INDUCED MUTATIONS IN GINGER

(Zingiber officinale R.)

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Induced mutations in ginger (Zingiber officinale Roscoe)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

Vellayani
20-12-1989.

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CERTIFICATE

Certified that this thesis, entitled "**Induced mutations in ginger (*Zingiber officinale* Roscoe)**" is a record of research work done independently by Sri. B.K. Jayachandran under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

INTRODUCTION

Kerala is blessed with a salubrious climate with abundant rain fall, warm humid atmosphere and fairly uniform temperature throughout the year. Because of the favourable climatic conditions, Kerala has become the leader in India with respect to production of various spice crops.

Ginger (Zingiber officinale Roscoe) grows well in warm and humid climatic conditions upto an altitude of 1500 m. It thrives well in a wide range of soil with good drainage, like sandy or clay loam, red loam or lateritic loam. Kerala is the largest ginger producing state accounting for about 25 per cent of the production in India (George, 1989). The area under the crop during 1986-1987 was 15,490 ha with a production of 43,598 tonnes, which gives an average productivity of 2815 kg/ha.

The national average yield of ginger is 2,182 kg/ha, the yield obtained by progressive farmer is 5,500 kg/ha, yield obtained in research stations is 8,000 kg/ha and the highest recorded yield is 11,500 kg/ha (Nair, 1989). Though several reasons can be cited for the yield gap, the major constraint in ginger production at present is the devastating diseases of soft rot and bacterial wilt caused by Pythium spp

and Pseudomonas solanacearum respectively. In severe cases yield loss due to these diseases will be more than 90 per cent. The use of plant protection chemicals to control the diseases has been of only very limited practical use.

The crop improvement work carried out in ginger so far had been confined to the collection of cultivars from the different localities and their yield evaluation. The conventional breeding programmes have been handicapped by the relatively shy flowering nature of most of the cultivars and the absence of seed set. Even if we succeed in the production of seeds, there is no scope for creating variability by cross breeding (for disease resistance), since resistance to soft rot and bacterial wilt has not been reported to be available in any of the cultivated ginger types. The only alternative suggested by Nair et al. (1982) is to induce variability through physical and chemical mutagens. Orton (1984) and Wenzel et al. (1987) recommended another relatively new technique of induced somaclonal variation for generation of biological diversity in vegetatively propagated plants. Since ginger is solely vegetatively propagated, the induced variability can be fixed immediately and true to type lines can be established through vegetative propagation. There is also the possibility of quick multiplication of large quantity of new planting material by tissue culture techniques.

The main advantage of mutation induction in vegetatively propagated crops is the possibility to change one or a few characters of an otherwise outstanding cultivar without altering the remaining and often unique part of the genotype (Broertjes and Van Harten, 1978). Many successful mutations in rhizomatous crops have been reported by Broertjes and Van Harten (1978). In Tamil Nadu, a high yield mutant of turmeric (Co.1) has been released (Shah et al., 1982). This is a vegetative mutant derived by X-ray irradiation of the rhizomes of the local 'Erode' type at a radiation dose of 5.0 krad. In ginger systematic induced mutagenesis is very scanty. Investigation in ginger with gamma rays at 0.7 to 2.0 krad indicated decrease in the quantitative traits as the doses increased in the vM_1 generation (Giridharan, 1984).

The present investigation aimed at studying the effects of mutagens on rhizome and shoot characters in the vM_1 generation, assessment of the extent of variability induced in the qualitative as well as quantitative characters (including earliness, resistance/tolerance to bacterial wilt and soft rot in the vM_2 generation), investigation of the effects of mutagens on flowering and seed set in the vM_1 and vM_2 generations and studying the vM_3 progenies of the desirable vM_2 plants.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Mutation breeding is one of the methods available to the Plant Breeders when the crop is amenable to vegetative propagation. The fact that 146 commercial mutants of various vegetatively propagated crops have been put on the market by 1977 (Broertjes and Van Harten, 1978) substantiates the above point. The rapid increase in the release of mutants in the recent years shows unmistakably that induced mutations are now being used successfully in plant breeding programmes (Sigurbjornsson, 1977 and Rangaswamy, 1986). The possibility of inducing mutation by X-rays was first suggested by De Vries, followed by Koernicke (1905) and Gager (1908). However the conclusive proof that ionising radiations induce mutations was presented by Muller (1927) in drosophila.

Reports of Stadler (1928), Gager and Blakeslee (1927) and Goodspeed (1929) indicated the use of ionising radiations for inducing mutations in plants. The historically important findings of this period were followed in the next three to four decades by investigations of a purely experimental nature, such as the sensitivity of the crop towards the mutagen, morphological variations and cytological

characteristics. According to Broertjes (1977), too many mutation experiments have been carried out in the past with no other objective than "to see what might come out". These early works did not contribute much to plant improvement. However, the seventies witnessed the practical utilization of induced mutations in a wide range of crops (Gregory, 1972).

Even before the discovery of the mutagenic effects of X-rays, the search for chemicals capable of causing mutations began (Auerbach, 1967). Early in the century, chemical mutagenesis was attempted by Schiemann (1912). Induction of mutations by means of treatments with mustard gas was demonstrated by Auerbach and Robson (1942, 1947) in England and Rapoport (1948) in the USSR. Since then, a number of chemicals possessing mutagenic properties have been identified and their effects studied. The reports on the induction of mutations in higher plants with chemical mutagens are numerous in the recent years.

The vegetatively propagated crops are a very suitable group of plants for the application of mutation breeding methods. The generally high degree of heterozygosity which causes a complex inheritance of genetic factors as well as the frequent polyploidy, both serious handicaps in the conventional methods of breeding, are advantageous in mutation breeding, as large variations can often be observed

in the irradiated populations. Further mutation is the only source of variability in sterile plants or in obligate apomicts.

The most promising aspect of mutation induction in the vegetatively propagated plants, compared to the cross-breeding methods, is the ability to change only a very few characters of an otherwise good cultivar without altering significantly the remaining and often unique genotype (Broertjes, 1977). Mutation breeding, therefore, must be considered as the obvious means to perfect the leading products of conventional plant breeding and as a possible shortcut for inducing desired genetic alterations in the outstanding cultivars. Broertjes (1977) described the methods of mutation induction applicable to vegetatively propagated crops. Broertjes and Van Harten (1978) reviewed the mutation work in various vegetatively propagated crops. The success of mutation breeding depends largely on the choice of appropriate plant materials, mutagens and selection procedures. Janick (1986) emphasised the need for artificial induction of mutations for creating changes that have not occurred naturally in asexually propagated plants.

In ginger, systematic mutation breeding works are very scanty. Preliminary research on the direct effects of treatment with physical mutagen indicated the nature of

sensitivity of the crop towards different doses of gamma rays (Gonzalez et al., 1969; Raju et al., 1980 and Giridharan, 1984).

Scope of induced mutations in ginger

Ginger (Zingiber officinale Roscoe) is exclusively propagated vegetatively by means of rhizomes and has been found to never set seed. Hooker (1894) described ginger as a species very rarely flowering and never setting seed. East (1940), Fryxell (1957) and Pillai et al. (1978) suspected that the lack of seed set in ginger was due to the phenomenon of self-incompatibility. Attempts were made to break the self-incompatibility and to induce seed set by hand pollination, bud pollination, removal of stigmatic surface before pollination and application of sucrose-boric acid germination medium to the stigma followed by pollination (Jayachandran and Vijayagopal, 1979). The treatments did not succeed to produce seeds.Clones of ginger have shown evidence of structural hybridity for interchanges and inversions. Only eight per cent normal pollen was observed by Ramachandran (1969) who suggested a chromosomal basis for the sterility. Significant linear regression between pollen sterility and chromosome aberrations at anaphase II was observed in cytological studies conducted by Ratnambal (1979). She concluded that chromosomal aberrations had a

significant influence in lowering the fertility of the cultivars. Nair et al. (1982) suggested that it would be worth attempting to manipulate the physiology of the plant to induce seed setting and also the possibility of inducing flowering and seed set through the use of growth substances.

Even if seed set in ginger is achieved, there is little scope for creating variability by the conventional methods of breeding for resistance to the serious diseases like soft rot and bacterial wilt, since sources of resistance have not yet been located in the cultivated ginger types. The alternate method is to induce variability by employing physical and chemical mutagens (Nair et al., 1982). The National Seminar on Ginger and Turmeric, held at the Central Plantation Crops Research Institute, Calicut, during 1980, recommended mutation breeding methods to incorporate disease resistance and other desirable characters in ginger (Anon, 1982). Ginger has the inherent advantage that any variability obtained by mutation can be fixed immediately and true-to-types could be multiplied through vegetative propagation. Very rapid multiplication of the new material is possible by tissue culture techniques (Nair et al., 1982) also.

The improvement of crop species through mutation should be pursued only with outstanding varieties. The use

of inferior varieties for this purpose has been proved to be of little value (Scarascia-Mugnozza, 1969).

The cultivar Rio-de-Janeiro introduced from Brazil for cultivation in Kerala and other ginger growing areas in India, has given much higher yields than the indigenous cultivars. However it is not very suitable for conversion to dry ginger. It is becoming popular, however, where ginger is used in the green form or for the extraction of oleoresin (Purseglove et al., 1981). The superiority of Rio-de-Janeiro in respect of yield and other characters which made the cultivar an acceptable one, has been pointed out by many workers (Thomas, 1966; Muralidharan, 1973; Muralidharan and Ramankutty, 1975; Purseglove et al., 1981 and Sreekumar et al., 1982).

Various plant parts, namely, bulbs, tubers, rhizomes, suckers, stem-cuttings, buds, leaves with or without petiole etc. have been treated with mutagens for induction of mutation in vegetatively propagated crops. In ginger which is exclusively propagated by rhizomes, Gonzalez et al. (1969), Raju et al. (1980) and Giridharan (1984) used the rhizomes for treatment with the mutagens.

Mutagens

For induction of the mutational events in plant

material, the breeder can choose between two groups of mutagenic agents, physical and chemical. The physical mutagens have been in use for many decades, whereas the systematic use of chemicals is relatively recent, starting around 1940 (Broertjes and Van Harten, 1978).

Physical mutagens

Physical mutagens are widely used for treating the different plant parts. Though a variety of ionizing radiations are available, only X-rays and gamma rays are generally used. Thermal or fast neutrons can also be used (Broertjes and Van Harten, 1978). There are also reports on the use of ultraviolet rays (Cline and Salisbury, 1966; Ikenga and Mabuchi, 1966) and radio isotopes (Jauhar, 1969). In ginger Gonzalez et al. (1969); Raju et al. (1980) and Giridharan (1984) used gamma rays for induction of mutations. The lowest dose of gamma rays used by Raju et al. (1980) in ginger was 2.0 krad, where 32 per cent rhizomes germinated. The details of survival were not available.

The LD_{50} was found to be below 2.0 krad. Giridharan (1984) observed that the LD_{50} worked in terms of germination, was between 1.5 and 2.0 krad. The data on survival were not available in this case also. The suitable effective radiation doses for induction of somatic mutations in vegetatively propagated crops and the plant material treated

with gamma rays have been furnished by IAEA (1977) and presented in Table 1.

Table 1. Suitable radiation doses and plant materials used for induction of somatic mutations

Crop	Plant material treated	Dose (krad)
Canna	Rhizomes	1.3
Dahlia	Tubers	1.5-2.5
Gladiolus	Corms	4.0
Banana	Corms	2.5-5.0
Potato	Tubers	2.0-3.0
Sweet potato	Detached leaves	3.0-4.0

Many workers have demonstrated the effectiveness of physical mutagens in inducing mutation in vegetatively propagated plants, such as sweet potato (Hernandez et al., 1964 and Kukimura and Takemata, 1975); yam (Koo and Cuevas Ruiz, 1964 and Abraham, 1970); dahlia (Broertjes and Ballego, 1967); potato (Jauhar and Swaminathan, 1967; Roer (1967) and Jauhar, 1969), tapioca (Vasudevan et al., 1967 and Nayar, 1975); colocasia (Vasudevan et al., 1968; Vasudevan and Jos 1988b; Jos and Vasudevan, 1989); garlic (Sklyar, 1973 and Zhila, 1975); coleus (Vasudevan and Jos, 1988 a); peppermint

(Mital et al., 1972 and Murray, 1972); pepper (Irulappan et al., 1982); grapes (Becker, 1989); sweet cherry (Saamin and Thompson, 1989); apple (Paprstein and Blazek, 1985); jasmine (Kumar, et al., 1983); rose (Kaicker and Swarup, 1972; Irulappan and Madhava Rao, 1982; Huang Shanwu and Chen Yanfang, 1986; Kaicker and Dhyani, 1985); canna (Nakornthap, 1965; Mukherjee and Khoshoo, 1970 and Desai and Abraham, 1974); tuberose (Gupta et al., 1974 and Sambandamurthi, 1983) and gladiolus (Misra, 1976).

Chemical mutagens

The use of chemicals for inducing mutation began since 1960 (Heslot, 1964) following the introduction of ethyl methane sulphonate (EMS). According to Heslot (1977) EMS is the most efficient member of alkylating agents (the most important group of chemicals having ability to induce mutation in cultivated plants). Alkylating agents react with DNA by alkylating the phosphate groups as well as the purine and pyrimidine bases. The most frequent event involving bases leads to the formation of 7-alkyl guanine. Chemical mutagens were expected to mutate specific genes. Heslot (1965) observed that such expectations were not realistic. Krishnaswamy (1968) observed that chemical mutagens like EMS, are capable of causing functional alterations in the genes in polyploid plants. They are able to induce mutations even in

auto-tetraploids which find phenotypic expression in the vM_2 generation itself. According to Mackey (1967),the alkylating agents have a reaction pattern more suited than that of ionising radiations, for breaking down the buffering characteristics of polyploid germplasm and for creating a maximum of genetic diversification and allelic interaction between homologue loci. However, Scarascia-Mugnozza (1969) opined that the chemical mutagens are more dependent on the genetic constitution of the plant than the ionizing radiations.

For inducing mutation in vegetatively propagated plants, chemical mutagens have been less frequently used due to the poor uptake and penetration of the chemicals in the vegetative parts (Bowen, 1965; Nybom, 1961 and Broertjes and Van Harten, 1978). Plant materials like bulbs, rhizomes and tubers treated with chemical mutagen are usually bulky. This makes it difficult to obtain reproducible results (Broertjes and Van Harten, 1978).

The treatment duration must be long enough to permit hydration and thorough infusion (Konzak et al., 1965). In the USSR, it is reported that the breeders concentrate on, or even use exclusively, chemicals for the induction of mutations (Broertjes and Van Harten, 1978). Amirov (1974), Dryagina (1974) and Dryagina and Limberger (1974) claimed

that chemical mutagens had a higher efficiency and output of mutations, if the duration of the treatment and the concentrations were well adjusted (IAEA, 1973).

Swaminathan (1965) observed that alkylating agents are more efficient than radiations for inducing point mutations; but less efficient for inducing chromosome aberrations. He, however, reported on several cases where EMS was as effective as gamma rays in inducing chlorophyll mutations in bread wheat. Ethyl methane sulphonate has been successfully used in vegetatively propagated crops such as chrysanthemum (Bowen, 1965); rose (Dommergues et al., 1967 and Kaicker and Swarup, 1972); mint (Kaul and Kak, 1973, 1975); apple (Broertjes and Van Harten, 1978) and mango (Sharma et al., 1983).

Effect of mutagens on plant growth

The mutagenic sensitivity of plants is usually assessed by parameters such as sprouting, survival, plant height, flowering behaviour and occurrence of chlorophyll chimeras and morphological variations.

Sprouting

The effect of mutagen on sprouting and/or germination has been reckoned as one of the reliable estimates of

seedling lethality, by several workers. In ginger, 5.0 krad gamma rays prevented total germination of the rhizomes (Gonzalez et al., 1969). Raju et al. (1980) recorded 32 per cent germination when ginger rhizomes were irradiated with 2.0 krad gamma rays. Graded decrease in the sprouting percentage was observed in ginger cultivars Rio-de-Janeiro and Maran, as the doses of gamma rays increased (Giridharan, 1984). At 2.0 krad, the sprouting percentage of the cultivars Rio-de-Janeiro and Maran was 33 and 19 per cent, respectively. Sprouting was completely inhibited by gamma rays at 4.0 krad and above doses.

Similar inhibitory effects on the germination have been reported in other vegetatively propagated crops by many workers. Sparrow and Christenson (1950) observed inhibitory effect of X-ray irradiation on sprouting of potato tubers. Uzenbaev and Nazernko (1969) observed delayed germination of canna rhizomes. In mango-ginger (Curcuma amada Roxb.) irradiated with gamma rays at 2.0 krad the germination percentage was 64, which reduced to 16 per cent at 5.0 krad (Raju et al., 1980). Radio-sensitivity studies undertaken by these scientists indicated that mango-ginger and ginger were more sensitive than turmeric. In costus (Costus speciosus) gamma rays at 3.0 krad reduced the sprouting of the rhizomes (Gupta et al., 1982). The percentage of sprouting decreased as the doses of gamma rays increased in tuberose. At 0.5

krad the sprouting percentage was 96 which reduced to 72 at 2.5 krad (Sambandamurthi, 1983).

The inhibitory effects of gamma rays on sprouting of sugarcane (Vijayalakshmi and Rao, 1960) cassava (Moh, 1963; Vasudevan et al., 1967 and Thamburaj et al., 1985) and chrysanthemum (Datta, 1988) have also been reported.

Sambandamurthi (1983) observed a trend of reduction in the percentage of sprouting as the doses of ethyl methane sulphonate (EMS) increased. At 15 mM concentration of the chemical, the sprouting percentage was 98 which reduced to 40 at 75 mM. In tapioca, the percentage of sprouting at 45 days after planting decreased with increase in the doses of EMS. The sprouting based on the percentage of control at 12.5 mM was 73. At higher concentrations of the chemical, the sprouting progressively reduced and reached 25 per cent at 75 mM (Thamburaj et al., 1985).

Survival

According to several reports the survival count is a better estimate of lethality than the percentage germination as it accounts for post-germination lethality also. Vasudevan et al. (1968) irradiated tubers of colocasia (Colocasia esculenta L.) with 0.5 to 10.0 krad gamma rays and found that some plants germinated normally but failed to

survive. In tuberose, sprouting of tubers took place at 4.0 krad gamma rays, but the sprouts did not grow further indicating that exposing tuberose above 2.0 krad will give no survival of plants (Gupta et al., 1974). Abraham and Desai (1976) considered the percentage of survival as a reliable estimate in bulbous ornamentals for studying their sensitivity to mutagens. In gladiolus post-germination lethality occured and 50 per cent survival of plants was obtained at 4.7 krad gamma rays. Reduction in survival resulted on gamma ray treatment at 3.0 krad in Costus speciosus (Gupta et al., 1982). Decrease in survival recorded after irradiation with gamma rays in nine rose cultivars (Datta, 1985b). In all the cultivars, the survival percentage decreased as the doses of gamma rays increased. At the highest dose employed (5.0 krad), the sprouted plants in two cultivars did not grow further and failed to survive. In tapioca, Thamburaj et al. (1985) observed that survival at 45th day of planting decreased with increase in the doses of gamma rays. At 0.5 krad gamma rays, the survival was 95.2 per cent and the same reduced to 7.5 per cent at 3.0 krad gamma rays. In gladiolus, 2.5 to 12.5 krad gamma rays exhibited reduction in the survival (Banerji and Datta, 1988). According to Datta (1988), survival reduction was noticed in chrysanthemum by gamma irradiation at a dose range of 1.5 to 2.5 krad.

Application of EMS in tuberose affected the survival of the treated specimens. Ninety eight per cent of the tuberose plants survived when a dilute concentration of 15 mM EMS was used. But at high concentration of 75 mM, only 32 per cent plants survived (Sambandamurthi, 1983). In tapioca, Thamburaj et al. (1985) observed that the survival count taken at 45th day decreased with increase in the concentration of EMS. At 12.5 mM EMS, survival was 78.3 per cent and the same reduced to 20.8 per cent at 75 mM of EMS.

Plant height

Plant height in ginger was found generally to decrease as the dose of gamma rays increased. The average height of the plants treated with 2.0 and 5.0 krad gamma rays was 6.5 and 3.0 cm respectively while the height of the control plants was 35.0 cm (Raju et al., 1980). Decrease in the plant height in ginger cultivars Rio-de-Janeiro and Maran was observed as a result of gamma ray treatment at doses of 0.7 krad to 2.0 krad (Giridharan, 1984). Gamma irradiation in mango-ginger revealed a decreasing trend in plant height as the doses increased. The mean height of the control plants was 40 cm which was reduced to 35 and 7 cm, respectively at 2.0 and 5.0 krad gamma rays (Raju et al., 1980). They further observed that the lowest dose of gamma rays applied

(2.0 krad) did not alter the height of the turmeric plants. However, at 5.0 krad the height was reduced to 18 cm from 60 cm recorded for the control. The plant height of Costus speciosus was found to be significantly reduced on exposure of the rhizomes to gamma rays. As a result of the gamma ray treatment at 3.0 krad a drastic reduction of height from 48.7 to 17.7 cm resulted.

Significant reduction in the height of tuberose treated with gamma rays at 0.5 krad to 2.0 krad was observed by Sambandamurthi (1983). The reduction in the height due to 2.0 krad gamma rays was 17 per cent of the height of the plants at the lowest dose of 0.5 krad. In gladiolus, gamma irradiation treatment resulted in the reduction of plant height (Raghava et al., 1988). Irradiation of plants with some hundreds of rads of fast neutrons induced changes in plant height in Poinsettia (Love, 1966, 1972). The average height of plants of all the cultivars of chrysanthemum was reduced due to irradiation with 1.0 krad to 2.5 krad gamma rays (Gupta and Jugran, 1983). In rose reduction of height resulted on irradiation with 3.0, 4.0 and 5.0 krad gamma rays (Datta, 1985 b) and with 3.0 and 4.0 krad gamma rays in recurrent gamma irradiation experiment (Datta, 1986). In cassava, Thamburaj et al. (1985) observed that the height of the plants at 45 days after planting decreased with increased dose of radiation. At 0.5 krad, the plant height was 18.2 cm

whereas the control plants recorded a height of 24.2 cm. At 3.0 krad, the height reduced to 5.5 cm.

With respect to plant height in tuberose an increasing trend was observed as the dosage of EMS was increased from 15 to 60 mM (Sambandamurthi, 1983) in vM_1 generation. EMS at 45 mM and 60 mM recorded increases of 14 and 12 per cent respectively of the control. However in the sensitivity study, he obtained contrary results showing decreasing plant height with increasing concentrations. In tapioca, the height of the plants measured 45 days after planting decreased with increase in the concentration of the EMS. At 12.5 mM EMS the height was 14.2 cm whereas the control plants recorded 21.2 cm height. At 75.0 mM, the height further reduced to 6.2 cm (Thamburaj et al., 1985).

Cholorophyll chimera

Chlorophyll deficient sectors have been observed as a result of gamma irradiation of the rooted cuttings of carnation (Buiatti et al., 1965). They found that the frequency of occurrence of chlorophyll deficient sectors per plant and branches was roughly proportional to the dose.

In tapioca, gamma rays at a wide range from 0.3 krad to 10.0 krad, produced chlorophyll deficient plants. A mutant obtained at 7.5 krad, produced a sectorial chimera

showing chlorophyll deficient leaves. The branch showing chlorophyll deficient leaves when vegetatively propagated gave rise to two branches of which one was normal. The other was a mutant which on further propagation was found to be "true breeding" (Vasudevan et al., 1967). Occurrence of chlorophyll deficient plants as a result of gamma ray irradiation at 0.5 krad to 10.0 krad in one of the strains of Colocasia esculenta was reported by Vasudevan et al.(1968). At higher doses, the majority of the plants were completely devoid of chlorophyll at the time of germination and survived only for some days. However, there was a few sprouts which were chlorophyll deficient at germination; but showed gradual recovery and development of chlorophyll with further growth. There were others which exhibited normal appearance at the time of germination but their growth was accompanied with partial or complete loss of chlorophyll. In the latter case, the plants failed to survive. Vasudevan et al. (1987) observed chlorinas and other leaf abnormalities in colocasia. Variation in leaf shape and colour has been observed in costus by Gupta et al. (1982) when gamma irradiation was resorted to. In ginger Giridharan (1984) reported appearance of yellow streaks as a result of gamma irradiation in the cvs. Rio-de-Janeiro and Maran.

Leaf variegations due to gamma irradiation have been

reported in crops propagated vegetatively, namely, canna (Nakornthap, 1965), colocasia (Vasudevan et al., 1968), mentha (Ono, 1971), banana (Valez and Maldonado, 1972) and tuberose (Gupta et al. 1974; Konzak, 1984 and Sambandamurthi, 1983).

Morphological abnormalities

Rhizomes of the young plants of canna were irradiated with gamma rays at 1.0, 1.5 and 2.7 krad. The treatments resulted in stunted plants with variegated leaves (Nakornthap, 1965).

Escober and Lopez (1970) treated sugarcane seed pieces with gamma rays to find out the effect of irradiation on the growth of the resultant plants. Abnormalities of the growing point, malformation of the leaves as well as stunting and reduction in the size of the stalk, were observed in the irradiated plants.

Growth reduction and drastic leaf aberrations were observed in banana when treated with gamma rays (Valez and Maldonado, 1972).

Shoot tip cuttings of three sweet potato cultivars were irradiated with 1.0, 2.0 and 3.0 krad gamma rays. Wrinkled and deformed leaves with reduced plant growth were observed in plants treated with 2.0 and 3.0 krad gamma rays

with the latter dose producing higher percentage of such plants. During the first three weeks of growth, most of the damaged plants produced wrinkled and deformed leaves; but they started to produce normal looking leaves during the fourth week. Differences in the response to gamma radiation have been observed among sweet potato cultivars (Pido and Engle, 1987).

Raju et al. (1980) observed formation of weak and elongated underground rhizomes in ginger on treatment with 2.0 krad gamma rays. In turmeric and mango-ginger, the same treatment showed almost normal growth; but the leaves showed abnormalities. Their rhizomes were stored separately and planted in the next year. They produced normal plants in the vM_2. The leaves at the lower nodes showed some morphological abnormalities.

The abnormalities noticed in the vM_2 generation of gamma iradiated (1.5 to 15.0 krad) population of mango were in the form of smaller and larger leaves, small lanceolate leaves, ovate leaves, deeply cut leaf margins, highly cupped leaf margins, leaves without apex, twisting of mid-ribs, bifurcation of the mid-rib, interveinal chlorotic streaks and patches, disturbed phyllotaxy and bifurcation of the shoot at the wrong point (Sharma et al., 1983).

Effect of mutagens in flowering

Hooker (1894) described ginger as a species very rarely flowering and never setting seed. The shy flowering nature and the probable reasons for the absence of seed set in ginger have been explained by many workers (East, 1940; Fryxell, 1957; Pillai et al., 1978; Jayachandran and Vijayagopal, 1979; Jayachandran et al., 1979; Velayudhan et al., 1983 and Usha, 1984). Very little systematic research undertaken to study the effect of mutagens on flowering and seed set. Giridharan (1984) studied the direct effect of radiation in flowering and seed set in the vM_1 generation of ginger and found that gamma irradiation at dose range of 0.7 to 2.0 krad had no favourable effect on flowering and seed set.

Many wrokers demonstrated the effect of gamma rays in modifying the flowering behaviour of rhizomatous and allied crops such as iris (Halevy and Shoub, 1965 and Hekstra and Broertjes, 1968), canna (Nakornthap, 1965; Mukherjee and Khoshoo, 1970), dahlia (Singh et al., 1970 and Das et al., 1975), tuberose (Gupta et al., 1974 and Sambandamurthi, 1983) and gladiolus (Misra, 1976; Banerji and Datta, 1988 and Raghava et al., 1988).

The studies on the effect of gamma rays on flowering of vegetatively propagated crops such as chrysanthemum

(Bowen, 1965; Gupta and Jugran, 1983 and Datta 1985 a), poinsettia (Love, 1966, 1972), rose (Lata and Gupta, 1971; Datta, 1985 b, 1986), pineapple (Nayar et al., 1978) etc. have yielded results indicating the feasibility of using physical mutagens to create variability.

Effects of mutagens on disease resistance

Ginger is affected by a number of diseases leading to varying degrees of crop damage and yield reduction. Soft rot is the most serious disease of ginger in India and in some other countries (Purseglove et al., 1981). Joshi and Sharma (1982) reported more than 50 per cent loss due to the infection of soft rot. Soft rot is caused by a few species of Pythium. Pythium aphanidermatum (Edson) Fitz., P. butleri Subram., P. complectens Braun., P. deliense Meurs., P. gracile (de Bary) Schrenk., P. graminicolum Subram., P. myriotylum Drechsler and P. vexans de Bary are important species causing soft rot. P. aphanidermatum (Edson) Fitz. was reported to be the principal species in India (Purseglove et al., 1981). P. vexans has been observed at an altitude of 1170 m above MSL in Wynad area in Kerala (Joshi and Sharma, 1982). The initial symptoms of the disease appear as light yellowing of the tips of the lower leaves which gradually spread down the leaf blade and leaf sheath along the margin. In the early stages, the middle portion of the lamina remains

green while the margins become yellow. The yellowing spreads to all the leaves followed by drooping, withering and drying of the plant. The infection spreads to the rhizomes resulting in the decay of the rhizomes and roots.

