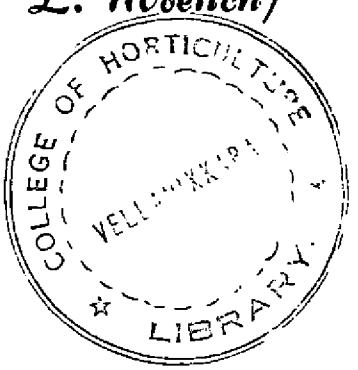
INDUCED MUTATIONS IN

BHINDI (*Abelmoschus esculentus L. Moench*)



BY

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (AGRICULTURAL BOTANY)

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COLLEGE OF AGRICULTURE

VELLAYANI, TRIVANDRUM.

1985

DECLARATION

I hereby declare that this thesis entitled "Induced Mutations in Bhindi" 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 any other similar title of any other University or Society.

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Vellayani,

26-1-1985

C E R T I F I C A T E

Certified that this thesis entitled "Induced Mutations in Bhindi" is a record of research work done independently by Kum. PILLAY MAHALEKSHMY KRISHNA under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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PILLAY MAHALEKSHMY KRISHNA

C O N T E N T S

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LIST OF TABLES

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LIST OF TABLES CONTD.

LIST OF FIGURES

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INTRODUCTION

INTRODUCTION

Biotechnology for tailoring better genic architecture enjoys the pride of place among scientific investigations all the world over, today. The discovery of the secrets of inheritance, its chemical basis and further on, the induction of variations in it has opened vistas of possibilities to man-kind. Reports indicate the kaleidoscopic variations that can be induced in crop species by mutagenic treatment programmes, since plant breeding is nothing but creation of genetic variation, followed by selection.

Treatment of plant materials with mutagens resulted in changes in the genetic apparatus, many of which were expressed in subsequent generations. This phenomenon was first brought to light by the works of Muller (1927) and Stadler (1928). Since then plant breeders have been utilizing this discovery to create variations in stagnant varieties. The variations can be induced in any character and consequently the mutant populations have to be subjected to careful selection procedures.

Mutations induced can be in quantitative (polygenic) characters also. Such variations may be of small magnitude

but can influence evolution greatly, over a long period of time. In fact, such mutations ocurring naturally contribute most to the progress of organic evolution. In mutation breeding, the breeder only amplifies the frequency of such occurrences. Here it may be noted that, for the very same reason too, the mutations induced by artificial processes differ from natural mutations, only in their frequency.

Physical mutagens include X-rays, beta rays, gamma rays, U-V rays, neutrons etc. These radiations act at the chromosomal level, producing random structural and numerical changes. Among the physical mutagens gamma rays have been observed to be the most potent agent and used widely for irradiating seeds and seed material. A common source of gamma rays is ^{60}Co.

The advantages of mutagenesis are its easiness of application, the recovery of great varieties with in a short period, possibilities of producing even specific changes in adapted varieties and its applicability to a wide range of crop species showing diverse methods and capabilities of reproduction. The conventional methods

of breeding viz. selection and crossing pose several difficulties. The potentialities offered by mutagenesis become very attractive in this context. It helps to produce larger genetic variation and this means the possibility of greater responses to selection and higher chances of improvement.

The recovery of mutations is however, dependent on several factors including genotype, type of mutagen, doses employed and many other modifying factors. It also depends on characters for which mutations are sought. Mutations in quantitative characters can be discovered only by statistical observations. Some crops also show less susceptibility. Dicot plants like bhindi with high chromosome number are not as easily mutated as monocots like barley, rice or wheat.

In India, bhindi is mainly grown as a vegetable crop. Bhindi originated in the Abyssinian centre. In India also, one meets with several of its wild forms. It requires warm growing season. Varieties of bhindi differ in the yield, duration, branching habit, pigmentation and disease resistance. One important breeding aim in the case of bhindi, has been to produce plant types resistant to the

dreaded virus disease called yellow vein mosaic. Though variety Pusa sawani is fairly mosaic tolerant none of the varieties satisfactorily escape damage by this pathogen. This gives scope for induced alterations in the existing genotypes especially in breaking the linkage that has been observed between the genes for high yield and susceptibility to the pathogen.

The present investigation was conducted to assess the extent of mutations induced by ^{60}Co gamma rays in bhindi. A local variety Anakomban was selected for the study. This variety is a high yielder, adaptible but susceptible to the virus.

The objectives of the work are as follows:-

1. To analyse the variability arising due to treatment with gamma ray mutagen.

ii. To estimate the direct effect of the mutagen in terms of M_1 sterility and lethality.

iii. To score out viable mutants in M_2, if any.

iv. To assess mutagenic effectiveness and efficiency to produce variability in M_2.

v. To study the extent of variability created in the important polygenic traits in the M_2 population with respect to selected sterility groups in the M_1 population.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The epoch of induced mutagenesis begins with the Nobel prize winning work in Drosophila (Muller, 1927) followed by similar results by Stadler (1928) on plants. Since then mutations have been reported by numerous workers in widely varying crops. A systematic review of these reports yet remains to be done.

Both physical and chemical mutagens have come into use for specific purposes, depending on their effect on the crop and requirements. In this investigation, since the mutagen used is a physical one, a review on the effects of ionizing radiation on crops, with emphasis on gamma rays, is presented.

Physical Mutagens

Radiations act at the chromosomal level producing random structural and numerical changes. The reviews made so far clearly demonstrate that specificity is difficult to attain due to the random aberrations created. Studies on the effect of different doses of physical mutagens revealed a dose-dependent linear increase in frequency of chlorophyll mutations (Siddiq, 1967; Siddiq and

Swaminathan, 1968; Yamaguchi and Miah, 1964; Nair, 1981). Swaminathan et al. (1970) found that mutagenic effectiveness in neutron treatment were 7 - 10 times greater than that in the case of gamma rays.

Use of Gamma rays

Mutation is an excellent means to increase genetic variability for subsequent selections. Gamma ray-induced variability was investigated by Sahai and Dalal (1973) in Safflower. Similar experiment on Solanum khasianum by Chuhan (1978) showed increased variability in the development of spines per unit leaf area.

Mutations induced by gamma rays for creating desirable combinations of traits have been reported in bread wheat (Goud, 1967c), rice (Singh, 1970), barley (Sethi, 1975), pigeon pea (Venkiteswarlu et al., 1978), chillies (Subash and Nizam, 1977, Khan et al., 1979) and green gram (Rathnaswamy et al., 1978). Parameters for measurement of radiosensitivity to gamma rays were suggested for cucurbits by Vishnoi and Joshi (1981). These included germination percentage and root and shoot length. Rai and Das (1978) reported sterility and yield reduction after gamma irradiation of dry linseed seeds. The problem of plant survival

after irradiation was discussed by many previous workers, including Ehrenberg et al. (1961) and Fujii and Matsumara (1958). Sinha and Godward (1972) observed the effect of different doses of gamma irradiation on plant survival and growth and found a general reduction in percentage of survival at higher doses.

Genotype in relation to mutations:

Mutagenic sensitivity to irradiation has been studied in several crops. It varies with the genetic constitution of the crop as reported by Gustafsson (1944, 1947, 1965); Gustafsson and Tedin (1954); Nilan (1956); Lamprecht (1956 and 1958); Gelin et al. (1958); Smith (1961); Sparrow (1961); Konzak et al. (1961a) and Sparrow et al. (1965).

The corollary that naturally follows this finding, that closely resembling genotype show greater similarity in their mutation frequency and spectrum, was reported by Enken (1966a, b). A study of mutagenic sensitivity is a pre-requisite for practical mutation breeding programmes since the sensitivity of seeds to mutagenic treatments is dependent on many factors including genetic factors. In fact Gregory (1960) opined that the important factor in production and recovery of mutations is the

genetic make-up of the plant material. Comparative studies among varieties of tomato (Bianchi et al., 1963) barley (Mikaelson and Brunner, 1968), soybean (Ukai and Yamashita, 1968) and rice (Siddiq and Swaminathan, 1968) showed variation in response to radiation among different genotypes indicating influence of genetic factors on radiosensitivity and recovery of mutations.

Effect of irradiation in Dicots

An important group of dicots used as experimental material is the vegetable crops. Most of the work has been on common vegetables like cucumber, chillies, tomato and bhindi.

Cucurbitaceous vegetable crops have been popular with mutation breeders especially cucumber. Campos and Walderice (1963) and Roy et al. (1971) conducted studies on the effect of ionizing radiations on cucumber. Gamma rays were used by Haq and Abidi (1972) on cucumber and they found that higher doses reduced survival of seedlings.

Chillies is another vegetable crop in which innumerable works have been done. X-ray irradiation was done by several workers on chillies (Raghavan and Venkitasubban 1970; Campos and Morgan, 1960). Morphological abnormali-

ties and chromosomal aberrations in M_1, following X-irradiation of seeds were reported by Sahib and Abraham (1970). Duration was reduced by 20-30 days in X-ray irradiated material, as reported by Subash and Nizam (1973). Meiotic abnormalities, induced by X-rays, were also studied by Subash and Nizam (1977).

Another solanaceous crop, in which much work has been done is tomato. Early reports on X-ray induced mutations in tomato was that of Johnson (1931); Mac Arthur (1934) and Young (1940). Lesley and Lesley (1956) observed delayed germination due to X-rays. Yu (1961) observed reduced survival at higher doses of X-rays on tomato. Georgiov (1966) and Davies (1962) conducted studies on radiosensitivity in tomato. Brock (1965a) isolated early maturing tomato mutants. Kaushik and Kalloo (1979) studied the extent of variability induced by gamma rays in comparison with chemicals. Similar comparative studies on mutagenic efficiency have been conducted by Majid (1975).

A good amount of mutation work has been done in peas and beans also. Altman (1923) and Bajaj and Saettler (1970) studied the effect of irradiation at higher doses in Phaseolus. Similar investigations had been conducted

in Trifolium (Jones and Plummer, 1960), fodder pea (Vasileva and Mehandzieva, 1969) and peas (Ananthaswamy et al., 1970). Chromosomal aberrations produced by irradiation were found to increase with dose, in cowpea, by Nair (1964). Sidorova and Kalinina (1967) also studied mitotic changes in pea. Chimerism had been noticed in irradiated population of peas (Blixt, 1961; Blixt et al., 1958), cowpeas (Nair, 1964) and french bean (Vishnuswarup and Gill, 1967). Chlorophyll mutations were comparatively rare in leguminous plants (Sjodin, 1962). Dwarf mutants have been isolated in french bean (Lamprecht, 1958) and Cicer (Athwal, 1963). Irradiation-reduced germinability in Cicer (Athwal, 1963) and field bean (Shirshov and Sain, 1967) has been reported, though, in some other cases, it produced no effect, as in pea (Olatunde et al., 1971) and cowpea (Nair, 1964). Similar contradictory results in other parameters were obtained in pea (Constantin and Love, 1968).

In bhindi, less work has been done compared to chillies or tomato. This may be because of its confinement, as an important vegetable crop, to the tropical areas. In fact, in several leading countries, bhindi is only a minor fibre yielding crop. Also, its high chromo-

some number renders it less susceptible to mutation treatments. But even in this crop a few reports are available by Asian workers. Mutations were induced by Kuwada (1972) in Abelmoschus by ^{60}Co gamma rays and X-rays to create resistance to Phytophthora. Characters such as plant height, stem thickness and number of nodes varies according to the type and dose of radiation. Patel (1967) isolated dwarf mutants in bhindi using X-rays. Increased variation in plant height, number of days taken for flowering and yield was obtained using gamma rays (Nandpuri et al., 1970). ^{60}Co gamma rays were used by Rao and Raj (1976) to study changes in morphological characteristics in bhindi. Similar studies had been conducted by Koshy and Abraham (1978). Inheritance of chlorina, an induced chlorophyll mutant, in okra was reported by Jambhale and Nerkar (1979, 1981). The chlorina mutant of A. esculentus obtained, was named Vaishali Vadhu.

Radiation effects in M_1

In the M_1 generation, it is possible to observe the direct effect of the mutagens. The twin effects, which come as direct effects, are lethality and sterility.

