INDUCTION OF GENETIC VARIABILITY IN KACHOLAM (*Kaempferia galanga* L.)

By

KANAKAMANY M. T.

THESIS

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DEPARTMENT OF PLANT BREEDING AND GENETICS
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DECLARATION

I hereby declare that this thesis entitled "**INDUCTION OF GENETIC VARIABILITY IN KACHOLAM (*Kaempferia galanga* L.)**" is a bonafide record of research work done by me during the course of research and that this 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.

KANAKAMANY M.T.

DR. K.M.N. NAMBOODIRI
Professor and Head (Retd.)
Dept. of Plant Breeding & Genetics
College of Horticulture, KAU.

'Narayaneeyam'
Shornur, Palakkad

CERTIFICATE

Certified that this thesis entitled "**INDUCTION OF GENETIC VARIABILITY IN KACHOLAM (*Kaempferia galanga* L.)**" is a record of research work done independently by Ms. **KANAKAMANY M.T.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Place : Vellanikkara
Date : 15-11-97

DR. K.M.N. NAMBOODIRI
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. **KANAKAMANY M.T.** a candidate for the Degree of **Doctor of Philosophy in Agriculture,** agree that this thesis entitled "**INDUCTION OF GENETIC VARIABILITY IN KACHOLAM (*Kaempferia galanga* L.)**" may be submitted by Ms. **KANAKAMANY M.T.** in partial fulfilment of the requirement for the Degree.

DR. K.M.N. NAMBOODIRI
(Chairman, Advisory Committee)
Professor and Head (Retd.)
Department of Plant Breeding and Genetics
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur

DR. ACHAMMA OOMMEN
(Member, Advisory Committee)
Associate Professor
Dept. of Plant Breeding & Genetics
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur

DR. K. PUSHKARAN
(Member, Advisory Committee)
Associate Professor & Head
Dept. of Plant Breeding & Genetics
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur

DR. V.K.G. UNNITHAN
(Member, Advisory Committee)
Associate Professor
Department of Agrl. Statistics
College of Horticulture
KAU, Vellanikkara

DR. N.K. VIJAYAKUMAR
(Member, Advisory Committee)
Associate Professor
College of Forestry
Kerala Agrl. University
Vellanikkara, Thrissur

6/2/98
(P.M. Salimath)
EXTERNAL EXAMINER

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INTRODUCTION

The Indian System of Medicine (ISM) is predominantly a plant based materia medica, making use of our native plants. It caters to almost the entire rural population of our country. The dependence on herbal medicines is growing even in urban communities. People in western countries are also showing increased interest and preference for organic drugs. This has necessitated the cultivation of important medicinal plants on a commercial scale and development of new varieties suited for this purpose.

Kacholam, *Kaempferia galanga* L. belonging to the family Zingiberaceae is a highly valued aromatic herb distributed throughout the plains of India. It finds its main use as a flavouring agent for rice. Rhizomes and leaves are employed as perfumes in cosmetics. The rhizome is used as a stimulant, expectorant, carminative and diuretic. The oil finds extensive use in flavouring confectionary, pharmaceuticals and many other allied industries. The commercial cultivation of this crop is gaining importance owing to its varied uses and the ease with which it can be grown in the tropics.

In spite of the medicinal importance of kacholam, not much work has been done in improving the genetic potential of this crop. Systematic breeding programmes have not been undertaken and no promising varieties have so far been reported. Improvement of this crop by conventional breeding methods is not possible for want of seed production. Seedlessness also contributes to the limited variability available within the crop. The multiplication of this crop is done entirely through vegetative means and so the variation has remained more or less fixed. Genetic improvement has always been difficult due to absence of sexual reproduction to generate genetic recombinants. Induced mutagenesis is the only way to generate large genetic variability in such crop plants.

The history of mutation is as old as the science of modern genetics. Plant mutation research has not only produced valuable results in terms of varieties, it also has stimulated progress in related sciences, such as plant genetics and plant physiology. Spontaneous mutations have played a considerable role in the improvement of vegetatively propagated crops. The greater constancy of these plants preserved through clonal propagation permits the detection of even slight phenotypical changes. Mutation is the only means to produce genetic variability in sterile crops. The main advantage of mutation induction in vegetatively propagated crops is the ability to change

one or a few characters of an otherwise outstanding cultivar, without altering the remaining part of genotype. The high degrees of heterozygosity and polyploidy in vegetatively propagated crops which are serious handicaps in conventional breeding are advantageous in mutation breeding, as large variations can often be observed in the irradiated plants. Ionising and non-ionising radiations and certain chemicals play a significant role for permitting favourable permanent changes thereby increasing scope for selection.

In the light of the above, the present study was proposed with the following objectives in view.

1. To identify the optimum dose of physical and chemical mutagens for the induction of variability in kacholam

2. To induce variability by physical mutagens like gamma rays and chemical mutagens like EMS

3. To isolate the desirable mutants from the population

4. Large scale propagation of selected mutants by *in vitro* techniques

5. Pollination studies to explore the possibility of seed set in kacholam.

Review of Literature

REVIEW OF LITERATURE

Kacholam, *Kaempferia galanga* L. is a monocotyledonous plant, belonging to the family Zingiberaceae of the order Zingiberales. It comes under the series Epigynae (Bentham and Hooker, 1884). Schumann (1904) divided the family into three tribes viz., Globeae, Hedychiae and Zingibereae. The genus *Kaempferia* comes under the tribe Hedychiae. There are four subgenera under the genus *Kaempferia* namely, *Sincorus, Protanthium, Monolophus* and *Stachyanthesis* (Hooker, 1892). The subgenera *Sincorus* includes 11 species other than *Kaempferia galanga*. The systematic position of *Kaempferia galanga* is as follows:

Systematic position

Class	:	Monocotyledons
Series	:	Epigynae
Order	:	Zingiberales
Family	:	Zingiberaceae
Sub family	:	Zingiberoideae
Tribe	:	Hedychiae
Genus	:	*Kaempferia*
Subgenus	:	*Sincorus*
Species	:	*galanga*

Kaempferia is believed to have originated in Southeast Asia, probably in Burma. The genus *Kaempferia* is widely distributed in the tropics and subtropics of Asia and Africa. There are 55 species in the genus *Kaempferia,* but only 10 are known in India of which *Kaempferia galanga* and *Kaempferia rotunda* are the economically important ones.

Kirthikar and Basu (1935) described the morphology of *Kaempferia galanga* L. as follows. The plant is a stemless herb with tuberous aromatic root stock which posses fleshy cylindric nonaromatic root fibres. Leaves are horizontally spreading and lying flat on the surface of the ground and having a length of 4.5 to 9.00 cm. They are deep green in colour, rotund, ovate, deltoid and acuminate. Leaf margins are neither thickened nor coloured. Inflorescence is a scape covered by a leathery sheath, directly arising from the rhizomes (Gamble, 1926). An inflorescence contains two to 13 buds of which the outer one opens first. Each flower is subtended by a bract and one or two bractioles. Flowers are bisexual, complete and zygomorphic. The floral parts are arranged in trimerous whorls. Perianth is in two whorls of calyx and corolla. Outer one is connate at the base and free at the apex. The inner whorl is tubular at the base forming a long tube and free at the apex. Of the inner whorl the posterior lobe is absent.

The outer whorl of stamens is represented by two staminodes which are situated at the base of the tubular perianth. The posterior stamen of inner whorl is the only fertile stamen. The other two stamens are united and form a large bilobed showy labellum, which is the most conspicuous part of the flower. The fertile stamen is bilobed and the connective forms a hood above the stamen. The anther lobes form a groove through which the style passes so that the stigma comes very close to the anthers.

Gynoecium consists of tricarpellary, syncarpous, inferior ovary with ovules in axile placentation. Style is long, about four centimetre in length and ends in spiny stigma.

In *Kaempferia galanga* flowering occurs only once in a year. Flowering starts in June and ends in September with peak during July-August months. The crop is harvested six months after planting. The tuberous rhizomes possess a camphoraceous odour with bitter aromatic taste. Steam distillation of rhizome will give 2.40 to 3.88 per cent (on dry weight) of volatile oil. Propagation of the crop is by the division of rhizomes.

In *Kaempferia,* genetic improvement is difficult due to absence of sexual reproduction to generate genetic recombination. Seedlessness also contributes to the

limited variability available within the crop. Mutation breeding under these circumstances seemed to offer unique opportunity for inducing desired genetic alterations in the outstanding cultivars.

2.1 Induced mutagenesis

Mutations are the basis of plant evolution and they help us for better understanding of plant growth and development. They provide us with the raw materials for the genetic improvement (Broertjes and Harten, 1978).

Mutation breeding makes use of the possibility of altering genes by exposing plant parts to mutagens. It is advantageous because the basic genotype of the variety is usually slightly altered, an improved character is added and the time required to breed the improved variety can be much shorter than when hybridisation is used to achieve the same results. A desired mutation can be recovered in a homozygous stage already in M_2 or M_3 generation as compared to the F_6 or F_7 generation in the case of hybridisation. Some workers have used mutagenic treatment in combination with hybridisation in order to increase the variability to widen the basis for selection (Krull and Frey, 1961). In sterile plants and in obligate apomicts mutations are the only source of variability (Nybom, 1961).

2.1.1 Mutagens

The breeder can choose between two groups of mutagenic agents, physical and chemical. Both kinds of mutagens are valuable. Among the physical mutagens, X-rays and gamma rays are extensively used by plant breeders. Among the chemical mutagens, ethyl methane sulphonate is generally the mutagen of choice.

2.1.1.1 Physical mutagens

Though a variety of ionising radiations are available only X-rays and gamma rays are generally used. The critical doses of gamma rays depended on the genotype of the irradiated plants. Favourable changes brought about by lower doses of gamma irradiation have been successfully exploited in vegetatively propagated crops (Desai and Abraham, 1974 and Raghava *et al.*, 1988).

In vegetatively propagated crops, the use of X-ray and gamma irradiation is the most practical method of inducing mutation in all the kinds of starting materials, such as whole plants, tubers, bulbs, rhizomes, cuttings and detached leaves. Low dosages of irradiation are preferred if the goal is to change only one gene in an otherwise undisturbed genetic background (Broertjes and Harten, 1978).

Mutations induced by gamma rays for creating desirable combination of traits have been reported in many ornamentals like carnation (D Amato *et al.*, 1964), crocus (Mitsukini and Arai, 1965), gladiolus (Buiatti and Tesi, 1968), *Polyanthes tuberosa* (Gupta *et al.*, 1974), chrysanthemums (Broertjes and Harten, 1978) and in plants like cassava (Vasudevan, *et al.*, 1967), potato (Upadhya and Purohit, 1973); turmeric (Reghupathy *et al.*, 1976), ginger (Raju *et al.*, 1980; Giridharan, 1984 and Jayachandran and Mohanakumaran, 1992), sweet potato (Kukimura and Kouyama 1982) and coleus (Vasudevan and Jos, 1988 and 1989). Generally gamma rays in lower dosage causes stimulatory effects (Gupta *et al.*, 1982) and higher dosage induces mutagenic changes (Ono, 1971).

The main bottlenecks in irradiation of vegetatively propagated plants, where vegetative parts have to be irradiated, are chimera formation and diplontic selection, both being complications caused by the multicellular nature of bud apex. The result is a relatively low mutation frequency and limited mutation spectrum, while selection procedures cannot be applied before the stable periclinal chimera stage has been reached. These difficulties can be largely restricted or avoided by the use of *in vivo* or *in vitro* adventitious bud techniques, by which large number of solid, non chimeral mutants can be produced if detached leaves or explants respectively, are irradiated before

regeneration of the adventitious shoots (Broertjes and Harten, 1978). Plant breeders generally prefer ionizing radiation because it is easily applicable, clean with good penetration and reproducibility and high mutation frequency (Broertjes and Harten, 1978).

After irradiation of plant or plant parts, a range of effects can be observed depending on the character and stage of material as well as on the radiation treatment given. Only a few plants reveal mutation effects, because the naturally occurring material when subjected to artificial changes responds very little on account of its well established stabilisation in constitution, in the course of natural evolution. Mutation effects may differ even within the same plant. The main effects of mutagens include physiological changes (primary injury), point mutations or gene mutations and chromosomal aberrations (Gaul, 1970). Primary injury is restricted to M_1 generation whereas the latter two are transferred to the succeeding generations. In chromosomal mutation all the plant features are affected, while in other types of mutations only few or single characters are affected.

Plant injury or lethality account for physiological damage and they can be chromosomal or extra chromosomal in origin. Mutagenic treatments with low physiological effects and strong genetic effects are desirable (Gaul, 1970). The

plant injury may vary depending on the genotype, type of mutagen, doses employed and various other modifying factors (Sparrow, 1961 and Gaul, 1970).

Sparrow (1961) and Evans (1962) have reported that cytological changes are also met with as a result of mutagenic treatments. The type of induced chromosomal mutations, their mitotic and meiotic behaviour and genetic consequences have been reported by Sparrow (1961). According to Gaul (1970) the mutagen induced sterility may be caused by chromosome mutation, factor mutation, cytoplasmic mutation and physiological effects, but chromosome mutations are the major origin.

Most of the radiations induced sterility in M_1 and further generations is haplontic (Muntzing, 1930) and EMS induced sterility appears to be diplontic in nature (Sato and Gaul, 1967).

2.1.1.2 Chemical mutagen

Chemical mutagens have a higher efficiency and output of mutations if the duration of treatment and the concentrations are well adjusted. In the case of vegetatively propagated plants, the main advantage is that once a good genotype is obtained it can be propagated and made use of directly (Broertjes and Harten, 1978).

The induction of genetic changes by means of mutagens has been well recognised for the past three decades. Schiemann tried the induction of mutations by means of chemical treatment as early as in 1912. Induction of mutation by means of chemical mutagens was demonstrated in England with mustard gas (Auerbach and Robson, 1947) and in Germany with urethreans (Oehlkers, 1943). Since then a number of chemicals possessing mutagenic properties have been identified and their effects studied.

Among the various chemical mutagens known, the alkylating agents have been found to be the most efficient in inducing mutations in a wide range of organisms (Auerbach, 1961). Within this group, ethyl methane sulphonate appears to be more efficient in producing mutations in several organisms including higher plants (Swaminathan, *et al.*, 1962). The mutagenic efficiency of ethyl methane sulphonate was first demonstrated by Heslot *et al.* (1959) in barley. Dryagina and Limberger (1974) claimed that chemical mutagens had a higher efficiency, if the duration of the treatment and the concentrations are well adjusted.

Swaminathan (1965) observed that alkylating agents were more efficient than radiations for inducing point mutations, but less efficient for inducing chromosomal aberrations. Ethyl methane sulphonate has been

successfully used in vegetatively propagated crops such as chrysanthemum (Bowen, 1965), rose (Kaicker and Swarup, 1972), mint (Kaul and Kak, 1973) and apple (Broertjes and Harten, 1978). Several research workers like Gustafsson (1963), Yamaguchi and Miah (1964), Konzak (1966), Sato and Gaul (1967), Siddiq (1967) and Soriano (1968) studied the effectiveness and efficiency of chemical mutagens in various plant species. The relatively low toxic and high genetic effects of ethyl methane sulphonate (Gaul, 1961) and its high mutagenic effectiveness as well as efficiency in higher plants (Konzak *et al.* 1961) led to its enhanced practical application. The effect of alkylating agents and their mechanism of action in biological test systems have been reviewed by Loveless (1966) and Lawley (1973).

2.1.2 Mutation frequencies

Information about frequencies of spontaneous and induced mutations in vegetatively propagated crops is rather scarce. Mutations are said to occur at random, many suggest differences in mutability between different loci and regions of chromosomes. For potato, Heiken (1958) has summarized mutation frequencies as, an average of one in 10^{-6} or 10^{-7} plants showing a spontaneous mutation for the leaf character and yellow margin. According to many studies polyploids exhibit lower mutation frequencies than diploids. In practice it appears that in polyploids mutagenic treatments lead to gross chromosomal damage with

a dominant expression for certain traits (Broertjes, 1976). Most authors express mutation frequencies for vegetatively propagated plants as the percentage of plants showing one or more mutations for number of visible characters.

The effect of mutagen treatment on different traits under study in some important vegetatively propagated crops is reviewed here.

2.1.2.1 Sprouting

Generally a delay in sprouting and reduction in germination percentage are noticed consequent to irradiation at higher doses as reported by Sparrow and Christenson (1950) in potato tubers, Vijayalakshmi and Rao (1960) and Jalaja (1971) in sugarcane, Vasudevan *et al.*,(1967) and Thamburaj *et al.*, (1985) in cassava, Vasudevan *et al.*, (1967) in colocasia, Gupta and Shukla (1971) in rose, Natrajan (1975) in turmeric and Gupta *et al.* (1982) in costus. Abraham (1970) reported in cassava that maximum sprouting of buds from irradiated stem cuttings was obtained at doses of less than 1.5 kR where as no sprouts were produced at all, at doses of five kR and more. The percentage of sprouting decreased as the dose of gamma rays increased in tube rose. At 0.5 kR the sprouting percentage was 96 which reduced to 72 at 2.5 kR (Sambandamurthi, 1983).

Gamma irradiation studies in *Kaempferia galanga* by Viswanathan *et al.* (1992) produced stimulatory effect on germination.

Summa Bai and Nayar (1992) observed in sweet potato that the gamma ray induced population took more number of days for sprout initiation and completion of sprouting. Giridharan and Balakrishnan (1992) noticed decreased sprouting percentage at increased dosages of gamma rays in ginger.

The sprouting of suckers of mentha was affected adversely when treated with EMS (0.01%) (Kaul and Kak, 1973). Sambandamurthi (1983) observed a trend of reduction in the percentage of sprouting as the doses of ethyl methane sulphonate (EMS) increased. In tapioca, the percentage of sprouting decreased with increase in the doses of EMS (Thamburaj *et al.*, 1985).

2.1.2.2 Survival

Vasudevan *et al.* (1968) irradiated colocasia with gamma rays and found that many plants germinated normally but failed to survive. Gupta *et al.* (1974) found that tube rose above two kR gives no survival of plants. In gladiolus, post germination lethality occurred and 50 per cent survival of plants was obtained at 4.7 kR gamma rays (Abraham and Desai, 1976).

Reduction in survival resulted on gamma ray treatment at higher doses of three kR in *Costus specious* (Gupta *et al.*, 1982) and tapioca (Thamburaj *et al.*, 1985). Survival decreased with increase in the concentration of EMS in tuberose (Sambandamurthi, 1983).

2.1.2.3 Flowering

The number of days for flowering was very much influenced by mutagenic treatments. Many workers demonstrated the effect of gamma rays in modifying the flowering behaviour of rhizomatous and allied crops such as canna (Nakornthap, 1965) iris (Broertjes, 1968), dahlia (Singh, 1970 and Das *et al.*, 1975), tube rose (Gupta *et al.*, 1974 and Sambandamurthi, 1983) and gladiolus (Misra, 1976 and Raghava *et al.*, 1988).

In the case of sugarcane, induction of nonflowering characters has been reported by Walker and Sisodia (1969) and Rao (1974). Volkov and Danko (1972) reported that in potato tuber treatment with physical and chemical mutagens led to increase in fertility. The flowering time in kalanchoe was very much influenced by mutation (Broertjes and Harten, 1978). Rao (1974) obtained sugarcane clones with little or no flowering when the cuttings were treated with three and five kR gamma rays followed by three cycles of propagation and selection for absence of flowering. Jagathesan (1977) could produce flowerless mutants in sugarcane.

In sweet potato flowering mutants were produced by Kukimura and Kouyama (1982). Flowering behaviour of ginger could not be altered by the levels of gamma irradiation (Giridharan and Balakrishnan, 1992).

2.1.2.4 Yield and quality attributes

In vegetatively propagated crops large number of useful types with high yield were obtained as a result of mutation.

In cassava (*Manihot esculenta*) Vasudevan *et al.* (1967) could observe mutants with high starch content and with decreased HCN content, which would enhance the industrial value of cassava. Abraham (1970) and Nayar (1975) obtained mutants for high yield in cassava. Moh (1976) obtained somatic mutants in cassava with high tuber yield.

Miu (1973) obtained cold tolerant sweet potato types when exposed to gamma rays. Kukimura and Takemata (1975) reported that mutants with increased as well as reduced sugar contents were obtained after treatments of shoots, dormant root tubers and seeds of sweet potato with Co^{60} gamma rays. The higher exposure of gamma rays was effective in increasing the tuber yield and tuber number in sweet potato (Suma Bai and Nayar, 1995).

In costus diosgenin content increased as a result of two kR gamma ray treatment whereas it decreased at 30 kR (Gupta *et al.*, 1982).

Reghupathy *et al.* (1976) obtained mutants of curcuma lines resistant to scales (*Aspidiotus hartii*) through gamma irradiation. Stimulatory and mutagenic effects of ionising radiations can be exploited commercially in turmeric (Shah *et al.*, 1982). Rangaswamy (1986) obtained mutants in *Curcuma longa* by X-irradiation, with orange yellow rhizomes with a high curing percentage and curcumin content.

Dormant single budded suckers of *Mentha arvensis* var. *Piperascens* were subjected to various X and gamma rays treatment, and mutants with improved oil constituents obtained (Kak and Kaul, 1979). Pavlovic *et al.* (1983) observed a positive correlation between irradiation dose and essential oil content in *Mentha piperata*. Nair (1969) isolated mutants with high essential oil content in lemongrass by gamma ray treatment.

Lata and Gupta (1971) and Irulappan (1979) were able to isolate some mutant clones of roses with qualitative and quantitative changes in their essential oils.

In ginger Giridharan (1984) found that the quality in terms of spice oil and oleoresin content was not altered by irradiation with gamma rays.

In banana significant variations in the quality of fruit were noticed with increase in dose of gamma rays in MV_2 and MV_3 generations (Radha Devi and Nayar, 1996).

2.1.2.5 Duration

Different crops behave differently to physical and chemical mutagens. The mutagenic treatments caused considerable effect in reducing the crop duration which was directly proportional to an increase in the doses/ concentrations of the mutagens (Moh, 1976). He could produce early maturing mutants in cassava due to mutagenic treatments with gamma rays and EMS

In sugarcane several early flowering mutants with higher cane girth, weight and sucrose content were obtained by Luo (1979). Vasudevan and Jos (1989) could produce photo insensitive coleus by gamma ray irradiation. Two early mutants which took 100 days after planting for harvesting, as against 150-160 days in normal plants were identified by them.

2.1.2.6 Morphological variants

A number of useful morphological variants have been reported in vegetatively propagated crops in general and ornamentals in particular.

The mutagenic treatment of *Saccharum* at the Sugarcane Breeding Institute, Coimbatore, resulted in a number of foliar abnormalities (Prasad, 1959). Vijayalakshmi and Rao (1960) irradiated species of *Saccharum* and hybrids and obtained several morphological mutants. Hrishi *et al.* (1968) found that the treatment of buds and growing meristems of sugarcane with different chemical mutagens produced morphological mutants. Mutants for glabrous leaf sheath in pubescent varieties of sugarcane Co-527 and Co-419 were observed by Jagadesan and Sreenivasan (1970). Escober and Lopez (1970) irradiated sugarcane seed pieces with gamma rays and produced abnormalities of the growing point, malformation of the leaves, as well as stunting and reduction in the size of the stalk. Jagadesan (1977) produced a number of variants in sugarcane like dwarfs, those that are flowerless, and those with glabrous leaves, increased girth rate and yield.

In potato, the gamma irradiation of tubers resulted in different aberrant types like light green types with several kinds of leaf deformity (Heiken, 1961). Kishore *et al.* (1963) and Jauhar (1969) also studied the effect of gamma irradiation in potato tubers and observed morphological differences in foliage shape, size and vigour of plants as well as tuber production. Roer (1967) induced somatic mutations in potato clones through gamma irradiation and observed some colour mutations. In potato

a few mutants of practical importance such as the yellow stained tuber mutants from an outstanding red skinned seedling (Broertjes and Harten, 1978) and tuber mutants in commercial varieties Kufri Sindhuri and Kufri Red (Jauhar and Swaminathan, 1967) are well known. Davies (1973) studied the effect of acute gamma irradiation on growth and yield of potato and found that the effects of radiation were considerably greater when experienced early in the life cycle of the crop.

Masima and Sato (1959) observed variations in leaves, stems and tubers in the X_1 generation of the X-ray irradiated young shoots of sweet potato. Hernandez *et al.* (1964) observed somatic mutations such as changes in skin colour and flesh colour of roots, due to irradiation of gamma rays in sweet potato. Soriano (1972) could irradiate buds of sweet potato with gamma rays (0.8-32.0 kR) and obtained leaf mutants with ovate, cordate and serrate types. Suma Bai (1989) found that in sweet potato the vine length and branch number per vine varied in treated population. The tuber number and weight of tubers per vine were found to be significantly increased by gamma ray exposure at two kR.

In cassava, Vasudevan *et al.* (1967) obtained viable morphological mutants by treating the stem cuttings with gamma rays at a dose range of four kR and seven kR, Moh (1976) obtained somatic mutants in cassava with four kR gamma rays.

Irradiation experiments had been conducted in roses by number of workers and mutations mostly for flower colour were produced in a number of varieties (Gupta, 1966). Gupta (1966) and Gupta and Shukla (1970) noted that, radiation treatments not only affected the flower shape and size in rose, but significant changes were produced in essential oil content also. Kaicker and Swarup (1972) observed the occurrence of deformed shoots with puckered, thickened and chlorophyll mosaic leaves and leaves with forked and united leaflets in five kR gamma rays irradiated Christian Dior Rose. Several new cultivars were raised by gamma irradiation of buds or scions of roses.

Desai (1973) exposed the cuttings of chrysanthemums to both acute and chronic gamma irradiation and observed acute exposure to be more effective than chronic ones. Gupta *et al.* (1974) induced and multiplied large number of flower colour mutants in chrysanthemums.

Raju *et al.* (1980) observed formation of weak and elongated underground rhizomes in ginger on treatment with 20 kR gamma rays. In turmeric and mango ginger, the same treatment showed almost normal growth, but the leaves showed abnormalities.

A stable mutant of gladiolus with shell pink floret was isolated from a wild rose in the one kR treatment and released as a variety under the name Shobha (Raghava *et al.*, 1988).

Rhizomes of young plants of canna irradiated with gamma rays at 1.0, 1.5 and 2.7 kR resulted in stunted plants with variegated leaves (Nakornthap, 1965). In Dahlia a large number of mutations for flower colour and form have been reported (Broertjes and Ballego, 1967). Broertjes and Harten (1978) induced 250 mutants in two cultivars of kalanchoe. Gupta *et al.* (1982) reported that in cactus the number of branches per plant increased at 1.5 kR but decreased at three kR.

In banana growth reduction and drastic leaf aberrations were observed when treated with gamma rays (Velez and Maldonado, 1972). Sharma and Mukherjee (1973) produced mutants in grape variegata by gamma irradiation of a Pusa seedless cutting. Donini (1976) presented several cherry scion mutants by the induction of X-rays, Ü-rays and thermal neutrons.

2.1.2.7 Chlorophyll deficient mutants

Chlorophyll mutants are most convenient for evaluating the genetic effects of mutagens in plants (Mitra and Bhowmik, 1997).

Buiatti *et al.* (1965) observed chlorophyll deficient sectors, as a result of gamma irradiation of the rooted cuttings of carnation. In tapioca Vasudevan *et al.* (1967) and in *Colocasia* Vasudevan *et al.* (1968) produced chlorophyll deficient plant as a result of gamma ray irradiation.

In other vegetatively propagated crops namely, canna (Nakornthap, 1965), colocasia (Vasudevan *et al.*, 1968), mentha (Ono, 1971) and tube rose (Gupta *et al.*, 1974, Konzak, 1966 and Sambandamurthi, 1983) leaf variations due to gamma ray irradiation have been reported. Giridharan and Balakrishnan (1992) reported appearance of yellow stripes as a result of gamma irradiation in the ginger cv. Rio-de-janeiro and Maran. In colocasia Vasudevan *et al.* (1987) observed chlorinas and other leaf abnormalities.

2.1.3 Heritability and genetic advance

Heritability in the broad sense refers to the relative proportion of genotypic variance to phenotypic variance. Coefficient of variation (CV) is used to compare the relative variables, when different metric traits are measured in different units. Dividing the standard deviation of the trait by mean renders the coefficient of variation independent of unit of measurement.

Lush (1937) and Johnson *et al.* (1955) developed accurate procedures for the calculation of genetic advance (GA) under specified intensities of selection which in metric traits largely depends on the heritability, phenotypic variability of the trait under selection and the selection differential expressed as phenotypic standard deviation.

Reddy (1980) estimated heritability in broad sense and GA in respect of cane yield, number, millable canes, single cane weight, length of millable cane and juice quality in ten varieties of sugarcane. He found the number of millable canes recorded maximum heritability and GA indicating that this character is less vulnerable to environmental influence and could be relied upon as one of the important criterion for selection.

Variability, broad sense heritability and genetic advance were estimated in a mutagen induced variable population by Khairwal *et al.* (1985) and reported that genotypic coefficient of variation was high for yield of cane per clone followed by tillers per clone and millable canes per clone.

2.1.4 Correlation studies

Correlation studies to determine the inter-relationship among various traits, are useful in making selection. Information on the association of plant characters with yield and also on the intercorrelations are available in crops like ginger and turmeric.

Nybe (1978) reported that in ginger length of leaf blade, length of petiole, leaf area index and number, length and girth of primary and secondary fingers were positively correlated with yield. According to Mohanty and

Sharma (1979) rhizome yield was positively and significantly correlated with number of stems, leaves, secondary rhizome fingers, tertiary rhizome fingers, total rhizome fingers, plant height, leaf breadth, girth of secondary rhizome fingers, number and weight of adventitious roots.

Nambiar (1979) estimated the inter correlation among the morphological characters and yield in turmeric and the results showed that number of tillers, plant height and number of fingers had high significant positive correlation with the yield. Number of fingers per plant, number of tillers per plant, height, rhizome length and dry matter percentage contributed four per cent towards yield of turmeric rhizome. Mukhopadhyay and Roy (1986) observed a high correlation between plant height and yield per plant at both the phenotypic and genotypic levels.

2.1.5 Path coefficient analysis

Path coefficient analysis is a standardized partial regression coefficient and as such measures the direct influence of one variable upon another and permits the separation of correlation coefficients into components of direct and indirect effects (Dewey and Lu, 1959).

In ginger path coefficient analysis (Ratnambal, 1979) revealed that the phenotypic correlation between yield of

rhizome and height of pseudostem was quite high and so also the direct effect of height towards the correlation. The direct effect of number of leaves on yield was found to be low. Eventhough length of leaf had a negative direct effect, it was compensated by a high positive correlation between plant height and final yield.

Path coefficient analysis in turmeric (Nambiar, 1979) indicated that plant height (of pseudostem) was a single important morphological character for which selection for yield could be made. The height of the plant and length of secondary fingers were the major contributors towards rhizome yield. Direct effects of number of leaves per tiller and girth of mother rhizome were positive whereas number of nodes per primary finger and petiole length had high negative direct effect on rhizome yield (Geetha, 1985). Mukhopadhyay and Roy (1986) recommended tillers per clump, leaves per shoot and plant height as selection criteria for improving yield.

Material and Methods

MATERIALS AND METHODS

The investigations reported herein on the "Induction of genetic variability in kacholam, *Kaempferia galanga* L." were undertaken in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period 1992-1995. Field experiments were conducted in the fields of Department of Plant Breeding and Genetics (22.5 m above MSL) and Agricultural Research Station, Mannuthy (1.5 m above MSL) situated between 10° 32' N latitude and 76° 10' E longitude.

3.1 Materials

Rhizomes of kacholam, *Kaempferia galanga* L. cv. Vellanikkara local, available in the AICRP on Medicinal and Aromatic plants of the College of Horticulture, Vellanikkara were made use of in the study.

Gamma irradiation was done in the Co^{60} gamma chamber available at the Radiotracer Laboratory attached to the College of Horticulture, Vellanikkara. The chemical mutagen used was ethyl methane sulphonate (EMS) obtained from Sisco Research Laboratories, Bombay. The details of the source, dose rate/half life and mode of action of mutagens are given in Table 1.

Table 1 Source, dose rate and mode of action of mutagens

Sl. No.	Mutagens	Source	Dose rate/ half life	Mode of action
1	Gamma rays	Radio Tracer Laboratory, College of Horticulture, Vellanikkara	5000 rads/minute	Ionization
2	Ethyl Methane Sulphonate $CH_3SO_2\ OC_2\ H_5$	Sisco Research Laboratory, Bombay	30 hrs.	Alkylation

3.2 Methods

Healthy and viable rhizomes of kacholam were cut into small bits of uniform size with two or three viable buds. They were subjected to different mutagenic treatments. Detailed studies on qualitative and quantitative aspects were made in MV_1, MV_2 and MV_3 generations.