The disease spreads either through diseased rhizomes or through oospores in the soil. The infected plant debris remaining in the field forms an important source of infection. The use of disease free rhizomes as well as seed and soil treatment with fungicides are recommended for controlling the disease. In practice, once the infection starts and favourable conditions like high moisture content of the soil with insufficient drainage occur in the field, high losses may result. Screening aimed at identification of disease tolerant/ resistant cultivars did not give encouraging results. Breeding for disease resistance seems to be the alternate method.

Bacterial wilt is another very serious disease in most of the ginger growing areas. The causative organism is Pseudomonas solanacearum. Two biotypes of this bacterium have been reported. The biotype 3 is present in India and the biotype 3 and 4 in Queensland (Joshi and Sharma, 1982). The first symptoms of the disease are yellowing and wilting of the lower leaves which quickly spread upwards. In the advanced stages, the base of the pseudostem becomes water-

soaked and readily breaks away from the rhizome. The vascular tissues become dark brown or black. The cut pseudostem and rhizome give a white, milky exudate (Purseglove et al., 1981). Planting of healthy rhizomes disinfected by immersion in a 0.6 per cent mercurial seed protectant for 90 minutes, crop rotation and soil fumigation with methyl bromide have been suggested as the control measures. In practice, once the infection starts, the control measures will not be of much help. In the absence of sources with in-built tolerance/resistance to bacterial wilt, induction of this character by mutation breeding has been suggested (Nair et al., 1982).

Breeding for disease resistance certainly represents the most important way to counteract the pathogens. Induced mutations are now being developed as a complementary tool in breeding for disease resistance (Borojevic, 1972). Breeding resistant varieties is in many cases the most economic and the least hazardous measure of managing crop plant diseases. Induced mutation could be useful to develop resistant varieties provided adequate screening techniques to detect the desired plant characters are available at hand (Micke, 1974).

Mutation rectification of an otherwise desired variety is preferred to ad-hoc mutation breeding programmes.

Concepts like the stage of selection, the environment of selection and the intensity of selection have changed during the last five years (Murty, 1983). The detection of a disease resistant mutant results generally from eye inspection of the symptoms and is assumed on the basis of the frequency and size of lesions caused by the pathogen (Micke, 1974). The first report on the induction of mutations for disease resistance is by Freisleben and Lein (1942). Working in Germany, they isolated a mutant in Haisa barley simultaneoulsy resistant to three races of powdery mildew, as a result of treatment with X-rays and after a survey of about 12,000 progenies.

Increased resistance to Phytophthora infestans was observed by X-ray irradiation in potato (Kishore et al., 1963). Resistance to Verticillium wilt was observed in the progenies when dormant stolons of peppermint (Mentha piperita) were irradiated with 500 to 6000 rads of X-rays (Murray, 1969). Seven highly resistant and five moderately resistant peppermint strains were obtained from 1,00,000 irradiated plants (Murray, 1969, 1971). A new strain 'Todd's Mitcham Peppermint' was finally registered by the Crop Science Society of America in 1972. The oil from this strain was found to be quantitatively as well as qualitatively about the same as the oil from the original Mitcham variety. This is one of the best examples of successful mutation breeding,

which could only be successful because of the efficient screening technique and the simple way in which peppermint propagates (from pieces of stolons) that reduced or even avoided the disadvantages of chimera formation (Broertjes and Van Harten, 1978). A trial for inducing disease resistance in sugarcane seed pieces (Escober and Lopez, 1970) with gamma rays did not give encouraging results. In Mentha arvensis, resistance to rust disease could be induced by gamma irradiation (Ono and Ikeda, 1970). Irradiation of one or two-node sections of the (dormant) rhizomes, stolons or sprigs of Bermuda grass varieties with 7-12 krad of gamma rays yielded mutants that showed high level of resistance to root knot nematode (Powell et al., 1974). Gamma irradiation of nodes of St. Augustine grass resulted in disease resistance and improvement in agronomic characteristics (Toler and Grisham, 1983).

Quality

Quality of ginger rhizomes are mainly determined by the content of volatile oil, non-volatile ether extract, fibre and starch. According to Jayachandran et al. (1980), the average percentage content of the above components in ginger cv. Rio-de-Janeiro were volatile oil (2.7) NVEE (8.3) fibre (6.6) and starch (40.2). Good quality ginger contains less fibre, but more volatile oil and NVEE.

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

Flowers of rose produced lesser oil when the buds were subjected to irradiation before budding (Lata and Gupta, 1971). Gamma ray treatment resulted in the isolation of mutants with high essential oil content in lemon grass (Nair, 1979), mint (Kaul et al., 1978; Kak and Kaul, 1979) and rose (Irulappan, 1979). In costus, diosgenin content increased as a result of 2.0 krad gamma ray treatment whereas it decreased at 3.0 krad (Gupta et al., 1982). Pavlovic et al. (1983) observed a positive correlation between irradiation dose and essential oil content in Mentha piperita.

In tuberose, though irradiation with gamma rays induced high percentage of concrete recovery, the yield of flowers was poor (Sambandamurthi, 1983).

MATERIALS AND METHODS

MATERIALS AND METHODS

The studies were undertaken in the Department of Horticulture, College of Agriculture, Vellayani, Thiruvananthapuram during the period from 1985 to 1989. Four experiments, namely, Standardisation of the doses of the mutagens; Effect of the mutagens in the vM_1 generation; Evaluation of the vM_2 generation and screening the vM_2 generation against bacterial wilt and soft rot and Evaluation of mutants in the vM_3 generation and inoculation studies on the vM_3 plants considered as tolerant/ resistant to bacterial wilt were included in the studies.

MATERIALS

Plant material

Ginger (Zingiber officinale Roscoe), belonging to the family Zingiberaceae, is a slender perennial herb which is grown as an annual. The cultivar Rio-de-Janeiro was used for the studies. The important features of this cultivar under the two agro- climatic situations are given in Table 2.

Mutagens

Physical and chemical mutagens were employed for the induction of mutation.

Physical mutagen

Gamma ray was the physical mutagen used. The irradiation was done at the Radio Tracer Laboratory, Kerala Agricultural University, Vellanikkara utilizing the Cobalt-60

Table 2. Important features of ginger cultivar Rio-de-Janeiro under two agro-climatic situations

Plant characters	Exhibited at Vellayani, Thiruvananthapuram	Exhibited at Ambalavayal, S. Wynad
Height (cm)*	66.4	48.6
Number of tillers/plant	19.6	9.5
Number of leaves/plant	259.0	103.3
Yield of rhizomes/plant (g)	348.0	306.6
Recovery of dry ginger (%)	14.6	18.0
Crude fibre (%)	6.6	5.8
Non volatile ether extract(NVEE)(%)	8.3	NA
Oleoresin (%)	NA	10.8
Starch (%)	40.2	NA
Volatile oil (v/w) (%)	2.7	NA
References :	Jayachandran and Sethumadhavan (1979) and Jayachandran *et al*. (1980)	Sreekumar *et al*.(1982)

*Height from the base of the plant to the leaf tip

gamma chamber. The source was operated at an intensity of 0.238 MR/hour and 0.205 MR/hour for the Experiment I and II, respectively.

Chemical mutagen

The chemical mutagen, ethyl methane sulphonate $CH_3SO_2-O-C_2H_5$ was used. The chemical was obtained from M/s Koch-Light Laboratories Ltd., (Bucks), England.

METHODS

Irradiation with gamma rays

Well-developed, disease-free and uniform rhizome bits weighing 5 to 7 g, with one viable bud each were used for the irradiation. Twenty rhizome bits were packed in muslin cloth bags and treated with gamma rays. The doses were adjusted by regulating the period of exposure to the rays. The irradiated rhizomes were planted in the field during the next day of the treatment.

Treatment with ethyl methane sulphonate (EMS)

The rhizome bits selected as above with one viable bud each were used for treatment with EMS. The rhizome bits were soaked in the desired concentration of the aqueous solution of EMS for eight hours at room temperature ($30 \pm 2^{\circ}$ C) with intermittent stirring and shaking. The treated rhizomes were

washed thoroughly in running water and planted in the field during the same day.

Experiment I: Standardisation of the doses of the mutagens

A field experiment was laid out in randomised block design with 11 treatments and three replications for standardising the doses of the physical mutagen. Another field experiment in randomised block design with 12 treatments and three replications was laid out to standardise the doses of the chemical mutagen. The doses of the gamma rays and EMS are given in Table 3.

Observations

The following observations were recorded.

Sprouting

The number of rhizomes sprouted was recorded 45 days after planting and expressed as percentage of the control. Emergence of green leaves above the ground level was taken as the criterion for sprouting.

Survival

Counts of the surviving plants were taken 150 days after planting and expressed as percentage of the control. All the healthy plants with green colour were considered as surviving.

Plant height

Plant height (cm) was measured from the ground

Table 3. Details of mutagenic treatments of the Experiment I

Sl. No.	Gamma rays Dose (krad)	Sl. No.	EMS Dose(mM)
1	Control (untreated)	1	Control (Water soaking 8 hrs. at room temp.)
2	0.50	2	8
3	1.00	3	16
4	1.50	4	32
5	2.00	5	48
6	2.50	6	64
7	3.00	7	80
8	3.50	8	96
9	4.00	9	112
10	4.50	10	128
11	5.00	11	144
		12	160

level to the base of the fully opened terminal leaf of the tallest tiller of the plant 150 days after planting and expressed as percentage of the control.

Experiment II: Effect of the mutagens in the vM_1 generation

Based on the results of Experiment I, five doses of gamma rays and five concentrations of EMS were selected for the experiment.

The treatment details of the Experiment II are presented in Table 4.

Table 4. Details of mutagenic treatments of the Experiment II

Sl.NO.	Treatment	Dose
1	Control (Absolute)	No treatment
2	Control	Water soaking (8 hrs. at room temp.)
3	Gamma ray	0.50 krad
4	Gamma ray	0.75 "
5	Gamma ray	1.00 "
6	Gamma ray	1.25 "
7	Gamma ray	1.50 "
8	EMS	2.00 mM
9	EMS	4.00 "
10	EMS	6.00 "
11	EMS	8.00 "
12	EMS	10.00 "

The field experiment was laid out in randomised block design with three replications. Two hundred single budded rhizome bits of uniform size per treatment were used.

The following observations were recorded.

Sprouting

The number of rhizomes sprouted 45 days after planting was recorded and expressed as percentage of the control. The rhizomes which sprouted thereafter were counted as "delayed". The number of rhizomes exhibiting delayed sprouting was recorded and expressed as percentage of the number of rhizomes planted, since delayed sprouting was not exhibited in the control.

Survival

The healthy plants with green colour were considered surviving and the survival count was taken 150 days after planting and expressed as percentage of the control.

Plant height

Plant height (cm) was measured at 30-day intervals from 60 days to 180 days after planting and expressed as percentage of the control.

Number of tillers per plant

The total number of tillers per plant was recorded at

30-day intervals from 60 days to 180 days after planting and expressed as percentage of the control.

Number of leaves per plant

The total number of leaves per plant was recorded 180 days after planting and expressed as percentage of the control.

Chlorophyll chimera

Plants with chlorophyll deficient portions on their leaves were recorded as chlorophyll chimeras. The number of plants with chlorophyll chimera was counted and expressed as percentage over the rhizomes sprouted.

Flowering

The number of plants which flowered in each treatment was recorded and expressed as percentage of the rhizomes sprouted.

Pollen fertility and seed set

Pollen fertility was studied using acetocarmine stain method and expressed as percentage. Flowers were observed periodically for verifying seed set.

Maturity period

The period from planting to harvest was recorded as the period taken for maturity. Yellowing and drying of the leaves were considered as the indications of maturity.

Rhizome yield

The rhizomes were cleaned immediately after the harvest and their fresh weight (g) recorded.

Experiment III (a): Evaluation of the vM_2 generation

Plants with distinct morphological differences from the standard and the randomly selected observational plants were raised as progeny rows in the vM_2. In the evaluation of vM_2 generation the following observations were recorded as in Experiment II.

Plant height

Number of tillers per plant

Number of leaves per plant

Flowering

Pollen fertility and seed set

Maturity period

Rhizome yield

The vM_2 generation was screened for distinct variations for plant characters including maturity period. Based on the expression of the different characters in the vM_2 generation, they were classified into three groups for each character (except for maturity period) as given below. For maturity period the plants were classified into two groups (Table 5).

Table 5. Criteria for classification of ginger plants into groups

Character	Classification	Criterion
Plant height (cm)	Tall	Above 55
	Medium	35 to 55
	Dwarf	Below 35
Tillers	High	Above 25
	Normal	10 to 25
	Low	Below 10
Leaves	High	Above 500
	Normal	150 to 500
	Low	Below 150
Yield (g)	High	Above 400
	Medium	100 to 400
	Low	Below 100
Maturity (months)	Early	Below 7 months
	Normal	7 to 8 months

Experiment III (b): Screening the vM_2 generation against bacterial wilt and soft rot

The vM_2 progenies of one replication were planted in previously selected intensive sick plots at the Regional Agricultural Research Station, Ambalavayal for screening against bacterial wilt and observations on the reaction to bacterial wilt were recorded and the plants were graded as per a modified scale adopted by Indrasenan *et al*. (1982). The number of plants affected by bacterial wilt were observed

and the plants that were found to be free from the disease 180 days after planting were classified as having tolerated/ survived bacterial wilt disease. Another part (one-third) of the vM_2 progenies were planted at the Instructional Farm, College of Agriculture, Vellayani for screening against soft rot disease. The plants were inoculated with homogenised soft rot disease specimen 60 days and 90 days after planting and observations on the reaction to soft rot were recorded.

Experiment IV (a): Study of the mutants in the vM_3

The probable mutant plants having desirable characters isolated in the vM_2 were carried forward to vM_3 progeny rows. The following observations were recorded as in Experiment II.

Plant height

Number of tillers per plant

Number of leaves per plant

Maturity period

Rhizome yield

The following observations were additionally made in the plants that inherited the mutant characters. Composite samples from each vM_3 mutant progeny were used for the analysis.

Recovery of dry ginger

Immediately after the harvest, the rhizomes were

cleaned thoroughly and the skin was scraped off. They were chopped into small pieces for easy drying and dried in a cross Flow Air Oven at 55 $\pm$ 2 °C to a constant moisture content of 10 per cent and the weight of the dry ginger recorded and expressed as percentage.

Moisture estimation

The moisture percentage of dried ginger rhizomes was determined by toluene distillation method (AOAC, 1975).

Volatile oil

The content of volatile oil was estimated by Clevenger distillation method (AOAC, 1975) and expressed as percentage (V/W) on dry weight basis.

Non-volatile ether extract (NVEE)

The content of non volatile ether extract was estimated by Soxhlet distillation method (AOAC, 1975) and expressed as percentage on dry weight basis.

Starch

The starch content was estimated by Lane-Eynon general volumetric method (AOAC, 1960) and expressed as percentage on dry weight basis.

Crude fibre

The crude fibre was estimated by the AOAC method (1975) and expressed as percentage on dry weight basis.

Experiment IV (b): Inoculation studies on the vM_3 progenies of plants that survived bacterial wilt in the vM_2

The vM_2 plants that survived bacterial wilt at the RARS, Ambalavayal were carried forward to vM_3 progeny rows at the Instructional Farm, College of Agriculture, Vellayani. The plants were inoculated with homogenised bacterial wilt disease specimen 60 days and 90 days after planting. The reaction of the plants to bacterial wilt disease was recorded.

RESULTS

RESULTS

Experiment I: Standardisation of the doses of the mutagens

Sprouting

The data on the percentage of sprouting of ginger rhizomes 45 days after planting presented in Table 6 indicate that as the doses of gamma rays and EMS increased the sprouting percentage decreased (Fig. 1 and 2). At higher doses, complete inhibition of sprouting resulted. Gamma rays at 2.50 krad and above as well as EMS at 32 m$\underline{M}$ and above completely inhibited the sprouting of ginger rhizomes. The LD_{50} in respect of sprouting of gamma ray treated rhizomes appeared to be between 0.50 and 1.00 krad and that for EMS appeared to be below 8 m$\underline{M}$.

Survival

The data on percentage survival 150 days after planting have been presented in Table 7. It can be seen that survival of ginger plants decreased with increasing doses of gamma rays and EMS (Fig. 1 and 2). At 2.0 krad gamma rays, only 2.0 per cent of the plants survived 150 days after planting.

As far as the chemical mutagen was concerned, the concentration of 32 m$\underline{M}$ was found to be completely lethal.

Table 6. Effect of gamma rays and EMS on sprouting 45 days after planting (ginger)

Mutagen	Dose (krad)	Sprouting (% of control)	Mutagen	Dose (mM)	Sprouting (% of control)
Control	(untreated)	100	Control	(water soaking)	100
Gamma ray	0.50	65	EMS	8	48
"	1.00	40	"	16	22
"	1.50	23	"	32	--
"	2.00	6	"	48	--
"	2.50	--	"	64	--
"	3.00	--	"	80	--
"	3.50	--	"	96	--
"	4.00	--	"	112	--
"	4.50	--	"	128	--
"	5.00	--	"	144	--
			"	160	--

FIG.1. EFFECT OF GAMMA RAYS ON SPROUTING AND SURVIVAL IN GINGER (EXPERIMENT-I)

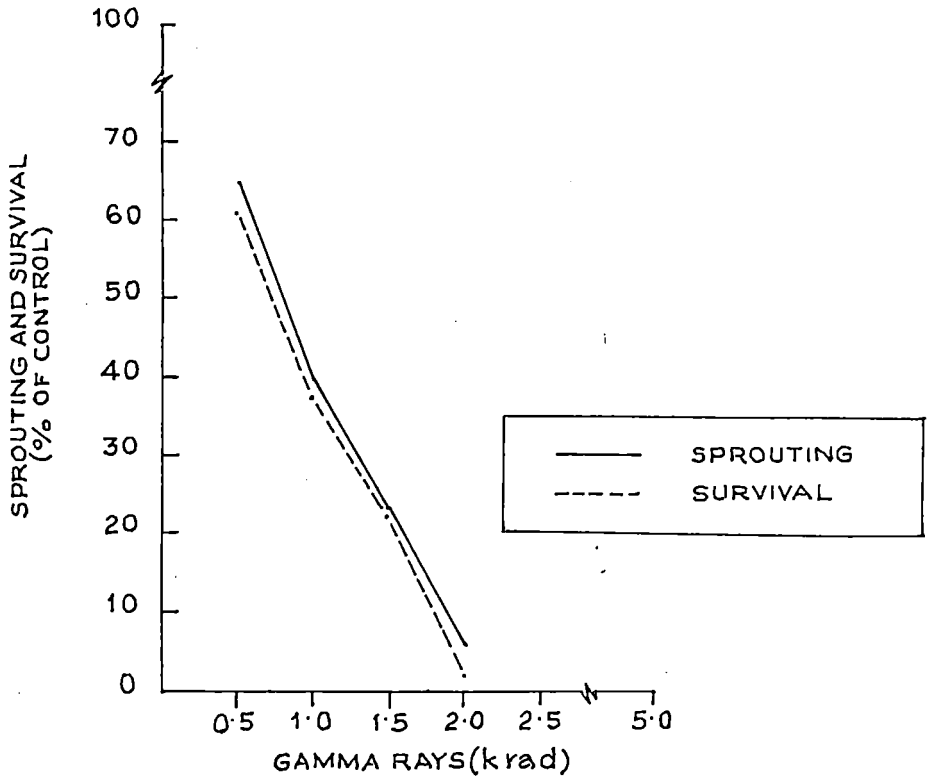


FIG.2. EFFECT OF EMS ON SPROUTING AND SURVIVAL IN GINGER (EXPERIMENT-I)

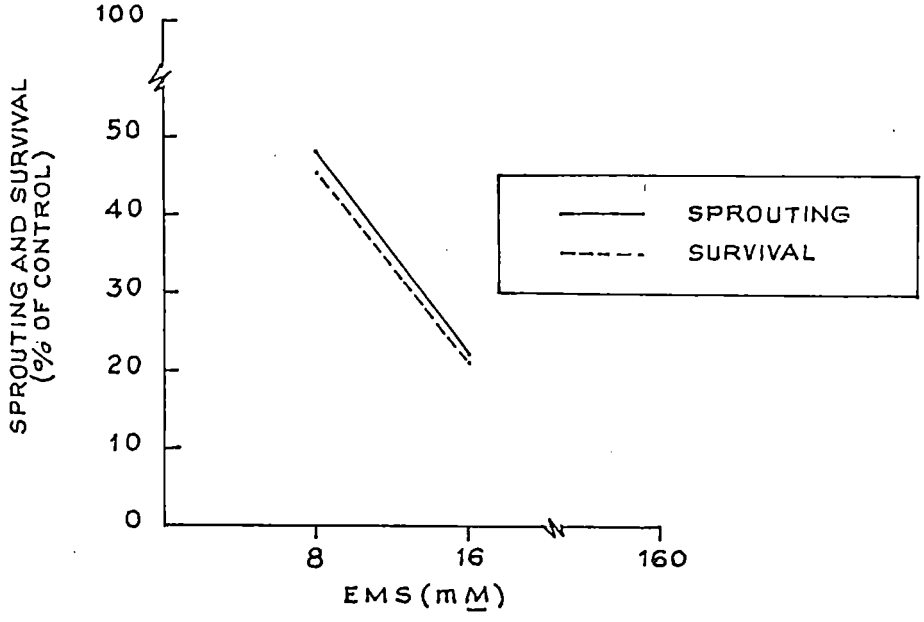


Table 7. Effect of gamma rays and EMS on survival 150 days after planting (ginger)

Mutagen	Dose (krad)	Survival (% of control)	Mutagen	Dose (mM)	Survival (% of control)
Control	(untreated)	100	Control	(water soaking)	100
Gamma ray	0.50	61	EMS	8	45
"	1.00	37	"	16	21
"	1.50	22	"	32	--
"	2.00	2	"	48	--
"	2.50	--	"	64	--
"	3.00	--	"	80	--
"	3.50	--	"	96	--
"	4.00	--	"	112	--
"	4.50	--	"	128	--
"	5.00	--	"	144	--
			"	160	--

Low survival (21 per cent) was observed at 16 m$\underline{M}$. The data suggest that the LD_{50}, based on survival, lies between 0.50 and 1.00 krad of gamma rays and below 8 m$\underline{M}$ of EMS.

Plant height

The data on plant height, recorded 150 days after planting, have been presented in Table 8. Plant height was found to decrease progressively with increasing doses of both the mutagens (Fig.3 and 4). Sixty five per cent reduction in height is seen induced by 2.00 krad gamma rays. EMS at 8 m$\underline{M}$ and 16 m$\underline{M}$ reduced the plant height by 45 and 64 per cent, respectively. The LD_{50}, on the basis of plant height, appeared to lie between 1.50 krad and 2.00 krad of gamma rays and between 8 m$\underline{M}$ and 16 m$\underline{M}$ of EMS.

Experiment II : Effect of the mutagens in the vM_1 generation

Sprouting and survival

The data on percentage sprouting 45 days after planting, delayed sprouting (75 to 135 DAP) and survival 150 days after planting respectively are presented in Table 9. The data indicate that the percentage sprouting decreased as the doses of physical and chemical mutagens increased (Fig.5 and 6). At 0.50 krad gamma rays, the sprouting percentage related to that of the control was 80, which reduced to 26 at 1.50 krad (Plates I and II). The LD_{50} in respect of sprouting seemed to be between 1.00 and 1.25 krad of gamma

Table 8. Effect of gamma rays and EMS on plant height, 150 days after planting (ginger)

Mutagen	Dose (krad)	Mean height (% of control)	Mutagen	Dose (mM)	Mean height (% of control)
Control	(untreated)	100	Control	(water soaking)	100
Gamma ray	0.50	84	EMS	8	55
"	1.00	67	"	16	36
"	1.50	56	"	32	--
"	2.00	35	"	48	--
"	2.50	--	"	64	--
"	3.00	--	"	80	--
"	3.50	--	"	96	--
"	4.00	--	"	112	--
"	4.50	--	"	128	--
"	5.00	--	"	144	--
			"	160	--

FIG. 3. EFFECT OF GAMMA RAYS ON PLANT HEIGHT 150 DAYS AFTER PLANTING IN GINGER (EXPERIMENT.I)

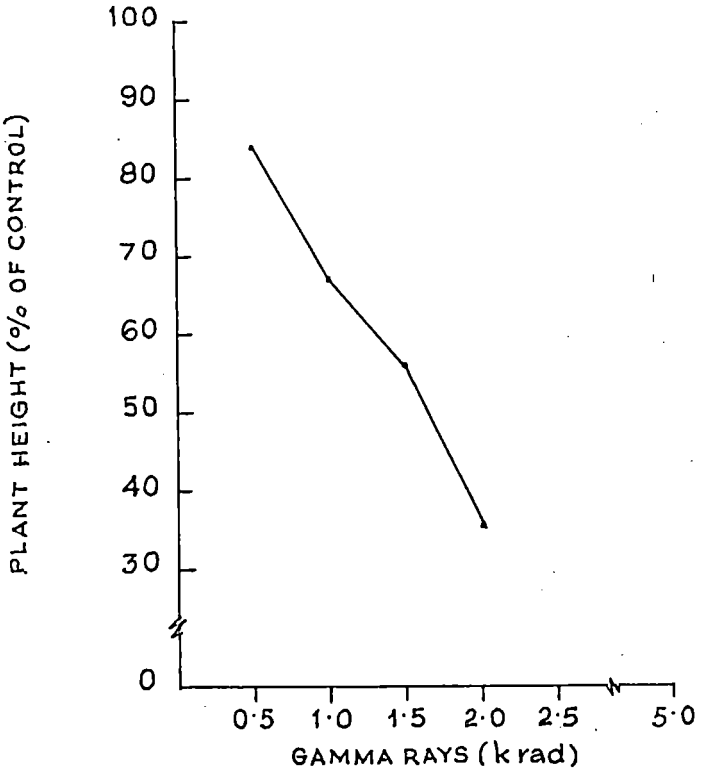


FIG. 4. EFFECT OF EMS ON PLANT HEIGHT 150 DAYS AFTER PLANTING IN GINGER (EXPERIMENT.I)

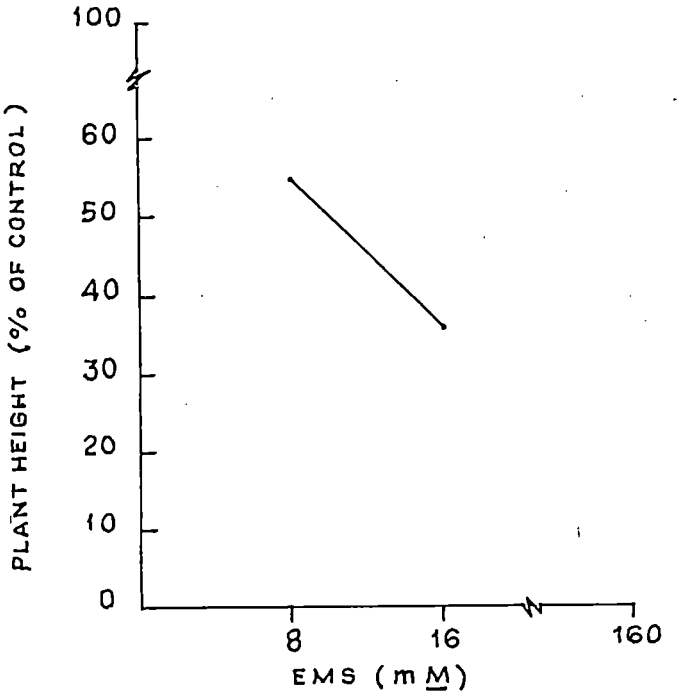


Table 9. Effect of mutagens on sprouting, delayed sprouting and survival, in the vM_1 generation (ginger)

Mutagen	Dose	Sprouting 45 DAP* (% of control)	Delayed sprouting (% of rhizomes planted)	Survival 150 DAP (% of control)
Control	(absolute)	100	-	100
Control	(water soaking)#	100	-	100
Gamma ray (krad)	0.50	80	-	76
"	0.75	72	0.5	63
"	1.00	61	0.2	57
"	1.25	46	1.5	42
"	1.50	26	-	18
EMS (mM)	2.00	81	0.2	79
"	4.00	80	0.7	78
"	6.00	73	0.2	72
"	8.00	71	0.5	69
"	10.00	52	-	50

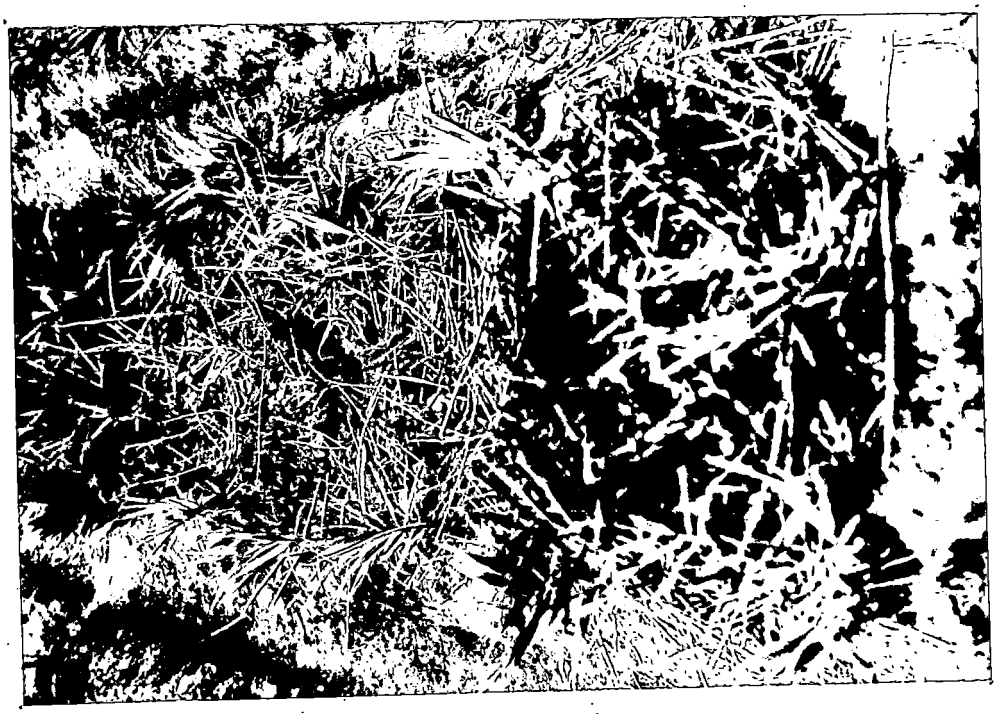
*DAP = days after planting.
#EMS treated plants were compared with control (water soaking)

PLATE No.I

Ginger plants (absolute control)

PLATE No.II

Ginger plants in the vM_1 generation (1.50 krad gamma rays)



rays. It can be seen from the table that at 2 mM EMS, 81 per cent rhizomes sprouted. At the highest concentration of EMS, namely 10 mM, only 52 per cent of the rhizomes sprouted (Plate III). The LD_{50} related to sprouting thus lies above 10 mM of EMS.