The lethality can occur at early stages of the crop resulting in poor germination, or at later stages. The net effect is a poor stand of the crop. Sterility can occur at any stage of the crop and will reduce the representation of the genotype in subsequent generations. Several workers have reported M_1 damages as due to different causes such as auxin destruction (Skoog, 1935; Smith and Kersten, 1942), production of diffusible growth retarding substances (Mackey, 1951), inhibition of DNA synthesis (Mikaelsen and Brunner, 1968), changes in enzyme specificity (Cherry and Hageman, 1961), delay in onset of first mitotic division (Natarajan et al., 1958) and inhibition of growth (Gaul, 1970).

The criteria for the determination of damage can be based on M_1 population or M_2 mutation frequency (Gaul, 1959). He listed out certain characters. These include germination per cent, height in field or lab conditions, root length, survival, fertility in terms of number of flowers or inflorescences per plant, number of seeds per fruit and number of fruits per plant. Observations on chlorophyll deficiencies as spots or streaks should be made. According to Gaul (1963) survivors are those plants that produce at least one spike regardless of whether seeds are produced or not.

Effect on germination per cent in M_1

Irradiation can produce varying effects on germination and sometimes no effect at all. Higher doses generally show delayed and reduced germination. This was observed in cotton (Ibragimov and Kovalchuk, 1962), rice (Borasio and Corbetta, 1964), field beans (Shirshov and Sain, 1967) and Phaseolus (Bajaj and Saettler, 1970). Low doses of X-rays hastened germination in cotton (Ibragimov and Kovalchuk, 1962), while no effect was observed on germination in cowpea (Nair, 1964), jute (Kundu et al., 1961) and pea (Olatunde et al., 1971), where low and moderate doses were employed.

Seedling survival:

Gaul (1959, 1963) observed correlation between seedling height and survival. The same was reported by Geard et al. (1967). Higher doses produced reduced survival in field bean (Shirshov and Sain, 1967), sorghum (Wu and Pi, 1968); potato (Mcrory and Grun, 1969), peas (Hangil'din, 1967), sunflower (Ljascenko, 1965) and Phaseolus (Teodoradze, 1968).

Expression on induced sterility

Sterility is a very important effect in the M_1. It acts as a sieve through which drastic mutations which are usually deleterious, if expressed, get screened out. In the case of physical mutagens, the chromosomal variations are structural aberrations and can cause considerable abnormalities during gamete formation. This will result in sterility.

Sparrow (1961) listed the different stages at which sterility can occur and the expressions of sterility leading to reduced reproduction potential. They are as follows:-

(1) severe stunting and poor flowering,

(2) formation of sterile flowers

(3) pollen abortion,

(4) embryo abortion at fertilization or before maturity and

(5) poor seed germination.

Gaul (1960) stated that M_1 sterility is a very good indication for the efficacy of the mutation treatment. In certain cases, this sterility can be transmitted to subsequent generations, especially in the case of radiations (Gaul and Mittelstencheid, 1960).

A linear increase in sterility with dose was reported in several crops, as barley (Gustafsson, 1944; Ehrenberg, 1955), sorghum (Kaukis and Webster, 1956) and jute (Kundu et al., 1961). But this linear trend stopped after a certain level. This had been reported in rice (Beckendam, 1961).

In other cases, fertility was increased with irradiation in potato (Jasina and Krisanova, 1966). In Phaseolus (Genter and Brown, 1941) there was no difference with treatment. Muller (1966) concluded that the achievements of increased mutation frequencies is limited by increased sterility of the M_1 plants and not by the increased M_1 lethality.

Nayar (1976) reported sterility in M_1 of irradiated Japonica and indica varieties. Very high or complete sterility was also reported in rice (Veluswamy, 1966). The causes of sterility in irradiated plants were explained by numerous workers as due to abnormalities in chromosomes or segmental interchange (Chang and Hsieh, 1957).

Mutations on Plant height and Plant type

Here also, different types of conclusions have been reached by workers, on different materials.

Greater variation in height was seen in paddy (Goud et al., 1967) and barley (Vasudevan et al., 1969). At much lower and higher levels, the variance did not differ much (Ota et al., 1962). Lack of marked effects in plant height following irradiation was reported by Kolstov and Kolstov (1925) in wheat. Similar results were obtained by Ozol and Peterson (1966) in maize. Both lower and higher doses can produce inhibition. Slight inhibition following lower doses was observed in wheat (Ananthaswamy et al., 1970) and higher doses inhibited Brassica (Kornicke, 1904), Phaseolus (Altman, 1923; Bajaj and Saettler, 1970) and french bean (Vishnuswarup and Gill, 1967). Lower doses produced stimulatory effect on tomato (Johnson, 1931), wheat (Shull and Mitchell, 1933) and Trifolium (Jones and Plummer, 1960).

Significant correlation between early mutants and plant height have been reported by Abrams and Frey (1964) and Oung (1965). Reduction of internode number, shortening of basal internodes or lengthening of upper internodes were observed on early maturing barley mutants (Ehrenberg et al., 1965) Mutants in plant growth habit have also been reported. Zacharias (1956) and Sjodin (1962) mentioned mutants with branches and leaves

projecting at different angles. Plant habit is determined by the action of several genes, regulating plant morphogenetic behaviour.

Even though height is a quantitative character and therefore show micro or 'Klein-mutationen' (Baur, 1924) visual detections of dwarfs are possible when some mutations are transgressive (Scarascia 1956). Some plant type mutations may be economically useful as they are linked positively with yield and can be used for indirect selection for yield. The erectoides mutant in barley, reported by Gustafsson (1947), belongs to this class. This character is controlled by several genes at many different loci (Hagberg, 1959).

Flowering and Fruiting

Mutants, with changes in flowering or ripening time, had been reported in numerous experiments. Early flowering and maturing mutations are, in general, less frequent than late ones. (Gustafsson, 1947; Kawai, 1964). Quantitative studies on variations of heading date on irradiated materials show that variation, in many instances is induced by earliness and lateness in nearly equal frequencies and in other cases more by lateness than by

earliness (Abrams and Velez-Fortuno, 1962; Abrams and Frey 1964.; Sakai and Suzuki, 1964). Aastveit (1965) reported that early heading mutations were induced less frequently in early heading varieties than late heading varieties.

Yielding capacity of early maturing mutants is generally reduced (Gustafsson, 1960; Gaul and Mittelstencheid, 1960). Plant height also, as mentioned earlier, show correlation with yield.

Irradiation increased earliness in wheat (Karapetjan, 1960); field bean (Shirshov and Sain, 1967), Soybean (Gotah, 1968) and produced the reverse effect in pea (Teretchenko, 1967) and Phaseolus (Bajaj and Saettler, 1970).

On fruit and seed setting, the effects were varied. No effect was reported by Nair (1964). A decreased setting was noted in Phaseolus (Bajaj and Saettler, 1970). There was increased variability in fruit set in rice (Sharma et al., 1967) and pea (Teretchenko, 1967).

Effect on yield:

Gregory (1955) observed, while working on peanuts, that there was an increase in genotypic variance of yield.

He obtained a depression in the mean. But in subsequent generations (M_4 and M_5) he isolated high yielding mutant lines. Similar results had been obtained in rice (Sakai and Suzuki, 1964), soybean (Rawlings et al., 1958), barley (Gaul, 1963), oats (Griffiths and Johnston, 1962) and wheat (Swaminathan, 1963). Characters such as oil and protein content do not appear to be depressed as easily as is yield, by X- ray treatment (Bilques et al., 1965; Williams and Hanway, 1961). Borojevic (1965) used thermal neutrons and X-rays to induce mutations in the fitness character, number of kernels per spike in wheat. She obtained a four-fold increase in variation and the means in M_2 and M_3 were equal to and higher respectively, than control. She suggested that the increase in M_3 means may have resulted from the purposeful elimination of all mutants, which produced abnormal spike morphology and fertility prior to the M_3 generation.

Improved yields following selection in irradiated populations had been reported for peanuts (Gregory, 1955, 56; Emery et al., 1965; Bilques et al., 1965); Lespedeza (Offutta, 1962), rice (Matsuo and Onozawa, 1961) and barley (Gaul 1961, '67).

Yield mutants are difficult to detect and positive yield mutants are rare. Gustafsson (1965) stated that positive mutations, in the homozygous state and under conditions optimal to the mother strain, surpassing in the production of vegetative matter as well as grain, are perhaps formed once in 500 to 1000 genotypical changes. Anderson and Olsson (1954) were able to obtain a high yielding mustard variety by X-ray irradiation. The superiority was confirmed by carrying out variability experiment in which both control and treated genotypes were represented equally and continued selection with equal intensity carried out over two or more generations.

Colour mutations in Segregating generations:

In segregating generations starting from M_2, variability studies are conducted to assess shifts in mean and frequency of phenotypes. These are done for quantitative characters like height, number of leaves, nodes, branches, heading date and yield. But an assessment of the efficiency and effectiveness of mutagen, in inducing mutations, is made based on the chlorophyll mutations in the M_2 population (Monti and Saccardo, 1968). But it is significant that the environmental conditions

and crop selected alter the chlorophyll deficient phenotypes. Mutations occurring per plant cannot be a good measure since the sector size can vary (Gaul, 1960).

Both frequency and spectrum of chlorophyll mutations are studied. Methods have been suggested to estimate frequency by Favret and Godak (1959), Yoshida (1962), and Sarvella et al. (1962). Most of the work has been on monocots like Triticale (Vettel, 1958), wheat (Goud, 1967c). A linear increase with dose is noticed as in wheat (Varghese and Swaminathan, 1968) and rice (Basu, 1969). Above a certain dose, the mutation frequency fell in rice (Kawai, 1964).

A wide spectrum of mutants ranging from albinos to viridis has been reported, but it varies in different species and varieties. Gustafsson (1947); Narayanan and Nair (1963) and Satpathy and Arnason, (1971) listed different types of chlorophyll mutants.

Colour changes are observed in flowers, stem nodes and peduncle. Colour changes in flowers have been observed in dicots like tobacco (Goodspeed, 1928), Trifolium (Bruns, 1954), Linum (Hoffman and Zoschke, 1955) and toria (Gupta, 1967).

Chimerism characterised by chlorophyll deficiency, alteration in anthocyanin pigmentation and pollen sterility have been noted in cowpea (Nair, 1964), rice (Balaram, 1968) and maize (Singh, 1971).

Other mutations

Mutations occurring in leaves, stem and new growth forms have been reported in mutant population or their progenies. Dwarf mutants had been observed in Solanum (Giles, 1956), rice (Bora and Rao, 1958), and Oats (Frey, 1965). Narrow leaf mutants were obtained in barley (Gustafsson, 1947) and distorted leaves in cowpea (Nair, 1964). Rosette tomato was reported by Butler (1954) in tomato.

Abnormal fruits were produced in tomato (Nilsson, 1950), Solanum (Giles 1956) and soyabean (Gotah, 1968). Variants in flower types had been reported in potato (Jasina and Krisanova, 1966) and strawberry (Pandey and Gupta, 1971).

Viable mutations:

Many of the visible mutations may be lethal but such mutants are fewer than viable ones (Vishnuswarup and Gill, 1967). Erectoides mutant in barley (Gustafsson, 1960) is an example of a viable mutant.

Others include awned mutants and amber grain in wheat (Varghese and Swaminathan, 1968).

Mutations in Quantitative characters:

Early reports on mutations were on qualitative characters and more often than not, produced visible differences on a large scale. Concomitant with these scattered early reports, description on damages and destructive effects of the irradiation treatment also appeared. Cytogenetical investigations in plants (Sax, 1943; Giles, 1956) disclosed numerically important relationship between the dose of X-ray and destruction to chromosomes. While changing nature through macro-mutations by using specific mutagens is appealing, there is little foundation for it in the laws of evolution as they apply to plant improvement. In contrast, the induction of many mutations, each with small effect on quantitative characters, permits the full operation of the lines of evolution and is therefore, the logical alternative to the induction of the generally degenerative macromutant (Gregory, 1957).

Spontaneous mutation for quantitative characters was described by Baur (1924); Stubbe (1934); East (1935)

and Schules and Sprague (1956). Experimental work on inducing mutations for quantitative characters was initiated by Scossiroli (1954).

