (1946 and 1948) established the high mutagenicity of epoxides and epimines in Drosophila. Alkylating agents were found to be superior than other chemical

in inducing mutations in a wide range of organisms ranging from unicellular to higher multicellular organisms (Auerbach, 1961). Among the alkylating agents, ethyl methane sulphonate appeared to be the outstanding one in inducing mutations in a wide variety of organisms including the higher plants. (Swaminathan et al. 1962).

The innumerable number of induced mutants released so far from different countries all over the world proves beyond doubt the suitability and excellency of induced mutation in crop improvement programme. It is much time saving compared to conventional plant breeding techniques. A desired mutation can be recovered in a homozygous state in M_2 or M_3 generation compared to F_6 or F_7 generation in the case of hybridization (Sigurbjornsson, 1970). The release of the wheat variety Sharbati Sonora by Swaminathan (1969) in three and a half years is a typical example for this. The work of Sears (1956) on transferring the resistance factor from the wild grass species Aegilops umbellulata to the cultivated wheat variety Chinese Spring by induced translocations has shown that new combinations can also be created by mutation breeding. According to Brock (1970) it is possible to induce any mutation that occurred naturally and probably, many which have either never occurred naturally or have been lost from the natural population. In pulses and oil seeds about 22 varieties have been developed.

Out of the 22, 15 have been used directly as mutants and seven have been utilized for crosses. In cereals alone about 137 varieties with improved characters have been released and 76 varieties in other crops. In almost all the crops, a steady increase in the number of mutants can be noticed. Inter-crossing of mutants can give rise to new transgressive variations particularly when the same character is governed by many mutant genes (Aastveit, 1966; Gaul, 1963 and Hagberg, 1959).

TYPES OF MUTAGEN

Mutagens are broadly classified into physical mutagens and chemical mutagens. Physical mutagens are being tried right from the beginning of this century while chemical mutagens are recent introductions (Broertjes and Van Harten, 1978). Physical mutagens mainly include radiations and chemical mutagens include various chemicals exhibiting mutagenic property.

(a) Physical Mutagens

The various types of radiations coming under physical mutagens include X-rays, gamma rays, U.V. rays, neutrons (fast, slow and thermal), beta particles, alpha particles and protons (Sparrow and Konzak, 1958). Gamma rays are of shorter wave length than X-rays and are commonly used in mutation breeding programmes. Mutation induced by gamma rays for creating desirable character combinations have been reported in bread

wheat (Goud, 1967), rice (Singh, 1970), barley (Sethi, 1975), pigeon pea (Venkiteswarulu et al. 1978), chillies (Subash and Nizam, 1977; Khan et al. 1979) and green gram (Rathnaswamy et al. 1978).

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 macro molecular structure of DNA. It is difficult to attain specificity with the physical mutagens because they cause random distribution of breaks over all the chromosomes. It is known (primarily from in-vitro studies) that radiation may cause the following alterations in DNA.

1. Rupture of the hydrogen bonds, which link the base pairs adenine - thymine, cytosine - guanine;
2. Induction of breaks in either or both of the DNA chains, between a sugar and a phosphate group;
3. Formation of links between adjacent thymine residues on a chain to form a dimer;
4. Cross-linking between the single strands of the helix or between a strand and another DNA molecule or between a strand and the protein (histone) associated with DNA in the chromosome.

Ionising radiations can introduce transcription errors into the precise information storage and retrieval system of the cell

and such errors result in mutations. Swaminathan et al. (1970) found that mutagenic effectiveness in neutron treatment were 7 to 10 times greater than that in the case of gamma rays.

(b) Chemical mutagens

Friese (1963) classified chemical mutagen as base analogue substitutes, dyes, acids, metals, and alkylating agents. The remarkable works of Gustafsson (1963), Yamaguchi and Miah (1964), Kawai and Sato (1965), Bhatia and Vander Veen (1965), Konzak et al. (1965), Gaul et al. (1966), Sato (1966), Sato and Gaul (1967), Siddiq et al. (1968), Soriano (1968), Kawai (1969) and Mikaelson et al (1971) gave a clear picture of the research work carried out to study the effectiveness and efficiency of chemical mutagens in various plants. The effect of alkylating agents and their mechanism of action on the biological 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. The mutagenic efficiency of EMS was demonstrated by Ehrenberg (1960). The relatively low toxicity and high genetic effects of EMS (Gaul, 1961) and its high mutagenic effectiveness and efficiency in higher plants (Konzak et al. 1965) provide scope for its exploitation in crop improvement programmes.

Chemical mutagens produce a non-random "localized" distribution of breaks in the chromosome which is due to the fact that definite chromosomes of the genome and definite regions of a chromosome break preferentially. In general, after treatment with chemicals more breaks are found in the heterochromatin of the chromosome than in euchromatin. It seems to be the characteristics of many chemicals to produce very small structural changes. They also produce small duplications more frequently (Ford, 1948).

Alexander and Stacy (1958) and Reiner and Zamenhof (1957) have identified alkylation of phosphate groups in the DNA as the primary reaction sites. The resulting instable phosphate triester can hydrolyse between the phosphate and the deoxyribose resulting in the back bone breakage. Brookes and Lawley (1961) have established that alkylation of purine and pyrimidine bases also occur. Alkylating agents appear to induce preferentially so called "transitions" but may also induce mutations by loss of a purine or of fragments of a greater length. Point mutations seem to require base alkylation which leads to a change in the nucleotide sequence through the exchange of a single base pair. The alkylated base will probably pair with a wrong partner giving rise to a "transition". The greater toxicity and chromosome breaking ability of bifunctional and polyfunctional alkylating agents may be due to their ability to induce cross-linkages in macro molecules including DNA.

(c) Physical versus chemical mutagens

In seed propagated plants chemical mutagens have yielded very high mutation frequencies and in most cases they were more efficient than ionising radiation (Kamra and Brunner, 1970). As the mode of action of the physical and chemical mutagens in the biological material is different, the spectrum of induced mutations and recoverable mutations varies depending on the mutagens used (Nilan, 1966; Smith, 1961). The spectrum of chlorophyll deficient mutants may depend upon the type of mutagen employed (Nilan and Konzak 1961; Ehrenberg et al. 1961 and Gustafsson, 1963). This brings out the fact that the mutation rates of specific loci may also vary depending on the type of mutagens employed in addition to other modifying factors. So the possibility of getting desired mutants can be boosted by the use of several mutagens in the mutation work.

It is characteristic for most chemical mutagens that they induce more chromosome fragments and fewer chromosome recombinations by comparison with ionizing radiations. It was found, for instance, that EMS produces in barley seeds at least five times more fragments per bridge than X-rays; more over, many fragments and bridges of chromatid type were found after EMS treatment, while they were scarcely found after X - irradiation (Sato and Gaul, 1967). While a random distribution of chromosome breaks is produced by physical mutagen, a regional specificity has been proved for many chemicals. Chemical mutagens produce

relatively more intra chromosomal than interchromosomal changes. Compared with radiation induced breaks chemical induced breaks tend to remain latent a longer time, and to open up later. For the occurrence of recombinations it is necessary that two breaks are present simultaneously in one cell. Recombinations cannot occur by potential breaks, which open in different cell cycles. The rarity of recombinations between different chromosomes can therefore be explained by the delayed effect of chemical mutagens.

DIRECT EFFECT OF THE MUTAGENS

It is possible to study the direct effect of the mutagen in the first generation (M_1 generation). Physical and chemical mutagens result in three types of these effects. They include

1. Physiological damage
2. Factor mutations (gene mutations) and
3. Chromosome mutations.

Physiological damage represent injuries created in the biochemical set up of the organism and they are restricted to M_1 generation. The physiological damage sets a practical limit to increase the dose. An end point is reached with 100% lethality. It is for this reason that doses of the mutagens are fixed which result in low plant injury with high genetic effects. For a given mutagenic treatment, there is a correlation between M_1 seedling height and survival on one

hand and M_1 , mutation frequency on the other hand (Gaul, 1959). Therefore a quantitative determination of M_1 injury should be a routine procedure in mutation breeding experiments. Gaul (1970) reported that for any breeding purpose mutagenic treatment with low physiological effects and strong genetic effects are desirable.

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 per plant
6. Number of florets per spike
7. Number of seeds per spike and
8. Fruits and or seeds per plant.

Gaul (1970) also reported that with increasing dose the values obtained for each of these above 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 that correlation permits the prediction of the killing rate produced by a definite dose. Factor and chromosome mutations are well defined changes of the genetic material and they are carried over to succeeding generations.

The M_1 effects noticed due to mutagen treatment include,

1. Reduced germination
2. Reduction in survival
3. Growth inhibition
4. Reduced fertility
5. Chlorophyll chimeras and
6. Other morphological and developmental abnormalities.

Germination of seeds

Generally a delay in germination and reduction in germination percentage are noticed consequent to irradiation at higher exposures. This was clearly demonstrated by Athwal (1963) in Cicere using X-ray treatment, by Shirshov and Shain (1966) using gamma rays in field beans and by Sidorova et al. (1966), Maslov and Stepanova, (1967) using gamma rays in peas. Similar reports were made by Alikhan et al. (1973) in red gram with gamma rays and by Bajaj and Saettler (1970) in phaseolus using gamma rays. In many bean seeds, 30 kR and 70 kR gamma exposures were found to decrease germination (Dahiya, 1973). A reduction in germination at higher doses of gamma rays and a complete suppression of germination at 60 kR and above were noticed in cowpea by Louis and Kadambavanasundaram (1973 a). In groundnut, Reddy et al. (1977) found that with gamma irradiation germination ranged from 24-57% compared to 98-100% in the control group. Reduction in germination with increase in dosage of gamma rays in pigeon pea was reported by Venkateswa et al. (1978).

Chemical mutagens like EMS, NMS, EI etc. induced reduction in germination with increase in concentrations in Pisum (Blixt and Gelin, 1965). Wellen sick (1965) observed that germination decreased rapidly with increase in concentrations of EMS in pea. Zoz and Kolotenkov (1968) noticed a decrease in germination in pea with longer exposure of N-nitroso - N ethyl urea (NEU). Borejko (1970) reported that various chemical mutagens reduced germination in soybean. Smutkupt (1973) found that 5-30 kR gamma rays did not affect seed germination in soybean. Ene et al. (1971) observed that DES treatment on bean seeds produced a slight inhibiting effect on germination which was dose dependent.

Survival of plants

The relationship between doses of mutagens and survival of plants was studied by several workers. Gladstones (1958) reported that increased doses from 0-125kR of X-rays in Blue Lupin resulted in decreased survival percentage and no plants survived above 80 kR treatment. Teodoradze (1966) noticed a decrease in survival with gamma irradiation in French bean and Soybean while 20 kR and above proved lethal for Phaseolus. Debeley and Zekurnov (1977) noticed that in sweet varieties of Lupin, 20 kR and for bitter varieties 30 kR of gamma rays were found to be lethal. Reddy et al. (1977) noted survival reduction in groundnut with gamma irradiation. Yamogata et al. 1965; Siddiq, 1967; Swaminathan et al. 1970 and Jananowskii, 1970; noticed a decline in survival rate with gamma rays of dosage

above 25 kR in pisum arvense and Vicia sativa. Constantin and Love (1967) observed that in green gram survival of plants decreased with increase of the mutagen. A significant reduction in plant survival with high doses of X-rays and neutrons was reported by various investigators including Ojomo and Chedda (1971) in cowpea. Mujeeb and Greig (1972) in Phaseolus vulgaris observed a progressive reduction in survival with the increase in dose of gamma irradiation. Fautrier (1976) reported that in Lucerne, no plants survived in treatments above 120 kR of gamma rays. Yadava and Chowdhury (1974) reported that 100 - 150 kR of gamma rays proved lethal in cyamopsis.

Wellensick (1965) reported that the percentage of healthy seedlings and full grown plants decreased rapidly with increasing concentrations of EMS (0.04 to 0.4%) in pea. Tarasenkov (1969) found the survival rate to be low in pea treated with NMU, DEU and NEU. Narasinghani and Kumar (1976) observed that the survival percentage was drastically reduced with 0.25 per cent EMS and 0.25 per cent MMS in cowpea.

Palaniswamy (1975) reported the effects of gamma rays and EMS on cowpea variety Co.2. A decrease in survival was noticed with increase in the dosage of gamma rays. The LD₅₀ value for gamma rays ranged from 40 - 50 kR and for EMS no lethal effects was noticed at concentrations ranging from 5 to 10 mM. The survival of seedlings was generally found to decrease with increasing doses of radiations and chemical mutagens (Rao and Ayengar, 1964). Alikhan et al. (1973) studied the effects of

X-rays, gamma rays, EMS and DES on germination and survival in redgram.

Plant height

Kwon and Im (1973) reported that height was retarded by 10-25 kR of gamma rays in soybean. Teretchenko (1966) noticed delayed seedling growth in pea by gamma irradiation. Gradual reduction in the height of seedlings was seen with increasing doses of gamma rays in cowpea by Louis and Kadambavanasundaram (1973 a). Sree Rangaswamy et al. (1973) observed that the green gram plants treated with gamma rays were shorter than the parents and those treated with 60 kR were the shortest. Decrease in seedling height was also reported by Shakoor et al. (1978) in mung bean with gamma rays and by Khanna and Makachandam (1980) in gram using gamma rays. Akilov (1966) noted an increase in plant height with increased dosage of gamma rays in soybean. Similar results were noted by Mujeeb (1974) in Cicer and by Chowdhury et al. (1977) in cluster bean. Reduction in plant height following gamma irradiation was reported in rice (El. Aishy et al. (1976); ragi (Goud et al. 1969 and Raveendran, 1976); wheat (Kapoor and Natarajan, 1970), sugar cane (Walker and Sisodia, 1969) and barley (Kapoor and Natarajan, 1970; Filev 1972; Kozachenko, 1974; L'vova and Konorovskaya, 1974).

Blixt and Gelin (1965) observed reduction in height with EMS, NMS and EI in Pisum. Wellensick (1965) reported that height decreased with increasing concentrations of EMS (0.04 - 0.4%) in pea. In sorghum, Ramulu (1974) reported gradual decrease in the mean of plant height as the concentrations of chemical

mutagens increased. Similar observation was made by Ayyamperumal (1977) in ragi. Narasinghani and Kumar (1976) following EMS treatment reported reduction in plant height in cowpea. So was the result obtained by Santos (1969) in mung bean. In pea, Bhojwani and Kaul (1976) reported that the plant height was significantly reduced following ethylene imine treatment.

Constantin et al. (1976) reported that in soybean seedling, height decreased as the dose of neutrons, gamma rays, EMS and DES increased. Similar results were obtained by Appa Rao and Jana (1976) in black gram following X-ray and EMS treatments. Maslov and Stepanova (1967) reported that plant height in peas was suppressed the most by gamma rays and EMS.

Growth rate

Reduction in growth rate is used as a reliable estimate of damage in several radiobiological experiments. Seedling growth inhibition by 50-62% at low doses of irradiation and almost completely at higher doses was reported in wheat (Ananthaswamy et al, 1971). Reduction in growth following gamma irradiation was reported in sorghum (Ramulu, 1974), ragi (Ayyamperumal 1977), rice (Nayar and Ninan, 1978) and bajra (Vijendra Das, 1978).

Rahman and Soriano (1972) showed an inverse relationship of seedling height and concentration of EMS in rice. In pearl millet reduction in growth rate was reported by Singh et al. (1978).

particularly with EMS. Magri-Allegra and Zannone (1965) found in comparison with EMS, EI produced much less growth reduction in vetch. Reduction in growth rate as affected by the different doses of chemical mutagens were reported by Scarsia et al. (1961) Konzak et al. (1961 a,b).

Cytological variations

Mutagenic treatments result in effects which can also be cytologically observed. There are a number of changes whose significance with respect to genetic integrity is vague or unknown such as chromosome piling, stickiness and clumping, abnormal chromosomal spiralization of chromonemata etc.

(a) Mitotic aberrations

The results of different mutagenic treatments can be compared only if the first mitotic cycle after seed germination is investigated. This is because, the process of diplontic selection will decrease the frequency of aberrant cells in the second, third, etc. mitotic cycle (cf. Sax, 1941). Although observations are possible at mitotic metaphase, in most plants anaphases are better suited for quantitatively recording chromosome mutations. In anaphase, two types of cytological damages can be determined, namely bridges and fragments. With ionising radiations, all or most of the bridges occur as double bridges (Caldecott and Smith, 1952). These paired bridges may lie (1) separate and more or less parallel. (2) apparently

touching, in which case they frequently lie across each other, or (3) occasionally interlocked. Acentric fragments also usually occur, despite the squashing technique, in pairs. The size of the fragments ranges from minute dots to rods which appear to be as long as whole chromosomes. Because of the appearance of double bridges and double fragments it can be deduced that the seed irradiation generally cause chromosome breaks. The cause of bridge formation is due to the fusion of two centromere-bearing chromosome fragments. It has been found that the frequency of these bridges is directly proportional to the dose of both X-rays and thermal neutrons (Caldecott et al. 1954; Gaul, 1963).

(b) Meiotic aberrations

When the development of the M_1 plants has proceeded to meiosis, spikes can be fixed in order to determine quantitatively chromosome mutations surviving to this stage. Diakinesis or metaphase I are usually the best to investigate. The only type of chromosome mutations that can be readily recognized in most plants are translocations. These occur in different ways, mostly as reciprocal translocations, that are recognized by ring or chain formation. In anaphase I and II of the M_1 generation no inversion bridges are to be found, or only in extremely rare cases. The absence of (visible) deficiencies in M_1 diakinesis or metaphase-I is not surprising, because it is expected that all larger deficiencies lead to cell death, and are therefore eliminated early from the cell population.

It has been found in barley that the translocation frequency determined in both mitosis and meiosis is the same and increases linearly with the radiation dose (Gaul, 1963; Caldecott and Smith, 1952; Caldecott et al. 1954). In mitotic studies on shoot tips, simple translocations are recorded. But in meiosis reciprocal translocations are recorded.

GENOTYPES IN RELATION TO MUTAGEN SENSITIVITY

A study of mutagen sensitivity is vital for practical plant breeding programmes since the sensitivity of the biological material to mutagen is dependent on many factors including genetic factors. As reported by Davidson (1960), Konzak et al. (1961 a,b) and Nilan (1956) the major factors that alter the genotypic sensitivity to mutagens include nuclear volume, water content, oxygen pressure, stage of development and hydrogen ion concentration. Sparrow et al. (1968) explained differences in radiosensitivity between different plant species mainly on the basis of differences in nuclear size and average interphase chromosome volume. It has been clearly demonstrated that there is an inverse relationship between radiosensitivity and DNA content. Data for the prediction of radiosensitivity of seeds in relation to total DNA content have been published by Osborne et al. (1963). There exists much evidence that genetic differences, even though they are as small as single gene differences, induce significant changes in radiosensitivity, which influence not only the total rate but also the spectrum of recoverable mutations (Gustafsson, 1944, 1947 and 1965; Gustafsson and Tedin, 1954; Nilan 1956; Lamprecht, 1956 and 1958; Gelin et al. 1958; Smith, 1961; Sparrow, 1961;

Konzak et al. 1961 a; Sparrow et al. 1965). Though the influence of a particular genotype on the mutation spectrum cannot be predicted, the choice of the parent material is a crucial event in any mutation breeding programme (Mackey, 1960 a,b). According to Gregory (1960) "the chief limiting factor in mutation production and mutants 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".

Definite information is available with regard to the influence of the ploidy level on the mutation spectrum. In contrast to diploid organisms a high degree of genes are reduplicated in polyploids, which increases their ability to bear gross chromosome aberrations. This results in the more frequent discovery of dominant and semidominant mutations. Phenotypic buffering is another property of polyploids which restricts mutability of many characters, especially those which are essential for the whole life of the plant. (e.g. process of chlorophyll formation). Thus, chlorophyll mutations decrease with the increasing level of ploidy (Stadler, 1929); but the total rate of mutation increases. Differences in mutagenic response also exist between species of the same level of ploidy and between varieties within the species. (Tsunewaki and Heyne, 1959;

Jagathesan and Swaminathan, 1961; Swaminathan, 1965).

Borojevic (1964) noticed differences in the frequency of mutation between varieties of Triticum aestivum sp. Vulgare and Enken (1966 a and 1966 b) concluded that the closer the varieties are in their genotypes, the greater is the similarity in their spectra and frequency of mutation.

Ramalingam (1980) reported that spectrum of mutations differed according to variety and mutagen and interaction between variety and dose of particular mutagen. A variety dependent variation was observed in the sensitivity to physical and chemical mutagens. Radiosensitivity of haploid plants was found to be higher than that of diploids (Tanaka, 1970). The diploids in turn were reported to be more sensitive than the respective auto tetraploids (Yamaguchi and Kobayashi, 1960; Yamaguchi, 1964 and Sree Rangaswamy, 1970).

Comparison among varieties of barley (Mikaelson and Brunner, 1968), tomato (Blanchi et al. 1963) and pea (Muqeab and Siddiqui, 1973) showed variation with respect to radiation response among different genotypes. Krishnaswami and Rathnam (1982) reported differential radiosensitivity to EMS exhibited by ten green gram cultivars. Difference in radiosensitivity was also reported in varieties of cucurbits (Vishnoi and Joshi 1981), safflower (Mallikarjunaradhya and Cowda, 1981) and tomato (Georgiev, 1966). In rice, marked intervarietal differences

radiosensitivity were recorded by Matsuo et al. (1958), Fuji (1962), Ukai (1967) and Mikaelson and Navaratha (1968) in rice. The varietal differences in radiosensitivity were also reported to be due to differences in chemical composition (Myttenaere et al. 1965) or due to differences in endogeneous levels of auxin and ascorbic acid (Goud et al. 1967). Radiosensitivity and mutability of different varieties of winter barley with respect to gamma rays were studied by Stefanov et al. (1979). In linseed Sinha et al (1978) studied the response of different genotypes to radiation. Virakkamath, B.C and Goud, J.V (1978) studied the sensitivity of sorghum genotypes to gamma irradiation and EMS treatments and concluded that the difference in mutation frequencies are attributed to difference in seed size and genetic constitution.

