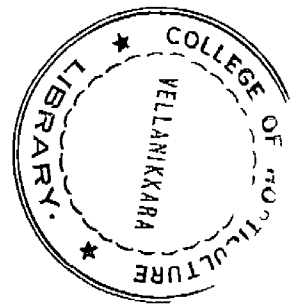
GENIC STATUS IN RELATION TO RADIO SENSITIVITY, MUTATION FREQUENCY AND SPECTRUM IN BHINDI



BY

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DECLARATION

I hereby declare that this thesis entitled "Genic status in relation to radiosensitivity, mutation frequency and spectrum in Bhindi" is a bonafide record of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title at any other University or Society.

Vellayani,
22nd January 1985.

(MARFEN ABRAHAM)

CERTIFICATE

Certified that this thesis entitled "Genic status in relation to radiosensitivity, mutation frequency and spectrum in Bhindi" is a record of research work by Smt. Mareen Abraham 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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INTRODUCTION

INTRODUCTION

The success of crop improvement programme depends on the amount of genetic variability available in the population and the efficiency of the selection techniques. Induced mutation has proved as a potent tool to increase variability which can either supplement or replace hybridization. However it should not be the end of a plant breeder's efforts. Such artificial induction of variability can serve as the basis for further improvement and the evolution of new varieties.

The work of Muller and Stadler almost 53 years ago gave birth to the mutation breeding technique which represents a departure from the conventional breeding procedures in agriculture. With the advent of this method the genotype and phenotype of living organisms are under human control and can be changed at times according to the needs. In fact it has opened a new era in plant breeding and is commonly used now-a-days for crop improvement in agriculture. This is amply demonstrated by the relatively large and steadily increasing number of mutant varieties which have been commercially released from different countries all over the world.

The successful exploitation of atomic radiations for inducing alterations in the base sequence of DNA is one of the most potent lines of contemporary breeding research.

The success of the green revolution, to a certain extent a product of induced mutations has already proved that radiations can be beneficially utilised for tailoring better varieties of crop plants. Artificially induced variations have been extensively studied and reported in almost all crop plants especially in seed propagated ones. These reports clearly show that all morphological and physiological characters within the species boundary and even beyond this, can be induced by mutation. Gottschalk (1969) in his work on Pisum sativum has clearly shown that mutations which are progressive in the evolutionary sense can be induced by different mutagenic agents. Most plant breeders now recognise mutation breeding as a potent tool for evolving new varieties. It is also much time saving, compared to conventional plant improvement methods. A desired mutation can be recovered in a homozygous state in M_2 or M_3 generation compared to F_6 or F_7 generations in the case of hybridization (Siguobjoranssen, 1970). The release of the wheat variety, 'Sharbati Sonora' by Swaminathan (1969a) in three and a half years is the most striking example of this. The work of Sears (1956) on transferring the resistance factor from the wild grass species, Aegilops umbellulata to the cultivated wheat variety Chinese Spring by induced translocations has shown that new combinations can also be created by mutation breeding. According to Brock (1970)

"we can induce any mutation that occurred naturally, and probably many which have either never occurred naturally, or have been lost from the natural population".

Sparrow et al. (1958) have reviewed the progress in mutation research during 1896 to 1955. The reports on induced mutations by Gottschalk (1960), Gaul (1964), Sparrow (1961), Sparrow et al. (1963), Gustafsson and Gadd (1965), Nilan (1956), Kawai (1962) and Swaminathan (1969 a,b) give a clear picture of the achievements made in the field of mutation breeding in various crop plants.

Study of mutagen sensitivity is a pre-requisite for initiating practical mutation breeding programme in any crop plant, as there is a positive correlation between susceptibility and yield of positive variants. The sensitivity of seeds to mutagenic treatment is dependent on various factors including genotype, type of mutagen, the dose and dose rate employed and many other modifying factors. The response of cells of higher plants to physical and chemical mutagens is influenced to varying degrees by numerous biological, environmental and chemical factors as reported by Kawai and Sato (1966). They further added that these factors modify the effectiveness and efficiency of mutagen in higher plants. Though it is not clearly understood why these factors influence mutations and chromosome

aberrations, it has been clearly demonstrated that many of these factors must be controlled in mutagen treatment in order to obtain reliable, repeatable and usually optimum results. The success of mutation breeding to a great extent depends on selection of variety to which mutagenesis is created.