3.2.1 Sensitivity studies

3.2.1.1 Gamma irradiation

In order to find out the optimum dose of gamma irradiation 13 samples of rhizomes were selected from the variety Vellanikkara local. From each sample 32 rhizome bits were irradiated at 13 different doses of gamma rays viz; 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 150, 200 and 300 Gy. The irradiated rhizome bits along with control

were planted immediately in zinc trays filled with sand. Germination counts were taken daily for 45 days from the date of sowing and percentage of germination worked out. These values of germination were then statistically analysed after angular transformation to find out the significance of difference between the treatments. From the results obtained the LD_{50} (the dose which gave 50 per cent and above mortality) was found out by employing the method of probit analysis. Based on the result thus obtained eight doses of gamma rays at regular intervals with LD_{50} as the highest dose were fixed as 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 Gy for further field trials.

3.2.1.2 EMS treatment

In order to find out the optimum duration of presoaking of rhizomes, concentration of chemical and duration of treatment, a preliminary laboratory test was conducted as detailed below:

Three durations of presoaking viz., one hour, two hours and three hours were tried with five concentrations of chemical viz., 0.5 per cent, 1.0 per cent, 1.5 per cent, 2.0 per cent and 2.5 per cent at three durations of treatment viz., six hours, eight hours and ten hours. Altogether there were 45 treatment combinations as listed below.

1 1 hour presoaking with 0.5 per cent EMS and 6 hours of treatment

2 1 hour presoaking with 0.5 per cent EMS and 8 hours of treatment

3 1 hour presoaking with 0.5 per cent EMS and 10 hours of treatment

4 1 hour presoaking with 1.0 per cent EMS and 6 hours of treatment

5 1 hour presoaking with 1.0 per cent EMS and 8 hours of treatment

6 1 hour presoaking with 1.0 per cent EMS and 10 hours of treatment

7 1 hour presoaking with 1.5 per cent EMS and 6 hours of treatment

8 1 hour presoaking with 1.5 per cent EMS and 8 hours of treatment

9 1 hour presoaking with 1.5 per cent EMS and 10 hours of treatment

10 1 hour presoaking with 2.0 per cent EMS and 6 hours of treatment

11 1 hour presoaking with 2.0 per cent EMS and 8 hours of treatment

12 1 hour presoaking with 2.0 per cent EMS and 10 hours of treatment

13 1 hour presoaking with 2.5 per cent EMS and 6 hours of treatment

14 1 hour presoaking with 2.5 per cent EMS and 8 hours of treatment

15 1 hour presoaking with 2.5 per cent EMS and 10 hours of treatment

16 2 hours presoaking with 0.5 per cent EMS and 6 hours of treatment

17 2 hours presoaking with 0.5 per cent EMS and 8 hours of treatment

18 2 hours presoaking with 0.5 per cent EMS and 10 hours of treatment

19 2 hours presoaking with 1.0 per cent EMS and 6 hours of treatment

20 2 hours presoaking with 1.0 per cent EMS and 8 hours of treatment

21 2 hours presoaking with 1.0 per cent EMS and 10 hours of treatment

22 2 hours presoaking with 1.5 per cent EMS and 6 hours of treatment

23 2 hours presoaking with 1.5 per cent EMS and 8 hours of treatment

24 2 hours presoaking with 1.5 per cent EMS and 10 hours of treatment

25 2 hours presoaking with 2.0 per cent EMS and 6 hours of treatment

26 2 hours presoaking with 2.0 per cent EMS and 8 hours of treatment

27 2 hours presoaking with 2.0 per cent EMS and 10 hours of treatment

28 2 hours presoaking with 2.5 per cent EMS and 6 hours of treatment

29 2 hours presoaking with 2.5 per cent EMS and 8 hours of treatment

30 2 hours presoaking with 2.5 per cent EMS and 10 hours of treatment

31 3 hours presoaking with 0.5 per cent EMS and 6 hours of treatment

32 3 hours presoaking with 0.5 per cent EMS and 8 hours of treatment

33 3 hours presoaking with 0.5 per cent EMS and 10 hours of treatment

34 3 hours presoaking with 1.0 per cent EMS and 6 hours of treatment

35 3 hours presoaking with 1.0 per cent EMS and 8 hours of treatment

36 3 hours presoaking with 1.0 per cent EMS and 10 hours of treatment

37 3 hours presoaking with 1.5 per cent EMS and 6 hours of treatment

38 3 hours presoaking with 1.5 per cent EMS and 8 hours of treatment

39 3 hours presoaking with 1.5 per cent EMS and 10 hours of treatment

40 3 hours presoaking with 2.0 per cent EMS and 6 hours of treatment

41 3 hours presoaking with 2.0 per cent EMS and 8 hours of treatment

42 3 hours presoaking with 2.0 per cent EMS and 10 hours of treatment

43 3 hours presoaking with 2.5 per cent EMS and 6 hours of treatment

44 3 hours presoaking with 2.5 per cent EMS and 8 hours of treatment

45 3 hours presoaking with 2.5 per cent EMS and 10 hours of treatment

Sixteen uniform sized rhizomes were treated as per the schedule given above with two replications. After the treatment, the rhizomes were washed thoroughly with water for an hour to remove traces of the chemical from the rhizomes. Rhizomes were then kept in zinc trays filled with sand for testing their germinability at room temperature. Germination counts were taken daily for 45 days and the percentage of germination worked out. These values of germination were then transformed into angular sines and statistically analysed to find out the significance of the difference between the treatments. From the results thus obtained, the LD_{50} was found out by employing the method of probit analysis. Based on the result thus obtained six concentrations of ethyl methane sulphonate (EMS) at regular intervals with LD_{50} as the

highest dose were arrived at as 0.25 per cent, 0.50 per cent, 0.75 per cent, 1.00 per cent, 1.25 per cent and 1.50 per cent for further studies at the field level.

3.2.2 Field studies

3.2.2.1 Effect of mutagens on MV_1 generation

Two field experiments were laid out one with gamma irradiated rhizomes and another with EMS treated rhizomes, using the variety Vellanikkara local.

Rhizomes were exposed to eight doses of gamma rays viz., 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 Gy along with control in a randomised block design replicated thrice. Seventy five bits of rhizomes were used for each treatment. The rhizomes were sown on the same day of treatment in raised beds at a spacing of 15 cm x 15 cm.

The solution of EMS was prepared with double distilled water without buffer. Six different concentrations of the solution viz., 0.25 per cent, 0.50 per cent, 0.75 per cent, 1.00 per cent, 1.25 per cent and 1.50 per cent along with a control (water treatment) constituted seven treatments. Seven samples of rhizomes with 48 bits in each were presoaked for one hour and treated with the chemical for a duration of six hours with three replications in a randomised block design. After six hours, the rhizomes were thoroughly washed in running water for one hour to

remove the traces of chemical from the rhizomes. The rhizomes were sown on the same day of treatment in raised beds of 15 cm x 15 cm spacing.

Both gamma irradiated and EMS treated experiments were laid out in the field attached to the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara. The soil was lateritic loam and the cultural, manurial and plant protection measures were done as per the Package of Practices Recommendations (1993) of the Kerala Agricultural University. Irrigation was provided uniformly as and when required.

The following observations were recorded in the MV_1 generation.

3.2.2.1.1 Germination

3.2.2.1.1a Days taken to start sprouting

Number of days taken for sprouting was calculated from the date of sowing to the date of emergence of sprouts above the ground level.

3.2.2.1.1b Days taken to complete sprouting

Number of days taken to complete sprouting was calculated from the date of sowing to the day after which no further sprouting was observed. The time between the first sprouting and the completion of sprouting in one treatment is taken as the duration of sprouting.

3.2.2.1.1c Germination percentage

Germination counts were taken daily from the date of sowing to the day after which no further sprouting was observed and total germination percentage was estimated from these values.

3.2.2.1.2 Lethality

3.2.2.1.2a Lethality on 90th day of sowing

Lethality of the plants was determined on the 90th day of sowing the rhizomes. The plants surviving in the field on that particular day were counted and the percentage value computed from the total germination percentage for each treatment.

3.2.2.1.2b Lethality on 135th day of sowing

The number of plants surviving in each treatment on 135th day of sowing was counted and percentage lethality computed relating this value with that of the total gemination per cent for each treatment.

3.2.2.1.2c Lethality at harvest

The number of plants surviving in each treatment at the time of harvest was counted. The percentage lethality at harvest was computed relating this value to that of the total germination percentage for each treatment.

3.2.2.1.3 Plant spread (cm)

Spread of the plant was measured on the 90th day of sowing of rhizomes, when the plants have fully established, and on 135th day of sowing when the plants were in their maximum vegetative phase.

3.2.2.1.4 Number of tillers per plant

Number of sprouts produced per plant was studied, 180 days after planting. The sprouts produced per rhizome were counted separately and the average number of sprouts produced per plant per treatment was determined.

3.2.2.1.5 Number of leaves per plant

The number of leaves produced per plant was counted at an interval of 45 days from the 45th day after sowing till senescence of leaves.

3.2.2.1.6 Length of leaf

The length of leaf was measured from the base to the tip of leaf on 135th day after sowing when the plants attained their maximum vegetative phase. Ten plants were randomly selected per treatment per replication and the third leaf from the centre was used for the observation, and the mean value was expressed in centimetre.

3.2.2.1.7 Breadth of leaf

The plants tagged for taking length of leaf were also used for this observation. The breadth of leaf was measured from the widest part of the leaf on 135th day after sowing. The mean of ten leaves was expressed in centimetre.

3.2.2.1.8 Number of rhizomes per plant

Number of rhizomes from each plant was recorded separately immediately after harvest.

3.2.2.1.9 Fresh weight of rhizomes

Total weight of rhizomes from individual plants from all the treatments were recorded immediately after the harvest.

3.2.2.1.10 Days to harvest

Number of days taken from sowing to maturity of rhizomes was calculated separately for all plants. The senescence (drying) of leaves was taken as the criterion for harvesting the plants.

3.2.2.1.11 Yield of dry rhizome

The fresh rhizomes were cut into small pieces and oven dried. The weight of rhizomes after drying was recorded and the percentage recovery of dry rhizomes was calculated.

3.2.2.1.12 Essential oil content of rhizomes

The essential oil content was estimated separately for each treatment. The oleoresin was extracted from dried, powdered rhizomes following the procedure given by Panicker *et al.* (1926).

The rhizomes were chopped, dried, powdered and 100 g powder was added to 200 ml hexane in a distillation flask and heated for one hour at 60-70°C in water bath. This was filtered and washed with hot hexane three or four times. The solvent was evaporated until constant oil weight was obtained.

3.2.2.2 Effect of mutagens on MV_2 generation

After the post harvest observations, the rhizomes were stored in shade under the soil for five months and then were planted in the field to study the MV_2 generation. The experiment was laid out in the fields of Agricultural Research Station, Mannuthy.

Rhizomes of individual plants from the MV_1 were grown in progeny rows. The identity of rhizomes was maintained by planting sib rhizomes in separate rows. A total of 7508 rhizomes were sown in MV_2 and all the observations as in MV_1 generation were recorded.

The plants were examined at frequent intervals to assess the chlorophyll deficient mutants and to isolate morphological variants. Screening and selection of plants were done in MV_2 generation.

3.2.2.3 Frequency distribution of variants in MV_2 generation

The treated population in MV_2 generation was screened for deviant phenotypes from normal for plant type, leaf, and rhizome characters. Based on the expression of the different quantitative characters in the MV_2 generation, they were classified into three groups for each character as given below:

Sl. No.	Quantitative character	Criteria	Classification
1	Plant spread (135th DAP)	Below 20cm	Low
		20-25 cm	Normal
		Above 25 cm	High
2	Number of leaves per plant (135th DAP)	Below 10	Shy
		10-15	Normal
		Above 15	Profuse
3	Leaf length	Below 12 cm	Short
		12-16 cm	Normal
		Above 16 cm	Long
4	Leaf width	Below 8 cm	Narrow
		8 - 11 cm	Normal
		Above 11 cm	Broad

5	Days to harvest	Less than 200 days	Short duration
		200-225 days	Normal
		More than 225 days	Long duration
6	Number of rhizomes per plant	Below 10	Shy
		10 - 15	Normal
		Above 15	Profuse
7	Fresh weight of rhizomes per plant	Below 40 g	Low
		40-60 g	Normal
		Above 60 g	High

The frequency of each group per treatment was calculated in percentage.

3.2.2.4 Effect of mutagens on MV_3 generation

Based on the variability expressed in MV_2 population for single character or combination of two or more characters simultaneously, 40 plants were selected from gamma ray and EMS treated MV_2 population, as deviants from control. The plants were harvested when fully matured. The rhizomes of the selected plants were dried in shade, and were carried forward to raise MV_3 generation in progeny rows. The planting, manuring and irrigations were done as described earlier. The following observations were taken.

1) Plant spread
2) Number of tillers per plant
3) Number of leaves per plant
4) Length of leaf
5) Breadth of leaf
6) Number of rhizomes per plant
7) Fresh weight of rhizomes
8) Days to harvest

The percentage inheritance of the characters of the selected plants of MV_2 was studied after growing them in progeny rows in MV_3.

3.2.3 Micropropagation

Viable and superior mutants isolated from MV_3 generation were subjected to *in vitro* propagation technique following the method suggested by Vincent *et al.* (1992) for their large scale multiplication.

Fresh rhizomes collected from the field are washed thoroughly in tap water, and cut into small pieces with single bud. Scale leaves and skin are removed and surface sterilised first with 50 per cent alcohol for two minutes and then with 0.1 per cent mercuric chloride solution for five minutes in laminar flow. It is washed repeatedly for five to six times in distilled water, dried and inoculated to half MS medium containing eight ppm boric acid and three per cent sucrose under light at 28°C.

3.2.4 Causes for failure of seed set

3.2.4.1 Pollination studies

To explore the possibility of widening the gene pool and to find out the causes of failure of seed set in kacholam different systems of pollination as shown below were tried and the seed set in each was assessed.

3.2.4.1.1 Artificial self pollination

By using pollen from same flower.

3.2.4.1.2 Artificial sibbing

By using pollen from separate flower of the same variety.

3.2.4.1.3 Artificial cross pollination

By using pollen from different cultivar.

3.2.4.1.4 Bud pollination

Pollination was done the previous day of flower opening.

3.2.4.1.5 Mentor pollination

By using a mixture of pollen grains, ie. from irradiated and normal plants.

3.2.4.1.6 Chemically aided pollination

Stigmatic surfaces were sprayed with eight per cent sucrose and boric acid and pollination was carried out by using pollen from separate plants

3.2.4.1.7 Removal of stigma and artificial pollination

The stigma was removed using a scalpel and pollen grains were applied on the cut surface of the style at the time of stigma receptivity.

Twenty flowers were randomly selected used for each pollination technique. Percentage of seed set was recorded during subsequent days of pollination.

3.2.4.2 Reproductive biology

In order to find out the causes for failure of seed set the reproductive biology of the crop was examined closely and the following observations were taken.

3.2.4.2.1 Days taken to first flower opening

Number of days taken to start flowering was recorded by counting the days taken from sowing to the first flower opening in a plant.

3.2.4.2.2 Time of anthesis

Flower opening was closely observed at five minutes interval from 12 midnight to 6.00 AM in five plants in each replication in every treatment.

3.2.4.2.3 Pollen fertility (Percentage)

Pollen grains collected on the day of anther dehiscence were stained with one per cent acetocarmine on slides and the fertility percentage was estimated as the percentage of stained pollen grains over the total number of pollen grains (Shivanna and Johri, 1985). About 250 pollen grains were studied from each treatment per replication.

3.2.4.2.4 Pollen size (μ m)

The diameter of pollen grains (μ m) was measured from 40 pollen grains per treatment per replication and the average value was worked out for each treatment.

3.2.4.2.5 Style length

Length of style was measured after anthesis of plants as mm and compared with the respective control.

3.2.4.2.6 Pollen viability (Percentage)

Pollen grains from all the treatments were collected and kept for germination in the following media, in an incubator at 25°C.

Media

1. Distilled water
2. 8% sucrose + 60 ppm boric acid
3. 15% sucrose + 60 ppm boric acid
4. 30% sucrose + 60 ppm boric acid
5. 8% sucrose + 60 ppm boric acid + 1% gelatin
6. 15% sucrose + 60 ppm boric acid + 1% gelatin
7. 30% sucrose + 60 ppm boric acid + 1% gelatin

The germinated grains were counted from five different fields per treatment per replication and the percentage of germination was calculated as:

$$\text{Percentage of germination} = \frac{\text{No. of pollen grains germinated}}{\text{Total number of pollen grain}} \times 100$$

3.2.5 Statistical analysis

The LD_{50} value was calculated by probit analysis method (Wardlaw, 1985). Analysis of variance was worked out for all the characters for each treatment studied in MV_1 and MV_2 generations as detailed by Panse and Sukhatme (1967). In the case of germination, lethality, pollen fertility and viability the percentage values were transformed into angular sines and subjected to statistical analysis. Variations observed for various traits in the

different treatments were expressed as coefficient of variation by the method followed by Snedcor and Cochran (1975). The correlation between MV_1 parent performance and MV_2 mean progeny performance was studied. The heritability and genetic advance expressed as percentage of mean was studied as suggested by Lush (1937). Simple correlation between yield and yield components and interrelationship of characters were studied as per the methods outlined by Panse and Sukhatme (1967). Path analysis was done for selected characters in selected treatments, where significant deviation from the control in association of characters was observed (Dewey and Lu, 1959).

RESULTS

Rhizomes of kacholam (*Kaempferia galanga* L.) were treated with gamma rays and EMS in different concentrations. The variability induced by the mutagens was evaluated for three generations. The results of the studies are presented below:

4.I Sensitivity studies

The efficiency of the mutagens was studied on the basis of the germination of rhizomes under laboratory conditions.

4.I.1 Gamma rays

Different doses of gamma rays were tried under laboratory conditions on germination of rhizomes and the results are presented in Table 2.

From the Table it was found that the germination percentages of rhizomes varied from zero to 87.5. Maximum germination was recorded by the control (87.5%). As the dose increased, the germination percentage decreased and none of the rhizomes germinated above 50 Gy gamma rays. On probit analysis of the data on germination of rhizomes, the LD_{50} was calculated to be 20 Gy. Therefore, eight doses of

Table 2 Effect of gamma rays on germination of rhizomes (laboratory conditions)

Treatments		Germination percentage	Percentage variation over control
Gamma irradiation (Gy)			
	Control	87.5 (69.30)	0
	5	78.1 (62.10)	10.74
	10	75.0 (60.00)	14.29
	15	62.5 (52.24)	28.60
	20	37.5 (37.76)	57.14
	30	37.5 (37.76)	57.14
	40	25.0 (30.0)	71.43
	50	25.0 (30.0)	71.43
	75	0	-
	100	0	-
	150	0	-
	200	0	-
	300	0	-

LD_{50} 20.0 Gy

Numbers in parenthesis denote transformed values

the mutagen at regular intervals with LD_{50} as the highest dose viz., 20.0, 17.5, 15.0, 12.5, 10.0, 7.5, 5.0 and 2.5 Gy were tried in the field trials.

4.I.2 EMS

The data on the effect of presoaking of rhizomes, concentrations of EMS and duration of treatment on germination of rhizomes obtained in the preliminary laboratory test are presented in Table 3.

The results presented in the above Table indicated that the three durations of presoaking viz., one hour, two hours and three hours tried in the present investigation did not differ significantly in the germination of rhizomes. Similar result was obtained in the case of three durations of chemical treatment viz., six hours, eight hours and ten hours. However the concentrations of the chemical tried viz., 0.5, 1.0, 1.5, 2.0 and 2.5 per cent produced significant difference on germination of rhizomes.

From the results, it was found that an increase in the concentration of chemical from 0.5 per cent brings a corresponding reduction in germination. The germination percentages at 0.5 per cent and one per cent EMS were 83.4 and 79.2 respectively, while the corresponding values for 1.5 per cent, 2.0 per cent and 2.5 per cent were extremely less. So for all the field trials, the rhizomes presoaked

Table 3 Effect of presoaking of rhizomes, concentration of EMS and duration of treatment on germination of rhizomes (Preliminary laboratory test)

Duration of presoaking (hours)	Treatments		Germination (%)
	Concentration of chemical (%)	Duration (hours)	
1	0.5% EMS	6	84.4 (66.74)
1	0.5% EMS	8	81.3 (64.38)
1	0.5% EMS	10	84.4 (66.74)
1	1.0% EMS	6	78.1 (62.10)
1	1.0% EMS	8	78.1 (62.10)
1	1.0% EMS	10	81.3 (64.38)
1	1.5% EMS	6	68.8 (56.04)
1	1.5% EMS	8	71.9 (57.99)
1	1.5% EMS	10	71.9 (57.99)
1	2.0% EMS	6	37.5 (37.76)
1	2.0% EMS	8	37.5 (37.76)
1	2.0% EMS	10	40.6 (39.58)
1	2.5% EMS	6	25.0 (30.00)
1	2.5% EMS	8	28.2 (32.08)
1	2.5% EMS	10	25.0 (30.00)
2	0.5% EMS	6	84.4 (66.74)
2	0.5% EMS	8	81.3 (64.38)
2	0.5% EMS	10	84.4 (66.74)
2	1.0% EMS	6	78.1 (62.10)
2	1.0% EMS	8	78.1 (62.10)
2	1.0% EMS	10	81.3 (64.38)
2	1.5% EMS	6	68.8 (56.04)
2	1.5% EMS	8	71.9 (57.99)
2	1.5% EMS	10	71.9 (57.99)
2	2.0% EMS	6	37.5 (37.76)
2	2.0% EMS	8	37.5 (37.76)
2	2.0% EMS	10	40.6 (39.58)

Contd....

Table 3 contd....

2	2.5% EMS	6	25.0 (30.00)
2	2.5% EMS	8	28.2 (32.08)
2	2.5% EMS	10	25.0 (30.00)
3	0.5% EMS	6	84.4 (66.74)
3	0.5% EMS	8	81.3 (64.38)
3	0.5% EMS	10	84.4 (66.74)
3	1.0% EMS	6	78.1 (62.10)
3	1.0% EMS	8	78.1 (62.10)
3	1.0% EMS	10	81.3 (64.38)
3	1.5% EMS	6	68.8 (56.04)
3	1.5% EMS	8	71.9 (57.99)
3	1.5% EMS	10	71.9 (57.99)
3	2.0% EMS	6	37.5 (37.76)
3	2.0% EMS	8	37.5 (37.76)
3	2.0% EMS	10	40.6 (39.58)
3	2.5% EMS	6	25.0 (30.00)
3	2.5% EMS	8	28.2 (32.08)
3	2.5% EMS	10	25.0 (30.00)

Numbers in parenthesis denote transformed values

for one hour and treated with the chemical for six hours were used since there was no significant difference among different durations of presoaking and chemical treatment. The concentrations of chemicals tried in the preliminary laboratory studies viz., 0.5, 1.0, 1.5, 2.0 and 2.5 per cent differed significantly on the effect of germination. On probit analysis of the data on germination the LD_{50} was calculated to be 1.5 per cent. Six concentrations of the chemical EMS at regular intervals with LD_{50} as the highest dose viz., 1.50, 1.25, 1.00, 0.75, 0.50 and 0.25 per cent were tried in the field trials.

4.2 Field studies

The mutagen treated population were compared with the respective control for each character and the results are presented below.

4.2.1 Effect of mutagens on MV_1 generation

The effect of different doses/concentrations of gamma rays and EMS on various growth and yield parameters in MV_1 generation is presented below.

4.2.1.1 Germination

The effect of different doses/concentrations of mutagens on germination of rhizomes under field conditions is furnished in Table 4.

Table 4 Effect of mutagens on the germination of rhizomes in MV_1 generation (Field conditions)

Treatments	Germination (%)	Percentage variation over control	No. of days to start sprouting (mean period in days)	Percentage varation over control	Duration (Mean days)	Percentage variation over control
Gamma irradiation (Gy)						
Control	89.3 (70.91)	0	29.3	0	12.0	0
2.5	82.7 (65.42)	-7.39	31.3	6.82	10.0	-16.67
5.0	81.3 (64.38)	-8.96	32.3	10.23	10.0	-16.67
7.5	81.3 (64.38)	-8.96	34.7	18.21	8.30	-30.83
10.0	80.0 (63.40)	-10.41	35.7	21.62	8.00	-33.33
12.5	78.7 (62.50)	-11.87	36.7	25.03	7.00	-41.67
15.0	78.7 (62.50)	-11.87	37.0	26.15	7.00	-41.67
17.5	76.0 (60.0)	-14.89	38.7	31.85	6.00	-50.00
20.0	73.3 (58.89)	-17.92	38.7	31.85	6.00	-50.00
CD (0.05)	(2.651)		0.858	-	(0.333)	-
EMS(%) Control	92.0 (73.57)	0	30.3	0	10.00	0
0.25	88.0 (69.73)	-4.35	31.7	4.6	8.00	-20.00
0.50	85.7 (67.78)	-6.85	31.7	4.6	8.00	-20.00
0.75	81.3 (64.38)	-11.63	34.3	13.2	7.30	-27.00
1.00	79.0 (62.73)	-14.13	34.7	14.5	6.70	-33.00
1.25	69.0 (56.17)	-25.0	37.3	23.1	5.30	-47.00
1.50	67.0 (54.94)	-27.17	37.7	24.2	5.00	-50.00
CD (0.05)	(1.041)		1.453	-	(0.593)	-

Numbers in parenthesis denote transformed values

4.2.1.1a Germination percentage

The comparison of the effects of different doses/concentrations of gamma rays and EMS has indicated that both the mutagens have almost the same effect in reducing the percentage of germination on increasing doses/concentrations. The percentage variation over control gave negative values in all the treatments. The mean values ranged from 73.3 (20.0 Gy) to 89.3 (control) in the case of gamma irradiation and 67.0 (1.5%) to 92.0 per cent (control) in the case of EMS treated population. The sprouting percentage significantly decreased with increasing doses/concentrations of mutagens in all the treatments.

4.2.1.1b Days taken to start sprouting

The number of days taken to start sprouting in gamma rays and EMS treated population is depicted in Table 4. Statistical analysis of the data showed significant variation among the treatments. Delayed sprouting was accounted especially at higher doses.

Germination started earlier in the control whereas it was significantly delayed in the treated rhizomes. Progressive delay in germination was noticed as the level of dosage increased. The mean values ranged from 29.3 (control) to 38.7 (17.5 and 20.0 Gy) in gamma ray exposed and 30.3 (control) to 37.7 (1.5%) in EMS treated population.

4.2.1.1c Duration of germination

The duration of germination (interval between first and last germination within a treatment) was found to be reduced with an increase in dose/concentration of mutagen (Table 4). Statistical analysis of the data showed significant variation among the gamma ray exposed as well as EMS treated plants. The percentage variation gave negative values in both the cases and the mean values ranged from six (17.5 and 20.0 Gy gamma rays) to 12 days (control) in gamma rays and five (1.5% EMS) to ten days (control) in EMS.

4.2.1.2 Lethality

The effect of gamma rays and EMS on lethality at 90 days after sowing, 135 days after sowing and at harvest is presented in Table 5.

Statistical analysis of the data showed significant variation in lethality in different doses/concentrations of mutagens. In the case of gamma rays the mean values at 90 days after sowing ranged from 14.7 per cent in control to 28.0 per cent in 20.0 Gy. In EMS treated plants the mean values ranged from 8.3 per cent in control to 35.4 per cent in 1.5 per cent concentration. The percentage of lethality increased with increase in doses/concentrations of mutagens.

Table 5 Effect of mutagens on the lethality (percentage) at different stages of growth in MV_1 generation

Treatments	Lethality (percentage)					
	90 DAS	Percentage variation over control	135 DAS	Percentage variation over control	At harvest	Percentage variation over control
Gamma irradiation (Gy)						
Control	14.7 (25.54)	0	14.7 (22.54)	0	14.7 (22.54)	0
2.5	20.0 (26.57)	36.1	20.0 (26.57)	36.1	20.0 (26.57)	36.1
5.0	21.3 (27.49)	44.9	21.3 (27.49)	44.9	21.3 (27.49)	44.9
7.5	21.3 (27.49)	44.9	21.3 (27.49)	44.9	21.3 (27.49)	44.9
10.0	21.3 (27.49)	44.9	21.3 (27.49)	44.9	21.3 (27.49)	44.9
12.5	24.0 (29.33)	63.3	24.0 (29.33)	63.3	24.0 (29.33)	63.3
15.0	25.3 (30.20)	72.1	26.7 (31.11)	81.6	26.7 (31.11)	81.6
17.5	25.3 (30.20)	72.1	28.0 (31.95)	90.5	28.0 (31.95)	90.5
20.0	28.0 (31.95)	90.5	30.7 (33.65)	108.8	30.7 (33.65)	108.8
CD (0.05)	(2.287)	-	(2.354)	-	(2.354)	-
EMS % Control	8.3 (16.74)	0	12.5 (20.70)	0	12.5 (20.70)	0
0.25	16.6 (24.04)	100.0	18.7 (25.62)	49.6	18.7 (25.62)	49.6
0.50	16.7 (24.12)	101.2	16.7 (23.34)	33.6	16.7 (23.34)	33.6
0.75	20.8 (27.13)	150.6	25.1 (30.07)	100.8	25.1 (30.07)	100.8
1.00	20.8 (27.13)	150.6	25.0 (30.0)	100.8	25.0 (30.0)	100.8
1.25	33.3 (35.24)	301.2	35.4 (36.51)	183.2	45.8 (42.59)	266.4
1.50	35.4 (36.51)	326.5	37.5 (37.76)	200.0	47.9 (43.80)	283.2
CD (0.05)	(6.610)	-	(6.366)	-	(5.760)	-

Numbers in parenthesis denote transformed values

In the case of gamma rays the mean values of lethality were same at 135 days after sowing and at harvest. The values ranged from 14.7 per cent in control to 30.7 per cent in 20.0 Gy. In EMS treated population, the mean values at 135 days after sowing ranged from 12.5 per cent in control to 37.5 per cent in 1.5 per cent concentration. At harvest the range was 12.5 per cent and 47.9 per cent.

4.2.1.3 Plant spread

The effect of gamma rays and EMS on plant spread recorded on 90 and 135th day after sowing is presented in Table 6.

Significant difference in plant spread was observed due to treatments. At 90 days after sowing maximum plant spread was noticeable at 7.5 Gy gamma rays (21.6 cm) which was followed by 5.0 and 2.5 Gy gamma rays with mean plant spread of 20.8 and 20.7 cm respectively. Control plants recorded 19.0 cm spreading (Plate 1). At 135 days after sowing the maximum plant spread of 25.7 cm was recorded at 7.5 Gy gamma rays (Plate 2), closely followed by treatments with 2.5 and 5.0 Gy gamma rays with 25.4 and 25.1 cm spreading respectively.