Gregory (1955) working on peanuts, a self pollinating legume, induced an increase in genotypic variance in the fitness character, yield of pods, by X-ray treatment of seeds. Results obtained in non-fitness characters, leaflet dimensions in peanuts (Loesch, 1964; Emery et al., 1964), seed size in rice oats and soybeans (Yamaguchi, 1962; Krull and Frey, 1961) showed that variability was increased. A shift in mean of quantitative characters following induced mutation was presented by Brock and Latter (1961) and Brock (1965 a,b). They theorised that 'random mutation would be expected to increase the variance and to shift the mean away from the direction of previous selection'. From a comprehensive programme of yield testing with barley , Gaul (1963, '65) found some mutant lineswith means less than the control and some with means almost equal to the control. Oka et al. (1958) showed that when morphological mutants were discarded the frequency distributions for plant height and heading date in rice were symmetrical. Bateman (1959) concluded

that polygenic variations are unidirectional and debilitating. Similarly Sakai and Suzuki (1964) also reported that in most cases, polygenes investigated by them mutated unidirectionally in a minus direction. Brock (1965) criticised this extreme view. Gregory (1965b) stated that the numbers of plus and minus mutations in the polygenic system are nearly equal. The magnitude of the phenotypic effect of a mutation gives minus effects and not its unidirectional character. Gregory also observed that the frequency of observed changes increases with decreasing magnitude of change.

Many plant species produce toxic, noxious or bad-tasting substances, reducing edibility and/or palatability. For eg. Melitotus albus, a white sweet clover contains large quantities of glucoside, producing bitterness. Large scale screening experiments for non-bitter plants failed but Scheibe and Hulsmaan (1958) and Micke (1958) were successful in selecting less bitter mutants after treatment.

Physical mutagens have been used by several workers in a variety of crops for mutating a wide range of quantitative characters. X-rays have been used in

Gossypium barbadense (Anderson and Olsson, 1954 and Al Didi, 1965), on groundnut (Gregory 1955, 1956a 1957), Glycine soja (Rawlings et al., 1958 and Humphrey, 1954) and tobacco (Gaul, 1961). Gamma rays were used in Arabdiopsis (Daly, 1960), tobacco (Ando and Vencovsky, 1967), sorghum (Barabas, 1965), wheat (Goud 1968) and Okra (Yashivar, 1975) for inducing mutation in quantitative characters.

Another mutagen, used to induce mutations in quantitative characters, is neutrons, both thermal and fast neutrons. Thermal neutrons were employed on Arabdiopsis thaliana (Brock 1957, Krull and Frey, 1961), Avena sativa (Frey, 1965), wheat (Borojevic,1965) and rice (Matsua and Onozawa, 1961). Ehrenberg et al. (1965) used fast neutrons to induce variation in quantitative characters of Hordeum vulgare and in Triticum aestivum by Goud (1968). Bhatia and Swaminathan (1962) used betarays on Triticum aestivum.

Since micro-mutations are difficult to identify in single plants, score values are given for deviations from normal. Large populations will have to be raised and measurements have to be taken at periodical intervals.

To minimise the characteristically high influence of environment on quantitative characters, special designs are adopted.

The experiment is given requisite replications also. Owing to non-additive gene effects and interaction between families and years, estimates from single experiments can be expected to be too high (Aastveit and Gaul, 1967).

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation, to evaluate critically the variability created due to gamma rays in a locally well adapted strain of bhindi, was carried out in the Department of Agricultural Botany, College of Agriculture, Vellayani. The experiment was conducted during 1982-1984.

A. MATERIALS

Selection of variety

The variety selected for mutagenesis was Anakomban. It is a well adapted local variety and is moderately high yielding. It comes to maturity in about 90-110 days.

Botanical description

The plants are tall (90-140 cm), branched and green stemmed. The flowers are yellow. The fruits are long and green in colour. Slight anthocyanin pigmentation is natural on the base of the main stem and underside of petioles.

This variety is adaptible to the local condition and is very popular.

Selection of seed material

About 1000 healthy seeds of apparently uniform size were carefully selected out from well matured dry pods.

Seven lots of 120 seeds each were taken, to represent the six treatments and control.

METHODS

Treatment of seed material

The seven treatments represent the following:

1. Control
2. Seeds exposed to 10 kR ^{60}Co-gamma rays
3. Seeds exposed to 20 kR ^{60}Co-gamma rays
4. Seeds exposed to 30 kR ^{60}Co-gamma rays
5. Seeds exposed to 40 kR ^{60}Co-gamma rays
6. Seeds exposed to 50 kR ^{60}Co-gamma rays
7. Seeds exposed to 60 kR ^{60}Co-gamma rays.

Gamma irradiation

The dry seeds (10-12 per cent moisture content) were irradiated at six different dose levels. The irradiation was done using a ^{60}Co-gamma cell installed at KAU Headquarters, Vellanikkara, the dose rate employed, being 0.3145 MR per hour.

Planting technique

The experiment was laid out in R.B.D., with seven treatments and four replications. The 120 seeds of each of the seven treatments were sown on the fifth day of

treatment at the rate of 30 seeds per replication, with proper randomisation. The seeds were soaked overnight to facilitate uniform pre-soaking.

The land was prepared for the crop with repeated diggings and bunds made to demarcate the replications. The crop was sown in the planting season of June-July. Pre-sowing irrigation was given to keep soil sufficiently moist. The recommendations, given by the Package of Practices published by the Kerala Agricultural University (1983), were followed for maintaining the crop. Accordingly during land preparation, fertilizers and manures were given as basal dressing at the rate of 12 tonnes farm-yard manure per hectare and ammonium sulphate 125 kg per hectare, superphosphate 50 kg per hectare and muriate of potash 50 kg per hectare. The seeds were sown at the moderate spacing of 40 cm between rows and 45 cm between plants.

<u>After cultivation and top dressing</u>

A further dose of ammonium sulphate at 125 kg/ha was applied one month after sowing. Irrigations were given at intervals of five to six days for the earlier part of the crop. This was necessitated by the very hot and prolonged summer of 1983. Regular weeding and earthing was done between rows.

Plant protection

Malathion (Cythion) 0.05 per cent was sprayed twice before fruit setting to control shoot borers.

All the field experiments in this work were conducted in the experimental area, attached to the Department of Agricultural Botany, College of Agriculture, Vellayani.

Assessment of the direct effect of the mutagen in the M_1 generation

The direct effect of ^{60}Co-gamma rays was studied. Observations were made on all the plants. The following characters were studied.

1. Germination percentage
2. Percentage survival of plants
3. Height and number of leaves on the 30th day of planting
4. Height, number of branches and leaves on the 60th and 90th days of planting
5. Height, number of branches and total number of nodes on 120th day of planting
6. Number of flowers buds produced/plant
7. Number of flowers produced/plant
8. Number of stigmatic lobes/flower
9. Number of mature fruits/plant
10. Fruit characteristics: length of fruits and number of locules

11. Sterility: (a) Number of sterile buds and sterile flowers

(b) Pollen and seed sterility and

12. Chlorophyll variations

Germination percentage

Total germination percentage was estimated from the values taken on the day after which no further germination was observed in the field.

Survival of plants

This was determined on the 90th day of sowing. The values were expressed as percentage values over the number of seeds germinated.

Height and number of leaves on 30th day

Measurement of height was made from the soil level to the tip of the shoot and expressed in cm. The number of leaves excluding the first two leaves was counted.

Height, number of leaves and branches on 60th and 90th day of planting

Observations on height and number of leaves were made at 30 days interval. From the 60th day, branches produced also were counted at the same interval.

Height, number of branches and total number of nodes at maturity

Final observations on height and number of branches were taken per plant. Total number of nodes on main stem and branches was also counted.

Number of buds produced

Periodical observations were taken to get the total number of buds produced by each plant in each treatment/ replication.

Number of flowers produced

The number of buds opening into flowers also was counted. Daily observations were made to get the required values.

Number of stigmatic lobes

The number of stigmatic lobes vary. The number of stigmatic lobes in two randomly selected flowers of each plant was taken in all the treatments of each replication.

Number of mature fruits

Number of flowers setting into mature fruits was counted.

Fruit characteristics

Two fruits per plant from all plants in each treatment per replication were collected and their length and number of locules were taken.

Sterility as number of sterile buds and flowers

Daily observations were made to assess M_1 sterility in bud and flower stages. For this, counts on number of buds failing to produce flowers and number of flowers that do not form fruits were taken and calculated in percentages.

Pollen sterility

Pollen sterility was assessed from the first and the third flowers of ten plants selected randomly from each treatment per replication. Pollen grains were collected early in the morning from 8-9 A.M., when normal anthesis occurs. Pollen sterility analysis was done using acetocarmine-glycerine staining technique. The anthers were dusted on a drop of the media to release the pollen grains. A minimum of five microscopic fields were observed per slide and one slide was prepared for each flower. Pollen grains evenly stained, of uniform size and oval or spherical in shape were counted as fertile and shrivelled and lightly stained as sterile. Pollen sterility was expressed as percentage over total number of grains.

Seed sterility

Seed sterility was taken from the first two healthy fruits of each plant. Shrivelled and discoloured seeds

were counted as sterile and fully developed, rounded seeds as fertile. Seed sterility was estimated as the percentage of sterile seeds to the total number of seeds in a fruit. Based on the sterility percentage M_1 plants were grouped under the following classes.

1. Below 5 per cent sterility
2. 6 to 15 " " (control group)
3. 16 to 25 " "
4. 26 to 35 " " and
5. Above 36 " "

Chlorophyll variations

Careful examination of each plant was done periodically from the day of germination for any chlorophyll deficiency, due to direct effect of mutagen.

All observations were expressed as mean value per replication for each treatment.

Observations on M_2 plants

Selection of M_1 plants for representation in the M_2 was done based on the seed sterility and number of branches. The five M_1 sterility classes include:

1) below 5% sterility
2) 6 to 15% sterility (control group)
3) 16 to 25% sterility
4) 26 to 35% sterility and
5) above 36% sterility.

M_1 branch categories

One plant each representing M_1 branch categories, below 2 branches/plant, 3 to 5 branches/plant and above 6 branches per plant in the sterility groups, 6 to 15 per cent and above 36 per cent were included for each treatment per replication. Uniformly dried seeds obtained from each of the selected plant, twenty seeds collected at random from the first two fruits, were sown in rows, each row representing one M_1 plant. Two seeds were sown per pit to get a large population and there were ten pits for each M_1 plant selected. The total population of M_2 for each M_1 plant was twenty. The cultivation practice followed for M_2 was same as recommended for the crop by Kerala Agricultural University. Proper randomisations of the treatments and the four replications were done. As far as possible special care was devoted to maintain uniform field conditions over the entire crop. The following observations were taken in M_2.

Observations taken in M_2

1. Chlorophyll mutations
2. Micro-mutations

Detailed observations on quantitative traits like

i. Height of plant, number of nodes and branches at 90 days sowing

ii. Number of fruits per plant

iii. Fruit characteristics: Weight, length and number of locules per fruit.

iv. Days to first flowering

v. Days to complete harvesting and

3. Incidence of yellow vein mosaic disease.

Chlorophyll mutations

Careful examination of each plant was made especially in the early hours of the day for chlorophyll deficiencies.

Micro-mutations

In taking observations on quantitative characters, the border plants and those with conspicuous morphological variations were excluded.

Height of plant and number of nodes

Final height of plants was taken following the same procedure as in M_1. Total number of branches produced per plant was also counted at the same time.

Number of fruits per plant

Number of fruits produced per plant was counted at crop maturity to get total yield per plant.

Fruit characteristics

Two healthy fruits were collected per plant for measurement of weight, length and number of locules of each fruit per plant.

Days to first flowering

Days taken to first flowering were noted in every plant per treatment per replication and days taken to flowering from the date of sowing were counted.

Days to complete harvesting

The total duration of the crop was assessed by counting number of days taken for the last fruit to mature in each plant per treatment.

Incidence of yellow vein mosaic

Number of plants showing vein clearing symptoms in every line was counted to assess the incidence of the disease in the mutated population.

Table 1. M_2 control group range for various characters

Characters	Plant height cm	No. of branches	No.of nodes	Days to flowering.	Days to final harvest	Total No.of fruits	Fruit length cm	Fruit weight g
Control group Range	70-100	1-3	12-18	40-50	80-95	2-5	11-16	12-20

Classification of M_2 phenotypes

Based on height of plants they were grouped under three different classes.

1) Dwarf (below 70 cm height, plants in the negative group)
2) Medium (70 to 100 cm height, control group)
3) Tall (above 100 cm height, plants in the positive group)

Based on number of productive branches per plant they were grouped under three different classes.