INDUCED MUTATIONS IN VEGETATIVELY PROPAGATED PLANTS

The problems and prospects associated with mutation breeding in vegetatively propagated plants differ from those of seed propagated plants. Their mode of reproduction is the main reason for this. The various spontaneous mutants we have, clearly indicates that induced mutations can improve vegetatively propagated plants to a larger extent. A recent example of the radiation induced mutant of great commercial value is that of Chrysanthemum morifolium Cv Horim. The interest in the application of mutation induction in vegetatively propagated plants is in addition a consequence of the large economic importance of many species of this group. This is clearly indicated by the fact

that crops like sugarcane, potato, sweet potato, tapioca, and several fruit crops including grapes are some among the world crops with the highest fresh weight production.

For various reasons vegetatively propagated crop plants are a very suitable group of plants for the application of mutation breeding methods. The high degree of heterozygosity which causes a complex inheritance of genetic factors as well as the frequent polyploidy both serious handicaps in conventional breeding are advantageous in mutation breeding. The importance of high degree of heterozygosity is that under heterozygous condition the frequency of desirable mutants is more than in homozygous crops. Polyploid nature of most of the vegetatively propagated plants is another feature that makes them suitable for mutation breeding. Under polyploid condition a high degree of genes are reduplicated which increases their ability to bear gross chromosome aberrations. Phenotypic buffering is another property of polyploids which restricts the mutability of many characters especially those which are essential for the whole life of the plant. (eg. process of chlorophyll formation).

Once a good genotype is obtained it may be propagated and made use of directly, without any homozygotization. Moreover the vegetative mode of propagation permits the use of chromosomal re-arrangements with phenotypical effects. In sexually propagated plants such rearrangements probably would be eliminated at or after meiosis. With the introduction of adventitious bud technique it is possible to get solid mutants in vegetatively propagated crop plants.

Ornamental plants are highly benefited by mutation. In this case since we are going for easily detectable characters like flower colour, size, etc, desirable mutants can be easily identified. In truly vegetatively propagated plants mutation is the only source of variability. The main advantage of mutation induction in this category of plant species is the possibility of changing one or a few characters of an otherwise outstanding cultivar without altering the remaining and often the unique part of the genotype.

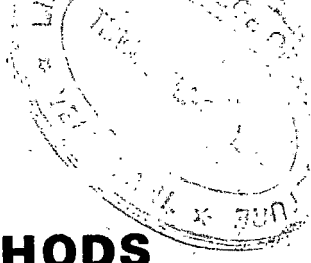
For inducing mutations in vegetatively propagated plants chemical mutagens are not usually considered mainly because the number of cases in which they have been applied successfully and in which they were better than physical mutagens has been small. The lack of success is probably a consequence of poor uptake and penetration of the chemical compound, (Bowen, 1965; Moes, 1966 and Nybom, 1961). Moreover bulky materials like bulbs, scions for grafting and plants are difficult to be treated with chemicals in a reproducible way. But it is somewhat surprising that in some countries like U.S.S.R. mutation breeders concentrate on or even use exclusively chemicals for inducing mutations as demonstrated by the work of Amirov (1974), Dryagina (1974) and Rapoport (1973). Successful application of chemical mutagens to pine apple was reported by Singh and Iyer (1974), Kaul and Kak (1973, 1975) to peppermint, and Mee et al. (1969) to sugarcane.

In general most mutation breeders prefer ionising radiations for mutating vegetative parts. Physical mutagens like X-rays, gamma rays etc. are widely used to induce mutations in all kinds of plant parts. Small specimens are more conveniently X-rayed. For vegetative parts that are bulky a ^{60}Co or ^{137}Cs source of 50-200 curie may be a more convenient one. Ultra violet rays are not generally preferred for irradiating vegetative parts due to their poor penetration capacity.

Various regions to be irradiated include shoot apices, bulbs, corms, buds, tubers etc. Treatment must be confined to the buds or parts of the plants from which the mutations are to be violated later. The results of Sparrow et al. (1961) consistently show that acute treatments give higher frequencies of somatic changes. Results of Schwemmer and Robbelen (1962) indicate that irradiation of growing plants may bring about mutation that do not occur after seed irradiation. Unrooted cuttings are more sensitive than plant parts with existing root and shoot meristems. Treatment just at the beginning of sprouting or bud swelling generally seems to be preferred compared with treatments of material in deep dormancy or in a more advanced stage. If the mutagenic treatment is carried out at a stage when the primordia consists of relatively few and undifferentiated cells it may reduce diplontic selection and chimerism.

Moh (1963) was the first to use radiation in Cassava. He irradiated nodes upto 4 kR of gamma rays and found that the LD₅₀ value was 3 kR. X-ray treatments of potatoes were performed for the first time by Jacobsen (1923), Johnson (1928, 1937) and Sprague and Lenz (1929). High energy protons were used recently by Tarasenko (1977), in potato and were found to be as effective as neutrons. Konkei No. 45 and Mariline - 2 are the two induced mutants reported in potato. In sweet potato, Miller (1935) was the first to use radiation. Kukimura and Takemata (1975) recently reported that mutants with increase as well as reduced sugar contents were obtained after treatment of shoots, dormant root tubers and seeds of sweet potato with ⁶⁰Co gamma rays.

Cardamom is a crop amenable to both seed and vegetative propagation. Very little attempt has been made so far to bring about genetic improvement in cardamom, due to their special mode of cultivation under high range conditions. Results of the mutations study conducted at the Cardamom Research station, Pampadumpara showed that seeds irradiated at 20 kR and above failed to germinate and there was a decrease in germination at doses of 10 kR and above. The LD₅₀ value was found to lie between 8-10 kR. At doses upto 4 kR (0.5, 1.00, 2.00 and 4 kR) Mysore showed maximum germination, while Malabar showed the least. In Vazhukka the germination was intermediate. Albinos were not observed among the seedlings.



MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation to assess the relative biological effectiveness of gamma rays and ethyl methane sulphonate (EMS) on cardamom varieties was carried out in the Department of Agricultural Botany, College of Agriculture, Vellayani during 1985 - 1986.

A. MATERIALS

Selection of variety

Three varieties of Elettaria cardamomum (L.) Maton, were chosen for the study. They include Malabar (V₁), Mysore (V₂) and Vazhukka (V₃). Malabar variety is characterized by plants of medium size, creeping or prostrate panicles with small and roundish capsules and leaves pubescent on the under surface. Plants of Mysore variety have robust growth, large leaves with smooth under surface, erect panicles with longish fruits larger in size than the Malabar. Vazhukka is supposed to be a natural cross between Malabar and Mysore. It has deep green leaves and is as robust as Mysore with semierect panicles and roundish long capsules.

Selection of seed material

Seeds were collected from fully ripe capsules of healthy and well grown adult plants maintained at the Cardamom Research Station, Pampadumpara. The ripe capsules immediately after

harvest were immersed in water and seeds gently pressed out of the capsules. The seeds were then mixed with coarse river sand in the ratio 1:3 and rubbed vigorously by hand for 5 - 10 minutes to remove the thin layer of mucilage adhering to the testa of the seeds. The extracted seeds were washed in four to five changes of water. After draining, seeds were mixed with ash and dried in shade for two days. Uniformly dried healthy seeds having uniform size and colour were selected for the mutagen treatment.

B. METHODS

(1) Gamma irradiation

Two hundred dry seeds (moisture content 12-15%) of each variety were exposed to 10 kR - 70 kR ^{60}Co gamma rays at an interval of 10 kR using a gamma cell installed at the Radio Tracer Laboratory, Vellanikkara. The dose rate employed was 60 kR/hr. The irradiated seeds were sown on the subsequent day of treatment. Two replications were maintained for each treatment.

Another set of two hundred seeds (moisture content 12-15%) of each variety were sown in germination beds under glass house condition. They were taken out of the soil on the 30th day of sowing and were exposed to gamma rays at 10 kR and 20 kR. Treated seeds were sown on the subsequent day in two replications for each treatment.

(2) EMS treatment

EMS solutions of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 per cent concentrations were prepared in glass distilled water immediately before use. Two hundred seeds of each variety were used for each concentration. The seeds having uniform size and colour were first soaked in 50% ether for ten minutes followed by soaking in 2% HNO_3 for five hours as detailed by Koloigi et al. (1973) for hastening germination. The seeds were then washed thoroughly in running distilled water to remove any trace of HNO_3 . The superficial water particles on the seeds were removed by gently pressing the soaked seeds within the folds of a blotting paper. EMS treatment was done immediately after pre-soaking.

The seeds were immersed in the mutagen solution for 6 hours with intermittent shaking. To facilitate uniform adsorption of the mutagen by the seeds, 15 ml of the solution was used. The treatment was done at room temperature. The treated seeds were washed thoroughly in distilled water and kept in running water for two hours. Corresponding controls were kept in distilled water for the same period as for the mutagenic treatment.

(3) Planting technique

The gamma ray and EMS treated seeds and control seeds (both dry and soaked) were sown in germination beds in two replications under controlled conditions in a glass house. The

germination beds were of 1m x 6m size with a height of 20 cm. The sand-soil mixture for bed preparation was made with cattle manure, river sand, and soil in the ratio 1:1:1. A fine layer of humus rich forest soil was spread over the beds. Seeds were sown in lines with a spacing of 20cm between the lines and the ground was gently pressed. Watering was carried out regularly with a rose can to keep the beds sufficiently moist.

On the 20th day of sprouting, the seedlings were transplanted to polythene bags filled with potting mixture. Potting mixture of uniform proportion was used to fill the polythene bags and facilities for prompt drainage were provided in the bags. These seedlings were also maintained under glass house conditions with proper irrigation. On the 90th day of transplanting to the polythene bags, the seedlings were transplanted to pots of uniform size filled with equal quantities of potting mixture to ensure better root as well as shoot growth and tillering.

(4) Observations

The direct effect of gamma rays and EMS on the three genotypes were studied with respect to different growth metrics. The observations taken include,

1. Days taken to start germination
2. Days taken to complete germination from the date of sowing
3. Days taken to complete germination from the date of first sprout
4. Rate of germination

5. Germination percentage
6. Seedling survival on the 20th day of sprouting
7. Survival of plants on the 70th day of transplanting in polythene bags.
8. Chlorophyll and other M_1 variants
9. Plant height and number of leaves per plant at 15 days interval
10. Number of tillers at 15 days interval from the 90th day of transplanting in polythene bags.
11. Leaf area (L x B) and
12. Cytological analysis.

1. Days taken to start germination

The date of first sprout was recorded for each treatment and the duration from the date of sowing till the date of first sprout in each treatment was calculated for obtaining the number of days taken to initiate germination.

2. Days taken to complete germination from the date of sowing.

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

3. Days taken to complete germination from the date of first sprout

The duration from the date of first sprout till the date of last sprout was calculated for each treatment.

4. Rate of germination

The number of seeds germinated daily per treatment was recorded from the date of first sprout in the early morning hours.

5. Germination percentage

Germination counts in the different treatments were taken from the 25th day of sowing in the early morning hours. Total germination percentage was estimated from the value taken on the day after which no further germination was observed.

6. Survival of Seedlings

Survival of seedlings was determined on the 20th day of sprouting and also on the 70th day of transplanting in polythene bags. The number of seedlings survived per treatment was counted and expressed in percentage values.

7. Chlorophyll and other M_1 variants

(a) Chlorophyll variants

Careful examination of each plant in all the treatments was done periodically in the early morning hours from the day of germination for the presence of any chlorophyll deficient plants. The spectrum of various types of mutants following Gustafsson (1940) and their frequency was calculated for each treatment.

(b) Other M_1 variants.

Careful screening of the population was done regularly for the presence of other morphological variants due to the dire

effect of the mutagen. Morphological variants including splitlamina, terminal bud splitting, and narrow leaved types were observed and their frequency calculated.

Growth metric analysis

Growth metrics were calculated based on plant height, number of leaves per plant, number of tillers per plant and leaf area. Observations were taken from a sample of ten healthy plants selected randomly from each treatment. Since sufficient population was not available in EMS treatments, soaked seed treatments and at exposures above 30 kR, they were not considered for growth metric analysis.

Plant height:

Plant height was measured in centimeter from the soil level to the tip of the shoot at 15 days interval from the date of transplanting in polythene bags till the sixth month. The average height for each treatment was calculated.

Number of leaves per plant

The number of leaves produced by the randomly selected plants each per treatment was counted and recorded at 15 days interval. Observations were taken for a period of six months from the date of transplanting in polythene bags. Counts of only the active leaves available were noted.

Leaf area per plant

The leaf area (length and breadth) of the terminal, mid

and basal leaves of the primary shoot of randomly selected plants for each treatment was measured and the average area taken for each treatment and analysed.

Number of tillers per plant

The number of tillers produced per plant at 15 days interval from the 90th day of transplanting till the sixth month was recorded and analysed.

Analysis on M_1 variants

Plant height

Based on the height of plants, they were classified under three different categories namely positive variants, control group and negative variants. The frequency of height variants falling under positive and negative sides of the control values for each treatment was computed and analysed statistically.

Number of leaves per plant, number of tillers per plant and leaf area per plant

As in the case of plant height variants, M_1 variants were classified into three categories positive, negative and control groups for number of leaves per plant, number of tiller per plant and also for leaf area per plant separately. The frequency of each group per treatment was calculated and significance tested following proper statistical procedures.

Cytological Analysis

Mitotic study of root tips was carried out for analysing the frequency of dividing cells and also the cytological aberrations created if any, following mutagenic treatment. Cytological analysis of root tips was carried out following Ramachandran (1969).

Root tips of the control and gamma ray treated plants (10 kR and 20 kR) were collected variety wise at 7.45 a.m. and chilled for an hour at 6-10°C in water. Immediately after chilling the root tips were fixed in Carnoy's fluid (alcohol, acetic acid and chloroform 3:1:1 ratio) for half an hour. Root tips were then hydrolysed in 1N HCl at 40°C for one hour just before staining with acetocarmine. Five slides each were thus prepared treatmentwise for each variety. In each slide ten fields were randomly observed. The total number of cells in each field, the cells under different stages of division and the chromosomal aberrations if any were studied in detail. The spectrum of cells under different stages and the frequency and rate of cell division were calculated for each variety under different treatments and analysed statistically.

Statistical Analysis

Data were analysed statistically as per the methods suggested by Fischer (1935). The angular transformation was applied to percentage values wherever found necessary.

(Snedecor, 1956). The transformation $\sqrt{X+1}$ was also applied in necessary cases. Data on germination percentage and rate of germination were subjected to the analysis of variance of the 18x3 factorial experiment in RBD. The break up of the total degrees of freedom was as follows:

<u>Source</u>	<u>Degrees</u>	<u>of</u>	<u>freedom</u>
Total		107	
Replications		1	
Varieties (V)		2	
Treatments (T)		17	
V X T		34	
<u>Dry seeds</u>			
Between mutagens		1	
Between levels of gamma rays		6	
Between levels of EMS		6	
Treated Vs. control - I		1	
<u>Soaked seeds</u>			
Treated Vs. control - II		1	
Between levels of gamma rays		1	
Soaked Vs. dry seed treatment		1	
Error		53	

Since higher doses of radiation (60 kR and 70 kR) and soaked seed treatments inhibited germination drastically, analysis was modified in the case of following tables (1) Days taken to start germination (2) Days taken to complete germination from the date of sowing. (3) Days taken to complete germination from the date of first sprout (4) Survival of seedlings (5) Survival of plants and (6) Frequency of chlorophyll deficient plants.

Analysis was done as a 13 x 3 factorial design in RBD. The break up of the total degrees of freedom was as follows:

<u>Source</u>	<u>Degrees</u>	<u>of</u>	<u>freedom</u>
Total		77	
Replications		1	
Treatments (T)		12	
Varieties (V)		2	
V X T		24	
Between gamma ray exposures		4	
Between EMS concentrations		6	
Between mutagens		1	
Control Vs. treated		1	
ERROR		38	

Analysis of growth metrics were done as a 4 x 3 factorial design in RBD. The outline of the analysis of variance table showing the source of variation and corresponding degrees of freedom is given below:

<u>Source</u>	<u>Degrees</u>	<u>of</u>	<u>freedom</u>
Total			23
Replications			1
Treatments (T)			3
Varieties (V)			2
V X T			6
Error			11

The frequency and spectrum of dividing cells under different treatments were analysed using CRD. The breakup of the total degrees of freedom is as follows:

<u>Sources</u>	<u>Degrees</u>	<u>of</u>	<u>freedom</u>
Total			44
Treatments			8
Error			36

RESULTS

RESULTS

Days taken to start germination

The number of days taken to start germination due to the influence of mutagens in three varieties of cardamom is presented in Table 1. The statistical analysis of data showed significant variation among treatments, between levels of both the mutagen, within mutagens between varieties, mutagen x variety interaction and control with various treatments.

The total number of days taken for germination ranged from 31.00 in V_2 control to 190.00 in 1.25% EMS treated V_3 population. In V_1 the effect due to gamma ray exposures was significant. The number of days taken for germination ranged from 32.50 in control and 40 kR exposures to 75.00 in 50 kR exposures. A significant increase in the number of days taken to start germination could be observed with increase in levels of gamma ray exposures, except at 40 kR. The effect due to EMS treatments was also found to be significantly different. The number of days taken to start germination ranged from 32.50 in control to 137.00 at 1.75% EMS concentrations. In higher doses of EMS namely 1.50 and 1.75% the number of days taken to start germination was much more (110.00 and 137.00 respectively) as compared to lower

Table 1

Number of days taken to start germination

<u>Variety</u> Mutagen	V ₁	V ₂	V ₃	General mean
Control	32.50 (5.70)	31.00 (5.57)	46.50 (6.85)	37.30 (6.04)
<u>Gamma ray</u>				
10 kR	33.50 (5.77)	43.50 (6.47)	49.50 (6.96)	42.10 (6.40)
20 kR	44.50 (6.66)	43.00 (6.54)	58.00 (7.56)	48.50 (6.92)
30 kR	68.00 (8.23)	49.50 (6.92)	72.00 (8.48)	63.10 (7.87)
40 kR	32.50 (5.70)	77.50 (8.80)	74.00 (8.60)	61.30 (7.70)
50 kR	75.00 (8.66)	82.00 (9.05)	130.00 (11.40)	95.66 (9.70)
<u>EMS</u>				
0.25%	78.00 (8.83)	50.00 (7.06)	47.00 (6.85)	58.30 (7.58)
0.50%	58.00 (7.62)	32.00 (5.70)	106.00 (10.30)	65.30 (7.85)
0.75%	82.00 (9.05)	72.00 (8.48)	99.00 (9.95)	84.30 (9.16)
1.00%	75.00 (8.66)	47.00 (6.90)	111.00 (10.50)	77.66 (8.70)
1.25%	48.00 (6.90)	104.00 (10.19)	120.00 (13.78)	114.00 (10.30)
1.50%	110.00 (10.50)	58.00 (7.60)	78.00 (8.60)	82.00 (8.97)
1.75%	137.00 (11.70)	49.00 (6.90)	166.00 (12.88)	117.30 (10.52)
General mean	67.20 (8.00)	56.80 (7.40)	94.50 (9.46)	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>	<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	19.76**	1.74	Mutagen x Variety	10.73**	1.74
Mutagens	29.31**	1.00	Between levels of gamma rays	23.10**	1.00
Varieties	70.66**	0.48	Between levels of EMS	19.07**	1.00
			Between mutagens	70.74**	0.41

* Significant at 5% level

concentrations. In general the EMS treated seeds took more number of days to start germination than irradiated ones.

In V_2 the influence of gamma rays was found to be significantly different. The number of days taken to start germination ranged from 31.00 in control to 82.00 in 50 KR. A significant increase in the number of days taken to start germination was noticed with increasing doses of gamma ray exposures. The influence of EMS treatments also produced significant difference among treatments. The number of days taken to start germination, ranged from 31.00 (control) to 104.00 (1.25%).

In V_3 the effect of gamma ray exposures differed significantly. The number of days taken to start germination ranged from 48.50 (control) to 130.00 (50 KR). With increasing doses of gamma rays, a significant increase in the number of days taken to start germination was also noticed. EMS also showed significant variation in the number of days taken to start germination. The number of days taken to start germination ranged from 48.50 (control) to 190.00 (1.25%). More number of days were taken by EMS treated seeds to start germination rather than irradiated ones.

A variety dependent significant variation in the number of days taken to start germination was noticed. Maximum mean number of days taken to start germination was noticed in V_3 (94.50) followed by V_1 (67.20) and V_2 (56.80).

Days taken to complete germination from the date of sowing

The number of days taken to complete germination from the date of sowing as influenced by mutagens in the three varieties is presented in Table 2. The statistical analysis of data showed no significant variation among treatments, between levels of both the mutagen, within mutagens, mutagen x variety interaction and control with various treatments. Significant variation was noticed only between varieties.

The total number of days taken to complete germination from the date of sowing ranged from 60.00 in V_2 (1.75% EMS concentration) to 268.00 in 40 kR gamma exposures of V_1 . In V_1 the effect due to gamma ray exposures showed no significant variation. The number of days taken to complete germination ranged from 165.00 in 10 kR exposures to 268.00 in 40 kR exposures. The effect due to the various doses of EMS was also not significant. The number of days taken to complete germination from the date of sowing ranged from 109.00 in 0.75% EMS concentrations to 191.00 in 1.25% EMS concentrations.