Bhindi or Okra (Abelmoschus esculentus, Moench) is considered as one of the most important vegetable crop in India due to their wide adaptability under a wide range of edaphic and soil conditions. Availability of its fruits during most of the seasons of the year and the relatively low cost of production made it one of the most favoured vegetable crop of the world. Bhindi is considered as a proteinaceous vegetable. Numerous varieties have been released by conventional breeding techniques in addition to a large number of local varieties under cultivation. But none of the varieties shows resistance or tolerance to the most dreadful disease of the crop variety, yellow vein clearing, a virus disease. This clearly demonstrates that the genes concerned with resistance to YVM is not available in natural population. The only alternative is to create variability by artificial means and direct selection procedures to isolate out resistant types. An attempt has been initiated in the Department of Agricultural Botany, College of Agriculture, Vellayani to create variability to isolate

out resistant types in this particular crop. During the course of this main objective, in the present study, an earnest attempt is made to test the varietal variations on their response to the most efficient physical mutagen, ^{60}Co gamma rays.

The main objectives of the present investigation include:

1. To test the mutagen sensitivity of Bhindi varieties based on M_1 lethality, injury and sterility,
2. To test the differential response of pure and hybrid seeds to mutagen, and
3. To assess the induced variability in M_2 for various polygenic traits in pure and hybrid seeds.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The idea of producing mutations artificially and using them for breeding was reported as early as 1901 by DeVries. In the first twenty five years of this century, numerous investigators tried to induce mutations in many different organisms by physical and chemical agents of varied nature. X-ray irradiation was applied to cells and chromosomes by Koernicke (1905) and Gagner (1908). However, the conclusive proof that ionizing radiations induce mutations was presented by Muller (1927) in Drosophila. This was closely followed by the successful experiments of Stadler (1928 a,b) in inducing mutations in barley and maize. Since then different kinds of radiations have been tried. Though these attempts were successful in some cases, the methods used were not sufficient to provide clear and convincing results (Gaul, 1964). A wide range of both physical and chemical mutagens is now available and it is therefore natural that several investigators have probed the relative advantages and disadvantages of the different mutagens (Swaminathan, 1969 a,b). However, most of the varieties developed by mutation breeding have arisen from material irradiated with ionizing radiation only (Sigurbjoroasson and Micke, 1969). In sexually propagated crops chemical mutagens have yielded very high mutation

frequencies and in most cases they were more efficient than ionizing radiations (Kamra and Brunner, 1970).

Varietal sensitivity

As reviewed and reported by Davidson (1960), Konzak et al. (1961 a,b) and Nilan (1956) the major factors that alter the genotypic sensitivity to mutagens include nuclear volume, water content, oxygen pressure, stage of development and hydrogen ion concentration. In recent years, the role of nuclear volume and chromosome content (DNA value) in determining the radiosensitivity of plant species has received a great deal of attention. It has been clearly demonstrated that there is an inverse relationship between radiosensitivity and DNA content. Data for the prediction of radiosensitivity of seeds in relation to total DNA content have been published by Osborne et al. (1963). Genetic differences eventhough they are as small as single gene differences, can induce significant changes in radiosensitivity. Gustafsson (1944, 1947, 1965), Gustafsson and Tedin (1954), Nilan (1956), Lamprecht (1956, 1958), Gelin et al. (1958), Smith (1961), Sparrow (1961), Konzak et al. (1961a) and Sparrow et al. (1965) clearly reported that any change in the genotypic level can induce significant changes in radiosensitivity which influence

not only the total rate, but also the spectrum of recoverable mutations. Ramalingam (1980) reported that spectrum of mutations differed according to variety and mutagen and interaction between variety and dose of a particular mutagen. A variety-dependent variation was observed in the sensitivity to physical and chemical mutagens.

A clear and specific prediction on the influence of a particular genotype on the mutation spectrum is not available as reported by Mackey (1960 a,b). Jagathesan and Swaminathan (1961) and Swaminathan (1965) reported a differential effect of mutagen between species of the same ploidy level and between varieties within the same species in various crops. Radiosensitivity of haploid plants was found to be higher than that of diploids (Tanaka, 1970). The diploids in turn were reported to be more sensitive than the respective autotetraploids (Yamaguchi and Kobayashi, 1960; Yamaguchi, 1964 and Sree Rangaswamy, 1970). Enken (1966 a,b) concluded that closer the varieties in their genotypes, greater is the similarity in their spectra and frequency of mutations.