In the case of EMS treated population, the maximum plant spread (17.2 cm) was noticed at 0.75 per cent EMS at 90 days after sowing which was significantly superior to

Table 6 Effect of mutagens on plant spread and number of tillers in MV_1 generation

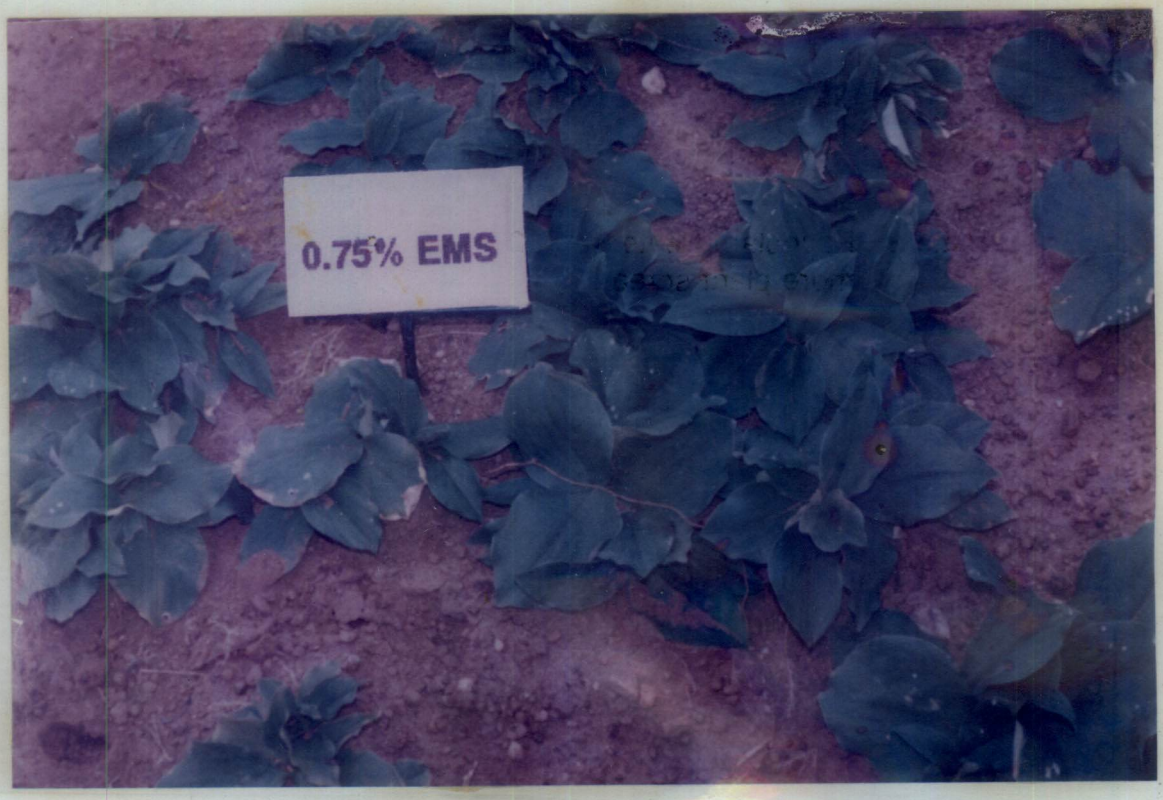
Treatments	Plant spread (cm)						No. of tillers		
	90 DAS			135 DAS					
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Gamma irradiation(Gy)									
Control	13.0-26.5	19.0	8.82	17.4-30.0	24.2	8.46	3-7	3.4	9.4
2.5	13.2-29.0	20.7	13.14	19.2-31.4	25.4	12.0	2-9	3.7	14.7
5.0	10.0-29.4	20.8	27.04	16.2-29.8	25.1	15.35	3-9	3.8	11.4
7.5	12.1-27.5	21.6	14.06	17.5-29.2	25.7	13.51	3-9	4.0	13.0
10.0	9.0-28.5	19.7	23.21	16.5-31.0	24.3	12.76	2-7	3.0	12.2
12.5	10.2-27.5	17.7	26.96	20.0-29.0	23.9	18.75	2-6	3.3	15.9
15.0	9.5-26.5	16.4	28.07	14.0-26.5	22.9	20.26	2-6	3.5	20.0
17.5	7.0-22.5	14.9	27.45	15.0-29.0	22.6	19.28	2-4	3.0	12.2
20.0	5.9-21.0	13.0	32.47	14.0-24.2	18.7	18.90	2-6	2.8	16.7
CD (0.05)		(1.860)			(2.808)			(0.470)	
EMS (%)									
Control	11.0-21.0	15.3	8.77	18.2-29.5	23.0	8.98	3-5	3.1	8.1
0.25	12.0-23.4	16.4	20.09	17.0-30.0	23.3	16.04	3-9	3.6	8.6
0.50	11.2-22.5	16.6	14.44	16.2-27.5	24.4	18.97	3-7	3.9	21.0
0.75	12.0-22.0	17.2	17.80	18.0-26.0	24.9	13.28	3-7	3.4	14.4
1.00	10.0-22.5	14.2	16.16	15.5-30.0	20.4	16.15	3-5	3.0	19.8
1.25	10.0-19.7	12.9	19.43	14.0-23.0	18.4	14.08	2-7	2.8	12.5
1.50	10.0-18.6	12.4	15.91	14.0-23.5	18.4	14.52	2-7	2.8	13.3
CD (0.05)		(1.150)			(1.610)			(0.363)	

Plate 1 Kacholam-control plants


Control

Plate 2 Kacholam treated with 7.5 Gy gamma rays having more plant spread

Plate 3 Kacholam treated with 0.75 per cent EMS having more plant spread


0.75% EMS

the control. This was followed by 0.5 per cent EMS with 16.6 cm spreading and 0.25 per cent EMS with 16.4 cm spreading which was on par with 0.75 per cent EMS. The lowest plant spread of 12.4 cm was noticed at the highest concentration of 1.5 per cent EMS and was on par with 1.25 per cent EMS with 12.9 cm spreading. At 135 days after sowing also the maximum spread of 24.9 cm was noticed at 0.75 per cent EMS (Plate 3), followed by and on par with 0.5 and 0.25 per cent EMS with 24.4 cm and 23.3 cm spreading respectively. The lowest plant spread of 18.4 was recorded by 1.25 and 1.5 per cent EMS.

The coefficient of variation (CV) was higher for all doses/concentrations of mutagens. The CV for plant spread recorded at 90 days after sowing ranged from 8.82 (control) to 32.47 (20.0 Gy) for gamma rays and 8.77 (control) to 20.09 (0.25%) for EMS. When the plant spread was recorded at 135 days after sowing the CV ranged from 8.46 (control) to 20.26 (15.0 Gy) and 8.98 (control) to 18.97 (0.5%) for gamma rays and EMS respectively.

4.2.1.4 Number of tillers per plant

The effect of gamma rays and EMS on number of tillers per plant is presented in Table 6.

Kacholam showed an increase in number of tillers per plant with increase in dosage of gamma rays upto 7.5 Gy (Table 6) and there on decreased with further increase in

dosage. However a reduction in number of tillers was observed from 10.0 Gy onwards on 180 days after sowing. The maximum number of tillers was produced at 7.5 Gy gamma irradiation (4.0) (Plate 4) followed by and on par with 5.0 and 2.5 Gy gamma rays with 3.8 and 3.7 tillers per plant respectively.

In the case of EMS treated population the highest value of 3.9 tillers was recorded at 0.5 per cent (Plate 5), followed by and on par with 0.25 per cent with mean value 3.6. The lowest value of 2.8 was recorded by 1.25 and 1.5 per cent EMS.

The coefficient of variation ranged from 11.4 at 5.0 Gy to 20.0 per cent at 15.0 Gy gamma rays, while a CV of 9.4 per cent was observed in the control. In the case of EMS treated population, the CV ranged from 8.1 (control) to 21.0 per cent (0.5% EMS).

4.2.1.5 Number of leaves per plant

The influence of gamma rays and EMS on number of leaves per plant is presented in Table 7.

At lower doses of gamma rays (2.5 Gy to 10.0 Gy) leaf production increased over control and at higher doses it decreased. A gradual reduction in mean number of leaves was observed from 12.5 Gy gamma rays in the three stages of

Plate 4 Kacholam treated with 7.5 Gy gamma rays having more tillers

Plate 5 Kacholam treated with 0.5 per cent EMS having more tillers


7.5 Gy


0.5% EMS

Table 7 Effect of mutagens on number of leaves in MV_1 generation

Treatments	Number of leaves											
	45 DAS			90 DAS			135 DAS			180 DAS		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Gamma irradiation(Gy)												
Control	4-6	5.2	11.67	6-14	13.6	6.75	8-17	14.0	9.01	10-19	15.0	7.70
2.5	4-7	5.5	16.09	7-20	14.2	17.67	11-20	14.5	10.66	11-24	15.5	14.88
5.0	4-7	5.7	18.65	8-19	14.4	25.90	9-19	15.2	9.99	9-20	16.1	15.86
7.5	4-7	5.9	16.92	6-19	14.8	24.78	6-19	16.7	14.50	12-21	17.2	18.76
10.0	3-6	5.6	18.33	7-19	14.4	19.08	9-19	14.6	15.48	8-19	14.6	18.66
12.5	3-6	4.0	18.25	6-17	12.0	21.14	7-17	12.7	25.50	8-18	13.0	17.09
15.0	3-7	3.6	24.43	7-17	8.6	28.21	8-17	12.3	27.19	8-21	14.5	21.79
17.5	3-7	3.5	14.29	5-13	6.9	12.33	5-17	10.4	18.89	8-20	11.9	23.66
20.0	2-4	3.1	19.97	3-9	4.9	20.34	7-13	8.0	18.67	7-16	9.9	21.34
CD (0.05)		(0.533)			(1.912)			(1.911)			(2.288)	
EMS (%) Control	4-6	4.1	6.13	10-18	13.4	8.53	11-18	14.5	6.10	11-19	15.0	7.30
0.25	4-6	4.3	10.90	10-19	13.8	17.38	11-19	15.4	10.23	14-20	16.2	11.32
0.50	3-5	4.9	19.05	11-17	14.4	24.00	12-19	16.7	19.57	12-19	16.3	15.90
0.75	4-6	5.3	13.90	10-17	14.8	14.00	13-19	17.1	8.51	14-19	17.9	9.00
1.00	3-5	3.7	5.14	6-16	9.6	12.79	9-16	12.3	14.51	12-17	14.0	15.21
1.25	3-5	3.4	13.15	5-12	7.3	17.81	7-12	8.7	12.36	9-12	10.7	10.50
1.50	3-5	3.2	12.65	4-9	5.0	23.23	7-11	8.3	12.81	9-12	10.5	9.12
CD (0.05)		(0.215)			(1.811)			(0.836)			(1.179)	

measurement viz., 45, 90 and 135 days after sowing. At 45th day after sowing the number of leaves varied from 3.1 (20.0 Gy) to 5.9 (7.5 Gy). At 90 days after sowing maximum number of leaves (14.8) was recorded at 7.5 Gy gamma rays and the minimum (4.9) by the highest dose 20.0 Gy. Same was the trend at 135 and 180 days after sowing with maximum number of leaves recorded by the 7.5 Gy dose and the minimum by 20.0 Gy.

The statistical analysis of the data on EMS treated population showed significant variation among treatments. At 45th day after sowing the number of leaves varied between 3.2 (1.5%) and 5.3 (0.75%). Same trend was seen with respect to the number of leaves produced on 90, 135 and 180 days after sowing. Treatments with 0.75 per cent EMS gave 14.8 leaves per plant (Plate 6) and treatments with 1.5 per cent EMS gave only five leaves per plant at 90 days after sowing. At 135 and 180 days after sowing the highest values of 17.1 and 17.9 were recorded by 0.75 per cent EMS and is significantly superior to the rest of treatments. The lowest values of 8.3 and 10.5 leaves per plant were recorded at 1.5 per cent EMS.

The mutagen treated population had higher CV than the control. The CV of number of leaves at 45 days after sowing ranged from 14.29 (17.5 Gy) to 24.43 (15.0 Gy) for gamma rays and 5.14 (1.0%) to 19.05 (0.5%) for EMS. The

Plate 6 Kacholam treated with 0.75 per cent EMS having more leaves


0.75% EMS

control recorded 11.67% CV in gamma rays and 6.13 in EMS at 45 days after sowing. At 90 days after sowing the maximum CV (28.21%) was recorded by 15.0 Gy gamma rays and 0.5 per cent EMS (24.0%). At the maximum vegetative phase of the crop at 135 days after sowing the highest percentage of CV (27.19) was recorded by 15.0 Gy gamma rays and 0.5 per cent EMS (19.57%). The CV ranged between 7.7 per cent (control) to 23.66 per cent (17.5 Gy) and 7.3 (control) and 15.90 per cent when treated with 0.5 per cent EMS at 180 days after sowing.

4.2.1.6 Leaf length

The effect of gamma rays and EMS on leaf length at the maximum vegetative phase of the crop is furnished in Table 8.

Statistical analysis of the data showed significant variation among treatments. Length of leaf in the case of gamma ray treated population ranged from 12.5 (20.0 Gy) to 14.9 cm (7.5 Gy and 2.5 Gy). This was followed by and on par with treatments of 5.0 Gy and 10.0 Gy gamma rays with leaf length of 14.8 and 14.7 cm respectively. The control plants recorded 13.8 cm length at the maximum vegetative phase. In the case of EMS treated population the leaf length ranged between 11.8 cm (1.5%) and 13.4 cm (0.75%).

Table 8 Effect of mutatagens on length and breadth of leaves in MV_1 generation

Treatments	Leaf length(cm)			Leaf breadth(cm)		
	Range	Mean	CV	Range	Mean	CV
Gamma irradiation (Gy)						
Control	11.0-16.2	13.8	9.61	9.0-12.9	10.8	12.43
2.5	11.1-17.8	14.9	13.51	8.8-12.3	10.8	13.07
5.0	11.7-18.1	14.8	13.62	8.2-12.7	11.1	14.60
7.5	11.3-17.5	14.9	11.70	7.0-12.6	11.2	18.33
10.0	12.0-17.6	14.7	13.59	8.0-12.5	10.8	15.77
12.5	10.5-16.0	13.5	12.40	7.4-12.5	10.4	13.68
15.0	10.0-18.5	13.5	19.22	8.5-11.6	9.9	18.36
17.5	10.0-16.2	12.9	14.41	7.4-12.7	9.4	15.46
20.0	10.4-14.7	12.5	12.69	6.0-10.8	6.2	13.80
CD (0.05)		(0.531)			(0.642)	
EMS (%)						
Control	11.0-16.7	12.9	9.2	8.2-10.9	9.5	9.1
0.25	10.5-14.7	12.9	10.2	8.3-12.2	9.7	11.9
0.50	11.0-14.9	13.0	14.9	7.5-12.6	10.8	13.5
0.75	10.0-14.8	13.4	9.5	8.3-12.0	10.9	13.5
1.00	11.0-15.1	13.3	9.5	8.1-9.9	9.1	6.6
1.25	10.1-15.6	12.1	12.6	7.0-11.0	8.9	11.1
1.50	10.9-13.0	11.8	5.7	6.0-9.5	6.2	9.5
CD (0.05)		(0.480)			(0.549)	

Gamma ray treated population had higher CV than control (Table 8). The CV of leaf length ranged from 9.61 (control) to 19.22 (15.0 Gy). In the case of EMS treated population, the CV for length of leaf ranged from 5.7 (1.5%) to 14.9 per cent (0.5%).

4.2.1.7 Leaf breadth

The effect of gamma rays and EMS on leaf breadth was also studied at the maximum vegetative phase of the crop (Table 8).

The breadth of leaves recorded on 135th day of planting showed significant variation among the treatments. The mean values ranged between 6.2 (20.0 Gy) and 11.2 cm (7.5 Gy gamma rays). At 135th day after planting the breadth of leaf was 6.2 cm when treated with 1.5 per cent EMS (Table 8). Plants treated with 0.75 per cent EMS recorded the highest breadth of 10.9 cm.

The gamma ray treated population had higher CV than the control (Table 8). The CV ranged between 12.43 (control) and 18.36 (15.0 Gy). In the case of EMS treated population, the CV for leaf breadth ranged from 6.6 (1.0% EMS) to 13.5 (0.5 and 0.75% EMS).

4.2.1.8 Number of rhizomes per plant

The influence of gamma rays and EMS on the number of rhizomes per plant is presented in Table 9.

Table 9 Effect of mutagens on number and fresh weight of rhyzomes in MV_1 generation

Treatments	Rhizome number			Fresh weight of rhizome(g plant^{-1})		
	Range	Mean	CV	Range	Mean	CV
Gamma irradiation(Gy)						
Control	9-21	14.2	10.57	20.0-88.6	47.9	18.39
2.5	7-25	15.2	12.48	13.4-91.4	56.1	29.74
5.0	9-27	15.7	26.65	25.6-90.0	58.5	31.73
7.5	10-28	16.7	24.06	25.0-84.1	58.6	31.27
10.0	8-29	16.4	30.96	18.0-111.1	58.0	34.47
12.5	8-27	15.4	30.42	16.8-109.3	53.3	35.88
15.0	4-28	14.5	43.03	12.6-81.2	47.2	49.25
17.5	4-17	10.6	37.70	16.6-78.1	41.6	23.42
20.0	4-16	8.3	30.22	16.2-79.5	40.5	32.33
CD (0.05)		(2.590)			(12.880)	
EMS (%)						
Control	7-16	13.9	8.42	43.4-77.2	45.8	5.81
0.25	8-22	15.4	22.33	20.0-63.7	57.0	30.89
0.50	8-20	15.8	26.69	36.5-73.5	57.7	37.33
0.75	8-21	16.1	25.73	38.2-78.1	59.8	19.86
1.00	9-27	16.0	24.75	40.1-78.3	59.6	20.32
1.25	10-18	12.0	19.95	16.5-67.9	44.2	25.11
1.50	4-13	8.9	18.51	28.5-64.3	40.5	22.98
CD (0.05)		(1.704)			(5.192)	

Positive shifts in the number of rhizomes per plant were observed due to gamma rays and EMS at lower doses. A gradual reduction in the number of rhizomes per plant was observed at higher doses/concentrations of mutagens. Maximum number of rhizomes (16.7) was reported by the rhizomes treated with 7.5 Gy gamma rays. The control gave 14.2 rhizomes per plant. Number of rhizomes show a gradual decline from 15.0 Gy onwards and reached a minimum of 8.3 at 20.0 Gy gamma rays. In the case of EMS treated population, maximum number of rhizomes (16.1) was recorded when treated with 0.75 per cent EMS and the minimum (8.9) when treated with 1.5 per cent EMS.

In gamma ray treated population, as the dose rate increased, the CV for number of rhizomes also increased (Table 9). The range was from 12.48 (2.5 Gy) to 43.03 (15.0 Gy). In the case of EMS, the treated population showed increase in CV than the control. The CV for number of rhizomes ranged between 18.51 (1.5%) and 26.69 (0.5% EMS).

4.2.1.9 Fresh weight of rhizomes

The influence of gamma rays and EMS on rhizome weight is depicted in Table 9.

The mean weight of rhizomes in gamma ray treated population ranged from 40.5 (20.0 Gy) to 58.6 (7.5 Gy) and

in EMS treated population from 40.5 (1.5%) to 59.8 (0.75%). As the dosage increased, the weight of rhizome also increased and reached the maximum at 7.5 Gy gamma rays. Thereafter it showed a decline and reached to 40.5 g at 20.0 Gy gamma rays. The same trend was followed in EMS treated population also.

The rhizome weight also showed increase in CV when treated with mutagens. In the case of gamma rays the maximum CV (49.25) was recorded at 15.0 Gy gamma rays. In the case of EMS treated population, the control plants recorded the lowest CV of 5.81 while plants treated with 0.5 per cent EMS recorded the highest CV of 37.33 per cent.

4.2.1.10 Crop duration

There were variations in the crop duration, due to mutagenic treatments (Table 10).

The mutagenic treatments caused considerable effect in reducing crop duration, which was directly proportional to an increase in the dose/concentration of mutagen. The maximum of 211 days were taken by the control plants, while they took only 180 days when exposed to 20.0 Gy gamma rays. The plants treated with 1.25, 1.5, and 1.0 per cent EMS took 199, 200 and 204 days respectively for harvest. The control took 216 days and was on par with 0.25 per cent EMS which took 214 days for harvest.

Table 10 Effect of mutagens on crop duration in MV_1 generation

Treatments	Crop duration (days)		
	Range	Mean	CV
Gamma irradiation (Gy)			
Control	205-220	211	2.12
2.5	190-220	206	4.19
5.0	190-210	201	3.85
7.5	180-210	191	3.75
10.0	180-210	196	4.54
12.5	180--210	190	3.89
15.0	180-200	191	3.87
17.5	170-195	182	2.95
20.0	170-195	180	2.87
CD (0.05)		(7.738)	
EMS (%)			
Control	200-228	216	0.84
0.25	205-225	214	1.33
0.50	200-220	208	2.17
0.75	200-215	207	1.45
1.00	200-210	204	1.85
1.25	190-215	199	2.31
1.50	190-210	200	1.36
CD (0.05)		(6.654)	

The CV for crop duration was more when treated with mutagens (Table 10). In the case of gamma rays, the range was between 2.12 (control) and 4.54 (10.0 Gy). In EMS treated plants, the values ranged between 0.84 (control) and 2.31 (1.25%).

4.2.1.11 Dry weight of rhizomes

Effect of mutagens on dry weight of rhizome is depicted in Table 11.

In the case of gamma rays there was no significant difference between treatments.

In the case of EMS treated plants, the maximum drying percentage (28.4) was recorded at one per cent EMS and minimum dry weight of 24.7 at 1.5 per cent EMS.

4.2.1.12 Oleoresin

The influence of gamma rays and EMS on oleoresin content of rhizomes is presented in Table 12.

The treatments did not differ significantly with respect to the oleoresin content in both gamma irradiated and EMS treated experiments.

Table 11 Effect of mutagens on dry weight of rhyzomes (percentage) in kacholam

Treatments	Dry weight (Percentage)	Percentage variation over control
Gama irradiation (Gy)		
Control	24.9	0
2.5	24.4	-2.00
5.0	25.5	2.41
7.5	26.3	5.62
10.0	26.9	8.03
12.5	26.3	5.62
15.0	26.5	6.43
17.5	26.3	5.62
20.0	24.9	0
CD (0.05)	NS	
EMS % Control	26.8	0
0.25	27.1	1.12
0.50	27.5	2.61
0.75	28.0	4.48
1.00	28.4	5.97
1.25	28.0	4.48
1.50	24.7	-7.84
CD (0.05)	0.838	

NS - Non-significant

Table 12 Effect of mutagens on oleoresin content of kacholam

Treatments	Oleoresin (%)	Percentage variation over control
Gama irradiation (Gy)		
Control	3.1	0
2.5	3.3	6.45
5.0	3.3	6.45
7.5	3.5	12.90
10.0	3.2	3.23
12.5	3.2	3.23
15.0	2.9	-6.45
17.5	3.0	-3.23
20.0	2.9	-6.45
	NS	
EMS % Control	3.1	0
0.25	3.3	6.45
0.50	3.0	-3.23
0.75	3.0	-3.23
1.00	2.7	-12.90
1.25	2.8	-9.68
1.50	2.9	-6.45
	NS	

4.2.2 Effect of mutagens on MV_2 generation

When the plants were advanced to MV_2 generation, the germination of rhizomes, lethality, the important yield attributes like plant spread, number of tillers, number of leaves, leaf length, leaf breadth, rhizome number, fresh weight of rhizomes and crop duration were studied. The mean of the characters along with range and coefficient of variation are presented in Tables 13 to 19.

From the results the same trend as in the case of MV_1 was noticed in MV_2 generation also.

4.2.2.1 Heritability

Induction of variability and subsequent exploitation for the economic traits should always be based with heritability, genetic advance and association among the economic characters. Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are more reliable in predicting the gain under selection than heritability estimates alone.

In the present study high estimates of heritability (broad sense) were noticed for all the characters studied. The maximum heritability of 96.27 per cent was noticed in the case of leaf length and the minimum of 64.50 per cent in the case of rhizome weight. Genetic advance expressed as percentage of mean was maximum (39.30) for number of leaves

Table 13 Effect of mutagens on the germination of rhizomes in MV_2 generation

Treatments	Germination (%)	Percentage variation over control	No. of days to start sprouting (mean period in days)	Percentage varation over control	Duration (Mean days)	Percentage variation over control
Gamma irradiation (Gy)						
Control	92.1 (73.68)	0	28.3	0	10.0	0
2.5	87.3 (69.12)	-5.21	30.7	8.48	9.0	-10.0
5.0	80.3 (63.65)	-12.81	31.7	12.01	9.0	-10.0
7.5	79.7 (63.22)	-13.46	33.7	19.08	7.3	-26.7
10.0	80.0 (63.43)	-13.14	35.3	24.73	7.0	-30.0
12.5	78.3 (62.24)	-14.98	35.7	26.15	6.0	-40.0
15.0	77.7 (61.82)	-15.64	36.7	29.68	5.0	-50.0
17.5	75.0 (60.0)	-18.57	37.3	31.80	5.0	-50.0
20.0	72.3 (58.24)	-21.50	38.0	34.28	4.7	-53.3
CD (0.05)	(3.950)		(0.905)		(0.456)	
EMS(%) Control	92.0 (73.57)	0	28.7	0	9.0	0
0.25	90.0 (71.57)	-2.17	30.1	4.88	8.0	-11.1
0.50	85.9 (67.94)	-6.63	30.5	6.27	8.0	-11.1
0.75	83.1 (65.73)	-9.67	32.4	12.89	7.0	-22.2
1.00	81.9 (64.82)	-10.98	34.1	18.82	6.0	-33.3
1.25	72.3 (58.24)	-21.41	36.3	26.48	5.0	-44.4
1.50	74.3 (59.54)	-19.24	37.9	32.06	4.7	-47.8
CD (0.05)	(1.730)		(0.905)		(0.388)	

Numbers in parenthesis denote transformed values

Table 14 Effect of mutagens on lethality expressed at different stages of growth (percentage) in MV_2 generation

Treatments	90 DAS	Percetage variation over control	135 DAS	Percentage variation over control	At harvest	Percentage variation over control
Gama irradiation (Gy)						
Control	14.7 (22.54)	0	14.7 (22.54)	0	14.7 (22.54)	0
2.5	21.4 (27.56)	45.58	23.8 (29.20)	61.90	23.8 (29.20)	61.90
5.0	26.1 (30.72)	77.55	26.1 (30.72)	77.55	26.1 (30.72)	77.55
7.5	18.0 (25.10)	22.45	20.0 (26.57)	36.05	22.0 (27.97)	49.66
10.0	26.0 (30.66)	76.87	26.0 (30.66)	76.87	26.0 (30.66)	76.87
12.5	23.9 (29.27)	62.59	23.9 (29.27)	62.59	23.9 (29.27)	62.59
15.0	17.8 (24.95)	21.09	20.0 (26.57)	36.05	20.0 (26.57)	36.05
17.5	24.4 (29.60)	65.99	28.3 (32.74)	92.52	28.3 (32.14)	92.52
20.0	28.0 (31.95)	90.48	28.0 (31.95)	90.48	28.0 (31.95)	90.48
CD (0.05)	(4.742)		(4.664)		(3.460)	
EMS % Control	17.4 (24.65)	0	17.4 (24.65)	0	17.4 (24.65)	0
0.25	19.1 (25.91)	9.77	19.1 (25.91)	9.77	19.1 (25.91)	9.77
0.50	29.2 (32.71)	67.82	29.2 (32.71)	67.82	29.2 (32.71)	67.82
0.75	22.6 (28.39)	29.89	22.6 (28.39)	29.89	22.6 (28.39)	29.89
1.00	20.3 (26.78)	16.67	20.3 (26.78)	16.67	20.3 (26.78)	16.67
1.25	34.7 (36.09)	99.43	34.7 (36.09)	99.43	34.7 (36.09)	99.43
1.50	34.7 (36.09)	99.43	34.7 (36.09)	99.43	34.7 (36.09)	99.43
CD (0.05)	(5.573)		(3.931)		(4.324)	

Numbers in parenthesis denote transformed values

Table 15 Effect of mutagens on plant spread and number of tillers in MV_2 generation

Treatments	Plant spread						No. of tillers		
	90 DAS			135 DAS					
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Gamma irradiation (Gy)									
Control	16.4-22.1	18.6	10.64	19.0-28.1	22.7	11.25	2-7	3.4	8.71
2.5	18.0-23.1	20.6	11.91	21.6-26.8	22.8	8.17	2-7	3.8	9.60
5.0	15.4-21.8	20.8	12.49	22.1-28.9	24.3	6.72	2-7	3.9	7.09
7.5	14.4-24.7	21.7	9.28	18.3-26.8	24.6	13.13	2-7	4.1	7.62
10.0	14.0-26.2	20.1	9.10	20.1-28.7	23.0	10.93	3-6	3.4	8.07
12.5	13.6-19.2	16.4	12.04	14.3-30.6	20.9	13.45	3-7	3.2	9.30
15.0	12.3-28.5	15.9	27.12	13.2-26.6	20.4	16.52	3-6	3.1	9.34
17.5	9.4-17.1	14.7	12.16	19.2-27.6	23.2	9.10	2-7	2.9	7.09
20.0	9.7-17.3	12.9	20.33	14.2-24.6	17.7	10.70	2-6	2.4	6.50
CD (0.05)		(0.723)			(1.004)			(0.312)	
EMS (%) Control	13.0-18.3	15.1	12.80	13.5-26.5	19.2	9.03	3-6	3.5	7.03
0.25	14.2-22.7	17.2	19.52	20.0-28.5	22.7	7.30	3-6	3.5	8.92
0.50	13.0-20.0	17.0	23.17	17.0-27.2	23.5	18.16	3-5	4.0	9.70
0.75	15.5-22.1	17.5	16.81	20.2-29.5	24.6	9.23	3-6	4.0	9.05
1.00	11.2-19.5	14.3	10.87	17.0-28.3	20.7	16.51	3-7	3.5	5.20
1.25	10.0-18.5	12.9	10.78	12.5-26.8	19.2	8.69	3-6	3.2	6.13
1.50	10.0-17.6	11.7	22.31	14.0-23.6	18.5	9.57	2-5	2.4	8.01
CD (0.05)		(1.189)			(2.082)			(0.197)	

Table 16 Effect of mutagens on number of leaves in MV_2 generation

Treatments	Number of leaves											
	45 DAS			90 DAS			135 DAS			180 DAS		
	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
Gamma irradiation (Gy)												
Control	4-6	4.7	7.67	12-17	12.5	9.33	13-16	14.5	7.06	15-21	17.0	12.01
2.50	4-7	5.4	11.85	9-17	13.1	12.02	13-17	15.4	14.83	14.20	18.1	20.36
5.00	4-7	5.8	14.08	8-18	13.9	9.04	10-19	16.6	20.75	11-22	18.8	20.17
7.50	4-8	5.9	9.18	8-19	14.1	12.82	10-19	16.8	23.58	12-22	18.8	19.09
10.0	4-7	5.1	14.20	8-19	14.1	10.02	10-19	15.6	21.26	11-24	18.7	20.16
12.5	3-7	4.3	10.55	8-17	12.2	11.07	10-18	13.0	26.05	11-18	13.3	17.08
15.0	3-6	3.4	17.18	7-18	12.1	15.11	8-15	10.3	27.22	10-17	11.8	20.24
17.5	2-6	3.3	7.03	5-14	7.0	12.04	7-16	9.5	23.94	10-16	11.4	13.32
20.0	2-5	3.3	12.42	3 -7	4.4	12.57	6-14	8.0	25.39	6-16	9.5	14.06
CD (0.05)		(0.376)			(0.502)			(1.504)			(1.353)	
EMS (%) Control	3-7	4.3	7.13	8-15	11.5	7.12	12-19	14.3	8.33	10-21	16.1	8.53
0.25	3-7	4.7	6.82	10-14	12.8	7.43	11-17	14.8	17.70	12-22	17.6	9.21
0.50	4-7	5.0	12.02	7-17	11.8	18.22	10-19	15.5	18.97	12-22	17.0	23.36
0.75	4-7	5.4	7.81	7-14	11.3	13.87	11-20	16.6	18.60	13-25	18.7	23.57
1.00	3-7	3.4	9.58	4-9	6.3	17.51	6-16	9.5	14.41	9-17	11.7	22.06
1.25	3-6	3.4	6.27	4-9	5.4	12.78	5-12	8.4	19.63	6-14	10.7	17.90
1.50	2-6	3.3	8.22	4-8	5.2	10.34	5-12	7.8	17.80	7-14	10.8	12.08
CD (0.05)		(0.596)			(0.878)			(0.895)			(0.837)	

Table 17 Effect of mutagens on length and breadth of leaves in MV_2 generation

Treatments	Leaf length (cm)			Leaf breadth (cm)		
	Range	Mean	CV	Range	Mean	CV
Gamma irradiation(Gy)						
Control	11.2-16.0	14.8	10.02	9.0-12.6	10.6	11.74
2.5	13.1-17.6	15.4	9.67	8.1-12.2	11.3	8.40
5.0	12.0-18.0	15.4	8.82	5.2-12.0	11.4	11.00
7.5	11.2-19.5	15.5	7.36	8.3-12.5	11.6	5.33
10.0	12.0-18.3	15.0	11.45	7.0-12.9	10.6	8.12
12.5	10.0-16.4	13.9	10.75	7.0-11.2	9.9	8.38
15.0	10.0-16.0	14.7	15.80	7.2-11.2	9.4	11.13
17.5	11.5-17.1	14.8	11.55	7.3-11.4	9.4	10.03
20.0	11.0-17.1	13.8	13.51	6.2-12.3	8.3	10.99
CD (0.05)		(0.553)			(0.412)	
EMS (%) Control	11.1-15.4	12.6	12.08	8.1-12.4	8.5	12.08
0.25	10.0-17.6	12.9	16.03	6.2-10.9	8.5	16.03
0.50	10.0-17.5	12.8	16.76	6.4-10.5	8.5	16.76
0.75	11.0-17.1	13.8	15.98	7.3-11.4	9.7	15.98
1.00	11.1-16.6	13.6	15.11	7.3-11.7	10.3	15.11
1.25	9.0-14.9	12.0	14.73	7.2-12.9	9.1	14.73
1.50	9.2-14.4	11.8	13.58	6.6-10.7	7.9	13.58
CD (0.05)		(0.894)			(0.445)	

Table 18 Effect of mutagens on number and fresh weight of rhizomes in MV_2 generations

Treatments	Rhizome number			Fresh weight of rhizome (g plant^{-1})		
	Range	Mean	CV	Range	Mean	CV
Gamma irradiation (Gy)						
Control	4-25	13.0	15.46	28.3-78.1	43.6	17.06
2.5	6-27	13.6	18.40	20.7-87.5	45.7	24.83
5.0	6-27	14.2	16.83	19.0-90.5	48.9	20.75
7.5	4-25	14.8	16.18	17.0-85.2	50.3	23.58
10.0	5-25	14.5	17.85	22.4-82.7	50.6	31.26
12.5	3-24	14.3	38.55	22.2-85.0	50.1	26.05
15.0	2-26	14.3	39.89	20.0-87.5	48.0	37.22
17.5	3-19	10.8	39.01	20.4-80.7	41.6	33.94
20.0	2-17	8.3	34.20	14.7-79.5	37.7	35.39
CD (0.05)		(1.241)			(5.977)	
EMS (%) Control	6-18	12.4	20.64	20.3-78.8	46.9	18.64
0.25	6-18	13.5	37.73	21.0-88.5	54.9	32.95
0.50	11-20	14.4	35.79	25.3-81.0	53.5	36.34
0.75	10-22	17.2	35.65	27.2-86.8	61.8	28.44
1.00	10-21	15.5	32.05	26.8-80.3	57.3	35.45
1.25	10-14	11.4	22.74	16.2-74.0	45.0	35.30
1.50	7-12	9.2	17.50	22.0-51.5	35.0	26.15
CD (0.05)		(2.108)			(7.418)	

Table 19 Effect of mutagens on crop duration in MV_2 generation

Treatments	Duration		
	Range	Mean	CV
Gamma irradiation(Gy)			
Control	205-228	213	1.98
2.5	190-230	205	4.53
5.0	190-224	204	3.68
7.5	188-216	201	4.41
10.0	180-220	199	3.09
12.5	180-215	195	4.64
15.0	190-210	195	4.60
17.5	180-205	192	4.92
20.0	180-210	188	4.13
CD (0.05)		(4.356)	
EMS (%) Control	180-225	220	2.33
0.25	180-220	215	3.68
0.50	180-220	215	4.43
0.75	180-220	211	3.57
1.00	190-215	208	4.65
1.25	190-210	206	3.04
1.50	190-210	201	4.86
CD (0.05)		(3.105)	

and the minimum (13.04) for leaf length. High estimates of heritability coupled with high genetic advance was noticed for number of leaves and rhizome number (Table 20 and Fig.1).