In V_2 also the influence of gamma rays was not significant. The number of days taken to complete germination from the date of sowing ranged from 143.00 (40kR) to 184.50 days (control). The treated population took lesser number of days than the control to complete germination. The effect of EMS treatments was also not significant. The number of days taken to complete germination from the date of sowing ranged from 60.00 in 1.75% EMS concentrations to 184.50 days in control.

Table 2

Number of days taken to complete germination from the date of sowing

Mutagen \ Variety	V ₁	V ₂	V ₃	General mean
Control	170.00 (12.83)	184.50 (13.21)	187.50 (13.35)	180.66 (13.13)
<u>Gamma rays</u>				
10 kR	165.00 (12.63)	160.50 (12.49)	171.50 (12.88)	165.66 (12.66)
20 kR	166.00 (12.70)	166.00 (12.64)	151.50 (11.99)	161.16 (12.44)
30 kR	165.50 (12.71)	176.50 (13.04)	114.50 (10.63)	152.16 (12.13)
40 kR	268.00 (16.37)	143.00 (11.95)	152.00 (12.32)	187.60 (13.55)
50 kR	183.00 (13.52)	155.00 (12.44)	163.00 (12.76)	167.00 (12.91)
<u>EMS</u>				
0.25%	166.00 (12.88)	78.00 (8.82)	166.00 (12.88)	136.60 (11.53)
0.50%	125.00 (11.07)	179.00 (13.37)	138.00 (11.74)	147.30 (12.10)
0.75%	109.00 (10.43)	89.00 (9.43)	137.00 (11.70)	111.66 (10.52)
1.00%	158.00 (12.56)	135.00 (11.61)	180.00 (13.41)	157.66 (12.53)
1.25%	191.00 (13.82)	126.00 (11.22)	220.00 (14.83)	179.00 (13.29)
1.50%	180.00 (13.41)	179.00 (13.37)	158.00 (12.56)	172.33 (13.12)
1.75%	179.00 (13.37)	60.00 (7.74)	171.00 (13.05)	136.60 (11.40)
General mean	171.19 (12.95)	140.80 (11.64)	162.00 (12.63)	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	1.69	-
Mutagens	1.62	-
Varieties	4.40*	1.25
Mutagen x Variety	1.50	-
Between levels of gamma rays	0.63	-
Between levels of EMS	2.15	-
Between mutagens	2.82	-
Control Vs. treated	1.22	-

* Significant at 5% level

(Transformed values are presented in parenthesis)

In V_3 the effect of gamma rays on the number of days taken to complete germination was not significant. The range was 114.50 days in 30 kR exposure to 187.50 days in control. In EMS treatments also there was no significant variation. The range was 137.00 days in 0.75% EMS concentrations to 220.00 days in 1.25% EMS concentrations.

A variety dependent significant variation in the number of days taken to complete germination was noticed. Maximum mean number of days taken to complete germination was noticed in V_1 (171.19) followed by V_3 (162.00) and V_2 (140.80).

Days taken to complete germination from the date of first sprout

The number of days taken to complete germination from the date of first sprout due to the influence of mutagens in three varieties is presented in Table 3. The statistical analysis of data showed no significant variation among varieties treatments, between levels of both the mutagens, within the mutagen, mutagen x variety interaction and control with various treatments.

The total number of days taken to complete germination from the date of first sprout ranged from 5.00 in 1.75% EMS treated V_3 population to 235.50 days in 40 kR under V_1 . In V_1 the influence of gamma ray exposures was found to produce no significant variation. The number of days taken to complete germination in V_1 from the date of first sprout ranged from

Table 3

Number of days taken to complete germination from the date of first sprout

17

Mutagen \ Variety	V ₁	V ₂	V ₃	General mean
Control	137.50 (11.45)	153.50 (11.86)	139.00 (11.46)	143.30 (11.59)
<u>Gamma rays</u>				
10 kR	131.50 (11.10)	117.00 (10.68)	122.00 (10.84)	123.50 (10.87)
20 kR	121.50 (10.69)	123.00 (10.80)	93.50 (9.24)	112.66 (10.07)
30 kR	97.50 (9.40)	127.00 (11.05)	42.50 (6.26)	89.00 (8.89)
40 kR	235.50 (15.35)	65.50 (8.09)	78.00 (8.83)	126.33 (10.75)
50 kR	108.00 (10.40)	73.00 (8.54)	28.00 (5.13)	69.66 (8.02)
<u>EMS</u>				
0.25%	88.00 (9.37)	28.00 (5.22)	119.00 (10.90)	78.30 (8.50)
0.50%	67.00 (8.10)	147.00 (12.12)	32.00 (5.64)	82.00 (8.60)
0.75%	27.00 (5.19)	17.00 (4.12)	38.00 (6.60)	27.00 (5.30)
1.00%	83.00 (9.11)	80.00 (9.38)	69.00 (8.31)	80.00 (8.90)
1.25%	43.00 (6.55)	22.00 (4.69)	30.00 (5.44)	31.66 (5.60)
1.50%	70.00 (8.37)	121.00 (11.00)	80.00 (8.94)	90.33 (9.43)
1.75%	42.00 (6.48)	11.00 (3.31)	5.00 (2.23)	19.32 (4.00)
General mean	96.26 (9.35)	84.07 (8.51)	67.38 (7.67)	

Analysis of Variance

<u>Source</u>	<u>F. value</u>	<u>CD value</u>
Treatments	0.11	-
Mutagens	0.22	-
Varieties	0.12	-
Mutagen x variety	0.05	-
Between levels of gamma rays	0.06	-
Between levels of EMS	0.20	-
Between mutagens	0.80	-
Control Vs. treated	0.43	-

97.50 days in 30 kR to 235.50 days in 40 kR. A general decrease in the number of days taken to complete germination was noticed with increase in level of exposures except at 40 kR. The effect due to EMS treatments also showed no significant variation. The number of days taken to complete germination ranged from 27.00 in 0.75% EMS treated population to 137.50 days in the control population. The EMS treated seeds took comparatively lesser number of days to complete germination compared to irradiated seed.

In V_2 there was no significant difference between the different levels of gamma ray exposures. The number of days taken to complete germination ranged from 65.50 days in 40 kR gamma exposure to 153.50 days in the control population. The higher exposures, 40 kR and 50 kR took comparatively lesser number of days to complete germination than others. The influence of the various EMS concentration was also found to be not significant. The number of days taken to complete germination ranged from 11.00 in 1.75% of EMS concentrations to 153.50 days in the control group.

In V_3 also gamma rays showed no significant variation. The number of days taken to complete germination ranged from 28.00 at 50 kR exposure to 139.00 in the control group. A decrease in the number of days taken to complete germination was found with increasing dose of gamma rays except at 40 kR(78.00). EMS treatments were also found to produce no significant variation in the number of days taken to complete germination from the date of first sprout. The number of days taken to complete germination

ranged from 5.00 in 1.75% of EMS to 139.00 in the control group.

Varietal variation showed no significant influence in the number of days taken to complete germination from the date of first sprout. The maximum number of days taken to complete germination was noticed in V_1 (96.26) followed by V_2 (84.07) and V_3 (67.38).

Rate of germination

The influence of the mutagens on percentage germination as on 60th day of sowing in different varieties is presented in Table 4. Analysis of the data showed significant variation among various treatments and also between the two mutagens. Dry seed treatments showed significant variation between the two mutagens and also in the case of the effect of treatment Vs control. When soaked seeds were exposed to different doses of gamma rays they also showed significant variation among different levels of gamma rays and also in control Vs treatments. Significant variation was also noted in the case of gamma ray treatments of soaked Vs dry s

In V_1 , germination percentage as on 60th day ranged from 0 (in majority of higher doses) to 27.50 (control in soaked seeds). In dry seeds germination was noted only in the case of control, 10-40 kR gamma ray exposures and in 0.5% and 1.25% EMS concentration. In the case of soaked seed treatments 10 kR alone gave 8% germination. The soaked seed control population showed significant increase in germination compared to 10 kR population.

Table 4 Rate of germination (percentages) as on 60th day of sowing under different doses of gamma rays and EMS

<u>Mutagen</u> \ <u>Variety</u>	<u>V₁</u>	<u>V₂</u>	<u>V₃</u>	<u>General mean</u>
<u>Dry seeds</u>				
Control - I	7.50 (15.11)	16.50 (20.90)	10.00 (14.70)	11.33 (16.87)
<u>Gamma rays</u>				
10 kR	6.00 (14.17)	11.00 (16.50)	9.00 (13.97)	8.66 (14.00)
20 kR	3.00 (9.82)	3.50 (10.50)	2.00 (7.19)	2.80 (9.18)
30 kR	1.00 (5.50)	1.00 (5.50)	0 (2.86)	0.66 (4.61)
40 kR	2.00 (8.13)	0 (2.86)	0 (2.86)	0.66 (4.61)
50 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	0 (2.86)	7.00 (15.30)	5.00 (12.85)	4.00 (10.30)
0.50%	4.00 (11.53)	6.00 (13.90)	0 (2.86)	3.33 (9.45)
0.75%	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
1.00%	0 (2.86)	4.00 (11.53)	0 (2.86)	1.33 (34.51)
1.25%	2.00 (8.13)	0 (2.86)	0 (2.86)	0.66 (27.70)
1.50%	0 (2.86)	6.00 (13.90)	0 (2.86)	2.00 (39.80)
1.75%	0 (2.86)	3.00 (9.82)	0 (2.86)	1.00 (31.11)
<u>Soaked seeds</u>				
Control - II	27.50 (31.59)	19.00 (25.83)	15.00 (22.77)	20.50 (26.73)
10 kR	8.00 (16.30)	0 (2.86)	6.00 (14.17)	4.66 (11.00)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>General mean</u>				
	3.30 (8.12)	4.20 (9.30)	2.61 (6.70)	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	4.61**	12.27
Mutagens	11.35**	7.08
Varieties	2.90	-
Mutagen x Variety	1.34	-
<u>Dry seeds</u>		
between levels of gamma rays	5.80	-
between levels EMS	2.01	-
between mutagens	153.16**	2.67
Control - I Vs. treated	30.64**	5.10
<u>Soaked seeds</u>		
between levels of gamma rays	9.65**	7.32
Control-II Vs. treated	74.11**	6.34
soaked Vs. dry treatment	31.52**	3.15

* Significant at 5% level
** Significant at 1% level

(Transformed values are presented in parenthesis)

In V_2 the percentage germination ranged from 0 (in majority of higher doses) to 19.00 % (in soaked seed control). The doses above 30kR failed to show any germination in dry seed irradiation, when it was nil in the case of the lowest exposure of soaked seed treatments. As regards EMS treatments, germination percentage was quite erratic. Here the maximum germination percentage was shown by 0.25% (7.00) followed by 0.50% and 1.50% (6.00).

In V_3 germination percentage ranged from zero (in majority of higher concentrations of both gamma rays and EMS) to 15.00 (Soaked seed control). In dry seed irradiation gamma ray exposure under 10 kR gave 9.00 percent germination when it was 2.00 per cent under 20 kR. All the doses above 20 kR failed to show germination. As regards EMS concentration only the lowest concentration (0.25%) gave any germination (5.00%). In all the doses above 0.25%, germination percentage was zero. Under soaked seed irradiation, when 10 kR gave 6.00 per cent germination it was zero in 20 kR.

In all the three varieties the control population gave a comparatively higher germination percentage both in dry and soaked seeds compared to all other doses. The soaked seeds gave a comparatively higher value compared to dry seeds in all the 3 varieties. In dry seeds, the germination percentage in control ranged from 7.50 (V_1) to 16.50 (V_2) when it was 15.00 (V_3) to 27.50 (V_1) in soaked seeds. In treated population maximum

germination was noted in V_2 compared to V_3 and V_1 . In EMS treatments V_2 was able to give a comparatively higher germination (6.00%) even at 1.50% concentration.

The influence of the mutagens on percentage germination as on 90th day of sowing in different varieties is presented in Table 5. Analysis of the data showed significant variation among various treatments and also between the mutagens. Significant variation was noticed between the different levels of gamma rays and also between the control and treated ones in both dry and soaked seeds. Significant variation was also noticed between dry and soaked seed treatments with gamma exposures.

In V_1 the percentage of germination as on 90th day ranged from 0 (at higher doses of EMS and gamma rays) to 31% (control in soaked seeds). The germination percentage in dry seeds was zero at 60 kR and 70 kR gamma exposures and also in EMS concentrations of 1.50 and 1.75 per cent. In soaked seed treatment, while the control and 10 kR showed 31 and 24 per cent germination respectively it was zero at 20 kR. The soaked seed control population showed significant increase in germination percentage in comparison with all other treatment except dry seed control population and 10 kR soaked seed treatment. In the case of dry seed treatment, there was significant increase in germination in control and 10 and 30 kR in comparison with all other treatments.

In V_2 the germination percentage ranged from 0 to 34.50 (in dry seed control). In dry seed treatment, there was no

Table 5 Rate of germination (percentage) on 90th day of sowing under different doses of gamma rays and EMS

variety Mutagen	V ₁	V ₂	V ₃	General mean
<u>Dry seeds</u>				
Control - I	23.50 (27.50)	34.50 (33.62)	39.00 (36.50)	32.33 (32.58)
<u>Gamma rays</u>				
10 kR	19.00 (25.47)	29.50 (31.21)	26.50 (25.90)	25.00 (27.53)
20 kR	9.50 (17.93)	13.50 (21.12)	14.50 (21.11)	12.50 (20.06)
30 kR	17.00 (24.34)	9.00 (17.40)	17.50 (24.66)	14.50 (22.14)
40 kR	4.00 (11.50)	2.00 (8.12)	2.00 (8.13)	2.60 (9.26)
50 kR	1.00 (5.73)	2.00 (8.12)	0 (2.86)	1.00 (5.57)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	4.00 (11.50)	16.00 (23.56)	9.00 (17.42)	9.60 (17.50)
0.50%	8.00 (16.40)	8.00 (16.40)	0 (2.86)	5.30 (11.90)
0.75%	4.00 (11.50)	11.00 (19.19)	0 (2.86)	5.00 (11.19)
1.00%	5.00 (12.90)	7.00 (15.30)	0 (2.86)	4.00 (10.33)
1.25%	4.00 (11.50)	0 (2.86)	0 (2.86)	1.30 (5.70)
1.50%	0 (2.86)	9.00 (17.40)	3.00 (9.82)	4.00 (10.03)
1.75%	0 (2.86)	4.00 (11.53)	0 (2.86)	1.30 (5.75)
<u>Soaked seeds</u>				
Control - II	31.00 (33.81)	21.00 (27.25)	22.00 (27.96)	29.67 (24.66)
10 kR	24.00 (29.30)	0 (2.86)	16.00 (23.55)	13.33 (18.58)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>General mean</u>				
	8.50 (14.10)	9.25 (14.70)	12.27 (8.30)	

Analysis of variance

Source	F. Value	CD Value
Treatments	4.09**	19.50
Mutagens	10.21**	11.31
Varieties	1.07	-
Mutagen x variety	1.20	-
<u>Dry seeds</u>		
Between levels of gamma rays	11.54**	11.30
Between levels of EMS	1.80	-
Between mutagens	2.53	-
Control - I Vs. treated	45.86**	8.27
<u>Soaked seeds</u>		
Between levels of gamma rays	13.82**	11.30
Control - II Vs. treated	26.00**	9.70
Soaked Vs. dry treatment	4.51**	5.00

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

germination at 60 kR and 70 kR gamma exposures and 1.25% EMS concentration. Germination was noted only in control group (21.00%) with respect to soaked seed treatment. In the case of dry seed treatment, the control group and 10 kR showed significant increase in germination compared to all other treatments.

In V_3 the germination percentage ranged from 0 to 39.00 (dry seed control population). In dry seed treatment germination percentage was zero at exposures above 40 kR. The germination percentage was zero at most of the EMS concentrations except at 0.25% and 1.5% with 9.00 and 3.00 per cent respectively. In soaked seed treatment while the germination percentage was zero at 20 kR, control group and 10 kR showed germination percentage of 22.00 and 16.00 respectively. The dry seed control population showed significant increase in germination compared to all other treatments.

Germination percentage was relatively higher in the control group in all the three varieties with respect to both dry and soaked seed treatments. Except in V_1 , the dry seed control population showed higher germination percentage than soaked seed control group. In dry seed, while the germination percentage in control population ranged from 39.00 (V_3) to 23.50 (V_1), it was 21.00 (V_2) to 31.00 (V_1) in soaked seed treatment. In treated population maximum germination was noted in V_2 compared to V_3 and V_1 .

In EMS treatments germination percentage was comparatively poor in V_3 .

The influence of the mutagens on percentage germination as on 120th day of sowing in different varieties is presented in Table 6. Analysis of the data showed significant variation among various treatments and also between the mutagens. In both dry and soaked seed treatment significant variation was noticed between different levels of gamma ray exposures and also between control and the treated ones.

In V_1 , germination percentage as on 120th day ranged from 0 to 35.50 per cent (dry seed control). In dry seed treatment germination was nil at exposures above 50 kR and in EMS concentration of 1.75%. In soaked seed treatment, while germination percentage was 32.00 (control group) and 25.50 (10kR), 20 kR showed no germination at all. Dry seed control population showed a significant increase in the germination percentage when compared with all other treatments except 10 kR gamma ray exposure of both dry and soaked seed treatment and also soaked seed control.

In V_2 germination percentage ranged from 0 to 36.50 (dry seed control group). In dry seed treatment germination was nil at 60 and 70 kR exposures. In soaked seed treatment, while control group showed 20.00 per cent germination, 10 and 20 kR exposures failed to show any germination. The dry seed control population showed a significant increase in germination compared with all

Table 6 Rate of germination on 120th day of sowing under different doses of

gamma rays and EMS (percentage)

<u>variety</u> Mutagen	V ₁	V ₂	V ₃	General mean
<u>Dry seeds</u>				
Control - I	35.50 (34.88)	36.50 (35.30)	41.00 (37.87)	37.66 (36.00)
<u>Gamma rays</u>				
10 kR	27.50 (30.88)	34.00 (34.00)	31.00 (28.53)	30.80 (31.14)
20 kR	18.00 (25.09)	18.00 (24.70)	15.00 (21.74)	17.00 (23.90)
30 kR	20.00 (26.55)	11.50 (19.77)	18.00 (25.06)	16.50 (23.80)
40 kR	4.00 (11.53)	4.00 (11.53)	4.00 (11.53)	4.00 (11.53)
50 kR	2.00 (8.12)	4.50 (12.22)	0 (2.86)	2.16 (7.73)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	8.00 (16.42)	16.00 (23.56)	12.00 (20.25)	12.00 (20.08)
0.50%	10.00 (18.42)	11.00 (19.34)	7.00 (15.30)	9.30 (17.68)
0.75%	9.00 (17.42)	13.00 (21.11)	8.00 (16.30)	10.00 (18.27)
1.00%	10.00 (18.42)	8.00 (16.42)	3.00 (9.80)	7.00 (14.89)
1.25%	8.00 (16.42)	7.00 (15.20)	0 (2.86)	5.00 (11.52)
1.50%	4.00 (11.53)	10.00 (18.40)	6.00 (14.17)	6.66 (14.71)
1.75%	0 (2.86)	4.00 (11.50)	0 (2.86)	1.33 (5.75)
<u>Soaked seeds</u>				
Control - II	32.00 (34.43)	20.00 (27.25)	22.00 (27.96)	24.60 (29.88)
10kR	25.50 (30.31)	0 (2.86)	20.00 (26.55)	15.16 (19.91)
20kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>General mean</u>	11.86 (17.33)	10.90 (16.77)	10.33 (15.12)	

Analysis of variance

<u>Source</u>	<u>F. Value</u>	<u>CD Value</u>
Treatments	3.44**	21.67
Mutagens	9.22**	12.51
Varieties	0.71	-
Mutagen x variety	0.71	-
<u>Dry seed</u>		
Between levels of gamma rays	11.79**	12.51
Between levels of EMS	2.14	-
Between mutagens	0.005	-
Control - I Vs. treated	38.48**	9.15
<u>Soaked seeds</u>		
Between levels of gamma rays	13.27**	12.51
Control - II Vs. treated	20.84**	10.83
Soaked Vs. dry treatment	0.60	-

* Significant at 5% level

** Significant at 1% level

other treatments except dry seed treatment of 10 kR gamma exposure (34%). The germination percentage in EMS treatments was erratic.

In V_3 , germination percentage ranged from 0 to 41.00 (dry seed control group). Exposures above 40 kR and EMS concentrations of 1.25 and 1.75 percent in dry treatment showed no germination. In soaked seed treatment, while control and 10kR showed 22.00 and 20.00 per cent respectively, 20 kR failed to show any germination. Dry seed control group showed significant increase in germination compared to all other treatments excluding 10kR of dry treatment (31.00%).

In both dry and soaked seed treatment, the control groups showed relatively higher germination percentage when compared to all other treatments, in the three varieties. The dry seeds showed a higher value compared to soaked seeds. In dry seeds the germination percentage in control population ranged from 35.50 (V_1) to 41.00 (V_3). In control population it ranged from 20.00 (V_2) to 32.00 (V_1) in soaked seed treatment. In treated population maximum germination was noted in V_2 (34.00%) compared to V_1 and V_3 . In EMS treatments of 1.75% V_2 showed 4 per cent germination while V_1 and V_3 showed nil. At 1.25% V_3 failed to show germination when it was 8.00 and 7.00 per cent under V_1 and V_2 respectively.

The influence of the mutagens on percentage germination as on 15th day of sowing in different varieties is presented in

Table 7. Analysis of the data showed significant variation among various treatments and also between the mutagens. In both dry and soaked seed treatments significant variation was noticed between the different levels of gamma rays and also between control and treated groups. Significant variation in dry seeds was also noticed among the different levels of EMS.