Delay in sprouting has been caused by the physical and chemical mutagens. During the period from 46 days to 74 days after planting, no further sprouting was recorded. Delayed sprouting (75 to 135 DAP) to limited extent has been produced by gamma ray and EMS treatments (Table 9 and Appendix I). The resultant plants were dwarfs, with single or a few tillers and reduced number of leaves, morphologically abnormal, pre-maturing with underdeveloped rhizomes weighing a few grams only (Appendix I). They were therefore, not counted as surviving plants.

The percentage survival decreased as the doses of the mutagens increased (Fig.5 and 6). At 0.50 krad gamma rays, 76 per cent plants survived 150 days after planting. The survival at 1.50 krad was reduced to 18 per cent. The LD_{50} appeared to be between 1.00 and 1.25 krad gamma rays. At 2 mM of EMS, the survival recorded was 79 per cent. As the doses of EMS increased further reduction in the survival count was observed. At 10 mM EMS, the survival was 50 per cent indicating that LD_{50} was 10 mM EMS.

PLATE No.III

Ginger plants in the vM_1 generation (10mM EMS)

FIG. 5. EFFECT OF GAMMA RAYS ON SPROUTING AND SURVIVAL IN THE VM_1 GENERATION (GINGER)

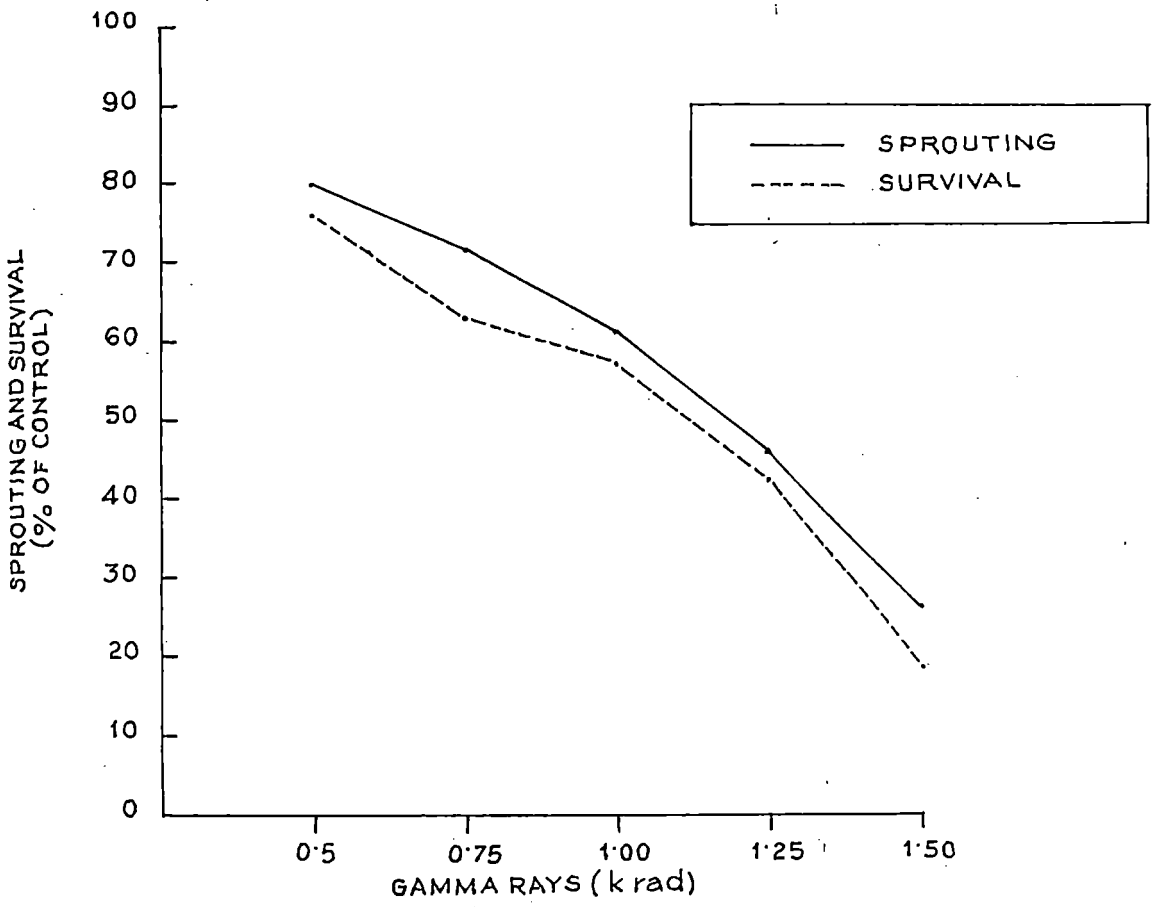
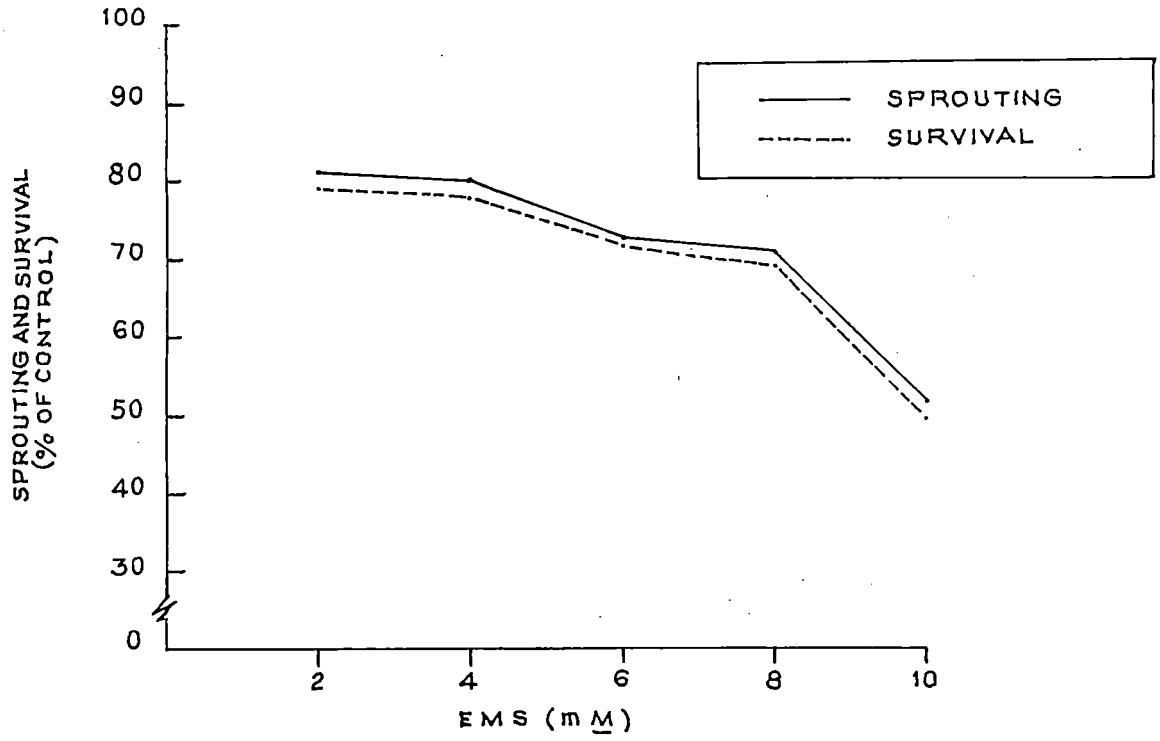


FIG. 6. EFFECT OF EMS ON SPROUTING AND SURVIVAL IN THE VM_1 GENERATION (GINGER)



Plant height

The data on the plant height from 60 to 180 days after planting are presented in Table 10. At 60 days after planting, the height was found to decrease as the doses of gamma rays increased. As the vegetative growth advanced (from 60 to 180 DAP), the height of the treated plants tended to reach that of the control plants (Fig. 7). The highest dose of gamma rays (1.50 krad) recorded the minimum plant height of 58 per cent of the control at 60 days after planting (Plates IV and V). The corresponding plant height when the growth period advanced to 180 days after planting was 86 per cent of the control. The data further indicate that at the lower doses also, the reduction in plant height observed at 60 DAP seemed to nullify as the growth advanced.

The chemical mutagen EMS at all concentrations was found to cause injury, as evidenced by the height of the plants recorded at 60 days after planting. Comparison of the data at 60 DAP and 180 DAP further revealed that recovery of plant height at different rates as the growth phase advanced was observed in the EMS treated plants (Table 10 and Fig. 8).

Tiller production

The data presented in Table 11 indicate that the treatments with gamma rays reduced the tiller production. At 60 days after planting, the lowest dose of gamma rays (0.50

Table 10. Effect of mutagens on plant height in the vM_1 generation (ginger)

Mutagen	Dose	Plant height (cm) at									
		60 DAP*		90 DAP		120 DAP		150 DAP		180 DAP	
		**Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control
Control	(absolute)	16.5	100	25.5	100	35.0	100	40.2	100	43.7	100
Control	(water soaking)#	20.4	100	29.4	100	37.5	100	42.4	100	45.6	100
Gamma ray (krad)	0.50	15.6	95	23.1	91	29.2	83	35.9	89	40.4	92
"	0.75	14.5	88	18.3	72	32.4	93	37.5	93	41.0	94
"	1.00	13.7	83	21.5	84	34.7	99	38.7	96	41.4	95
"	1.25	11.6	70	18.9	74	25.8	74	33.3	83	38.3	88
"	1.50	9.5	58	14.9	59	23.2	66	31.6	79	37.5	86
EMS (mM)	2.00	15.4	75	22.2	76	32.6	87	36.2	85	38.7	85
"	4.00	16.8	82	25.5	87	38.3	102	41.6	98	43.9	96
"	6.00	17.1	84	24.6	84	36.2	97	38.2	90	39.6	87
"	8.00	16.1	79	24.0	82	33.7	90	40.4	95	44.9	98
"	10.00	12.1	59	19.7	67	28.2	75	32.4	76	35.2	77

* DAP = Days after planting
**Mean of thirty observational plants
EMS treated plants were compared with control (water soaking)

FIG. 7. EFFECT OF GAMMA RAYS ON PLANT HEIGHT IN THE VM_1 GENERATION (GINGER)

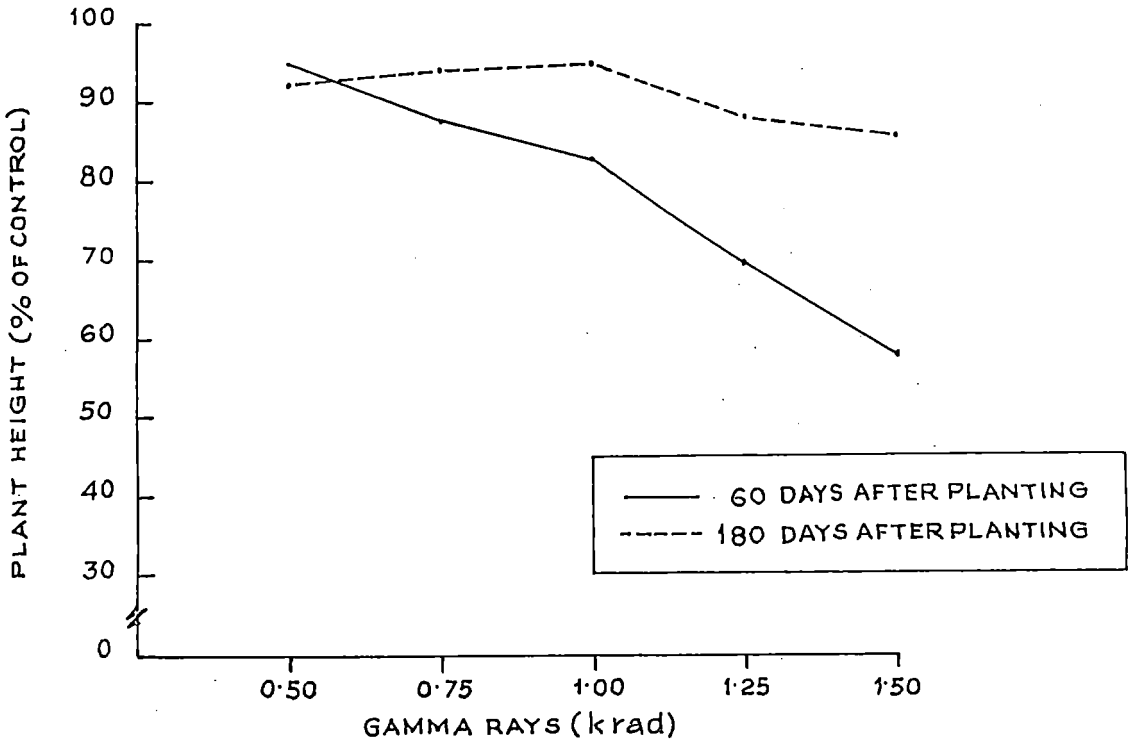


FIG. 8. EFFECT OF EMS ON PLANT HEIGHT IN THE VM_1 GENERATION (GINGER)

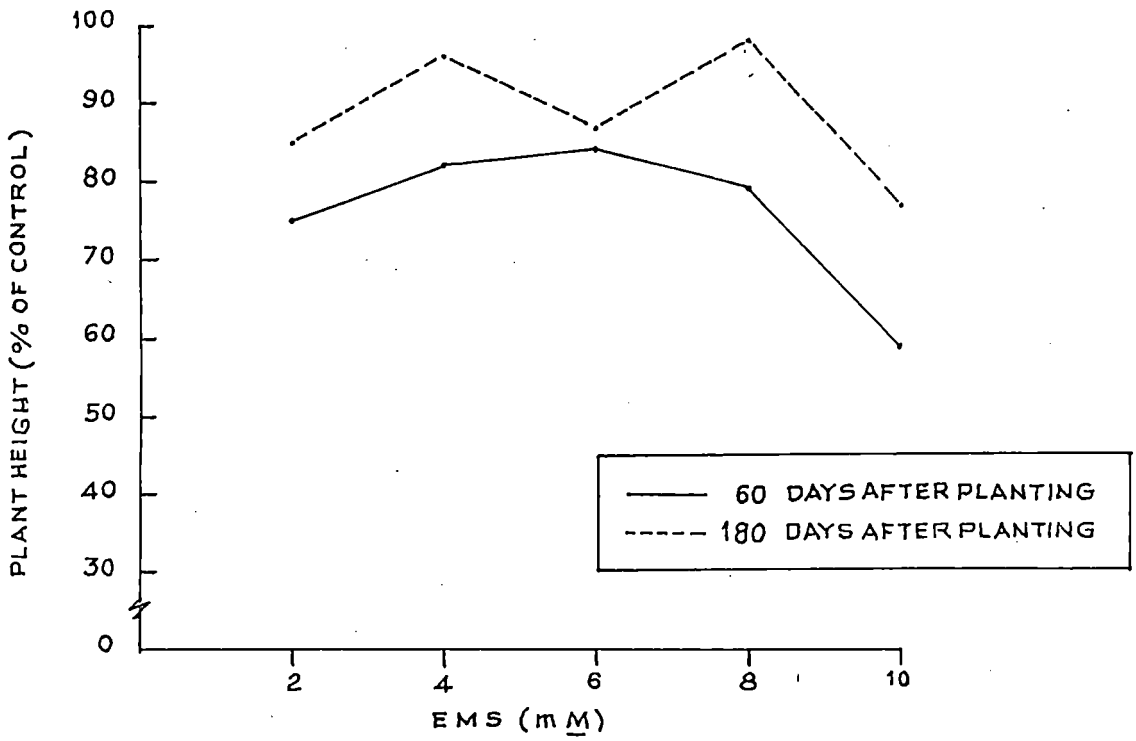


PLATE No.IV

Ginger plant (normal) at 105 days after planting

PLATE No.V

Ginger plant (dwarf) at 105 days aften planting (1.50 krad gamma rays)

Table 11. Effect of mutagens on tiller production in the vM_1 generation (ginger)

Mutagen	Dose	Number of tillers per plant at									
		60 DAP*		90 DAP		120 DAP		150 DAP		180 DAP	
		Mean**	% of control	Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control
Control	(absolute)	2.9	100	5.7	100	9.5	100	12.3	100	15.1	100
Control	(water soaking)#	3.3	100	6.3	100	10.9	100	14.4	100	18.0	100
Gamm ray (krad)	0.50	2.4	83	5.1	89	8.5	89	11.1	90	13.6	90
"	0.75	2.4	83	4.9	86	8.8	93	11.7	95	14.6	97
"	1.00	2.4	83	4.9	86	9.1	96	12.3	100	15.5	103
"	1.25	2.2	76	4.2	74	8.0	84	10.9	89	13.8	91
"	1.50	1.3	45	2.5	44	7.6	80	11.4	93	15.3	101
EMS (mM)	2.00	2.8	85	4.1	65	8.0	73	10.9	76	13.6	76
"	4.00	3.1	94	6.0	95	10.2	94	13.3	92	16.4	91
"	6.00	2.8	85	4.8	76	9.4	86	12.9	90	16.3	91
"	8.00	2.5	76	5.0	79	8.7	80	11.5	80	14.2	79
"	10.00	2.0	61	4.4	70	8.4	77	11.4	79	14.5	81

*DAP = Days after planting
**Mean of thirty observational plants
#EMS treated plants were compared with control (water soaking)

krad) reduced the tillering to 83 per cent of the control. The effect of 0.75 krad and 1.00 krad on reduction of tillering was similar to that of 0.50 krad. As the dose increased further, drastic reduction in tillering was evident. At 1.50 krad, the tillering at 60 days after planting was only 45 per cent of the control. As the growth phase advanced, the adverse effect of gamma ray treatments on tillering was found to show recovery (Fig.9).

The data presented in Table 11 indicate that the treatment with EMS affected the tiller production. The lowest dose of EMS (2 mM) reduced the tillering slightly (85 per cent of the control) at 60 days after planting. As the concentration increased to 10 mM the tiller production was 61 per cent of the control. The recovery rate of tillering capacity was high at this dose, as evidenced by the data at 180 days after planting where 81 per cent tillering has been observed (Table 11 and Fig. 10).

Leaf production

The data presented in Table 12 indicate that leaf production was affected by the radiation treatments. At 60 days after planting, the total number of leaves produced by the 1.50 krad treated plants was only 25 per cent of the control.

During the advanced stages of growth, the inhibitory

FIG. 9. EFFECT OF GAMMA RAYS ON TILLER PRODUCTION IN THE VM_1 GENERATION (GINGER).

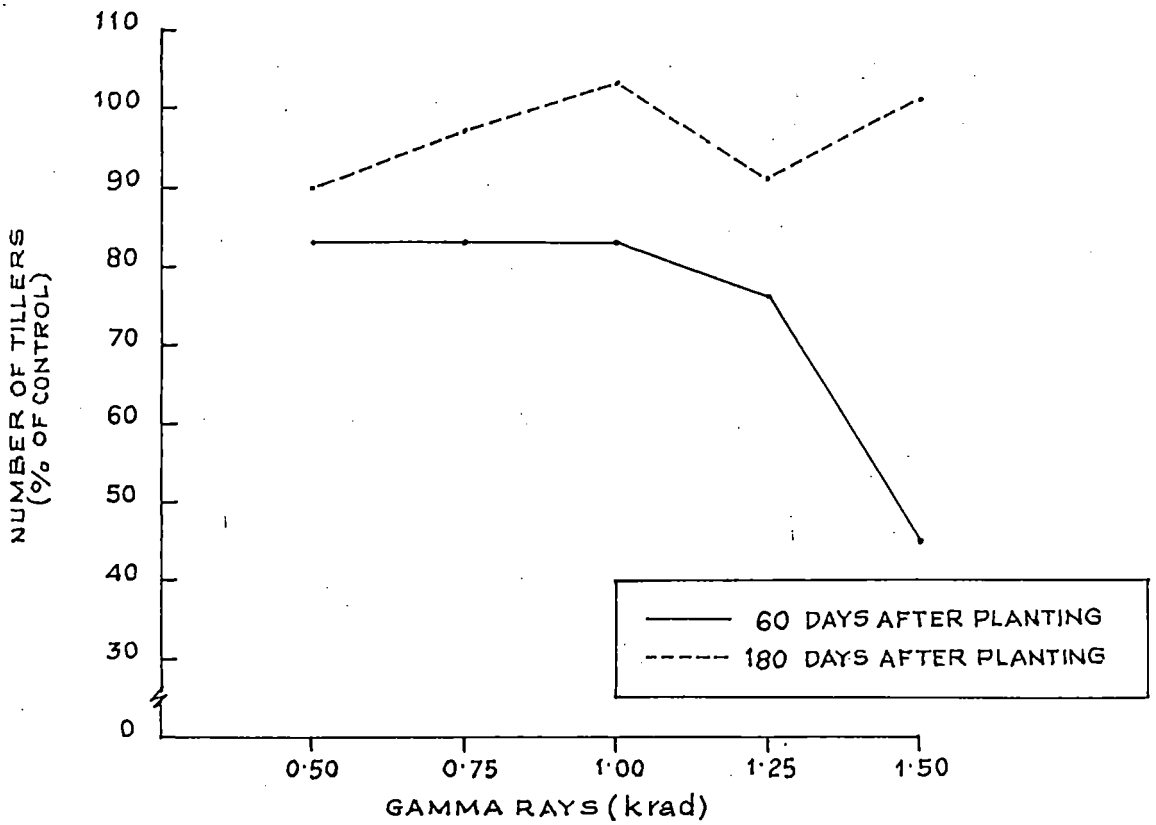


FIG. 10. EFFECT OF EMS ON TILLER PRODUCTION IN THE VM_1 GENERATION (GINGER).

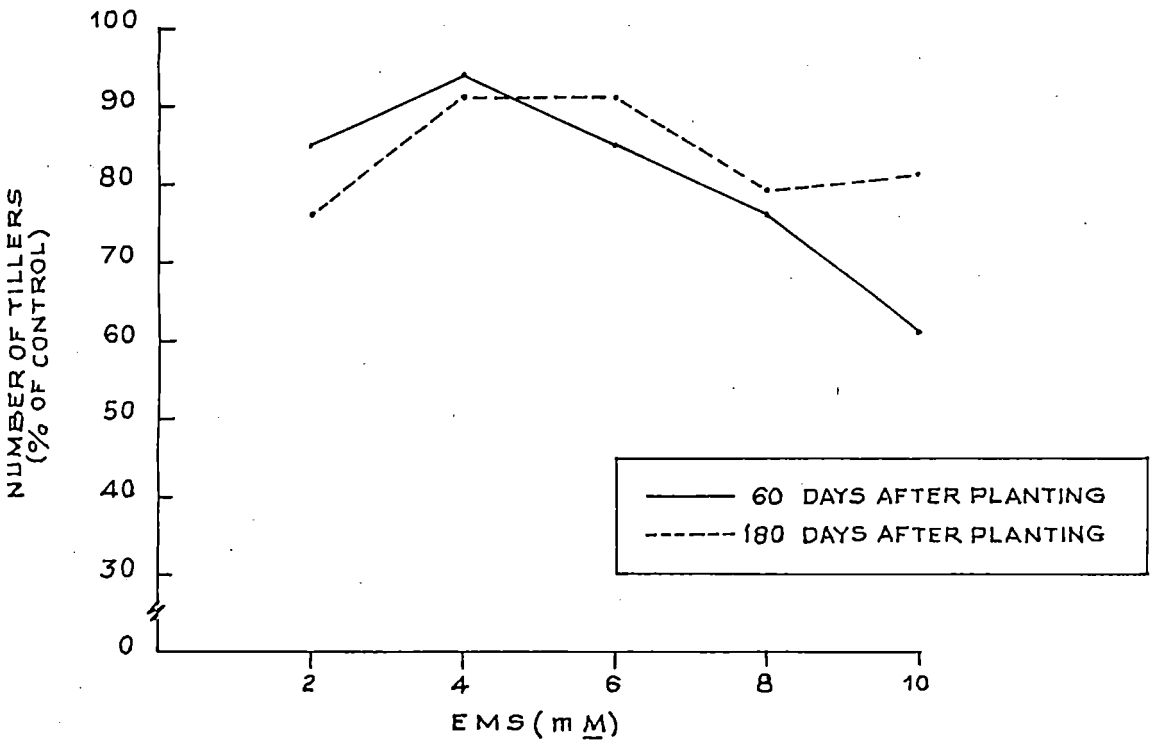


Table 12. Effect of mutagens on leaf production in the vM_1 generation (ginger)

Mutagen	Dose	Number of leaves per plant at									
		60 DAP*		90 DAP		120 DAP		150 DAP		180 DAP	
		Mean**	% of control	Mean	% of control	Mean	% of control	Mean	% of control	Mean	% of control
Control	(absolute)	16	100	45	100	100	100	133	100	215	100
Control	(water soaking)#	18	100	51	100	127	100	173	100	294	100
Gamma ray (krad)	0.50	11	69	40	89	85	85	113	85	196	91
"	0.75	10	63	30	67	98	98	134	101	226	105
"	1.00	10	63	33	73	100	100	139	105	235	109
"	1.25	7	44	27	60	71	71	100	75	202	94
"	1.50	4	25	14	31	67	67	103	77	208	97
EMS (mM)	2.00	13	72	22	43	71	56	101	58	205	70
"	4.00	17	94	47	92	118	93	158	91	260	88
"	6.00	13	72	35	69	106	83	151	87	252	86
"	8.00	10	56	37	73	87	69	118	68	204	69
"	10.00	7	39	29	57	82	65	115	66	200	68

*DAP = Days after planting

**Mean of thirty observational plants

#EMS treated plants were compared with control (water soaking)

effect of radiation on the production of leaves was seen diminished. Recovery of leaf production capacity was more evident at 1.50 krad than at the lower levels of radiation, since 97 per cent leaves as compared to the control were recorded at 180 days after planting (Fig. 11).

EMS at all concentrations was found to reduce leaf production. EMS at 2 mM reduced the leaf production (72 per cent of control) and at the highest concentration of EMS, namely, 10 mM the leaf production was reduced drastically (39 per cent of the control). The inhibitory effect on leaf production caused by EMS was found to diminish as the maturity advanced. The nullification of the inhibitory effect was found to be maximum at 10 mM EMS (Fig. 12).

Chlorophyll chimera

Plants with chlorophyll deficient portions on their leaves were observed in physical as well as chemical mutagen treatments (Appendix I, Table 13 and Plates VI a to h).

In general the physical mutagen produced more number of plants with chlorophyll chimeras(70), compared to the chemical mutagen (19). Treatment with 0.75 krad gamma rays produced the maximum number (20) of chlorophyll chimeras while the minimum number (8) was produced by 1.50 krad. In the case of chemical mutagen, lowest concentration of 2 mM generated the maximum number (7) of chlorophyll chimeras and EMS (6 mM and 8 mM) produced the minimum number (2).