1) Non branching (No branches/plant, negative group)
2) Medium branching (1 to 3 branches/plant, control group)
3) High branching (above 3 branches/plant, positive group)

Similarly in the case of all other characters, the M_2 plants were classified under three heads, ie.

1) Plants falling in the negative group
2) Plants falling in the control group and
3) Plants falling in the positive group

The frequency of each group per treatment was calculated in percentages and significance tested following proper statistical techniques.

Statistical analysis

Analysis of variance of the data was done following Fischer (1935). The experiment was in 7 x 4 R.B.D. with six exposures and one control laid out in four replications, for both the generations. In both generations, mean values were taken for each character per treatment for each repli-

cation. The data collected in percentages were transformed by the angular or $\sin^{-1}$ transformation proposed by Snedecor (1956), before conducting their analysis of variance.

The analysis of variance table for both generations followed this outline:

Source	Degrees of freedom
Total	27
Block	3
Treatment	6
Error	18

χ^2 analyses of frequency of variants from control range also were conducted following Fischer's method (1935) based on observation in control plants.

In the M_2, there were 20 plants each for each sterility group per exposure per replication. Statistical analysis for frequencies was done for a 6 x 5 x 3 split-split plot analysis with 4 replications, 6 treatments, 5 sterility groups and 3 phenotypic classes. The outline of the analysis of variance table is as follows:

Source	Degrees of freedom
Total	359
Replication	3
Treatment (T)	5
Error (I)	15
Sterility groups (S)	4
T x S Interaction	20
Error (2)	72
Phenotypic classes (P)	2
T x P	10
S x P	8
Error (3)	220

RESULTS

RESULTS

Direct effect of the Mutagen:

The direct effect of the mutagen is seen in the first generation of mutagenesis. It is best estimated in terms of lethality, injury and sterility. In the present investigation, to assess the direct effect of the mutagen, characters such as germination and survival, height of plants, number of leaves and number of branches per plant at different intervals, flowering and fruit setting percentages, number of fruits per plant, fruit characteristics such as length of fruits and number of locules per fruit and pollen and seed sterility were taken into consideration.

i) Germination

The data on germination, in percentages, over total number of seeds sown per treatment are presented in Table 2. The statistical analysis of the data showed no significant difference among the different exposures employed. The germination percentage ranged from 99.08 per cent in 10 kR exposure to 90.23 per cent in 60 kR exposure. At 30 kR exposure 96.83 per cent and at 40 kR 93.18 per cent germination was obtained. Untreated seeds showed cent per cent germination while treated seeds

Table 2. Effect of gamma rays on germination, survival and plant height in M_1 generation.

Treatment	Germination percentage	Survival percentage	Height (cm)			
			30 days after planting	60 days after planting	90 days after planting	final stage of crop
T_1	100	100	18.31	57.25	78.57	78.92
T_2	99.08	94.99	17.18	49.7	78.19	77.88
T_3	99.25	98.48	18.02	48.64	72.28	76.25
T_4	96.83	95.23	18.28	52.42	73.19	77.88
T_5	93.18	91.66	16.66	51.21	73.82	76.46
T_6	97.73	91.63	15.97	48.77	74.98	76.25
T_7	90.23	90.08	15.75	46.62	67.93	75.73
F value	1.80	1.38	1.50	0.88	1.52	0.78

showed a tendency for decrease in the percentage of germination with an increase in the dose.

2) Survival:

The survival of plants under different exposures is presented (Vide Table 2). Analysis showed no significant difference in the survival percentage among the different exposures. However, compared to control, the treated plants showed a decreased survival percentage. Control population showed cent per cent survival. The survival percentage ranged from 90.08 per cent for 60 kR to 98.48 per cent for 20 kR exposure. Thus direct effect of mutagen in M_1 in terms of survival was obtained as a slight decrease from control population depending on exposure.

3.1) Height of plants (30 days after planting)

The mean value of plant heights taken in cm, 30 days after planting is presented in Table 2. Statistical analysis of the data showed no significant difference among the various exposures. The mean values ranged from 18.28 cm in 30 kR to 15.75 cm in 60 kR exposure. The control group showed the highest mean value of 18.31 cm

and the highest dose of 60 kR produced plants of lowest mean height, 15.75 cm. Thus a tendency for decreasing plant height was observed as the exposure increased to the higher dose.

3.2) Height of plants (60 days after planting)

The data on plant height taken at 60 days after planting are shown as mean values per treatment (Vide Table 2.). The values did not differ significantly from each other. The mean height of plants ranged from 57.25 cm for control to 46.62 cm for the highest exposure of 60 kR. Here also higher doses produced a reduction in mean plant height in comparison with lower exposures and control.

3.3) Height of plants (90 days after planting)

Mean heights of plants for the seven treatments including control are given in Table 2. Statistical analysis showed that the treatments did not differ significantly in their mean plant height. Nevertheless the control group showed the highest value in comparison with the other treatments with a mean of 78.57 cm. The lowest mean plant height was observed for the highest exposure of 60 kR. This treatment produced plants with mean plant height of 67.93 cm. Thus the plant height

mean values ranged from 78.19 cm to 67.93 cm for the treated plants, higher doses of the mutagen showing a tendency to reduce the height of plants.

3.4) Height of plants at harvest

The plant height measured at the final stage of the crop is expressed as mean values in Table 2. The data showed that the values did not differ significantly for the different exposures. The mean values ranged from 78.92 cm for control to 75.73 cm for the highest exposure of 60 kR. The treatments showed a very narrow range of 77.88 cm to 75.73 cm for 30 kR and 60 kR respectively. This indicated that the mutagen did not affect final height of the plants in any considerable way in the M_1 generation.

4.1) Number of leaves (30 days after planting)

Statistical analysis of data (Table 3) showed that in M_1, the plants belonging to the different treatments did not differ significantly in the number of leaves produced. But exposure to the mutagen seemed to reduce the number of leaves produced, as a regular decrease was observed as the dose increased. The mean value was

Table 3. Effect of gamma rays on growth rate in M_1 generation

Treatment	Mean number of leaves			Total number of leaves produced	Mean number of branches	
	30 days after planting	60 days after planting	90 days after planting		60 days after planting	90 days after planting
T_1	6.48	27.09	26.35	55.87	4.35	4.53
T_2	5.84	27.06	25.41	49.86	3.76	4.32
T_3	4.98	26.59	22.32	46.33	3.74	4.11
T_4	4.99	24.48	21.18	46.18	3.48	3.56
T_5	4.93	24.40	22.19	46.38	2.84	3.22
T_6	4.83	23.88	20.32	45.71	2.22	3.16
T_7	4.71	23.09	18.68	41.45	2.09	3.07
F value	1.27	1.16	2.54	2.69*	4.08*	3.58*
CD	-	-	-	11	0.87	0.848

* Significant at 5% level

consequently the highest for the control group plants which produced 6.48 leaves per plant on an average. The other treatment groups produced 5.84, 4.98, 4.99, 4.93 and 4.83 leaves under 10, 20, 30, 40 and 50 kR respectively, the lowest mean (4.71) being obtained for the highest exposure of 60 kR.

4.2) Number of leaves (60 days after planting)

Data on the mean number of leaves produced by plants in each treatment are given in Table 3. Statistical analysis showed that the number of leaves produced did not differ significantly with different treatments. The control plants produced the highest number of leaves (27.09) 60 days after planting. For the treatments, the means ranged from 27.06 for 10 kR to 23.09 for 60 kR. As noted in other cases, the lowest mean was obtained for the highest exposure with a regular decreasing trend as the dose increased.

4.3) Number of leaves (90 days after planting)

Data on mean number of leaves per plant (90 days after planting) are presented (Vide Table 3.) The values did not differ significantly. But there was a dose dependent fall in number of leaves produced per treatment.

The untreated plants showed the highest mean (26.35) and the lowest (18.68) was obtained from the highest dose (60 kR). The other exposures showed a decline with means of 25.41, 22.32, 21.18, 22.19 and 20.32 for 10 kR, 20, 30, 40 and 50 kR respectively.

4.4) Total number of leaves produced

Observations made on the total number of leaves produced per plant given in Table 3 showed that as the dose increased, the number of nodes produced, decreased and the mean values differed significantly from each other. The number of leaves ranged from 55.87 for the control to 41.45 for the highest exposure of 60 kR. The mean values obtained were 49.86, 46.33, 46.18, 46.38 and 45.71 at doses 10, 20, 30, 40 and 50 kR respectively. The highest dose of 60 kR thus produced considerable decrease in the total number of leaves produced and all the exposures showed much less number of leaves in comparison with control plants.

5.1) Number of branches (60 days after planting)

The data on mean number of branches produced in each treatment are presented in Table 3. The statistical analysis showed that they did differ significantly

for the various exposures. The mean values ranged from 4.35 for control to 3.76 in 10 kR, 3.74 in 20 kR, 3.48 in 30 kR, 2.84 in 40 kR, 2.22 in 50 kR and 2.09 in 60 kR. Thus here also the results showed a decreasing trend in the number of branches with increasing exposures.

5.2) Number of branches (90 days after planting)

The number of branches produced per plant (Table 3) differed significantly for the various levels of exposures. The control population showed the highest mean (4.53), the rest of the treatments showing a progressive decline from 4.32 in 10 kR, 4.11 in 20 kR, 3.56 in 30 kR, 3.22 in 40 kR and 3.16 in 50 kR. The lowest mean (3.07) was obtained with the highest exposure; the lower doses of 10 and 20 kR produced significantly higher number of branches compared to the higher doses of 50 and 60 kR.

6. Total number of buds

The mean number of flower buds produced per plant in each treatment is given in Table 4. When statistically analysed, the values did not differ significantly for the different treatments. But the number of buds decreased from control to the highest dose. The range extended

Table 4. Effect of gamma rays on floral and fruit characters in M_1 generation.

Treatments	Mean number of buds	Mean number of flowers	Mean number of stigmatic lobes	Mean number of fruits	Mean number of locules	Mean number of fruits (cm)
T_1	18.82	6.25	7.40	6.03	7.53	17.39
T_2	17.86	6.20	7.43	5.57	7.54	16.87
T_3	17.69	6.07	7.86	5.53	7.56	16.56
T_4	16.74	5.92	7.47	5.47	7.46	16.41
T_5	16.73	5.87	6.91	5.43	7.40	16.15
T_6	16.71	5.84	7.19	5.45	7.46	16.10
T_7	16.61	5.39	7.48	5.20	7.55	15.78
F value	0.90	0.86	0.90	0.74	0.26	2.03

from 18.82 for control to 16.61 for the highest exposure (60 kR). The progressive decline in bud production for the different exposures could be seen from their mean values - 17.86 in 10 kR, 17.69 in 20 kR, 16.74 in 30 kR, 16.73 in 40 kR, 16.71 in 50 kR and 16.61 in 60 kR.

7. Number of flowers per plant

The mean number of flowers produced per plant for each treatment did not differ significantly (Table 4). The highest mean number of flowers was obtained for the control group (6.25). The other treatments showed a decrease in the number of flowers, commensurate with the dose used. The values ranged from 6.2 in 10 kR to 5.39 in 60 kR; as usual the lowest value being obtained for the highest dose. The other dose levels of 20,30, 40 and 50 kR had mean values of 6.07, 5.92, 5.87 and 5.44 respectively.

8. Number of stigmatic lobes

The mean numbers of stigmatic lobes were analysed statistically for the different treatments. The data are given in Table 4. The values ranged from 7.86 in 20 kR to 6.91 in 40 kR. Control plants showed a mean value of 7.4. The seven treatments including control did not differ significantly in their values. The highest dose

of 60 kR showed a mean number of 7.48 stigmatic lobes per flower while 10, 30 and 50 kR exposures gave 7.43, 7.47 and 7.19 respectively.

9. Number of fruits (Yield)

The yield per plant is represented as number of fruits produced. The mean data on yield with respect to each treatment are given in Table 4. The yield per plant did not differ significantly. The highest mean yield was obtained for the control plants (6.03) while the lowest mean was obtained for 60 kR exposure. Though there was no significant variance, the means showed a declining trend with increasing exposures. The 10, 20, 30, 40 and 50 kR exposures gave mean yields of 5.57, 5.53, 5.47, 5.43 and 5.45 fruits per plant respectively. Thus except in the case of 50 kR the mean yield for each treatment was lower than the adjacent higher dose employed. The range of variation was however, not great, extending from 6.03 in control to 5.2 fruits per plant in the highest dose of 60 kR.