4.2.2.2 Association of characters

A knowledge on the degree of association among the quantitative characters would help the breeder to select those characters which are positively correlated with yield and elimination of characters which are negatively correlated with yield. Correlation coefficient is a statistical measure which is used to find out the degree and direction of relationship between two or more variables. It is represented by `r' . A positive value of `r' shows that the changes of two variables are in the same direction. When `r' is negative the movements are in opposite direction.

The association of important yield attributes with yield was studied and the results are presented in Tables 21 to 36.

4.2.2.2.1 Gamma irradiation

4.2.2.2.1a Control

The simple correlation coefficient between rhizome yield and its components were significant and positive for all the characters except duration. The association between yield and number of rhizomes was strong with the

Table 20 Heritability (broad sense) and genetic advance as percentage of mean

Characters	Heritability (percentage)	Genetic Advance (percentage of mean)
Number of leaves (135 DAS)	94.24	39.30
Tiller number (180 DAS)	95.37	19.49
Leaf length	96.27	13.04
Leaf breadth	95.17	18.56
Plant spread (135 DAS)	80.69	14.98
Rhizome number	90.63	37.36
Rhizome yield	64.50	18.68

X_1 - Number of leaves

X_2 - Number of tillers

X_3 - Leaf length

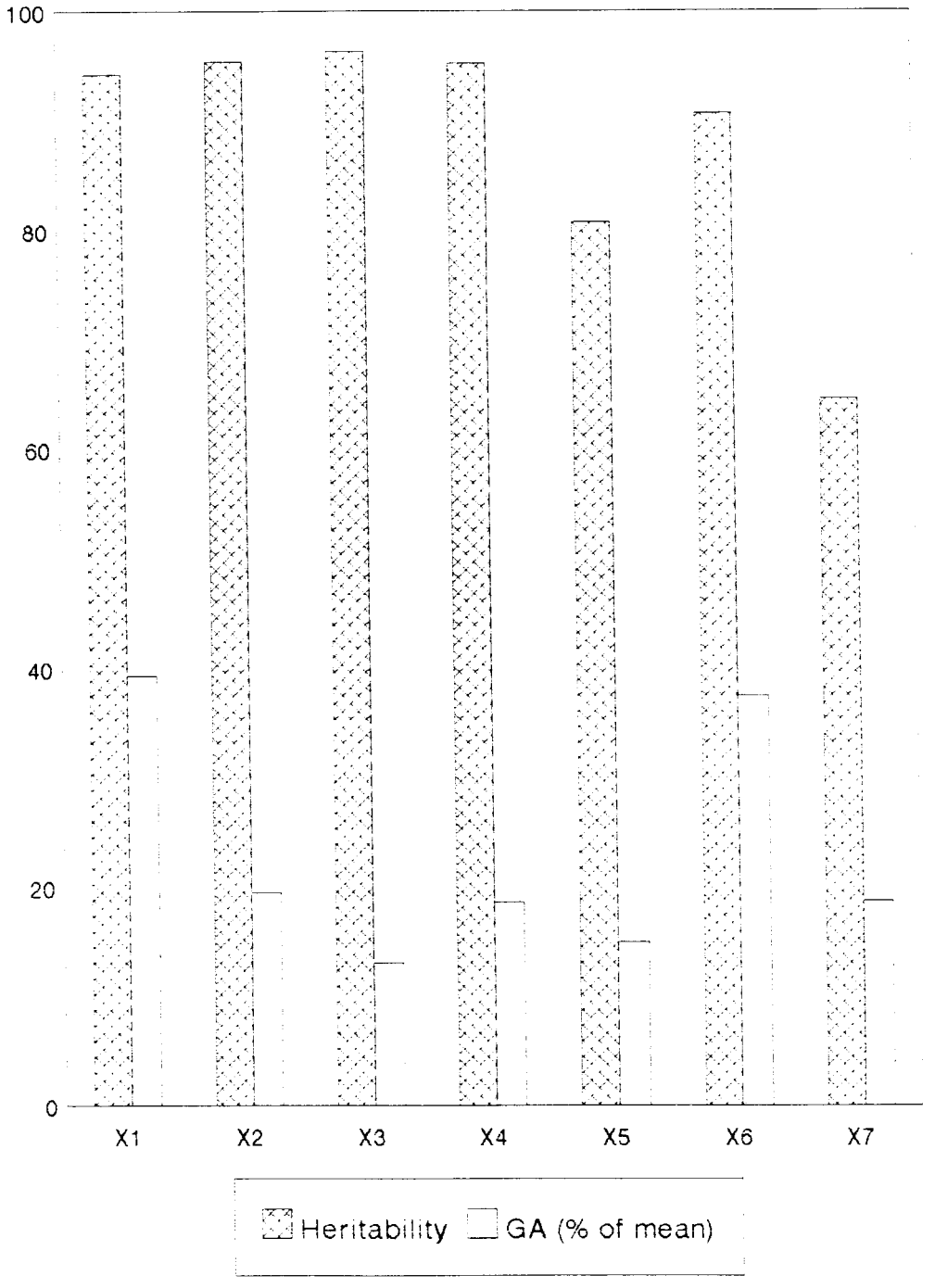
X_4 - Leaf breadth

X_5 - Plant spread

X_6 - Rhizome number

X_7 - Rhizome yield

Fig.1 Heritability and genetic advance as percentage of mean in kacholam



highest `r' value of 0.993. The interrelationship among the yield components also exhibited positive and significant relationship except between number of leaves and leaf breadth (0.197) leaf breadth and duration (0.292); and duration and number of rhizomes with `r' value 0.329 (Table 21 and Fig.2)

4.2.2.2.1b 2.5 Gy

The correlation coefficients between yield and components were positive and significant except the correlation of yield with number of tillers as well as duration. The correlation coefficient was of larger magnitude between plant spread and rhizome yield (0.738). The number of leaves with number of tillers, leaf breadth and duration; leaf breadth with number of tillers; leaf length with leaf breadth and plant spread with rhizome number also exhibited significant positive correlation (Table 22).

4.2.2.2.1c 5.0 Gy

There was positive significant correlation of yield with leaf length (0.810), leaf breadth (0.799), number or rhizomes (0.674), plant spread (0.625) and number of leaves (0.593). The interrelationship among the yield components also exhibited positive association between the component characters (Table 23).

Table 21 Correlation coefficients between rhizome yield and components in kacholam (gamma experiment control)

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.505**	1.000						
Leaf length (X3)	0.373*	0.590**	1.000					
Leaf breadth (X4)	0.197	0.735**	0.381*	1.000				
Plant spread (X5)	0.495**	0.665**	0.548**	0.639**	1.000			
Duration (X6)	0.542**	0.561**	0.474**	0.292	0.582**	1.000		
Rhizome No. (X7)	0.440*	0.420*	0.419*	0.400*	0.505**	0.329	1.000	
Rhizome yield (X8)	0.495**	0.495**	0.465**	0.347*	0.503**	0.332	0.993**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

X_1 - Number of leaves

X_2 - Number of tillers

X_3 - Leaf length

X_4 - Leaf breadth

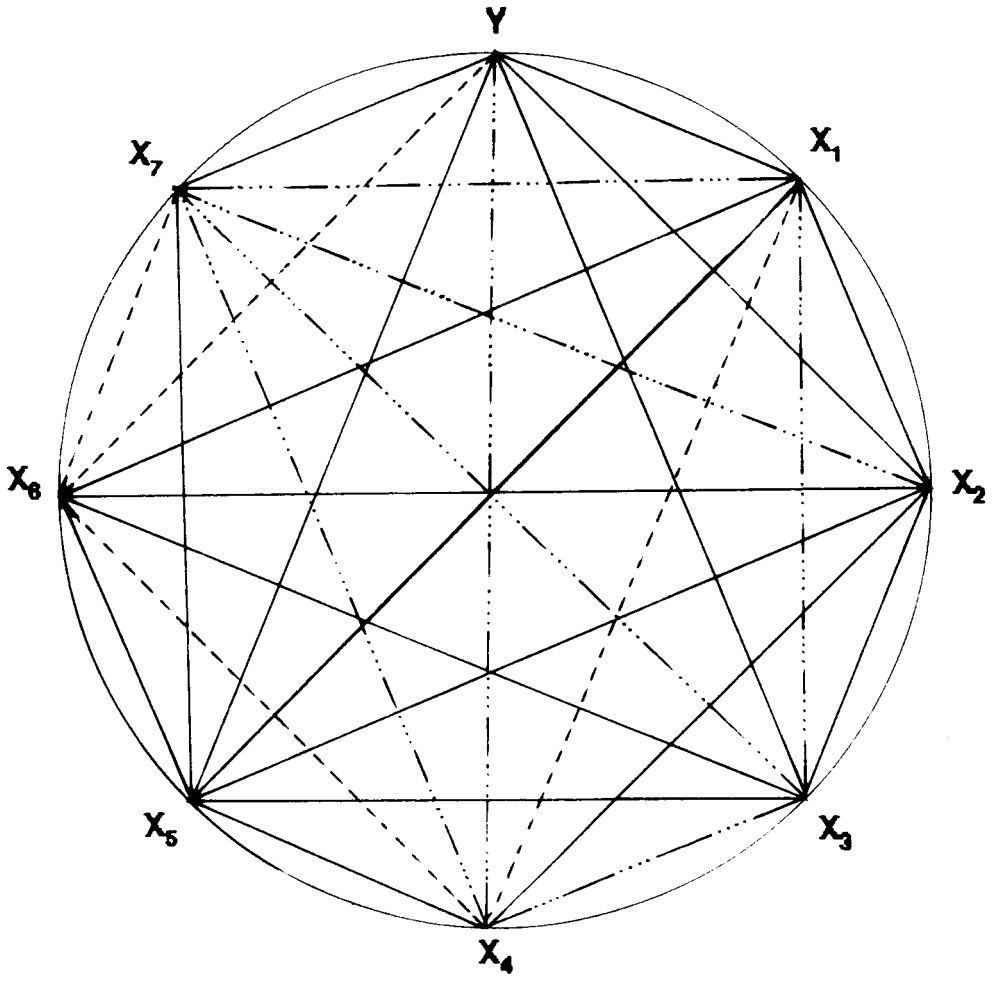
X_5 - Plant spread

X_6 - Duration

X_7 - Rhizome number

Y - Rhizome yield

Fig.2 Correlations among yield attributes in kacholam (gamma experiment - control)



——— Significant positive correlation (0.01 level)

—·····—····· Significant positive correlation (0.05 level)

- - - - - - Positive correlation

Table 22 Correlation coefficients between rhizome yield and components in 2.5 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.480**	1.000						
Leaf length (X3)	0.040	0.296	1.000					
Leaf breadth (X4)	0.717**	0.502**	0.382*	1.000				
Plant spread (X5)	0.246	0.228	0.497**	0.327	1.000			
Duration (X6)	0.366*	-0.045	-0.048	0.111	0.304	1.000		
Rhizome No. (X7)	0.300	0.073	0.460**	0.319	0.739**	0.180	1.000	
Rhizome yield (X8)	0.357*	0.328	0.376*	0.481**	0.738**	0.223	0.725**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 23 Correlation coefficients between rhizome yield and components in 5.0 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.148	1.000						
Leaf length (X3)	0.437*	0.394*	1.000					
Leaf breadth (X4)	0.592**	0.296	0.780**	1.000				
Plant spread (X5)	0.324	0.133	0.603**	0.612**	1.000			
Duration (X6)	0.085	0.210	0.177	0.277	0.332	1.000		
Rhizome No. (X7)	0.414*	0.321	0.495**	0.516**	0.517**	0.120	1.000	
Rhizome yield (X8)	0.593**	0.311	0.810**	0.799**	0.625**	0.334	0.674**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

4.2.2.2.1d 7.5 Gy

There was positive and significant association of rhizome yield with leaf breadth (0.817), leaf length (0.799), plant spread (0.762), number of rhizomes (0.674), number of tillers (0.597) and number of leaves (0.501). The interrelationship among the component characters exhibited positive relationship except for the association of number of leaves with crop duration, number of tillers and plant spread. The association of crop duration with rhizome number, leaf length, leaf breadth and plant spread also remained non significant (Table 24 and Fig. 3).

4.2.2.2.1e 10.0 Gy

The correlation coefficient between yield and its components were positive and significant except for the correlation of yield with leaf length (0.305) and number of tillers (0.240). The association of yield with number of rhizomes had the highest `r' value of 0.855. The inter-relationship among the yield components also exhibited positive association for all the component characters (Table 25).

4.2.2.2.1f 12.5 Gy

The correlation coefficient between yield and its components were positive and significant. The inter relationship among the component characters also maintained

Table 24 Correlation coefficients between rhizome yield and its components in 7.5 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.328	1.000						
Leaf length (X3)	0.425*	0.484**	1.000					
Leaf breadth (X4)	0.543**	0.457**	0.559**	1.000				
Plant spread (X5)	0.305	0.515**	0.433*	0.883**	1.000			
Duration (X6)	-0.083	0.416*	-0.122	-0.030	0.189	1.000		
Rhizome No. (X7)	0.384*	0.502**	0.499**	0.514**	0.420*	0.055	1.000	
Rhizome yield (X8)	0.501**	0.597**	0.799**	0.817**	0.762**	0.061	0.674**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

X_1 - Number of leaves

X_2 - Number of tillers

X_3 - Leaf length

X_4 - Leaf breadth

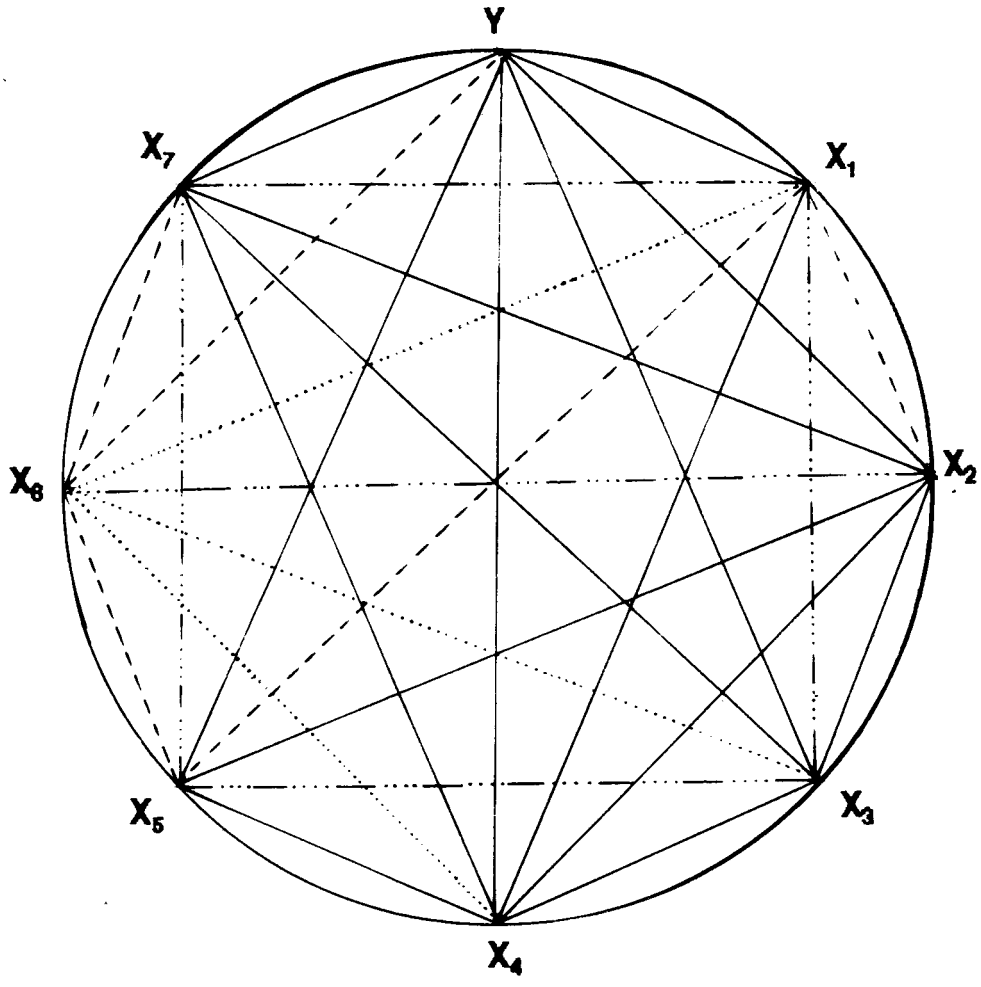
X_5 - Plant spread

X_6 - Duration

X_7 - Rhizome number

Y - Rhizome yield

Fig.3 Correlations among yield attributes in 7.5 Gy gamma rays treated kacholam



——— Significant positive correlation (0.01 level)

—·—·— Significant positive correlation (0.05 level)

- - - - - - - Positive correlation

..... Negative correlation

Table 25 Correlation coefficients between rhizome yield and components in 10.0 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.509**	1.000						
Leaf length (X3)	0.373*	0.024	1.000					
Leaf breadth (X4)	0.586**	0.440*	0.256	1.000				
Plant spread (X5)	0.399*	0.457**	0.238	0.668**	1.000			
Duration (X6)	0.490**	0.544**	0.033	0.543**	0.447*	1.000		
Rhizome No. (X7)	0.406*	0.162	0.061	0.616**	0.179	0.414*	1.000	
Rhizome yield (X8)	0.561**	0.240	0.305	0.788**	0.410*	0.603**	0.855**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

this association, except for the correlation of leaf breadth with leaf length (0.249), number of leaves (0.205) and number of tillers (0.018). Association of duration with rhizome number also remained non significant (Table 26).

4.2.2.2.1g 15.0 Gy

The correlation between yield and its components indicated positive significant relationship with component characters except for plant spread. The interrelationship indicated the positive association of all the component characters (Table 27).

4.2.2.2.1h 17.5 Gy

The correlation of yield with the component characters indicated, maximum relationship, with number of rhizomes ($r = 0.876$). The relationship of yield with the other component characters also remained positive and significant. The interrelationship of the component characters maintained this relationship except for characters like number of leaves with leaf length (0.244), plant spread and number of tillers (0.338) and number of leaves (0.237) and plant spread with leaf breadth (0.322) and duration (0.253) and duration with leaf length (Table 28).

Table 26 Correlation coefficients between rhizome yield and its components in 12.5 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.503**	1.000						
Leaf length (X3)	0.470**	0.431*	1.000					
Leaf breadth (X4)	0.205	0.018	0.249	1.000				
Plant spread (X5)	0.504**	0.456**	0.442*	0.517**	1.000			
Duration (X6)	0.775**	0.492**	0.545**	0.387*	0.547**	1.000		
Rhizome No. (X7)	0.687**	0.545**	0.634**	0.342	0.392*	0.227	1.000	
Rhizome yield (X8)	0.735**	0.446*	0.580**	0.539**	0.624**	0.384*	0.763**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 27 Correlation coefficients between rhizome yield and components in 15.0 Gy gamma ray treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.239	1.000						
Leaf length (X3)	0.248	0.407*	1.000					
Leaf breadth (X4)	0.356*	0.266	0.437*	1.000				
Plant spread (X5)	0.119	0.149	0.141	0.344*	1.000			
Duration (X6)	0.072	0.128	0.264	0.168	0.115	1.000		
Rhizome No. (X7)	0.207	0.518**	0.641**	0.471**	0.443*	0.552**	1.000	
Rhizome yield (X8)	0.363*	0.673**	0.529**	0.368*	0.265	0.709**	0.759**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 28 Correlation coefficients between rhizome yield and components in 17.5 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.184	1.000						
Leaf length (X3)	0.244	0.620**	1.000					
Leaf breadth (X4)	0.558**	0.359*	0.510**	1.000				
Plant spread (X5)	0.237	0.338	0.544**	0.322	1.000			
Duration (X6)	0.355*	0.428*	0.325	0.586**	0.253	1.000		
Rhizome No. (X7)	0.553**	0.350*	0.768**	0.485**	0.516**	0.475**	1.000	
Rhizome yield (X8)	0.552**	0.556**	0.805**	0.595**	0.585**	0.432*	0.876**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

4.2.2.2.1i 20.0 Gy

The correlation coefficients of yield with the component characters indicated positive significant relationship (Table 29). The interrelationship of yield components indicated positive significant association except for the association of number of leaves with leaf breadth (0.271), tillers (0.269), and rhizome number (0.219); duration with number of rhizomes (0.302) and tillers (0.154) and leaf breadth with rhizome number (0.269).

4.2.2.2.2 EMS

4.2.2.2.2a Control

There was positive significant correlation of rhizome yield with plant spread (0.852), number of rhizomes (0.795), leaf length (0.752), leaf breadth (0.747) and number of leaves (0.663). Correlation of rhizome yield with number of tillers (0.255) and crop duration (0.040) gave positive response but not significant.

The association of number of leaves with leaf length, leaf breadth, plant spread and rhizome number is positive and significant. Association of number of tillers with leaf breadth, plant spread and number of rhizomes remains positive and significant. The correlation of leaf length, leaf breadth and plant spread with component characters remained positive and significant, except the association with duration (Table 30 and Fig.4).

Table 29 Correlation coefficients between rhizome yield and components in 20.0 Gy gamma rays treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.269	1.000						
Leaf length (X3)	0.556**	0.377*	1.000					
Leaf breadth (X4)	0.271	0.361*	0.722**	1.000				
Plant spread (X5)	0.418*	0.578**	0.503**	0.517**	1.000			
Duration (X6)	0.336	0.154	0.574**	0.535**	0.478**	1.000		
Rhizome No. (X7)	0.219	0.375*	0.455**	0.269	0.353*	0.302	1.000	
Rhizome yield (X8)	0.478**	0.531**	0.651**	0.599**	0.516**	0.584**	0.621**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 30 Correlation coefficients between rhizome yield and components in kacholam (EMS experiment control)

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.254	1.000						
Leaf length (X3)	0.535**	0.282	1.000					
Leaf breadth (X4)	0.625**	0.609**	0.641**	1.000				
Plant spread (X5)	0.677**	0.795**	0.651**	0.687**	1.000			
Duration (X6)	0.020	0.130	0.038	0.152	0.194	1.000		
Rhizome No. (X7)	0.661**	0.673**	0.624**	0.555**	0.671**	0.222	1.000	
Rhizome yield (X8)	0.663**	0.255	0.752**	0.747**	0.852**	0.040	0.795**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

X_1 - Number of leaves

X_2 - Number of tillers

X_3 - Leaf length

X_4 - Leaf breadth

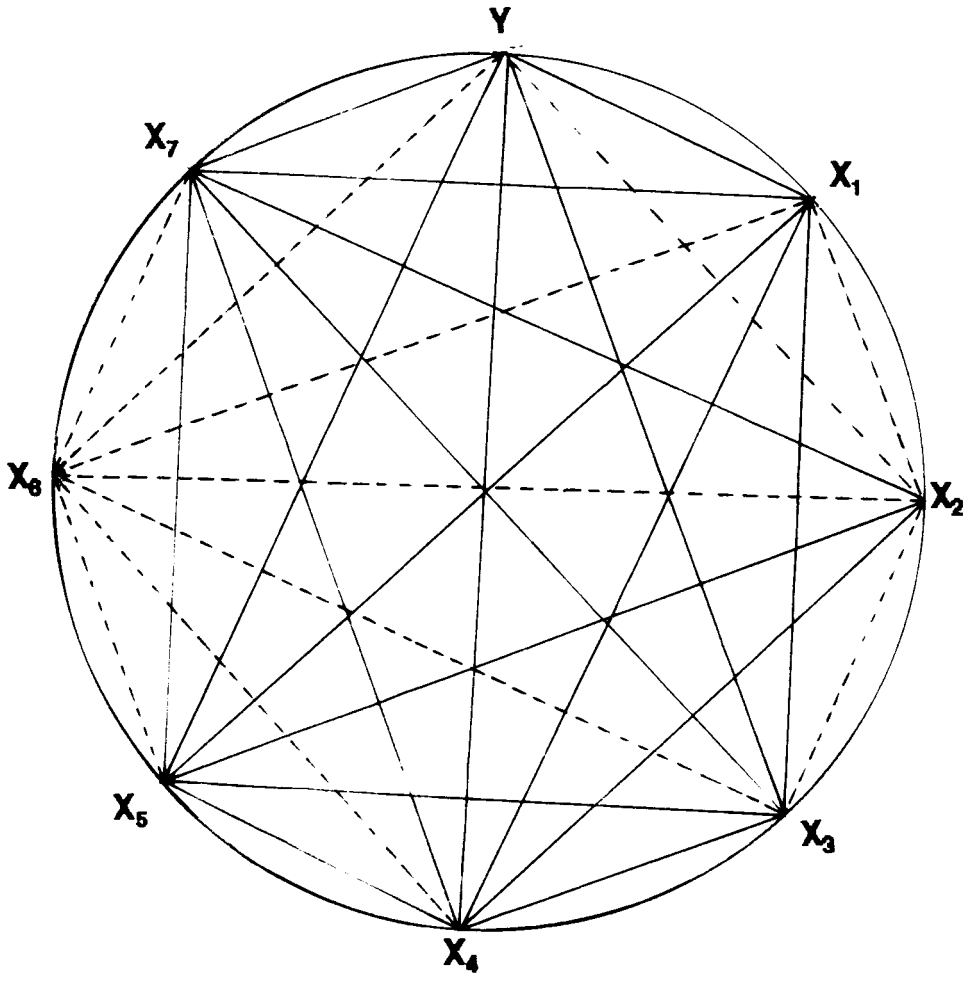
X_5 - Plant spread

X_6 - Duration

X_7 - Rhizome number

Y - Rhizome yield

Fig.4 Correlations among yield attributes in kacholam (EMS experiment - control)



——— Significant positive correlation (0.01 level)

- - - - - - Positive correlation

4.2.2.2.2b 0.25% EMS

There was positive significant association of rhizome yield with number of leaves (0.767) and number of rhizomes (0.749). The correlation coefficient of yield with leaf length (-0.284) and leaf breadth (-0.024) gave negative values. There was strong positive association of number of rhizomes with number of leaves (0.722) (Table 31).

4.2.2.2.2c 0.50% EMS

The correlation of yield with component characters indicated positive significant association. The inter-relationship between component characters also remained positive and significant (Table 32).

4.2.2.2.2d 0.75% EMS

The correlation of yield with the component characters shown positive significant association (Table 33 and Fig.5). The inter-relationship between the characters also indicated the same response except for number of tillers and duration (0.279).

4.2.2.2.2e 1.0% EMS

The correlation coefficient of yield with the component characters exhibited positive significant association (Table 34). The interrelationship between the component characters also indicated positive significant association except between number of tillers with duration (0.344) and number of leaves (0.342).

Table 31 Correlation coefficients between rhizome yield and components in 0.25% EMS treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.438*	1.000						
Leaf length (X3)	-0.012	-0.253	1.000					
Leaf breadth (X4)	0.132	0.012	-0.042	1.000				
Plant spread (X5)	0.208	0.225	0.153	-0.244	1.000			
Duration (X6)	0.248	0.285	-0.286	-0.021	0.183	1.000		
Rhizome No. (X7)	0.722**	0.347*	-0.215	0.115	0.044	0.026	1.000	
Rhizome yield (X8)	0.767**	0.223	-0.284	-0.024	0.167	0.237	0.749**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 32 Correlation coefficients between rhizome yield and components in 0.50% EMS treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.640**	1.000						
Leaf length (X3)	0.689**	0.696**	1.000					
Leaf breadth (X4)	0.617**	0.621**	0.621**	1.000				
Plant spread (X5)	0.617**	0.657**	0.655**	0.460**	1.000			
Duration (X6)	0.783**	0.637**	0.596**	0.786**	0.593**	1.000		
Rhizome No. (X7)	0.552**	0.513**	0.528**	0.432*	0.891**	0.560**	1.000	
Rhizome yield (X8)	0.636**	0.706**	0.526**	0.744**	0.712**	0.790**	0.738**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 33 Correlation coefficients between rhizome yield and components in 0.75% EMS treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.498**	1.000						
Leaf length (X3)	0.382*	0.399*	1.000					
Leaf breadth (X4)	0.520**	0.542**	0.579**	1.000				
Plant spread (X5)	0.719**	0.776**	0.428*	0.588**	1.000			
Duration (X6)	0.458**	0.279	0.350*	0.463**	0.361*	1.000		
Rhizome No. (X7)	0.706**	0.589**	0.355*	0.643**	0.692**	0.369*	1.000	
Rhizome yield (X8)	0.674**	0.572**	0.591**	0.741**	0.743**	0.590**	0.759**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

X_1 - Number of leaves

X_2 - Number of tillers

X_3 - Leaf length

X_4 - Leaf breadth

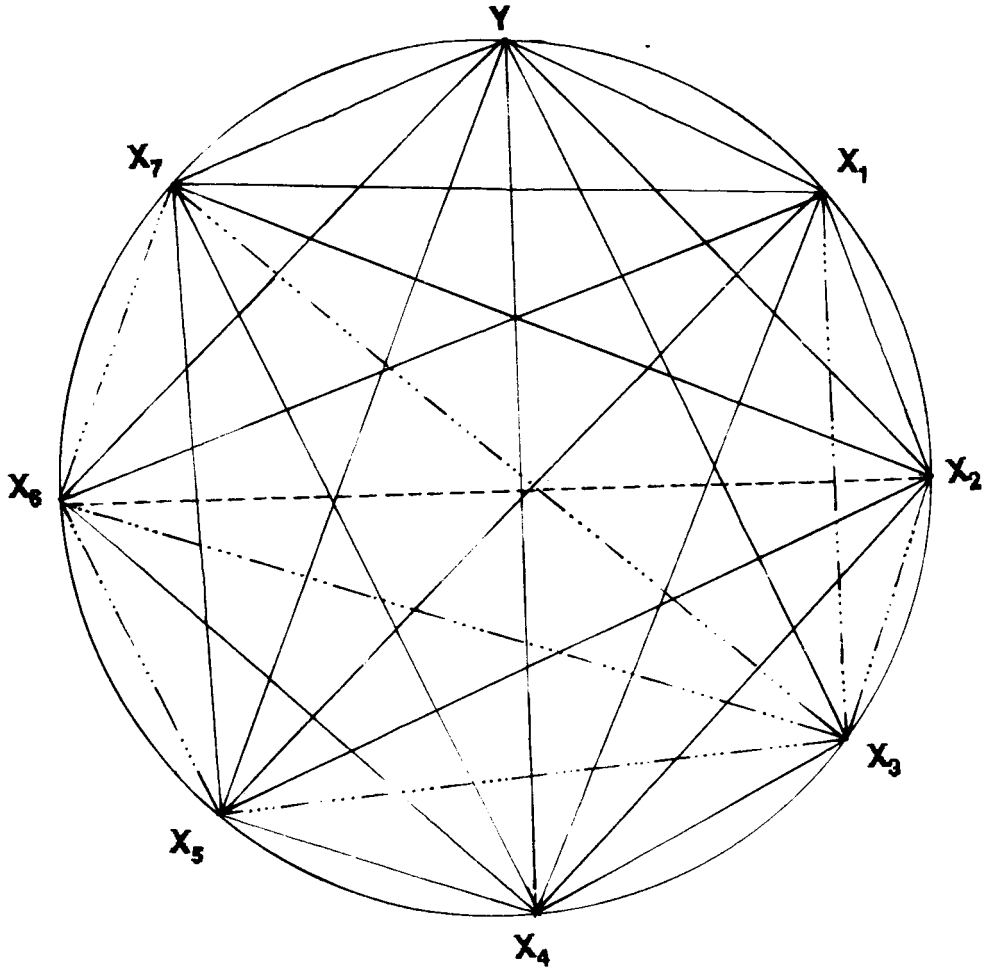
X_5 - Plant spread

X_6 - Duration

X_7 - Rhizome number

Y - Rhizome yield

Fig.5 Correlations among yield attributes in 0.75 per cent EMS treated kacholam



——— Significant positive correlation (0.01 level)

—·····— Significant positive correlation (0.05 level)

– – – – – Positive correlation

Table 34 Correlation coefficients between rhizome yield and components in 1.00% EMS treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.342	1.000						
Leaf length (X3)	0.704**	0.376*	1.000					
Leaf breadth (X4)	0.761**	0.459**	0.727**	1.000				
Plant spread (X5)	0.659**	0.518**	0.829**	0.705**	1.000			
Duration (X6)	0.713**	0.344	0.664**	0.498**	0.631**	1.000		
Rhizome No. (X7)	0.638**	0.474**	0.660**	0.713**	0.550**	0.526**	1.000	
Rhizome yield (X8)	0.934**	0.410*	0.714**	0.793**	0.616**	0.708**	0.742**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

4.2.2.2.2f 1.25% EMS

The correlation of yield with component characters indicated positive significant response except with duration (0.290) which indicated positive but not significant response. The inter-relationship between component characters indicated positive significant association except number of tillers with leaf length (0.337) and duration (0.045) (Table 35).