FIG.11. EFFECT OF GAMMA RAYS ON LEAF PRODUCTION IN THE νM_1 GENERATION (GINGER)

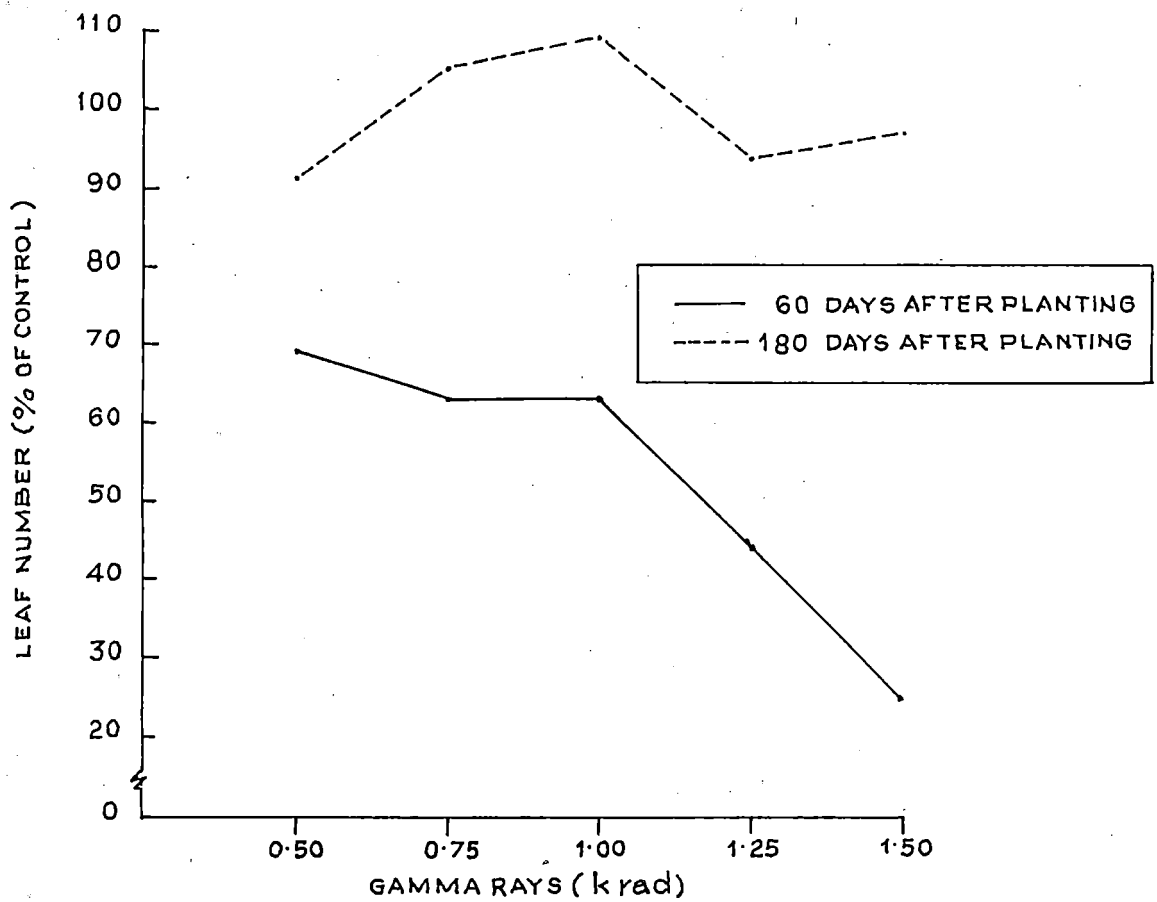


FIG.12. EFFECT OF EMS ON LEAF PRODUCTION IN THE νM_1 GENERATION (GINGER)

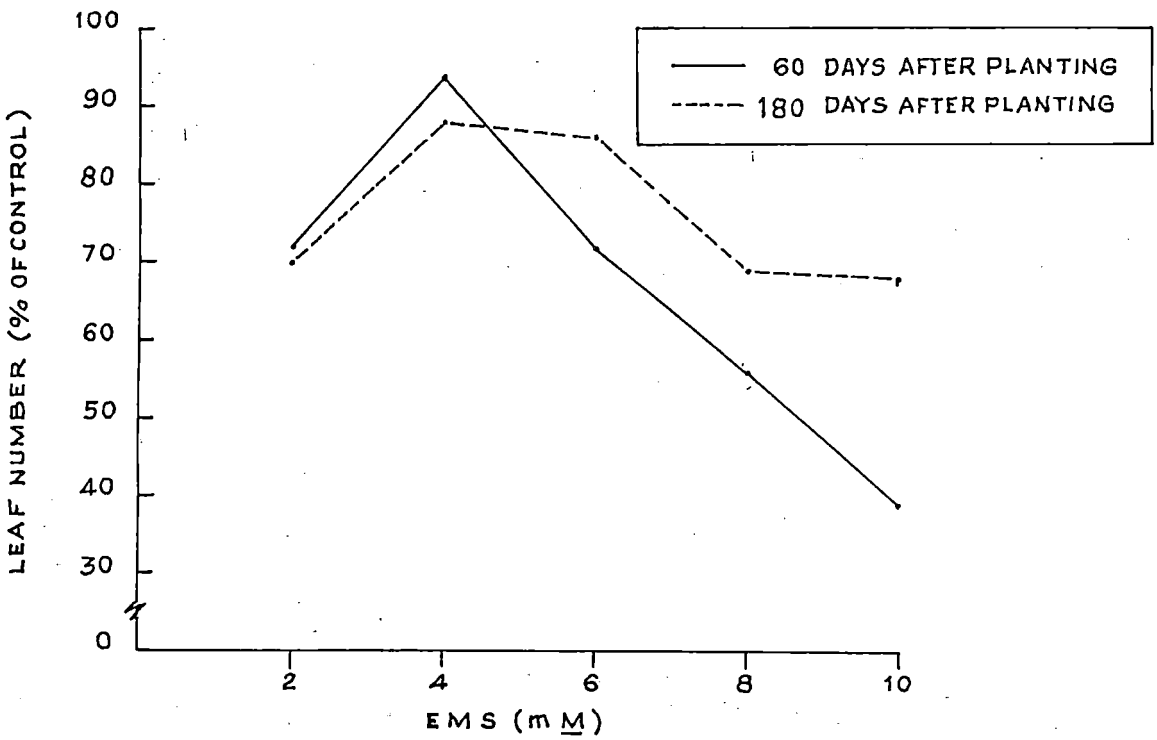


Table 13. Effect of mutagens on chlorophyll chimera in the vM_1 generation (ginger)

Mutagen	Dose	Plants exhibited chlorophyll chimera	
		No.	% of rhizomes sprouted
Control	(absolute)	--	--
Control	(water soaking)	--	--
Gamma ray (krad)	0.50	10	2.5
"	0.75	20	5.0
"	1.00	15	4.7
"	1.25	17	6.5
"	1.50	8	6.0
EMS (mM)	2.00	7	1.6
"	4.00	5	1.1
"	6.00	2	0.5
"	8.00	2	0.5
"	10.00	3	1.1

PLATE No. VI(a)

Ginger plant with chlorohyll chimera in the vM_1 generation (0.50 krad gamma rays)

PLATE No.VI(b)

Ginger plant with chlorophyll chimera in the VM_1 generation (0.75 krad gamma rays)

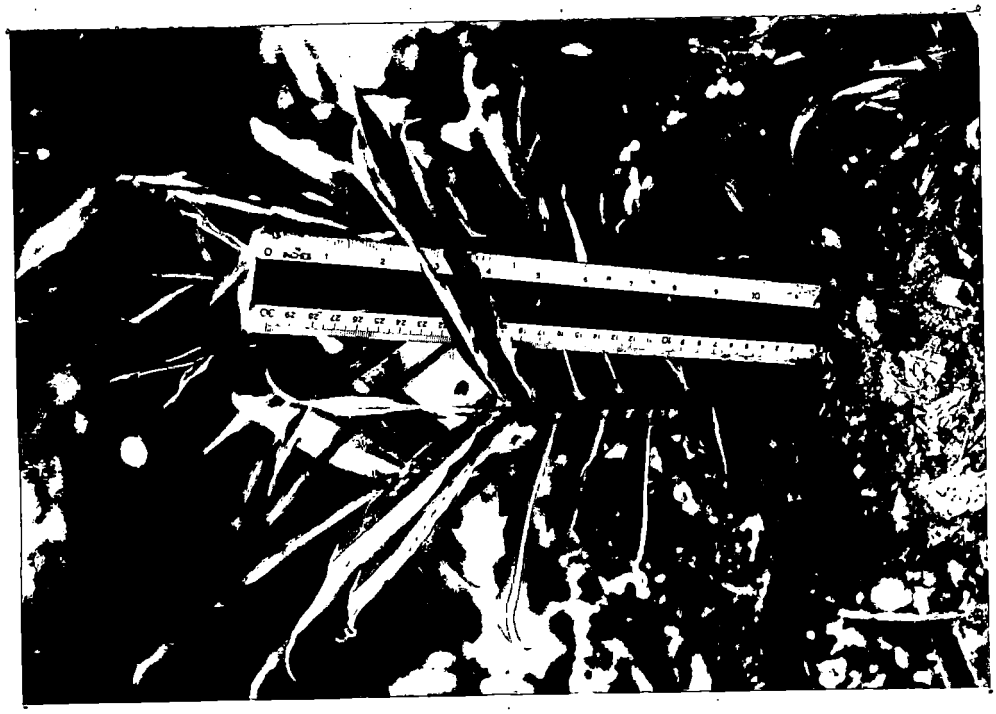


PLATE No.VI(c)

Ginger plant with chlorophyll chimera in the vM_1 generation (1.00 krad gamma rays)

PLATE No.VI(d)

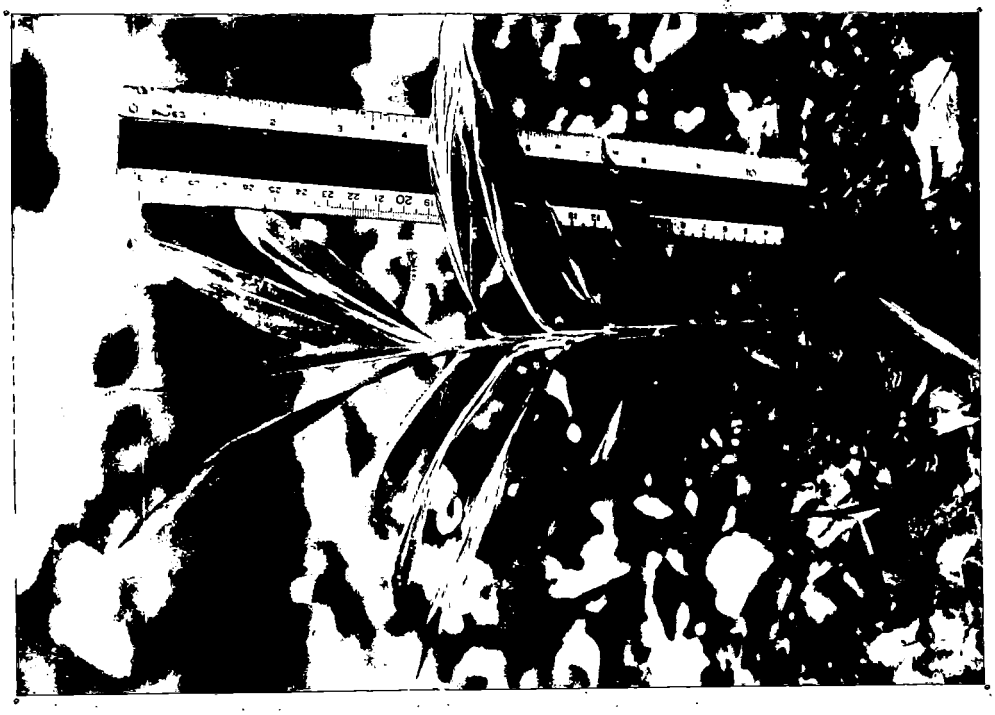
Ginger plant with chlorophyll chimera in the vM_1 generation (1.25 krad gamma rays)

PLATE No.VI (e)

Ginger plant with chlorophyll chimera in the vM_1 generation (1.50 krad gamma rays)

PLATE No.VI(f)

Ginger plant with chlorophyll chimera in the vM_1 generation (2 m$\underline{M}$ EMS)



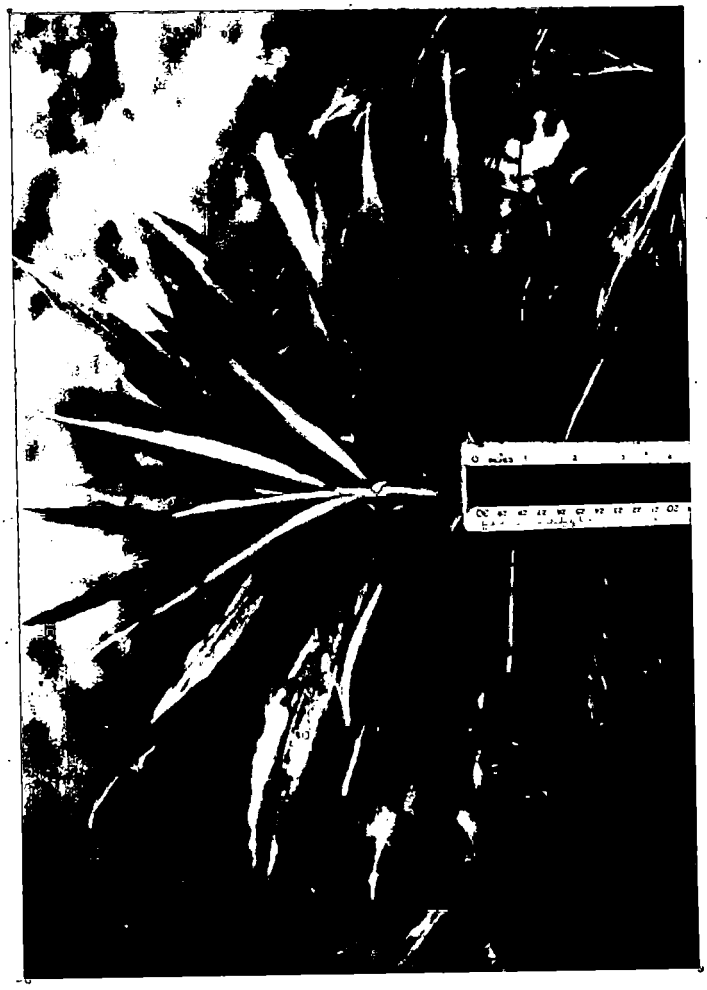
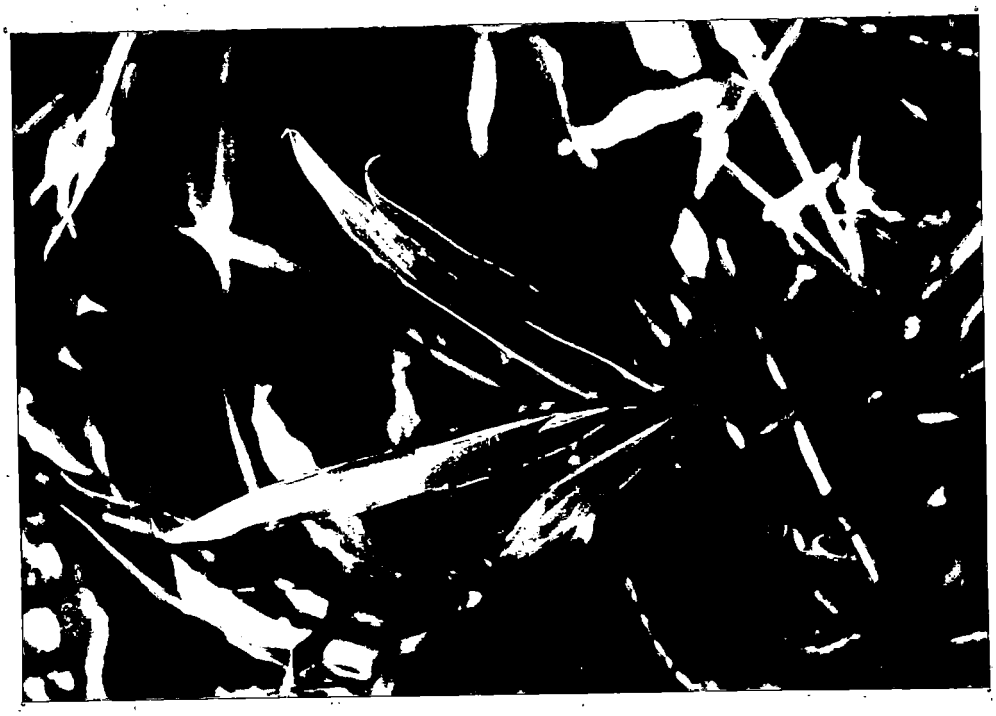


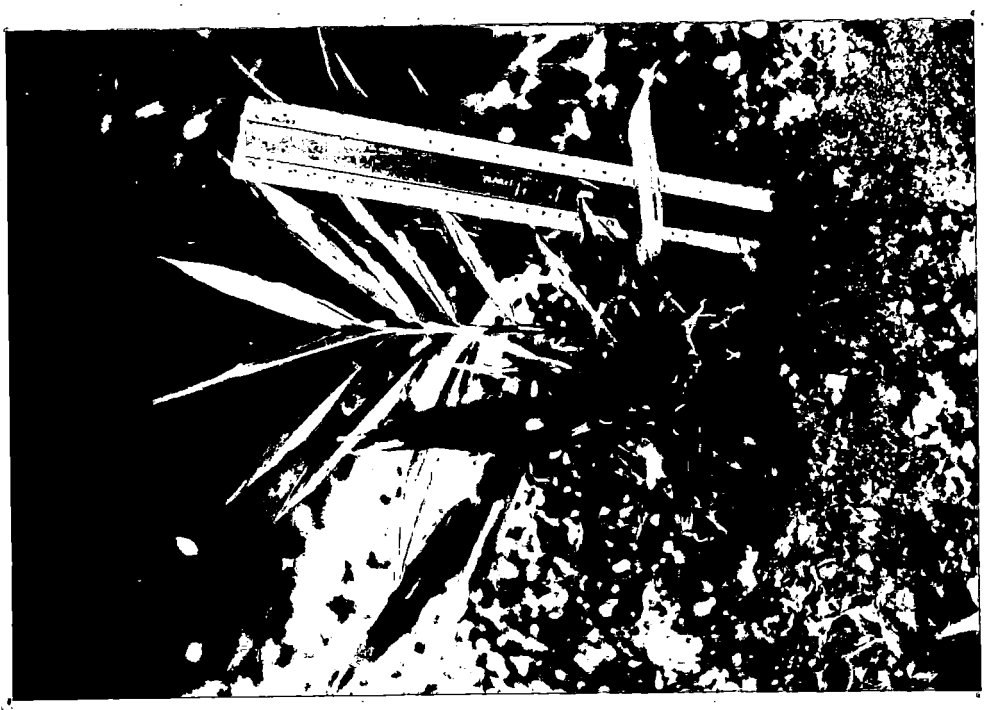
PLATE No. VI (g)

Ginger plant with chlorophyll chimera in the vM_1 generation (4 m$\underline{M}$ EMS)

PLATE No. VI (h)

Ginger plant with chlorophyll chimera in the vM_1 generation (10 m$\underline{M}$ EMS)





Flowering and pollen fertility

Flower production in the control and treated plants was observed to be very limited. A few plants from the control, the gamma ray treated plants at 1.00 krad (Plate VII) and the EMS treated plants at 6 and 8 mM produced flowers. The percentage of plants that produced flowers ranged from 0.4 to 0.9 (Table 14). The flowering started 95 days after planting and was completed by 120 days. The plants treated with lower and higher doses of gamma rays and lower and higher concentrations of EMS did not produce flowers. The pollen fertility data indicate that there is little difference in the fertility status as a result of the mutagen treatments. Periodical observation of the flowers indicated that the mutagen treatments were ineffective in influencing seed production.

Maturity period

The harvesting stage in the treated plants, as indicated by the yellowing and drying of the leaves of the healthy plants, was found to be normal and similar to that of the control plants. But the morphologically abnormal and weak plants in some of the treatments dried up early (Appendix I).

Rhizome yield

Reduced rhizome production was observed in the mutagen

PLATE No. VII

Ginger plant with inflorescence in the vM_1 generation (1.00 krad gamma rays)

PLATE No.VIII

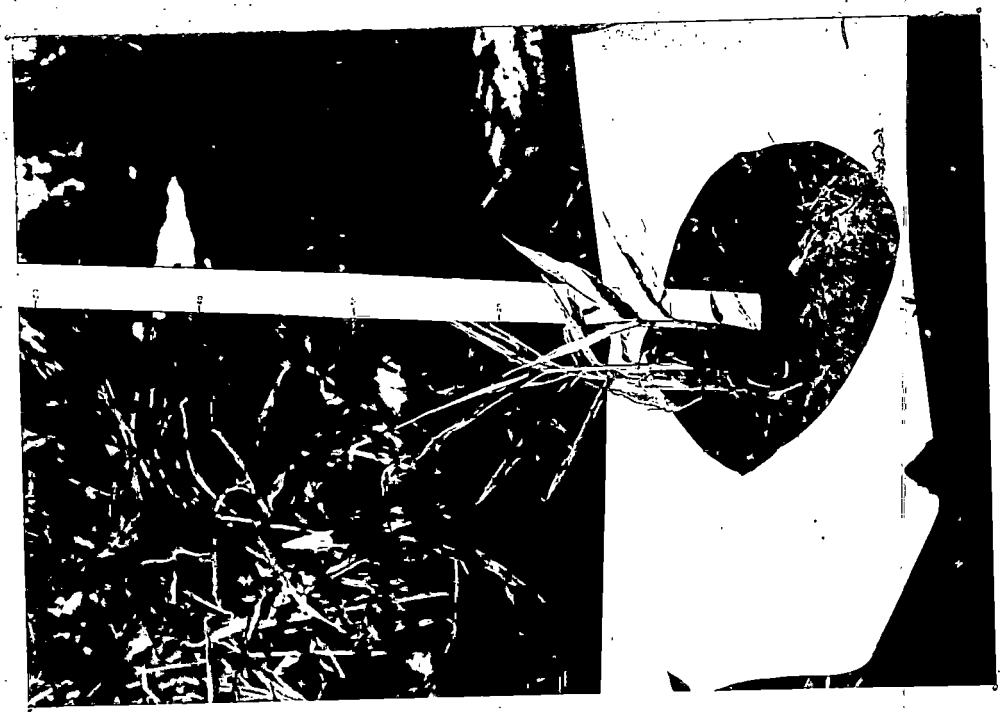
Ginger plant with shy tillering in the vM_2 generation (1.00 krad gamma rays)

PLATE No. IX

Ginger plant with profuse tillering in the vM_2 generation (0.50 krad gamma rays)

PLATE No. X

Ginger plant with profuse tillering and high yield in the vM_2 generation (4m<u>M</u> EMS)



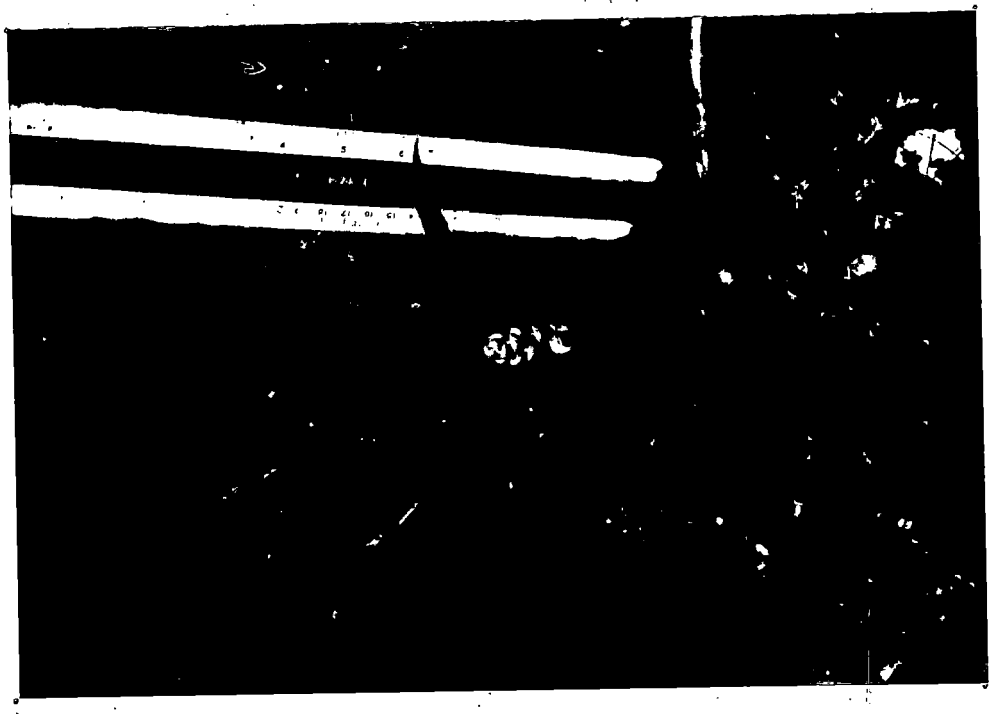


Table 14. Effect of mutagens on flowering and pollen fertility in the vM_1 generation (ginger)

Mutagen	Dose	Plants flowered (No.)	Plants flowered over survival (%)	Pollen fertility (%)
Control	(absolute)	3	0.6	22
Control	(water soaking)	2	0.4	22
Gamma ray (krad)	0.50	-	-	-
"	0.75	-	-	-
"	1.00	3	0.9	21
"	1.25	-	-	-
"	1.50	-	-	-
EMS (mM)	2.00	-	-	-
"	4.00	-	-	-
"	6.00	2	0.5	22
"	8.00	2	0.5	23
"	10.00	-	-	-

Table 15. Effect of mutagens on rhizome yield in the vM_1 generation (ginger)

Mutagen	Dose	Mean rhizome yield per plant* (g)	Mean rhizome yield (% of control)
Control	(absolute)	165	100
Control	(water soaking)#	184	100
Gamma ray (krad)	0.50	161	98
"	0.75	160	97
"	1.00	156	95
"	1.25	140	85
"	1.50	118	72
EMS (mM)	2.00	180	98
"	4.00	181	98
"	6.00	159	86
"	8.00	156	84
"	10.00	128	70

*Mean of 30 observational plants
#EMS treated plants were compared with control (water soaking)

treatment (Table 15). At 1.50 krad gamma rays the per plant yield obtained was 72 per cent of that of the control and at 1.25 krad, 85 per cent. The treatment (10 mM EMS) produced 70 per cent of the yield of the control plants. At lower concentrations of EMS, namely, 2 mM and 4 mM, the yield reduction observed was minimum (less than 2 per cent of the yield of the control).

Experiment III (a): Evaluation of the vM_2 generation

Plant height

It can be seen from the data presented in Table 16 that the mean plant height in general exhibited a negative shift from that of the control. However, 4 mM EMS appeared to be an exception by producing taller plants. Among the gamma ray treatments, the height decreased as the doses of gamma rays increased. The EMS treatments also generally decreased the plant height as the concentration increased, except for 4 mM EMS where the plants were taller than the control (112 per cent of the control).

Wide variation in plant height (5.0 to 90.0 cm) was observed among the population. Plants with the minimum height of 5.0 cm were observed in the vM_2 population of 1.50 krad gamma ray treated plants where the mean height was also minimum (67 per cent of the control). Maximum plant height

Table 16. **Plant height 180 days after planting in vM_2 generation (ginger)**

Mutagen	Dose	Plant height		
		Mean* (cm)	Mean (% of control)	Range (cm)
Control	(untreated)	42	100	35-51
Gamma ray (krad)	0.50	40	95	10-85
,,	0.75	39	93	5-70
,,	1.00	37	88	17-58
,,	1.25	30	71	11-41
,,	1.50	28	67	5-44
EMS (mM)	2.00	40	95	7-68
,,	4.00	47	112	7-90
,,	6.00	35	83	6-59
,,	8.00	35	83	12-60
,,	10.00	30	71	6-64

* Mean of the surviving plants

of 90.0 cm was seen among the vM_2 population of 4 m$\underline{M}$ EMS treated plants where the mean height was also the maximum (112 per cent of the control).

Tiller production

The mean tiller production exhibited both positive and negative shifts from the control under gamma ray and EMS treatments (Table 17). Gamma rays at 0.50, 0.75 and 1.00 krad increased tiller production to 138, 150 and 131 per cent of the control, respectively. At higher doses of 1.25 and 1.50 krad gamma rays, the mean tiller production was found to decrease, 94 and 81 per cent of the control, respectively. EMS at 4, 6 and 8 m$\underline{M}$ increased the mean number of tillers to 23, 19 and 18 respectively (144, 119 and 113 per cent of control). However at the highest concentration (10 m$\underline{M}$), the tiller production was reduced to 75 per cent of the control.

Wide variation was found in the tiller production capacity of the mutagen treated plants (Plates VIII to X). The maximum number of tillers (70 tillers/ plant) was recorded at 0.75 krad gamma rays and the minimum (2 tillers /plant), at 0.50, 1.25 krad gamma rays and 10 m$\underline{M}$ EMS treated plants.