Gregory (1960) stated that the chief limiting factor in mutation production and mutation recovery is the genic constitution of the experimental organism and not the type of mutagen used. Thus for the plant breeder a knowledge of

what might be called mutant expectations in his material may be made important than a resolution of the mechanism of mutation changes at the submicroscopic level.

Comparison among varieties of tomato (Biachi et al., 1963), barley (Mikaelson and Brunner, 1968) and pea (Mukeeb and Siddiqui, 1973) showed variation in respect to radiation response among different genotypes indicating the influence of genetic factors on radiosensitivity. Krishnaswami and Rathnam (1982) reported differential radiosensitivity to EMS exhibited by ten greengram cultivars. Gamma irradiation of greengram varieties indicated variation in the mutagenic sensitivity in the M_1 generation (Ratnaswamy et al., 1978). Difference in radiosensitivity was also reported in varieties of cucumbits (Vishnoi and Joshi, 1981), Safflower (Mallikarjunaradhya and Gowda, 1981) and Tomato (Georgiov, 1960).

Davees (1962) studied the genetic control of radiosensitivity in tomato using growth measurements and other characters. Biachi et al. (1963) have also conducted experiments with tomato varieties. Marked intervarietal differences in radiosensitivity were recorded by Matsuo et al. (1958), Fuji (1962), Ukai (1967) and Mikaelson and Navaratna (1968) in rice. The varietal differences in radiosensitivity were also reported to be due to differences in chemical composition (MyHenaere et al., 1965) or due to

differences in endogenous levels of auxin and ascorbic acid (Goud et al., 1967).

Chromosome constitution and pollen/seed sterility affected by Mutation treatment

The absorption of ionizing radiation in a bacterial, plant or animal cell has long been known to produce a variety of structural aberrations in chromosomes which are visible under the microscope. As a result of extensive works done by various workers it has been repeatedly shown that apart from the genetically transmissible changes, irradiation causes a number of cytological abnormalities. Formation of micronuclei, restitution nuclei, multinucleate cells, multipolar spindles, as well as abnormal contraction of chromosomes and stickiness of chromosomes may be mentioned as some of the important cytological abnormalities. The frequencies and kinds of aberrations have shown to be effective yardsticks for the cytological assay of radiation damage.

Goodspeed (1928) reported failure of some chromosome to pair after irradiation. Levitsky and Asaration (1932) in Crepis, Vicia and Secale and Muller and co-workers (1937, 1938) in Drosophila observed chromosome breakage due to X-ray irradiation. Stadler (1932) reported chromosome breakages in maize.

Lea and Calchecide (1942) found in Tradescantia that

chromatid breaks and isochromatid breaks were induced at the prophase, while chromosome breaks and chromosome interchanges were induced at the interphase. Sax (1943) had reported that the primary genetic event in irradiation is the breakage of chromosome thread. Muller (1954) stated that the frequency of gross structural changes varied as a power of the dose higher than one when X-rays or gamma rays are applied in ordinary doses since with such irradiation the broken ends which unite are usually produced independently arising from breaks and the products, the structurally changed chromosomes, therefore represents a concentration of effects.

Sparrow (1961) reported chromosome stickiness and clumping as an immediate result of irradiation with high doses. It has been found in barley that the translocation frequency determined by both mitosis and meiosis is the same and increases linearly with radiation dose (Gaul, 1963; Calbult and Smith, 1952; Caldecott et al., 1954). Budakina and Scapova (1965) observed ring formation along with bivalents in 10 kR gamma irradiated Triticum dicoccum. Evans (1967) concluded that radiation does not immediately break chromosomes and may not produce direct breakages of chromosomes at all. Vasileva and Mikhanzhiev (1972) reported an increase in chromosome aberrations with the increase in gamma irradiation doses in different varieties of pea.

In soya bean it was reported that the frequency of root tip cells with structural chromosome re-arrangements increased with increase in concentration and dosage of mutagens, and the most frequent form of chromosome aberrations after chemical mutagen treatment was lagging and use of X-rays and gamma rays produced bridges and fragments. (Mashkin and Prokudina, 1974). Structural chromosome re-arrangements were observed in mitosis and meiosis after treatment of pea seeds with gamma rays and EMS by Rekhmatulla and Gostimskii (1976). They also found that the frequency of chromosome aberrations to be considerably greater after treatment with gamma rays. In Capsicum annum after irradiating with 0-40 kR gamma rays meiotic abnormalities like stickiness, clumping and breakages of chromosomes, formation of multivalents and univalents, non-orientation of chromosomes on the metaphase plate, unequal chromosome separation and laggards occurred at frequencies proportional to dosage.