4.2.2.2.2g 1.50% EMS

The correlation of yield with component characters have indicated the positive significant relationship except with number of tillers (0.281). The number of tillers with duration (-0.136) gave negative but not significant response. The association between number of rhizomes and yield was high with `r' value 0.845 (Table 36).

4.2.2.3 Path Coefficient Analysis

Based on the high correlations of yield and components, path coefficient analysis was carried out to elicit further information through direct and indirect effects of important yield attributes. The results are presented in Tables 37 to 42.

Table 35 Correlation coefficients between rhizome yield and components in 1.25% EMS treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.676**	1.000						
Leaf length (X3)	0.611**	0.337	1.000					
Leaf breadth (X4)	0.657**	0.381*	0.783**	1.000				
Plant spread (X5)	0.499**	0.407*	0.770**	0.869**	1.000			
Duration (X6)	0.524**	0.045	0.672**	0.549**	0.516**	1.000		
Rhizome No. (X7)	0.542**	0.425*	0.698**	0.695**	0.764**	0.437*	1.000	
Rhizome yield (X8)	0.532**	0.377*	0.581**	0.641**	0.629**	0.290	0.552**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 36 Correlation coefficients between rhizome yield and components in 1.50% EMS treated kacholam

Characters	X1	X2	X3	X4	X5	X6	X7	X8
No. of leaves (X1)	1.000							
Tillers (X2)	0.198	1.000						
Leaf length (X3)	0.729**	0.273	1.000					
Leaf breadth (X4)	0.788**	0.240	0.715**	1.000				
Plant spread (X5)	0.452**	0.410*	0.700**	0.443*	1.000			
Duration (X6)	0.624**	-0.136	0.497**	0.536**	0.148	1.000		
Rhizome No. (X7)	0.606**	0.150	0.778**	0.704**	0.463**	0.529**	1.000	
Rhizome yield (X8)	0.718**	0.281	0.891**	0.789**	0.620**	0.657**	0.845**	1.000

* Significant at 0.05 level

** Significant at 0.01 level

Table 37 Path coefficient analysis showing direct effect of characters on yield under different mutagenic treatments

Treatments	No. of leaves	Leaf length	Leaf breadth	Plant spread	Rhizome No.	Residual effect
Gamma irradiation(Gy)						
Control	0.0863	0.2288	0.1308	-0.1249	0.7415	0.2933
2.5	0.0864	0.1101	0.3058	0.5051	0.2751	0.4518
5.0	0.1701	0.4600	0.1469	0.0636	0.2855	0.4201
7.5	0.1015	0.4802	0.0436	0.5349	0.0831	0.3488
10.0	0.0601	0.1394	0.4018	0.3008	0.2625	0.2784
12.5	0.3108	0.0640	0.2328	0.3238	0.3882	0.4611
15.0	0.1789	0.0679	0.0817	0.1139	0.7770	0.5763
17.5	0.0449	0.2044	0.1407	0.2568	0.5291	0.3408
20.0	0.3410	0.1811	0.0895	0.0993	0.4219	0.5357
EMS (%) Control	0.0747	0.1395	0.1147	0.2469	0.7379	0.6551
0.25	0.6553	0.2428	0.0456	0.0433	0.2527	0.3940
0.50	0.0423	0.0896	0.4047	0.0641	0.4323	0.4400
0.75	0.1452	0.1275	0.3044	0.3920	0.4742	0.5073
1.00	0.6236	0.1275	0.0695	0.0113	0.2452	0.2942
1.25	0.1752	0.1126	0.0619	0.4802	0.1714	0.4237
1.50	0.2510	0.3237	0.2170	0.1675	0.1290	0.3013

Table 38 Path coefficient analysis showing indirect effect of number of leaves via other characters on yield under different mutagenic treatments

Treatments	Leaf length	Leaf breadth	Plant spread	Rhizome No.
Gamma irradiation (Gy)				
Control	0.1207	0.0411	-0.0264	0.3635
2.5	-0.0058	0.2225	0.1453	0.0981
5.0	0.2043	0.0915	0.0233	0.1097
7.5	0.2354	-0.0228	0.1596	0.0409
10.0	0.0487	0.2474	0.1748	0.1348
12.5	-0.0430	0.0184	0.0798	0.2480
15.0	0.0300	-0.0316	-0.0245	0.2700
17.5	0.0612	0.0877	0.0999	0.2958
20.0	0.1002	0.0426	0.0442	0.0917
EMS (%)				
Control	-0.0776	-0.0178	0.0663	-0.0774
0.25	0.0087	-0.0052	0.0095	0.1898
0.50	0.0732	0.2682	0.0478	0.2834
0.75	0.0584	0.1476	0.1819	0.1208
1.00	0.0967	0.5500	-0.0085	0.1564
1.25	0.0854	0.0464	0.3147	0.1133
1.50	0.2630	0.1771	0.0857	0.1036

Table 39 Path coefficient analysis showing indirect effect of leaf length via other characters on yield under different mutagenic treatments

Treatments	No. of leaves	Leaf breadth	Plant spread	Rhizome No.
Gamma irradiation (Gy)				
Control	0.0455	0.0477	-0.0779	0.4322
2.5	0.0045	0.1149	0.2657	0.1268
5.0	0.0755	0.1200	0.0389	0.1208
7.5	0.0497	-0.0283	0.2541	0.0476
10.0	0.0210	0.0989	0.0712	0.0257
12.5	0.2090	0.0595	0.1435	0.2465
15.0	0.0790	-0.0385	-0.0192	0.4877
17.5	-0.0134	0.0746	0.1542	0.4155
20.0	0.1887	0.0727	0.0521	0.1943
EMS (%)				
Control	0.0415	-0.0012	-0.0729	0.0475
0.25	-0.0235	0.0002	0.0061	-0.0588
0.50	-0.0345	0.3149	0.0515	0.3012
0.75	0.0665	0.1913	0.1462	0.0886
1.00	0.4730	0.0484	-0.0088	0.1593
1.25	0.1329	0.0488	0.3671	0.1204
1.50	0.2040	0.1546	0.1152	0.1011

Table 40 Path coefficient analysis showing indirect effect of leaf breadth via other characters on yield under different mutagenic treatments

Treatments	No. of leaves	Leaf length	Plant spread	Rhizome No.
Gamma irradiation (Gy)				
Control	0.0271	0.0834	-0.0623	0.3574
2.5	0.0629	-0.0414	-0.0580	-0.0508
5.0	0.1060	0.3758	0.0401	0.1447
7.5	0.0532	0.3114	0.4852	0.0531
10.0	0.0370	0.0343	0.2268	0.9043
12.5	0.0245	-0.0164	0.1690	0.1364
15.0	0.0691	0.0320	-0.0401	0.3805
17.5	-0.0280	0.1083	0.1257	0.2783
20.0	0.1621	0.1470	0.0574	0.1279
EMS (%)				
Control	0.0116	-0.0014	-0.0262	0.0405
0.25	0.0745	0.0012	0.0108	0.0213
0.50	-0.0280	0.0697	-0.0526	0.3569
0.75	0.0704	0.0802	0.1689	0.1016
1.00	0.4931	0.0887	-0.0085	0.1643
1.25	0.1313	0.0887	0.4211	0.1228
1.50	0.2048	0.2306	0.0727	0.0972

Table 41 Path coefficient analysis showing indirect effect of plant spread via other characters on yield under different mutagenic treatments

Treatments		No. of leaves	Leaf length	Leaf breadth	Rhizome No.
Gamma irradiation(Gy)					
Control		0.0183	0.0427	0.0653	0.3025
2.5		0.0249	-0.0580	0.1175	0.2049
5.0		0.0625	0.2817	0.0928	0.1428
7.5		0.0303	0.2281	-0.0395	0.0403
10.0		0.0349	0.0330	0.3030	0.1741
12.5		0.0766	-0.0284	0.1215	0.1603
15.0		0.0385	0.0114	0.0288	0.3603
17.5		-0.0175	0.1229	-0.0689	0.2849
20.0		0.1516	0.0951	0.0517	0.1479
EMS (%)	Control	-0.0201	-0.0412	-0.0122	-0.1713
0.25		0.1436	-0.0339	0.0113	-0.0212
0.50		-0.0315	0.0719	0.3317	0.3712
0.75		0.0905	0.0639	0.1760	0.1300
1.00		0.4661	0.0995	0.0520	0.1289
1.25		0.1148	0.0861	0.0543	0.1340
1.50		0.1284	0.2226	0.0943	0.0590

Table 42 Path coefficient analysis showing indirect effect of rhizome number via other characters on yield under different mutagenic treatments

Treatments	No. of leaves	Leaf length	Leaf breadth	Plant spread
Gamma irradiation (Gy)				
Control	0.3635	0.1207	0.0411	-0.0264
2.5	0.0981	-0.0058	0.2225	0.1453
5.0	0.1097	0.2043	0.0915	0.2330
7.5	0.0409	0.2354	-0.0228	0.1596
10.0	0.1348	0.0487	0.2474	0.1348
12.5	0.2480	-0.0430	0.4996	0.0917
15.0	0.2700	0.0300	0.0612	0.1002
17.5	0.2958	-0.0316	0.0877	0.0426
20.0	0.0917	0.2700	0.0999	0.0442
EMS (%) Control	-0.0774	-0.0776	-0.0178	0.0663
0.25	0.1898	0.0087	0.0052	0.0095
0.50	0.2834	0.0732	0.2682	0.0478
0.75	0.1208	0.0584	0.1476	0.1819
1.00	0.1564	0.0967	0.0550	-0.0085
1.25	0.1133	0.0854	0.0464	0.3147
1.50	0.1036	0.2630	0.1771	0.0857

4.2.2.3.1 Gamma irradiation

4.2.2.3.1a Direct effects

Among the five component characters involved in the path coefficient analysis, the number of rhizomes exerted the highest positive direct effect (0.7415) on rhizome yield in the control population (Fig.6). The trend remained same when rhizomes were treated with 12.5, 15.0, 17.5 and 20.0 Gy gamma rays. Plant spread had maximum direct effect on yield at 2.5 Gy and 7.5 Gy gamma rays. At 5.0 Gy gamma rays leaf length exerted maximum direct effect and at 10.0 Gy leaf breadth had the maximum direct effect on yield (Table 37).

4.2.2.3.1b Indirect effects

Number of leaves, leaf length, leaf breadth, plant spread and number of rhizomes per plant had indirect effect on rhizome yield (Tables 38 to 42). Number of leaves exhibited indirect effects via length of leaf, leaf breadth, plant spread and rhizome number. Similarly leaf length, leaf breadth, plant spread and rhizome number also exerted indirect effects through the component traits. The effects were low in magnitude in the control population. At 7.5 Gy gamma ray treated population, the indirect effect of leaf breadth via plant spread is of larger magnitude (0.4852) (Table 40 and Fig.7). Similarly at 10.0 Gy the indirect effect of leaf breadth via rhizome number was larger in magnitude (0.9043) (Table 40). Indirect effects

X_1 - Number of leaves

X_2 - Leaf length

X_3 - Leaf breadth

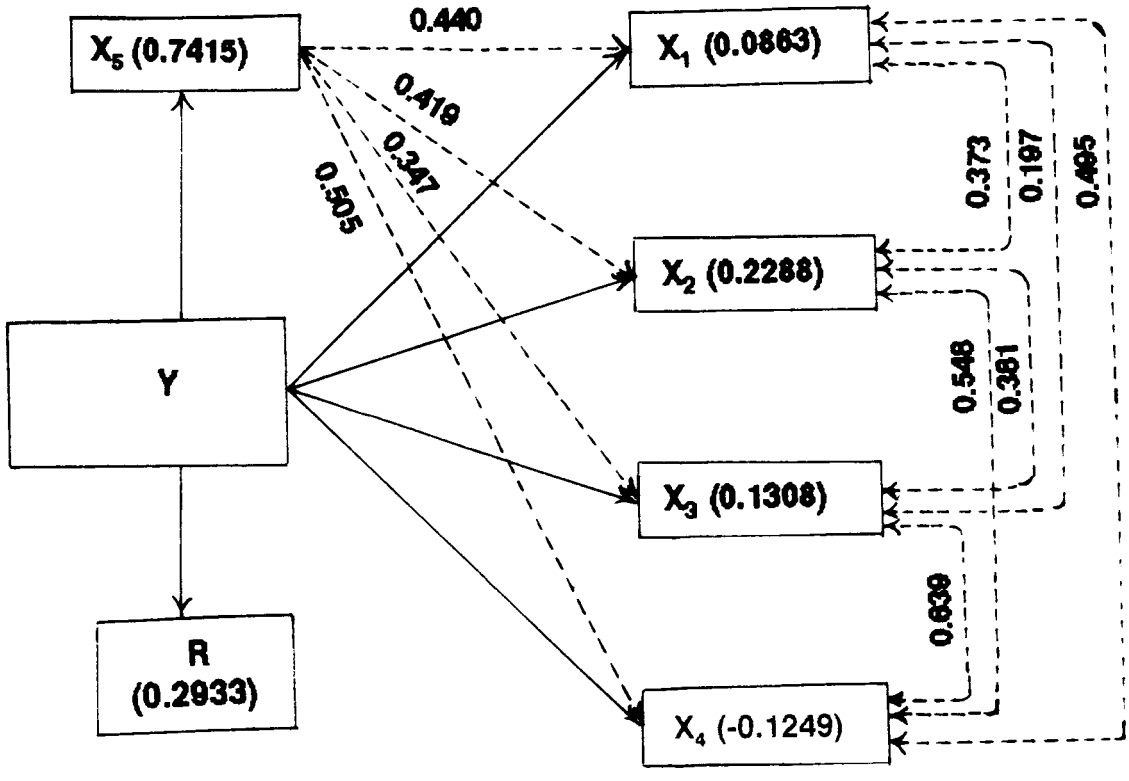
X_4 - Plant spread

X_5 - Rhizome number

Y - Rhizome yield

R - Residual effect

Fig.6 Path diagram showing direct and indirect effects of component characters on yield in kacholam (gamma experiment - control)



X_1 - Number of leaves

X_2 - Leaf length

X_3 - Leaf breadth

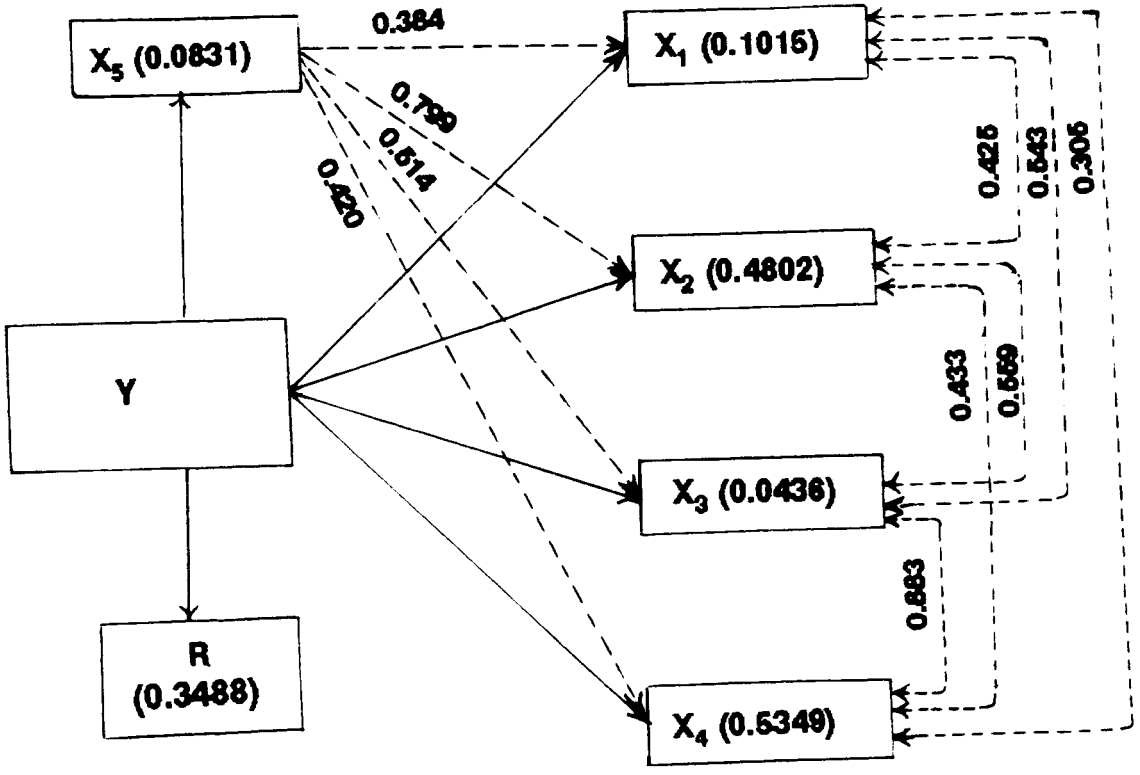
X_4 - Plant spread

X_5 - Rhizome number

Y - Rhizome yield

R - Residual effect

Fig.7 Path diagram showing direct and indirect effects of component characters on yield in 7.5 Gy gamma rays treated kacholam



of leaf length via rhizome number at 15.0 and 17.5 Gy gamma ray treated population is positive and of larger magnitude.

4.2.2.3.2 EMS

4.2.2.3.2a Direct effects

Rhizome number exhibited maximum direct effect (0.7379) on yield in the control as well as 0.5 and 0.75 per cent EMS treated population (Fig. 8 and 9). Number of leaves exerted maximum direct effect on yield when treated with 0.25 and one per cent EMS and leaf length had maximum direct effect when treated with 1.5 per cent EMS. Plant spread had maximum direct effect when treated with 1.25 per cent EMS (Table 37).

4.2.2.3.2b Indirect effects

Number of leaves exhibited indirect effect through leaf length, leaf breadth, plant spread and rhyzome number. Leaf length, leaf breadth, plant spread and rhizome number also exhibited indirect effects through component characters, in the control but of low magnitude. The mutagen treated population also maintained this relationship. When treated with one per cent EMS the indirect effect of plant spread through number of leaves is more (0.4661). Indirect effects of leaf breadth through number of leaves, plant spread and rhizome number also remained more when treated with 1.0, 1.25 and 0.5 per cent EMS (Tables 38 to 42).

X_1 - Number of leaves

X_2 - Leaf length

X_3 - Leaf breadth

X_4 - Plant spread

X_5 - Rhizome number

Y - Rhizome yield

R - Residual effect

Fig.8 Path diagram showing direct and indirect effects of component characters on yield in kacholam (EMS experiment - control)

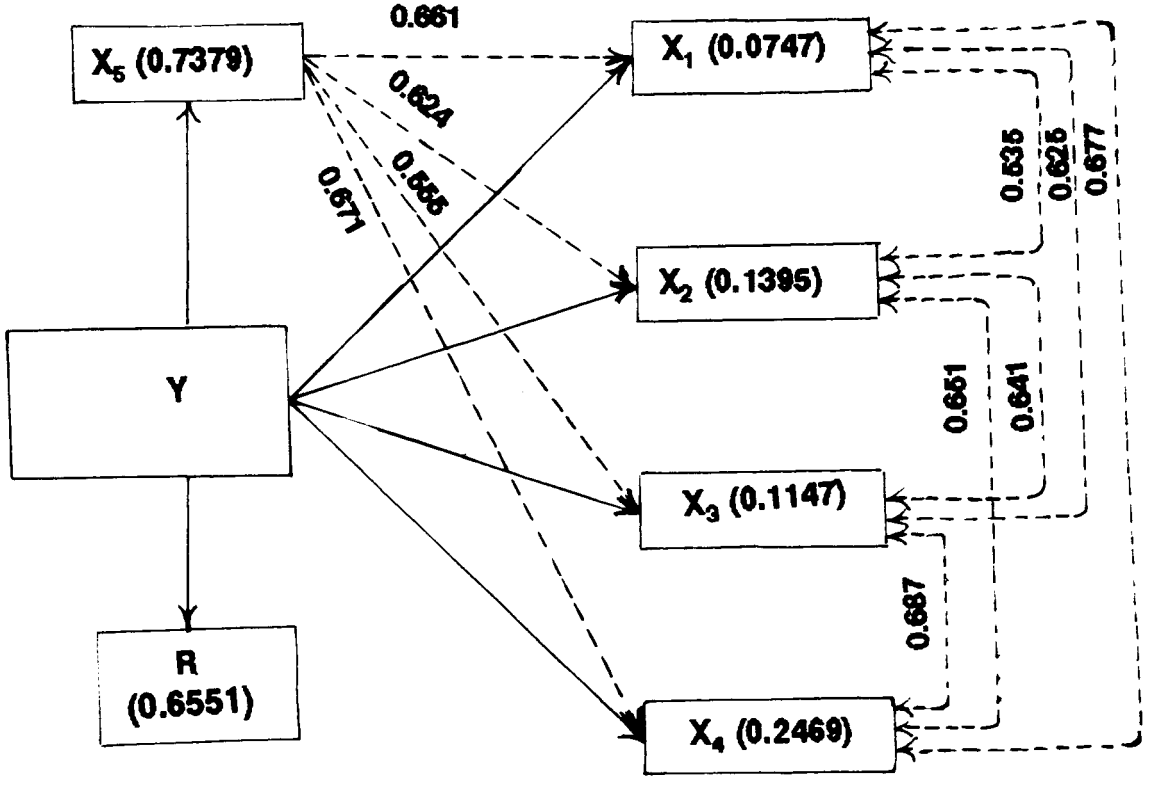
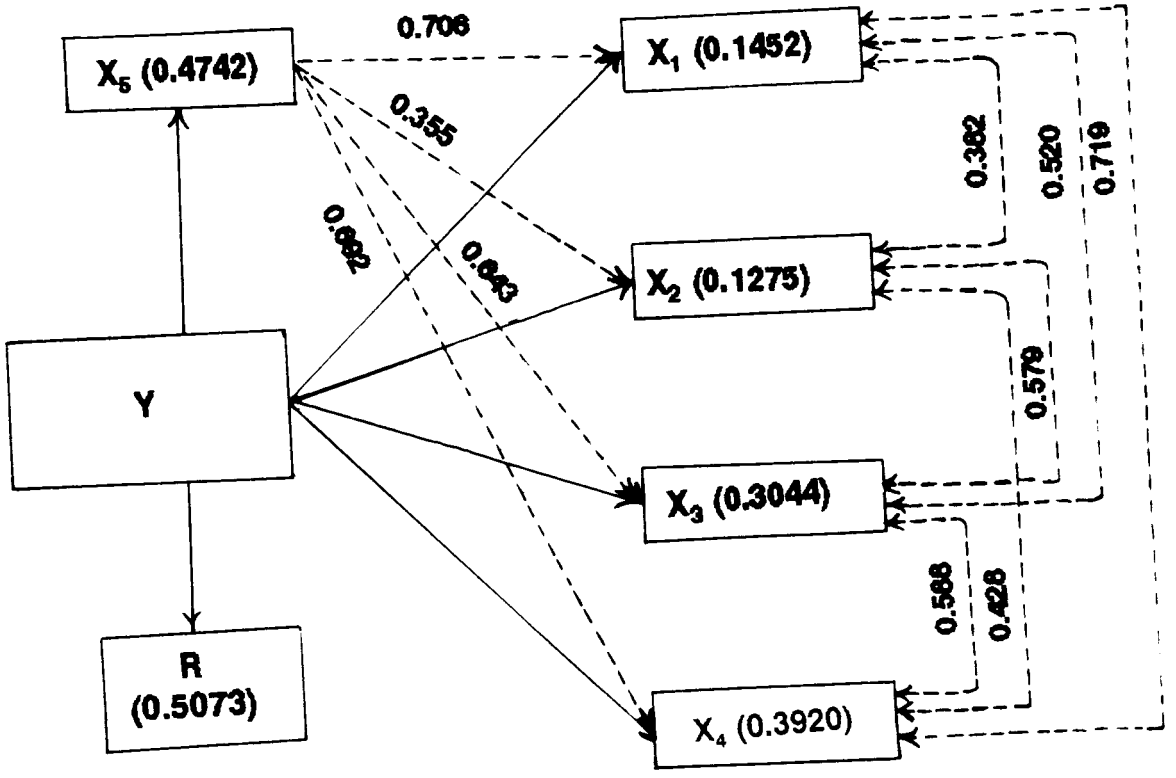


Fig.9 Path diagram showing direct and indirect effects of component characters on yield in 0.75 per cent EMS treated kacholam



4.2.2.4 Parent progeny correlation between MV_1 and MV_2

The parent progeny correlation between MV_1 and MV_2 generations in respect of eight characters viz., number of leaves, number of tillers, leaf length, leaf breadth, plant spread, rhizome number, rhizome yield and crop duration was estimated for all the treatments. The data on the correlation coefficients are presented in Table 43.

4.2.2.4.1 Number of leaves

Correlation between MV_1 and MV_2 generations in the control for number of leaves was not significantly different from control (Table 43). This trend was least affected by mutagen treatments except gamma ray treatment at 2.5 Gy (0.390). Gama rays at 15.0 Gy caused negative correlation (-0.350).

4.2.2.4.2 Number of tillers

There was significant correlation in number of tillers between MV_1 and MV_2 generation in the control (0.413) as well as in 2.5 Gy (0.378) and 10.0 Gy gamma rays (0.417). But in the case of EMS the there was no significant correlation between the control as well as mutagen treatments (Table 43).

Table 43 Parent progeny correlation for number of leaves, number of tillers, leaf length, leaf breadth, plant spread, number of rhizomes, rhizome weight and duration in kacholam

Treatments	LS No.	Tiller No.	Leaf length	Leaf breadth	Plant spread	Rhizome No.	Rhizome weight	Duration
Gamma irradiation (Gy)								
Control	-0.041	0.413*	0.009	0.207	0.066	0.133	0.030	0.238
2.5	0.390*	0.378*	0.400*	0.187	0.155	0.008	0.325	0.116
5.0	0.208	0.009	0.029	0.255	0.144	0.115	0.083	0.253
7.5	0.052	0.133	-0.430*	0.181	0.233	0.115	0.317	0.324
10.0	0.231	0.417*	0.116	0.142	0.284	0.236	0.546*	0.609*
12.5	0.121	0.073	0.067	-0.382*	0.206	0.160	0.073	0.052
15.0	-0.350*	0.120	0.139	0.219	-0.375*	-0.449*	0.206	0.329
17.5	0.035	0.300	0.259	0.288	0.018	0.085	0.023	0.007
20.0	0.032	0.013	0.165	0.067	0.0216	0.083	0.179	0.265
EMS (%)								
Control	0	0.066	0.523*	0.069	0.046	0.337	0.263	0.054
0.25	0.182	0.292	0.150	0.078	0.080	0.031	0.231	0.036
0.50	0.034	0.131	0.106	0.100	0.169	0.068	0.082	0.049
0.75	0.343	0.048	0.080	0.321	0.248	-0.383*	0.052	0.037
1.00	0.103	0.065	0.184	0.131	0.040	0.116	0.012	0.064
1.25	0.047	0.139	0.015	0.039	0.096	0.059	0.285	0.246
1.50	0.167	0.172	0.026	0.388*	0.183	0.133	0.052	0.151

* Significant at 0.05 level

4.2.2.4.3 Leaf length

The control plants of gamma rays, did not show any significant correlation between MV_1 and MV_2 in respect of leaf length. But the treated plants of 2.5 Gy gamma rays showed significant positive correlation (0.400), while gamma rays at 7.5 Gy caused negative correlation (-0.430).

In the case of EMS, treated population, the control plants showed positive significant correlation between MV_1 and MV_2, but the treatments altered this trend (Table 43).

4.2.2.4.4 Leaf breadth

There was no significant correlation between parent and progeny in the untreated control (Table 43) in respect of breadth of leaves. Gamma ray treatment at 12.5 Gy caused a negative correlation (-0.382), while EMS at 1.5 per cent caused significant positive correlation (0.388).

4.2.2.4.5 Plant spread

There was no significant positive correlation between the parent and progeny in the untreated control in respect of plant spread (Table 43). EMS treatments did not alter this trend, while gamma rays at 15.0 Gy caused a negative correlation (-0.375).

4.2.2.4.6 Rhizome number

There was no significant positive correlation in number of rhizomes, between parent and progeny in the control as well as in the gamma ray and EMS treated population. Gamma rays at 15.0 Gy (-0.449) and EMS at 0.75 per cent (-0.383) caused significant negative correlation (Table 43).

4.2.2.4.7 Rhizome weight

There was no significant correlation between MV_1 and MV_2 in the control for yield of rhizomes (Table 43). EMS treatment did not alter this trend. But gamma rays at 10.0 Gy (0.546) level caused significant positive correlation between the parent and the progeny.

4.2.2.4.8 Duration

There was no significant correlation in duration between MV_1 and MV_2 generation in control, as well as in EMS treatments (Table 43). But gamma rays at 10.0 Gy caused significant positive correlation.

4.2.2.5 Frequency distribution of variants in MV_2 generation

Plants exposed to gamma rays and EMS were grouped into three classes with respect to each of the growth parameters studied viz., number of leaves at maximum vegetative phase, plant spread (135 DAS), length and breadth of leaves, number and fresh weight of rhizomes per plant and crop duration (Table 44).

Table 44 Frequency distribution of variants in MV_2 generation

Treatments/ characters	Number of leaves			Plant spread (cm)			Leaf length (cm)		
	< 10	10-15	> 15	< 20	20-25	> 25	<12	12-16	> 16
Gamma irradiation (Gy)									
2.5	0	64.71	35.29	35.00	65.00	0	0	87.50	12.50
5.0	0	58.82	41.18	0	13.33	86.67	0	68.63	31.37
7.5	0	73.68	26.32	1.75	91.23	7.02	5.26	91.23	3.51
10.0	0	63.16	36.84	0	84.21	15.79	0	96.49	3.51
12.5	0	87.72	12.28	29.82	21.06	49.12	3.51	96.49	0
15.0	43.86	56.14	0	40.35	59.65	0	0	100.00	0
17.5	58.82	39.22	1.96	3.92	84.32	11.76	1.96	90.20	7.84
20.0	91.11	8.89	8.89	86.67	13.33	0	13.33	84.45	2.22
EMS (%)									
0.25	0	66.67	33.33	0	66.67	33.33	28.21	69.23	2.56
0.50	0	66.67	33.33	33.33	61.11	5.56	30.00	69.33	6.67
0.75	0	78.79	21.21	0	90.38	9.62	15.38	79.49	5.13
1.00	66.67	27.27	6.06	48.48	48.49	3.03	12.12	84.85	3.03
1.25	96.30	3.70	0	70.37	25.93	3.70	55.56	44.44	0
1.50	87.50	12.50	0	87.50	12.50	0	54.17	45.83	0

Contd...

Table 44 contd....