Leaf production

The mean number of leaves per plant recorded 180 days

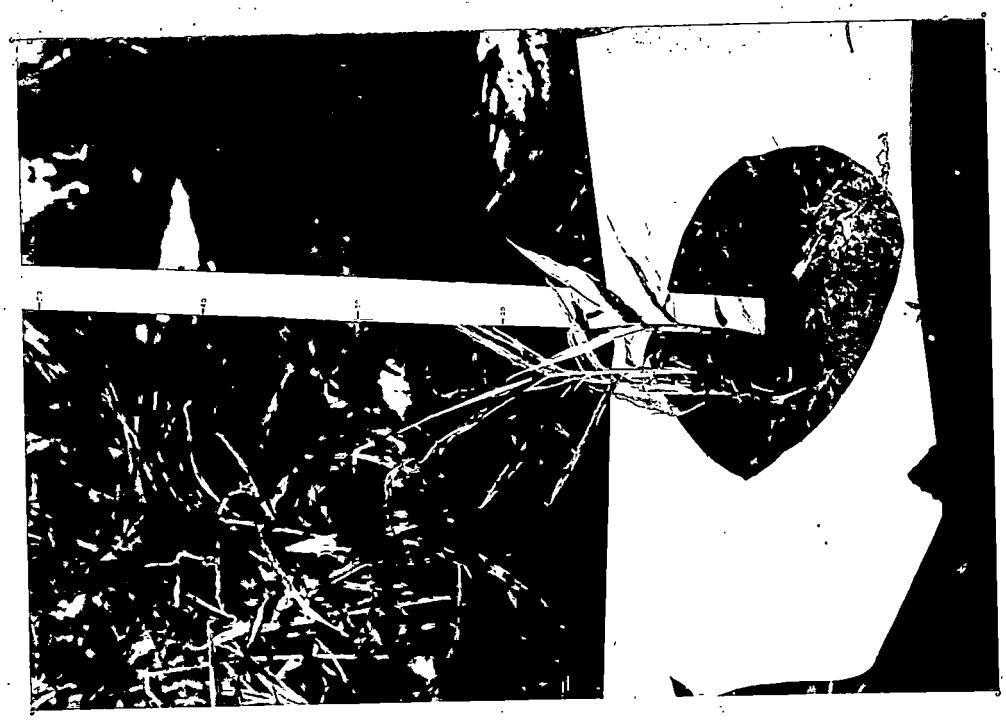


Table 17. **Tiller production 180 days after planting in vM_2 generation (ginger)**

Mutagen	Dose	Tiller production		
		Mean* (No)	Mean (% of control)	Range
Control	(untreated)	16	100	7-21
Gamma ray (krad)	0.50	22	138	2-62
,,	0.75	24	150	3-70
,,	1.00	21	131	3-40
,,	1.25	15	94	2-24
,,	1.50	13	81	4-17
EMS (mM)	2.00	16	100	6-52
,,	4.00	23	144	5-55
,,	6.00	19	119	3-52
,,	8.00	18	113	2-44
,,	10.00	12	75	2-46

* Mean of the surviving plants

PLATE No. IX

Ginger plant with profuse tillering in the vM_2 generation (0.50 krad gamma rays)

PLATE No. X

Ginger plant with profuse tillering and high yield in the vM_2 generation (4m$\underline{M}$ EMS)

Table 18. **Leaf production 180 days after planting in vM_2 generation (ginger)**

Mutagen	Dose	Leaf production		
		Mean* (No)	Mean (% of control)	Range
Control	(untreated)	230	100	148-420
Gamma ray (krad)	0.50	358	156	38-1426
,,	0.75	280	122	39-1188
,,	1.00	336	146	54-882
,,	1.25	192	84	48-432
,,	1.50	155	67	28-272
EMS (mM)	2.00	230	100	96-1040
,,	4.00	405	176	100-1320
,,	6.00	258	112	45-989
,,	8.00	216	94	26-746
,,	10.00	135	59	25-404

* Mean of the surviving plants.

after planting showed higher values than the control at the lower doses of gamma rays (Table 18). At 0.50, 0.75 and 1.00 krad gamma rays the leaf production was 156, 122 and 146 per cent of the control, respectively. At the higher doses (1.25 and 1.50 krad gamma rays), leaf production was found decreased (84 and 67 per cent of the control). EMS at 4 and 6 mM concentration increased the leaf production. At higher concentrations, EMS exhibited a negative trend in leaf production. Wide variation in the number of leaves was observed as a result of mutagen treatments. The minimum leaf number was 25 and the maximum was 1426 as against a range of 98 to 420 in the control plants.

Flowering and pollen fertility

Flower production in the vM_2 generation was found to be very limited. Unlike in the case of vM_1 generation, the gamma ray and EMS treated plants flowered (Table 19). It can be seen from the data that only 5 per cent of the control plants produced flowers. The data further indicate that 3.0 to 7.0 per cent of the treated plants flowered. The minimum flowering (3.0 per cent) was observed under 1.25 and 1.50 krad gamma rays and the maximum (7.0 per cent) under 0.75 krad gamma rays and 6 mM EMS. The studies revealed that mutagens did not exhibit any effect on the pollen fertility, which ranged from 23 to 24 per cent. Periodical observation

Table 19. Flowering and pollen fertility in vM_2 generation (ginger)

Mutagen	Dose	Plants flowered (%)	Pollen fertility (%)
Control	(untreated)	5	24
Gamma ray (krad)	0.50	6	24
"	0.75	7	24
"	1.00	4	23
"	1.25	3	24
"	1.50	3	24
EMS (mM)	2.00	6	23
"	4.00	5	24
"	6.00	7	24
"	8.00	4	24
"	10.00	4	24

of the flowers confirmed the absence of seed set in the vM_2 generation.

Maturity period

A few plants were found to be early maturing by about one month. The details of plants that exhibited earliness are presented in Appendix II.

Rhizome yield

The data presented in Table 20 indicate yield increase to the tune of 113 and 104 per cent of the control at the lower doses of gamma rays (0.50 and 0.75 krad). Progressive reduction in yield was observed as the doses of the mutagen increased. The minimum mean yield of 72 g/ plant was recorded at the highest dose of 1.50 krad of gamma rays. The reduction in yield worked out to 59 per cent of the control.

At lower doses (2 and 4 m$\underline{M}$), EMS induced an increase in the rhizome yield (107 and 121 per cent of the control). But doses over 4 m$\underline{M}$ exhibited an adverse effect on the rhizome yield, reduction in yield being more in higher doses of the chemical.

Variation observed in the rhizome yield was large. The minimum yield of 1.0 g was recorded at 6 m$\underline{M}$ EMS whereas the maximum yield of 1320 g was obtained from 2 m$\underline{M}$ EMS treated plants. The next higher yield (1050 g) was obtained

Table 20 . **Rhizome yield in vM_2 generation (ginger)**

Mutagen	Dose	Yield of rhizomes per plant		
		Mean* (g)	Mean (% of control)	Range (g)
Control	(untreated)	175	100	80-210
Gamma ray (krad)	0.50	197	113	8-700
,,	0.75	182	104	4-709
,,	1.00	140	80	4-421
,,	1.25	84	48	3-134
,,	1.50	72	41	3-89
EMS (mM)	2.00	187	107	2-1320
,,	4.00	212	121	10-1050
,,	6.00	132	75	1-495
,,	8.00	120	69	4-399
,,	10.00	75	43	2-409

* Mean of the surviving plants

at 4 m<u>M</u> of EMS. A perusal of the data shows that the chemical induced wider variations.

Selection of mutants for vM_3 study

Based on the variability expressed in the vM_2 plants for one character or combination of two or more characters simultaneously, 98 possible mutants were selected for vM_3 study and were classified into 22 groups and presented in Table 21. The data indicate that the treatment with gamma rays produced 40 mutants and EMS 58 mutants. Among the doses of gamma rays, the lower dose (0.75 krad) induced 55 per cent mutants recovered from the radiation treatments. In the case of chemical mutagen treatments also the lower dose, namely, 4m<u>M</u> EMS yielded 55 per cent of the mutants.

Data presented in Table 22 delineate the distribution of the selected mutants into six groups, considering single character at a time irrespective of the fact that a plant selected for a particular character (say tallness) might carry variation for other characters (say more tillers, early maturity etc.) also simultaneously. The lower doses of gamma rays (0.50 krad and 0.75 krad) produced 83 per cent of the mutants selected. Similarly 2 m<u>M</u> and 4 m<u>M</u> of EMS induced 78 per cent of the selected mutants.

Table 21. Classification of vM_2 mutants selected for vM_3 study

Sl. No.	Mutant groups	No. of possible mutants under Gamma rays (krad) 0.5	0.75	1.0	1.25	1.5	Total	EMS (mM) 2	4	6	8	10	Total	Grand Total
1.	ta								2	1			3	3
2.	d		4	1		1	6				1	1	2	8
3.	ti									1	1	1	3	3
4.	y		1				1							1
5.	e	3	3				6	2	4	3	1	1	11	17
6.	ta ti									1	1	1	3	3
7.	ta 1		1				1		1				1	2
8.	ta y								4				4	4
9.	ta e		1				1							1
10.	d ti					1	1		1				1	2
11.	d e		3		1		4							4
12.	ti 1	1	2				3	1			1		2	5
13.	ti y		1				1					1	1	2
14.	ti e				1		1			1			1	2
15.	y e								1				1	1
16.	ta ti 1	3	2				5			1	1		2	7
17.	ta ti y								1			1	2	2
18.	ta 1 y								3				3	3
19.	d ti 1			1			1							1
20.	d ti y		1				1							1
21.	ti 1 y	2	2				4	1	1				2	6
22.	ta ti 1 y	2	1	1			4	2	14				16	20
	TOTAL	11	22	3	2	2	40	6	32	8	6	6	58	98

ta=tall; d=dwarf; ti=more tillers; y=high yield; e=early maturity; tal=tall-more leaves

Table 22. Classification of vM_2 mutants (single character wise)

Mutagen	Gamma rays (krad)						EMS (mM)						Grand Total
dose -> / character ↓	0.5	0.75	1.0	1.25	1.50	Total	2.0	4.0	6.0	8.0	10.0	Total	
Tall	5	5	1	–	–	11	2	25	3	1	2	33	44
Dwarf	–	8	2	1	2	13	–	–	1	1	1	3	16
More Tillers	8	9	1	1	1	20	4	17	5	3	4	33	53
More Leaves	8	8	2	–	–	18	4	20	1	1	–	26	44
Early Maturity	3	7	–	2	–	12	2	5	4	–	1	12	24
High yield	4	6	1	–	–	11	3	24	–	–	2	29	40
Total	28	43	7	4	3	85	15	91	14	6	10	136	221

Experiment III (b): Screening the vM_2 generation against bacterial wilt and soft rot

Reaction to bacterial wilt disease

The data on the number of rhizomes planted and sprouted, plants affected by bacterial wilt and plants that tolerated/survived bacterial wilt are presented in Table 23. At the final harvest 108 plants exhibited tolerance/field survival against bacterial wilt including 15 plants under control. The maximum survival (9.5 per cent) was under 6 mM EMS treatment, followed by 0.50 krad gamma ray treatment (8.9 per cent). None of the plants subjected to 0.75 krad gamma ray treatment survived. Among the control plants, 5.6 per cent survived the bacterial wilt disease.

Reaction to soft rot disease

The data on the number of rhizomes planted and sprouted and plants affected by soft rot are presented in Table 24. The data indicate that in the control and mutagen treated populations, incidence of the disease was severe resulting in the total destruction of plants at 180 days after planting.

Experiment IV (a): Study of the mutants in the vM_3

In the vM_2, suspected mutants were grouped according to the characters exhibited by them. Altogether 98 progenies

Table 23. **Reaction of vM_2 generation against bacterial wilt disease (ginger)**

Mutagen	Dose	Rhizomes Planted (No.)	Rhizomes sprouted (No.)	Plants affected by B.W.* (No.)	Palnts which tolerated/survived bacterial wilt	
					(No.)	% over rhizomes sprouted
Control	(untreated)	328	269	254	15	5.6
Gamma ray (krad)	0.50	242	192	175	17	8.9
,,	0.75	192	154	154	-	0
,,	1.00	177	134	131	3	2.2
,,	1.25	126	92	88	4	4.3
,,	1.50	107	70	66	4	5.7
EMS (mM)	2.00	308	247	228	19	7.7
,,	4.00	491	408	396	12	2.9
,,	6.00	219	158	143	15	9.5
,,	8.00	264	185	175	10	5.4
,,	10.00	170	117	108	9	7.7

*Bacterial wilt

Table 24. **Reaction of vM_2 generation against soft rot disease (ginger)**

Mutagen	Dose	Rhizomes planted (No.)	Rhizomes sprouted (No.)	Plants affected by soft rot (No.)	Plants tolerated/ survived soft rot (No.)
Control	(untreated)	240	208	208	-
Gamma ray (krad)	0.50	146	117	117	-
,,	0.75	172	139	139	-
,,	1.00	140	107	107	-
,,	1.25	84	61	61	-
,,	1.50	78	55	55	-
EMS (mM)	2.00	206	165	165	-
,,	4.00	258	212	212	-
,,	6.00	279	221	221	-
,,	8.00	193	145	145	-
,,	10.00	124	94	94	-

could be identified which showed either single mutant character or combination of mutant characters (Appendix II). Appendix III contains the mean performance of the vM_3 progeny of each vM_2 mutant (un-confirmed) and the range of variation observed in the progeny rows. The percentage of progenies that expressed the distinct variation as well as the percentage of progenies that expressed all the mutant characters are also computed.

The performance of the progenies of the selected vM_2 plants, as described in Table 21 is presented below.

Tall mutants

Three vM_2 plants (59, 66 and 86) selected for tallness were carried forward to vM_3 generation. Progenies of the Plants 59 and 66 did not express the character. Twenty five per cent of the progeny of Plant 86 inherited the mutant character-tallness (Appendix III).

Dwarf mutants

Out of the progenies of eight dwarf vM_2 mutant (un-confirmed) plants, those of 36 and 97 did not express the dwarf character in vM_3. The progenies appeared to be normal. The progeny of Plant 40 did not survive after three months of planting. Progenies of the other plants (15,16,17,18 and 92) expressed dwarfness in varying degrees, ranging from 33 to 86 per cent (Appendix III).

Tiller mutants

Three vM_2 plants 80, 91 and 96 from EMS 6,8 and 10 m*M* respectively which produced large number of tillers were designated as tiller mutants. Their progenies were evaluated for inheritance of the character. While the progeny of Plant 91 did not express the character, twenty five per cent of the progeny of Plant 80 and six per cent of the progeny of Plant 96 expressed the character.

Yield mutants

One vM_2 Plant (19) from 0.75 krad gamma ray treatment could be identified as high yielding based on the rhizome yield in vM_2 generation. The study of its progeny revealed that the high yielding character was not expressed in the subsequent generation.

Early mutants

In the vM_2 generation, 17 early maturing plants were identified as (un-confirmed) early mutants, three from 0.50 krad gamma ray and three from 0.75 krad gamma ray treatments, two from 2 m*M*, four from 4 m*M*, three from 6 m*M*, one from 8 m*M* and one from 10 m*M* EMS treatments. The vM_3 studies indicated that the progenies of ten early mutants (2, 6, 20, 29, 46, 57, 67, 82, 83 and 88) had normal maturity period. While all the progenies of plants 4, 14 and 98 expressed earliness, only a certain percentage of the progenies of Plants 45, 63, 68 and 85 expressed the character (Appendix III).

Tall-tiller mutants

The height of the plants and the number of tillers per plant were considered for classifying the plants as tall-tiller mutants. Three vM_2 plants (81, 89 and 95) obtained from 6, 8 and 10 m$\underline{M}$ EMS treatment were included for vM_3 studies. The progenies of the Plants 81 and 95 did not express the characters. Considering the characters independently, it appeared that 22 per cent of the progeny of Plant 89 and 33 per cent of the progeny of Plant 95 expressed tallness and more tillering, respectively. However none of the progenies was observed with both the mutant characters.

Tall-leaf mutants

Only two plants, the Plant 24 from 0.75 krad gamma ray and the Plant 69 from 4 m$\underline{M}$ EMS treatment were located in vM_2 with the mutant (un-confirmed) characters tallness and more leaves. In vM_3 , while none of the progeny of 69 expressed the characters, 14 per cent of the progeny of 24 expressed the leaf mutation having produced more number of leaves.

Tall-yield mutants

Based on height of plants and rhizome yield tall-yield mutants were identified. Plants 48,65,70,and 76 were identified in vM_2 as mutants (un-confirmed) for tallness and high yield. Progeny studies in vM_3 revealed that the progenies of Plants 48, 65 and 76 failed to express the

characters. However 18.0 per cent progeny of Plant 70 were found to express both the characters.

Tall-early mutants

Plants with tallness and earliness in maturity were considered as tall-early mutants. Only one Plant (13) in the 0.75 krad gamma ray treatment was located as possible mutant for tiller and early maturity in vM_2 . The evaluation of the progeny indicated that tallness was not expressed in vM_3 . However, 67 per cent vM_3 plants expressed earliness.

Dwarf-tiller mutants

Two dwarf and profuse tillering plants (39 and 84) from 1.50 krad gamma rays and 6 m$\underline{M}$ EMS, respectively were identified as possible mutants in vM_2 generation. The examination of the progeny of Plant 39 indicated that profuse tillering habit was not expressed in vM_3 , but dwarfness found expression in 71 per cent of the progeny. Although none of the progeny of Plant 84 carried both the mutant characters, 38 per cent were dwarf mutants and 13 per cent, tiller mutants.

Dwarf-early mutants

Plants with dwarfness and early maturity were grouped as dwarf-early mutants. Both dwarf and early maturity attributes were exhibited by the Plants 21, 30 and 31 in 0.75 krad gamma ray treatment and the Plant 37 in 1.25 krad gamma

ray treatment. The progeny of the Plant 21 expressed both the characters to the tune of 33 and 100 per cent respectively in the vM_3 generation. The corresponding values for the progeny of the Plant 30 was 44 and 44. The progenies of the Plants 31 and 37 expressed only dwarfness (in 50 per cent and 14 per cent plants, respectively).

Tiller-leaf mutants

The number of tillers and the number of leaves per plant were considered for classifying the plants as tiller-leaf mutants. Plants with more number of tillers and leaves were identified from 0.50 krad gamma rays (8), 0.75 krad gamma rays (26 and 27), 2 m$\underline{M}$ EMS (41) and 8 m$\underline{M}$ EMS (90). Study of the progenies of the plants indicated that 17 per cent progeny of Plant 8 and 60 per cent progeny of Plant 26 expressed both the characters. The progeny of 27 did not express any character.

Tiller-yield mutants

Plants with more tillers and high yield were grouped as tiller-yield mutants. The Plant 23 of 0.75 krad gamma ray treatment and the Plant 94 of 10 m$\underline{M}$ EMS treatment were identified as possible tiller-yield mutants. The high yield recorded in vM_2 was not seen expressed in the vM_3 . However, 12 per cent progeny of 23 and ten per cent progeny of 94 expressed the high tillering habit.

Tiller-early mutants

Based on more tillering and early maturing attributes plants were grouped as tiller-early mutants. Two plants in the vM_2 generation exhibited the characters. Sixty seven per cent progeny of Plant 38 expressed more tillering, while none of the progeny was early maturing. The earliness of Plant 79 was seen expressed in the vM_3 progeny, while only five per cent progeny showed more tillering. Thus five per cent progeny of Plant 79 appeared to possess both the characters.

Yield-early mutant

One vM_2 plant (54) was selected from 4 m$\underline{M}$ EMS treatment for high rhizome yield and early maturity. The evaluation of the vM_3 progeny revealed that earliness was not inherited, but the high yielding character was expressed by four per cent progeny.

Tall-tiller-leaf mutants

Plants with tallness, profuse tillering and production of larger number of leaves were considered as tall-tiller-leaf mutants. Three Plants (1,7 and 9) from 0.50 krad gamma rays, two Plants (12 and 25) from 0.75 krad gamma rays, one Plant (52) from 4m$\underline{M}$ EMS and one plant (87) from 6 m$\underline{M}$ EMS were selected. Progenies of Plants 1, 9, 12 and 87 expressed the three characters, together (tallness, more tillers and more leaves) to the tune of 12, 14, 18 and 33 per cent,

respectively. These characters together were not seen expressed by any of the progenies of the Plants 7, 25 and 52.

Tall-tiller-yield mutants

Tallness, production of more tillers and high yield of rhizomes were exhibited together by one vM_2 Plant (60) from 4 mM EMS treatment and one Plant (93) from 10 mM EMS treatment. None of the progenies of the Plants (60 and 93) expressed the three characters together in the vM_3 generation. However, 17 per cent progenies of Plant 60 showed more tillering coupled with high yield.

Tall-leaf-yield mutants

Three plants (47, 64 and 74) from among the 4 mM EMS treated vM_2 plants were identified as possible mutants for tallness, production of more leaves and high rhizome yield. Among the progeny of the Plant 74 , eight per cent expressed tallness, 44 per cent, more leaf production and 20 per cent, high yield. The three characters together were seen expressed only by four per cent progeny. Twelve per cent progenies each of Plant 47 and Plant 64 expressed more leaves. Six per cent progenies each of two plants expressed high yield but none expressed the tall character.

Dwarf-tiller-leaf mutants

Dwarfness and production of more tillers and leaves together were exhibited by one Plant (35) from the vM_2

population of 1.00 krad gamma ray treatment. In the progeny more tillers and more leaves were not seen produced eventhough 67 per cent progeny expressed dwarfness.

Dwarf-tiller-yield mutants

Dwarfness, production of more tillers and high yield were shown by one Plant (28) in the vM_2 population of 0.75 krad gamma ray treatment. The characters were not found expressed together in the vM_3 .

Tiller-leaf-yield mutants

Plants with profuse tillering, large number of leaves and higher rhizome yield were classified as tiller-leaf-yield mutants. Two radiation treatments, namely, 0.50 krad and 0.75 krad gamma ray, yielded two Plants each (5,10 and 32,33) showing all the three mutant characters in the vM_2 gneration. Chemical mutagen treatments, namely, 2 m$\underline{M}$ and 4 m$\underline{M}$ EMS yielded one Plant each (43 and 53, respectively). Nine and seven per cent progenies of the Plants 5 and 10 expressed the three characters together in the vM_3 generation. Fourteen per cent progeny of Plant 32 and three per cent progeny of Plant 33 expressed all the three characters together in the vM_3 . The EMS treatments although induced production of more tillers, more leaves and high yield in vM_2 generation did not cause expression of the characters together in their vM_3 progenies.

Tall-tiller-leaf-yield mutants

The expression of tallness as well as ability to produce larger number of tillers, leaves and high yield was used for locating the possible mutants with economic value. The gamma ray treatments yielded four possible mutants, namely, Plants 3 and 11 from 0.50 krad, the Plant 22 from 0.75 krad and the Plant 34 from 1.00 krad treatments. The EMS treatments yielded 16 possible mutants, namely, Plants 42 and 44 from 2.00 mM and Plants 49, 50, 51, 55, 56, 58, 61, 62, 71, 72, 73, 75, 77 and 78 from 4 mM treatments. The vM_3 studies indicated that in 4.0 to 21.0 per cent of the progenies of the Plants 3, 42, 44, 71, 73 and 77 there was combined expression of four characters.

Study of quality attributes in vM_3 generation of the possible mutants

Table 25 contains the data on dryage, volatile oil, non-volatile ether extract, starch and crude fibre with respect to the progenies of plants identified in the vM_2 as possible mutants. Recovery of dry ginger ranged from 13.0 to 15.4 per cent as against 15.2 per cent in the control plants. The maximum dryage was recorded by progenies of Mutants 41 (tiller-leaf mutant),92 (dwarf-mutant) and 77 (tall-tiller-leaf - yield mutant).

Table 25. **Drying percentage, content of volatile oil, non volatile ether extract (NVEE), starch and crude fibre in the vM_3 progenies of the possible mutants identified in the vM_2**

Mutant No.	Drying (%)	Volatile oil V/W(%)	NVEE (%)	Starch (%)	Crude fibre (%)
1 (N)*	15.3	3.0	8.0	40.3	6.5
3 (N)	15.2	3.0	8.0	40.4	6.5
4 (E)**	13.2	3.2	10.3	36.7	7.7
5 (N)	15.0	3.1	8.1	40.4	6.2
8 (N)	15.3	2.7	8.2	40.4	6.2
9 (N)	15.2	2.9	8.2	40.3	6.2
10 (N)	15.2	3.0	8.2	40.3	6.4
12 (N)	15.3	3.0	8.1	40.4	6.5
14 (E)	13.1	3.1	10.4	37.0	7.6
15 (N)	15.1	2.9	8.0	40.0	6.3
16 (N)	15.2	2.9	8.1	40.0	6.5
17 (N)	15.2	3.0	8.1	39.9	6.5
18 (N)	15.2	3.0	8.1	40.3	6.5
21 (E)	13.5	3.2	10.5	36.6	7.6
26 (N)	15.5	2.9	8.0	39.9	6.2
30 (E)	13.0	3.2	10.3	36.8	7.9
32 (N)	15.4	3.3	10.3	40.0	6.2
33 (N)	15.1	3.1	8.1	40.4	6.4
41 (N)	15.4	2.7	8.0	40.3	6.4
42 (N)	15.3	3.0	8.2	40.3	6.2
44 (N)	15.1	3.0	8.0	39.9	6.2
45 (E)	13.0	2.9	9.2	36.5	7.8
63 (E)	13.2	3.3	10.5	36.9	7.8
68 (E)	13.2	3.3	10.6	37.0	7.9
70 (N)	15.2	3.0	8.0	40.3	6.4
71 (N)	15.1	3.0	8.1	40.0	6.5
73 (N)	15.1	3.0	8.1	40.4	6.4
74 (N)	14.2	2.7	7.5	38.8	6.2
77 (N)	15.4	3.3	10.1	39.9	6.2
79 (E)	13.2	3.1	10.4	36.5	7.6
80 (N)	15.2	3.0	8.0	39.9	6.3
85 (E)	13.2	3.1	10.6	37.0	7.6
86 (N)	15.0	2.9	8.1	40.0	6.2
87 (N)	15.1	2.9	8.1	40.0	6.2
90 (N)	15.3	3.1	8.2	40.3	6.5
92 (N)	15.4	3.3	9.2	40.3	6.3
96 (N)	15.0	3.0	8.1	40.0	6.4
Control	15.2	2.9	8.1	40.1	6.3

* (N)=Normal with respect to maturity
**(E)=Early maturity

The content of volatile oil ranged from 2.7 to 3.3 per cent among the vM_3 progenies, as against 2.9 per cent in the control plants. The progenies of Mutant 74 recorded the minimum volatile oil content of 2.7 per cent while the progenies of Mutants 63 and 77 gave the maximum of 3.3 per cent. It can be seen from the data that early maturing mutants appeared to yield more volatile oil than others including the control.

The non volatile ether extract (NVEE) varied from 7.5 to 10.6 per cent in the vM_3 progenies as against 8.1 per cent in the control. The Progeny of Mutant 74 gave the minimum NVEE (7.5 per cent) while the progenies of the Mutants 68 and 85 gave the maximum NVEE (10.6 per cent). The early maturing mutants appeared to record more NVEE percentage (9.2 to 10.6) than the others including the control (8.0 to 10.1).

In the vM_3 progenies, the starch content ranged from 36.5 to 40.4 per cent, as against 40.1 per cent in the control plants. The data further reveal that the early maturing mutants contained lower amounts of starch.

The crude fibre in the vM_3 progenies ranged from 6.2 to 7.9 per cent. The results of the chemical analysis show that the early maturing mutants had comparatively higher proportion of crude fibre ranging from 7.6 to 7.9 per cent,

while others including the control recorded a range of 6.2 to 6.5 per cent.

Experiment IV (b): Inoculation studies on the vM_3 progenies of plants that survived bacterial wilt in the vM_2

Inoculation studies revealed that out of the 426 vM_3 plants obtained from the vM_2 generation maintained at the sick field of the Regional Agricultural Research Station, Ambalavayal, 78 vM_3 plants appeared to be free from the disease (Table 26). Five out of the 25 control plants were also seen unaffected.

Table 26. Reaction of vM_3 generation against bacterial wilt disease (ginger)

Treatment	Number of plants treated	Number	
		Diseased	Survived
Inoculation	426	348	78
Control	25	20	5

DISCUSSION

DISCUSSION

The role of mutation breeding in the improvement of vegetatively propagated crops has been increasingly realised in the recent years. Genetic improvement of such crops (which, in addition to being vegetatively propagated, exhibit very limited seed set) through methods involving crossing is limited due to obvious difficulties. Further, the plants are generally highly heterozygous and often polyploids. These cause complicated segregation patterns and make the detection of useful recombinants rather difficult. Further, incompatibility and other barriers to crossing, which exist in some of these crops, render the task of the Breeders extremely difficult.

Induction of mutation and exploitation of desirable mutants obviously are the means for producing genetic variability in vegetatively propagated (sterile) and obligatory apomictic crop plants.

Seed production and propagation of ginger through sexual methods have not been reported. Ginger is, therefore, universally propagated by vegetative means. As such, the use of mutations for inducing variability assumes greater

importance. That heterozygosity of the crop has remained more or less fixed unlike in the seed propagated plants, is an advantage. According to Orton (1984) and Wenzel et al. (1987), the relatively new technique of induced somaclonal variation can be employed for generation of biological diversity in vegetatively propagated plants.

The success in mutation breeding largely depends on our understanding of the process of induction and recovery of the mutants and of the screening methods for evaluating the desired mutants. In ginger, systematic attempt on induction of mutation are very scanty and the methodologies for induction and recovery of the mutants are yet to be standardised. In the present studies, emphasis was given for understanding the details of induction of variability and recovery of the desirable mutants. An attempt has been made to throw light on the basic aspects of induced mutagenesis in ginger and to open out new avenues in the genetic improvement of the crop. One physical mutagen (gamma ray) and one chemical mutagen (EMS) were employed. The results of the studies are discussed in this chapter.

Standardisation of the doses of mutagens

Basic data on the sensitivity of the plant material to the mutagens are essential for prescribing the optimum dose of the mutagens that would enable recovery of desirable

mutants. Several parameters have been used for determining the sensitivity of crop plants to different mutagens. Sambandamurthi (1983) considered sprouting, survival and extent of growth reduction as the parameters useful for assessing the sensitivity of tuberose bulbs to mutagenic treatments.

Considering the findings on the crops related to ginger, sprouting, survival and height of the plants were evaluated for assessing the sensitivity of ginger rhizomes to gamma ray and EMS treatments.