In V_1 the percentage germination as on 150th day of sowing ranged from 0 to 37.50 (dry seed control). In dry seed treatment, 60 kR and 70 kR exposures showed no germination at all. In soaked seed treatment, the control group and 10kR showed germination percentage of 32.00 and 25.50 respectively while it was zero at 20kR. The dry seed control population showed a significant increase in germination with respect to all other treatments excluding 10 kR of dry seed treatment and soaked seed control group. In dry seed treatment, control group and 10 kR showed a significant increase in the rate of germination with respect to all other treatments.

In V_2 the germination percentage ranged from 0 to 37.00 (dry seed control). In dry seed treatment there was no germination above 50kR and in soaked seeds, treated population showed no germination at all. Dry seed control population, and 10kR of dry seed treatment showed significant increase in germination percentage as compared to all other treatments. In EMS treatments, 0.25% and 0.50% showed significant increase in germination compared to 1.75% (4.00%).

In V_3 , the germination percentage ranged from 0 to 43.00 (dry seed control). In dry seed treatment, germination percentage

Table 7 Rate of germination (percentage) as on 150th day of sowing under different

doses of gamma rays and EMS

Mutagen \ Variety	V ₁	V ₂	V ₃	General mean
<u>Dry seeds</u>				
Control - I	37.50 (36.64)	37.00 (35.70)	43.00 (39.70)	39.16 (37.40)
<u>Gamma rays</u>				
10 kR	34.00 (33.54)	35.00 (34.92)	31.00 (28.52)	33.33 (33.00)
20 kR	19.00 (25.80)	27.50 (31.57)	20.50 (26.83)	22.33 (28.07)
30 kR	21.00 (27.20)	14.00 (21.90)	20.00 (26.55)	18.30 (25.25)
40 kR	6.00 (14.17)	6.50 (14.60)	5.50 (13.54)	6.00 (14.12)
50 kR	4.00 (11.53)	7.00 (15.30)	2.00 (8.12)	4.30 (11.64)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	10.00 (18.40)	16.00 (23.56)	14.00 (21.96)	13.30 (21.30)
0.50%	11.00 (19.34)	16.00 (23.56)	13.00 (21.10)	13.30 (21.30)
0.75%	9.00 (17.30)	13.00 (21.11)	12.00 (20.19)	11.33 (19.57)
1.00%	11.00 (19.30)	12.00 (20.25)	7.00 (15.20)	10.00 (18.30)
1.25%	9.00 (17.40)	9.00 (17.40)	0 (2.86)	6.00 (12.57)
1.50%	4.00 (11.54)	10.00 (18.40)	7.00 (15.29)	7.00 (15.08)
1.75%	3.00 (9.82)	4.00 (11.53)	0 (2.86)	2.33 (8.07)
<u>Soaked seeds</u>				
Control - II	32.00 (34.40)	21.00 (27.25)	22.00 (27.96)	25.00 (29.88)
10 kR	25.50 (30.32)	0 (2.86)	20.00 (26.55)	15.16 (19.91)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>General mean</u>				
	13.11 (18.75)	12.60 (18.26)	12.05 (17.00)	

Analysis of variance

Source	F. Value	CD Value
Treatments	4.09**	20.28
Mutagens	11.07**	11.71
Varieties	0.51	-
Mutagen x variety	0.81	-
<u>Dry seeds</u>		
Between levels of gamma rays	15.35**	11.70
Between levels of EMS	2.50*	11.70
Between mutagens	0.01	-
Control - I Vs. treated	41.63**	8.50
<u>Soaked seeds</u>		
Between levels of gamma rays	15.15**	11.70
Control - II Vs. treated	23.78**	10.14
Soaked Vs. dry treatment	0.078	-

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

was zero in exposures above 50 kR. EMS treatments of 1.25% and 1.05% also showed zero germination. The dry seed control population showed a significant increase in germination percentage when compared to all other treatments.

In all the three varieties, the control population gave a comparatively higher germination percentage both in dry and soaked seeds compared to all other doses. The dry seeds gave a comparatively higher value than soaked seeds in all the three varieties. In dry seeds the germination percentage in control group ranged from 37.00 (V_2) to 43.00 (V_3) whereas in soaked seeds it was 21.00 (V_2) to 32.00 (V_1). In treated population maximum germination was noted in V_2 (35.00%) compared to V_3 and V_1 .

The influence of the mutagens on percentage germination as on 180th day of sowing in different varieties is presented in Table 8. Analysis of the data showed significant variation among various treatments and also between mutagens. Significant variation in germination percentage was noticed between different levels of gamma rays and also in the case of the effect of treatment vs control in both dry and soaked seed treatments. Significant variation was also noticed between different levels of EMS in the case of dry seed treatments.

In V_1 the germination percentage ranged from 0 to 39.00 (dry seed control). In dry seed treatment 60 and 70kR exposures

Table 8 Rate of germination (percentage) as on 180th day of sowing under different doses of gamma rays and EMS

Mutagen \ Variety	V ₁	V ₂	V ₃	General mean
<u>Dry seeds</u>				
Control - I	39.00 (37.83)	38.00 (36.68)	47.00 (42.93)	41.33 (39.15)
<u>Gamma rays</u>				
10 kR	38.50 (38.34)	35.00 (34.92)	33.00 (32.12)	35.50 (35.12)
20 kR	19.50 (26.17)	30.50 (31.38)	24.00 (29.30)	24.66 (29.62)
30 kR	21.00 (27.28)	14.00 (21.96)	20.00 (26.55)	18.33 (25.24)
40 kR	7.00 (15.33)	6.50 (14.66)	6.00 (14.17)	6.50 (14.72)
50 kR	5.00 (12.90)	8.00 (16.42)	4.50 (12.22)	5.83 (13.85)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	15.00 (22.76)	16.00 (23.56)	19.00 (25.82)	16.66 (24.05)
0.50%	11.00 (19.34)	18.00 (25.06)	13.00 (21.11)	14.00 (21.83)
0.75%	9.00 (17.42)	13.00 (21.11)	12.00 (20.19)	11.32 (19.57)
1.00%	14.00 (21.96)	12.00 (20.25)	9.00 (17.42)	11.66 (19.88)
1.25%	9.00 (17.42)	9.00 (17.42)	0 (2.86)	6.00 (12.57)
1.50%	5.00 (12.85)	12.00 (20.25)	10.00 (18.42)	9.00 (17.18)
1.75%	6.00 (14.17)	4.00 (11.53)	4.00 (11.53)	4.66 (12.41)
<u>Soaked seeds</u>				
Control - II	32.00 (34.40)	21.00 (27.25)	22.00 (27.16)	25.00 (29.88)
10 kR	25.50 (30.31)	0 (2.86)	20.00 (26.55)	15.16 (19.91)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>General mean</u>	14.59 (19.84)	13.16 (18.66)	13.52 (18.76)	

Analysis of variance

Source	F. Value	CD. Value
Treatments	5.20**	18.28
Mutagens	14.25**	10.55
Varieties	0.32	-
Mutagen x variety	0.96	-
<u>Dry seeds</u>		
Between levels of gamma rays	20.70**	10.55
Between levels of EMS	2.53*	10.55
Between mutagens	0.09	-
Control - I Vs. treated	53.73**	7.72
<u>Soaked seeds</u>		
Between levels of gamma rays	18.67**	10.55
Control - II Vs. treated	29.30**	4.60
Soaked Vs. dry treatment	1.09	-

* Significant at 5% level
 ** Significant at 1% level

(Transformed values are presented in parenthesis)

failed to produce any germination. In soaked seeds, while control and 10 kR showed a germination percentage of 32.00 and 25.50 there was no germination at 20 kR. In dry seed treatment, significant variation in germination percentage was noticed in dry seed control and 10 kR treatment when compared with all the other treatments.

In V_2 the percentage of germination ranged from 0 to 38.00 (dry seed control). Exposures above 50 kR failed to germinate in the case of dry seed treatment. The control group alone showed germination (21.00%) in the case of soaked seed treatment. In dry seed treatments control population, 10 and 20 kR exposures showed a significant increase in germination percentage compared with all other treatments.

In V_3 , the percentage germination ranged from 0 to 47.00 (dry seed control). Germination was nil in exposures above 50kR and also at 1.25% EMS in dry seed treatment. In soaked seed treatment, when control group and 10kR gave 22.00 and 20.00 per cent germination respectively, it was zero at 20 kR exposure. Dry seed control population and 10 kR showed a significant increase in germination percentage when compared to all other treatments. In dry seed treatment control group and exposures upto 30 kR showed a significant increase in germination when compared with rest of the exposures (above 30kR). In EMS treatments, 0.25% showed a significant increase in the germination percentage compared with 1.25 (0) and 1.75% (4.00).

In all the three varieties the control population gave a comparatively higher germination percentage both in dry and soaked

seeds compared to all other doses. The dry seeds gave a relatively higher value in all the three varieties. In dry seeds the germination percentage in control population ranged from 38.00 (V_2) to 47.00 (V_3) and that of the soaked seeds ranged from 21.00 (V_2) to 32.00 (V_1). In treated population maximum germination was noted in V_1 (38.50).

The influence of the mutagens on percentage germination as on 210th day of sowing in different varieties is presented in Table 9. Analysis of the data showed significant variation among various treatments and also between the mutagens. Both dry and soaked seed treatments showed significant variation in germination percentage among different levels of gamma rays and also in the effect of control Vs soaked seeds.

In V_1 germination percentage as on 210th day ranged from 0 to 43.50 (dry seed control). Germination was nil at exposures above 50 kR in the case of dry seed treatment. In soaked seed treatment while germination was nil at 20 kR, control and 10kR showed a germination of 32.00 and 25.50 per cent respectively. Dry seed control population showed significant increase in germination when compared with all other treatments. In dry seed treatment, control and 10 kR exposure was found to have significant increase in the germination percentage when compared to all the rest. In EMS treatments it was erratic.

In V_2 , the percentage of germination ranged from 0 to 40.00 (dry seed control). Germination was nil at exposures above 50kR

Table 9 Rate of germination (percentage) as on 210th day of sowing under different doses of gamma rays and EMS

Mutagen \ Variety	V ₁	V ₂	V ₃	General mean
<u>Dry seeds</u>				
Control - I	43.50 (40.98)	40.00 (38.33)	47.50 (43.29)	43.66 (40.87)
<u>Gamma rays</u>				
10 kR	40.00 (39.21)	38.00 (37.33)	34.00 (33.33)	37.33 (36.62)
20 kR	25.00 (29.76)	31.00 (33.68)	24.50 (29.65)	26.83 (31.03)
30 kR	21.00 (27.25)	15.00 (22.77)	20.00 (26.55)	18.66 (25.52)
40 kR	8.00 (16.42)	6.50 (14.66)	6.00 (14.17)	6.83 (15.08)
50 kR	5.50 (13.50)	8.00 (16.42)	4.50 (12.22)	6.00 (14.06)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	15.00 (22.76)	16.00 (23.56)	19.00 (25.82)	16.66 (24.05)
0.50%	11.00 (19.34)	19.00 (25.82)	13.00 (21.11)	14.33 (22.09)
0.75%	9.00 (17.42)	13.00 (21.11)	12.00 (20.19)	11.33 (19.57)
1.00%	14.00 (21.96)	12.00 (20.25)	10.00 (18.42)	12.00 (20.21)
1.25%	11 (19.30)	9.00 (17.42)	5.00 (12.85)	8.33 (16.50)
1.50%	6.00 (14.17)	12.00 (20.25)	10.00 (18.42)	9.33 (17.62)
1.75%	6.00 (14.17)	4.00 (11.53)	4.00 (11.53)	4.66 (12.41)
<u>Soaked seeds</u>				
Control - II	32.00 (34.43)	21.00 (27.25)	22.00 (27.96)	25.00 (29.88)
10 kR	25.50 (30.31)	0 (2.86)	20.00 (26.50)	15.16 (19.91)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
General mean	15.13 (20.55)	13.50 (18.99)	13.97 (19.48)	

Analysis of variance

Source	F. Value	CD. Value
Treatments	6.37**	16.70
Mutagens	18.00**	9.66
Varieties	0.57	-
Mutagen x variety	0.90	-
<u>Dry seeds</u>		
Between levels of gamma rays	26.95**	9.66
Between levels of EMS	2.25	-
Between mutagens	0.21	-
Control - I Vs. treated	70.88**	7.07
<u>Soaked seeds</u>		
Between levels of gamma rays	22.27**	9.66
Control - II Vs. treated	34.95**	8.36
Soaked Vs. dry treatment	2.48	-

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

dry seed treatments and also in the treated groups of soaked seeds. A significant increase in germination was noticed at 10 and 20kR gamma rays and dry seed control in comparison with the rest of the treatments.

In V_3 the percentage of germination ranged from 0 to 47.50 (dry seed control). Germination was nil at exposures above 50 kR in the case of dry seed treatment. In soaked seed treatment germination was nil at 20kR, when control population and 10kR showed a germination percentage of 22.00 and 20.00 respectively. Dry seed control population showed significant increase in germination percentage when compared with all other treatments. Dry seed under 10kR showed significant increase in germination percentage when compared to all other treatments except dry seed control. In the case of dry seed treatment, 20 and 30 kR exposure were found to have significant increase in germination when compared with all other treatments except 10kR and control.

In all the three varieties the control population gave a comparatively higher germination percentage both in dry and soaked seed treatment. The dry seeds gave a comparatively higher value in all the 3 varieties. In dry seeds, the germination percentage in control population ranged from 40.00 (V_2) to 47.50 (V_3) and in soaked seeds it ranged from 21.00 (V_2) to 32.00 (V_1). In treated population maximum germination of 40.00 per cent was noticed in V_1

The influence of the mutagens on the germination percentage as on 240th day of sowing in different varieties is presented in Table 10. Analysis of the data showed significant variation among various treatments and also between the two mutagens. In both dry and soaked seeds, a significant variation was noticed between the various doses of gamma rays and also in the case of the effect of treatment Vs control. Significant variation was also noticed between different levels of EMS in the case of dry seed treatment.

In V_1 the germination percentage ranged from 0 to 45.00 (dry seed control). In dry seed treatment germination percentage was zero at exposures above 50kR. In soaked seed treatment 32.00 per cent germination was noticed in the control and 10kR exposure gave 25.50 per cent while 20kR failed to produce any germination. Dry seed control and 10kR (dry seed) showed significant increase in germination percentage when compared to all other treatments.

In V_2 the germination percentage ranged from 0 to 42.00 (dry seed control). The higher exposures (60 and 70 kR) showed no germination in the case of dry seed treatment. In soaked seeds, germination was noticed only in the control group (21.00%). The dry seed treatment under control and 10 and 20kR showed significant increase in the percentage of germination when compared to all other treatments.

EMS treatments of 0.25 and 0.50% concentrations showed significant increase in germination percentage when compared with

Table 10 Rate of germination (percentage) as on 240th day of sowing under different
doses of gamma rays and EMS

<u>Variety</u> <u>Mutagen</u>	V_1	V_2	V_3	General mean
<u>Dry seeds</u>				
Control - I	45.00 (41.90)	42.00 (39.83)	48.00 (43.63)	45.00 (41.80)
<u>Gamma rays</u>				
10 kR	41.00 (39.79)	38.50 (37.70)	35.50 (34.80)	38.33 (37.40)
20 kR	27.00 (30.97)	35.00 (36.00)	25.00 (30.00)	29.00 (32.32)
30 kR	21.00 (27.25)	18.00 (25.00)	20.00 (26.50)	19.66 (26.30)
40 kR	8.00 (16.40)	6.50 (14.66)	6.00 (14.17)	6.83 (15.08)
50 kR	5.50 (13.54)	8.00 (16.42)	4.50 (12.20)	6.00 (14.06)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	15.00 (22.76)	16.00 (23.56)	19.00 (25.82)	16.66 (24.05)
0.50%	11.00 (19.30)	19.00 (25.80)	13.00 (21.11)	14.33 (22.09)
0.75%	9.00 (17.40)	13.00 (21.11)	12.00 (20.19)	11.33 (19.57)
1.00%	14.00 (22.00)	12.00 (20.25)	10.00 (18.42)	12.00 (20.21)
1.25%	11.00 (19.34)	9.00 (17.42)	7.00 (15.30)	9.00 (17.35)
1.50%	6.00 (14.17)	12.00 (20.25)	10.00 (18.40)	9.33 (17.62)
1.75%	6.00 (14.17)	4.00 (11.53)	4.00 (11.53)	4.66 (12.41)
<u>Soaked seeds</u>				
Control - II	32.00 (34.43)	21.00 (27.25)	22.00 (27.16)	25.00 (29.88)
10 kR	25.50 (30.31)	0 (2.86)	20.00 (26.55)	15.16 (19.91)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
General mean	15.30 (20.69)	14.10 (19.35)	14.20 (19.74)	

Analysis of variance

<u>Source</u>	<u>F. Value</u>	<u>CD Value</u>
Treatments	7.18**	16.10
Mutagens	20.40**	9.30
Varieties	0.46	-
Mutagen x variety	0.94	-
<u>Dry seeds</u>		
Between levels of gamma rays	31.19**	9.29
Between levels of EMS	2.34**	9.29
Between mutagens	0.06	-
Control - I Vs. treated	81.17**	6.8
<u>Soaked seeds</u>		
Between levels of gamma rays	24.04**	9.29
Control - II Vs. treated	37.74**	8.05
Soaked Vs. dry treatment	3.36	-

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

1.75% EMS concentration. A significant increase in germination was also noticed at 0.50% compared to 1.25% concentration.

In V_3 the germination percentage ranged from 0 to 48.00 (dry seed control). In dry seed treatment germination was nil at 60 and 70kR exposures. In soaked seeds, 22 and 20 per cent germination was noticed in control and 10kR exposures respectively whereas 20kR failed to produce any germination. Dry seed control population and 10kR exposure of dry seed treatment was found to have a significant increase in germination percentage when compared to all other treatments.

In all the three varieties the control population gave a comparatively higher germination percentage both in dry and soaked seeds. The dry seeds gave a comparatively higher value in all the three varieties. In dry seeds, the germination percentage in control population ranged from 42.00 (V_2) to 48.00 (V_3) and in soaked seeds it ranged from 21.00 (V_2) to 32.00 (V_3). In treated population maximum germination was noticed in V_1 (41.00%).

The influence of the mutagens on the germination percentage as on 27th day of sowing in different varieties is presented in Table 11. Analysis of the data showed significant variation among various treatments and also between the mutagens. In both dry and soaked seeds, a significant variation was noticed between the various doses of gamma rays and also in the case of the effect of treatment Vs control.

Table 11 Rate of germination (percentage) as on 270th day of sowing under different doses of gamma rays and EMS

Variety Mutagen	V ₁		V ₂		V ₃		General mean	
<u>Dry Seeds</u>								
Control - I	45.00	(41.95)	45.00	(41.95)	49.00	(44.32)	46.33	(42.71)
<u>Gamma rays</u>								
10 kR	41.00	(39.80)	38.50	(37.70)	35.50	(34.80)	38.33	(37.46)
20 kR	27.00	(30.90)	35.00	(36.00)	25.00	(30.00)	29.00	(32.32)
30 kR	21.00	(27.25)	20.00	(26.55)	20.00	(26.55)	20.33	(26.70)
40 kR	10.50	(19.89)	6.50	(14.66)	6.00	(14.17)	7.66	(15.91)
50 kR	5.50	(13.54)	8.00	(16.40)	4.50	(12.22)	6.00	(14.06)
60 kR	0	(2.86)	0	(2.86)	0	(2.86)	0	(2.86)
70 kR	0	(2.86)	0	(2.86)	0	(2.86)	0	(2.86)
<u>EMS</u>								
0.25%	15.00	(22.70)	16.00	(23.56)	19.00	(25.82)	16.66	(24.04)
0.50%	11.00	(19.34)	19.00	(25.82)	13.00	(21.11)	14.32	(22.09)
0.75%	9.00	(17.42)	13.00	(21.11)	12.00	(20.19)	11.33	(19.50)
1.00%	14.00	(22.00)	12.00	(20.25)	10.00	(18.42)	12.00	(20.21)
1.25%	11.00	(19.30)	9.00	(17.42)	7.00	(15.30)	9.00	(17.35)
1.50%	6.00	(14.17)	12.00	(20.25)	10.00	(18.42)	9.33	(17.62)
1.75%	6.00	(14.17)	4.00	(11.53)	4.00	(11.53)	4.66	(12.41)
<u>Soaked Seeds</u>								
Control - II	32.00	(34.40)	21.00	(27.25)	22.00	(27.90)	25.00	(29.80)
10 kR	25.50	(30.31)	0	(2.86)	20.00	(26.55)	15.16	(19.91)
20 kR	0	(2.86)	0	(2.86)	0	(2.86)	0	(2.86)
General mean	15.50	(20.83)	14.30	(19.55)	14.27	(19.78)		

Analysis of Variance

Source	F ₁ Value	CD ₁ Value
Treatments	7.85**	15.54
Mutagens	22.37**	8.90
Varieties	0.49	-
Mutagen x Variety	1.02	-
<u>Dry Seeds</u>		
Between levels of gamma rays	33.56**	8.97
Between levels of EMS	2.51	-
Between mutagens	0.01	-
Control - I Vs. treated	93.49*	6.56
<u>Soaked Seeds</u>		
Between levels of gamma rays	25.82**	8.97
Control. II Vs. treated	40.53**	7.70
Soaked Vs. dry treatment	4.00	-

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

In V_1 the germination percentage ranged from 0 to 45.00 (dry seed control). In dry seed treatments germination percentage was zero at 60 and 70kR. In soaked seeds, while control and 10kR showed germination percentage of 32.00 and 25.50 respectively there was no germination in 20kR. Dry seed control and 10kR showed significant increase in germination percentage when compared with all other treatments.

In V_2 the germination percentage ranged from 0 to 45.00 (dry seed control). The higher exposures of 60 and 70kR failed to produce any germination in the case of dry seed treatment. In the case of soaked seeds, only the control group showed germination. The dry seed control and 10 and 20kR showed significant increase in germination percentage when compared to all other treatments.