Reduction in fertility of M_1 plants is a reliable parameter to assess the effectiveness of mutagenic treatment as reported by Kiwi (1962). A linear dependence of decreased pollen and seed fertility on mutagen dose was reported by Beachell (1957); Chang and Hsich (1957) and Singh (1970). Pollen sterility which occurred in most plants resulted from cumulative effects of aberrant meiotic

stages and physiological and genetic damage caused by chromosome breakage (Rao and Lakshmi, 1980). Baukowska and Rhymza (1970) observed that fertility was reduced in proportion to the dose of gamma rays in Phaseolus vulgaris. Bekendam (1961) in Rice had indicated a decrease in fertility with increasing dose upto a certain level beyond which there is however a saturation effect.

Singh (1970) observed that in rice gamma rays induced a high frequency of translocations and this might be correlated with pollen sterility. Vasileva and Mekhanzhiev (1972) reported an increase in chromosome aberrations with the increase in gamma irradiation doses in different varieties of pea. Bansal and Singh (1972) studied a polypetalous mutant of NP-46A, which breeds true and which was induced by X-rays.

Effect of Mutagens in the M_1 generation

Mutagens disturb the normal biological organisation of an organism and this is expressed in a number of ways. The low dosages do not show any severe effects but high doses produce gross visible disturbances. These effects are seen in the M_1 generation in six categories (1) reduced germination (2) reduction in survival (3) growth inhibition (4) reduced fertility (5) Chlorophyll chimeras and (6) other morphological and developmental abnormalities.

Germination of seeds

Delayed and reduced germination is a common feature at higher dose levels and rates as reported by Athwal (1963) in Cicers using X-ray treatment. Shrishov and Shain (1966) using gamma irradiation in field beans and Sidorova et al., 1966; Maslov and Stepanova, 1967 in Pea confirmed the above results. Dahiya (1973) reported that among the different gamma irradiation treatments 30 and 70 kR decreased germination of the treated mung bean seeds. Louis and Kadambavanasundaram (1973a) reported a reduction in germination percentage and an increase in delay for germination following gamma irradiation of cowpea seeds.

Survival of plants

The survival of seedlings was generally found to decrease with increasing doses of radiations and chemical mutagens (Rao and Ayengar, 1964). Yamagata et al., 1965; Siddiq, 1967; Swaminathan et al., 1970 and Jananowskii, 1970; obtained a sharp fall in survival rate with gamma ray dosage above 25 krad in Pisum arvense and Vicia sativa. A significant reduction in plant survival with high doses of X-rays and neutrons was reported by various investigators including Ojomo and Chheda (1971) in cowpea. Mujeeb and Grig (1972) in Phaseolus vulgaris observed a progressive reduction in survival with the increase in dose of gamma irradiation. Fautrier (1976) reported that in Lucerne, no plant

survived in treatments above 120 krad of gamma rays. Dabaley and Zekurnov (1977) noticed that in sweet variety of Lupin 20 krad and for bitter varieties 30 krad of gamma rays were found to be lethal.

Plant height

Teretchenko (1966) noticed delayed seedling growth in pea by gamma irradiation. Akilov (1966) noted an increase in plant height with increased dosage of gamma rays in soyabean. Though the height reduction was conspicuous at seedling stage, Louis and Kadambavanasundaram (1973a) found the plant height at maturity to be uniform over the treatments after gamma irradiation of cowpea seeds at different doses. Sreerangaswamy et al. (1973) observed that the greengram plants treated with gamma rays were shorter than the parents and those treated with 60 krad were the shortest. Decrease in seedling height in gram was noticed by Khanna and Makachandam (1980).

Mutations in the M_2 generation

Gustafsson (1947) stated that chlorophyll disorganisation is one of the many effects of irradiation. An increase in the frequency of chlorophyll mutations with increasing doses of radiations was reported by several investigators in rice. Mutation frequency reached a maximum at moderate doses of X-rays and gamma rays and decreased at

high doses (Matsuo et al., 1958; Masima and Kawai, 1959) Gailey and Telbert (1958) reported that very high doses of gamma irradiation to the extend of 50 krad did cause disorganisation of chlorophyll.