Sprouting was found to decrease with increase in the dose of gamma rays, reaching the minimum of 6.0 per cent (as against control) at 2.0 krad. At the higher doses (2.5 krad to 5.0 krad), none of the rhizomes sprouted.

In their studies, Raju et al. (1980) observed 32.0 per cent sprouting at 2.0 krad gamma rays as against 5.0 per cent (6.0 per cent of the control) obtained under the present investigations. Raju et al. (1980) observed sprouting even at higher doses of 5.0 and 10.0 krad gamma rays while in the present studies no sprouting was observed at doses of 2.5 krad and above. Based on their work, doses of 20.0 krad and above have been reckoned as lethal by Raju et al. (1980). In the present studies it has been observed that doses above 2.5

krad as lethal. Since details such as the cultivar, the number of buds present in the rhizome bits etc., have not been specified by Raju et al. (1980), comparison of their results with those of the present studies is not meaningful. The conclusion of the present investigation that exposure of "well-developed disease-free and uniform rhizome bits with one viable bud each to gamma rays at 2.5 krad and above is lethal", therefore, has to be accepted. The observations of Giridharan (1984) that gamma ray doses of 3.0 krad and above are lethal to ginger (cultivar Rio-de-Janeiro) support the findings of the present studies.

With respect to sprouting of irradiated ginger rhizomes, the LD_{50} was found to be between 0.50 and 1.00 krad. A perusal of the data presented by Raju et al. (1980) indicates the LD_{50} for ginger (cultivar unspecified) to be below 2.0 krad gamma rays while those presented by Giridharan (1984) indicate the LD_{50} for ginger cultivar Rio-de-Janeiro to be between 1.0 krad and 1.5 krad in sensitivity study and 1.5 krad and 2.0 krad in the subsequent field trials. The objection raised earlier regarding the data presented by Raju et al. (1980) hold good in this context also. The findings of the present studies indicate that the LD_{50} for ginger cultivar Rio-de-Janeiro can be considered as a dose between 0.5 and 1.0 krad.

The chemical mutagen (EMS) also decreased the sprouting of the ginger rhizomes as the dose increased. While the sprouting at 8 mM EMS treatment was 48 per cent of the control, it was reduced to 22 per cent at 16 mM concentration. No sprouting was observed at 32 mM and at the higher doses tried (48 to 160 mM). Since effect of treatments with EMS has not been reported in ginger, results obtained with EMS in other crops have been examined. In garlic sprouting of cloves was found to be decreased with an increase in the concentration of EMS (Choudhary and Dnyansagar, 1980). In tuberose, a progressive reduction in the sprouting of the bulbs with increasing doses of EMS was reported by Sambandamurthi (1983). At 15 mM concentration of the chemical, the sprouting percentage was 98, which reduced to 40 at 75 mM. In tapioca, a progressive reduction in the sprouting (percentage) was observed as the concentration of EMS was increased (Thamburaj et al., 1985). These findings support the results of the present studies.

The survival of the resultant plants has been considered as a better estimate than the sprouting/germination of the treated material for assessing the sensitivity of the crop to different mutagen treatments as it accounts for post-germination lethality also. According to Abraham and Desai (1976), the percentage survival is a reliable parameter for assessing the

sensitivity of ornamentals like tuberose, dutch amaryllis and gladiolus to mutagen. In the present studies, the percentage of surviving plants decreased with increasing doses of gamma rays. At 0.5 krad gamma rays 61 per cent of the plants survived 150 days after planting. As the dose of gamma rays increased to 2.0 krad, the survival decreased to 2.0 per cent of the control. A similar trend in the survival of tuberose subjected to gamma rays has been reported by Sambandamurthi (1983). At 0.5 krad gamma rays, 96 per cent of the control plants survived 100 days after planting. Drastic reduction of survival percentage (from 96 to 22) occurred at 2.5 krad gamma rays.

With respect to survival of irradiated ginger plants, the LD_{50} was found to be between 0.50 and 1.00 krad. The data presented by Sambandamurthi (1983) in tuberose indicate the LD_{50} based on survival 100 days after planting to be 2.0 krad gamma rays.

The chemical mutagen (EMS) at the lower doses (8 m$\underline{M}$ and 16 m$\underline{M}$) gave 45 and 21 per cent survival, respectively suggesting that the LD_{50} was below 8 m$\underline{M}$. A decreasing trend of survival was noticed in EMS treated garlic as the doses of EMS increased (Choudhary and Dnyansagar, 1980). In tuberose, Sambandamurthi (1983) observed that the survival based on control was 64 per cent when treated with 60 m$\underline{M}$ EMS which

reduced to 32 per cent when treated with 75 mM EMS. Therefore in tuberose the LD_{50} based on survival lies between 60 mM and 75 mM of EMS.

Besides sprouting and survival of the plants, the growth parameters also indicate the sensitivity/effectiveness of the treatments. The data on the height of the plants recorded 150 days after planting indicated a progressive reduction with increase in the doses of both the mutagens. The height of ginger plants treated with 0.5 krad gamma rays was 84 per cent over the control whereas at higher dose viz., 2.0 krad, the height reduced to 35 per cent over the control. Raju et al. (1980) and Giridharan (1984) observed a similar trend of height reduction as a result of gamma ray treatments. The average height of plants treated with 2.0 and 5.0 krad gamma rays was 6.5 and 3.0 cm, respectively while the height of the control plants was 35.0 cm (Raju et al., 1980). They observed height reduction consequent on gamma ray treatments in mango-ginger and turmeric also. Decrease in the plant height in ginger cultivar Rio-de-Janeiro was observed as a result of gamma ray treatment at doses of 0.7 krad to 2.0 krad (Giridharan, 1984). Plant injury in the form of height reduction was observed by gamma ray treatment in vegetatively propagated crops like tuberose (Sambandamurthi, 1983), gladiolus (Raghava et al., 1988),

chrysanthemum (Gupta and Jugran, 1983) and rose (Datta, 1985b, 1986). The findings of the present investigator are in agreement with the findings of the investigators referred to above.

In the present study, EMS treatment at 8 mM and 16 mM concentrations reduced the height by 45 and 64 per cent of that of the control, respectively. In garlic Choudhary and Dnyansagar (1980) observed that EMS (0.30 to 0.75 per cent) was found to decrease sprout height. The observations of Sambandamurthi (1983) that EMS treatment at 15 mM and 75 mM concentrations in a sensitivity study in tuberose reduced the height by 96 and 63 per cent of that of the control, respectively, support the findings of the present studies.

Effect of the mutagens in the vM_1 generation

The data on the sprouting of the treated rhizomes and the survival of the resultant plants in the vM_1 generation revealed that both the characters decreased as the doses of mutagens increased. The general trend was as observed in the experiment for standardisation of the doses. However, the vM_1 generation registered higher values for sprouting and survival (Table 9) compared to the values for sprouting and survival recorded in the studies for standardisation of the doses (Tables 6 and 7) for both the mutagens.

In the experiment for standardisation of the doses of mutagens, the sprouting percentage obtained for gamma ray treatment (0.50 krad) was 65. In the vM_1 generation, the corresponding figure for the same dose was 80. At higher dose of gamma ray treatment (1.0 and 1.5 krad) in the vM_1 the same trend for sprouting was observed. EMS 8mM resulted in 48 per cent sprouting compared to the control in the dose standardisation studies, whereas the same concentration of EMS gave 71 per cent sprouting compared to the control in the vM_1 generation.

Broertjes and Van Harten (1978) thought it difficult to get reproducible results on treating bulky materials like bulbs,rhizomes, tubers and other vegetative parts, especially with chemical mutagens. The difference in sprouting and survival counts between the preliminary experiment (aimed at standardisation of the doses) and vM_1 generation can be explained as due to this factor. It may, however, be pointed out that the trend observed in the vM_1 is in agreement with the results reported by Sambandamurthi (1983) in tuberose and Thamburaj et al. (1985) in tapioca.

The data on survival count at 150 days after planting suggest that the post-germination lethality was more in the gamma ray treated plants than in the EMS treated plants (Table 9). A similar trend of exhibiting more post-

germination lethality among gamma ray (0.5 krad to 2.5 krad) treated plants of tuberose compared with EMS (15mM to 75 mM) treated plants was reported by Sambandamurthi (1983). Gorden and Weber (1955) and Skoog (1935) had attributed, the reduction in the survival percentage as due to a drop in the auxin level. Read (1959) and Sparrow (1961) found chromosomal aberration as the major cause for reduction in survival. The higher post-germination lethality in physical mutagen treatment might be due to its effects on inducing large chromosomal aberrations and random distribution, resulting in continuous somatic elimination as against very small structural changes observed in the chemical treatments (Gaul, 1977).

The data on the plant height (Table 10) measured at 60 days after planting showed a progressive reduction with increase in the radiation doses. Giridharan (1984) in ginger cultivars Rio-de-Janeiro and Maran noticed graded decrease in plant height as the irradiation doses increased from 0.7 krad to 2.0 krad gamma rays. The trend of decrease in height as the irradiation doses increased was recorded by Sambandamurthi (1983)in tuberose, Raghava et al. (1988) in gladiolus, Gupta and Jugran (1983) in chrysanthemum, Datta (1985 b) in rose and Thamburaj et al. (1985) in cassava. In the present investigation as the vegetative growth advanced

(from 60 DAP to 180 DAP) the height of the treated plants tended to reach that of the control plants. The highest dose of gamma rays (1.50 krad) recorded the minimum plant height (58 per cent of that of the control) at 60 days after planting. The corresponding plant height when the growth period advanced to 180 days after planting was 86 per cent of the control. The data further indicate that in the lower doses (1.25 krad, 1.00 krad and 0.75 krad gamma rays) also, the reduction in plant height observed at 60 DAP seemed to nullify as the growth advanced.

The chemical mutagen EMS at all concentrations was found to cause injury as evidenced by the reduction in the height of plants recorded at 60 days after planting. At higher doses of EMS the extent of injury was higher as in the case of physical mutagen treatments. It appeared that later in the growth phase the plants recovered from the injury as indicated by the data recorded at 180 days after planting.

The height reduction as a result of mutagen treatment can be interpretted in cytological, physiological, biochemical and anatomical view points such as interference in normal mitosis and mitotic aberrations (Wertz, 1940), inhibition in the rate of assimilation and consequent changes in the nutrient level of plants (Ehrenberg, 1955) and inactivation of vital enzymes especially those concerned with

respiration (Casarett, 1968). Various other explanations were also offered for the reduced growth at various stages following mutagenic treatments such as auxin destruction (Skoog, 1935; Smith and Kersten, 1942), inhibition of auxin synthesis (Gordon, 1954), failure of assimilatory mechanism (Quastler and Baer, 1950), production of diffusible growth retarding substance (Mackey, 1951), changes in the specific activity of enzymes (Haskins and Chapman, 1956; Cherry and Lessman, 1967; Eno, 1967), delay in the onset of first mitosis (Natarajan, 1958)and inhibition of DNA synthesis (Mikaelson, 1968). In the present investigation the apparent recovery of vM_1 plants from injury as the growth advanced might be due to the growth of uninjured meristematic cells which suppressed the injured ones as growth proceeded.

The data on tiller production (Table 11) indicate that the gamma ray treatments reduced the tiller production at 60 days after planting. At this stage, the lowest dose of gamma rays (0.50 krad) resulted in 83 per cent tillers when compared with control. Drastic reduction of tillering was noticed at the highest dose (1.50 krad) which was 45 per cent of the control. As the growth phase advanced (from 60 to 180 DAP) the adverse effect of gamma ray treatment on tillering was found to show recovery. One of the reasons for the recovery may be due to the rapid growth of unaffected tissues of the treated rhizomes which replaced the affected one in

the later stages of plant growth. This can also be due to the tendency of the crop to produce more tillers at later stages of growth when the initial effects of the treatments subsided. According to Davies (1974) the increase in vegetative growth occurs not by direct stimulation but as a consequence of radiation injury elsewhere in the plant, it is likely that increased tillering is intiated by the damage to the primary growth meristems.

The continuous moisture supply ensured by monsoon showers and irrigation during dry period may be one of the reasons for tiller production even at the later stages of plant growth irrespective of the treatments (Table 11).

Giridharan (1984) observed, in ginger cultivar Rio-de-Janeiro, more tiller production as a result of gamma ray treatments at 0.7 and 1.0 krad during 180 days after planting. However, the highest dose of 2.0 krad gamma rays reduced the number of tillers produced. In tuberose the number of suckers per plant decreased gradually as the doses of gamma rays increased from 0.5 krad to 1.5 krad. At the highest dose of gamma rays (2.0 krad) a drastic reduction by 48 per cent was observed. The drastic reduction of tillers observed in the present investigation as a result of the highest dose of gamma rays (1.50 krad) at 60 days after planting was comparable to the drastic reduction of suckers

of tuberose noticed by Sambandamurthi (1983) with 2.0 krad gamma ray treatment which was the highest dose used in the vM_1 generation. Natarajan (1975)also revealed decrease in tiller production as the doses of gamma rays increased in turmeric cultivars.

The reduction in the number of tillers in the present studies may be due to the direct effect of radiation treatments on the growing points which are responsible for tiller production. Contrary to this Giridharan (1984) observed more tillers in ginger cultivars Rio-de-Janeiro and Maran at lower doses of gamma rays (0.7 to 1.5 krad). However, he observed reduced tiller production at the highest dose of gamma rays (2.0 krad).

The data presented in Table 11 indicate that EMS at all doses also affected tiller production at 60 days after planting. As the growth phase advanced from 60 DAP to 180 DAP tiller production progressed and the plants tended to show a recovery.

In tuberose Sambandamurthi (1983) observed more sucker production than control for lower doses of EMS and lesser sucker production for higher doses of EMS. As against the trend observed in tuberose with respect to sucker production the result of the present studies revealed a decrease in the

trend of tiller production as the concentration of EMS increased. Since data on sucker production in tuberose during different growth periods is not available a comparative analysis about the possible recovery or nullification of the effect of the treatment on sucker production at different growth periods in tuberose and ginger is not attempted.

Leaf production in ginger was found to be affected by mutagen treatments (Table 12). At 60 days after planting a decreasing tendency of leaf production was noticed as the doses of gamma rays increased. At the highest dose of gamma rays (1.50 krad) the total leaves were only 25 per cent of the control. As the growth advanced the inhibitory effect of radiation on the production of leaves was seen diminished. Recovery of leaf production was more evident at 1.50 krad gamma rays than at the lower levels of radiations. Two radiation doses (0.75 krad and 1.00 krad gamma rays) at 180 days after planting gave more leaves compared to the control as a result of recovery effect.

Giridharan (1984) indicated a reduction in leaf production as a result of radiation treatments. Reduction in the number of leaves was noted in gamma irradiated tuberose (Gupta et al, 1974; Sambandamurthi, 1983). In chrysanthemum lower dose of gamma rays increased, the number of leaves

whereas the highest dose of 2.50 krad decreased the leaf production (Datta, 1985a). According to Gupta, et al. (1982) reduction in the number of leaves was indicated as a result of gamma ray treatments in Costus speciosus.

The data presented in Table 12 indicate that EMS treatments, reduced the leaf production in ginger at 60 days after planting. The inhibitory effect on leaf production caused by the chemical mutagen was found to diminish as the growth advanced. Reduction in the number of leaves in tuberose plants treated with EMS (60 mM and 75 mM)was reported by Sambandamurthi (1983). As opposed to the present findings at lower doses (15mM to 45mM) there was no reduction in leaf number in tuberose. The results obtained by Sambandamurthi reveals that the effect of EMS on leaf production depends on the dose used. In ginger, the number of leaves appeared to depend on the doses of EMS treatments especially during initial stages of growth period.

Plants with chlorophyll deficient portions on their leaves were observed in physical as well as chemical mutagen treatments (Table 13 and Appendix I). In general, gamma ray treatments produced more number of plants with chlorophyll chimera (70) compared to the EMS treatments (19). Treatment with 0.75 krad gamma rays produced the maximum number (20) of chlorophyll chimeras while the minimum number (8) was

produced by 1.50 krad. In the case of chemical mutagen, the lowest concentration of 2mM generated the maximum number (7) of chlorophyll chimeras and EMS (6mM and 8mM) produced the minimum number (2). In ginger Giridharan (1984) observed yellow streaks as a result of radiation treatments in cultivars Rio-de-Janeiro and Maran. In colocasia occurance of chlorophyll deficient plants was reported by Vasudevan et al.(1968) as a result of gamma irradiation. Variation in leaf shape and colour were observed in costus (Gupta et al, 1982) when gamma irradiation was resorted to. Leaf variegation due to gamma irradiation was also reported in tuberose (Sambandamurthi, 1983). Nayar and Rajendran (1987) observed light green leaves in tapioca as a result of radiation treatment. The occurrence of chlorophyll chimera in the present investigation can be due to chromosomal aberrations, change in the route of auxin synthesis, distribution or disruption of mineral metabolism or accumulation of free amino acids as concluded by Gupta et al.(1982). Laxmi et al.(1980) considered that chimera formation in leaves as a result of gamma irradiation might be due to the multi-cellular nature of the tissues treated. Nuclear and/or plastid mutations were thought to cause variegations in leaves (Kirk and Bassett, 1967).

Flower production in ginger during the season (vM_1 generation) was observed to be very limited among treated as

well as control plants (Table 14). The shy flower production nature exhibited in the present studies in ginger was also reported earlier by Hooker (1894), East (1940), Fryxell (1957), Pillai et al. (1978), Jayachandran and Vijayagopal (1979), Jayachandran et al. (1979), Velayudhan et al.(1983) and Usha (1984). The plants treated with lower and higher doses of both gamma rays and higher dose of EMS did not produce flowers. Stray flowering was observed in other treatments and in control plants. It therefore appears difficult to draw valid conclusions on the effect of mutagen on flowering.

Pollen fertility data (Table 14) indicate that there is little difference in the pollen fertility status as a result of the mutagen treatments. Giridharan (1984) also recorded almost a similar trend in respect of pollen fertility.

The harvesting stage in the treatment plants, as indicated by the yellowing and drying of the leaves was appeared to be normal and similar to that of the control plants. However some plants appeared to produce the apparent symptoms of early maturity. These plants were morphologically abnormal and weak (Appendix I).

Rhizome yield of ginger was found to be reduced by all

doses of gamma rays and EMS (Table 15). At 1.50 krad gamma rays mean rhizome yield obtained on per plant basis was 72 per cent of that of the control and at 1.25 krad, 85 per cent. The 10 mM EMS treatment produced 70 per cent of the rhizome yield of the control. The lowest concentration of EMS (2 mM and 4 mM) appeared to have reduced the rhizome yield very slightly.

In an irradiation study Giridharan (1984) found reduction in yield as the irradiation doses increased from 0.7 krad to 2.0 krad gamma rays.

The low yield obtained in the investigation could be attributed to the reduction caused by the gamma rays on plant growth. Raju et al. (1980) also reported weaker and elongated underground rhizomes in ginger due to 2.0 krad gamma rays. In Costus speciosus irradiation of rhizome with gamma rays resulted in decreased yield (Gupta et al., 1982).

All radiation doses and all concentrations EMS adversely affected tiller and leaf production and height especially during the early stages of growth. As the growth period advanced the plants could more or less recover from the adverse effect noted during early stages in respect of the above characters. However, the recovery of growth parameters achieved during the later stages of growth did not appear to have sufficient contribution to the rhizome

development. This can be the reason for low yield resulted at higher doses of gamma rays and EMS irrespective of the fact that the plants could recover from the shock of mutagen treatments later in their growth period.

Evaluation of the vM_2 generation

For various reasons vegetatively propagated crops are a very suitable group of plants for the application of mutation breeding methods. 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 and often unique part of the genotype. Moreover mutations are the only source of variability in sterile plants like ginger. Nevertheless, mutation breeding of vegetatively propagated plants is associated with several major bottle-necks. A mutation is a single-cell event. The multicellular nature of the apex causes complicated problems like chimera formation and diplontic selection. The result is a relatively low mutation frequency and probably a limited mutation spectrum while selection procedures cannot be applied before a stable periclinal chimera stage has been reached. If, therefore, multicellular apices are irradiated, measures should be taken to promote an increase in sector size and to obtain complete periclinal chimeras as soon as possible. Selection and

further propagation could then be begun. Dermen (1967) observed that periclinal chimerism was a common situation in mutated (vegetatively propagated) plants after one or a few cycle of vegetative propagation.

Gupta and Jugran (1983) projecting valid arguments concluded that screening for somatic mutation should not be confined to vM_1 but it should be continued in vM_2 and subsequent vegetative generations for the success of mutation breeding programme.

In the present investigation vM_2 generation was studied in comparison with the treated plants in the vM_1 generation and the untreated plants. It can be seen from the data presented in Table 16 that the mean plant height exhibited, in general, a negative shift from that of the control. However, 4 mM EMS appeared to be an exception, in having produced taller plants. The mean values in respect of height of plants of vM_2 generation were comparable with those of the vM_1 generation (Table 16 and Table 10). A decreasing tendency of height as the doses of gamma rays and EMS increased was the general tendency in both vM_1 and vM_2 plants. In the vM_2 generation the height reduction noticed as a result of higher doses of gamma rays and EMS was more pronounced.

The range of variation in respect of plant height as a result of gamma rays and EMS treatment was wide. Plants with very dwarf nature (5 cm) were observed in the 0.75 krad gamma ray treated population of the vM_2 generation. Tall plants (90 cm) were resulted from 4m*M* EMS. Generally the range of variation was more in lower doses of both the mutagens (Table 16). An analysis of the plant height variation due to the mutagen treatments indicates that lower doses of gamma rays (0.50 krad to 1.00 krad) and lower doses EMS (2m*M* and 4m*M*) induce greater variation in plant height in ginger cultivar Rio-de-Janeiro. Studies by Sanjeeviah (1967), Gupta and Jugran (1983) Sambandamurthi (1983), Datta (1985 b), Thamburaj *et al*. (1985) and Raghava *et al*. (1988) also showed same trend.

The mean number of tillers in the vM_2 progenies of mutagen treated plants, indicated transgression to either side of the control (Table 17). Gamma rays at 0.50, 0.75 and 1.00 krad increased tiller production whereas reduction in tiller production was noticed at higher doses of gamma rays (1.25 and 1.50 krad). A comparison of the tiller production of vM_1 and vM_2 plants at 180 days after planting revealed that in the doses, 0.50 and 0.75 krad gamma rays low mean values observed in the vM_1 generation shifted to higher mean values, in vM_2generation.

EMS at 4, 6 and 8 m*M* increased the mean number of tillers to 23,19 and 18, respectively (144, 119 and 113 per cent of the control). However at the highest concentration (10 m*M*) the mean tiller production was reduced to 12 (75 per cent of the control). A comparison of vM_1 and vM_2 data (Table 11 and Table 17) reveals that the general tendency is that vM_2 plants show higher values of mean number of tillers per plant compared to vM_1 plants except in 10 m*M* EMS where a reduction of tillers was noticed.

Similar results were reported and discussed by several workers. Singh *et al.* (1970), Borojevic (1966) and Scossiroli (1965) attributed this change to the elimination of negative genes and lethals in field crops. Another explanation offered by Gaul *et al.*(1969) was the effect of natural selection. Sambandamurthi (1983) considered that the elimination of deliterious mutants may be responsible for the upward shift in the mean values observed in the vM_2 generation on tuberose. The recovery effect from the vM_1 injuries due to mutagenic treatments was considered to be another possible cause for increased mean values in the vM_2 by Borojevic (1966).

Wide variation was found in the tiller production capacity of the mutagen treated plants. The maximum number of tillers (70 tillers per plant) was recorded at 0.75 krad

gamma rays and the minimum (2 tillers per plant), at 0.50, 1.25 krad gamma rays and 10 mM EMS treated plants. The wide variability observed in the present investigation indicates the ability of gamma rays and EMS to induce variability in the character in ginger. Creation of variability for quantitative traits due to mutagen treatments was reported by Gregory (1955), Rawlings et al.(1958), Bhaskaran and Swaminathan (1962), Gaul et al. (1966), Goud (1967), Shroff (1974), Conger et al. (1976), Kumar and Das (1977), Rao and Siddiq (1976) and Ravi et al. (1979). Considering the mean values and the range of variation 0.50 krad to 1.00 krad gamma rays and 2 mM to 6mM EMS seem to be the desirable dose ranges.

Leaf production, recorded 180 days after planting in the vM_2 generation, at 0.50, 0.75 and 1.00 krad gamma rays, was 156, 122 and 146 per cent of the control respectively. At the higher doses (1.25 and 1.50 krad gamma rays) leaf production was found to decrease (84 and 67 per cent of the control). A comparison with the vM_1 generation at 180 days after planting (Tables 12 and 18) reveals a similar positive trend of leaf production in vM_1 and vM_2 generation at lower doses of gamma rays. At higher doses in both generations a negative trend was observed among gamma ray treated population.

EMS, at 4 and 6mM concentrations increased the leaf production. At higher concentrations (8 and 10mM) EMS exhibited a negative trend in leaf production. The increase in leaf production in lower doses and the decrease in leaf production in higher doses of both the mutagens appeared to be due to their direct effect on the production of tillers.

Wide variation in the number of leaves was observed under radiation and chemical mutagen treatments. The minimum leaf number was 25 and the maximum, 1426 as against a range of 98 to 420 in the control plants. It might appear that the relatively lower doses of gamma rays ranging from 0.50 krad to 1.0 krad and EMS from 2 mM to 6 mM can be the safe range of doses of the mutagens for induction of greater variations as far as the number of leaves are concerned.

Flower production in the vM_2 generation occurred only in a very limited number of plants. Unlike in the case of vM_1 generation, the irradiated and the EMS treated plants flowered (Tables 14 and 19). The minimum flowering (3.0 per cent) was observed in 1.25 and 1.50 krad gamma ray treated plants and the maximum (7.0 per cent) at 0.75 krad gamma rays and 6 mM EMS treatments. Among control plants only five per cent plants flowered. This factor and the general scanty flowering behaviour of the vM_2 generation make it difficult to draw valid conclusions about the effect of mutagens on

flowering in the vM_2 generation. Hooker (1894), East (1940), Fryxell (1957), Pillai et al. (1978), Velayudhan et al.(1983) and Usha (1984) also recorded shy flowering habit of ginger. As in the vM_1 studies the mutagenic treatments did not show any effect on pollen fertility (Tables 14 and 19).

The mean rhizome yield in vM_2 indicated shifts in both directions, positive and negative, the lower doses giving positive shift and higher doses negative shift (Table 20). Compared with the vM_1, while the mean yields in the lower doses remain almost steady, they showed negative shift in vM_2 in the higher doses. The range of variation was also limited in higher doses of mutagens compared to the lower doses. The maximum yield recorded by plants subjected to higher doses was only 495 g as against the highest yield 1320g. Most of the vM_2 plants at higher doses of mutagens fell in the lower half of the spectrum of variation. It therefore appears that as far as yield is concerned dose ranges of 0.50 to 0.75 gamma rays and 2 mM to 4 mM EMS are more effective in inducing wider variations and higher mean values. This result is in agreement with the data presented by Raju et al. (1980), Gupta et al. (1982) and Giridharan (1984) who reported adverse effect on yield at higher doses of mutagen treatments.

Screening the vM_2 generation against bacterial wilt and soft rot

Breeding for disease resistance certainly represents the most important way to counteract the pathogens. In many crop plants resistance to pathogens has been reported to be under genetic control. In majority of the cases disease resistance range from total immunity to varying degrees of susceptibility. Immunity is normally simply inherited whereas field resistance is quantitative. The categories of disease resistance are vertical, being mostly monogenic and durable or horizontal which is quantitative, being the combinations of genic and cytoplasmic interaction (Murty, 1983). While the genetic control of many diseases of crop plants is known (eg. rice and wheat) no information is available on the genetic control either of bacterial wilt or of soft rot in ginger. This is mainly because of the sterility of the crop and the vegetative propagation followed for perpetuation. Indrasenan et al. (1982) studied reaction of different types of ginger to bacterial wilt caused by Pseudomonas solanacearum at the Regional Agricultural Research Station, Ambalavayal and graded Rio-de-Janeiro as highly susceptible (40 per cent and above incidence). The vM_2 progenies studied in the intensive sick fields of Regional Agricultural Research Station, Ambalavayal (Table 23) revealed that the incidence of disease ranged from 90.5 to 100% in different treatments including control plants. It,

therefore, appears that none of the treated plants exhibited resistance to the disease. The total infection of vM_2 generation screened for soft rot tolerance/resistance (Table 24) at the Instructional Farm, Vellayani confirmed that the induction of mutation did not contribute plants that are tolerant/resistant to soft rot disease. The fact that none of the plants showed resistance to bacterial wilt and soft rot diseases does not rule out the possibility of inducing resistance in ginger through repeated, intensive and large scale mutation breeding programmes.

Similar early works for the induction of mutation in potato tuber also did not yield disease resistant types (Upadhya et al., 1974). However, successful induction of mutation for resistance against diseases in vegetatively propagated plants have been reported. In peppermint a Verticillium resistant mutant was evolved (Murray, 1969). Broertjes and Van Harten (1978) cited this as one of the best example of successful mutation breeding and indicated the reason for this success as the simple way in which peppermint propagates (from pieces of stolons) that reduced or even avoided the disadvantage of chimera formation. But in ginger, due to the multicellular nature of the buds used for propagation, the chance of chimera formation prevails to a great extent.