In V_3 , the germination percentage ranged from 0 to 49.00 (dry seed control). In dry seed treatment germination percentage was zero at exposures above 50kR. In soaked seeds, while the control and 10kR exposure produced germination percentage of 22.00 and 20.00 respectively 20kR exposure produced none. Dry seed control population showed significant increase in germination percentage when compared with all other treatments. In dry seed treatment 10kR showed significant increase in germination percentage when compared with all other treatments except with dry seed control population. In dry seed treatment 20 and 30kR exposures were found

to have significant increase in germination in comparison with rest of the higher exposures.

In all the three varieties the control population gave a comparatively higher germination percentage both in dry and soaked seeds compared to all other doses. The soaked seeds gave a comparatively lower value than dry seeds, in all the varieties. In dry seeds, the germination percentage in control population ranged from 45.00 (V_1 and V_2) to 49.00 (V_3). In soaked seeds the range was 21.00 (V_2) to 32.00 (V_1). In treated population maximum germination percentage was noted in V_1 compared to V_2 and V_3 .

Germination percentage

The influence of varieties, mutagens and their doses on the germination percentage of seeds is presented in Table 12. The statistical analysis of the data showed significant variation between treatments, and among different doses of mutagens. Dry seed treatment showed significant variation among the different levels of gamma rays, levels of EMS and also in the case of the effect of treatment Vs control. When soaked seeds were exposed to different doses of gamma rays they also showed significant variation among the different levels of gamma rays and in control Vs treatments. Significant variation was also noted in the case of gamma ray treatment soaked Vs dry seeds.

Germination percentage ranged from zero (60 and 70 kR of V_1 , V_2 and V_3 in dry seed treatment; 10kR in V_2 and 20 kR in all

Table 12 Germination percentage as influenced by varieties, mutagens and their doses

Mutagen \ variety	variety			General mean
	V ₁	V ₂	V ₃	
Dry seeds				
Control - I	45.00 (41.95)	45.00 (41.95)	49.00 (44.32)	46.33 (42.71)
<u>Gamma rays</u>				
10 kR	41.00 (39.79)	38.50 (37.70)	35.50 (34.88)	38.33 (37.46)
20 kR	27.00 (30.97)	35.00 (36.00)	25.00 (29.90)	29.00 (32.32)
30 kR	21.00 (27.25)	20.00 (26.55)	21.00 (27.25)	20.66 (27.02)
40 kR	10.50 (18.89)	6.50 (14.66)	6.00 (14.17)	7.66 (15.91)
50 kR	5.50 (13.54)	8.00 (16.40)	4.50 (12.22)	6.00 (14.06)
60 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
70 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>EMS</u>				
0.25%	15.00 (22.76)	16.00 (23.56)	19.00 (25.82)	16.66 (24.05)
0.50%	11.00 (19.34)	19.00 (25.82)	13.00 (21.11)	14.33 (22.09)
0.75%	9.00 (17.42)	13.00 (21.11)	12.00 (20.19)	11.33 (19.57)
1.00%	14.00 (21.90)	12.00 (20.25)	10.00 (18.42)	12.00 (20.21)
1.25%	11.00 (19.34)	9.00 (17.42)	7.00 (15.29)	9.00 (17.35)
1.50%	6.00 (14.17)	12.00 (20.25)	10.00 (18.42)	9.33 (17.62)
1.75%	6.00 (14.17)	4.00 (11.53)	4.00 (11.53)	4.66 (12.41)
<u>Soaked seeds</u>				
Control - II	32.00 (34.40)	21.00 (27.25)	22.00 (27.96)	25.00 (29.88)
10 kR	25.50 (30.31)	0 (2.86)	20.00 (26.55)	15.16 (19.91)
20 kR	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
General mean	15.52 (20.83)	14.30 (19.55)	14.30 (19.82)	

Analysis of variance

Source	F. Value	CD. Value
Treatments	7.87**	15.53
Mutagens	22.42**	8.97
Varieties	0.48	-
Mutagen x Variety	1.03	-
<u>Dry seeds</u>		
Between levels of gamma rays	33.71**	8.90
Between levels of EMS	2.50*	8.90
Between mutagens	0.000	-
Control - I Vs. treated	93.45**	6.56
<u>Soaked seeds</u>		
Between levels of gamma rays	25.84**	8.90
Control - II Vs. treated	40.56**	7.76
Soaked Vs. dry treatment	4.04*	3.99

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

varieties of soaked seed treatment) to 49.00 (control in V_3 of dry seed treatment). In V_1 germination percentage was found to differ significantly due to the influence of radiations and EMS. The range was 0 (higher doses in dry seed treatment and 20kR in soaked seeds) to 45.00 (dry seed control population). In dry seed treatment, there was no germination in 60 and 70kR exposures. In the irradiated population, control and 10kR showed a significant increase in germination percentage when compared to all other exposures. A general decrease in germination percentage was noticed with increase in level of gamma ray exposures. In EMS treatments, 1.50% and 1.75% concentrations showed a significant decrease in germination percentage when compared to 0.25% concentration. In comparison with the control, all the EMS treated population showed a significant reduction in germination. EMS treated population showed lesser germination percentage when compared to irradiated population. In soaked seed treatment, control and 10kR showed a significant increase in germination percentage when compared to EMS treatments and higher doses (above 20kR) of gamma ray exposures in dry seeds. Dry seed control showed a significant increase in germination percentage when compared to soaked seed control.

In V_2 there was a significant variation in germination percentage due to the influence of gamma rays and EMS. The range of germination percentage was 0 (60 and 70kR in dry seed treatment and 10 and 20kR in soaked seed treatment) to 45.00 (dry seed control). In dry seed treatments, there was no germination in 60 and 70kR.

Control, 10kR and 20kR showed a significant increase in germination percentage when compared to all other exposures. A decrease in germination percentage was noticed with increase in dose of gamma ray exposures. In EMS treatments, there was a significant reduction in germination percentage when compared with the control population. A significant reduction in germination percentage was noticed in 1.75% EMS concentration compared to 0.25%, 0.50% and 0.75%. In soaked seed treatment, only the control population showed germination (21.00%).

In V_3 the germination percentage range from 0 (60 and 70kR of dry seed treatment and 20kR of soaked seed treatment) to 49.00 (control of dry seed treatment). A significant variation was noticed in the germination percentage due to the influence of gamma rays and EMS. In dry seed treatment, the germination percentage was found to decrease with increase in the dose of gamma ray exposures. A significant increase in germination percentage was noticed in the control when compared to all other exposures. A significant increase in germination percentage was found in 10kR exposure when compared to all other exposures except control population. In EMS treatments, a significant reduction in germination percentage was noticed in all the concentrations when compared to the control population. A significant reduction in germination percentage was noticed in 1.25% and 1.75% in comparison with 0.25% EMS concentrations. A significant increase in germination percentage was noticed in 0.50% EMS concentrations when compared

1.75% EMS concentration.

In soaked seed treatment, germination was nil in 20kR. A significant reduction in germination percentage was noticed in soaked seed control when compared with dry seed control.

A variety dependent insignificant variation was noticed in germination percentage. Maximum mean germination percentage was noticed in V_1 (15.52) followed by V_2 and V_3 (14.30 in both).

Seedling Survival

The percentage survival of seedlings on 20th day of sprouting as influenced by the two mutagens in three varieties is presented in Table 13. The statistical analysis of data showed significant variation between treatments, between the mutagens, within the mutagen, between levels of EMS, between varieties and control with various treatments. The percentage survival of seedlings on 20th day of sprouting ranged from 0 (1.75% in V_1 , V_2 and V_3) to 100 (0.75% in V_1 and 1.00% in V_3).

In V_1 the effect due to gamma ray exposures was not significant. The percentage survival of seedlings ranged from 71.36 (40kR) to 91.66 (50kR). The effect due to EMS treatment was found to differ significantly. The percentage survival of seedlings ranged from 0 (1.75%) to 100 (0.75%). A significant reduction in percentage survival was noticed at 0.25%, 1.50% and 1.75% in comparison with other concentrations.

In V_2 also the effect due to irradiation was not significantly different. The percentage survival of seedlings on the 20

Table 13

Seedling survival on 20th day of sprouting (Percentage)

Variety Mutagen	V ₁	V ₂	V ₃	General mean
Control	87.70 (73.70)	83.65 (66.90)	78.82 (62.70)	83.39 (67.70)
<u>Gamma rays</u>				
10 kR	80.49 (64.50)	89.56 (71.12)	86.88 (73.15)	85.64 (69.59)
20 kR	90.00 (75.27)	79.98 (64.00)	76.62 (63.80)	82.20 (67.70)
30 kR	82.99 (66.23)	77.50 (61.69)	63.33 (52.72)	74.60 (60.22)
40 kR	71.36 (57.62)	87.50 (73.55)	83.33 (65.87)	80.73 (65.68)
50 kR	91.66 (76.50)	68.75 (56.09)	77.50 (61.69)	79.30 (64.76)
<u>EMS</u>				
0.25%	86.60 (68.51)	93.75 (78.19)	63.88 (53.41)	81.41 (66.70)
0.50%	91.66 (76.50)	84.44 (66.90)	84.52 (66.81)	86.87 (70.09)
0.75%	100.00 (87.13)	84.52 (66.81)	85.70 (72.38)	90.07 (75.44)
1.00%	92.85 (77.44)	91.66 (76.50)	100.00 (87.13)	94.83 (80.35)
1.25%	61.66 (52.54)	55.00 (47.86)	25.00 (23.92)	47.22 (41.44)
1.50%	66.66 (54.70)	66.66 (55.42)	30.00 (32.88)	54.44 (47.67)
1.75%	0 (2.86)	0 (2.86)	0 (22.86)	0 (2.86)
General mean	77.20 (64.11)	74.07 (60.61)	65.80 (55.33)	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	8.18**	28.00
Mutagens	22.65**	16.16
Varieties	4.76*	7.76
Mutagen x variety	1.24	-
Between levels of Gamma rays	0.70	-
Between levels of EMS	41.11**	16.16
Between mutagens	18.63**	6.70
Control Vs. treated	3.65*	12.00

* Significant at 5%

** Significant at 1%

(Transformed values are presented in parenthesis)

of sprouting ranged from 69.75 (50kR) to 89.56 (10kR). The effect due to EMS treatment was found to differ significantly. The percentage survival of seedlings ranged from 0 (1.75%) to 93.75 (0.25%). There was a significant reduction in the percentage survival of seedlings at 1.25%, 1.50% and 1.75% concentration, when compared to all other treatments.

In V_3 , also as in the case of the other two varieties radiation treatment showed no significant reduction in survival percentage. The percentage survival of seedlings on the 20th day of sprouting ranged from 63.33 (30kR) to 86.88 (10kR). There was significant variation in the percentage survival of seedlings due to the effect of EMS treatments. The percentage survival of seedlings ranged from 0 (1.75%) to 100 (1.00%). A significant reduction in survival percentage was met with in 1.25%, 1.50% and 1.75% when compared with other concentrations. A significant increase in percentage survival was noticed in 1.00% when compared with other doses except in 0.50% and 0.75%.

A significant variation was noticed in the percentage survival of seedlings on the 20th day of sprouting due to the influence of variety. A maximum mean percentage survival was noticed in V_1 (77.20) followed by V_2 (74.07) and V_3 (65.80).

Survival percentage as on 70th day of transplanting

Table 14 represents the percentage survival of plants on 70th day of transplanting as influenced by the different mutagens in three varieties. The statistical analysis of data showed

Table 14

Survival of plants on 70th day of transplanting (Percentage)

Variety Mutagen	V ₁	V ₂	V ₃	General mean
Control	53.19 (46.90)	64.59 (54.02)	65.93 (54.67)	61.23 (51.86)
<u>Gamma rays</u>				
10 kR	41.46 (39.73)	65.33 (54.31)	75.40 (66.28)	60.73 (53.44)
20 kR	67.22 (55.11)	58.28 (49.70)	52.15 (46.23)	59.21 (50.36)
30 kR	62.23 (52.06)	65.00 (53.75)	52.16 (46.23)	59.79 (50.68)
40 kR	47.72 (43.67)	71.25 (57.81)	66.66 (54.70)	61.87 (52.06)
50 kR	73.33 (59.05)	56.25 (48.59)	67.50 (55.36)	65.69 (54.34)
<u>EMS</u>				
0.25%	66.95 (54.93)	63.75 (56.09)	47.77 (43.69)	61.15 (51.57)
0.50%	73.33 (59.05)	63.33 (52.72)	69.03 (56.17)	68.56 (55.98)
0.75%	77.50 (61.69)	69.03 (56.17)	68.57 (56.24)	71.70 (58.03)
1.00%	71.42 (58.42)	66.66 (55.42)	80.00 (63.40)	72.69 (59.08)
1.25%	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
1.50%	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
1.75%	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
General mean	48.79 (41.48)	49.88 (42.09)	49.62 (42.43)	

Analysis of variance

<u>Source</u>	<u>F. Value</u>	<u>CD Value</u>
Treatments	17.67**	20.39
Mutagens	53.62**	11.77
Varieties	0.10	-
Mutagen x variety	1.16	-
Between levels of Gamma rays	0.31	-
Between levels of EMS	86.82**	11.77
Between mutagens	110.15**	4.87
Control Vs. treated	11.10**	8.66

** Significant at 1% level

(Transformed values are presented in parenthesis)

significant variation between treatments, between mutagens, within the mutagen, between the levels of EMS and control with various treatments. The percentage survival of seedlings on 70th day of transplanting ranged from 0 (in EMS above 1%) to 80.00 (1% in V_3).

In V_1 the influence of gamma rays on the percentage survival of plants was not significant. The percentage survival of plants as on 70th day of transplanting ranged from 41.46 (10KR) to 73.33 (50KR). The effect of EMS treatments on the percentage survival of plants was found to differ significantly and it ranged from 0 (at concentrations above 1.00%) to 77.50 (at 0.75%). At higher concentrations of EMS, the survival percentage was found to be zero.

In V_2 also gamma ray exposures showed no significant reduction in survival of plants. The percentage survival of seedlings on the 70th day of transplanting ranged from 56.25 (50KR) to 71.25 (40KR). The effect of EMS treatments on the percentage survival of plants was found to differ significantly. It ranged from 0 (at concentration above 1.00%) to 69.03 (0.75% concentration).

In V_3 also the influence of gamma rays was not significant. The percentage survival of seedlings on 70th day of transplanting ranged from 52.15 (20KR) to 75.40% (10KR). The influence of EMS treatments was found to be significant. The survival percentage of plants on 70th day of transplanting ranged from 0 (at concentrations above 1.00%) to 80 (1.00% concentration). A significant

increase in percentage survival was noticed at 1.00% concentration when compared with the control group and 0.25% EMS treated population.

The influence of variety on the percentage survival of plants on the 70th day of transplanting was found to be insignificant. Mean percentage survival of V_1 , V_2 and V_3 was 48.79, 49.88 and 49.62 respectively.

Plant height

Data regarding mean plant height as influenced by varieties, mutagens and their doses, at different time intervals is presented in Table 15-1. The statistical analysis of data showed significant variation among varieties on 90th day and 135th day. No significant variation was noticed among levels of the mutagen, between treatment and variety into dose effect interaction in all the four different time interval and also between varieties on 45th day and 165th day.

On 45th day the mean plant height ranged from 5.60 cm under 30kR in V_1 and V_2 to 12.65 cm under 20kR in V_3 . In V_1 the influence of different doses of gamma ray exposures on mean plant height was not significant. Mean plant height in V_1 ranged from 5.60 cm in 30kR to 10.15 cm in 10kR. When a decrease in height was noticed at 20 (8.50cm) and 30kR (5.60cm) compared to control, 10kR gave an increase in mean height.

In V_2 the effect of radiation was found to produce no significant variation in mean plant height. A decrease in mean plant height was noticed with increasing doses of gamma ray

Table 15-1 Mean plant height(cm) as influenced by varieties, mutagens and their doses at different time intervals

Variety Mutagen	Time interval															
	45th day				90th day				135th day				165th day			
	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean
Control	9.77	9.45	11.00	10.07	14.77	19.38	31.82	21.99	27.52	40.47	57.60	41.86	37.80	52.70	65.50	52.02
10 kR	10.15	8.22	9.52	9.30	17.69	13.35	24.35	18.46	28.55	23.72	48.92	33.73	36.67	30.82	60.90	42.80
20 kR	8.50	8.22	12.65	9.76	17.22	13.65	17.82	16.23	28.57	28.20	34.67	30.48	34.10	37.70	42.60	38.14
30 kR	5.60	5.60	8.12	6.50	9.70	10.52	18.89	13.04	15.77	17.77	29.77	21.10	22.60	24.80	35.57	27.69
General mean	8.52	7.89	10.32		14.84	14.23	23.22		25.10	27.50	42.70		32.81	36.52	51.14	

<u>Analysis of variance</u>			<u>Analysis of variance</u>			<u>Analysis of variance</u>			<u>Analysis of variance</u>		
Source	F. Value	CD Value	Source	F. Value	CD Value	Source	F. Value	CD value	Source	F value	CD value
Treatments	1.44	-	Treatments	2.01	-	Treatments	2.03	-	Treatments	1.65	-
Mutagens	3.00	-	Mutagens	2.31	-	Mutagens	3.06	-	Mutagens	2.87	-
Varieties	2.30	-	Varieties	5.50*	9.40	Varieties	5.06*	18.64	Varieties	3.50	-
Mutagen x variety	0.41	-	Mutagen x variety	0.70	-	Mutagen x variety	0.51	-	Mutagen x variety	0.42	-

* Significant at 5% level.

exposures in comparison with the control. The mean plant height ranged from 5.60 cm (30kR) to 9.45cm (control). In V_3 , no significant variation was produced by different doses of gamma r on mean plant height. The mean plant height ranged from 8.12cm (30kR) to 12.65cm (20kR).

A variety dependent insignificant variation in mean value was noticed. Variety V_3 showed the maximum average mean height of 10.32 cm followed by V_1 (8.52cm) and V_2 (7.89 cm).

On 90th day the mean plant height ranged from 9.70 cm (30kR of V_1) to 31.82 cm (in the control group of V_3). In V_1 , the influence of radiation on mean plant height was not significantly effective. The mean plant height ranged from 9.70 cm at 30kR exposure to 17.69 cm at 10kR exposures. An increase in mean plant height was noticed at 10kR (17.69 cm) and 20kR (17.22 cm) when compared with the control (14.77cm). In V_2 also gamma ray exposures was not able to give a significant variation in the mean plant height. The mean plant height ranged from 10.52 cm (30kR) to 19.39 cm (control population). A reduction in mean plant height was noticed at 10,20 and 30kR when compared with the control population. In V_3 also the effect was not significant on mean plant height. The mean plant height ranged from 17.82 cm (20kR) to 31.82 cm (in control). A general reduction in mean plant height was noticed in the irradiated population when compared

with the control population. A variety dependent significant variation was produced in the mean plant height. Variety V_3 had the maximum mean plant height (23.22 cm) followed by V_1 (14.84 cm) and V_2 (14.23 cm).

The mean plant height on the 135th day of growth ranged from 15.77 cm (30kR of V_1) to 57.60 cm (control population of V_3). Here also the gamma ray exposures were not able to give a significant variation in the mean plant height. In V_1 the mean plant height ranged from 15.77 cm (30 kR) to 28.57 cm (20 kR). At 10 kR and 20 kR exposures, an increase in mean plant height was noticed when compared with the control population. In V_2 the mean plant height ranged from 17.77 cm (30kR) to 40.47 cm (control population). The treated population was found to exhibit a decrease in mean plant height compared with the control population.

In V_3 , the mean plant height ranged from 29.77 cm (30 kR) to 57.60 cm (control population). A decrease in mean plant height with increase in the level of gamma ray exposures was noticed.

A variety dependent significant variation, in mean height was noticed on 135th day of growth. Variety V_3 showed the maximum mean plant height of 42.70 cm followed by V_2 (27.50 cm) and V_1 (25.10 cm).

The mean plant height on 165th day ranged from 22.60 cm (30kR of V_1) to 65.50 cm (control of V_3).

There was no significant variation in the mean plant height due to the influence of gamma ray. In V_1 the mean plant height ranged from 22.60 cm (30 kR) to 37.80 cm (Control population). There was a general reduction in mean plant height with increase in dose of gamma rays. In V_2 , the mean plant height ranged from 24.80 cm (30 kR) to 52.70 cm (control population). A general decrease in mean plant height was noticed in the treated population compared with the control population. In V_3 the mean plant height ranged from 35.57 cm (30 kR) to 65.50 cm (control population). The mean plant height was found to decrease with increase in dose of gamma ray exposures.

Phenotypic frequency of plant height variants

The frequency distribution of plant height variants as affected by gamma rays in three varieties of cardamom at four different time intervals is represented in Table 15-2 and the results of statistical analysis in Table 15-2-A. Statistical analysis of the data showed no significant variation among treatments, doses of mutagen, varieties and also in varieties into doses interaction.