10. Number of locules per fruit

Mean values of locule number per fruit in each treatment (Table 4) did not differ significantly for the different treatments. The highest mean value was obtained

in 20 kR (7.56) and the lowest in 40 kR (7.40). The control plants showed a mean value of 7.53 locules. Both 30 kR and 50 kR gave identical values of 7.46 while 10 kR and the highest dose of 60 kR gave 7.54 and 7.55 respectively.

11. Mean length of fruits

The data on mean length of fruits in the different treatments in M_1 are represented in Table 4. Analysis of variance showed that the treatments did not differ greatly with respect to this character. But a regular trend was observed, the highest mean length being obtained for the control group (17.39). The range for the exposures extended from 16.87 in 10 kR to 15.78 in 60 kR. A progressive decline in mean fruit length as a result of direct effect of increasing levels of exposure could be seen in the values of 16.87 for 10 kR, 16.56 for 20 kR, 16.41 for 30 kR, 16.15 for 40 kR and 16.1 for 50 kR.

12. Number of sterile buds per plant

The mean numbers of sterile buds to total number of buds produced per plant, expressed as a percentage, are given in Table 5. This character also was not significantly different for the different treatments. The

Table 5. Effect of gamma rays on sterility in M_1 generation.

Treatment	Mean percentage of sterile buds	Mean percentage of sterile flowers	Mean pollen sterility %	Mean seed sterility percentage
T_1	57.14	8.53	4.85	9.96
T_2	58.96	9.42	12.93	16.63
T_3	59.24	9.26	12.77	18.55
T_4	61.25	11.35	15.72	20.72
T_5	61.15	13.70	18.51	24.13
T_6	63.25	13.13	19.81	27.22
T_7	64.38	14.09	22.51	32.43
F value	1.65	0.92	13.22*	15.79*
CD	-	-	4.88	4.64

* Significant at 5% level

Values ranged from 57.14 per cent for control plants to 64.38 per cent in 60 kR. There was a dose dependent increase in the sterility percentage. The 10,20,30, 40 and 50 kR exposures gave 58.96 per cent, 59.24 per cent, 61.25 per cent, 61.15 per cent and 63.25 per cent sterile buds per plant respectively.

13. Percentage of sterile flowers

Number of flowers produced that failed to set fruits (vide Table 5) was not significantly different for the various treatments. They ranged from 8.53 per cent for control to 14.09 for the highest exposure of 60 kR. The doses tended to produce more sterile flowers as the exposure increased, affecting the reproductive capacity of the treated plants. The 50 and 40 kR exposures produced 13.13 per cent and 13.7 per cent flower sterility respectively.

14. Pollen sterility

Pollen sterility is also expressed as a percentage to total number of pollen grains in selected samples. The sterility values (Table 5) for pollen grains, when statistically analysed showed that they differed signi-

ficantly in the different treatments. The control group showed a sterility percentage of 4.85 per cent only. Even the lowest dose of 10 kR produced a significantly higher sterility percentage of 12.93. There was a general increase in sterility per cent with dose. The 20 kR treatment gave a sterility value of 12.77 per cent, slightly less than 10 kR treatment. The rest of the exposures showed a steady increase with 15.72 per cent in 30 kR, 18.51 in 40 kR and 19.81 in 50 kR. The highest percentage of sterile pollen grains was obtained in 60 kR exposure (22.51).

15. Seed sterility

Direct effect of mutagen was also estimated in terms of percentage of sterile seeds produced in each treatment (Table 5). The mean percentage values were statistically analysed and were found to differ significantly for the different treatments especially with respect to control. The seed sterility percentage for control group was only 9.96. The highest exposure (60 kR) gave a significantly higher sterility percentage (32.43%) in comparison to the other exposures. All the exposures were significantly different from control. The sterility percentages increased with an increase in

dose. Thus 10,20, 30, 40 and 50 kR produced respectively sterility percentages of 16.63, 18.55, 20.72, 24.13 and 27.22.

16. Other M_1 variants

Fig. 10 shows two leaves borne on the same petiole. It was a variant of rare occurrence observed in the M_1 population. Similarly there were branches emerging at the same node, fused upto a certain point, before separating into branches at the tip (Fig.11). A few plants in the higher exposures (50 and 60 kR) showed the stem splitting at the apex to continue as two branches thereafter (Fig.12).

Flowers and fruits formed from them also showed this type of fusion, though the instances were rare. Fig. 13 shows two buds borne on the same stalk and Fig.14 the fused fruits formed from such buds.

Certain plants in 60 kR showed a change in plant habit as a result of the direct effect of the mutagen. Such plants showed a weak stem with a tendency to a tendril habit (Fig.20 and Fig.21).

Treated plants also produced two fruits (Fig.15, 16 and 17)or three fruits (Fig.18 and 19) at the same node.

Table 6. Character expression induced by gamma rays in M_2.

Treatment	Final height in cm	Mean number of branches per plant	Mean number of leaves per plant
T_1	78.48	1.35	16.88
T_2	77.88	1.27	16.33
T_3	77.78	1.26	15.86
T_4	73.20	1.23	15.67
T_5	77.78	1.27	15.30
T_6	72.56	1.13	15.42
T_7	70.55	1.04	15.03
F value	1.53	0.94	1.07

Table 7. Mean character expression induced by gamma rays in M_2.

Treatment	Days to flowering	Days to complete harvest	Total number of fruits/plant	Mean length of fruits (cm)	Mean weight of fruits (g)
T_1	45.84	83.90	2.96	13.74	13.49
T_2	45.37	80.28	2.95	13.64	13.48
T_3	45.47	79.35	2.87	13.38	12.95
T_4	45.80	79.48	2.77	13.38	12.95
T_5	44.04	79.27	2.70	13.34	12.89
T_6	44.16	79.23	2.84	13.29	12.86
T_7	44.00	79.19	2.73	13.27	12.88
F value	1.33	1.09	0.79	2.03	1.31

Table 8. χ^2_{10} analysis data on the frequency of mutants in M_2 generation

Characters	Plant height	No. of branches	No. of leaves	Days to flowering	Days to final harvest	Yield of fruits	Length of fruits	Fruit weight
Computed χ^2_{10} values	71.33*	41.53*	23.88*	64.01*	68.98*	26.42*	41.78*	20.35*

* Significant at 5% level

Effects of gamma ray in M_2 generation

The induced mutational analysis on polygenic characters made in the present investigation include:

i. Plant height,
ii. Mean number of leaves per plant,
iii. Number of branches per plant,
iv. Days taken to flowering,
v. Days to complete maturity,
vi. Number of fruits per plant,
vii. Length of fruits and
viii. Weight of fruits.

i. Plant height

Mean plant heights as influenced by different exposures of gamma rays in M_2 are given in Table 6. It was found that the seven treatments tested did not differ significantly in their heights. But the higher exposures produced a dwarfing effect, as these plants showed lower mean plant heights. The mean values ranged from 78.48 cm for control to 70.55 in 60 kR. Except 40 kR (77.78 cm), the other exposures gave lower means, depending on dose.

The χ^2 value (71.33) for the frequency distribution of M_2 plants in the three phenotypic classes for

plant height are given in Table 8. The χ^2 value was significant showing that the mutagen had created plant height variants from the control range.

The mean frequency (percentage) distribution of plant height variants under the five sterility groups is given in Table 9-1. Split-split plot analysis done, showed that neither exposures nor sterility groups differed significantly, but the phenotypic classes produced significant difference in the distribution. Among the phenotypic classes, the largest mean frequency was obtained in the control group (48.36) and the least for the positive variants (10.25). Transformed values of frequency distribution in interactions and ANOVA results are given in Table 9-2, 9-3 and 9-4. None of the interactions were significant. The highest frequency was obtained in the highest sterility group under 60 kR exposure (Table 9-2) and the lowest in 20 kR. In the S x P interaction, the highest frequency was obtained in the lowest sterility group in the control range.

2. Number of leaves per plant

Mean numbers of leaves produced per plant are given in Table 6. The means did not differ significantly.

But there was a dose dependent decline in the mean value. The control group showed the highest mean (16.88). The mean was lowest (15.03) in 60 kR exposure. The 10 kR exposure plants had a mean of 16.33 while all the rest of the treatments had means less than 16.00 cm.

χ^2 value for frequency distribution in the three phenotypic classes (Table 8) was significant for number of leaves produced per plot. Table 10-1 gives the percentage distribution of plants in the three phenotypic classes under the five M_1 sterility categories. Mean frequency under each phenotypic class differed significantly. The interaction means are given in Table 10-2, 10-3 and 10-4. The treatments also differed significantly. The exposure vs sterility and exposure vs phenotypic interactions were not significant. The highest frequency in T x S interaction was obtained in the 50 kR exposure in the highest sterility group of >36 per cent sterility (Table 10-2). The S x P interaction was found to be significant. For all sterility groups, the frequency in the control group was more than that of the other variants. The highest frequency was obtained in the second sterility class. Negative variants were fewer than positive variants for all the sterility groups.

3. Number of branches

Mean values are given in Table 6. They did not differ significantly. The control group showed the highest number of branches per plant (1.35). The mean values in the other higher exposures ranged from 1.27 in 10 kR and 40 kR to 1.04 in 60 kR. The mutagen had a reducing effect on the number of branches per plant as seen from the mean values of 20 kR (1.26), 30 kR (1.23) and 50 kR (1.13).

χ^2 analysis showed that the mutagen had produced significant variation in the frequency of positive and negative variants from the control range (Table 8). Exposures and sterility mean frequencies did not show significance (Table 11-2, 11-3) but phenotypic classes showed significant difference in the means. Interactions were not significant. In the exposure Vs M_1 sterility class interaction, the highest frequency was obtained in the 20 kR treatment in the highest sterility class (Table 11-2). Among the phenotypic classes, the control group had the highest frequency for all the treatments and sterility groups. The frequency of negative variants was more than the positive variants in all exposures and sterility classes.

4. Days to flowering

The mean number of days taken from planting to first flowering is given in Table 7. Statistical analysis of the means showed that they did not differ significantly for the seven treatments including control. The time taken to maturity however decreased as dose increased. Control plants took the maximum days (45.84 days) to flower compared to the treated plants. The minimum days were taken by the 60 kR exposure with a mean of 44 days. The other exposures had means ranging from 45.8 in 30 kR to 44.04 in 40 kR.

χ^2 distribution of the frequency was significant for this polygenic character (Table 8). For all exposures, the positive variants were in higher frequencies than negative variants. Mean percentage frequency distribution under each M_1 sterility group is given in Table 12-1. Only phenotypic classes showed significance in their means (Table 12-3). Interaction between exposure Vs M_1 sterility showed no significant difference. The mean frequencies for the interaction are given in Table 12-2. The highest frequency was noted in the 30 kR exposure in the medium sterility group (16-25%). Number of positive

variants was more than negative variants in all the exposures. The second sterility group alone produced more negative variants than positive variants (Table 12-4).

5. Days to complete harvest

The duration of the crop under different treatments is given in Table 7. The mean values did not differ significantly for the treatment. They ranged from 83.90 days for control to 79.19 days in 60 kR. There was a regular decline in duration with higher exposures. Thus 10, 20, 30, 40 and 50 kR exposures showed durations of 80.28, 79.35, 79.48, 79.27 and 79.23 respectively.

The frequencies of the variants under the three phenotypic classes weresignificant (Table 8). Mean frequency (percentage) distribution of variants under the five M_1 sterility groups is presented in Table 13-1. Phenotypic classes alone gave means that were significantly different. The lowest frequency was noted in the case of positive variants.

The interactions were not significant. Interaction means of T x S, T x P and S x P are presented in Table 13-2, 13-3 and 13-4. Highest frequency in T x P interaction was obtained in the 30 kR exposure in the highest steri-

lity group. For all the exposures and sterility groups, the number of positive variants were much less compared to negative variants.

6. Yield in number of fruits

Mean yield per plant corresponding to each treatment is presented in Table 7. The yield was measured in terms of the number of fruits produced per plant. The treatments did not differ significantly. The yield ranged from 2.7 in 40 kR to 2.96 in control. The highest dose of 60 kR gave mean yield of 2.73 fruits per plant.

The χ^2_{10} value for frequency distribution of yield variants was significant (26.42). The frequency of negative variants was higher than positive variants for all the exposures (Table 14-3). Here also, the phenotypic classes showed significantly different frequencies.