Study of the mutants in the vM_3

The main bottlenecks in mutation breeding 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 the bud apex and the fact that mutation is a one cell event. The result is a low mutation frequency and probably a limited mutation spectrum. Many workers have suggested that the selection process cannot be applied before a stable periclinal chimera stage has been reached. Moh and Alan (1973) suggested that chimera cuttings of cassava should be grown for two or more generations to "purify" the mutated sector. Jagathesan (1979) while discussing the utilization of genetic variability in sugarcane breeding, emphasised the necessity for followup of the mutation generations upto vM_4 or vM_5 or till stability was established. Rao (1979) was also of the opinion that mutated progenies of sugarcane should be grown for 3 to 4 vegetative generations for the selection to be successful.

Out of the 98 mutants identified in the vM_2 generation and brought to vM_3 , many failed to carry either all or some of the mutations. Apparently in the vM_3 plants which failed to express the mutant character (exhibited in the vM_2), it is probable that the somatic sieve might have operated

through diplontic selection. Abraham and Desai (1976) pointed out that the low recovery of mutations in the vegetatively propagated plants was due to diplontic selection. Jagathesan (1979) concluded that many of the mutants either did not exhibit the mutant character in the subsequent generations or broke down due to basic chimeral nature.

Taking one character into consideration at a time, out of 44 tall vM_2 plants only 20 vM_2 families exhibited the character. This worked out to 45.4 per cent. In the vM_3 plant basis only 16.6 per cent inherited tallness. Dwarfness was inherited to 75.0 per cent of the vM_2 families and 55.0 per cent of the vM_3 plants. More tillers, more leaves and high yield also showed a similar trend. This reduction in the percentage of mutation in the vM_3 plants may be due to the elimination of the mutated sector through diplontic selection and due to the inability of some of the mutated plants to survive upto maturity. It is expected that the vM_3 mutants might have approached near stability.

The content of volatile oil, non-volatile ether extract, starch and crude fibre influence the quality of ginger greatly. Ginger rhizomes which gives more drying percentage is valuable in the dry ginger market. Ginger rhizomes with more volatile oil and NVEE are extremely

suitable for volatile oil and oleoresin extraction industry. Good quality ginger contains less fibre but more starch.

Giridharan (1984) did not observe any difference in volatile oil and oleoresin content of ginger rhizomes of Rio-de-Janeiro and Maran cultivars after irradiating with 0.7 to 2.0 krad gamma rays. The dryage was also unaffected. Lata and Gupta (1971) found reduced volatile oil in rose as a result of irradiation. In lemongrass gamma ray treatment resulted in the isolation of mutants with high essential oil content (Nair, 1979). Similar trends were obtained by Kaul et al. (1978) in mint and Irulappan (1979) in rose. In costus, diosgenin content increased as a result of 2.0 krad gamma ray treatment where as it decreased at 3.0 krad (Gupta et al., 1982). Pavlovic et al. (1983) observed a positive correlation between irradiation dose and essential oil content in Mentha piperita.

In the present investigation it appears that the volatile oil content and NVEE in the early maturing mutants were higher than control plants (Table 25). However, the drying percentage of these early maturing progenies were less and therefore the total recovery of the volatile oil and the NVEE may probably be more or less the same. Similar trends of having higher volatile oil content in early harvested ginger rhizomes were reported by Nybe and Nair (1978) and

Jayachandran et al. (1980). This suggests that the early maturing plants studied, might not have accumulated sufficient dry matter resulting in greater relative values of volatile oil and NVEE when expressed in percentage. The relative high content of crude fibre and low content of starch in these early maturing mutants can also be discussed in similar lines.

The isolation of plants (32 and 77) with high rhizome yield, dry ginger recovery and quality rhizomes in terms of higher volatile oil and non volatile ether extract in the vM_3 generation in the present investigation strongly suggests the possibility of evolving desirable cultivars by appropriate methods of induction and recovery of mutants.

Inoculation studies on the vM_3 generation of plants identified as tolerant/resistant to bacterial wilt in the vM_2

In the vM_3 generation which was inoculated with homogenised bacterial wilt disease specimen 18 per cent of plants were found to be unaffected by bacterial wilt. Indrasenan et al. (1982) graded ginger varieties as highly susceptible if more than 40 per cent population were affected by bacterial wilt. It was also noted that none of the vM_3 families were completely unaffected. Based on the grading of Indrasenan et al. (1982) none of the vM_3 plants appeared

to be resistant/tolerant. The fact that some of the control plants were also free of the disease suggests that the unaffected vM_3 plants appears to be escapes.

A recent development in breeding disease resistance is by the use of cell culture techniques. In potato, sugarcane, maize, dianthus etc. successful selection for disease resistance was done (Wenzel, 1987). A system that start with single cells can help to circumvent chimerism thereby increase the chance for finding valuable mutants.

The high genetic variability observed for cultures *in vitro* as well as correlations observed sometimes between *in vitro* and *in vivo* response of a host to pathogen support the use of *in vitro* approaches in resistance breeding. However, although substantial progress in dual growth of host and pathogen has been made, it is still not certain that future progress in tissue culture provide a simplified experimental system. More basic research is required for a wider application for progress in unconventional breeding technique, to overcome empirical and pragmatic strategies. A constructive understanding of regulatory processes acting early in the higher plant when changed by a pathogen is needed, and until such results are available, the trial and error approach may be followed (Wenzel, 1987). Therefore, repeated, intensive and large scale induction and continuous

screening for disease resistance may be the most feasible approach at present.

As mentioned elsewhere in the discussion the major barriers in the improvement of vegetatively propagated plants where vegetative plant parts have to be irradiated, are the chimera formation and diplontic selection. These are the complications caused by the multicellular nature of bud apex and the fact that mutation is a single cell event. This perforce results in a relatively low mutation frequency and probably a limited mutation spectrum. Stable periclinal chimeras can not be expected in the early generations and selection has to wait till such time where stable periclinal chimera stage has been reached. These difficulties can be overcome to a large extent by the use of *in vivo* or *in vitro* adventitious bud technique as described by Broertjes *et al.* (1968). This technique is based on the phenomenon that the apex of the adventitious buds, such as may be found at the base of the petiole of the detached leaves, originate from only one epidermal cell. Consequently adventitious plantlets either are completely normal or are complete solid mutants. In otherwords, chimera formation does not take place. Moreover diplontic selection is restricted to the very initial stage of bud formation.

Ginger being a sterile crop mutation breeding is the

only method of inducing variability where vegetative parts have to be subjected to mutagenic treatments. The results of the investigations reveal that induction of variability in ginger is possible but somatic sieve is very active and a serious diplontic drift is the consequence. Further studies are required for assessing the possibility of using *in vivo* and *in vitro* adventitious bud techniques and somaclonal variations.

Another suggestion is to irradiate or treat the rhizome buds at the earliest possible stage of development in order to give a mutated cell the best chance to take part in the formation of the rhizome (Broertjes and Van Harten, 1978). Mutagenic treatment should therefore take place immediately after harvest when no visible bud can be detected on the rhizome since buds are in ontogenetically young stage of development. Normally in our conditions ginger is harvested during December-January and sown during April-May, after nearly 3 to 4 months of storage. In the present study such stored rhizome bits were treated immediately prior to planting. The method of treatment of the rhizomes immediately following harvest may be tried in future studies. To facilitate this, artificial conditions as provided by green houses or the conditions for raising summer crop is essentially required. This type of ginger cultivation irrespective of the season will also help to avoid mortality

occuring during the storage period of the rhizomes, in between generations.

If these conditions can be taken care of it is worthwhile trying mutagen treatment of ginger rhizomes immediately after harvest and raising subsequent generations, without storage of the seed material, to get greater recovery of solid mutants.

SUMMARY

SUMMARY

Investigations were carried out during 1985-1989 for studying the effect of mutagens on the vegetative and flowering characters, and the yield of rhizomes in the vM_1 generation, for assessing the extent of variability in the vM_2 generation and for studying the vM_3 progenies of the selected vM_2 plants of ginger (Zingiber officinale Roscoe) cultivar Rio-de-Janeiro.

Ten doses of gamma rays from 0.5 krad to 5.0 krad and eleven doses of ethyl methane sulphonate (EMS) from 8 mM to 160 mM were used for the dose standardisation study.

The study revealed that the percentage of sprouting and survival, and the height of the plants decreased as the doses of the mutagens increased. Gamma rays at 2.50 krad and above as well as EMS at 32 mM and above completely inhibited the sprouting of ginger rhizomes. The LD_{50} for sprouting and survival appeared to be between 0.50 and 1.00 krad gamma rays and below 8 mM EMS.

Based on the dose standardisation study five gamma ray doses (0.50, 0.75, 1.00, 1.25 and 1.50 krad) and five doses of EMS (2, 4, 6, 8 and 10 mM) were selected for induction of

mutations in ginger rhizomes.

In the vM_1 generation, the percentage of sprouting and survival decreased as the doses of mutagens increased. Delayed sprouting, to a limited extent, was observed in the gamma ray (2.2 per cent of the rhizomes planted) and EMS (1.6 per cent of the rhizomes planted) treatments. Such plants exhibited stunted growth and abnormalities, including underdeveloped rhizomes.

At 60 days after planting, the height of the plants decreased with increase in the doses of the mutagens. As the vegetative growth phase advanced (from 60 to 180 DAP), recovery in plant height at different rates was observed.

Tiller production was reduced as a result of the treatments with mutagens to the extent of 45 per cent of the control in 1.50 krad gamma rays and 61 per cent in 10 mM EMS, at 60 days after planting. Leaf production also showed a similar trend. As the growth phase advanced, the plants showed recovery from the adverse effects.

Plants with chlorophyll deficient portions on their leaves were observed among the mutagen treated population. In general, gamma rays produced more number of plants with chlorophyll chimera than EMS.

Only a few plants (0.4 to 0.9 per cent) flowered in the treatments and the control. Periodical observation of the flowers revealed that the treatments did not influence pollen fertility and seed set.

Rhizome yield was affected by the mutagen treatments, which was dose-dependant. Morphologically abnormal and weak plants, and those exhibiting delay in sprouting, dried up early and caused considerable reduction in yield.

Evaluation of vM_2 generation, in general, revealed a decreasing tendency with respect to plant height, as the doses of the mutagens increased. Large variation in plant height (5 to 90 cm) was observed in the population.

The mean number of tillers in the vM_2 indicated transgression to either side of the control. The vM_2 plants showed higher mean values, compared to the vM_1 plants. Wide variation was observed in tiller and leaf production, indicating the ability of the mutagens to induce wide variability.

Only very few plants in the vM_2 generation flowered. As in the vM_1 generation, the treatments did not influence pollen fertility.

The mean rhizome yield in the vM_2 generation indicated

shifts in both the directions, the lower doses of the mutagens giving positive shifts and the higher doses, negative shifts. The variation in rhizome yield ranged from 1.0 to 1320.0 g. Dose range of 0.50 to 0.75 krad gamma rays and 2 to 4 mM EMS appeared to be comparatively more effective in inducing wider variations and high mean values with respect to rhizome yield than the other ranges.

Based on the variability expressed in vM_2 generation for one character or combination of two or more characters simultaneously, 98 probable mutants were selected for vM_3 study in progeny rows. They were classified into 22 groups.

Out of the 98 mutants, 40 were selected from the gamma ray treatments and 58 from the EMS treatments. More than half of the mutants resulted from the lower doses of 0.75 krad gamma rays and 4 mM EMS. These results indicate that the lower doses of the mutagens are more favourable for induction of mutations in ginger than the higher doses.

The selected 98 mutants were classified into six groups, considering one character at a time, irrespective of the fact that a plant selected for a particular character might carry variation for other characters also simultaneously.

Screening of the vM_2 generation against bacterial wilt

disease at the Regional Agricultural Research Station, Ambalavayal, revealed that none of the treated plants possessed resistance to disease.

Screening of the vM_2 plants against soft rot disease at the Instructional Farm, Vellayani did not reveal any tolerant/resistant plants.

The fact that none of the plants showed resistance to bacterial wilt and soft rot diseases does not rule out the possibility of inducing resistance in ginger through repeated, intensive and large scale mutation breeding programmes. The possibility of utilization of somaclonal variation and *in vitro* screening methods for disease resistance will have to be explored in ginger.

Out of 98 mutants identified in the vM_2 generation and brought to vM_3 , many failed to carry either all or some of the mutant character. This low recovery of mutations in the vM_3 generation has been explained as due to the elimination of the mutated sector through diplontic selection and due to the inability of some of the mutated plants to survive upto maturity. Follow up of mutation generation upto vM_4, vM_5 or till stability is achieved has been considered necessary.

Quality analysis of the dried ginger rhizomes revealed that the early maturing mutants gave a high percentage

content of volatile oil and NVEE. However, the dryage was less and therefore, the total recovery of the volatile oil and the NVEE may probably be more or less the same. The few mutants identified with more yield and drying percentage, and more volatile oil and NVEE content, appear to be promising. Isolation of such plants strongly suggests the possibility of evolving desirable cultivars by appropriate methods of induction and recovery of the mutants.

Inoculation studies on the vM_3 plants that survived bacterial wilt disease in the vM_2 revealed that the survived plants might be escapes.

The results of the investigations reveal that induction of variability is possibile; but isolation of solid mutants poses some problems. The multi-cellular nature of the buds treated with the mutagens result in chimera formation and undergoes diplontic selection. Further studies are required for assessing the possibility of using *in vivo* and *in vitro* adventitious bud techniques, somaclonal variations and *in vitro* screening for obtaining disease resistant material in ginger.

Treatment of the rhizomes with mutagens, immediately after harvest when buds are in ontogenetically young stage of development and raising vM_2 and subsequent generations immediately after each harvest are suggested.

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*Originals not seen.

APPENDICES

Appendix I: Sprouting, growth and yield parameters, chlorophyll chimera, morphological abnormalities and crop duration in the vM_1 generation (ginger)

Plant No.	Nature of sprouting	Plant height (cm)	Tillers/ plant	Leaves/ plant	Nature of chloro-phyll chimera	Nature of morpholo-gical abnormality	Rhizome yield/ plant (g)	Nature of rhizome development	Crop duration
1	2	3	4	5	6	7	8	9	10
Gamma rays (0.50 krad)									
1	Normal	43	9	98	Greenish-yellow streaks in 3 leaves of one tiller	Normal	80	Normal	Normal
2	Normal	10	1	6		Leaves crinkled and cupped together, dwarf	84	Normal	Normal
3	Normal	35	10	27	White streaks in 4 leaves of one tiller	One tiller with 3 branches	34	Normal	Normal
4	Normal	3	2	6		Very dwarf	4	Under-developed	Early
5	Normal	5	1	4		Very dwarf	9	Under-developed	Early
6	Normal	20	7	47		Dwarf	5	Under-developed	Early
7	Normal	10	1	6		Dwarf	1	Under-developed	Early
8	Normal	16	2	8		Dwarf	6	Under-developed	Early
9	Normal	14	2	10	Yellow-white colouration in one leaf of one tiller.	Dwarf	2	Under-developed	Early

...2/-

1	2	3	4	5	6	7	8	9	10
10	Normal	25	7	61	Yellow-white lines in two leaves in one tiller	Dwarf	38	Normal	Normal
11	Normal	20	7	53	Normal	Dwarf	4	Under-developed	Early
12	Normal	20	4	22		Dwarf	7	Under-developed	Early
13	Normal	21	7	50	White thick streaks in two leaves in one tiller	Dwarf	8	Under-developed	Early
14	Normal	33	16	125	White streaks in one leaf in one tiller	Dwarf	129	Normal	Normal
15	Normal	32	14	98	Yellow colour in one leaf in one tiller	Dwarf	20	Under-developed	Early
16	Normal	48	10	123	White thin stripes in five leaves of one tiller		81	Normal	Normal
17	Normal	39	13	104	Yellow white streaks in three leaves in five tillers		66	Normal	Normal
18	Normal	5	1	3		Very dwarf	2	Under-developed	Early
19	Normal	4	1	2		Very dwarf	4	Under-developed	Early
20	Normal	42	12	107	Two long streaks in one leaf on either side		68	Normal	Normal
21	Normal	13	9	64		Dwarf	10	Under-developed	Early
22	Normal	17	4	31		Dwarf	7	Under-developed	Early

...3/-

1	2	3	4	5	6	7	8	9	10
23	Normal	20	3	23		Dwarf	12	Under-developed	Early
24	Normal	16	3	21		Dwarf	8	Under-developed	Early
Gamma rays (0.75 krad)									
1	Normal	43	14	120	Yellow-white streaks in one leaf in one tiller		176	Normal	Normal
2	Normal	56	13	180	Yellow-white streaks in one leaf in one tiller	Tall	249	Normal	Normal
3	Normal	49	12	122	Yellow-white streaks in one leaf in one tiller		179	Normal	Normal
4	Normal	51	7	77	Greenish-yellow streaks in one leaf in 3 tillers		110	Normal	Normal
5	Normal	54	23	201	Yellow streaks in 2 leaves of 2 tillers		503	Normal	Normal
6	Normal	54	7	75	Yellow streaks in 2 leaves in 1 tiller		140	Normal	Normal
7	Normal	19	5	56	Yellow colour in 1 full leaf of one tiller	Dwarf	2	Under-developed	Early
8	Normal	47	14	210	White streaks in 2 leaves in 1 tiller		282	Normal	Normal
9	Normal	52	16	240	White streaks in 2 leaves in 1 tiller		225	Normal	Normal
10	Normal	34	6	64	Yellow-white streak in 1 leaf in 1 tiller		12	Normal	Normal
11	Normal	28	4	40	White streaks in 4 tillers	Dwarf	6	Under-developed	Early

...4/-

1	2	3	4	5	6	7	8	9	10
12	Normal	23	6	76	Narrow and broad white-yellowish streaks in 1 tiller	Dwarf	3	Under-developed	Early
13	Normal	50	18	290	-		260	Normal	Normal
14	Normal	16	4	40		Dwarf	1	Under-developed	Early
15	Delayed (90 DAP)	12	1	8		Dwarf	1	Under-developed	Early
16	Normal	21	3	28		Dwarf	3	Under-developed	Early
17	Normal	20	2	21		Dwarf	6	Under-developed	Early
18	Normal	16	2	18		Dwarf	2	Under-developed	Early
19	Normal	31	8	67		Dwarf	2	Under-developed	Early
20	Normal	27	7	57	White streaks in one leaf in one tiller	Dwarf	2	Under-developed	Early
21	Normal	18	4	33		Dwarf	110	Normal	Normal
22	Normal	12	2	10		Dwarf	18	Under-developed	Early
23	Normal	13	6	31		Dwarf	1	Under-developed	Early
24	Normal	40	18	180	White streaks in 1 leaf of 1 tiller		144	Normal	Normal
25	Normal	16	4	23		Dwarf	2	Under-developed	Early
26	Normal	22	4	36		Dwarf	4	Under-developed	Early
27	Normal	24	3	35		Dwarf,narrow leaf	19	Under-developed	Early

...5/-

1	2	3	4	5	6	7	8	9	10
28	Normal	50	26	360		More tillers	240	Normal	Normal
29	Normal	17	6	37	Yellow-white streaks in 3 leaves in 1 tiller	Dwarf	2	Under-developed	Early
30	Normal	12	6	32		Dwarf	3	Under-developed	Early
31	Normal	12	6	32		Dwarf	1	Under-developed	Early
32	Normal	13	6	33		Dwarf	30	Normal	Normal
33	Normal	36	4	40	Yellow-white streaks in 1 leaf in 1 tiller		1	Under-developed	Early
34	Normal	29	12	108		Dwarf, narrow leaf	15	Under-developed	Early
35	Normal	37	8	82	Slight white-yellow colouration in 1 leaf in 1 tiller		8	Under-developed	Early
36	Delayed (90 DAP)	7	1	4		Very dwarf	1	Under-developed	Early
37	Normal	27	4	30	All leaves of 2 tillers white thick streaks	Dwarf	34	Normal	Normal
38	Delayed (120 DAP)	4	1	3		Very dwarf	3	Under-developed	Early
39	Normal	14	4	25		Dwarf	4	Under-developed	Early
40	Normal	7	3	20		Very Dwarf	1	Under-developed	Early
41	Normal	10	2	10		Dwarf	3	Under-developed	Early
42	Normal	7	1	8		Very dwarf	5	Under-developed	Early

...6/-

1	2	3	4	5	6	7	8	9	10
43	Normal	34	15	180	Greenish-yellow streaks in 1 leaf in 3 tillers		20	Under-developed	Early
44	Normal	56	15	190	Yellow streaks in 2 leaves of 2 tillers	Tall	99	Normal	Normal
45	Normal	18	7	43		Dwarf	8	Under-developed	Early
46	Normal	27	13	120		Dwarf, narrow leaf	16	Under-developed	Early
47	Normal	11	7	39		Dwarf	3	Under-developed	Early
48	Normal	20	7	64		Dwarf	1	Under-developed	Early
49	Normal	20	5	57		Dwarf	8	Under-developed	Early
50	Normal	23	7	61		Dwarf	4	Under-developed	Early
51	Normal	60	9	117		Tall	54	Normal	Normal
Gamma rays (1.00 krad)									
1	Normal	20	8	48		Dwarf	2	Under-developed	Early
2	Normal	18	4	32		Dwarf	4	Under-developed	Early
3	Normal	13	3	20	Thick yellow-white streaks in 5 leaves in 1 tiller	Dwarf	10	Under-developed	Early
4	Normal	23	4	43		Dwarf	6	Under-developed	Early
5	Normal	26	4	31		Dwarf	9	Under-developed	Early

...7/-

1	2	3	4	5	6	7	8	9	10
6	Normal	61	20	205	White streaks in 1 tiller	Tall	321	Normal	Normal
7	Normal	14	8	47		Dwarf	3	Under-developed	Early
8	Normal	18	3	26		Dwarf	8	Under-developed	Early
9	Normal	27	3	24	One tiller completely white and later dried	Dwarf	10	Under-developed	Early
10	Normal	18	3	21		Dwarf	5	Under-developed	Early
11	Normal	20	8	39		Dwarf	6	Under-developed	Early
12	Normal	20	1	11		Dwarf	1	Under-developed	Early
13	Normal	17	6	27		Dwarf	5	Under-developed	Early
14	Normal	58	9	85		Tall	44	Partly under-developed	Normal
15	Normal	32	10	101	Narrow white line in 2 leaves in 1 tiller	Dwarf	18	Under-developed	Normal
16	Normal	32	3	31	Narrow white line in 2 leaves in 1 tiller	Dwarf	2	Under-developed	Early
17	Normal	18	8	45		Dwarf	5	Under-developed	Early
18	Normal	36	9	89	Dull white streaks in one leaf in one tiller		40	Normal	Normal
19	Normal	52	31	470	Yellow-white streaks in many leaves of 2 tillers	More tillering	129	Normal	Normal

...8/-

1	2	3	4	5	6	7	8	9	10
20	Normal	28	5	42	Dispersed streaks on all leaves of all tillers	Dwarf	2	Under-developed	Early
21	Normal	28	7	50	Green and yellow colour in 2 leaves	Dwarf,4 tillers dried up	3	Under-developed	Early
22	Normal	35	6	46	Yellow streaks in 2 leaves of 1 tiller		18	Normal	Normal
23	Normal	34	8	52	White thick line in 2 leaves of 1 tiller		10	Under-developed	Early
24	Normal	20	3	30		Dwarf,2 tillers dried up	1	Under-developed	Early
25	Normal	46	18	244	Yellow-white prominent streaks in 2 leaves of 2 tillers		182	Normal	Normal
26	Normal	37	11	118	Majority of leaves in 4 tillers half area white colouration		60	Normal	Normal
27	Normal	14	5	27		Dwarf	3	Under-developed	Early
28	Normal	3	1	3		Very dwarf	3	Under-developed	Early
29	Normal	7	4	14		Very dwarf	2	Under-developed	Early
30	Normal	3	1	4		Very dwarf dried immediately	0	Under-developed	Early
31	Normal	17	2	17	Yellow streaks in 2 leaves in 1 tiller	Dwarf	4	Under-developed	Early
32	Normal	30	13	160		Dwarf	14	Under-developed	Early

...9/-

1	2	3	4	5	6	7	8	9	10
33	Normal	16	3	30		Dwarf	1	Under-developed	Early
34	Normal	16	3	19		Dwarf	2	Under-developed	Early
35	Normal	14	6	32	Greenish-yellow streaks in 1 leaf in 3 tillers	Dwarf	1	Under-developed	Early
36	Delayed (135 DAP)	2	1	1		Very dwarf	0	Under-developed	Early
37	Normal	19	5	28		Dwarf	2	Under-developed	Early
38	Normal	50	21	240		Tall	112	Normal	Normal
39	Normal	12	3	29		Dwarf	1	Under-developed	Early
40	Normal	8	1	6		Very dwarf	5	Under-developed	Early
41	Normal	16	4	31		Dwarf	6	Under-developed	Early
42	Normal	21	4	36		Dwarf	12	Under-developed	Early
43	Normal	5	1	6		Very dwarf	2	Under-developed	Early
44	Normal	5	1	3		Very dwarf	1	Under-developed	Early
45	Normal	6	2	6		Very dwarf	1	Under-developed	Early
46	Normal	24	13	173		Dwarf	128	Normal	Normal
Gamma rays (1.25 krad)									
1	Delayed (110 DAP)	5	1	4	Yellow colour in 1 leaf	Very dwarf dried	5	Under-developed	Early

...10/-

1	2	3	4	5	6	7	8	9	10
2	Normal	14	4	24		Dried	1	Under-developed	Early
3	Normal	10	3	14		Dwarf, dried	1	Under-developed	Early
4	Normal	58	24	250		Tall	304	Normal	Normal
5	Normal	25	7	47	Thick white streaks in 2 leaves in 2 tillers	Dwarf	4	Under-developed	Early
6	Normal	23	7	56		Dwarf, dried	1	Under-developed	Early
7	Normal	23	8	75		Dwarf	8	Under-developed	Early
8	Normal	13	4	26		Dwarf	2	Under-developed	Early
9	Normal	37	5	56	White streaks in 4 leaves in 1 tiller		10	Under-developed	Early
10	Normal	36	4	62	Thick yellow white streaks in 9 leaves of 1 tiller		60	Normal	Normal
11	Normal	58	28	247		Tall,more tillers	135	Normal	Normal
12	Delayed (75 DAP)	13	4	26		Dwarf	4	Under-developed	Early
13	Delayed (120 DAP)	12	1	9		Dwarf	1	Under-developed	Early
14	Delayed (135 DAP)	2	1	3		Very dwarf	1	Under-developed	Early
15	Delayed (95 DAP)	9	1	9		Very dwarf	2	Under-developed	Early
16	Delayed (120 DAP)	4	2	6		Very dwarf	15	Under-developed	Early
17	Normal	13	2	17		Dwarf	1	Under-developed	Early

...11/

1	2	3	4	5	6	7	8	9	10
18	Normal	40	11	102	White streaks in 1 tiller		151	Normal	Normal
19	Normal	28	4	39	Yellowish-green thick streaks in 3 leaves in 1 tiller	Dwarf	4	Under-developed	Early
20	Delayed (120 DAP)	8	1	6		Very dwarf	2	Under-developed	Early
21	Delayed (125 DAP)	2	1	2		Very dwarf	1	Under-developed	Early
22	Normal	12	3	15	Yellow-white streaks in 5 leaves in 1 tiller	Dwarf	1	Under-developed	Early
23	Normal	11	5	18		Dwarf	2	Under-developed	Early
24	Normal	50 & 23	3	26		1 tiller tall, 2 tillers dwarf	3	Under-developed	Early
25	Normal	3	2	4		Very dwarf	4	Under-developed	Early
26	Normal	35	17	160	White-streaks in 4 leaves of 1 tiller	12 tillers dried up	124	Partly normal	Normal
27	Normal	13	5	20		Dwarf	1	Under-developed	Early
28	Normal	12	3	10		Dwarf	2	Under-developed	Early
29	Normal	19	11	69		Dwarf	5	Under-developed	Early
30	Normal	16	10	53		Dwarf	6	Under-developed	Early
31	Normal	24	5	53		Dwarf	19	Normal	Normal
32	Normal	42	24	214	Yellow streaks in 5 leaves of 1 tiller		124	Normal	Normal
33	Normal	26	10	84	Yellow-white streaks in 7 leaves	Dwarf	19	Under-developed	Early

...12/-

1	2	3	4	5	6	7	8	9	10
34	Normal	17	6	47		Dwarf	3	Under-developed	Early
35	Normal	9	2	8		Very dwarf	18	Under-developed	Early
36	Normal	21	8	41		Dwarf	2	Under-developed	Early
37	Normal	8	2	11		Very dwarf	3	Under-developed	Early
38	Normal	37	14	110	Orange yellow colour in 50% portion of 1 tiller		18	Under-developed	Normal
39	Normal	21	7	54	Yellow-white streaks in 5 tillers	Dwarf	1	Under-developed	Early
40	Normal	25	10	71	Yellow-white streaks in 3 tillers	Dwarf	8	Under-developed	Early
41	Normal	18	3	29		Dwarf	1	Under-developed	Early
42	Normal	23	6	59	Greenish-yellow streaks in 1 leaf in 2 tillers	Dwarf	4	Under-developed	Early
43	Normal	19	4	20	Yellow streaks in 2 leaves of 2 tillers	Dwarf	2	Under-developed	Early
44	Delayed (90 DAP)	9	3	19		Very dwarf	3	Under-developed	Early
45	Normal	14	4	31	Yellow colour in 1 full leaf of a tiller	Dwarf	5	Under-developed	Early
46	Normal	23	3	31		Dwarf	1	Under-developed	Early
47	Normal	17	4	24	Thick yellow white streaks in 2 leaves in 1 tiller	Dwarf	62	Normal	Normal