Both positive and negative variants were created by the different doses of gamma rays. The minimum mean frequency of zero for negative variants in plant height was observed in 30 kR of V_2 and the maximum (3.00) in

Table 15-2

Frequency distribution of plant height variants at different growth phases

Variety	Mutagen	Time interval											
		45th day			90th day			135th day			165th day		
		-ve Variants	Control group	+ve variants	-ve Variants	Control group	+ve variants	-ve variants	Control group	+ve variants	-ve variants	Control group	+ve variants
V ₁	Control	2.00	6.50	1.50	3.00	5.50	1.50	3.00	5.50	1.50	2.00	5.00	3.00
	10 kR	1.50	6.50	2.00	1.00	6.50	2.50	1.50	6.50	2.00	1.00	7.50	1.50
	20 kR	2.50	5.50	2.00	1.50	7.00	1.50	1.00	6.50	2.50	1.00	7.50	1.50
	30 kR	1.00	7.00	2.00	2.00	6.00	2.00	1.00	7.00	2.00	1.50	6.50	2.00
V ₂	Control	1.00	8.50	0.50	1.50	7.00	1.50	1.50	6.50	2.00	1.50	5.50	3.00
	10 kR	1.00	8.00	1.00	0.50	7.00	2.50	0.50	7.00	2.50	1.50	6.00	2.50
	20 kR	1.00	8.00	1.00	1.00	7.50	1.50	1.50	7.00	1.50	2.00	6.00	2.00
	30 kR	0.00	7.50	2.50	1.50	6.50	2.00	1.00	6.50	2.50	1.00	8.00	1.00
V ₃	Control	1.50	7.00	1.50	1.00	7.00	2.00	1.00	8.00	1.00	0.50	8.00	1.50
	10 kR	1.50	7.00	1.50	1.50	7.00	1.50	1.50	7.00	1.50	1.00	8.00	1.00
	20 kR	1.50	7.50	1.00	1.50	6.50	2.00	2.00	7.50	0.50	2.00	7.00	1.00
	30 kR	2.00	6.50	1.50	3.00	6.00	1.00	1.50	7.00	1.50	2.50	7.00	0.50

Table: 15-2-A. Statistical analysis of frequency distribution of plant height variants at different growth phases

Variety Mutagen	Time interval															
	45th day				90th day				135th day				165th day			
	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean
Control	-0.50	-0.50	0.00	-0.33	-1.50	0.00	1.00	-0.166	-1.50	0.50	0.00	-0.33	1.00	1.50	1.00	1.16
10 kR	0.50	0.00	0.00	0.16	1.50	2.00	0.00	1.16	0.50	2.00	0.00	0.83	0.50	1.00	0.00	0.50
20 kR	-0.50	0.00	-0.50	-0.33	0.00	0.50	0.50	0.33	1.50	0.00	-1.5	0.00	0.50	0.00	-1.00	-0.16
30 kR	1.00	2.50	-0.50	1.00	0.00	0.50	-2.00	-0.50	1.00	1.50	0.00	0.83	0.50	0.00	-2.00	-0.50
general mean	0.12	0.50	-0.25		0.00	0.75	-0.12		0.37	1.00	-0.37		0.62	0.62	-0.50	

Analysis of variance			Analysis of variance			Analysis of variance			Analysis of variance		
Source	F. Value	CD Value	Source	F Value	CD Value	Source	F Value	CD Value	Source	F Value	CD Value
Treatments	1.87	-	Treatments	0.87	-	Treatments	1.00	-	Treatments	1.33	-
Mutagens	2.86	-	Mutagens	1.10	-	Mutagens	0.88	-	Mutagens	2.35	-
Varieties	1.35	-	Varieties	0.62	-	Varieties	1.50	-	Varieties	2.42	-
Mutagen x Variety	1.55	-	Mutagen x Variety	0.84	-	Mutagen x Variety	0.86	-	Mutagen x Variety	0.46	-

the control of V_1 and 30kR of V_3 on the 90th day and also in the control population of V_1 on the 135th day. Positive variation ranged from 0.50 (control of V_2 on 45th day, 20kR of V_3 on 135th day and 30kR of V_3 on 165th day) to 3.00 in the control population of V_1 and V_2 on 165th day.

On 45th day the negative variants ranged from 0 (30kR of V_2) to 2.50 (20kR of V_1). The positive variants ranged from 0.50 (control of V_2) to 2.50 (30kR of V_2). In V_1 there was negative shift in 20kR (-0.50) and control population (-0.50), whereas positive shift was noticed in 10kR (0.50) and 30kR (1.00). While 10 and 20kR showed no positive or negative shifts in V_2 , control population showed negative shift (-0.50) and 30kR showed positive shift (2.50). In V_3 there was no positive shift while 20kR and 30kR showed negative shifts (-0.50 in both).

On 90th day the negative variants ranged from 0.50 (10kR of V_2) to 3.00 (control of V_1 and 30kR of V_3). The positive variants ranged from 1.00 (30kR of V_3) to 2.50 (10kR of V_1 and V_2). In V_1 while control population showed negative shift (-1.50) and 10kR showed positive shift (1.50), there was no shift in 20 and 30kR. In V_2 , all the treated population showed a positive shift while the control showed no shift. In V_3 , while there was no shift at 10kR, a negative shift was noticed in 30kR and positive shifts in the control and 20kR exposures.

On 135th day, the frequency of negative variants ranged from 0.50 (10kR of V_2) to 3.00 (control of V_1). The positive variants ranged from 0.50 (20kR of V_3) to 2.50 (20kR of V_1 , 10kR and 30kR of V_2). In V_1 , while control population alone showed a negative shift, a positive shift was noticed in all the treated population. In V_2 while all the treated and the control population showed positive shift, there was no shift in 20kR. In V_3 , 20kR alone showed a negative shift (-1.50).

On 165th day the negative variants ranged from 0.50 (control of V_3) to 2.50 (30kR of V_3). The positive variants ranged from 0.50 (30kR of V_3) to 3.00 (control of V_1 and V_2). In V_1 all the treatments showed a positive shift. In V_2 while control population and 10kR showed a positive shift, no shift was noticed in 20 and 30kR. In V_3 10kR showed no shift. But the control population showed a positive shift followed by a negative shift in 20 and 30kR.

Number of leaves per plant

Data regarding mean leaf number as influenced by varieties and mutagens, at different time interval is presented in Table 16. The statistical analysis of the data showed significant variation among levels of the mutagen on the 45th, 135th and 165th day and also between varieties on 90th, 135th and 165th day of growth. No significant variations were noticed among levels of the mutagen on 90th day, between varieties on 45th day, and also between treatments and variety into dose effect interaction on 45th, 90th

Table: 16-1 Number of leaves per plant at different growth phases as influenced by varieties, mutagens and their doses

Variety Mutagen	Time interval															
	45th day				90th day				135th day				165th day			
	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean
Control	4.35	4.00	5.00	4.42	4.60	5.70	7.80	6.05	6.10	8.40	10.50	8.30	7.80	11.00	12.05	10.28
10 kR	4.10	3.50	4.10	4.00	4.70	4.20	6.25	5.05	6.10	5.70	7.90	6.55	7.20	7.10	8.85	7.71
20 kR	4.35	3.60	4.10	4.02	5.10	4.25	5.90	5.06	5.75	7.00	8.00	6.86	6.50	8.30	8.50	7.80
30 kR	3.00	3.05	4.00	3.30	3.50	3.80	5.30	4.16	4.65	4.50	6.20	5.11	5.00	5.10	7.10	5.70
General mean	4.00	3.50	4.30		4.50	4.50	6.30		5.60	6.38	8.12		6.60	7.80	9.11	

<u>Analysis of variance</u>			<u>Analysis of variance</u>			<u>Analysis of variance</u>			<u>Analysis of variance</u>		
<u>Source</u>	<u>F Value</u>	<u>CD Value</u>	<u>Source</u>	<u>F Value</u>	<u>CD Value</u>	<u>Source</u>	<u>F Value</u>	<u>CD Value</u>	<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	2.45	-	Treatments	2.14	-	Treatments	2.55	-	Treatments	2.81	-
Mutagens	4.96*	0.94	Mutagens	2.50	-	Mutagens	4.60*	2.70	Mutagens	6.70**	3.16
Varieties	4.00	-	Varieties	6.55*	1.80	Varieties	5.60*	2.35	Varieties	3.98*	2.70
Mutagen x Variety	0.69	-	Mutagen x Variety	0.46	-	Mutagen x Variety	0.51	-	Mutagen x Variety	0.48	-

* Significant at 5%

** Significant at 1%

135th and 165th day.

On 45th day, the mean leaf number ranged from 3 (30kR of V_1) to 5.00 (Control population of V_3). The effect of radiation on mean leaf number was found to have significant variation. In V_1 the mean leaf number ranged from 3.00 (30kR) to 4.35 (20kR and control population). A significant reduction in mean leaf number was noticed at 30 kR when compared to the remaining treatments. In V_2 , the mean leaf number ranged from 3.05 (30kR) to 4.00 (control population). A significant reduction in the mean leaf number was noticed in the treated population when compared with the control population. In V_3 the mean leaf number ranged from 4.00 (30kR) to 5.00 (control population). A significant reduction in the mean leaf number was noticed at 30kR when compared with the control population. Maximum number of leaves was noticed in V_3 (4.30) followed by V_1 (4.00) and V_2 (3.50).

On 90th day mean leaf number ranged from 3.50 (30kR of V_1) to 7.80 (control population of V_3). There was no significant variation in the mean leaf number due to the influence of gamma ray exposures. In V_1 the mean leaf number ranged from 3.50 (30kR) to 5.10 (20kR). In V_2 the range was 3.80 (30kR) to 5.70 (control population). A general reduction in the mean leaf number was noticed in the treated population in comparison with the control population. In V_3 the mean leaf number ranged from 5.30 (30kR) to 7.80 (control population). A decrease in mean leaf number with increase in the dose of gamma ray exposure was

noticed. A variety dependent significant variation was noticed in the mean leaf number. Maximum mean leaf number was noticed in V_3 (6.30) which was significantly superior to the other two varieties.

On 135th day the mean leaf number ranged from 4.50 (30kR of V_2) to 10.50 (control population of V_3). The influence of gamma ray exposures was able to give significant variation in the number of leaves per plant. In V_1 the mean leaf number ranged from 4.65 (30kR) to 6.10 (control group and 10kR). The leaf number was found to be lower at 20kR and 30kR when compared to the rest of the treatments. In V_2 , the mean leaf number range from 4.50 (30kR) to 8.40 (control population). A significant reduction in the leaf number was noticed at 10 and 20kR compared with the control population. In V_3 the mean leaf number ranged from 6.20 (30kR) to 10.50 (control population). The mean leaf number at 30kR showed significant reduction when compared to the control population. A variety dependent significant variation was noticed and the maximum leaf number was found in V_3 (8.12) which was significantly superior to V_1 (5.60).

On 165th day, the mean leaf number ranged from 5.00 (30kR) to 12.05 (control population of V_3). The effect of gamma ray exposures was found to produce significant variation in the mean leaf number. In V_1 the mean leaf number ranged from 5.00 (30kR) to 7.80 (control population). There was a decrease in the mean leaf number with increase in the dose of gamma ray exposures.

In V_2 the mean leaf number ranged from 5.10 (30kR) to 11.00 (control population). There was a significant reduction in mean leaf number at 10 and 30kR exposures compared with the control population. In V_3 the mean leaf number ranged from 7.10 (30kR) to 12.05 (control population). A significant reduction in the mean leaf number was noticed in the treated population with increase in the dose of gamma ray exposure compared with the control population. A variety dependent significant variation was noticed in the mean leaf number. Maximum mean leaf number was noticed in V_3 (9.11) followed by V_2 (7.80) and V_1 (6.60).

Phenotypic frequency of leaf number variants

The frequency distribution of leaf number variants as affected by gamma rays in the three varieties of cardamom at four different time intervals is represented in Table 16-2, and the result of statistical analysis in Table 16-2-A. Statistical analysis of the data showed no significant variation between treatments, between mutagen, between varieties and in variety into doses interaction.

Both positive and negative variants were created by the different doses of gamma rays. The frequency of negative variants ranged from zero (10kR of V_3 on 45th day, 30kR of V_2 on 90th day, 10 kR and 20kR of V_1 on 165th day) to 2.50 (20kR of V_3 on 135th day). The frequency of positive variants ranged from zero (20kR of V_1 on 45th day, 30kR of V_1 on 135th day) to 2.50 (control of V_1 on 45th day).

Table 16-2

Frequency distribution of leaf number variants at different growth phases

		Time interval											
variety	Mutagen	45th day			90th day			135th day			165th day		
		-ve variants	Control group	+ve variants	-ve variants	Control group	+ve variants	-ve variants	Control group	+ve variants	-ve variants	Control group	+ve variants
V ₁	Control	1.50	6.00	2.50	2.00	6.50	1.50	1.00	7.50	1.50	1.50	7.50	1.00
	10 kR	0.50	8.50	1.00	1.50	7.50	1.00	0.50	8.00	1.50	0.00	8.00	2.00
	20 kR	0.50	9.50	0.00	1.50	7.50	1.00	1.00	7.50	1.50	0.00	8.00	2.00
	30 kR	1.00	7.50	1.50	1.00	7.50	1.50	1.50	8.50	0.00	0.50	8.00	1.50
V ₂	Control	0.50	8.00	1.50	0.50	8.50	1.00	0.50	7.50	2.00	1.00	8.00	1.00
	10 kR	1.50	7.50	1.00	1.00	7.50	1.50	1.50	7.50	1.00	2.00	6.50	1.50
	20 kR	0.50	9.00	0.50	0.50	7.50	2.00	1.50	7.00	1.50	1.50	7.00	1.50
	30 kR	1.00	8.00	1.00	0.00	8.50	1.50	0.50	8.50	1.00	0.50	9.00	0.50
V ₃	Control	0.50	8.50	1.00	1.00	7.00	2.00	1.50	7.50	1.00	1.00	8.00	1.00
	10 kR	0.00	8.00	2.00	1.00	7.00	2.00	1.00	8.00	1.00	1.00	7.00	2.00
	20 kR	0.50	9.00	0.50	0.50	9.00	0.50	2.50	6.50	1.00	1.50	7.00	1.50
	30 kR	0.50	9.00	0.50	1.00	7.00	2.00	0.50	8.50	1.00	0.50	9.00	0.50

Table 16-2-A Statistical analysis of frequency distribution of leaf number variants at different growth phases

Variety Mutagen	Time interval															
	45th day				90th day				135th day				165th day			
	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean	V ₁	V ₂	V ₃	General mean
Control	1.00	1.00	0.50	0.83	-0.50	0.50	1.00	0.33	0.50	1.50	-0.50	0.50	-0.50	0.00	0.00	-0.16
10 kR	0.50	-0.50	2.00	0.66	-0.50	0.50	1.00	0.33	1.00	-0.50	0.00	0.16	2.00	-0.50	1.00	0.83
20 kR	-0.50	0.00	0.00	-0.16	-0.50	1.50	0.00	0.33	0.50	0.00	-1.50	-0.33	2.00	0.00	0.00	0.66
30 kR	0.50	0.00	0.00	0.16	0.50	1.50	1.00	1.00	-1.50	0.50	0.50	-0.16	1.00	0.00	0.00	0.33
General mean	0.37	0.12	0.62		-0.25	1.00	0.75		0.12	0.37	-0.37		1.12	-0.12	0.25	

<u>Analysis of variance</u>			<u>Analysis of variance</u>			<u>Analysis of variance</u>			<u>Analysis of variance</u>		
<u>Source</u>	<u>F. Value</u>	<u>CD value</u>	<u>Source</u>	<u>F Value</u>	<u>CD value</u>	<u>Source</u>	<u>F value</u>	<u>CD value</u>	<u>Source</u>	<u>F value</u>	<u>CD value</u>
Treatments	0.48	-	Treatments	0.50	-	Treatments	1.82	-	Treatments	0.88	-
Mutagens	0.60	-	Mutagens	0.30	-	Mutagens	0.89	-	Mutagens	0.67	-
Varieties	0.23	-	Varieties	1.61	-	Varieties	1.20	-	Varieties	1.90	-
Mutagen x variety	0.50	-	Mutagen x variety	0.23	-	Mutagen x variety	2.50	-	Mutagen x variety	0.65	-

On 45th day the frequency of negative variants ranged from 0 (10kR of V_3) to 1.50 (10kR of V_2). The positive variants ranged from 0 (20 kR of V_1) to 2.50 (control of V_1). In V_1 all the treatments showed a positive shift except 20kR which showed a negative shift (-0.5). In V_2 while control population showed a positive shift (1) and 10kR showed a negative (-0.50), 20 and 30kR gave only control types. In V_3 , there was no negative shift. While control and 10kR showed a positive shift, no shift was noticed in 20 and 30 kR.

On 90th day the frequency of negative variants ranged from 0 (30kR of V_2) to 2 (control of V_1). The frequency of positive variants ranged from 0.50 (20kR of V_3) to 2.00 (20 kR of V_2 , control and 10kR of V_3). In V_1 all the treatments showed a negative shift except 30kR which showed a positive shift (0.50). In V_2 all the treatments showed a positive shift. In V_3 all the treatments showed positive shift except 20kR which gave only control types.

On 135th day the frequency of negative variants ranged from 0.50 (10kR in V_1 , control and 30kR of V_2 , and 30kR of V_3) to 2.50 (20kR of V_3). The frequency of positive variants ranged from 0 (30kR of V_1) to 2 (control of V_2). In V_1 all the treatments showed a positive shift except 30kR which showed a negative shift (-1.50). In V_2 20kR showed no shift. There was a negative

shift in 10kR and a positive shift in the control and 30kR treatments. In V_2 while control population and 20kR showed a negative shift, 30kR showed a positive shift. There was no shift in 10kR treatment.

On 165th day the frequency of negative variants ranged from 0 (10kR and 20kR of V_1) to 2.00 (10kR of V_2). The frequency of positive variants ranged from 0.50 (30kR of V_2 and V_3) to 2.00 (10kR and 20kR of V_1 and 10kR of V_3). In V_1 , there was a negative shift in the control while all the other treatments showed a positive shift. In V_2 , only 10kR showed a shift which was negative. In V_3 also there was shift only in 10kR which was positive.

Leaf area

Data regarding mean leaf area as influenced by varieties and mutagens on 165th day of sowing is presented in Table 17-1. The statistical analysis of data showed no significant variation among varieties, between treatments, between mutagens and in the case of variety into dose effect. The leaf area ranged from 12.54 (30kR of V_1) to 63.49 sq. cm. (control of V_3). The influence of gamma rays on mean leaf area was not significant.

In V_1 the mean leaf area ranged from 12.54 (30kR) to 38.00 sq. cm (control population). A general decrease in mean leaf area was noticed with increase in the dose of the mutagen. In V_2 mean leaf area ranged from 17.33 (30kR) to 44.11 sq. cm (control population). The treated population showed a reduction in mean leaf area when compared to the control population. In V_3 mean leaf area ranged from 27.30 (30kR) to 63.49 sq. cm. (control

Table: 17-1 Leaf area (sq.cm.) per plant as influenced by
different varieties mutagens and their doses

Mutagen \ Variety	V ₁	V ₂	V ₃	General mean
	Control	39.09	44.11	63.49
10 KR	34.61	20.88	50.00	35.16
20 KR	29.29	34.83	36.71	33.47
30 KR	12.54	17.33	27.30	19.05
General mean	28.63	29.18	44.37	

Analysis of varienco

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	1.37	-
Mutagens	2.90	-
Varieties	2.15	-
Mutagen x variety	0.31	-

population). The mean leaf area was found to decrease with increase in dose. A variety dependent variation was noticed in leaf area per plant. Maximum mean leaf area was noticed in V_3 (44.37 sq.cm) following by V_2 (29.18 sq.cm) and V_1 (28.63 sq.cm).
phenotypic frequency of leaf area variants

The frequency distribution of leaf area variants as affected by gamma rays in the three varieties as on 165th day is represented in Table 17-2 and the results of statistical analysis in Table 17-2-A. Statistical analysis of the data showed no significant variation among treatments, doses of mutagen, varieties and also in varieties into dose interaction.

Both positive and negative variants were created by the different doses of gamma rays. The frequency of negative variants ranged from 1.00 (control group of V_2 and V_3) to 3.00 (control in V_1 and 20 and 30kR of V_3). The frequency of positive variants ranged from 1.00 (10kR of V_1 , 30kR of V_2 and V_3) to 2.50 (10 and 20kR of V_2). In V_1 all the treatments showed a negative shift except at 30kR which showed no shift. In V_2 all the treatments showed a positive shift except 30kR which showed a negative shift (-0.50). In V_3 all the treatments showed a negative shift except in control population which showed a positive shift.

Tiller number

Data regarding mean tiller number as influenced by varieties and mutagens on 165th day of sowing is presented in Table 18-1

Table: 17-2 Frequency distribution of leaf area variants as influenced by the different mutagens

Variety	Mutagen	+ive variants	control group	+ive variants
V ₁	Control	3.00	5.00	2.00
	10 kR	2.50	6.50	1.00
	20 kR	2.50	6.00	1.50
	30 kR	2.00	6.00	2.00
V ₂	Control	1.00	7.00	2.00
	10 kR	2.00	5.50	2.50
	20 kR	2.00	5.50	2.50
	30 kR	1.50	3.50	1.00
V ₃	Control	1.00	7.00	2.00
	10 kR	2.50	5.50	2.00
	20 kR	3.00	5.50	1.50
	30 kR	3.00	6.00	1.00

Table: 17-2-A Statistical analysis of frequency distribution of leaf area variants

<u>Variety</u> <u>Mutagen</u>	<u>V₁</u>	<u>V₂</u>	<u>V₃</u>	<u>General mean</u>
Control	-1.00	1.00	1.00	0.33
10 kR	-1.50	0.50	-0.50	-0.50
20 kR	-1.00	0.50	-1.50	-0.66
30 kR	0.00	-0.50	-2.00	-0.83
General mean	-0.87	0.37	-0.75	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	1.40	-
Mutagens	1.08	-
Varieties	2.55	-
Mutagen x variety	1.16	-

Table: 18-1 Number of tillers per plant as influenced by different varieties mutagens and their doses

<u>Variety</u> <u>Mutagen</u>	<u>v₁</u>	<u>v₂</u>	<u>v₃</u>	<u>General</u> <u>mean</u>
Control	0.85	1.10	1.35	1.10
10 kR	0.75	0.45	0.60	0.60
20 kR	1.10	0.50	0.45	0.68
30 kR	0.30	0.35	0.45	0.33
General mean	0.72	0.62	0.76	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	1.67	-
Mutagens	3.90*	0.69
Varieties	0.32	-
Mutagen x variety	0.96	-

* Significant at 5% level

The statistical analysis of data showed significant variation between doses of the mutagens. But the variation between treatments, between varieties and also in the case of variety into dose effect was not significant. The mean tiller number ranged from 0.20 (30kR of V_1) to 1.35 (control of V_3). The influence of gamma ray exposures on the mean tiller number was found to be significant.