The interaction means are presented in Tables 14-2, 14-3 and 14-4. They showed no significant difference. The highest frequency was obtained for the T x S combination with 20 kR exposure in the 26-35 per cent sterility group. In all the sterility groups, the negative variants occurred at higher frequency than the positive variants.

Frequency was highest in the control range. The lowest frequency was obtained in the third phenotypic class in the third sterility group (16-25%) as shown in Table 14-4.

7. Fruit length

Mean length of fruits was not significantly different for the different treatments (Table 7). The length ranged from 13.74 cm for control to the lowest value of 13.27 in 60 kR. The length decreased with dose. The exposures 20 and 30 kR produced fruits with same mean length (13.38 cm). The 10 kR exposure gave mean length of fruits (13.64 cm) comparable to control. Frequency distribution represented by χ^2 showed significance with respect to the three phenotypic classes (Table 8). Mean frequency (percentage) distribution of variants is given in Table 15-1 and transformed values of interaction means in Table 15-2, 15-3 and 15-4. Phenotypic classes alone had significant means. Negative variants showed higher frequency in all the exposures except the 60 kR exposure (Table 15-3). In the T x S interaction table (Table 15-2) the highest frequency was obtained in the 60 kR exposure in the lowest sterility group. In the sterility groups, the phenotypic classes showed highest frequency in the control range.

The interaction mean frequencies did not differ significantly.

8. Weight of fruits

The mean fruit weight in the M_2 ranged from 13.49 g for control plants to 12.86 in 50 kR (Table 7). The mean values did not differ significantly. The mutagen tended to reduce the fruit weight as all treated plants gave less mean weight than control. The lowest exposure, 10 kR, had a mean fruit weight of 13.48 g which is only slightly less than control. Both 20 and 30 kR showed the same mean weight of fruits (12.95 g). χ^2 value was significant for this character. Mean frequency (percentage) of variants from control, in both directions is given in Table 16-1 and transformed values of interaction means in 16-2, 16-3 and 16-4. Split-split plot analysis showed that neither sterility groups nor exposures differed significantly. Phenotypic classes showed significantly differing means (Table 16-3). The positive variants were very few compared to control and negative variants. Interactions were also non-significant.

DISCUSSION

DISCUSSION

The present investigation on mutation breeding in Abelmoschus esculentus L. Moench. was undertaken with a view to finding out the effects of the mutagen on the crop as observed in the M_1 and M_2 generations. For the programme, a well adapted local variety, Anakomban was used as the basic material for irradiation. Although it is not possible to give a conclusive picture regarding all the changes brought by the mutagen, a brief discussion on the direct effect of the mutagen in M_1 and the variation created in the polygenic traits in the M_2 is useful in judging the extent and direction of change produced in bhindi, by mutation. A general idea regarding the effect of the selected mutagen at six different levels can be obtained.

I. Effect of irradiation in M_1 generation

(1) Seed germination:

The seed germinates following complex physical and chemical changes, the course of which can be influenced by the application of the mutagen. This can lead to variations in germinability of the seeds. In the present investigation, it was found that the percentage germina-

bility did not differ significantly for the different treatments. Yu (1961) observed that germinability of tomato seeds remained unaffected, irrespective of the doses employed. This is in conformity with the present finding. Similar results were obtained by Olatunde et al. (1971) in pea. Yet, there was a consistent decrease of germinability with dose in other cases. This inverse relationship has been observed by several workers including Fujii and Matsumara (1958). Rangaswamy (1969) and Wu and Pi (1968) reported a reduction in germination with increase in dose in cereal crops like paddy, sorghum and pearl millet. Sree Ramulu (1970) also observed that mutagen treatment delayed and hindered germination in sorghum. Bhaskaran (1959) found that three species of wheat showed reduced germination percentage with increased X-ray doses. Goud et al. (1967) also reported a similar decreasing trend. Gustafsson and Gadd (1965) found that germination in Poa sp decreased due to mutagens. Other workers reported the same finding in non_cereal crops too. Gregory (1955, 1968), Bilques and Martin (1961) and Giles and De Winck (1969) reported it in pea nuts. Other workers include Roy et al. (1971) in Cucumis sativus, Lesley and Lesley (1956) in tomato, Bohera and Patnaik (1979) in Amaranthus and Majid (1975) in tomato.

This mutagen effected reduction in germination has been attributed to several causes. It could be due to varied response of irradiation on the chromosome complements or initiation of cell divisions or due to greater time lapse between irradiation and planting. Skoog (1935) and Smith and Kersten (1942) attributed it to the destruction of auxins. Gordon and Webber (1955) and Gordon (1957) suggested that it could be due to inhibition of auxin synthesis rather than destruction of synthesised auxins. Gross chromosomal breakage was supposed to be the cause of the reduced germination in Trifolium subterraneum, subjected to X rays and thermal neutrons by Brock (1965b). Other mutation breeders like Sinha and Godward (1972) working in Lens culinaris attributed it to physiochemical disturbances or chromosomal aberrations. Another suggestion was mooted by Chauhan and Singh (1975) that physiological effects can reduce germinability. Venkiteswarlu et al. (1978) also attributed the reduced germination in pigeon pea to physiological effect of X rays. Mitotic impairment could influence or disrupt meristematic activity in seeds (Cherry and Hageman, 1961).

Survival of plants

Post-germination mortality is determined by the percentage of plants surviving in the treated population. This is a good estimate of the direct effect of the mutagen along with sterility. This toxic effect results from profound nuclear and chromosomal alterations that are irreversible. In the present investigation, the survival percentage in all the exposures was lower than in control. The control showed cent per cent survival. Though the treated plants showed lower survival percentage a slight increase was observed for 20 and 30 kR exposures. This is in conformity with the observations of Jacob (1949) in jute. An inversely proportional relationship between survival and dose was observed by Yu (1961) in tomato. McCrory and Grun (1969) and Olatunde et al. (1971) reported the same trend in vegetables like potato and peas.

Cytological explanations are given for the reduction in the survival percentage with increased doses of radiation. Konzak et al. (1965) attributed the decrease in survival percentage to the reduced cell growth resulting from cytological abnormalities

and also due to the decrease in the synthesis of auxins and other physiological changes. Mitotic abnormalities due to irradiation results in the structural changes in the chromosomal complements. This interferes with the normal growth and development of organs which might have led to the fall in survival percentage with increased doses. Others who have reported a mutagen dependent variation in survival are D' Amato et al. (1962) in wheat, Tomohira et al. (1964) in Capsicum and Datura and Sahib and Abraham (1970) in Capsicum.

The slight increase in the survival percentage in 20 and 30 kR treatments indicates a tendency of a recovery process or an increased resistance of plants to the drastic conditions of treatments, at an early stage. At lower doses, lethality was reduced. This is in conformity with the findings of Beard (1957) in rice and Micke (1958) in Melitotus.

Plant growth

The growth rate in plants is governed by the rate of internal metabolic processes of the system

which, to a certain extent is influenced by the external factors. Treatments, with radiations, are known to affect growth. In the present investigation, growth rate was estimated, in terms of height and number of branches and leaves at periodical intervals. All these characters showed a general reduction with increase in dose (Fig. 1,2,3).

Height is brought about by shoot growth which is mostly due to cell elongation. Final height did not differ significantly with treatments but showed proportional reduction with increased doses of mutagen except 20 kR. Reports on similar results have been obtained by Kuzin (1956) in pea and Nair (1964) in cowpea. The inhibition of growth by irradiation can be interpreted from physiological, biochemical and anatomical view points. The other characters like number of branches and leaves also decreased with the increase in dose, in the present investigation. This could be due to upsetting of oxidation-reduction process in the cells, inactivation of vital enzymes especially those concerned with respiration or inhibition in the rate of assimilation and consequent changes

in the nutrient levels of plants (Ehrenberg, 1955). In the case of number of leaves and branches, there was a significant decrease for the treated plants, especially in higher doses.

Periodical observations of height, number of branches and leaves helped to determine the rate of growth. They showed that rate of growth was reduced by the mutagens. Caldecott and Smith (1952) observed a reduction in growth of barley plants following seed irradiation with X-rays. Similar results were obtained by Konzak et al. (1961a) in wheat and Woodstock and Justice (1967) in maize, wheat, sorghum and radish. Growth rate reduction could be due to auxin destruction (Smith and Kersten, 1942). Pele and Howard (1955) suggested that the possible interference of irradiation with the synthesis of new DNA could be the cause, while Evans and Sparrow (1961) opined that the influence of ionising radiations on growth can be attributed basically to the genic loss due to chromosomal aberrations. Evans et al. (1957) and Evans and Scott (1964) reported mitotic delay as the major cause of growth retardation in irradiated populations, resulting in reduced growth

rate. Respiratory cycle is the main metabolic source of energy for growth. Under these circumstances, the variation in the growth of plants due to the effect of irradiation, is not difficult to explain. Anantha-swamy et al. (1971) observed inhibition of seedling growth in gamma irradiated wheat seeds and suggested that the adverse effect of seedlings might be due to specific effects on certain respiratory system operating during crop growth. Sinha and Godward (1972) pointed out that growth inhibition at higher doses may be due to chromosomal aberration or both. Nilavao (1936) has reported that X-ray irradiation of sprouting barley seeds caused a reduction in the catalase and amylase activity which influences the growth in a reductional way. Quastler et al. (1952) found that growth inhibition in mung beans following irradiation was not due solely and directly to radiation effects on mitosis, but also due to induced physiological changes.

The role of DNA in reducing growth in the mutagen treated materials, has been discussed by several authors like Sinha and Godward (1972). Pele and Howard (1955) based on their studies on X-rayed seeds,

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

Effect on reproductive activity

Both number of buds and flowers produced were reduced with increase in dose. Thus the mutagen reduced the reproductive capacity of the plants, especially at higher doses. The stigmatic lobes increased in number in lower doses and in the highest dose. This could have been due to enhancing effect of the mutagen at these doses while medium doses tended to inhibit lobe formation.

Abscission of flower buds and low bud production were reported by several workers. In the present investigation also, production of buds that failed to bloom and poor fruit setting have been observed at an increasing rate for higher doses. Gunckel and Sparrow (1961) found that abscission of flower buds occurred in tomato, tobacco, snapdragon and other plants at higher dosages in the gamma field. Bianchi et al. (1963) in tomato and Iqbal (1972) in Capsicum annuum observed delayed and retarded flowering. Radhammal (1972) reported profuse flowering but negligible fruit set due to meiotic abnormalities.

Yield

Number of fruits was progressively reduced with increase in exposures, although the means did not differ significantly for the exposures. This is in conformity with the result reported by Tedin (1954) in legumes. Caldecott et al. (1954) reported reduction in yield in M_1. Gottschalk (1965) and Jana (1962) also found that yield was decreased in M_1 plants. Many workers reported similar results in both cereal and non-cereal crops. Reduction in yield following higher doses of irradiation

has been reported in Arachis hypogaea (Bora et al., 1961), Phaseolus mungo (Jana, 1962), Linum usitassimum (Tomohira et al., 1964), Lycopersicon esculentum (Yamakawa and Sparrow, 1966), Lens culinaris (Sinha and Godward, 1972), rice (Nayar, 1976), wheat (Soossiroli, 1966a, 1966b). Caldecott et al. (1954) reported that this reduction in yield could be due to radiation induced structural changes in chromosomes, involving translocations. Higher doses may decrease auxin production resulting in an inhibition of flower bud production itself. Sree Ramulu (1970) based on his studies on sorghum, using X-rays, reported that the cause of reduction in yield can be attributed to reduced pollen fertility, resulting in inviability of the microspores due to meiotic disturbances. Sato and Gaul (1967) suggested imbalanced genetic constitution of micro or megaspores to be the possible reason for reduction in yield.

Sterility

In all forms of sterility analysed in the present investigation there was a dose dependent increase with exposures. This includes the most important indication

of successful mutagenesis. Both seed (Fig. 23) and pollen sterility showed significant difference and higher exposures greatly reduced fertility.

Pollen sterility reflects the functioning of reproductive systems. A dose dependent increase in sterility percentage was observed by Beachell (1957); Chang and Hsieh (1957) and Singh (1970). Others who reported the same results include Venkiteswarlu et al. (1978); Rai and Das (1978); Sahai and Dalal (1973); Sato and Gaul (1967); Morris (1952) and Singh and Roy (1971).