Treatments/ characters	Leaf length (cm)			Number of rhizomes			Fresh weight of rhizomes (g plant^{-1})			Duration (days)		
	< 8	8-11	> 11	< 10	10-15	> 15	< 40	40-60	> 60	< 200	200-225	> 225
Gamma irradiation(Gy)												
2.50	10.42	66.66	22.92	17.65	50.98	31.37	35.29	43.14	21.57	21.05	75.44	3.51
5.00	15.69	68.62	15.69	21.57	50.98	27.45	43.14	30.19	26.67	40.35	59.65	0
7.50	18.33	71.14	10.53	14.04	45.61	40.35	40.35	42.11	27.54	35.09	64.91	0
10.0	5.26	84.21	10.53	12.28	45.61	42.11	24.56	50.88	24.56	56.14	43.86	0
12.5	42.11	57.89	0	17.54	52.64	29.82	29.82	40.36	29.82	53.33	46.67	0
15.0	10.53	89.47	0	24.56	47.37	28.07	33.33	35.09	31.58	60.34	39.66	0
17.5	13.73	86.27	0	47.06	43.14	9.80	60.78	29.42	9.80	56.14	43.86	0
20.0	55.56	37.77	6.67	62.22	26.67	11.11	68.89	22.22	8.89	65.39	34.61	0
EMS (%)												
0.25	41.03	58.97	0	20.51	64.10	15.39	17.95	48.72	33.33	41.03	58.97	0
0.50	36.67	63.33	0	0	80.00	20.00	20.00	46.67	33.33	50.00	50.00	0
0.75	2.56	97.44	0	0	38.46	61.54	10.26	53.84	35.90	92.31	7.69	0
1.00	3.03	96.97	0	0	57.88	42.42	15.15	36.37	48.48	48.48	51.52	0
1.25	3.70	92.60	3.70	0	100.0	0	33.33	40.74	25.93	48.15	51.85	0
1.50	54.17	45.83	0	58.33	41.67	0	66.67	33.33	0	66.67	33.33	0

4.2.2.5.1 Number of leaves

The major type of mutation affecting leaf character was change in number of leaves. Normal plants had 10-15 leaves per plant. Plants producing less than 10 and more than 15 leaves were noticed in the mutagen treated population (Table 44).

The frequency of normal plants in the gamma ray treated population ranged from 8.89 per cent (20.0 Gy) to 87.72 per cent (12.5 Gy). Zero (2.5,5.0,7.5,10.0,12.5) to 91.11 per cent (20.0 Gy) produced less than 10 leaves per plant, while zero (15.0 Gy) to 41.18 per cent (5.0 Gy) were with more than 15 leaves.

In the case of EMS treated population 3.70 per cent (1.25%) to 78.79 per cent (0.75%) of plants were normal. When treated with 1.25 and 1.50 per cent EMS, there was no plant with more than 15 leaves, but majority were with less than 10 leaves (96.3% and 87.5% respectively). When treated with one per cent EMS three classes viz., less than 10 (66.67%), normal (27.27%) and more than 15 (66.67) were noticed.

4.2.2.5.2 Plant spread

The frequency of plants with low plant spread (<20 cm) ranged from zero (5.0 and 10.0 Gy) to 86.67% (20.0 Gy) in the case of gamma rays and that of EMS treated plants, the

range was zero (0.25%, 0.75%) to 87.50 (1.5%). The frequency of plants with normal plant spread (20-25 cm) ranged from 13.3 per cent (20.0 Gy, 5.0 Gy) to 91.23 per cent (7.5 Gy). In the case of EMS treated population the range was 12.5 (1.5%) to 90.38 per cent (0.75%). Frequency of plants with high plant spread (>25 cm) ranged from zero (2.5, 15.0 and 20.0 Gy) to 86.67 (5.0 Gy) in gamma rays and zero (1.5%) to 33.3 per cent (0.25%) in EMS treated plants (Table 44).

4.2.2.5.3 Leaf length

In gamma rays zero (2.5, 5.0, 10.0 and 15.0 Gy) to 13.33 per cent (20.0 Gy) of plants were with short leaves while in the case of EMS, the range was 12.12 (1.0%) and 55.56 (1.25% EMS). Hundred per cent of 15.0 Gy and 68.63 per cent of five Gy gamma ray treated population were normal. When treated with 1.25 and one per cent EMS, the percentages of normal plants were 44.44 and 84.85 respectively. The frequency of plants with long leaves were zero (12.5 and 15.0 Gy gamma rays) and 31.37 (5.0 Gy gamma rays). In the case of EMS also the frequency of plants with long leaves were zero (1.25 and 1.5%) and 6.67 per cent (0.5% EMS)(Table 44).

4.2.2.5.4 Leaf breadth

The frequency of plants with narrow leaves ranged between 5.26 per cent (10.0 Gy) to 55.56 per cent (20.0 Gy) in gamma rays and 2.56 per cent (0.75%) to

54.17 (1.50%) in EMS treated population. Again 37.77 per cent (20.0 Gy) to 89.47% (15.0 Gy) of gamma ray treated plants and 45.83 per cent (1.50%) to 97.44 per cent (0.75%) of the EMS treated ones were with normal leaves. Frequency of plants with broad leaves (>11 cm) ranged from zero (12.5, 15.0 and 17.5 Gy) to 22.92 per cent (2.5 Gy) in gamma ray treated population and zero (0.25, 0.50, 075, 1.00 and 1.50%) to 3.7 per cent (1.25%) in EMS treated population (Table 44).

4.2.2.5.5 Number of rhizomes

The range of plants producing less than 10 rhizomes was from 12.28 (10.0 Gy) to 62.22 per cent (20.0 Gy) for gamma ray treatments and from zero (0.5, 0.75, 1.0, 1.25) to 58.33 (1.5%) for EMS treatments. The gamma ray treated plants which had 10 to 15 rhizomes ranged from 26.67 per cent (20.0 Gy) to 52.64 (12.5 Gy) while the range was 38.46 per cent (0.75%) to 100 per cent (1.25%) for EMS treatments. Plants with more than 15 rhizomes per plant were 9.8 per cent (17.5 Gy) to 42.11 per cent (10.0 Gy) in the MV_2 progenies raised from gamma ray and zero (1.25% and 1.50%) to 61.54 (0.75%) in EMS treated plants (Table 44).

4.2.2.5.6 Fresh weight of rhizomes

The frequency of poor yielders with less than 40 g rhizome yield ranged between 24.56 (10.0 Gy) and 68.89 per cent (20.0 Gy) in gamma rays and between 10.26 (0.75%) and 66.67 (1.50%) in EMS treated population. The frequency

of normal plants (40-60 g) ranged between 22.22 per cent (20.0 Gy) and 50.88 per cent (10.0 Gy) in gamma rays and 33.33 (1.5%) and 53.84 (0.75%) in EMS treated population. High yields (>60 g/plant) were recorded for 8.89 per cent (20.0 Gy) to 31.58 per cent (15.0 Gy) in gamma ray treated population. The range for EMS treated high yielding population varied from 0 (1.5%) to 48.48 per cent (1.0%)(Table 44).

4.2.2.5.7 Crop duration

The frequency of short duration plants (<200 days) was between 21.05 per cent (2.5 Gy) and 65.39 (20.0 Gy) in gamma rays and 41.03 (0.25) and 92.31 (0.75%) in EMS. There were no long duration plants (>225 days) in the MV_2 progenies raised from plants treated with gamma rays and EMS, except in 2.5 Gy gamma rays (3.51%). A range from 34.61 per cent (20.0 Gy gamma rays) to 75.44 per cent (2.5 Gy) of the gamma ray treated plats was normal. In the case of EMS the range was 7.69 (0.75%) to 58.97 (0.25%) (Table 44).

4.2.2.6 Morphological variants

Plants with cluster of leaves, crinkled leaves, long slender rhizomes and short round rhizomes were observed in MV_1 and MV_2 population (Table 45). Among the gamma ray treated plants 2.71 (MV_1) and 3.23 (MV_2) per cent and among the EMS treatments 1.67 (MV_1) and 1.95 (MV_2) per cent were with cluster of leaves. Plants possessing crinkled leaves

Table 45 A comparison of morphological variants in MV_1 and MV_2 population

Treatments	Morphological variants (%)							
	Cluster of leaves		Crinkled leaves		Long slender rhizome		short round rhizome	
	MV_1	MV_2	MV_1	MV_2	MV_1	MV_2	MV_1	MV_2
Gamma irradiation(Gy)								
2.5	0	0	3.33	4.17	1.67	1.67	3.33	3.33
5.0	3.33	6.67	1.67	1.67	0	0	1.67	2.50
7.5	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
10.0	5.00	5.0	1.67	4.17	3.33	3.33	1.67	1.67
12.5	0	0	0	0	3.33	6.67	3.33	3.33
15.0	5.00	5.83	0	0	0	0	0	0
17.5	1.67	1.67	3.33	3.33	0	0	0	0
20.0	5.00 (2.71)	5.00 (3.23)	1.67 (1.67)	2.50 (2.19)	1.67 (1.46)	3.33 (2.08)	3.33 (1.88)	7.50 (2.50)
EMS (%)								
0.25	3.33	3.33	0	0	1.67	1.67	3.33	4.17
0.50	1.67	1.67	0	0	0	0	3.33	4.17
0.75	1.67	1.67	3.33	3.33	1.67	2.50	1.67	1.67
1.00	0	0	1.67	1.67	3.33	4.17	6.67	6.67
1.25	1.67	3.33	3.33	3.33	0	0	5.0	5.83
1.50	1.67 (1.67)	1.67 (1.95)	1.67 (1.67)	1.67 (1.67)	1.67 (1.39)	1.67 (1.67)	3.33 (3.89)	4.17 (4.45)

were also observed in the mutagen treated population. Of the total population 1.67 (MV_1) and 2.19 (MV_2) per cent of gamma irradiated and 1.67 (MV_1 and MV_2) per cent in EMS treatments showed this type of leaf character. Variations in rhizome size and shape could be observed due to mutagenic treatments (Plates 7, 8 and 9). The percentages of long slender rhizomes in gamma ray treated populations were 1.46 (MV_1) and 2.08 (MV_2) and that of EMS were 1.39 (MV_1) and 1.67 (MV_2). The percentages of plants with short round rhizomes were 1.88 (MV_1) and 2.50 (MV_2) per cent of gamma ray treated population and 3.89 (MV_1) and 4.45 (MV_2) per cent of EMS treated population.

4.2.2.7 Chlorophyll mutants

The variegation noted on leaves due to chlorophyll deficiency was considered as chlorophyll mutants (Plates 10 and 11). Such mutants were observed to occur in MV_1 and MV_2 generation at the initial stages of development. Gamma rays at 10.0 Gy and EMS at 1.50 per cent did not produce any chlorophyll mutants (Table 46).

4.2.3 Effect of mutagens on MV_3 generation

Based on the variability expressed in MV_2 population for single character or combination of two or more characters simultaneously, 40 probable mutants (31 from gamma irradiated and nine from EMS treated) were identified and classified under thirteen groups (Table 47). The

Plate 7 Rhizomes of kacholam - control


Control

Plate 8 Rhizomes of kacholam treated with 7.5 Gy gamma rays

Plate 9 Rhizomes of kacholam treated with 0.75 per cent EMS


7.5 Gy

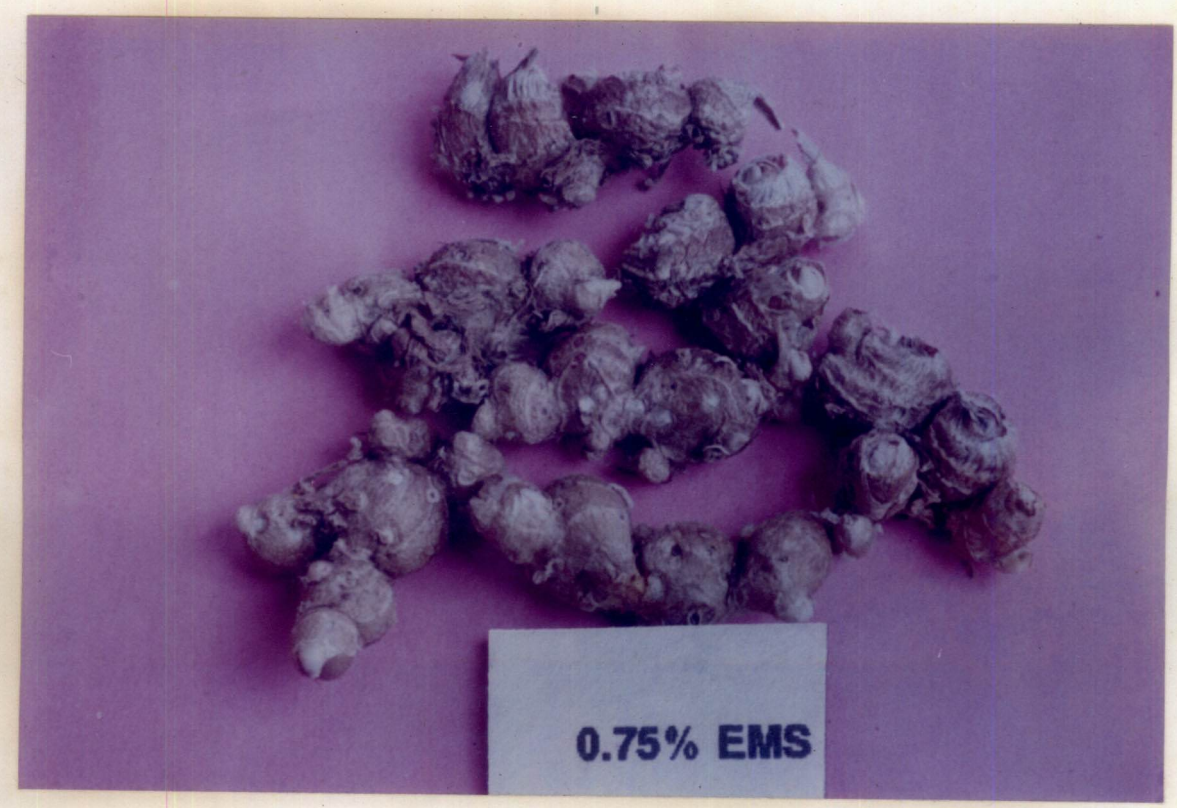

0.75% EMS

Plate 10 Chlorophyll deficient kacholam (15.0 Gy gamma rays)

Plate 11 Chlorophyll deficient kacholam (0.25 per cent EMS)


15.0 Gy


0.25%EMS

Table 46 A comparison of chlorophyll deficient mutants in MV_1 and MV_2 population

Treatments	Chlorophyll deficient mutants (percentage)	
	MV_1	MV_2
Gamma irradiation (Gy)		
2.5	3.33	1.11
5.0	0	0
7.5	3.33	1.48
10.0	0	0
12.5	5.00	0.37
15.0	1.67	0.74
17.5	1.67	0.74
20.0	6.67	0.74
EMS (%)		
0.25	6.67	2.22
0.50	3.33	1.11
0.75	3.33	1.11
1.00	10.00	0.55
1.25	6.67	3.33
1.50	0	0

Table 47 Classification of probable mutants in MV_2 geneation

Mutant groups	Number of variants under														
	Gamma rays (Gy)								EMS (percentage)						
	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	0.25	0.50	0.75	1.00	1.25	1.50	Total
I	-	1	1	-	-	1	2	-	-	-	1	-	-	-	6
II	-	1	1	-	2	-	-	-	-	-	-	-	2	-	6
III	-	-	1	-	-	-	-	-	-	-	-	1	-	-	2
IV	-	1	-	-	-	1	-	-	-	-	-	1	-	-	3
V	-	-	1	1	-	1	-	1	-	-	1	-	-	-	5
VI	-	1	-	1	-	-	3	1	-	-	-	-	-	-	6
VII	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
VIII	-	-	1	-	-	-	-	1	-	-	-	-	-	-	2
IX	-	-	1	1	-	1	-	-	-	-	-	1	-	1	5
X	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
XI	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
XII	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
XIII	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
TOTAL	-	4	6	4	4	4	5	4	-	-	2	3	3	1	40

I High leafy mutants
II Tiller mutants
III Long leaf mutants
IV Broad leaf mutants
V Plant spread mutants
VI Early mutants
VII Plant spread, long leaf mutants
VIII Plant spread broad leaf
IX Plant spread more leaves broad leaf mutants
X Rhizome number, yield and early mutants
XI Long leaf, plant spread, rhizome number and yield
XII High leaf, tiller, rhizome number and yield
XIII Plant spread, rhizome number, yield and early mutants

selected plants were positive variants as compared the control with respect to the yield attributing characters like number of leaves, tillers, leaf length, leaf breadth, plant spread, rhizome number, yield and duration (Table 48). The third vegetatively propagated generation was raised to test further the performance of the selected entries from MV_2 generation.

4.2.3.1 High leafy mutants

Six plants were located in MV_2 mutagen treated population as high leafy mutants with more than 20 leaves per plant. In MV_3, progenies of all the mutagen treated plants except plant number 2107 expressed the character. A total of 57 per cent of the progeny of plant number 3119 produced large number of leaves (Table 49). These plants can be considered as variants since they are not fully expressing the character.

4.2.3.2 Tiller mutants

Six plants of mutagen treated MV_2 population having more than seven tillers per plant were selected. In MV_3, progenies of plants selected from plant number 734 failed to express the trait which can be considered as an unstable mutant. All other plants expressed the character (Table 49).

Table 48 Rhizome yield and yield attributes in the selected plants in MV_2 population

Sl. No.	Plant No.	Number of leaves (135 DAS)	Number of tillers (135 DAS)	Leaf length (cm)	Leaf breadth (cm)
1	2107	21	6	19.7	10.7
2	2016	20	6	18.4	11.2
3	1020	20	6	17.5	11.5
4	3119	21	5	18.6	10.6
5	1738	20	6	19.3	11.1
6	874	20	6	19.2	9.8
7	1127	18	7	17.5	9.4
8	1520	19	7	18.1	10.1
9	3315	19	8	18.0	10.6
10	734	18	7	19.4	10.5
11	1571	17	7	19.0	9.8
12	3370	19	8	19.2	9.1
13	3218	17	5	21.5	10.0
14	1022	17	6	22.0	9.4
15	1772	19	6	18.4	12.7
16	912	17	5	18.6	12.2
17	3170	18	6	18.5	12.4
18	2374	18	6	17.2	9.8
19	1888	17	6	18.1	9.9
20	1084	18	6	18.2	8.6

Contd....

Table 48 contd...

Sl. No.	Plant No.	Number of leaves (135 DAS)	Number of tillers (135 DAS)	Leaf length (cm)	Leaf breadth (cm)
21	1373	17	6	19.7	10.6
22	3115	17	5	19.2	11.1
23	822	18	6	18.4	10.2
24	2118	19	6	18.1	9.4
25	2124	18	5	17.6	9.3
26	2363	17	5	17.2	9.4
27	2098	18	5	18.0	10.2
28	1422	18	6	18.2	10.1
29	1324	18	6	22.0	11.4
30	2180	18	5	18.6	12.1
31	978	19	6	19.7	12.3
32	1821	21	6	19.0	12.0
33	3572	20	6	17.2	12.2
34	3210	22	5	18.1	12.4
35	1322	21	6	19.6	12.1
36	1010	21	6	19.0	12.2
37	914	19	6	18.0	10.1
38	1063	18	5	22.0	10.6
39	3373	22	8	18.9	10.5
40	2215	18	5	19.1	11.3

Contd....

Table 48 contd....

Sl. No.	Plant No.	Plant spread (cm)	Rhizome number	Rhizome yield (g plant^{-1})	Duration (days)
1	2107	22.3	14	64.5	195
2	2016	24.6	17	70.3	195
3	1020	20.8	18	68.5	195
4	3119	20.7	19	62.9	195
5	1738	22.1	14	67.6	195
6	874	23.2	13	71.8	192
7	1127	20.5	9	78.4	192
8	1520	19.4	18	59.3	195
9	3315	22.0	17	62.2	200
10	734	24.5	15	65.5	200
11	1571	20.0	15	69.0	195
12	3370	20.2	12	78.0	195
13	3218	18.3	14	64.2	200
14	1022	21.7	15	65.0	200
15	1772	19.8	11	71.3	195
16	912	20.4	12	70.0	200
17	3170	22.0	13	63.0	195
18	2374	25.2	16	72.6	195
19	1888	25.4	10	70.8	200
20	1084	25.0	19	75.0	200

Contd....

Table 48 contd...

Sl. No.	Plant No.	Plant spread (cm)	Rhizome number	Rhizome yield (g plant^{-1})	Duration (days)
21	1373	26.5	17	78.3	192
22	3115	26.0	18	72.2	192
23	822	23.7	14	75.8	185
24	2118	20.8	19	63.5	190
25	2124	20.4	17	60.6	185
26	2363	20.5	15	59.8	185
27	2098	19.6	16	66.9	185
28	1422	18.9	12	70.5	190
29	1324	25.2	11	72.0	195
30	2180	25.8	17	75.0	200
31	978	25.3	13	78.3	192
32	1821	25.7	18	76.2	195
33	3572	26.0	15	75.1	195
34	3210	25.3	16	74.3	195
35	1322	25.8	14	71.6	195
36	1010	26.1	12	70.3	200
37	914	23.7	22	89.5	190
38	1063	25.9	21	91.0	190
39	3373	20.0	20	82.5	185
40	2215	26.4	24	85.7	185

4.2.3.3 Long leaf mutants

Two plants with extra long leaves (more than 20 cm) were identified as mutants from gamma rays and EMS treated population. The character was transferred to the progenies of the selected plants (Table 49).

4.2.3.4 Broad leaf mutants

Three plants from MV_2 generation were located with extra broad leaves (more than 12 cm). The progenies of plant numbers 912 and 3170 expressed the character of broad leaves (Table 49). Progenies of plant number 1772 did not express the character and can be considered as a variant.

4.2.3.5 Plant spread mutants

Five plants were identified from MV_2 population as mutants with more than 25 cm spreading. The progenies evaluated showed that 100 per cent (Plant No. 2374 and 3115), 57 per cent (Plant No. 1373), 40 per cent (Plant No. 1084) and 36 per cent (Plant No. 1888) of the progenies inherited the character which showed that only plant numbers 2374 and 3115 were stable mutants (Table 49).

4.2.3.6 Short duration mutants

Six plants of gamma rays identified as short duration having less than 190 days duration were ear marked to study the inheritance of the character. All the progenies of the selected plants expressed the character (Table 49).

Table 49 Percentage inheritance of characters of selected MV_2 plants

Sl. No.	Plant No.	Mutant character/ characters	Number of plants in MV_3	Number that expressed the mutant character	Percentage of inheritance
1	2107	High leaf	8	0	0
2	2016	"	7	7	100
3	1020	"	7	7	100
4	3119	"	7	4	57
5	1738	"	6	6	100
6	874	"	6	6	100
7	1127	Tiller	6	6	100
8	1520	"	7	7	100
9	3315	"	9	9	100
10	734	"	6	0	0
11	1571	"	7	7	100
12	3370	"	4	4	100
13	3218	Long leaf	7	7	100
14	1022	"	5	5	100
15	1772	Broad leaf	7	0	0
16	912	"	5	5	100
17	3170	"	6	6	100
18	2374	Plant spread	8	8	100
19	1888	"	11	4	36
20	1084	"	10	4	40

Contd....

Table 49 contd...

Sl. No.	Mutant No.	Mutant character/ characters	Number of plants in MV_3	Number that expressed the mutant character	Percentage of inheritance
21	1373	Plant spread	7	4	57
22	3115	"	7	7	100
23	822	Early	8	8	100
24	2118	"	7	7	100
25	2124	"	7	7	100
26	2363	"	6	6	100
27	2098	"	6	6	100
28	1422	"	6	6	100
29	1324	Plant spread - long leaf	6	6	100
30	2180	Plant spread - broad leaf	6	0	0
31	978	"	7	0	0
32	1821	Plant spread - high leaves - broad leaf	5	0	0
33	3572	"	6	0	0
34	3210	"	7	4	57
35	1322	"	7	0	0
36	1010	"	9	6	67
37	914	Rhizome number - yield early	10	3	30
38	1063	Long leaf - plant spread - rhizome number - yield	8	3	38
39	3373	High leaves - tiller - rhizome number - yield	9	2	22
40	2215	Plant spread - rhizome number - yield - early	8	4	50

4.2.3.7 Plant spread - long leaf mutants

Only one plant with more than 25 cm plant spread and 20 cm leaf length was ear marked in MV_2. In MV_3 generation all progenies of the selected plant inherited the characters together (Table 49).

4.2.3.8 Plant spread - broad leaf mutants

Two plants were located in MV_2 with mutant plant spread and broad leaf characters. But the progenies failed to express the characters together. Hence these plants can be considered as variants (Table 49).

4.2.3.9 Plant spread - high leaves - broad leaf mutants

Five plants exhibiting these traits were selected from MV_2. Fifty seven per cent of the progenies of 3210 and 67 per cent of the progenies of 1010 expressed all the characters together. Others failed to express the traits (Table 49).

4.2.3.10 Rhizome number - yield and early mutants

A single plant from gamma rays treated population was identified with more than 20 rhizomes and 80 g rhizome weight per plant and maturing in less than 190 days. MV_3 studies indicated that 30 per cent of the progenies of the plant exhibited all the mutant characters together (Table 49).

4.2.3.11 Long leaf - plant spread - rhizome number and yield mutants

Plant number 1063 was identified with more than 20 cm leaf length, 25 cm spreading, producing more than 20 rhizomes with 80 g fresh weight of rhizomes in MV_2. This was carried forward to MV_3 to study the inheritance of traits and 38 per cent of the progenies expressed all the four characters together (Table 49).

4.2.3.12 High leaf - tiller - rhizome number and yield mutants

A single plant from EMS treated MV_2 population with mutant characters was identified and 22 per cent of MV_3 progenies expressed all the four characters together (Table 49).

4.2.3.13 Plant spread - rhizome number - yield - and early mutants

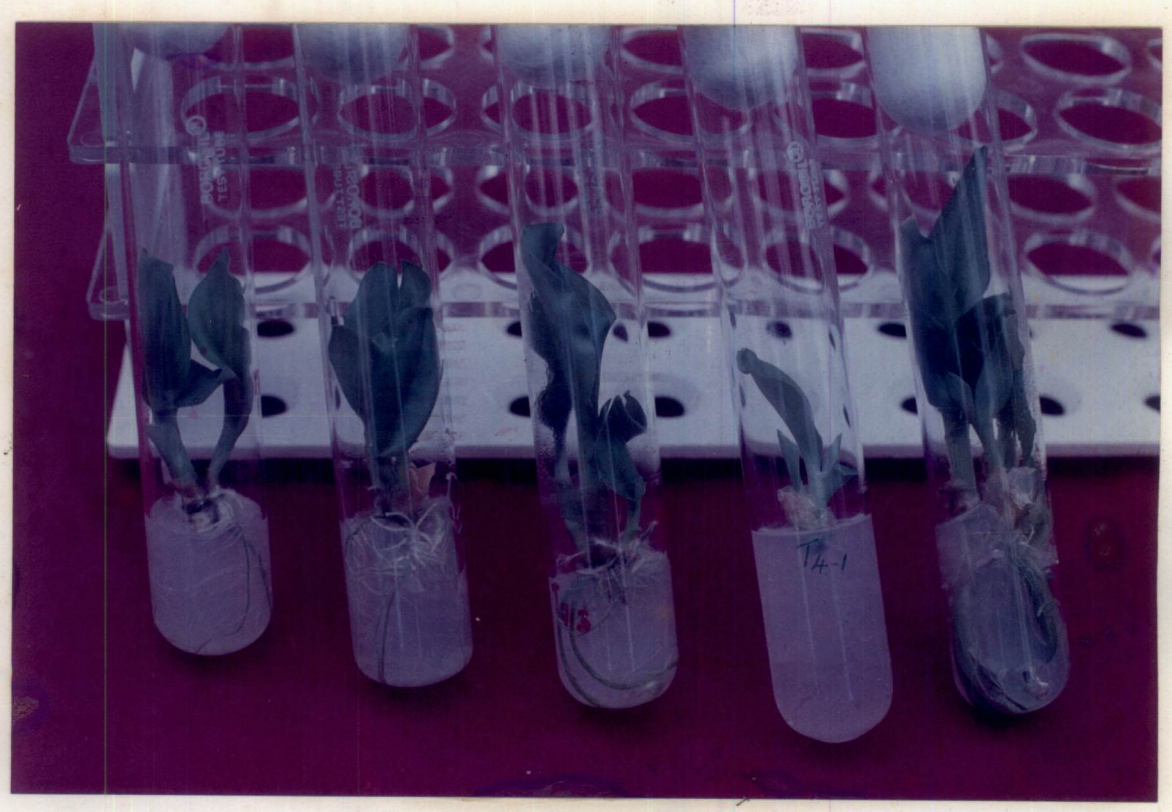
A single plant was identified in MV_2 population with four mutant characters together and 50 per cent of the progenies expressed all the characters together in MV_3 (Table 49).

4.2.4 Micropropagation

Axillary buds isolated from rhizomes of *Kaempferia galanga* indicated direct shoot proliferation when cultured in MS medium (Plate 12). All the regenerates were deep green and healthy in appearance. Healthy roots were also induced with the multiplication of shoots (Plate 13).

Plate 12 Different stages of micropropagation in kacholam

Plate 13 Micropropagated kacholam ready for planting out


T4-1

Small roots were visible from the basal part of the regenerated shoots after 20 to 30 days of culture. The rooted plantlets isolated from cultures were transferred to garden pots and kept in the green house till they become fully developed (Plate 14).

4.2.5 Causes for failure of seed set

4.2.5.1 Pollination studies

In order to find out the causes for failure of seed set in *Kaempferia galanga* different pollination techniques like artificial self pollination, artificial sibbing, artificial cross pollination, bud pollination, mentor pollination, chemically aided pollination and removal of stigma and artificial self pollination were employed to overcome the barriers in seed set. All the techniques failed to give any positive result on seed set.

4.2.5.2 Reproductive biology

The influence of mutagens on the reproductive biology of the crop was examined and the results are presented in Tables 50 and 51.

4.2.5.2.1 Number of days for flowering

The influence of mutagens on flowering is depicted in Table 50. Statistical analysis of the data showed significant difference among treatments.

Plate 14 Micropropagated kacholam in pots

Number of days for flowering decreased with increase in doses/concentrations of mutagens. It showed a variation from 52.0 days (15.0 Gy gamma rays) to 58.9 in untreated control. In the case of EMS treated population also the maximum number of days for flowering was in control (58.4) which was followed by and on par with 0.25 per cent EMS treatment. The rhizomes treated with the highest dose of 1.5 per cent EMS took only 52.4 days for flowering and was on par with 1.25 per cent which needed 52.8 days for flowering. The percentage variation over control gave negative values in all the treatments.

4.2.5.2.2 Time of anthesis

The influence of gamma rays and EMS on time of anthesis is depicted in Table 50.

There was no significant difference between the different treatments. From the Table it was found that in the case of control, 2.5 Gy and 5.0 Gy gamma rays treated population, the flower opening took place at 3.50 hours. As the dose level increased, there was variation in the time of anthesis. In plants treated with 7.5, 10.0 and 17.5 Gy gamma rays, the flower opening took place at four hours and in the rest of treatments the opening time was 4.03 hours.

Table 50 Effect of mutagens on floral characteristics in MV_1 generation of kacholam

Treatments	Days for flowering		Time of anthesis		Pollen fertility		Pollen size		Style length	
	Mean	Percentage variation over control	Mean (hours)	Percentage variation over control	Mean (%)	Percentage variation over control	Mean (µm)	Percentage variation over control	Mean (mm)	Percentage variation over control
Gamma irradiation (Gy)										
Control	58.9	0	3.50	0	70.5 (57.10)	0	108.8	0	45.0	0
2.5	56.2	-4.58	3.50	0	62.3 (52.12)	-11.63	107.5	-1.20	46.6	1.33
5.0	54.2	-7.98	3.50	0	59.0 (50.18)	-16.31	107.0	-1.65	45.2	1.78
7.5	53.7	-8.83	4.0	4.35	56.6 (48.79)	-19.72	106.8	-1.84	45.8	1.78
10.0	53.4	-9.34	4.0	4.35	51.9 (46.09)	-26.38	104.8	-3.68	46.2	2.67
12.5	53.1	-9.85	4.03	5.65	50.8 (45.46)	-27.94	102.4	-5.88	46.1	2.44
15.0	52.0	-11.71	4.03	5.65	47.9 (43.80)	-32.06	102.3	-5.97	46.3	2.89
17.5	52.6	-10.70	4.0	4.35	48.0 (43.85)	-31.91	101.6	-6.62	46.6	3.56
20.0	52.3	-11.21	4.03	5.65	46.9 (43.22)	-33.48	101.3	-6.89	47.0	4.44
CD (0.05)	(0.9546)		(0.2401)		(1.7199)		(5.1586)		NS	

Table 50 contd....