In V_1 the mean tiller number ranged from 0.20 (30kR) to 1.10 (20kR). A significant increase in mean tiller number under 20kR was noticed compared to all other treatments. In V_2 the mean tiller number ranged from 0.35 (30kR) to 1.10 (control). The treated population showed a significant decrease in mean tiller number compared to the control population. In V_3 , the mean tiller number ranged from 0.45 (20kR and 30kR) to 1.35 (control population). The treated population showed a significant decrease in mean tiller number compared to the control population.

A variety dependent variation, which was not significant was noticed in the mean tiller number. Maximum tiller number was noticed in V_3 (0.76) followed by V_1 (0.72) and V_2 (0.60).

Phenotypic frequency of tiller number variants

The frequency distribution of tiller number variants as affected by gamma rays in the three varieties as on 165th day of

Table: 18-2 Frequency distribution of tiller number variants
as influenced by the different mutagens

Variety	Mutagen	-ive variants	control group	+ive variants
V ₁	Control	5.00	5.00	0.00
	10 kR	6.00	4.00	0.00
	20 kR	4.50	5.00	0.50
	30 kR	8.00	2.00	0.00
V ₂	Control	2.50	7.00	0.50
	10 kR	7.00	3.00	0.00
	20 kR	7.00	2.50	0.50
	30 kR	7.00	3.00	0.00
V ₃	Control	2.50	7.00	0.50
	10 kR	4.00	6.00	0.00
	20 kR	7.00	2.50	0.50
	30 kR	7.00	2.50	0.50

Table: 18-2-A Statistical analysis of frequency distribution
of tiller number variants

Variety \ Mutagen	V ₁	V ₂	V ₃	General mean
Control	-5.00	-2.00	-2.00	-3.00
10 kR	-6.00	-7.00	-4.00	-5.60
20 kR	-4.00	-6.50	-6.50	-5.60
30 kR	-8.00	-7.00	-6.50	-7.16
General mean	-5.75	-5.62	-4.75	

V
Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	1.44	-
Mutagens	3.33	-
Varieties	0.43	-
Mutagen x variety	0.84	-

sowing is represented in Table 18-2 and the results of statistical analysis in Table 18-2-A. Statistical analysis of the data showed no significant variation among treatments, doses of mutagen, varieties and also in varieties into dose interaction.

Both positive and negative variants were created by the different doses of gamma rays. The frequency of negative variants ranged from 2.50 (V_2 and V_3 control) to 8.00 (30kR of V_1). The frequency of positive variants was zero and 0.50. In all the three varieties all the treatments showed a negative shift.

Frequency of chlorophyll deficient plants under different treatments

The frequency of chlorophyll deficient plants as influenced by various mutagenic treatments in three varieties is presented in Table 19. The statistical analysis of data showed significant variation between treatments, between mutagens within the mutagens, between levels of EMS, and control with various treatments. The frequency of chlorophyll deficient plants ranged from 0 (in control and lower doses) to 67.50 (in V_1 of 0.75% EMS treated population).

In V_1 the influence of gamma rays on the frequency of chlorophyll deficient plants was not significant. The frequency of chlorophyll deficient plants ranged from 0 (in control) to

Table 19

Frequency of chlorophyll deficient plants under different treatments (Percentage)

Variety Mutagen	V_1	V_2	V_3	General mean
Control	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
<u>Gamma rays</u>				
10 kR	9.75 (18.05)	20.00 (26.55)	14.91 (22.40)	14.88 (22.33)
20 kR	24.99 (29.95)	16.92 (24.14)	23.33 (27.54)	21.74 (27.21)
30 kR	9.54 (17.98)	10.00 (18.42)	9.54 (17.98)	9.69 (18.13)
40 kR	14.54 (22.04)	20.00 (21.04)	33.33 (35.24)	22.62 (26.11)
50 kR	10.00 (14.70)	12.50 (20.69)	12.50 (16.42)	11.66 (17.27)
<u>EMS</u>				
0.25%	0 (2.86)	12.50 (16.40)	5.55 (11.16)	6.01 (10.15)
0.50%	10.00 (14.70)	0 (2.86)	8.33 (13.47)	6.11 (10.34)
0.75%	67.50 (55.36)	16.66 (19.05)	7.14 (12.52)	30.43 (28.98)
1.00%	0 (2.86)	16.66 (19.05)	0 (2.86)	5.55 (8.26)
1.25%	0 (2.86)	0 (2.86)	25.00 (23.90)	8.33 (9.88)
1.50%	33.33 (28.78)	0 (2.86)	0 (2.86)	11.11 (11.50)
1.75%	0 (2.86)	0 (2.86)	0 (2.86)	0 (2.86)
General mean	13.81 (16.61)	9.63 (13.82)	10.70 (14.78)	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	2.00*	31.85
Mutagens (T)	3.48**	18.39
Varieties (V)	0.37	-
Mutagen x variety	1.39	-
Between levels of Gamma rays	0.88	-
Between levels of EMS	2.87*	18.39
Between mutagens	13.99**	7.61
Control Vs. treated	7.02*	13.53

* Significant at 5% level

** Significant at 1% level

(Transformed values are presented in parenthesis)

24.99 (20kR). The influence of EMS treatments on the frequency of chlorophyll deficient plants was found to differ significantly. The frequency ranged from 0 (in control, 0.25%, 1.00%, 1.25% and 1.75%) to 67.50 (0.75%). A significant increase in the frequency of chlorophyll deficient plants was noticed in 0.75% EMS treated population when compared to all other treatments.

In V_2 also the influence of gamma rays was significant. The frequency of chlorophyll deficient plants ranged from 0 (in the control group) to 20.00 (in 10 and 40kR). There was significant variation in the frequency of chlorophyll deficient plants due to the influence of EMS treatments. The frequency ranged from 0 (in 0.50%, 1.25%, 1.50%, 1.75% and in the control group) to 16.66 (in both 0.75 and 1.00).

In V_3 , the influence of gamma ray exposures on the frequency of chlorophyll deficient plants was as in V_1 and V_2 . The frequency ranged from 0 (in control group) to 33.33 (in 40kR). The influence of EMS treatments on the frequency of chlorophyll deficient plants was found to be significant. The frequency ranged from 0 (in control population, 1.00, 1.50% and 1.75%) to 25% (in 1.25%).

CYTOLOGICAL ANALYSIS

Frequency of mitotically active cells under different treatments

The frequency of mitotically active cells as influenced by various treatments is presented in Table 20. The statistical

Table: 20 Statistical analysis of frequency of dividing cells
under different treatments (Percentage)

Variety Mutagen	V ₁	V ₂	V ₃	General m
Control	10.76 (19.12)	7.80 (16.02)	8.27 (16.58)	8.94 (16.
10 KR	11.40 (19.38)	9.61 (17.86)	3.82 (11.12)	8.27 (16.
20 KR	8.02 (16.27)	11.94 (19.90)	3.62 (10.90)	7.86 (16.
General mean	10.06 (19.10)	9.70 (17.88)	5.23 (13.40)	

Analysis of variance

<u>Source</u>	<u>F Value</u>	<u>CD Value</u>
Treatments	6.34**	5.09

** Significant at 1% level

(Transformed values are presented in parenthesis)

analysis of data showed significant variation among treatments.

The frequency of mitotically active cells ranged from 3.62 (20kR in V_3) to 11.94 (20kR in V_2). The influence of gamma rays on the frequency of mitotically active cells in the three varieties was found to differ significantly. In V_1 10 kR showed maximum frequency (11.40) followed by control (10.76) and 20kR (8.02). In V_2 20kR showed maximum value (11.94) followed by 10kR (9.61) and control (7.80). In V_3 control population showed a maximum frequency of 8.27 followed by 3.82 in 10kR followed by 20kR (3.62). In general, V_1 showed maximum frequency of mitotically active cells (10.06) followed by V_2 (9.70) and V_3 (5.23).

Spectrum of dividing cells under different phases of mitotic cycle

Analysis on different phases of the mitotic cells due to various treatments is presented in Table 21. The statistical analysis of the data showed significant variation among treatment in prophase in all the three varieties. No significant variation was noticed among treatments in metaphase, anaphase and telophase due to the influence of gamma rays.

In V_1 the spectrum of the mitotic cells under different phases due to the influence of various treatments ranged from 0.26 (20kR in telophase) to 2.58 (10kR in prophase). Maximum number of cells under prophase was noticed at 10kR (2.58) which was greater than in control (2.42) and significantly superior to

Table: 21 Statistical analysis of spectrum of dividing cells under different treatments

Variety	Treatment	Division stages in mitosis							
		Prophase		Metaphase		Anaphase		Telophase	
V ₁	Control	2.42	(1.66)	1.10	(1.42)	1.32	(1.49)	0.56	(1.23)
	10 kR	2.58	(1.87)	1.48	(1.56)	1.44	(1.53)	0.28	(1.12)
	20 kR	1.04	(1.40)	1.42	(1.53)	1.30	(1.50)	0.26	(1.12)
V ₂	Control	1.22	(1.42)	1.14	(1.45)	1.84	(1.65)	0.50	(1.22)
	10 kR	3.12	(2.02)	1.10	(1.42)	1.28	(1.49)	0.28	(1.12)
	20 kR	3.68	(2.15)	0.92	(1.37)	1.02	(1.41)	1.28	(1.23)
V ₃	Control	3.54	(2.12)	1.52	(1.56)	1.52	(1.53)	0.10	(1.04)
	10 kR	1.40	(1.54)	0.90	(1.36)	0.82	(1.33)	0.16	(1.07)
	20 kR	1.48	(1.57)	0.88	(1.35)	0.38	(1.16)	0.16	(1.07)

Analysis of Variance

Source	F Value	CD Value
Treatments	6.35**	0.45

Analysis of Variance

Source	F Value	CD Value
Treatments	0.64	-

Analysis of Variance

Source	F Value	CD Value
Treatments	1.42	-

Analysis of Variance

Source	F Value	CD Value
Treatments	1.32	-

** Significant at 1% level

(Transformed values are presented in parenthesis)

20kR (1.04). The influence of gamma rays on the number of cells under prophase was found to differ significantly. Maximum number of cells under metaphase was noticed at 10kR (1.48) followed by 20kR (1.42) and control (1.10). The number of cells under anaphase was found to be maximum at 10kR (1.44) followed by control (1.32) and 20kR (1.30). The control group showed maximum number of cells under telophase (0.56) followed by 10kR (0.28) and 20kR (0.26).

In V_2 the influence of gamma rays on the number of cells under prophase was found to differ significantly. Maximum number of cells under mitotic prophase was noticed in 20kR (3.68) which was significantly superior to the control (1.22) and 10kR (3.12). Maximum number of cells under metaphase was noticed in the control (1.14) followed by 10kR (1.10) and 20kR (0.92). The control population was found to have maximum number of cells under anaphase (1.84) followed by 10kR (1.28) and 20kR (1.02). Maximum number of cells under telophase was noticed at 20kR (1.28) followed by control group (0.50) and 10kR (0.28).

In V_3 also the influence of gamma rays on the number of cells under mitotic prophase was found to differ significantly. The control population was found to have maximum number of cells under prophase (3.54) which was significantly superior to 20kR (1.48) and 10kR (1.40). The maximum number of mitotic cells

under metaphase was noticed in the control (1.52) followed by 10 (0.90) and 20kR (0.88). The control group was found to have maximum number of mitotically active cells under anaphase (1.52) followed by 10 (0.82) and 20 kR (0.38). The number of mitotically active cells under telophase was found to be 0.16 in both 10kR and 20kR population which was slightly greater than in control population (0.10).

DISCUSSION

DISCUSSION

The present investigation which forms a preliminary work under the broad area of induced mutations in cardamom was undertaken with a view to find out the relative biological effectiveness of gamma rays and EMS on three cardamom cultivars. The induction of mutations by physical and chemical agents is invariably accompanied by the production of undesirable changes in the biological materials. Most part of these undesirable changes are resulting from chromosome structural changes and toxicity due to the direct effect of the mutagen. The direct effect of mutagens in M_1 include lethality, injury and sterility. For a particular mutagenic treatment there exists a correlation between M_1 damage and M_2 mutation frequency (Gaul, 1959). Efficient treatments producing greater proportion of mutations to damages are essential for an economic mutation breeding programme. It is rather difficult to give a complete picture of all the M_1 effects that have been created in the three popular cardamom cultivars. However, a brief account on the direct effects of both the mutagens and their relative efficacy as evident from the results of the present investigation are discussed below.

Germination

In the present investigation, the various doses of gamma rays and EMS produced a significant delay in germination in all

the three cultivars of cardamom. A similar delay in germination in the mutagen treated population as was noted in the present investigation was also reported earlier by Gregory (1955, 1968) in *Pisum* and Favret (1963) and Gaul (1967) in barley. Subsequent investigations confirmed the delay in germination due to mutagen treatment as observed in cow pea (Louis and Kadambavanasundaram, 1973 a) and in tomato due to X-ray treatment (Lesley and Lesley, 1956). Similar report was also made by Sree Ramulu (1970) in sorghum. The delay in germination could be attributed to the mitotic impairment which disrupts resistance activity in seeds as postulated earlier by Cherry and Hageman (1961).

The delay in germination was found to increase with increase in levels of the mutagen. This is in accordance with the observations made by Athwal (1963) in *Cicer* using X-rays, Shirshov and Shain (1966) using gamma rays in field beans and Sidorova et al. (1966) and Maslov and Stepanova (1967) using gamma rays in peas. Similar reports were also made by Alikhan et al. (1973) in redgram using gamma rays and Bajaj and Saettler (1970) in *Phaseolus* using gamma rays.

In V_1 and V_3 , EMS treated population showed more delay in germination compared to irradiated ones. The effect of EMS in delaying germination of seeds has been clearly demonstrated by Vanderveen and Hilderling (1965) in tomato, Osone (1966) and

Sree Ramulu (1970) in rice and Chandra Sekhar and Reddi (1971) in sorghum. This kind of delay in germination seems to be caused by physiological damages brought about by chromosomal and extra chromosomal factors. Scerascia (1956) studied the effects of radiation in four cultivars of Nicotiana tabacum and found that the delay in germination was due to chromosome aberrations. On the contrary Gaul et al. (1966) observed that in barley the EMS treatment delayed seed germination due to the direct physiological damage caused by alkylating agents and also due the indirect effect of 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.

During the course of the present investigation it has been noted that in all the three varieties there was a reduction in germination percentage consequent to mutagenic treatment. Reduction in germination percentage as a result of mutagen treatment was reported earlier by many mutation workers in various crops including Gustafson and Gadd (1965) in Poa pratensis, Rangaswamy (1969) in Sorghum, Roy et al. (1971) in Cucumis sativus, Bohera and Patnaik (1979) in Amaranthus sp. and Masjid (1975) in Lycopersicon sp. In both the mutagens a progressive reduction in percentage of germination was observed with increase in dose level. Fuji an

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. Rangaswamy (1969) and Wu and Pi (1968) reported a reduction in germination with increase in dose in cereal crops like paddy, sorghum and pearl millet. Reduction in germination as was noted in the present investigation was reported by Sree Ramulu (1970) with both physical and chemical mutagens in sorghum. Goud et al. (1970) also reported a decreasing trend with increase in dosages. Gregory (1955, 1968), Bilques and Martin (1961) and Giles and Dewink (1969) also reported the same trend in peanut with radiation doses.

A drastic reduction in germination in EMS treatments compared to gamma rays was noted in the present investigation. This is in general agreement with the studies conducted on rice (Rao and Ayengar, 1964; Ganeshan, 1970; Siddiqu and Swaminathan 1968 and Yamagata et al; 1965) that chemical mutagens such as DES, EMS and NMU will drastically reduce germination compared to physical mutagens. In bajara, Singh et al. (1978) reported that gamma rays had little or no effect on germination whereas EMS resulted in drastic reduction.

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 Leus culinaris following gamma ray treatment and attributed the reduction to disturbances caused at physico-chemical level of the cells or acute chromosomal damage or both. Venkateswarlu et al. (1978) noticed reduced germination in pigeon pea, following irradiation and suggested that it may be due to threshold physiological effect of X-rays in this species. The physiological effect of mutagens in inhibiting germination was also reported by Chauhan and Singh (1975). According to them 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) in corn.

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 ionising 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.

Chemical mutagens are also known to cause reduction in germination of seeds (Siddiq and Swaminathan, 1968; Chandrasekhar and Reddi, 1971; Rao and Ayengar, 1964 and Sree Ramulu, 1970). 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 found to be due to the formation of acids upon hydrolysis which in turn reduce the p^H of the medium making it toxic (Freeze - Gertzen et al. 1963).

Contradictory reports in which germinability of gamma irradiated seeds were better as compared to the control have been reported by Swarup and Gill (1968) and Rukmanskee (1973) in French bean, Mujeeb (1974) in Cicer sp. Khan and Meshim (1978) in green gram. Mujeeb (1974) reported earlier germination in gram at low doses of gamma rays. It has also been reported by various investigators that the seed germination is not affected by low doses of ionizing radiations. (Sjodin, 1962; Wellensick, 1965 and Ojomo and Chheda, 1971). Sjodin (1962) proposed a physiological explanation for this observation. The first phase of germination is the swelling of cells by hydration followed by enzymatic activation and metabolism. The materials and

energy necessary for this initial growth are already available in the seed. So the young embryo has no need to synthesis new substances but only to activate those already stored in the cotyledons. This stage of germination is unaffected by radiations, and so the damage to embryo which might arise from ionizing radiations result only in post germination mortality.

Survival

Post germination lethality is determined by the percentage of plants surviving in the treated population. This is a good estimate to assess the direct effect of the mutagen along with sterility. In the present investigation it has been noted that the various treatments tested showed significant variation in survival percentage depending on the mutagens, their doses and the varieties concerned. On comparing the effect of gamma rays and EMS it could be clearly noted that the survival was more in gamma ray treated population compared to that of the EMS treated ones.

The present investigation is in line with the reports made by Tomohira et al. (1964) in Capsicum sp. and Datura sp. Matsumura (1966) and Chaudhary (1978) in wheat, Sahib and Abraham (1972) in Capsicum sp., Venkateswaralu et al. (1978) in pigeon pea. A mutagen dependent variation in survival percentage as was noted in the present investigation has been reported by D' Ama to et al. (1962) in wheat. Similar reports were made by Yu (1961) in tomato, Mc Crory and Crun (1969) and Olatunde et al. (1971) in certain vegetable crops. Narasinghani and Kumar (197

observed that the survival percentage was drastically reduced with 0.25 per cent EMS and 0.25 per cent MMS in cow pea.

Cytological explanations are given for the reduction in the survival percentage with increased doses of radiation. 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. Mitotic abnormalities due to irradiation results in the structural changes in the chromosomal complements. This interferes with the normal growth and development of organs which might have led to the fall in survival percentage with increased doses.

Rate of growth in plants

The growth rate in plants is governed by the rate of internal metabolic processes of the system which to a certain extent is influenced by the external factors. Treatments with mutagens are known to affect growth and growth rate. In the present investigation growth rate was estimated by observing plant height, number of leaves, leaf area and number of tillers at different time intervals. A general trend in the direction of shift in mean values for the entire growth period seen in the present investigation for plant height, number of leaves, leaf area and tiller number due to the effect of the three doses of gamma rays in three varieties is presented in table 22.

Table 22 Direction of shift in mean value for plant height, leaf number, leaf area and tiller number per plant.

		10 kR	20 kR	30 kR
Plant Height	V_1	+	+	+
	V_2	+	+	+
	V_3	0	-	-
Leaf Number	V_1	+	+	+
	V_2	-	+	+
	V_3	+	-	+
Leaf area	V_1	-	-	0
	V_2	+	+	-
	V_3	-	+	-
Tiller number	V_1	-	-	-
	V_2	-	-	-
	V_3	-	-	-

In V_1 and V_2 a general enhancement in mean plant height was noticed in all the treatments. In V_3 , 20 kR and 30 kR exposed population showed a decrease in mean plant height while 10 kR showed steady value.

A reduction in plant height as a result of mutagen treatment as was noted in certain treatments in the present investigation has been reported by several workers. Sakai and Susuki (1964) after X - irradiation in rice reported that mutation of polygenes responsible for quantitative characters like plant height occur in most cases unidirectionally in negative direction. Caldecott et al. (1952) observed a reduction in growth of barley seedlings following X-irradiation of seeds. In the present investigation higher levels of exposures reduced plant height in all the three varieties (Fig. 18, 19). Wood stock and Justice (1967) after studies in maize, 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 also reported by Roy et al. (1971) in Cucumis sativus with X - irradiation and Venkateswarlu et al. (1978) in pigeon pea after gamma ray treatment. Increase in mean values for plant height as a result of mutagen treatment, as has been noted in some cases in the present study, has also been reported by several workers. Kumar and Das (1977) studied induced polygenic variations in Brassica for plant height following treatments with gamma rays and thermal neutrons.

In V_1 there was a general increase in the mean leaf number whereas in V_2 and V_3 both positive and negative shifts were observed. In V_2 negative shift in the mean leaf number was noticed at 10 kR, and at 20 kR in V_3 .

A complete negative shift was observed in V_1 with respect to mean leaf area except at 30 kR where there was neither positive nor negative shift. In V_2 and V_3 both positive and negative shifts were noticed. Negative shift was noticed only at 30 kR in V_2 while in V_3 10 kR and 30 kR showed a negative shift in mean leaf area.