The induced sterility may be explained on cytological terms. Gaul (1970) pointed out that it could be caused by chromosome mutations, factor mutations, cytoplasmic mutations or due to physiological effect. Of these, chromosome mutations may be the main cause. As the dose level increased, the deleterious effects of irradiation were more marked in the chromosome complement. Katayama (1963) found a direct correlation between M_1 sterility and frequency of translocation in rice. In the case of gamma rays, Singh (1970) observed that they induced a high frequency of trans-

locations in rice. A high frequency of chromosome stickiness and pollen sterility induced by gamma rays was reported by Rao and Rao (1977). Katiyar and Roy (1974) analysed gamma ray induced chromosome aberration and pollen sterility in Capsicum annuum. The pollen sterility could be the result of cumulative effects of aberrant meiotic stages and physiological and genetic damage caused by chromosome breakage following formation of antimetabolic agent in the cell (Rao and Lakshmi, 1980). Nishimura and Kurakami (1952) have stated that the pollen sterility in barley after X-ray irradiation of seeds is partially due to the multi-valent association of chromosomes. Bora et al. (1961) are of the opinion that the high pollen sterility in Arachis hypogaea following X-ray and neutron irradiation is due to inversion and other chromosomal abnormalities such as non-orientation at metaphase II and laggards at anaphase II. Nerkar (1977) observed increase in sterility with increasing dose of gamma rays which was consistent with the increase in translocation and meiotic abnormalities in Lathyrus sativus.

Basu (1962) has opined that the total pollen sterility observed by him in X-rayed plants of jute cannot be attributed to the chromosomal abnormalities alone. Sudhakaran (1971a) has concluded that the pollen sterility which is proportional to the dosage of gamma rays in Vinca rosea seems to be the cumulative result of various aberrant meiotic stages observed in these plants, as well as the physiological and genetic damage induced probably by the breakage of chromosomes, through the formation of antimetabolic agents in the cells. Katiyar and Roy (1974; 1976) suggested that gamma ray induced pollen sterility in some cucurbits and Capsicum annuum was dose dependent and always higher than the detectable meiotic abnormalities. According to them, therefore it can be assumed that besides the observable meiotic abnormalities, some undetectable changes play important roles in the induction of pollen sterility.

Effects on plant morphology and plant organs.

a) Leaves:

The size, shape, number of lobes, lobe margins and symmetry of leaves raised after mutagen treatment were found to be altered.

Some plants produced leaves that were of marked difference in size from control (Fig.4,5 and 6). The shape of leaves also varied. Number of lobes varied, some leaves showing no lobes at all (Fig.7). The lobes had deeper serrations in some cases while others had smoother margins (Fig.8). Leaves were produced that had a roughter texture than normal (Fig.9).

Modifications in leaf size and shape have been reported on similar lines in many plant genera as a consequence of mutagen treatments. Singh et al. (1939) reported variation in shape and size of leaves of Gossypium hirsutum following X-ray irradiation. Schwartz (1954) noticed that following irradiation of dry maize seeds, the leaves showed reduction in size corresponding to an increase in dose of radiation. Patel and Datta (1960) observed narrow leaves following X-ray treatment in Corchorus capsularis. Sahib and Abraham (1972) noticed narrow leaves in X-irradiated chilli plants. Raghuvanshi and Singh (1974) observed crumpled leaves and dissected margins in Trigonella foenum-graceum following gamma ray treatment. Koshy and Abraham (1978) noticed progressive reduction in size, distorted shape, irregular lobing and change in texture of leaves in Abelmoschus esculentus following gamma ray treatment.

Irvine (1940) held the view that abnormalities observed in leaves after irradiation could be due to the disturbances of phytochromes as a result of irriadiation. Meiselman et al. (1961) stated that the irradiation-induced abnormalities such as reduction in the number and size or deformation of leaves might be due to chromosomal aberrations. Moh (1962) attributed reduction of leaves of coffee plants to chromosomal deficiency. Haber and Foard (1964) concluded that the reduction in size of leaves in gamma-irradiated wheat seedlings might be attributed largely to the radiation-induced mitotic inhibition rather than to the other actions of radiation.

b) Dichotomy

Stem, branch and petiole dichotomy was observed in the irradiated population. As a result of dichotomy, bifurcation of the organ occurs. It is well known that this results from the death of the apical cells in irradiated materials and regeneration of two apices. Nettancourt and Contant (1966) noted the occurrence of fasciation and bifurcation of stem in tomato as a regular feature following chronic gamma irradiation. Singh and Mitra (1967) observed bifurcation with X-ray

treatment in Hibiscus species. Gamma irradiation caused stem dichotomy in apple and peaches (Lapins et al., 1969). In maize, Chandramouli (1970) observed dichotomous branching among the irradiated population. Koshy and Abraham (1978) observed dichotomy of the stem in Abelmoschus esculentus L. Moench. following gamma irradiation.

Mackey (1951) stated that bifurcation of stem could be explained on the basis of regeneration of affected meristem in barley. Bishop and Aalders (1955) attributed it to the delayed expression of some chromosomal effect. Kuehnert (1962) explained it as due to enlargement of the central cells of Tunica along a vertical axis followed by periclinal divisions of the cells. This results in the displacement of activity from the centre to the flanks of the apex. As a result, two new apical meristem could develop. In the present study also, it seems probable that disturbances in auxin synthesis and destruction of terminal meristem followed by development of two apices might have resulted in dichotomy. In the case of leaves also, bifurcationof petiole and appearance of two leaflets at the same node has been reported by Raghuvanshi and Singh (1974), in Trigonella foenum-

graceum. The cause of this may be the same as for the occurrence of stem dichotomy. Fig. 11 shows bifurcation of branch, Fig. 10 Petiole dichotomy and Fig.12 stem-splitting at the apex.

c) Fruits

In the higher exposures the treated plants produced two (Fig. 15, 16 and 17) or three fruits (Fig. 19 and Fig. 18) at the same node. Also double fruits were produced in certain plants from fused buds (Fig.13 and Fig.14). This bears similarity to observations of Sparrow et al. (1965) in Nicotiana and David et al. (1968) in lettuce. Doubling tendency aberrations like double spike, double peduncles and double kernels were observed by Sethi and Gill (1969) in barley following gamma ray treatments. Koshy and Abraham (1978) noticed twin fruits ("Siamese twins") in the M_1 generation of bhindi.

Fruits also varied in size and number of locules (Fig.22). The length of fruits reduced with increase in dose. This could have been due to the inhibitory action of the mutagen on growth.

During the present study, it has also been observed that changes like twin fruits and other abnormalities were not heritable. The specific changes which lead to the initiation of such changes are still unknown but could be due to physiological disturbances or hormonal imbalances, created due to the direct effect of the mutagen.

Effects on M_2 generation.

In this investigation, emphasis was given for the extent of variability induced in M_2, in economically important characters. In crop plants, most of the productive traits like height, maturity, duration, yield, fruit weight etc. are controlled by polygenic systems. The mutations affecting quantitative characters also occur spontaneously in nature (East, 1935). He also showed that such mutations occur both in the negative and positive directions. Many workers have analysed and reported radiation effects on polygenic systems. Induced mutations affecting polygenic characters have been reported by Oka et al. (1958) in rice, Gregory (1955) in groundnut, Rawlings et al. (1958) in soy bean and Gaul and Mittlestencheid (1961) in barley.

Gamma rays induced a reduction in mean values over control in height of plants, days to flowering, duration, number of branches and leaves per plant, yield and length and weight of fruits in M_2. The means however, did not differ significantly. The height of plants showed a slight increase for 40 kR. Similar result was also obtained in the case of number of branches per plant. In the case of mean number of leaves produced, a dose dependent reduction was seen. The 50 kR treatment however, showed a slight increase over the previous treatment. In all the characters, a declining trend was observed as doses increased. The frequency distribution was assessed in the control range and in the positive and negative direction. For all the characters analysed, it showed significant variation. The phenotypic classes were significant. For all characters, negative variants were more numerous than positive variants, except in the case of number of leaves and days to flowering. This must have produced the reduction in mean values. In the case of number of leaves and days to flowering, the magnitude of earliness may have resulted in the net reduction in mean values.

A reduction in mean values as was noted in the present investigation, has been noted by Bhatia and

Swaminathan (1962); Scossiroli (1966), Borojevic (1969), Borojevic and Borojevic (1968) in wheat, Brock (1967) in Arabdiopsis and Gaul (1964a, 1967) in barley. In extensive studies performed by Scossiroli (1966a,b) and Scossiroli et al. (1966) on wheat, this effect was shown in the same population for a large number of characters. Sakai and Suzuki (1964) found in rice, after X-irradiation that, mutation of polygenes responsible for quantitative characters (plant height, heading date, number of tillers and the like) was found to occur in most cases unidirectionally in the minus direction. These authors, based on the results from various sources, concluded that induced mutations occur in the minus direction especially for productivity. Gaul (1970) has pointed out that in most instances, the mean values of mutagen treated populations are lower than in untreated populations. The effect of irradiations on the means, according to him, is due to detrimental mutations occurring more frequently than favourable ones. The detrimental mutations are selected against. As was obtained in this investigation, no significance was observed in the reduction of means by Oka et al. (1958), Yamaguchi (1964), Bhatt et al. (1961) in wheat and Bhatia and Van der Veen (1965) in Arabdiopsis.

Frequency distributions of the different classes of phenotypes for all characters were significant, showing the production of positive and negative variants. Goud (1967a) observed that in hexaploid wheat, the frequency curves showed a shift in the mean towards the negative direction for tiller number and yield, with skewed distribution. Oka et al. (1958) concluded that in X-rayed progeny of rice, mutations of polygenes could occur in plus as well as minus directions in the same frequency. Matsuo and Onozawa (1961) concluded from irradiation experiments on rice that mutations of polygenes could occur in plus as well as minus directions for heading date, stem length and grain yield. Griffiths and Johnston (1962) also reported the same trend. However, they obtained only minus mutations for yield. Mutations were found to occur symmetrically in plus and minus directions in rice by Miah and Yamaguchi (1965). Gaul (1965) working on barley, suggested that the induced polygenic mutations do not follow any particular direction. Gregory (1956 b) opined that both negative and positive mutants appeared but the frequency in the negative direction was greater, producing an overall negative shift in the mean. Swaminathan (1966 b) stated that the previous selection

history greatly influences the symmetry of the induced genetic variation and the direction of the shift in the mean. Similar reports were given by Rao and Siddiq (1977) in rice.

Earliness was induced by the mutagen in the M_2 generation. But both early and late variants have been obtained. The data collected by Wittmer (1960) on the flowering time of tobacco after X-ray treatments of the seeds indicate that genetic variability is greater in irradiated seeds than control. Goud (1967 b) and Rajendran (1975) observed increased variability in most of the characters due to irradiation. Ahmed and Goud (1978) based on their studies on sunflower suggested that in irradiated populations, though mean is reduced, variance is enlarged.

In the present investigation a very few plants were obtained showing chlorophyll deficiency in the early developed leaves. Fig. 25 and 26 show leaves with chlorophyll deficiencies. Hussein et al. (1974); Swaminathan et al. (1970); Mikaelson et al. (1971) and Nayar (1976) found induction of chlorophyll mutations with gamma ray treatment, but reported that the frequency and

spectrum were very low and narrow compared to other mutagens like EMS. This could have been due to lack of specificity of gamma rays to certain regions of the chromosomes, where location of genes relating to chlorophyll development occurs (Natarajan and Upadhyay, 1964). Chowdhary (1978) based on his studies on bread wheat suggested that the physical mutagens may be much less efficient in inducing mutations of genes in the proximal segments of the chromosomes, which control chlorophyll development. In the present study also, lack of specificity of action might have contributed to the low frequency of chlorophyll mutations. Favret (1960) and Ryan and Heslot (1963) also demonstrated this randomness in action of physical mutagens which may have produced the low frequency of chlorophyll mutations.

In the present investigation, some variations have been observed in pigmentation also. In some cases anthocyanin pigmentation was seen throughout the stem, petioles, petals and fruits while yet other plants showed only green colouration. This is unlike the partial pigmentation of Anakomban. Radiation is reported to alter the pigment system in several plants (Sagawa and Mehlquist 1957a; Love, 1966). Reduction in the

concentration of anthocyanin pigments has been reported by Love and Malone (1967); Furuya and Galston (1965). Sparrow et al. (1968) suggested that enzymes are required for the formation and destruction of pigments, which may be affected by irradiation.