EMS Control (%)	58.4	0	3.50	0	71.9 (57.99)	0	110.6	0	44.0	0
0.25	58.1	-0.51	3.50	0	67.7 (55.37)	-5.84	108.3	-2.08	44.2	0.45
0.50	55.5	-4.97	4.0	4.35	65.6 (54.09)	-8.76	106.0	-4.16	44.5	1.14
0.75	55.0	-5.82	4.05	6.52	59.5 (50.48)	-17.25	104.7	-5.33	45.6	3.64
1.00	54.2	-7.19	4.05	6.52	57.8 (49.49)	-19.61	103.3	-6.60	46.0	4.55
1.25	52.8	-9.59	4.10	8.70	49.2 (44.54)	-31.57	101.7	-8.05	46.6	5.91
1.50	52.4	-10.27	4.10	8.70	43.0 (40.98)	-40.19	101.3	-8.41	46.7	6.14
CD (0.05)	(0.5299)		(0.0501)		(1.8521)		(5.5789)		NS	

Numbers in parenthesis denote transformed values

In control and in plants treated with 0.25 per cent EMS, the flower opening was comparatively earlier and took place at 3.50 hours. At 1.25 per cent and 1.5 per cent EMS flower opening took place at 4.10 hours. At 0.5 per cent the flower opening took place at 4.00 hours. At 0.75 and 1.0 per cent EMS flowers open at 4.05 in the morning.

4.2.5.2.3 Pollen fertility

Pollen fertility among the different doses/ concentrations of mutagens as assessed by stainability in acetocarmine ranged from 46.9 (20.0 Gy) to 70.5 per cent (control) in gamma rays and 43.0 per cent (1.5%) to 71.9 (control) in EMS treated plants (Table 50).

The percentage variation over control gave negative values in all the treatments.

4.2.5.2.4 Pollen size

Size of pollen grains as influenced by different doses/concentrations of mutagens are furnished in Table 50.

Statistical analysis of the data showed significant difference among the treatments in respect to both the mutagens tried. A gradual reduction in the size of pollen grains was observed with increase in doses/concentrations of mutagens. Size of pollen grains ranged from 101.3 µ m when exposed to 20.0 Gy gamma rays to 108.8 µ m in untreated control. In the case of EMS treated population

the maximum size of pollen grains of 110.60 μ m was recorded by the control and was significantly superior to the treatments with 0.75, 1.0, 1.25 and 1.5 per cent EMS and was on par with the rest of the treatments 0.25 and 0.5 per cent EMS.

4.2.5.2.5 Style length

Observation on style length measured after anthesis of plants treated with different doses/concentrations of mutagens, along with relative percentage over control are presented in Table 50.

A gradual increase in style length with increase in dosage/concentration was observed. The control plants recorded 45 mm style length. When exposed to 20.0 Gy gamma rays the style length was 47 mm. A style length of 46.7 mm was observed when treated with 1.5 per cent EMS which was followed by and on par with 1.25 and 1.0 per cent EMS with 46.6 and 46.0 mm respectively.

4.2.5.2.6 Pollen viability

Viability of pollen grains was estimated in different germination media and the results are presented in Table 51. The control plants recorded maximum viability of pollen grains (52.4%) in medium containing eight per cent sucrose, 60 ppm boric acid and one per cent gelatin and minimum (20.1%) in distilled water. The same trend was observed in mutagen treated plants (Table 51).

Table 51 Effect of mutagens on pollen viability in different germination media

Treatments	Pollen germination							
	Distilled water		8% S + 60 ppm BA		15% S + 60 ppm BA		30% S + 60 ppm BA	
	Mean	Percentage variation over control	Mean	Percentage variation over control	Mean (hours)	Percentage variation over control	Mean (%)	Percentage variation over control
Gamma irradiation (Gy)								
Control	20.1 (26.64)	0	38.0 (38.06)	0	38.6 (38.41)	0	42.3 (40.57)	0
2.5	17.9 (25.03)	-10.95	30.5 (33.52)	-19.74	34.5 (35.97)	-10.62	41.7 (40.22)	-1.42
5.0	17.2 (24.50)	-14.43	28.1 (32.01)	-26.05	32.6 (34.82)	-15.54	41.2 (39.93)	-2.60
7.5	15.8 (23.42)	-21.39	29.6 (32.90)	-22.11	30.7 (33.65)	-20.47	40.8 (39.70)	-3.55
10.0	15.4 (23.11)	-23.38	30.7 (33.65)	-19.21	29.3 (32.77)	-24.09	37.5 (37.76)	-11.35
12.5	13.3 (21.39)	-33.83	24.9 (29.93)	-34.47	26.5 (30.98)	-31.35	33.6 (35.43)	-20.57
15.0	12.6 (20.79)	-37.31	23.7 (29.13)	-37.63	27.9 (31.88)	-27.72	32.4 (34.70)	-23.40
17.5	11.8 (20.05)	-41.29	19.1 (25.91)	-49.74	23.0 (28.66)	-40.41	27.5 (31.63)	-35.99
20.0	9.1 (17.56)	-54.73	15.4 (23.11)	-59.47	20.8 (27.13)	-46.11	23.7 (29.13)	-43.97
CD (0.05)	(3.405)		(3.7326)		(4.2987)		(2.7664)	

Contd...

Table 51 contd.....

EMS Control (%)	19.1 (25.91)	0	33.7 (35.49)	0	37.2 (37.52)	0	40.8 (39.70)	0
0.25	14.8 (22.63)	-22.51	31.3 (34.02)	-7.17	38.2 (38.17)	-12.77	38.6 (38.41)	-5.56
0.50	14.1 (22.06)	-26.18	29.3 (32.77)	-13.12	34.4 (35.91)	-7.46	35.6 (36.63)	-12.84
0.75	13.7 (21.72)	-28.27	23.4 (28.93)	-30.61	29.2 (32.71)	-21.45	33.7 (35.49)	-17.39
1.00	12.6 (20.79)	-34.03	22.2 (28.11)	-34.17	25.3 (30.20)	-31.82	30.0 (33.21)	-26.63
1.25	11.2 (19.55)	-41.36	20.3 (26.78)	-39.94	24.5 (29.67)	-34.08	28.0 (31.95)	-31.46
1.50	9.2 (17.66)	-51.83	18.3 (25.35)	-45.73	20.5 (26.92)	-44.71	22.3 (28.18)	-44.56
CD (0.05)	(2.725)		(2.4655)		(4.0467)		(1.5090)	

Contd....

Table 51 contd....

Treatments	Pollen germination					
	8% S + 60 ppm BA + 1% GA		15% S + 60 ppm BA + 1% GA		30% S + 60 ppm BA + 1% GA	
	Mean	Percentage variation over control	Mean	Percentage variation over control	Mean	Percentage variation over control
Gamma control	52.4 (46.38)	0	46.4 (42.94)	0	23.8 (29.20)	0
Irradiation (Gy)						
2.5	50.1 (45.06)	-4.39	44.8 (42.02)	-3.45	24.1 (29.40)	1.26
5.0	47.8 (43.74)	-8.78	42.7 (40.80)	-7.97	22.3 (28.18)	-6.30
7.5	46.6 (43.05)	-11.07	42.3 (40.57)	-8.84	22.2 (28.11)	-6.72
10.0	45.4 (42.36)	-13.36	41.2 (39.93)	-11.21	20.7 (27.06)	-13.03
12.5	41.7 (40.22)	-20.42	40.8 (39.70)	-13.58	20.4 (26.85)	-14.29
15.0	41.1 (39.87)	-21.56	40.8 (39.70)	-13.58	19.5 (26.21)	-18.07
17.5	39.2 (38.76)	-25.19	36.6 (37.23)	-21.12	18.8 (25.70)	-21.01
20.0	33.1 (35.12)	-36.83	28.8 (32.46)	-37.93	14.5 (22.38)	-39.08
CD (0.05)	(2.402)		(2.166)		(2.3683)	

Contd....

Table 51 contd....

EMS Control (%)	49.9 (44.94)	0	44.2 (41.67)	0	25.4 (30.26)	0
0.25	47.9 (43.80)	-4.08	42.6 (40.76)	-3.78	23.0 (28.66)	-9.45
0.50	45.7 (42.53)	-8.49	41.7 (40.22)	-5.72	22.1 (28.04)	-12.99
0.75	42.8 (40.86)	-14.31	39.5 (38.94)	-10.63	17.9 (25.03)	-29.53
1.00	38.5 (38.95)	-22.94	37.6 (37.82)	-14.93	17.6 (24.73)	-30.71
1.25	35.3 (36.45)	-29.33	34.6 (36.03)	-21.87	16.5 (23.97)	-35.04
1.50	31.8 (34.33)	-36.44	33.0 (35.06)	-25.28	15.6 (23.18)	-38.58
CD (0.05)	(2.462)		(2.465)		(2.9804)	

Numbers in parenthesis denote transformed values

Discussion

DISCUSSION

Mutation breeding has been suggested as one of the promising breeding techniques in the improvement of vegetatively propagated crops. The distinct advantage in breeding through induced mutations is the ability to change one or a few characters of an otherwise superior genotype without affecting its desirable characters. In this sense, this method can be considered as supplementary to the conventional breeding methods, and is the only method to produce genetic variability in vegetatively propagated, sterile and apomictic crops.

Kacholam, *Kaempferia galanga* L. is an important medicinal herb distributed in the tropics and subtropics. It is one of the most important rhizomatous herbs in use in pharmaceutical and cosmetic industry. No information is available in this crop regarding the possibilities of induction of mutation through physical and chemical mutagens for creating variability.

Kacholam, is reported to be sterile and hence always propagated by vegetative means. Hence the existing variability is very much limited. In the absence of variability and due to the existence of varying degrees of

sterility, the conventional breeding methods will have little value in the improvement of this crop. Mutation breeding thus offers unique opportunity for improvement of kacholam through induction of genetic variability and any desirable change observed in the mutant can be perpetuated by vegetative means.

During the last two to three decades, several new varieties have been developed by induced mutations in vegetatively propagated crops. In addition to the new varieties, thousands of mutants with outstanding desirable characteristics like earliness, high yield, high content of sugar, protein and oil, disease and insect resistance and tolerance to environmental stress have been developed through induced mutation.

Gamma rays have been successfully used to develop mutants in ornamentals like chrysanthemum (Bowen *et al.*, 1962), gladioli (Buiatti *et al.*, 1965), rose (Chan, 1966; Gupta and Shukla, 1971; and Lata and Gupta, 1971) and in many other vegetatively propagated crops (Broertjes and Harten, 1978). Though chemical mutagens have not been widely used to induce mutation in vegetatively propagated crops, the results obtained so far are quite promising.

The present investigations were undertaken to create genetic variability in *Kaempferia galanga* variety

Vellanikkara local, using gamma rays and EMS. Kacholam exhibited differences in days taken for sprouting, sprouting percentage, duration of sprouting, lethality, floral characteristics, yield and yield attributes with different doses of gamma rays as well as different concentrations of EMS. The results obtained from the present study are discussed in the following sections.

5.1 Sensitivity studies

Information on the sensitivity of plant material to the mutagen is essential to arrive at the optimum dose/concentration of mutagens. Several parameters have been used for determining the sensitivity of crop plants to different mutagens. Sambandamurthi (1983) in tuberose and Jayachandran and Mohanakumaran (1992) in ginger considered sprouting and survival as the parameters useful for assessing the sensitivity to gamma rays and EMS. Considering these findings in the present investigation, the efficiency of the mutagens was studied on the basis of germination of *Kaempferia galanga* rhizomes under laboratory conditions.

Mutagen treatment of rhizome bits resulted in decreased sprouting with increase in dosage (Plate 15). Similar results were also observed by Roer (1967) in potato and Jalaja (1971) in sugarcane. The percentage of germination revealed that the LD_{50} for *Kaempferia galanga*

Plate 15 Kacholam treated with 20.0 Gy gamma rays


20.0 Gy

was around 20.0 Gy gamma rays, since a dose of 20.0 Gy reduced the sprouting to 50 per cent.

Sambandamurthi (1983) observed 2.5 kR gamma rays as the optimum dose for sprouting in tuberose. The LD_{50} for the percentage of sprouting of cassava sets was between 1.5 and 2.0 kR gamma rays (Thamburaj *et al.*, 1985). According to Giridharan (1984) the LD_{50} for ginger cultivar Rio-de-janeiro was found to be between 1.0 and 1.5 kR while Jayachandran and Mohanakumaran (1992) observed the LD_{50} to be between 0.5 and 1.0 kR in ginger.

There was gradual inhibition of sprouting as the concentration of EMS increased . Similar results have been observed in other vegetatively propagated crops like garlic (Choudhary and Dnyansagar, 1980), tuberose (Sambandamurthi, 1983), cassava (Thamburaj *et al.*, 1985) and in ginger (Jayachandran and Mohanakumaran, 1992). In kacholam, the sprouting was 50 per cent at 1.5 per cent EMS and above 2.5 per cent EMS no sprouting could be observed. Hence for EMS, the LD_{50} value for sprouting was taken as 1.5 per cent.

5.2 Effect of mutagens on MV_1 and MV_2 generations

In the present investigation, the germination percentage on 45th day of planting was found to decrease with increase in doses/concentrations of mutagens. The

result is in accordance with the findings of Roer (1967) in potato, Mukherjee and Khoshoo (1970) in canna, Jalaja (1971) in sugarcane, Gupta *et al.* (1982) in costus and Giridharan (1984) in ginger. Jasina and Kirsanova (1966) reported that in potato the lower doses stimulated sprouting. The effect of gamma irradiation on *Kaempferia galanga* L. has been studied by Viswanathan *et al.* (1992) and it has been found that irradiation treatments at lower dosages viz., 0.5, 0.75 and 1.0 kR produced stimulatory effect on the germination and period taken for germination. Inhibitory effects on these characters were observed at higher doses.

The lower germination rate due to mutagenic treatment might be attributed to an inactivation of auxin levels in the plant with increasing exposures as reported by Skoog (1935). According to Sparrow (1961) mutation treatment caused chromosomal aberrations which adversely affected the cell division. Failure of assimilatory mechanism, production of diffusible growth retarding substances (Mackay, 1951), inhibition of auxin synthesis (Gordon, 1954) and changes in specific activity of the enzymes (Haskins and Chapman, 1956) also could have contributed for the reduced sprouting.

Delayed sprouting was accounted especially at higher doses, due to mutagenic treatments. The result is in

agreement with the findings by Vijayalakshmi and Rao (1960) and Jalaja (1971) in sugarcane, Vasudevan *et al.* (1967) in colocasia and Gupta and Shukla (1971) in rose. The delay in sprouting might be due to the influence of gamma rays and EMS on hormones and plant growth regulators in higher plants. The number of days taken for completion of sprouting also showed variation with respect to different mutagenic treatments. The duration of sprouting was significantly lower in the mutagen treated plants, as compared to control. Contrary to these results, sweet potato took more number of days to complete sprouting when treated with gamma rays (Suma Bai, 1989).

The percentage lethality was more in mutagen treated population compared to the control. The increase in percentage lethality was more at higher doses/concentrations of mutagens. As reported by Skoog (1935) the lower survival rate might be due to the inactivation of auxin level in the plant with increasing exposures. Sparrow (1961) observed chromosomal aberration which affected adversely the cell division causing reduction in the survival rate. According to Sato and Gaul (1967) the reduction in survival might be due to physiological disturbances. Mikaelsen (1968) noticed that the inhibition of DNA synthesis was responsible for reduction in survival at higher doses/concentrations of mutagens.

The mutagenic treatments at higher doses/concentrations reduced the plant spread considerably than in the control. The reduction in plant spread was more pronounced with the higher doses/concentrations of mutagens.

At lower doses of gamma rays and lower concentrations of EMS there was an increase in the plant spread making them photosynthetically more efficient. This indicated the possibility of altering the leaf area through mutation. Plant spread at maximum vegetative phase of the crop was responsible for highest rhizome yield as evidenced from the study.

The mutagenic treatments have caused the shift in means of number of tillers per plant from that of control in both positive and negative directions. At lower doses of gamma rays there was an increase in number of tillers per plant and at higher doses the number of tillers was reduced. The studies of Sambandamurthi (1983) in tuberose and Natarajan (1975) in turmeric revealed that as the doses of gamma rays increased the production of tillers decreased. The present study indicated that EMS at all concentrations affected tiller production in both positive and negative directions. Sambandamurthi (1983) observed increased sucker production at lower concentration of EMS, and less sucker production at higher concentration in tuberose, when compared to control.

The reduction in number of tillers of kacholam might be due to the retarded growth and development of the plant as a result of higher doses/concentrations of gamma rays and EMS. According to Cherry and Leasman (1967), Endo (1967) and Mikaelsen (1968) delay in the onset of first mitosis and inhibition of DNA synthesis could be attributed to the reduced growth.

Leaf production was found to be affected by mutagen treatment. An increase in leaf production was observed at lower doses/concentrations, while higher doses/concentrations resulted in a reduction in leaf production. Similar trend was observed by Irulappan (1979), Thamburaj *et al.* (1985) and Datta (1988) where the lower doses of gamma rays increased the number of leaves whereas higher doses decreased the leaf production. Gupta *et al.* (1974 and 1982); Sambandamurthi (1983) and Giridharan (1984) observed reduction in leaf production as a result of radiation treatment.

EMS treatment reduced the leaf production at higher concentration, while at lower concentration leaf production was more than that in control plants. But no such increase at lower concentration was obtained in tuberose as opined by Sambandamurthi (1983). The number of leaves appeared to depend on the concentration of EMS treatment in kacholam which is in agreement with the

reports by Jayachandran and Mohanakumaran (1992) in ginger. The reduction in number of leaves, leaf length and breadth as a result of mutagen treatment of high doses/concentrations might be due to a reduction in the general growth rate which can be attributed to the interference in normal mitosis. Other explanations such as auxin destruction (Skoog, 1935), inhibition of auxin synthesis (Gordon, 1954), failure of assimilatory mechanism (Quastler and Baer, 1950), production of diffusible growth retarding substance (Mackay, 1951), changes in the specific activity of enzymes (Haskins and Chapman, 1956; Cherry and Leasman, 1967; Endo, 1967), delay in the onset of first mitosis (Natarajan, 1958) and inhibition of DNA synthesis (Mikaelson, 1968) also accounted for the reduced growth at various stages following mutagenic treatment.

Positive shifts in the number as well as weight of rhizomes per plant were observed due to gamma rays and EMS at lower doses/concentrations. Higher doses/concentrations caused gradual reduction in the number and weight of rhizomes per plant.

In ginger Giridharan (1984) reported reduction in yield at higher doses of gamma rays, while reduction in yield at all doses of gamma rays and EMS was observed by Jayachandran and Mohanakumaran (1992). In *Costus speciosus* irradiation of rhizome with gamma rays resulted in decreased yield (Gupta *et al.*, 1982).

Abraham (1970) and Nayar (1975) obtained mutants for high yield in cassava and Kukimura and Kouyama (1982) in sweet potato, through gamma irradiation which lend support to the present findings. Similar increase in yield was reported by Natarajan (1975) in turmeric who obtained high yielding mutants using gamma rays.

The rhizome yield exhibited drastic decrease at higher doses/concentrations of mutagens. At higher doses of gamma rays and higher concentrations of EMS, there was negative shifts to different yield attributing characters and this cumulative negative shifts of the different yield traits might be the possible reason for the decrease in rhizome yield. In other words the positive shifts to the different yield attributing characters at lower doses of gamma rays as well as lower concentration of EMS were responsible for increase in yield.

Identification of short duration type was another important aspect of the study. In kacholam, the crop duration ranged from six to eight months. Development of types with a shorter growing period which would fit into the cropping system assumed top priority under Kerala condition. In kacholam, variation in crop duration was noticed due to mutagenic treatments. The mutagenic treatments caused considerable effect in reducing the crop duration which was directly proportional to an increase in

dose/concentration of mutagen. Early maturing mutants were obtained due to mutagenic treatments in cassava as reported by Moh (1976).

Treatment with gamma rays increased the dry weight of rhizomes as compared to control with the exception of 2.5 Gy which had decreased dry weight. The maximum driage of rhizomes was recorded at one per cent and minimum at 1.5 per cent EMS.

In the present study the oleoresin content of rhizomes was higher at lower doses/concentrations of mutagens. Mutagens at higher doses/concentrations resulted in negative shifts in oleoresin. Mutants with alterations in starch content were observed by Vasudevan *et al.* (1967) in cassava. Kaul and Kak (1973) isolated mutant clones of *Mentha* spp. having increased menthol content. In cassava, mutants with lower hydrocyanic acid was obtained due to gamma irradiation by Moh (1976). Kukimura and Kouyama (1982) obtained mutants with differences in starch content in sweet potato.

The present studies revealed that the highest values for yield and yield attributing characters were obtained for the treatment with 7.5 Gy gamma rays. Among the EMS treatments, 0.75 per cent registered highest yield. The treatments with 20.0 Gy gamma rays and 1.5 per cent EMS

resulted in lowest yield. At higher doses of gamma rays and higher concentrations of EMS, there were negative shifts of all the yield attributing characters where as with lower doses/concentrations of mutagens positive shifts were observed.

In any crop improvement programme wide spectrum of variability is a basic prerequisite. Mutation breeding has been attempted to induce variability in several crops especially vegetatively propagated crops, where only limited variability is available, thereby improving the chances of selection. Coefficient of variation is a statistical measure which give the extent of variability in a population. The effectiveness of mutagenic treatments can be assessed by estimating the coefficient of variation in the mutagen treated population. In the present study, the mutagenic treatments had increased coefficient of variation compared to the untreated population in respect of most of the characters studied viz., plant spread, number of leaves, leaf length, leaf breadth, duration, number and yield of rhizomes. This is in agreement with the findings of Gregory (1955), Bhaskaran and Swaminathan (1962), Goud (1967), Shrof (1974), Conger *et al.* (1976), Kumar and Das (1977), Rao and Siddiq (1977), Ravi *et al.* (1979), Natarajan (1975) and Thamburaj *et al.* (1985).

Considering the efficacy of different doses/ concentrations of mutagens in creating variability, gamma rays at 15.0 Gy and EMS at 0.5 per cent were most effective in inducing variability for rhizome yield and yield attributes in kacholam.

The study of mean and variability of mutagen treated population showed that adequate variability had been generated for all the yield attributing characters. Thus mutation breeding holds good for widening the gene pool in kacholam, for the crop improvement programme. Increased coefficient of variation provides scope for selection in desired direction as reported by Goud (1967) but the response to selection for various quantitative characters has not been commensurate with the increased coefficient of variation observed in the treated population.

5.2.1 Heritability

A good amount of variability and appreciably high magnitude of heritability with genetic advance in respect of yield and important yield attributes offer scope for identifying good types in this crop, on the basis of *per se* performance.

Estimates of heritability (broad sense) ranged from 64.50 per cent (rhizome weight) to 96.27 per cent (leaf length). In general high estimates of heritability

was observed for all the characters. The genetic advance expressed as percentage of mean ranged from 13.04 (leaf length) to 39.30 (number of leaves).

High estimates of heritability (broad sense) coupled with high genetic advance was noticed for number of leaves and number of rhizomes which indicated that there is considerable scope for genetic improvement with respect to these traits.

5.2.2 Correlation and path analysis

In any plant breeding programme, the main objective is the development of elite crop varieties through genetic upgrading of economic traits. The basic information which a breeder usually requires as a prerequisite in any breeding programme is the extent of variability present in the particular crop. The variability present at the exploitational level in this crop is meagre since it is vegetatively propagated. Induction of variability and subsequent exploitation for the economic traits should always be based on heritability, genetic advance and association among the economic characters. The association analysis based on the correlation coefficient of various components with yield may not give a true picture of the relative merits or demerits of each of the components to final yield. Since an individual component may have a direct influence in the improvement of yield or may have

influenced through other components or both an assessment of the merit of each character by analysing direct and indirect effect of each character towards yield is a valuable information for isolating the mutants thus created.

This type of correlation and path analysis was effectively used to assess the effect of yield components in vegetatively propagated crops like turmeric (Natarajan, 1975); rose (Irulappan, 1979); cassava (Thumburaj *et al.*, 1985) and tuberose (Sambandamoorthi, 1983).

It is interesting to note that the correlation coefficient between yield and yield components in the untreated control of both the experiments, except duration and number of tillers (in EMS only) had significant positive correlation with yield. Rhizome number, followed by plant spread has the highest correlation with yield in the untreated control of gamma ray experiment whereas plant spread followed by rhizome number has the high correlation with yield in the EMS experiment. In the association level the trend of rhizome number with yield is the highest in the controls of both the experiments. But the direct effect of plant spread with yield is negative only in the control of gamma ray experiment. In the treatment with a dose of 7.5 Gy gamma rays, there is positive direct effect with plant spread instead of its negative effect in control.

The deviation in the correlation and association of economic traits when compared to the control could be attributed as a genetic variability induced by the mutagen. The maximum yield was obtained in the 7.5 Gy dose, wherein very high correlation coefficient and high direct effect was noticed in the case of plant spread with yield. At the same time the association of rhizome number with yield and its correlation coefficient with yield was very low. This change in the plant architecture for higher yield could be attributed as the economically viable mutation. This trend could also be taken as a selection criterion for exploiting economically viable mutant from treatment with 7.5 Gy.

In the case of EMS treated population, maximum yield was obtained in the treatment with 0.75 per cent EMS. The best yield attributing character was rhizome number, followed by plant spread. The same trend was seen in the control, viz., plant spread followed by rhizome number. This has indicated that the alterations in the plant architecture so as to improve the yield is rather difficult by EMS.

5.2.3 Parent progeny correlation

In the present study, the correlation of MV_2 population on their respective MV_1 generation was estimated to assess how far the genetic variability generated in MV_1 by the mutagens had been inherited by the subsequent generation.

There was no significant correlation between MV_1 and MV_2 in the control except for tiller number (gamma irradiation) and leaf length (EMS). But the mutagenic treatments altered this association.

5.2.4 Frequency distribution of variants in MV_2 generation

Frequency distribution of variants in MV_2 generation indicated higher frequency of positive variants at lower doses/concentrations and higher frequency of negative variants at higher doses/concentrations of mutagens in respect of all the traits studied viz., number of leaves at maximum vegetative phase, plant spread (135 DAS), length and breadth of leaves and number and yield of rhizomes. This is due to higher number of positive variants at lower doses/concentrations of mutagens and more number of negative variants at higher doses/concentrations of mutagens.

5.2.5 Morphological variants and chlorophyll deficient mutants

From the MV_2 population several variants could be isolated based on the vegetative and rhizome characters.

Variegation in leaves might be produced by nuclear and plastid mutation. Sparrow (1961) observed that leaf abnormalities could be due to chromosomal breakage, disrupted auxin synthesis and accumulation of free amino

acids. Abnormalities of leaves may be due to inhibition of DNA synthesis (Gaul 1970), disturbance in production and distribution of growth substances (Gordon 1954), mineral deficiencies, disturbances of phosphate metabolism and accumulation of free amino acids (Jauhar 1969). Leaf abnormalities consequent to irradiation might be due to chromosomal aberrations, changes in route of auxin synthesis, disruption of mineral metabolism or accumulation of free amino acids (Gupta *et al.*, 1982). According to Jauhar (1969), the chimera formation in leaves due to mutagenic treatment might be due to the multicellular nature of tissue. Abraham and Desai, (1976) and Sambandamurthi, (1983) also obtained similar results. Induction of variation in leaves of bougainvillaea by gamma irradiation was reported by Abraham and Desai (1977).

Production of chlorophyll mutant leaves with yellow and white streaks is a matter of interest in ornamental horticulture. In the present study variation in leaf size and shape could be observed due to mutagenic treatments (Plate 16). Variation in leaf shape and colour has been observed in colocasia (Vasudevan *et al.*, 1968) and banana (Velez and Maldonado, 1972 and Gupta *et al.*, 1982).

Among the two mutagens, viz., gamma rays and EMS, the latter was found to be the most potent in the induction of

Plate 16 Variegations in leaf size and shape of kacholam

chlorophyll mutants in MV_1 and MV_2. As reported by Ehrenberg *et al.* (1961) the high frequency of EMS mutants might be due to the preferential reaction of ethyl group with DNA, possibly with guanine component.

Mutagenesis has been one of the most important tools for bringing about variation in plants. The frequency of induced chlorophyll mutation in MV_2 generation has been considered to be more reliable among the different indices for estimating the potency of mutagen due to greater accuracy in screening (Mackay, 1951).

Chlorophyll mutations are not only considered as a test to assess the effectiveness and efficiency of mutagens, but also as indicators to predict approximately the size of vital factor mutation (Gaul, 1964).

5.3 Effect of mutagens on MV_3 generation

The performance of the progenies of the selected MV_2 plants revealed that many plants failed to carry either all, or some of the mutation to MV_3. This may be due to chimeric nature of planting material and hence these plants can be considered as variants. Abraham and Desai (1976) pointed out that the low recovery of mutation in the vegetatively propagated plants was due to diplontic selection. According to Jagathesan (1979) many of the mutants either did not exhibit the mutant character in the

subsequent generations or were broken down due to basic chimeral nature. The reduction in the percentage of mutation in the MV_3 plants may be due to the elimination of the mutated sector through diplontic selection and due to inability of some of the mutated plants to survive upto maturity (Jayachandran and Mohanakumaran 1992).

The present investigations indicated that study of two more generations is necessary to obtain stable lines in *Kaempferia galanga*. Jagathesan (1979) emphasized the necessity for follow up of the mutation generation upto MV_4 or MV_5 or till stability was established, for the selection to be successful in sugarcane.

5.4 Micropropagation

Micropropagation involves cell culture systems of a range of explant tissues and most micropropagations are achieved from organised tissues by multiplication of meristem and axillary buds. In many cases it provides an opportunity to maintain true to type plant species and the propagation systems can produce large number of plants from a single clone. The results obtained have revealed that the axillary bud explants of kacholam have the potential to induce multiple shoots as well as roots in the same media. Similar results have also been reported in *Kaempferia galanga* by Vincent *et al.* (1992). The present results confirmed that *Kaempferia galanga* can be micropropagated readily.

5.5 Causes for failure of seed set in kacholam

In the present investigation, to explore the possibility of widening the gene pool and to overcome the barriers in fruit set and seed set different pollination techniques were employed. Seed set could not be obtained through hand pollination, bud pollination, pollination with mixed pollen, chemically aided pollination and removal of stigma and artificial self pollination.

The influence of mutagens on the reproductive biology of the crop was also studied in detail. Flower production in kacholam was limited in the mutagen treated as well as in the control population. Gamma ray treatment resulted in lower flowering duration, than the control. EMS also had a similar influence as gamma rays and brought down the mean values.

Pollen fertility data indicated a reduction in the fertility in the mutagen treated plants which was proportional to an increase in doses/concentrations of mutagens. Giridharan (1984) and Jayachandran and Mohanakumaran (1992) indicated that there was little difference in the pollen fertility status as a result of the mutagen treatment. Size of pollen grains also reduced as the doses/ concentrations of mutagens increased. Length

of style was increased as doses/concentrations of mutagens increased. Pollen germination was maximum in control, in all the germination media tested. Mutagen treated plants showed a decrease in pollen germination which was progressive as the doses/concentrations increased. The present study indicated that self incompatibility is not responsible for failure of seedset in kacholam. This needs further confirmation. As reported by Ratnambal (1979) due to continuous vegetative propagation, the species might have lost its need for sexual reproduction, and the complete sterility is not creating any barrier for its survival.

Summary

SUMMARY

Induction of genetic variability in kacholam, *Kaempferia galanga* L. to isolate desirable mutants of economic importance was attempted through induced mutagenesis with gamma rays and ethyl methane sulphonate (EMS). The gamma irradiation was done in the CO^{60} gamma chamber, available in the Radiotracer laboratory attached to the College of Horticulture, Vellanikkara. The chemical mutagen ethyl methane sulphonate was obtained from Sisco Research Laboratories, Bombay. Various studies including standardisation of dose/concentration of mutagen, induction of variability, frequency analysis of the variants, estimation of heritability, correlation studies, path coefficient analysis, pollination techniques to find out the causes for failure of seed set and *in vitro* propagation techniques were conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1992-95.

The effects of mutagens were assessed based on yield and yield parameters like number of leaves, plant spread, number of tillers, leaf length, leaf breadth, rhizome number, yield and crop duration. The performance of the treated plants were evaluated for three generations and the

mean performance was assessed based on yield and yield attributing characters. The salient results are presented below:

1. The exposure of rhizomes above 20.0 Gy gamma rays and 1.5 per cent EMS reduced the sprouting to 50 per cent and so LD_{50} value for gamma rays was calculated as 20.0 Gy and for EMS as 1.5 per cent.