All the treatments in the three cultivars showed a complete negative shift with respect to mean tiller number. A reduction in mean value for number of tillers following X - irradiation has been reported by Sakai and Susuki (1964) in rice. Gamma rays and EMS treatments in rice has led to a significant reduction in mean number of tillers in M_2 and M_3 generations (Nayar, 1976). Goud (1967) in hexaploid wheat observed a shift in mean towards the negative direction for tiller number. On the other hand, Bateman (1959) observed that the means for tillers per plant in rice increased after irradiation and suggested that the overall effect of polygenic mutation in rice was unidirectional. The mean number of branches in M_2 and later generations were found to be increased due to treatment with gamma rays and thermal neutrons as was reported by Kumar and Das (1977) in some species of Brassica.

The explanations offered for the delay and reduction in growth rate are many. Smith and Kerstan (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 interferences 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 Evan 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

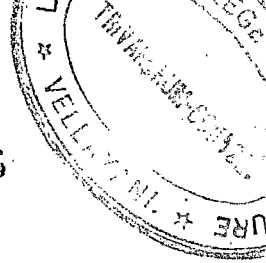
effect on certain respiratory system operating during crop growth. Sinha and Godward (1972) pointed out that growth inhibition at higher doses may be due to gross cellular injury either to genic controlled bio-chemical and physiological processes or due to chromosomal aberration or both.

Pollard (1964) postulated that irradiation inhibits the transcription of DNA and leads to a decrease in messenger RNA which should cause a decrease in protein synthesis and growth. Decrease in mitosis, irregular cell enlargement and degeneration of nuclei with progressively increasing dosages can be the cause of reduced growth. Conger et al. (1969) after exposing barley seeds to radiation found that damage to plant height and to chromosomes are closely correlated even within a treatment. 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.

Effect on morphological traits of plant organs

(a) Leaves

In the present investigation a few plants were found to possess leaves with altered size and shape due to radiation treatment. The leaves were relatively narrow with deep



serrations (Fig. /4). Seedlings with split lamina were also noticed consequent to EMS treatments (Fig. /3). Modification in leaf size and shape have been reported on similar lines in many plant genera as a consequence of mutagen treatments. Singh et al. (1939) reported variation in shape and size of leaves of Gossypium hirsutum following X-ray irradiation. Schwartz (1954) noticed that following irradiation of dry maize seeds, the leaves showed reduction in size corresponding to an increase in dose of radiation. Patel and Datta (1960) observed narrow leaves following X-ray treatment in Corchorus capsularis. This is in accordance with the present investigation using gamma rays. Narrow leaves were also reported in chillies following X-ray treatment by Sahib and Abraham (1972). Raghuvanshi and Singh (1974) observed crumpled leaves and dissected margins in Trigonella foenum - graceum following gamma ray treatment. Koshy and Abraham (1978) noticed progressive reduction in size, distorted shape, irregular lobe and change in texture of leaves in Abelmoschus esculentus following gamma ray treatment.

Irvine (1940) held the view that abnormalities observed in leaves after irradiation could be due to the disturbances of phytochromes as a result of irradiation. Meiselman et al. (1961) stated that the irradiation induced abnormalities such as reduction in the number and size or deformation of leaves might be due to chromosomal aberrations. Moh (1962) attributes

reduction of leaves of coffee plants to chromosomal deficiency. Haber and Foard (1964) concluded that the reduction in size of leaves in gamma irradiated wheat seedlings might be attributed largely to the radiation - induced mitotic inhibition rather than to the other actions of radiation.

(b) Dichotomy

Stem dichotomy was observed in most of the EMS treated seedlings (Fig. 16). As a result of dichotomy, bifurcation of the shoot occurs. Nettaancourt and Contant (1966) noted the occurrence of fasciation and bifurcation of stem in tomato as a regular feature following chronic gamma irradiation. Singh and Mitra (1967) observed bifurcation with X-ray treatment in Hibiscus species. Gamma irradiation caused stem dichotomy in apple and peaches (Lapins et al. 1969). In maize Chandramouli (1970) observed dichotomous branching among the irradiated population. Koshy and Abraham (1978) observed dichotomy of the stem in Abelmoschus esculentus following gamma irradiation.

Mackey (1951) stated that bifurcation of stem could be explained on the basis of regeneration of affected meristem in barley. Bishop and Aalders (1955) attributed it to the delayed expressions of some chromosomal effects. Kuehnert (1962) explained that it may be due to enlargement of the central cells of tunica along a vertical axis followed by, periclinal divisions of the cells. This results in the displacement of

activity from the centre to the flanks of the apex. As a result two new apical meristem could develop. In the case of leaves also bifurcation of petiole and appearance of two leaflets at the same node has been reported by Raghuvanshi and Singh (1974), in Trigonella foenumgraceum. (Fig. 16) shows stem dichotomy.

(c) Chlorophyll deficient plants

In the present investigation, chlorophyll deficiency was noticed at lower doses in certain plants consequent to the mutagenic treatments. Frequency of chlorophyll deficient plants was more in the irradiated population compared to EMS treated population. The different types of chlorophyll deficient plants observed include striata (Fig 15) albino and variegata. The striata type was found in greater frequency than the other two types.

Gustafsson (1947) stated that chlorophyll disorganisation is one of the many effects of irradiation. An increase in the frequency of chlorophyll mutation with increasing doses of radiations was reported by several investigators in rice. Mutation frequency reached a maximum at moderate doses of X-rays and gamma rays and decreased at higher doses (Matsuo et al, 1958 and Masima and Kawai, 1959). Gailey and Telbert (1958) reported that very high doses of gamma irradiation to the extent of 50 kR did cause disorganisation of chlorophyll. Alikhan and Veeraswamy (1974) studied the effect

of gamma rays and EMS in red gram and found that chlorophyll mutations were maximum at 24 kR and 70 mM treatments respectively. The frequency of chlorophyll mutations recoverable in a mutagenic experiment is a good indication of the effectiveness and efficiency of mutagenic treatment (Monte, 1968). Louis and Kadambavanasundaram (1973 b) reported the occurrence of albino, xantha, and viridis mutants in cowpea following gamma irradiation. Chekalin (1977) obtained wide spectrum of chlorophyll mutations in Lathyrus sativus following gamma irradiation and treatment with different chemical mutagens, the most frequent being chloro - viridis.

Cytological effects

In the present investigation, gamma ray treatment was found to affect mitotic cell division by way of reducing the frequency of cells under active cell division stages. A general reduction in the frequency of cell division was also noticed. A study of the cytological changes in Capsicum annum under the influence of chemical mutagens like EI, NMU, NEU, was conducted by Galukyan (1968). Iqbal (1969, 1970 and 1972) studied the extent of cellular damage and responses of shoot apices subsequent to radiation damage. The effect of X-rays on the mitotic activity and frequency of structural rearrangements in the chromosomes in the root cells of the species Capsicum annum was studied by Terzyan et al. (1974). A general survey of cellular changes due to mutagen has been presented by

various authors (Sparrow, 1961; Catchside, 1945; Darlington and La Cour, 1945; Evans, 1962; Gustafsson and Von Wettstein, 1958 and Swanson, 1957). They reviewed the types of induced chromosomal mutations, their mitotic and meiotic behaviour and genetic consequences.

Ionising radiations bring about a number of cytological damages like chromosome paling, stickiness and clumping, fragmentation, bridge formation, abnormal chromosomal spiralization of chromonemata etc. These cytological damages consequent to irradiation may be the reason for reduction in the frequency of mitotic cell division.

SUMMARY

SUMMARY

The present investigation was carried out in the Department of Agricultural Botany, College of Agriculture, Vellayani during 1985-86. This experiment was taken up as a preliminary trial in the broad area of induced mutations in cardamom (Elettaria cardamomum (L.) Maton). The direct effect of ^{60}Co -gamma rays and ethylmethane sulphonate (EMS) on three varieties of cardamom (Malabar, Vazhukka, and Mysore) was assessed with respect to various growth metrics. The experiment was laid out in RBD with two replications. Data were collected on

1. Days taken to start germination, complete germination from the date of sowing and to complete germination from the date of first sprout
2. Rate and percentage of germination
3. Seedling survival as on 20th day of sprouting and survival as on 90th day of sprouting
4. Plant height and number of leaves per plant at 15 days intervals
5. Leaf area (L x B) and number of tillers per plant as on the 6th month of growth
6. Chlorophyll and other M_1 variants, and
7. Cytological analysis (Frequency and spectrum of mitotically active cells under different treatments).

The tabulated data were analysed statistically. Direct effects of gamma rays and EMS were assessed based on various growth metrics, frequency and spectrum of chlorophyll deficient plants

and by cytological analysis. The various doses of gamma rays and EMS produced a significant delay in germination in all the three varieties of cardamom. The delay in germination was found to increase with increase in level of the mutagen. In V_1 and V_3 EMS treated population showed more delay in germination compared to irradiated ones. In all the three varieties there was a reduction in germination percentage consequent to mutagenic treatment. A progressive reduction in the percentage of germination was noticed with increase in dose level in both the mutagens. EMS induced a more drastic reduction in germination compared to gamma ray exposures.

The irradiated population was found to have a better survival percentage compared to the EMS treated population. A general enhancement in plant height was noticed consequent to mutagenic treatment in V_1 and V_2 whereas a reduction was noticed in V_3 at higher exposures (20 and 30 kR). In V_1 there was a general increase in the mean leaf number whereas in V_2 and V_3 both positive and negative shifts were observed. Negative shift in mean leaf number was noticed at 10 kR in V_2 and at 20 kR in V_3 . A complete negative shift was noticed with respect to mean tiller number in all the three varieties following mutagenic treatment. Chlorophyll deficiency was noticed at lower doses in certain plants consequent to mutagenic treatments. Chlorophyll deficient plants were found more frequently in the irradiated population compared to the EMS.

The different types of chlorophyll deficient plants noticed include striata, albina, and variegata, with a higher frequency of striata types. Irradiation induced plants with altered leaf size and shape. The leaves were narrow with deep serrations. EMS induced seedlings with split lamina and stem dichotomy were also observed.

Radiation treatment was found to affect mitotic cell division. Cytological analysis showed a general reduction in the frequency of cell division and also in the frequency of cells under different phases of mitotic cycle.

The present investigation clearly demonstrated that the cardamom varieties respond differentially for different mutagens and their doses. The shift in mean value both in negative and positive direction for various growth metrics indicate that a positive selection response can be created in cardamom by induced mutagenesis. Detailed analysis on segregating generation for various micromutational events is suggested based on the present investigation.

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ILLUSTRATIONS

**Fig. 1-4 Growth variation as on 45th day of
transplanting
(Variety Malabar)**



Fig - 1



Fig - 2



Fig - 3



Fig - 4

**Fig. 5-8 Growth variation as on 45th day of
transplanting
(Variety Mysore)**

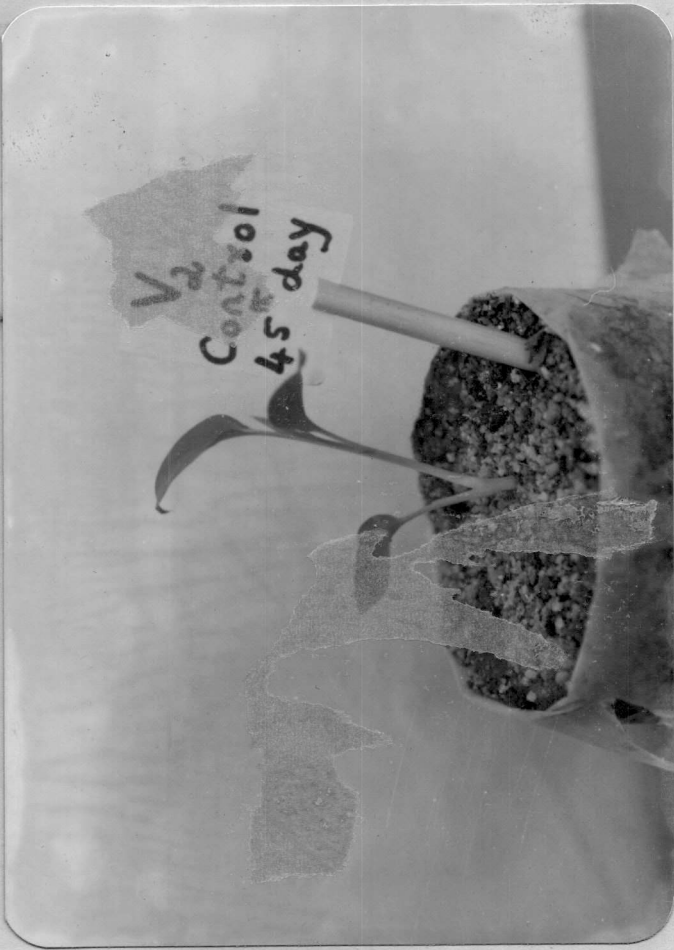


Fig - 5



Fig - 6



Fig - 7

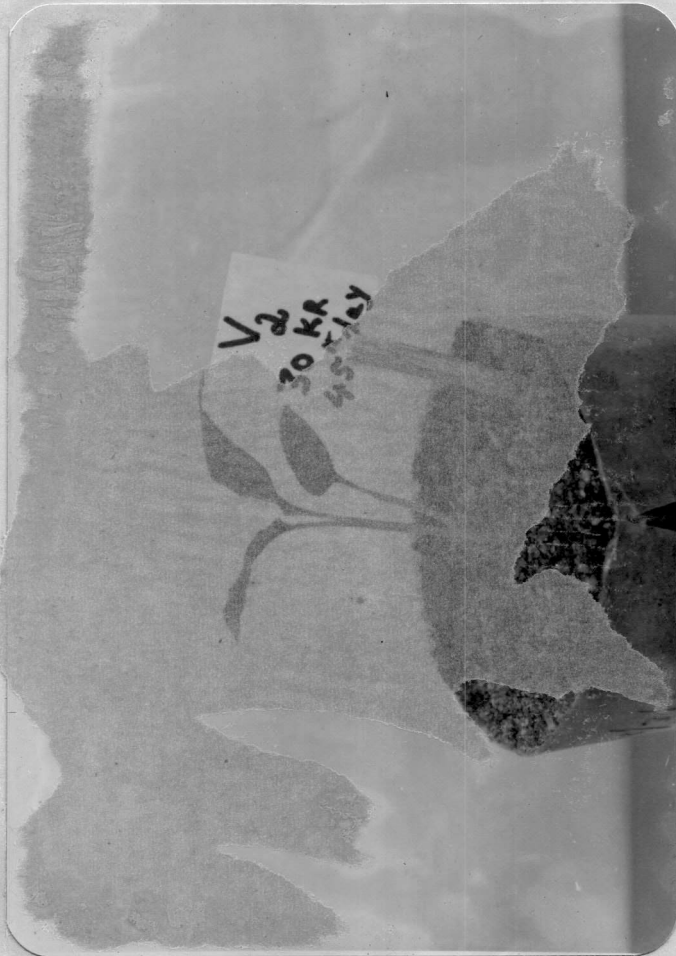


Fig - 8

Fig. 9-12 Growth variation as on 45th day of
transplanting
(Variety Vazhukka)



Fig - 9



Fig - 10



Fig - 11



Fig - 12

Fig. 13 seedling with split lamina

Fig. 14 plant with narrow leaves and deep serration

Fig. 15 Chlorophyll variant (striata type)



Fig - 13

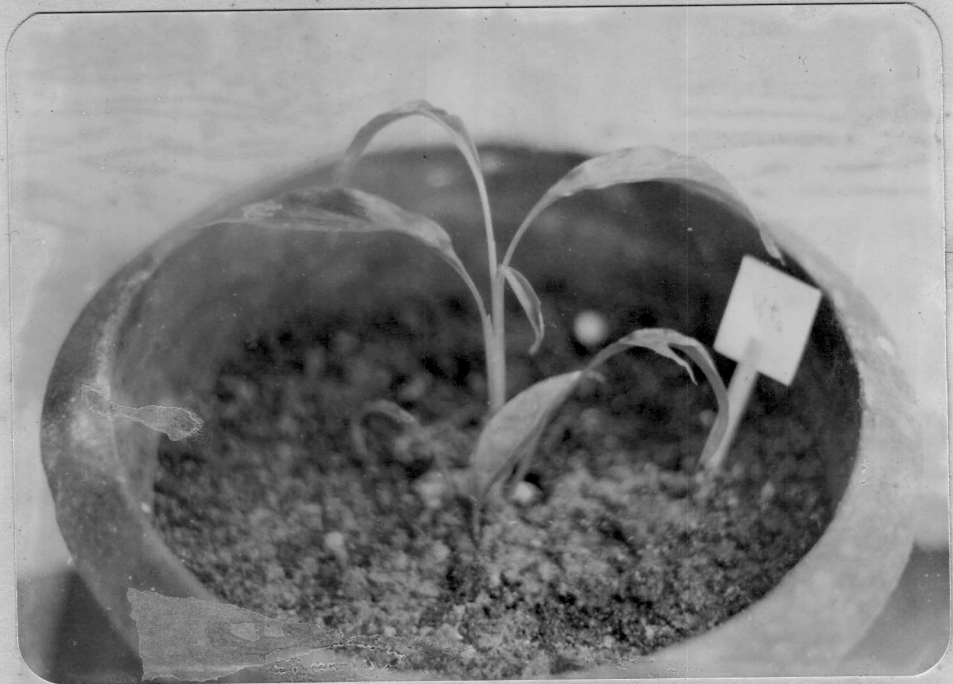


Fig - 14

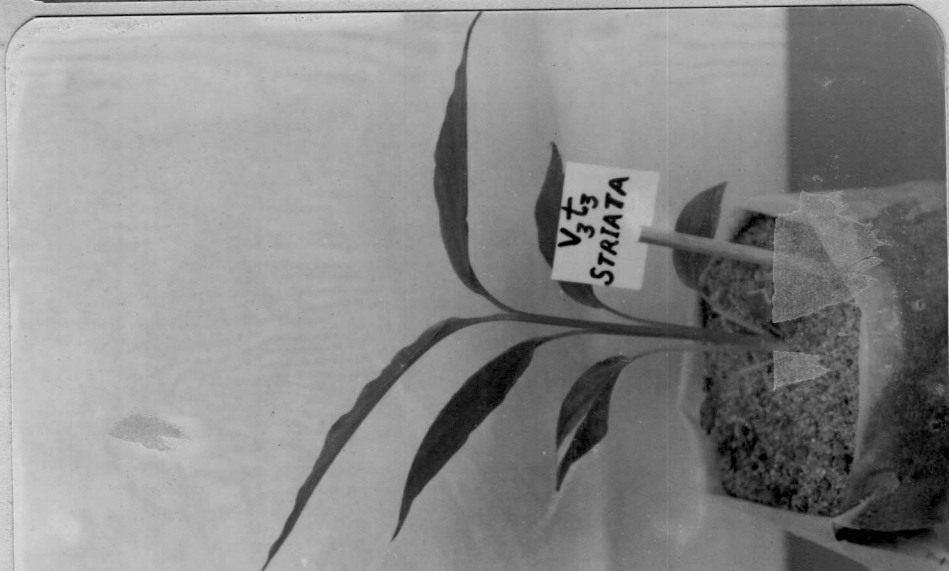


Fig - 15

Fig. 16 stem dichotomy.

Fig. 17 EMS induced growth retardation



Fig - 16



Fig - 17

Fig. 18 Gamma ray induced plant height reduction

(Right to left control, 10kR, 20kR & 30 KR)

Fig. 19 Gamma ray induced plant height reduction

at higher exposures

(Right to left control, 40kR & 50 kR)



Fig - 18



Fig - 19

ABSTRACT

The direct effect of ^{60}Co -gamma rays and ethylmethane sulphonate (EMS) on three varieties of cardamom namely Malabar, Mysore and Vazhukka was studied during the course of present investigation. The experiment was carried out at the Department of Agricultural Botany, College of Agriculture, Vellayani during the year 1985-1986.

Dry seeds were exposed to gamma rays at dose levels ranging from 10-70 kR at 10 kR interval and the pre-soaked seeds for 16 hours were treated with EMS concentrations ranging from 0.25% to 1.75% at an interval of 0.25%. Soaked seeds were also exposed to gamma rays with 10 and 20 kR. The experiment was laid out in RBD with two replications. Direct effect of the mutagens was assessed by analysing the effect of various growth metrics like number of days taken to start germination, number of days taken to complete germination from the date of sowing and also from the date of first sprout, rate of germination, germination percentage, survival percentage, growth rate based on plant height, leaf number, leaf area and tiller number, frequency of chlorophyll deficient plants and cytological effects. The data collected were analysed statistically. Since sufficient population was not available at higher doses of both the mutagens they were not considered for growth metric analysis.

A significant delay in germination was brought about in Malabar, Mysore and Vazhukka by the various doses of gamma rays and

EMS. The delay in germination was found to be dose dependent. A progressive reduction in germination percentage was produced with increase in dose level in both the mutagenic treatments. EMS induced more drastic reduction in germination compared to gamma rays. The survival percentage differed significantly depending on the mutagens, their doses and the varieties concerned.

Regarding growth rate, in all the three varieties characters like plant height, leaf number and leaf area produced shifts in both negative and positive directions but negative shift alone was noticed in the case of tiller number per plant.

Chlorophyll deficient plants like striata, albina, and variegata were produced consequent to mutagenic treatments. Narrow leaves with deep serrations were noticed in gamma irradiated population. EMS treatments induced morphological variants such as seedlings with split lamina and stem dichotomy.

Mitotic study of root tips was carried out for analysing the frequency of dividing cells and also the cytological aberrations created if any following mutagenic treatments. Root tips of control, 10 kR and 20 kR exposures were chosen for cytological study. A general reduction in the frequency of mitotic cell division was noticed consequent to radiation treatments in all the three varieties.