It is known in various crops that genetic differences, even though they are as small as single gene differences, can induce significant changes in radio sensitivity. This can influence the total rate and spectrum of recoverable mutations. This has been the opinion of several workers including Gustafsson (1944, 1947 and 1965); Gustafsson and Tedin (1954); Nilan (1956); Lamprecht (1956 and 1958); Sparrow (1961) and Sparrow et al. (1968). Mackey (1960 a,b) clearly demonstrated that although nobody is able to predict the influence of a particular genotype on the mutation spectrum, the choice of the parent material certainly plays a most decisive role in any mutation breeding programme. Besides it is also dependent on mutagen selected, conditions of mutagenesis and several other factors.

The results of the present study indicate clearly a change in mean in both M_1 and M_2 and a signi-

ficant increase in variability in both negative and positive directions in M_2. This shows scope for selection of desirable mutants both in negative and positive directions from the segregating population, depending on character and need of economically useful and viable mutant types.

SUMMARY

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The present investigation was carried out in the Department of Agricultural Botany, College of Agriculture, Vellayani during 1983-84. It was a preliminary trial in the broad area of 'Induced mutations in bhindi' (Abelmoschus esculentus L. Moench.) and aims at a general assessment of the effect of different doses of ^{60}Co-gamma rays in a well adapted bhindi variety, Anakomban, both in M_1 and M_2 generations.

Data were collected on various characters in M_1 and M_2 and analysed statistically following Fischer (1935). In the M_2 χ^2 analysis and split-split plot analyses were done to assess the frequency distribution based on chosen phenotypic classes under five M_1 sterility groups.

I. Effects on M_1 generation

1. A linear decrease in the germination percentage was observed as the dose level increased, compared to control. The lower doses also produced nominal reduction in germination.

2. Survival of plants increased with decrease in dose level. While control plants gave cent per cent survival, there was largest percentage of lethality in

the highest doses.

3. Height of plants at monthly intervals was measured. The data showed that the mutagen mostly reduced the plant growth depending on the exposure.

4. Mean number of leaves per plant produced by each treatment at monthly intervals was analysed. Here too reduction in number of leaves produced by treated plants was noted in comparison to control. The total number of leaves produced differed significantly between the exposures and showed a dose-dependent decrease.

5. Number of buds produced in each treatment did not differ significantly but there was a decrease in the bud production with increase in dose. This indicated the direct effect of the mutagen on the reproductive capacity of the M_1 plants.

6. Number of flowers also showed a similar decrease with increase in dose, the least mean number of flowers per plant being produced in the highest dose.

7. The number of stigmatic lobes produced by the flowers in each treatment increased for the lower doses and the highest dose. Here too the means did not differ significantly.

8. Yield per plant was reduced although not significantly for treated plants.

9. Fruit characteristics like mean fruit length and number of locules were analysed. Fruits were reduced in length due to direct effect of the mutagen and the smallest fruits were obtained in the highest dose. With regard to the number of locules produced, the lower doses enhanced the mean number as also the highest dose.

10. Data were collected on number of sterile buds and sterile flowers and pollen and seed sterility. In all the cases, it was observed that sterility increased with doses. Pollen sterility was significantly greater for the exposures compared to control, especially for the higher doses. Similar results were also obtained for seed sterility.

11. Other variants in M_1

Besides dwarfing, plants also differed in the shape and size of leaves produced. Some plants produced leaves with distinct lobes while others had lobeless leaves in comparison to control. The margin of lobes also were more serrated in some plants.

Dichotomy of petioles, branches and stem was observed in several plants in the two higher doses. Fruit abnormalities in the form of double fruits and production of two or more fruits at the same node were also obtained. Variants in plant habit were noticed, some plants showing weak stems.

II. Studies on M_2

Effect of gamma rays on polygenic traits like plant height, number of branches and leaves, days to flowering and complete harvest, yield and mean length and weight of fruits were analysed.

1. Plant height: The mean values did not differ significantly but the frequency distribution in the three phenotypic classes showed significance.

2. Number of branches in the M_2 did not differ significantly. However, there was a progressive decline in the mean value depending on dose. The frequency distribution also was significant. The interactions were not significant.

3. Number of leaves produced per plant also decreased with increase in dose. χ^2 value was significant. The frequency distribution in both treatments and the phenotypic classes was significant.

4. The mean number of days to flowering decreased as doses increased except in the 20 and 30 kR. The existence of variants from the control group in both directions was evidenced by the significant χ^2 value. Interactions of exposures with sterility groups and phenotypic classes were not significant.

5. Duration of crop was analysed. It was found from the data that the higher exposures reduced crop duration in M_2. Significant number of variants, especially shorter duration types than normal were obtained. The phenotypic classes showed significantly different frequencies.

6. Yield of fruits also was reduced for higher doses; frequency distribution of variants from control range was significant.

7. Fruit characteristics like fruit length and average fruit weight were analysed. It was found that several variants were produced in both positive and negative range but means did not differ significantly.

8. Chlorophyll mutations and variation in pigmentation:

In the M_2, close observation of individual plants was done for chlorophyll mutations. Only a very few plants showed chlorophyll deficiency in the earlier leaves.

Pattern of pigmentation also varied, some plants appearing entirely green or red unlike the normal colouring of the selected variety.

The consistent reduction in means in both M_1 and M_2 populations for important polygenic traits like plant growth, duration and yield and the significant variation in positive and negative segregants suggests scope for a positive response to selection and improvement in this particular crop variety. Selections can be made for directional change as both positive and negative variants have been produced. It also indicates that most of the characters can be altered by gamma rays. The present work relates to the study of the general mutagenic effect on the crop, as seen in the first two generations. Selection of desirable types and carrying forward to later generations are suggested as the future line of work in recovering viable mutations of economic value.

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ILLUSTRATIONS

Fig.1. Plant height variation in M_1 under low exposures (control on right side)

Fig.2. Plant height variation in M_1 under medium exposures (control on right side)

Fig.3. Plant height variation in M_1 under high exposures (control on right side)

Figure 1.

Figure 2.

Figure 3.

Fig.4. Leaf size variants among treatments in M_1 (Control on extreme left top).

Fig.5. Leaf size variants among treatments in M_1 (Control on extreme left top)

Fig.6. Leaf size variants among treatments in M_1 (Control on extreme left top).

Figure 4.

Figure 5.

Figure 6.

Fig. 7. Variation in number of lobes in M_1
(Control on extreme left top)

Fig. 8. Variation in leaf lobe margins in M_1
(Control on extreme left top)

Fig. 9. Variants for leaf texture in M_1
(Control on extreme left top)

Figure 7.

Figure 8.

Figure 9.

Fig.10. Petiole dichotomy.

Fig. 11. Splitting of the branch.

Fig.12. Bifurcated stem apex.

Figure 11.

Figure 12.

Fig. 13. Fused buds in M_1

Fig. 14. Double fruits produced from fused buds.

Fig. 15. Two fruits produced at the same node (M_1)

Fig. 16. Two fruits produced at the same node (M_1)

Fig. 17. Two fruits produced at the same node (M_1)

Figure 15.

Figure 17.

Figure 15.

Figure 17.

Fig. 18. Three fruits produced at the same node in M_1.

Fig. 19. Three fruits produced at the same node in M_1.

Figure 19.

Fig. 20. Weak-stemmed variant, for plant habit in M_1 (control on right side)

Fig. 21. Weak-stemmed variants for plant height (M_1)

Figure 21.

Fig. 22. Variation in fruit size, length and number of locules (M_1)

Fig. 23. Sterile seeds produced in M_1 (fertile seeds from control at the bottom)

Fig. 24. M_1 fruit without any seedset in comparison with normal fruit.

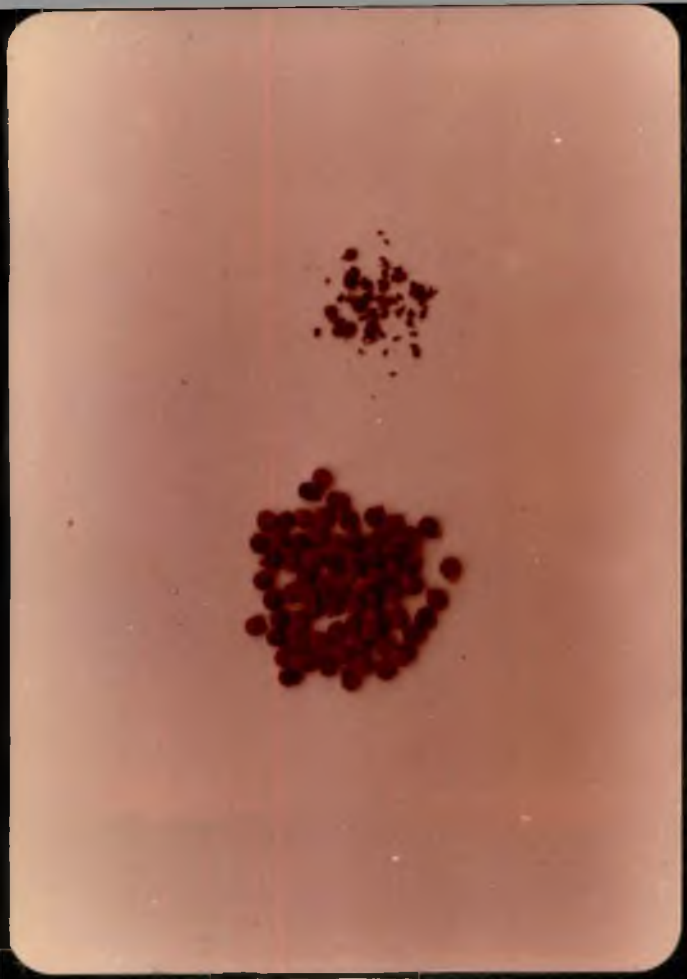

Figure 23.

Figure 24.

Fig. 25. Leaf showing chlorophyll deficiency in M_2.

Fig. 26. Leaves showing chlorophyll deficiency in M_2.

Figure 25.

Figure 26.

INDUCED MUTATIONS IN

BHINDI (*Abelmoschus esculentus L. Moench*)

BY

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ABSTRACT OF THE THESIS
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ABSTRACT

Mutations induced in bhindi (Abelmoschus esculentus L. Moench) Var. Anakomban by the most potent physical mutagen, ^{60}Co-gamma rays were studied using six doses. The investigation was carried out at the Department of Agricultural Botany, College of Agriculture, Vellayani, during the year 1982 - '84. The direct effect of the mutagen was assessed in the M_1 generation and the extent of variability in the M_2 with special reference to polygenic and economically important characters. The doses employed were 10 to 60 kR at an interval of 10 kR exposures.

Direct effect of the mutagen was assessed by studying the germination percentage, survival, growth rate, number of buds, flowers and fruits per plant, number of stigmatic lobes per flower, fruit length and number of locules per fruit, sterile buds, percentage fruit set and pollen and seed sterility.

In the M_2, observations were made on plant height, number of branches and leaves, day to flowering, duration of crop, yield and fruit characteristics like length and weight. Besides computing mean, frequency distribution of variants from the control range also was

determined. Careful observation of each M_2 plant was made for chlorophyll deficiencies especially in the early seedling stage.

Statistical analysis of the data collected clearly demonstrated a dose dependent variation in means for all the characters analysed. Germination was reduced, though not significantly. There was a significant difference between exposures for the pollen and seed sterility percentages. The other characters also showed a declining trend with increasing exposures although the means did not differ significantly.

Variants in leaf size, leaf lobes, leaf shape, stem and petiole dichotomy and double fruits also were observed in the M_1 generation.

In the M_2, the polygenic traits studied did not differ significantly for the mean expression of characters. But χ^2 analysis done on the frequency distribution of phenotypes in positive, negative and control group showed significant variation, for all the characters. The phenotypic classes under five M_1 sterility, classes showed significant variation in their mean frequencies in all the characters. Treatment mean frequencies were significant for number of leaves produced. Interactions were also insignificant for all the characters.

The M_2 population showed a very few plants with chlorophyll deficiencies in a few early formed leaves. Variations in pigmentation also was seen whereby some green and red pigmented plant types were observed. The fall in means and significant pollen and seed sterility in the M_1 and the significant frequency distribution of positive and negative variants in M_2 demonstrate the success of the mutagen treatment and the scope for a positive response to selection for isolation of beneficial mutants in succeeding generations.