2. The sprouting percentage of rhizomes was significantly decreased with increasing doses/concentrations of mutagens.

3. Progressive delay in germination was noticed as the level of dose/concentration increased.

4. Duration of germination was found to be reduced with increase in doses/concentrations of mutagens.

5. The percentage lethality was higher in mutagen treated population than the control.

6. Positive shifts in the mean number of leaves, plant spread, number of tillers, leaf length, leaf breadth, number of rhizomes and the fresh weight of rhizomes were observed due to treatment with gamma rays and EMS at lower dosages.

7. A gradual reduction in the mean number of leaves, plant spread, number of tillers, leaf length, leaf breadth, number of rhizomes and the fresh weight of rhizomes was observed at higher doses/concentrations of mutagens.

8. The mutagenic treatments caused considerable reduction in crop duration, which was directly proportional to an increase in the dose/concentration of mutagens.

9. Higher yield and best yield attributing characters were obtained for the treatment with 7.5 Gy gamma rays and 0.75 per cent EMS.

10. All the mutagen treated plants except 2.5 Gy gamma rays exhibited increased percentage of dry weight of rhizome than control. In the case of EMS treated plants, the maximum drying percentage was recorded at one per cent EMS.

11. Oleoresin content of rhizomes was increased at lower doses/concentrations of mutagens. There was an increase of 12.90 per cent at 7.5 Gy gamma rays and 6.45 per cent at 0.25 per cent EMS over the control.

12. The mutagenic treatments had increased coefficient of variation compared to the untreated population in respect of most of the yield attributes.

13. Gamma rays at 15.0 Gy and EMS at 0.5 per cent were most effective in inducing variability for rhizome yield and yield attributes.

14. High estimates of heritability (broad sense) was observed for all the characters studied, the highest being exhibited by leaf length (96.27%).

15. Number of leaves expressed maximum genetic gain (39.30%) while leaf length expressed the minimum (13.04%).

16. Correlation coefficient between yield and its components indicated significant positive association of yield with number of leaves, tillers, leaf length, plant spread and rhizome number in the untreated control.

17. The mutagenic treatments showed alterations in the association of yield and component characters when compared to the control.

18. Parent (MV_1) progeny (MV_2) correlation revealed no significant association between the yield traits in control. Mutagenic treatments altered this association.

19. Path coefficient analysis of important yield attributes indicated that the number of rhizomes had the maximum direct effect on yield in the untreated control as well as in treatments with 12.5, 15.0, 17.5 and 20.0 Gy gamma rays and 0.25 and 0.75 per cent EMS.

    Alterations in plant architecture for higher yield is possible with 7.5 Gy gamma rays.

    Change in plant architecture, so as to improve the yield is rather difficult by EMS treatment.

20. Frequency distribution of variants in MV_2 generation indicated higher frequency of positive variants at lower doses/concentrations and higher frequency of negative variants at higher doses/concentrations of mutagens in respect of all the traits studied.

21. Chlorophyll deficient mutants were observed to occur in MV_1 and MV_2 generation at the initial stages of development. Gamma rays at 5.0 Gy and 10.0 Gy and EMS at 1.5 per cent did not produce any chlorophyll mutants.

22. Based on the variability expressed in MV_2 population for one or more characters, 40 probable mutants were selected and classified under 13 groups.

23. The performance of the progenies of the selected MV_2 plants revealed that, many plants failed to carry either all or some of the traits to MV_3.

24. Micropropagation studies revealed that axillary bud explants have the potential to induce multiple shoots as well as roots in half MS, eight ppm boric acid and three per cent sucrose under light at 28°C.

25. Number of days for flowering decreased with increase in doses/concentrations of mutagens.

26. Mutagenic treatments had no significant impact on time of anthesis.

27. Pollen fertility (percentage) decreased with increase in doses/concentrations of mutagens.

28. There was gradual reduction in the size of pollen grains with increase in doses/concentrations of mutagens.

29. The ideal medium for pollen viability in kacholam was found to contain eight per cent sucrose, 60 ppm boric acid and one per cent gelatin.

30. A gradual increase in style length with increase in doses/concentrations of mutagens was observed.

31. Artificial self pollination, artificial sibbing, artificial cross pollination, bud pollination, mentor pollination, chemically aided pollination and removal of stigma and artificial pollination failed to give seed set in kacholam.

References

REFERENCES

Abraham, A. 1970. Breeding work on tapioca (cassava) and few other tropical tuber crops. *Tropical Root and Tuber Crops Tomorrow.* Univ. Hawaii, p.76-78

Abraham, V. and Desai, B.M. 1976. Biological effectiveness of fast neutrons and gamma rays in some bulbous ornamentals. *Indian J. Genet.* **36**(2): 230-237

Abraham, V. and Desai, B.M. 1977. A radiation induced Bougainvillea mutant. *Curr. Sci.* **46**(10): 351-352

Auerbach, C. 1961. Chemicals and their effects. *Mutat. Plant Breed.* **891**: 120-144

Auerbach, C. 1967. The chemical production of mutations. *Science.* **158**: 1141-1147

Auerbach, C. and Robson, J.M. 1947. The production of mutation by chemical substances. *Proc. R. Soc. Edinb.* **62**: 271-283

Bentham, G. and Hooker, J.D. 1884. *Genera Plantarum Vol.III,* Reeve and Co., Loudini, p.1007

Bhaskaran, S. and Swaminathan, M.S. 1962. Chromosome aberrations, changes in DNA content and frequency and spectrum of mutations induced by X-rays and neutrons in polyploids. *Radiat. Bot.* **1**: 166-181

Bowen, H.J.M. 1965. Mutations in horticultural chrysanthemums. *Radiat. Bot.* 5 : 695-700

Bowen, H.J.M., Cawse, P.A. and Dick, M.J. 1962. The induction of sports in chrysanthemums by gamma radiation. *Radiat. Bot.* 1: 297-303

Broertjes, C. 1968. Dose-rate effects in *Saintpaulia*. *Mutation Breeding Newsletter.* 2: p.63-71

Broertjes, C. 1976. Mutation breeding of auto-tetraploid Achimenes cultivars. *Euphytica.* 25: 297-304

Broertjes, C. and Ballego, J.M. 1967. Mutation breeding of *Dahlia variabilis*. *Euphytica.* 16: 171-176

Broertjes, C. and Van Harten, A. M. 1978. *Application of mutation breeding methods in the improvement of vegetatively propagated crops.* Elsevier Scientific Publishing Co. New York, p. 1-313

Buiatti, M., Ragazzini, R. and Tognoni, F. 1965. Effects of gamma irradiation on Gladiolus. *Radiat. Bot.* 5: 97-98

*Buiatti, M. and Tesi, R. 1968. Gladiolus improvement through radiation induced somatic mutation. *Laboratorio di Mutagenesi e differenziamento del.* C.N.R., Pisa, p.1-6

Chan, A.P. 1966. Chrysanthemum and rose mutations induced by X-rays. *Proc. Amer. Soc. Hort. Sci.* 88: 613-620

Cherry, J.H. and Leasman, K.J. 1967. Comparison of nucleic acids in maize shoots and epicotyl. *Amer. J. Bot.* **54**: 181-188

Choudhary, A.D. and Dnyansagar, V.R. 1980. Effect of physical and chemical mutagens on morphological parameters in garlic. *J. Indian Bot. Soc.* **59**: 202-206

Conger, B.V., Skinner, L.W. and Skold, L.N. 1976. Variability of components of yield induced in soybeans by seed treatment with gamma radiation. *Crop Sci.* **16**: 233-236

D'Amato, F., Moschini, E. and Pacini, L. 1964. Somatic mutations in carnations induced by gamma rays. *Caryologis.* **17**(1): 93-101

Das, P.K., Dube, S., Ghosh, P. and Dhua, S.P. 1975. Mutation breeding in Dahlia. *Indian J. Ornamental Hort.* **6**(2): 3-8

Datta, S.K. 1988. Gamma ray induced mutant of chrysanthemums. *J. Nuclear Agric. Biol.* **14**: 131-133

Davies, C.R. 1973. Apparent stimulation of vegetative growth by acute gamma-irradiation in crop plants. *Stimulation Newsletter.* **6**: 17-23

Desai, B.M. 1973. Report on mutation experiments on ornamentals at Bhabha Atomic Research Centre, Trombay. *2nd workshop on Floriculture,* Agri-Hort. Society, Calcutta, p. 29-31

Desai, B.M. and Abraham, V. 1974. Radiation induced mutants in canna. *Proceedings of symposium on the use of radiations and radio isotopes in studies of plant productivity,* Pant Nagar, p.214-217

Dewey, D.R. and Lu, K.H. 1959. A correlation and path coefficient analysis of components of crested wheat - grass seed production. *Agron. J.* **51**: 515-518

Donini, B. 1976. Breeding methods and applied mutagenesis in fruit plants. *Proc. Workshop Eur. Comm.* - Israel, Assoc. Euratom-ITAL, Wageningen, p.445-478

Dryagina, I.V. and Limberger, G.E. 1974. A new method for treating perennial fruit trees with chemical mutagens. Moscow Univ. *Biol. Sci. Bull.* **29**(6): 50-53

Ehrenberg, L., Gustaffson, A. and Lundqvist, U. 1961. Viable mutants induced in barley by ionizing radiations and chemicals. *Hereditas.* **47**: 243-282

Endo, T. 1967. Comparison of the effects of gamma rays and maleic hydrazide on enzyme systems of maize seeds. *Radiat. Bot.* 7: 35-40

Escober, J.T. and Lopez, M.E. 1970. A preliminary study on the gamma irradiation of sugarcane seed pieces. *Int. Sugar J.* 72-240

Evans, H.J. 1962. Chromosome aberrations induced by ionizing radiations. *Int. Rev. Cytol.* **13**: 221-222

Gamble, J.S. 1926. *Flora of presidency of Madras.* Vol.III (Ed. Fischer, E.C.), Adlard and Son Limited, London, p.577

Gaul, H. 1961. Studies on diplontic selection after X- ray irradiation of barley seeds. *Proceedings of Symposium on Effects of Ionizing Radiation on Seeds*, Karlsrube, IAEA, Vienna, 117-136

Gaul, H. 1964. Mutations in plant breeding. *Radiat Bot.* **4**(3): 155-232

Gaul, H. 1970. Mutation effects observable in the first generation. *Proceedings of Symposium on Manual Mutation Breeding,* IAEA, Vienna, p.85-95

Geetha, U. 1985. A comparison of path coefficient analysis and principal component analysis in turmeric. M.Sc. (Ag. Stat.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala

Giridharan, M.P. 1984. Effect of Gamma Irradiation in Ginger (*Zingiber officinale* Rosc.), M.Sc.(Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala

Giridharan, M.P. and Balakrishnan, S. 1992. Gamma ray induced variability in vegetative and floral characters of Ginger. *Indian Cocoa, Arecanut and Spices Journal.* 15(3): 122

Gordon, S.A. 1954. Occurence, formation and inactivation of auxins. *Ann. Rev. Plant Physiol.* 5: 341-378

Goud, J.V. 1967. Induced polygenic mutations in hexaploid wheats. *Radiat. Bot.* 7: 321-331

Gregory, W.C. 1955. X-ray breeding of peanut (*Arachis hypogaea* L. *Agron J.* 47: 396-399

Gupta, M.N. 1966. Induction of somatic mutations in some ornamental plants. *Proceedings of All India Symposium on Horticulture.* The Royal Agri-Horticultural Society of India, Calcutta, 107-114

Gupta, M.N., Laxmi, V., Dixit, V.S. and Srivastava, S.N. 1982. Gamma ray induced variability in *Costus speciosus. Progve. Hort.* **14**(4): 193-197

Gupta, M.N. and Shukla, R. 1970. Mutation breeding of garden roses: studies on induction of somatic mutations after gamma irradiation of bud wood. *Prog. Hort.* 2(3): 69-83

Gupta, M.N. and Shukla, R. 1971. Mutation breeding of garden roses. *J.Pl. Breed.* 21(3): 129-136

Gupta, M.N., Sumiran, R. and Shukla, R. 1974. Mutation breeding of tuberose (*Polianthes tuberosa* L.). *Proceedings of Symposium on Use of Radiations and Radio-isotopes in Studies of Plant Productivity,* Pantnagar, p.169-179

Gustafsson, A. 1963. Productive mutations induced in barley by ionizing radiations and chemical mutagens. *Hereditas.* **50**: 211-263

Haskins, F.A. and H.W. Chapman, 1956. Effects of irradiation, maleic hydrazide, temperature and age on enzyme activity in seedlings of corn (*Zea mays* L.), *Physiologia Pl.* **9**: 355-362

Heiken, A. 1958. Aberrant types in potato. *Acta. Agr. Scand.* **8**: 319-358

Heiken, A. 1961. Induction of somatic changes in *Solanum tuberosum* by acute gamma irradiation. *Hereditas.* **47**: 606-614

Hernandez, T.P., Hernandetz,T. and Miller, J.C. 1964. Frequency of somatic mutations in several sweet potato varieties. *Proc. Amer. Soc. Hort. Sci.* **85**: 430-433

*Heslot, H., Ferrary, R., Levy, R. and Monard, C. 1959. Recherches Sur les substances mutagenes esters sulphoniques et Sulphuriques. *Compt. rend.***248**:729-732

Hooker, J.D. 1892. *Flora of British India VI.L.* Reeve and Co. Ltd., London, p.792

Hrishi, N., Krishnamurthy, T.N. and Marimuthammal, S. 1968 Studies on mutagenesis in sugarcane - Induction of visible and micromutations in sugarcane by chemical mutagens. *Proc. Indian Acad. Sci.* **68**: 181-189

Irulappan, I. 1979. Studies on induced mutations in Edward Rose (*Rosa bourboniana* Desp.), Ph.D. thesis, TNAU, Coimbatore, India

Jagathesan, D. 1977. Induction and isolation of mutants in sugarcane. *Mutatation Breeding Newsletter.* **9**(1):5-6

Jagathesan, D. 1979. Utilization of genetic variability in sugarcane breeding. *Proceedings of the symposium on the Role of Induced Mutations in Crop Improvement.* Dept. of Genetics, Osmania Uni., Hyderabad, p.478-495

Jagathesan, D. and Sreenivasan, T.V. 1970. Inuced mutations in sugarcane. *Indian J. agric. Sci.* **40**: 165-172

*Jalaja, N.C. 1971. Studies on the radiosensitivity of *Saccharum* species., Ph.D. thesis, University of Madras

Jasina, I.M. and Kirsanova, E.V. 1966. Production of hereditary changes in potato by means of gamma rays. *Genetics.* **1**: 53-58

Jauhar, P.P. 1969. Morphological and physiological effects of radiations and radioisotopes on potato, *Solanum tuberosum* L. *Indian J. agric. Sci.* **39**: 88-100

Jauhar, P.P. and Swaminathan, M.S. 1967. Mutational rectification of specific defects in some potato variety. *Curr. Sci.* **36**: 340-342

Jayachandran, B.K. and Mohanakumaran, N. 1992. Effect of gamma ray irradiation on ginger. *South Indian Hort.* **40**(5): 283-288

Johnson, H.W., Robinson, H.F. and Constock, R.E. 1955. Genotypic and phenotypic correlations in soybean and their implications in selection. *Agron. J.* **47**:477-482

Kaicker, U.S. and Swarup, V. 1972. Induced mutation in roses. *Indian J. Genet.* **32**: 257-265

Kak, S.N. and Kaul, B.L. 1979. Induced mutations in the improvement of *Mentha species. Proceedings of Symposium on The Role of Induced Mutations in Crop Improvement.* Dept. of Atomic Energy, Govt. of India, p. 349-354

KAU,1993. *Package of Practices Recommendations "Crops"* Kerala Agricultural University, Directorate of Extention, Mannuthy, Kerala, p.211

Kaul, B.L. and Kak, S.N. 1973. Improvement of *Mentha arvensis* L. through induced mutations. *Advancing Frontiers in Cytogenetics*. Hindustan Publishing Corporation (India), Delhi, p.189-195

Khairwal, I.S., Singh, S. and Paroda, R.S. 1985. Genetic variability created by physical and chemical mutagens for some quantitative characters in sugarcane. *Indian J. agric. Sci.* **55**(5): 362-364

Kirthikar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants*. 2nd ed., Lalit Mohan Basu, Allahabad, p.2427

Kishore, H., Pushkarnath and Singh, G. 1963. The effects of gamma irradiation on potato tubers. *Indian Potato J.* 5: 86-92

Konzak, C.F. 1966. Qualitative mutations and plant breeding. *Int. Rice Commn. Newsl.* **15**(1): 20-21

Konzak, C.F., Nilan, R.A., Harle, J.R. and Heiner, R.E. 1961. Control of factors affecting the response of plants to mutagens. *Brookhaven Symp. Biol.* **14**: 128-157

Krull, C.F. and Frey, K.J. 1961. Genetic variability in oats following hybridization and radiation. *Crop. Sci.***1**: 141-146

Kukimura, H. and Kouyama, Y. 1982. Studies on mutation breeding in sweet potato (*Ipomoea batatas* (L) Lam.). *Proceedings of Symposium on Induced Mutations in Vegetatively Propagated Plants II*, IAEA, Vienna, 11-15

Kukimura, N. and Takemata, T. 1975. Induced quantitative variation of gamma rays and ethylene imine in tuber bearing plants. *Gamma Field Symp.* **14**: 25-38

Kumar, P.R. and Das, K. 1977. Induced quantitative variation in self-incompatible forms in *Brassica*. *Indian J. Genet.* **37**: 5-11

Lata, P. and Gupta, M.N. 1971. Effects of gamma rays on stem cuttings of essential oil bearing *Rosa* spp. Flavour Ind. 2(7): 421-425

Lawley, F.D. 1973. Reaction of MNUA with p^{32} labelled DNA, evidence for formation of phospho tri esters. *Chem. Biol. Interactions*, 7: 127-130

Loveless, A. 1966. *Genetic and allied effects of alkylating agents.* Better-worths, London, p.270

Luo, C.C. 1979. Induction of early high sugar mutants of sugarcane. *Taiwan Sugar Research Institute Report.* **85**: 1-11.

Lush, J.L. 1937. *Animal Breeding Plans.* Iowa State College Press. Ames. Iowa. p.473

Mackay, J. 1951. Neutron and X-ray experiments in barley. *Hereditas.* 37: 421-464

Masima, I. and Sato, H. 1959. X-ray induced mutation in sweet potato. *Japan J. Breed.* 8: 233-237

Mikaelsen, K.1968. Comparison between chromosome aberrations induced by ionizing radiations and alkylating agents at different stages of mitosis in barley seed. *Proceedings of Symposium on Mutation Plant Breeding.* IAEA, Vienna, p.287-290

Misra, R.L. 1976. A colour mutant in *Gladiolus* L. variety Picardy. *Sci. Cult.* **42**(11): 569-571

Mitra, P.K. and Bhowmik, G. 1997. Gamma radiation and EMS treatment of Black cumin cultivars for Mutational Bioassays. *Indian J. Genet.* **57**(2): 158-160

*Mitsukini, Y. and Arai, K. 1965. The effect of radiation on crocus. *Nippon Univ. J.. Radiat. Med. Biol.* **1**: 22-30

Miu, T.S. 1973. An inquiry into indication of cold tolerance among radiation induced lines of sweet potato. *Genetics.* 74: 2-11

Moh, C.C. 1976. Screening for acyanogenic somatic mutations in cassava (*Manihot esculenta* Crantz.), *Mutation Breeding Newsletter.* 8: 10-11

Mohanty, D.C. and Sharma, Y.N. 1979. Genetic variability and correlation for yield and other variables in ginger germplasm. *Indian J. agric. Sci.* **49**: 250-253

Mukherjee, I. and Khoshoo, T.N. 1970. Genetic evolutionary studies on cultivated cannas IV: Parallelism between natural and induced somatic mutations. *Radiat. Bot.* **10**(4): 351-364

Mukhopadhyay, S. and Roy, K. 1986. Correlation and path analysis in turmeric. *Indian Agricst.* **30**: 113-115

Muntzing, A. 1930. Outlines to a genetic monograph of the genus *Galeopsis* with special reference to the nature and inheritance of partial sterility, *Hereditas.* **13**: 185-341

Nakornthap, A. 1965. Radiation-induced somatic mutations in the ornamental. *Canna. Radiat. Bot.* **5**(Suppl.): 707-712

Nambiar, M.C. 1979. Morphological and cytological investigations in the genus *Curcuma* Linn. Ph.D. thesis, University of Bombay

Natarajan, A.T. 1958. A cytogenetical study of the effect of mutagens on plants with special reference to the induction of mutations, Ph.D. thesis, Delhi Univ., India

Natrajan, S.T. 1975. Studies on the yield components and gamma ray induced variability in turmeric (*Curcuma longa* L.). M.Sc.(Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, India

Nair, N.V. 1969. Considerations on overcoming intrasomatic selection during mutation breeding of vegetatively propagated plants. *Theor. Appl. Genet.* **39**: 99-103

Nayar, G.G. 1975. Improving tapioca by mutation breeding. *J. Root Crops*, **1**(2): 55-58

Nybe, E.V. 1978. Morphological studies and quality evaluation of ginger types (*Zingiber officinale* Rosc.), M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala

Nybom, N. 1961. The use of induced mutations for the improvement of vegetatively propagated plants. *Mutatation Plant Breeding.* **891**: 252-294

*Oehlkers, F. 1943. Die Auslosungvon Chromosomenmutalonen in der Meiosis durch Einwirkung Von Chemikalien, *Z. indukt. Abstamm. -u. VererbnLehre.* **81**: 313-341

Ono, S. 1971. Studies on the radiation breeding in the genus *Mentha* . Effective irradiation techniques to induce mutation in mint. *Sci. Rep. Fac. Agric. Okayama Univ.* **37**: 9-15

Panicker, P.M.B., Rao, B.S.and Simonsen, J.L. 1926. The essential oil from the rhizome of *Kaempferia galanga* L. *J. Indian Inst. Sci.* **9A**: 133

Panse, V.G. and Sukhatme, P.V. 1967. *Statistical methods for Agricultural Workers*, ICAR, New Delhi, p. 347

*Pavlovic, N.K., Stojanovic, N. and Milutinovic, M. 1983. Effect of different irradiation doses of mint (*Mentha piperata* L.) quality. *Arhiv za poliopriveredne* Nanke, **44**(154): 249-254

*Prasad, P.R.J. 1959. Studies on the effects of ionizing radiations in plants with special reference to sugarcane. M.Sc. thesis, Madras University.

Radha Devi, D.S. and Nayar, N.K. 1996. Gamma ray induced variation in fruit quality of Banana (MUSA (AAB) Nendran). *J. Trop. Agric.* **34**(1): 51-53

Raghava, S.P.S., Negi, S.S., Sharma, T.V.R.S. and Balakrishnan, K.A. 1988. Gamma ray induced mutants in gladiolus. *J. Nuclear Agric. Biol.* **17**: 5-10

Raju,E.C., Patel, J.D. and Shah, J.J. 1980. Effect of gamma irradiation in morphology of leaf and shoot apex of ginger, turmeric and mango-ginger. *Proc. Indian Acad. Sci.* (Plant Sci.) **89**(3): 173-178

Rangaswamy, S. 1986. Applied mutation research in field crops at Tamil Nadu Agricultural University. *Mutation Breeding Newsletter.* **28**: 13-14

Rao, G.M. and Siddiq, E.A. 1977. Induced variation for yield and its components in rice. *Indian. J. Genet.* **37**(1): 12-21

Rao, P.S. 1974. Mutation breeding for non-flowering in sugarcane. *Mutatation Breeding Newsletter.* 3: 9

Ratnambal, M.J. 1979. Cytogenetical studies in ginger (*Zingiber officinale* Rosc.), Ph.D. thesis, University of Bombay

Ravi, I.L., Minocha and Avtar Singh, 1979. Induced mutations for quantitative traits in lentil. *Proceedings of Symposium on The Role of Induced Mutations in Crop Improvement.* Dept. of Atomic Energy, Govt. of India, p. 414-419

Rawlings, J.O., Hanway, D.G. and Gardner, C.O. 1958. Variation in quantitative characters of soybeans after seed irradiation. *Agron. J.* **40**: 524-528

Reddy, C.R. 1980. Studies on heritability and genetic advance in sugarcane. *Maharashtra Sug.* 5(6): 15-18

Reghupathy, A., Santharam, G., Balasubramanian, M. and Arumugam, R. 1976. Occurence of the scale *Aspidiotus hartii* C. (*Diaspididae Homoptera*) on different types of turmeric, *Curcuma longa* Linn. *J. Pln.Crops.* **4**(2):80

Roer, L. 1967. Mutation in potatoes induced by gamma irradiation. *Euphytica.* **16**: 283-292

Sambandamurthi, S. 1983. Studies on induced mutations in tuberose (*Polianthes tuberosa* L.), Ph.D. thesis , Tamil Nadu Agrl. University, Coimbatore, India

Sato, M. and Gaul, H. 1967. Effect of ethyl methane sulphonate on the fertility of barley. *Radiat. Bot.* **7**: 7-15

*Schiemann, E. 1912. Mutation bei *Aspergillus niger, Z. indukt., Abetammange. Vererbungel.* **8**: 1

*Schumann, K. 1904. Zingiberaceae. In Engler's. *Pflanzenreich.* 4(46): 468

Shah, H.A., Seemanthini, R., Arumugam, R., Muthuswami, S. and Khader, 1982. Co-I Turmeric- A high yielding mutant turmeric . *S. Indian Hort.* 30(4): 276-277

Sharma, R.L., Mukherjee, S.K. 1973. Some anatomical features of a radiation induced grape variegata. *Vitis.* 12(3): 189-191

Shivanna, K.R. and Johri, B.M. 1985. *The Angiosperm pollen : structure and function.* Wiley Eastern Ltd., New Delhi, p.365

Shrof, V.N. 1974. Effect of mutagens on polygenic traits in cotton. *Proceedings of Symposium on Use of radiations and Radioisotopes in studies of plant productivity.* FAO/IAEA, Vienna, p.303-343

Siddiq, E.A. 1967. Induced mutations in relation to the breeding and phytogenetic differentiation of *Oryza sativa.* Ph.D. thesis, IARI., New Delhi

Singh, U. 1970. Chronic gamma irradiation induced resistance to red rot disease in sugarcane Var. Co-997. *Sugarcane Pathol. News.* 5: 24

Skoog, F. 1935. The effect of X-irradiation on auxin and plant growth. *J. Cell. Comp. Physiol.* 7: 227-270

Snedecor, W.G. and Cochran, G.W. 1975. *Statistical Methods* Oxford and IBH Publ. Co., New Delhi,p. 593

Soriano, J.D. 1968. Modifying the effects of fast neutrons in rice seeds by post-treatment with chemical mutagens. *Proceedings of Symposium on Neutron Irradiation of Seeds*, IAEA, Vienna, p.55-62

Soriano, J.D. 1972. Effect of gamma radiation on buds of sweet potato (*Ipomoea batatas* L.). *Nat. Appl. Sci. Bull.* **24**(1-2): 41-53

Sparrow, A.M. 1961. Types of ionizing radiation and their cytogenetic effects. *Mutat. Plant Breed.* **891**: 55-119

Sparrow, A.H and Christensen, E. 1950. Effects of X-ray, neutron and chronic gamma irradiation on growth and yield of potato. *Am. J. Bot.* **37**: 667

Suma Bai, D.I. 1989. Genic manipulations in sweet potato adopting induced mutations. Ph.D thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Suma Bai, D.I. and Nayar, N.K. 1992. Gamma ray induced growth rate variation in sweet potato genotypes. *Agricultural Science Digest.* **12**(3): 144-146

Suma Bai, D.I. and Nayar, N.K. 1995. Gamma ray induced variation for yield in sweet potato. *J.Trop. Agric.* **33**: 70-71

Swaminathan, M.S. 1965. A comparison of mutation induction in diploids and polyploids. *Proceedings of Symposium on The use of induced mutations in plant breeding.* FAO/IAEA, Tech. Meeting Report, Oxford, p. 619-641

Swaminathan, M.S., Chopra, V.L. and Bhaskaran, S. 1962. Chromosome aberrations, frequency and spectrum of mutations induced by ethyl methane sulphonate in barley and wheat. *Indian J. Genet.* 22: 192-207

Thamburaj, S., Muthukrishnan, C.R. and Irulappan, I. 1985. Studies on sensitivity of cassava buds to gamma rays and EMS. *S. Indian Hort.* 33(1): 13-17

Upadhya, M.D. and Purohit, A.N 1973. Mutation induction and screening procedure for physiological efficiency in potato. *Proceedings of Symposium on Induced Mutations in Vegetatively Propagated Plants.* FAO/IAEA, p.61-66

Vasudevan, K. and Jos, J.S. 1988. Gamma ray induced mutants in *Coleus. Mutation Breeding Newsletter.*32: 5

Vasudevan, K. and Jos, J.S. 1989. Gamma ray induced day length tolerant mutants in coleus. *Mutation Breeding Newsletter.* 34: 5-6.

Vasudevan, K., Nair, S.G., Jos, J.S. and Magoon, M.L. 1967. Radiation induced mutations in cassava. *Indian J. Hort.* 24(1-2): 95-98

Vasudevan, K., Nair, S.G., Jos, J.S. and Magoon, M.L. 1968. Radiation induced mutations in *Colocassia esculenta* (L.). *Indian J. Hort.* 25: 66-69

Vasudevan, K., Jos, J.S. and Unnikrishnan, M. 1987. Gamma ray induced mutations in Taro (*Colocasia esculenta) Bangladesh J. Nuclear Agric.*5 : 41-46

Velez, F.J. and Maldonado, A.C. 1972. The use of radiation in breeding banana (*Musa sapientum* L.). *Proceedings of Symposium on Induced mutation and plant improvement,* FAO/IAEA, p.485-489

Vijayalakshmi, V. and Rao, J.T. 1960. Effects of gamma rays on germination and growth in some species and hybrids of *Saccharum*. *Curr. Sci.* 29: 397-398

Vincent, K.A., Mathew, K.M. and Hariharan, M. 1992. Micropropagation of *Kaempferia galanga* L.- a medicinal plant. *Pl. Cell Tissue and Organ Culture.* 28: 229-230

Viswanathan, T.V., Sunil, K.P., Mahato, K.C. and Manuel, J. 1992. Effect of gamma irradiation on the M_1 generation of Kaempferia (*Kaempferia galanga* L.). *South Indian Hort.* 40: 146-150

*Volkov, V.D. and Danko, G.V. 1972. The use of chemical mutagens to obtain initial material for potato breeding. *Khim. mutagenez i sozdaric, selekts. materiala.* Moscow, USSR, Nauka, p.277-280

Walker, D.I.T. and Sisodia, N.S. 1969. Induction of non-flowering mutant in sugarcane (*Saccharum sp.*). *Crop Sci.* 9: 551-552

Wardlaw, A.C. 1985. *Practical Statistics for experimental biologists.* John Wiley, p.107-110

Yamaguchi, H. and Miah, M.A.J. 1964. Mutational mosaicism induced by X, gamma rays and ethylene imine in rice. *Radio isotopes.* 13: 471-476

* Originals not seen

INDUCTION OF GENETIC VARIABILITY IN KACHOLAM (*Kaempferia galanga* L.)

By

KANAKAMANY M. T.

ABSTRACT OF THE THESIS

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DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 654

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ABSTRACT

The present study "Induction of genetic variability in kacholam, *Kaempferia galanga* L." was undertaken in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during 1992-95. Rhizomes of *Kaempferia galanga* cv. Vellanikkara local were treated with eight doses of gamma rays (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 Gy) and six concentrations of EMS (0.25, 0.50, 0.75, 1.0, 1.25 and 1.50%) and MV_1, MV_2 and MV_3 generations were evaluated.

LD_{50} of gamma rays was 20.0 Gy and that of EMS 1.5 per cent.

The highest values for yield and yield attributing characters were obtained for 7.5 Gy gamma rays and 0.75 per cent EMS.

Gamma rays at 15.0 Gy and EMS at one per cent were most effective in inducing variability for rhizome yield and yield attributes.

High estimates of heritability (broad sense) coupled with high genetic advance was observed for number of leaves and rhizome number and direct selection for improvement of these traits will be effective.

Correlation coefficient between yield and its components indicated significant positive association of yield with number of leaves, tillers, leaf length, plant spread and rhizome number in the untreated control.

Mutagenic treatments induced alterations in the association between rhizome yield and components.

Path coefficient analysis of important yield attributes indicated that alterations in plant architecture for higher yield is possible with 7.5 Gy Gamma rays. Change in plant architecture so as to improve the yield is rather difficult in EMS.

High frequency of positive variants at lower doses and high frequency of negative variants at higher doses were observed.

Mutant characters present in MV_2 were not completely expressed in all MV_3 plants.

In vitro studies revealed that axillary bud explants have the potential to induce multiple shoots as well as roots in Murashige Skoog (MS) medium supplemented with boric acid and sucrose.

Different pollination techniques failed to induce seed set in kacholam.