...13/-

1	2	3	4	5	6	7	8	9	10
Gamma rays (1.50 krad)									
1	Normal	16	7	51	White streaks in 1 leaf	Dwarf	12	Under-developed	Early
2	Normal	13	4	29		Dwarf, dried	2	Under-developed	Early
3	Normal	37	10	93	White-yellowish streaks (7 leaves) and mosaic type white-yellowish colouration (6 leaves)		76	Normal	Normal
4	Normal	10	2	14		Dwarf	5	Under-developed	Early
5	Normal	35	18	197	Yellowish colouration in 50% area of 7 leaves in 3 tillers		70	Normal	Normal
6	Normal	22	8	75	All tillers white-yellowish colouration	Dwarf	41	Normal	Normal
7	Normal	20	4	34	White dispersed colour in 5 leaves each of 2 tillers	Dwarf	22	Under-developed	Early
8	Normal	22	8	81	Very slight white colouration in 3 leaves of 1 tiller	Dwarf	10	Under-developed	Early
9	Normal	37	22	194	Yellow streaks in 2 leaves of 2 tillers		42	Normal	Normal
10	Normal	26	5	47	White yellow streaks in 5 leaves in 1 tiller	Dwarf	26	Normal	Normal
EMS(2mM)									
1	Normal	15	2	12		Dwarf	1	Under-developed	Early
2	Normal	23	4	34		Dwarf	4	Under-developed	Early

1	2	3	4	5	6	7	8	9	10
3	Normal	23	6	47		Dwarf and dried	3	Under-developed	Early
4	Normal	20	2	11		Dwarf and dried	4	Under-developed	Early
5	Normal	14	5	29		Dwarf and dried	4	Under-developed	Early
6	Normal	24	2	32	Yellowish-green streaks in 9 leaves of 1 tiller	Dwarf	3	Under-developed	Early
7	Normal	18	6	34		Dwarf	3	Under-developed	Early
8	Normal	20	2	12		Dwarf	14	Under-developed	Early
9	Normal	59	26	111		Tall,more tillers	110	Normal	Normal
10	Normal	10	4	31		Dwarf and dried		-	-
11	Normal	24	6	40	White streaks in 2 tillers	Dwarf	4	Under-developed	Early
12	Delayed (90 DAP)	12	1	8		Dwarf	2	Under-developed	Early
13	Normal	19	7	35		Dwarf	4	Under-developed	Early
14	Normal	36	11	161	White narrow longitudinal streaks at border, midrib region of 1 tiller		15	Under-developed	Early
15	Normal	27	16	151	White streaks in 1 tiller	Dwarf, 9 tiller dried up	4	Under-developed	Early
16	Normal	29	2	18		Dwarf, narrow leaf	3	Under-developed	Early
17	Normal	35	15	180	Yellow colour in 1 leaf of 1 tiller		158	Normal	Normal

...15/-

1	2	3	4	5	6	7	8	9	10
18	Normal	24	12	87		Dwarf	3	Under-developed	Early
19	Normal	17	14	127	Yellow-white streaks in 3 leaves of 1 tiller	Dwarf	24	Normal	Normal
20	Normal	22	2	116		Dwarf	44	Normal	Normal
21	Normal	17	9	78		Dwarf	52	Normal	Normal
22	Normal	49	30	340		More tillers	212	Normal	Normal
23	Normal	37	26	344		More tillers	195	Normal	Normal
24	Normal	11	4	18		Dwarf	3	Under-developed	Early
25	Normal	11	2	10		Dwarf	2	Under-developed	Early
26	Normal	14	7	27		Dwarf	1	Under-developed	Early
27	Normal	17	2	20		Dwarf	3	Under-developed	Early
28	Normal	11	4	17		Dwarf	4	Under-developed	Early
29	Normal	22	4	33		Dwarf	2	Under-developed	Early
30	Normal	14	2	10		Dwarf	1	Under-developed	Early
31	Normal	24	6	47		Dwarf	15	Under-developed	Early
32	Normal	20	4	39	Yellow-white streaks in 3 leaves in 1 tiller	Dwarf	4	Under-developed	Early

...16/-

1	2	3	4	5	6	7	8	9	10
EMS (4mM)									
1	Normal	24	18	105		Dwarf	106	Normal	Normal
2	Normal	57	14	140		Tall	204	Normal	Normal
3	Delayed (90 DAP)	14	2	14		Dwarf	2	Under-developed	Early
4	Delayed (75 DAP)	19	1	9	Narrow leaves with yellow-greenish streaks in 3 leaves	Dwarf	4	Under-developed	Early
5	Normal	45	5	100	3 leaves white greenish thick streaks in 1 tiller		114	Normal	Normal
6	Normal	29	3	33		Dwarf	14	Under-developed	Early
7	Normal	70	31	480		Tall	340	Normal	Normal
8	Normal	58	8	82		Tall	170	Normal	Normal
9	Normal	59	22	321		Tall	170	Normal	Normal
10	Normal	19	3	52		Dwarf	8	Under-developed	Early
11	Normal	22	6	69		Dwarf	18	Under-developed	Early
12	Normal	12	2	10		Narrow, dwarf, unhealthy	10	Under-developed	Early
13	Normal	33	26	150		Dwarf, more tillers	13	Under-developed	Early
14	Normal	40	16	202	White streak in margin 2 leaves in 1 tiller	1 tiller bunchy top	156	Normal	Normal

...17/-

1	2	3	4	5	6	7	8	9	10
15	Normal	18	2	18		Dwarf	2	Under-developed	Early
16	Normal	9	3	11		Very dwarf	7	Under-developed	Early
17	Normal	2	1	2		Very dwarf	1	Under-developed	Early
18	Normal	12	3	10		Dwarf	4	Under-developed	Early
19	Normal	18	7	33		Dwarf	4	Under-developed	Early
20	Normal	27	6	42		Dwarf	12	Under-developed	Early
21	Normal	23	3	21		Dwarf	1	Under-developed	Early
22	Normal	56	15	210	Yellow-white streaks in 2 leaves of 1 tiller	Tall	164	Normal	Normal
23	Normal	17	5	37		Dwarf	1	Under-developed	Early
24	Normal	22	3	33		Dwarf	10	Under-developed	Early
25	Delayed (115 DAP)	9	1	7		Very dwarf	6	Under-developed	Early
26	Normal	13	2	11		Dwarf	1	Under-developed	Early
27	Normal	66	23	286		Tall	490	Normal	Normal
28	Normal	42	41	413		More tillers	220	Normal	Normal
29	Normal	22	9	98	Yellow-green streaks in 1 tiller	Dwarf	30	Normal	Normal
30	Normal	35	11	130		Narrow leaf	10	Under-developed	Early

...18/-

1	2	3	4	5	6	7	8	9	10
31	Delayed (110 DAP)	25	1	6		Dwarf	4	Under-developed	Early
32	Normal	19	2	20		Dwarf	14	Under-developed	Early
33	Normal	27	5	49		Dwarf	4	Under-developed	Early
34	Normal	59	35	338		Tall	320	Normal	Normal
EMS(6mM)									
1	Normal	12	2	12		Dwarf	2	Under-developed	Early
2	Normal	18	1	12		Dwarf	3	Under-developed	Early
3	Normal	27	4	51		Dwarf	6	Under-developed	Early
4	Normal	17	3	40		Dwarf, dried	2	Under-developed	Early
5	Normal	17	2	17		Dwarf	10	Under-developed	Early
6	Normal	60	21	188		Tall	258	Normal	Normal
7	Delayed (125 DAP)	4	1	1		Very dwarf, dried	0	-	-
8	Normal	14	2	12		Dwarf, dried	0	-	-
9	Normal	12	1	8		Dwarf, dried	0	-	-
10	Normal	10	4	25		Dwarf	2	Under-developed	Early
11	Normal	4	1	4		Very dwarf	1	Under-developed	Early
12	Normal	47	12	170	Yellow-white streaks in 6 leaves of 1 tiller		198	Normal	Normal

...19/-

1	2	3	4	5	6	7	8	9	10
13	Normal	46	26	140		More tillers	156	Normal	Normal
14	Normal	11	1	7	Slight white streaks in 2 leaves of 1 tiller	Dwarf, dried up	0	-	-
15	Normal	6	1	5		Very dwarf	2	Under-developed	Early
16	Normal	38	27	228		More tillers	156	Normal	Normal
17	Normal	23	13	91		Dwarf, dried	8	Under-developed	Early
18	Normal	12	4	35		Dwarf	4	Under-developed	Early
19	Normal	10	4	14		Dwarf	2	Under-developed	Early
20	Normal	3	1	4		Very dwarf	3	Under-developed	Early
21	Normal	13	7	42		Dwarf	6	Under-developed	Early
22	Normal	20	4	43		Dwarf	8	Under-developed	Early
23	Normal	12	1	10		Dwarf	1	Under-developed	Early
24	Normal	45	28	208		More tillers	146	Normal	Normal
25	Normal	28	11	99		Dwarf	8	Under-developed	Early
EMS (8 mM)									
1	Delayed (105 DAP)	3	2	7		Very dwarf	0	-	-
2	Normal	32	4	37		Dwarf, dried	2	-	-

20/-

1	2	3	4	5	6	7	8	9	10
3	Delayed (80 DAP)	8	3	18	4 leaves yellow greenish streaks	Very dwarf & dried	0	-	-
4	Delayed (80 DAP)	9	2	10		Very dwarf	2	Under-developed	Early
5	Normal	30	9	95		Dwarf	6	Under-developed	Early
6	Normal	24	6	56		Dwarf	3	Under-developed	Early
7	Normal	16	3	23	6 leaves yellow-white thick streaks	Dwarf	1	Under-developed	Early
8	Normal	57	16	170		Tall	160	Normal	Normal
9	Normal	25	5	40		Dwarf	8	Under-developed	Early
EMS (10 mM)									
1	Normal	23	3	11	White thin streaks in 1 tiller	Dwarf	5	Under-developed	Early
2	Normal	33	15	180	1 leaf white-greenish streaks in 1 tiller	Dwarf	180	Normal	Normal
3	Normal	59	26	320	White-greenish streaks in 1 leaf of 1 tiller	Tall	204	Normal	Normal
4	Normal	15	2	19		Dwarf	2	Under-developed	Early
5	Normal	14	1	8		Dwarf	3	Under-developed	Early
6	Normal	66	22	318		Tall	496	Normal	Normal

DAP = Days after planting

Appendix II: Plant characters of selected vM_2 mutants and distinct variation used for selection (ginger)

Plant No.	Plant Height (cm)	Tiller/plant (No.)	Leaves/plant (No.)	Rhizome yield/plant (g)	Distinct variation used for selection of mutants
1	2	3	4	5	6
Gamma rays (0.50 krad)					
1	58	37	675	340	Tall, more tillers and leaves
2	46	20	444	336	Early
3	85	55	1426	700	Tall, more tillers and leaves, high yield
4	43	20	360	180	Early
5	54	30	767	420	More tillers and leaves, high yield
6	52	22	485	189	Early
7	64	62	1235	343	Tall, more tillers and leaves
8	52	34	745	325	More tillers and leaves
9	64	58	1255	343	Tall, more tillers and leaves
10	44	50	760	808	High yield, more tillers and leaves
11	57	28	715	580	Tall, High yield, more tillers and leaves
Gamma rays (0.75 krad)					
12	60	32	672	310	More tillers, tall, more leaves
13	61	18	359	170	Tall, early
14	36	13	151	212	Early

...2/-

1	2	3	4	5	6
Gamma rays (0.75 krad) Contd.					
15	18	20	128	340	Dwarf
16	16	13	102	306	Dwarf
17	20	18	340	180	Dwarf
18	19	18	233	351	Dwarf
19	40	18	340	476	High yield
20	40	18	309	148	Early
21	31	14	152	218	Dwarf, early
22	70	60	1188	709	Tall, more tillers and leaves, high yield
23	46	34	499	510	More tillers, high yield
24	60	24	739	303	Tall, more leaves
25	62	40	788	320	Tall, more tillers and leaves
26	51	28	580	296	More tillers and leaves
27	47	28	613	304	More tillers and leaves
28	21	28	132	580	Dwarf, more tillers, high yield
29	50	8	160	181	Early
30	33	18	270	351	Early, dwarf
31	33	8	60	108	Early, dwarf
32	52	40	806	410	High yield, more tillers and leaves
33	42	70	642	431	More tillers and leaves, high yield

...3/-

1	2	3	4	5	6
Gamma rays (1.00 krad)					
34	58	40	840	410	Tall, more tillers and leaves, high yield
35	28	34	522	346	Dwarf, more tillers and leaves
36	26	10	158	203	Dwarf
Gamma rays (1.25 krad)					
37	23	12	107	73	Dwarf, early
38	40	30	427	189	More tillers, early
Gamma rays (1.50 krad)					
39	19	26	309	174	Dwarf, more tillers
40	15	20	227	90	Dwarf
EMS(2mM)					
41	52	42	937	342	More tillers and leaves
42	68	30	657	545	Tall, more tillers and leaves, high yield
43	47	30	548	495	More tillers and leaves, high yield
44	57	52	1040	1320	Tall, more tillers and leaves, high yield
45	46	10	119	280	Early
46	50	7	106	91	Early

...4/-

1	2	3	4	5	6
EMS (4mM)					
47	65	25	562	680	High yield, tall, more leaves
48	64	18	394	790	Tall, high yield
49	70	37	816	738	Tall, more tillers and leaves, high yield
50	75	26	607	606	Tall, more tillers and leaves, high yield
51	59	40	669	589	Tall, more tillers and leaves, high yield
52	60	36	684	385	Tall, more tillers and leaves
53	50	35	772	515	More tillers and leaves, high yield
54	51	24	455	440	High yield, early
55	70	32	656	680	Tall, more tillers and leaves, high yield
56	57	31	651	470	Tall, more tillers and leaves, high yield
57	42	22	312	185	Early
58	65	40	758	790	Tall, more tillers and leaves, high yield
59	59	12	160	125	Tall
60	62	28	495	510	Tall, more tillers, high yield
61	72	50	1110	660	Tall, more tillers and leaves, high yield
62	69	30	788	405	Tall, more tillers and leaves, high yield
63	43	10	126	160	Early
64	63	23	524	510	Tall, more leaves, high yield
65	67	20	472	460	Tall, high yield
66	67	12	228	128	Tall

...5/-

1	2	3	4	5	6
67	42	25	370	220	Early
68	51	14	313	225	Early
69	75	20	506	380	Tall, more leaves
70	67	20	485	681	Tall, high yield
71	83	36	1010	985	Tall, more tillers and leaves, high yield
72	70	51	1180	893	Tall, more tillers and leaves, high yield
73	60	26	507	680	Tall, more tillers and leaves, high yield
74	78	24	576	987	Tall, more leaves, high yield
75	57	55	1238	1050	Tall, more tillers and leaves,high yield
76	62	16	331	445	Tall, high yield
77	73	52	1320	710	Tall, more tillers and leaves, high yield
78	90	38	998	835	Tall, more tillers and leaves, high yield
EMS(6mM)					
79	37	30	438	360	Early, more tillers
80	42	36	469	350	More tillers
81	56	28	413	170	Tall, more tillers
82	52	16	304	152	Early
83	52	20	396	248	Early
84	17	32	249	222	Dwarf, more tillers
85	46	18	262	240	Early
86	59	20	270	207	Tall

...6/-

1	2	3	4	5	6
87	57	52	989	395	Tall, more tillers and leaves
EMS (8mM)					
88	36	15	202	110	Early
89	60	36	320	387	Tall, more tillers
90	48	44	746	180	More tillers and leaves
91	54	30	294	399	More tillers
92	16	8	90	50	Dwarf
EMS (10mM)					
93	64	38	497	405	Tall, more tillers, high yield
94	54	46	414	409	More tillers, high yield
95	56	33	251	350	Tall, more tillers
96	55	30	233	202	More tillers
97	19	7	59	56	Dwarf
98	40	25	202	39	Early
Control	42	18	412	195	

Appendix III: Performance of vM_3 progenies of selected vM_2 mutants (ginger)

vM_2 mutant Sl. No.	Distinct variation used for section of mutants in vM_2	Performance of progenies for the distinct variation in vM_3		Progenies which expressed the distinct variation in vM_3 (%)	Progenies which expressed all mutations (%)
		Mean	Range		
1	2	3	4	5	6
Gamma rays (0.50 krad)					
1	Tall	50 cm	34-64 cm	19	
	More tillers	26	13-50	46	12
	More leaves	529	193-1050	54	
2	Early	Normal	Normal	0	0
3	Tall	51 cm	28-66 cm	21	
	More tillers	21	10-35	17	
	More leaves	371	135-814	21	4
	High yield	180 g	42-517 g	4	
4	Early	Early	Early to normal	83	83
5	High yield	192 g	38-507 g	9	
	More tillers	20	7-45	27	9
	More leaves	427	150-1148	36	
6	Early	Normal	Normal	0	0
7	More tillers	13	8-15	0	
	Tall	32 cm	25-39 cm	0	0
	More leaves	197	132-270	0	

...2/-

1	2	3	4	5	6
8	More leaves	362	162-945	17	17
	More tillers	18	12-45	17	
9	More tillers	22	6-55	18	18
	Tall	41 cm	22-57 cm	9	
	More leaves	410	36-115	18	
10	High yield	227 g	35-640 g	7	7
	More tillers	22	10-50	40	
	More leaves	426	131-970	40	
11	Tall	42 cm	24-56 cm	9	0
	High yield	113 g	20-420 g	9	
	More tillers	13	4-25	0	
	More leaves	251	54-524	9	
Gamma rays (0.75 krad)					
12	More tillers	14	10-35	14	14
	Tall	42 cm	30-58 cm	21	
	More leaves	308	150-630	14	
13	Tall	38 cm	30-51 cm	0	0
	Early	Early	Early-Normal	67	
14	Early	Early	Early-Normal	86	86
15	Dwarf	28 cm	18-36 cm	86	86
16	Dwarf	29 cm	21-42 cm	77	77
17	Dwarf	36 cm	26-43 cm	50	50

...3/-

1	2	3	4	5	6
18	Dwarf	39 cm	29-50 cm	33	33
19	High yield	84 g	10-111 g	0	0
20	Early	Normal	Normal	0	0
21	Dwarf Early	35 cm Early	20-46 cm Early	33 100	33
22	Tall More tillers High yield More leaves	38 cm 30 318 g 532	27-46 cm 10-50 95-670 g 135-900	0 57 43 57	0
23	High yield More tillers	149 g 19	50-325 g 7-28	0 12	0
24	More leaves Tall	332 43 cm	27-840 32-54 cm	14 0	0
25	Tall More tillers More leaves	43 cm 17 338	38-48 cm 3-25 63-488	0 0 0	0
26	More tillers More leaves	26 507	18-30 297-653	60 60	60
27	More tillers More leaves	18 322	15-20 225-420	0 0	0
28	Dwarf More tillers High yield	43 cm 17 138 g	37-51 cm 10-22 85-200 g	0 0 0	0

...4/-

1	2	3	4	5	6
29	Early	Normal	Normal	0	0
30	Early	Early	Early-Normal	44	44
	Dwarf	37 cm	30-42 cm	44	
31	Dwarf	44 cm	30-60 cm	7	0
	Early	Normal	Normal	0	
32	More tillers	25	12-45	43	14
	More leaves	485	182-1019	29	
	High yield	270 g	80-540 g	14	
33	More tillers	16	4-32	3	3
	More leaves	295	54-722	7	
	High yield	153 g	40-460 g	3	
Gamma rays (1.00 krad)					
34	Tall	40 cm	26-52 cm	0	0
	More tillers	7	2-10	0	
	More leaves	122	39-210	0	
	High yield	52 g	19-128 g	0	
35	Dwarf	35 cm	26-46 cm	67	0
	More tillers	12	7-20	0	
	More leaves	181	53-360	0	
36	Dwarf	37 cm	36-42 cm	0	0

...5/-

1	2	3	4	5	6
Gamma rays (1.25 krad)					
37	Dwarf	47 cm	23-55 cm	14	0
	Early	Normal	Normal	0	
38	More tillers	28	20-33	67	0
	Early	Normal	Normal	0	
Gamma rays (1.50 krad)					
39	More tillers	15	10-20	0	0
	Dwarf	33 cm	27-44 cm	71	
40	Dwarf	Failed to survive			
EMS (2mM)					
41	More tillers	26	10-45	38	38
	More leaves	558	166-1084	50	
42	Tall	51 cm	30-67 cm	55	18
	More tillers	23	10-45	45	
	More leaves	498	97-1193	45	
	High yield	233 g	82-466 g	18	
43	More tillers	27	12-30	33	0
	More leaves	441	202-710	33	
	High yield	248 g	198-392 g	0	

...6/-

1	2	3	4	5	6
44	Tall	42 cm	17-66 cm	7	
	More tillers	21	4-60	29	5
	More leaves	399	36-1396	26	
	High yield	231 g	18-985 g	14	
45	Early	Early	Early-Normal	74	74
46	Early	Normal	Normal	0	0
EMS (4mM)					
47	Tall	38 cm	18-51 cm	0	
	More leaves	231	68-525	12	0
	High yield	112 g	19-403 g	6	
48	Tall	40 cm	26-52 cm	0	0
	High yield	92 g	19-195 g	0	
49	Tall	37 cm	28-53 cm	0	
	More tillers	14	3-20	0	0
	More leaves	258	41-450	0	
	High yield	97 g	30-300 g	0	
50	Tall	42 cm	20-57 cm	13	
	More tillers	18	7-45	8	0
	More leaves	362	95-1080	33	
	High yield	279 g	40-712 g	38	

...7/-

1	2	3	4	5	6
51	Tall	38 cm	27-44 cm	0	
	More tillers	21	10-45	8	
	More leaves	395	150-896	15	0
	High yield	196 g	48-590 g	8	
52	Tall	30 cm	19-38 cm	0	
	More tillers	15	10-18	0	0
	More leaves	225	150-338	0	
53	More tillers	16	5-30	8	
	More leaves	281	33-720	12	0
	High yield	140 g	25-328 g	0	
54	High yield	142 g	32-520 g	4	
	Early	Normal	Normal	0	4
55	Tall	36 cm	11-55 cm	4	
	More tillers	11	1-22	0	
	More leaves	189	8-528	4	0
	High yield	97 g	4-270 g	0	
56	Tall	40 cm	21-53 cm	0	
	More tillers	18	12-28	7	
	More leaves	320	95-540	14	0
	High yield	111 g	40-318 g	0	
57	Early	Normal	Normal	0	0
58	Tall	38 cm	25-47 cm	0	
	More tillers	15	8-21	0	
	More leaves	283	132-420	0	0
	High yield	144 g	70-205 g	0	

...8/-

1	2	3	4	5	6
59	Tall	42 cm	30-55 cm	0	0
60	Tall More tillers High yield	31 cm 17 200 g	25-39 cm 10-28 84-532 g	0 17 17	0
61	Tall More tillers More leaves High yield	39 cm 17 304 166 g	30-57 cm 8-32 108-816 32-410 g	11 11 22 11	0
62	Tall More tillers More leaves High yield	45 cm 18 385 172 g	35-57 cm 7-24 76-720 36-275 g	8 0 23 0	0
63	Early	Normal & Early	Early-Normal	50	50
64	Tall More leaves High yield	38 cm 290 152 g	23-51 cm 107-620 38-480 g	0 12 6	0
65	Tall High yield	45 cm 142 g	28-50 cm 79-300 g	0 0	0
66	Tall	47 cm	32- 55 cm	0	0
67	Early	Normal	Normal	0	0
68	Early	Early	Early-Normal	41	41

...9/-

1	2	3	4	5	6
69	Tall	30 cm	20-40 cm	0	
	More leaves	124	16-288	0	0
70	Tall	43 cm	18-59 cm	18	
	High yield	207 g	10-520 g	18	18
71	Tall	42 cm	27-57 cm	5	
	More tillers	23	5-40	45	
	More leaves	410	59-810	20	5
	High yield	261 g	20-803 g	20	
72	Tall	45 cm	27-58 cm	3	
	More tillers	20	4-47	23	
	More leaves	354	30-935	16	0
	High yield	178 g	40-685 g	6	
73	Tall	54 cm	42-67 cm	39	
	More tillers	19	4-50	16	
	More leaves	365	53-1003	16	11
	High yield	212 g	30-806 g	11	
74	Tall	47 cm	25-59 cm	8	
	More leaves	429	51-1200	44	4
	High yield	308 g	13-790 g	20	
75	Tall	35 cm	19-48 cm	0	
	More tillers	16	5-40	17	
	More leaves	237	38-750	17	0
	High yield	105 g	25-380 g	0	
76	Tall	36 cm	28-44 cm	0	
	High yield	97 g	20-140 g	0	0

...10/-

1	2	3	4	5	6
77	Tall More tillers More leaves High yield	47 cm 23 475 274 g	16-68 cm 1-55 10-1143 5-670 g	29 50 50 29	21
78	High yield Tall More tillers More leaves	Affected by soft rot completely.			
EMS(6mM)					
79	Early More tillers	Early 14	Early 6-28	100 5	5
80	More tillers	14	4-32	25	25
81	Tall More tillers	37 cm 12	23-48 cm 8-21	0 0	0
82	Early	Normal	Normal	0	0
83	Early	Normal	Normal	0	0
84	Dwarf More tillers	39 cm 17	29-54 cm 6-30	38 13	0
85	Early	Early	Early-Normal	81	81
86	Tall	43 cm	37-60 cm	25	25

...11/-

1	2	3	4	5	6
87	Tall	51 cm	33-65 cm	27	
	More tillers	24	13-35	40	20
	More leaves	492	200-985	33	
EMS (8mM)					
88	Early	Normal	Normal	0	0
89	Tall	52 cm	35-57 cm	22	0
	More tillers	22	18-31	33	
90	More tillers	27	14-34	40	20
	More leaves	472	327-812	27	
91	More tillers	18	12-24	0	0
92	Dwarf	31 cm	29-38 cm	75	75
EMS (10mM)					
93	Tall	40 cm	24-52 cm	0	
	More tillers	15	6-20	0	0
	High yield	118 g	50-205 g	0	
94	More tillers	20	7-45	10	0
	High yield	146 g	40-285 g	0	
95	Tall	36 cm	21-52 cm	0	0
	More tillers	14	7-25	0	
96	More tillers	16	7-40	6	6

...12/-

1	2	3	4	5	6
97	Dwarf	38 cm	35-40 cm	0	0
98	Early	Early	Early	100	100

Control (Mean values) : Height = 41.5 cm
Tillers = 15.2
Leaves = 202.0
Yield = 158 g

INDUCED MUTATIONS IN GINGER

(Zingiber officinale R.)

BY

B. K. JAYACHANDRAN

ABSTRACT OF THE THESIS
Submitted in partial fulfilment of the requirement
for the degree

DOCTOR OF PHILOSOPHY

Faculty of Agriculture
Kerala Agricultural University

Department of Horticulture
COLLEGE OF AGRICULTURE
Vellayani, Thiruvananthapuram

1989

ABSTRACT

Investigations in ginger cv. Rio-de-Janeiro, were carried out during 1985-89 for studying the effect of gamma rays and ethyl methane sulphonate (EMS) on the growth, yield and flowering in the vM_1 generation, for assessing the variability including tolerance/resistance to bacterial wilt and soft rot diseases in the vM_2 and for studying the vM_3 progenies of the desirable vM_2 plants.

Dose standardisation studies using 10 doses of gamma rays (from 0.5 to 5.0 krad) and 11 doses of EMS (from 8 to 160 m$\underline{M}$) revealed that the LD_{50} for sprouting and survival was between 0.5 and 1.0 krad gamma rays and below 8 m$\underline{M}$ EMS.

For the vM_1 study, five doses each of gamma rays (0.5 to 1.5 krad) and EMS (2 to 10 m$\underline{M}$) were used. Delayed sprouting occurred to a limited extent. Sprouting, survival, plant height, number of tillers and leaves, and rhizome yield decreased as the doses of the mutagens increased. In general, there was a tendency for recovery of growth parameters as the growth phase advanced. The number of plants with chlorophyll chimera was more in the radiation treatments. Flower production was not sufficient to draw valid conclusions.

In the vM_2 generation, plant height exhibited a negative shift. Tiller, leaf and rhizome production, at the lower doses of the mutagens in general, exhibited positive shifts and at the higher doses, negative shifts. Wide range of variability was observed with respect to these characters. Pollen fertility was not seen influenced by the treatments.

Screening the vM_2 plants against bacterial wilt and soft rot diseases did not enable the isolation of tolerant/resistant material.

Study of the mutants in the vM_3 revealed that majority of the plants failed to express all or some of the characters. A few plants with more yield and dryage, and more volatile oil and NVEE content, were located.

The studies indicated that though the range of variability induced is high, recovery of the mutants is very low; probably due to the multicellular nature of the apices of the rhizomes treated, and the consequent chimera formation and diplontic selection. Follow up of the mutation generation upto vM_4 or vM_5 or till stability is achieved and avoiding storage of the rhizomes between the generations have been considered necessary. Repeated, intensive and large scale induction and continuous screening for disease resistance is worth attempting.

Using *in vivo* and *in vitro* adventitious bud techniques, somaclonal variation, *in vitro* screening for disease resistance, induction of mutation immediately after the harvest when buds are in ontogenetically young stage of development, and raising of vM_2 and subsequent generations without storage of seed rhizome irrespective of the season, are areas suggested for future research.