The environmental conditions under which the seedlings are grown, alter or even induce certain chlorophyll deficient phenotypes. Thus Hansel (1968) and Nilan et al. (1963) found that unless M_2 seedlings of all treatments were grown under identical conditions, difference in mutation frequencies between treatments may not be valid. Popovic and Zecovic (1965) studying mutation changes in winter barley due to gamma irradiation observed a change in ear colour in some plants. They also recorded several chlorophyll mutants. Kasyanenko and Timofeyev-Resovsky (1967) in Arabidopsis thaliana L. found that when treated with Co^{60} all the 130 chlorophyll mutants were single recessive and six were considered to be of particular interest in that, one had increased pigmentation, but less assimilation rate, another with reduced pigmentation, and greater vitality etc. Alikhan and Veeraswamy (1974) studied the effects of gamma rays and EMS in red gram and found that chlorophyll mutations were maximum at 24 krad and 70 mm treatments respectively.

The frequency of chlorophyll mutations recoverable in

a mutagenic experiment is a good indication of the effectiveness and efficiency of mutagenic treatment (Monte, 1963).

Differences in the spectrum of mutations induced by physical and chemical mutagens were reported by several investigators. Bekendam (1961); Chao and Chai (1961); and Basu and Basu (1969) reported that in rice following irradiation, the albinos predominated in the chlorophyll mutation spectrum followed by viridis and Xantha. Louis and Kadambavanasundaram (1973b) reported the occurrence of albino, xantha and viridis mutants in cowpea following gamma irradiation. Chekalin (1977) obtained wide spectrum of chlorophyll mutations in Lathyrus sativus following gamma irradiation and treatment with different chemical mutagens, the most frequent being chloro-viridis.

Induced mutations on polygene traits

Almost all economically important characters in plants are known to be governed by polygenes. The expression 'Micro-mutation' is used to mean mutations in polygenes governing quantitative characters leading to small changes in phenotypes. East (1935) has pointed out that the deviations forming the fundamental materials of evolution are the small variations mentioned by Darwin. Baur (1929) in his paper on the means, origin and inheritance of racial differences in Antirrhinum introduced the term "Klein-mutationen" which Gregory (1969) interpreted as synonymous

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

Following the successful experiences of Gregory (1955) in the usefulness of mutation tool for groundnut improvement, breeders in different crop plants resorted to the micromutation technique to improve quantitatively inherited characters like yield and its components. Sax (1955) reported that yield can be increased by certain stimulatory doses of radiations, which may be due to higher mitotic activity of mutants (Tedorodza et al., 1977; Javeed Iqbal, 1979). Radiations produce more chromosome mutations which are seived off during meiosis while EMS is known to produce comparatively more point mutations (Ehrenberg et al., 1959). Experiments of Humphery (1954) and Rawlings et al. (1958) on induced mutations in soyabean clearly showed that the estimates of genetic variations for yield, plant height, maturity time and seed size on the average were five times as large as those of the controls, giving a better chance for selection.

A general reduction of mean and a significant skewness distribution after mutagenic treatments was reported by Scossiyoli (1965); Goud (1967a) and Minocha et al. (1977).

Kumar and Das (1977) have also agreed with the above report and explained the cause as the action of ionizing radiation on chromosomal and extra-chromosomal parts of the cell.

Increase in variance following mutagenic treatment was a common feature observed in quantitative characters as reported by several investigators (Oka et al., 1958; Batiman, 1959; and Matsue and Onozawa, 1961). Oka et al. (1958) and Ota et al. (1962) reported an increase in variance with increasing doses of mutagen, but Yamaguchi (1960) observed an opposite effect. Yamaguchi (1964) confirmed that variance did not increase linearly with the radiation dose. On the other hand, Miah and Bhatti (1968) reported that the variance decreased at higher doses. Sakai and Suzuki (1964) and Tanaka (1968) found that distribution of variance for certain characters was stunned and therefore, stated that the mutation of polygenes occurred mostly in a negative direction. Swaminathan (1966a) was of opinion that the directions of incidence of micro-mutations was strongly influenced by the previous selection history of the variety.

Brock et al. (1972) has reported that the increased variability in mutagen treated population is found to be largely due to increase in genetic components. Borojevic and Borojevic (1968) reported that genetic variability

for several quantitative characters increased in irradiated population of Triticum aestivum.

The induced genetic variability proved also to be suitable for artificial selection on specific quantitative traits. The experimental work initiated by Scossiroli (1964) and followed by Clayton and Robertson (1955, 1964) and Kitagawa (1967) proved to be fundamental to this point of view.

As a general rule induced mutations can be successfully used to create any sort of useful variations in quantitatively inherited characters. The classical work of Gustafsson (1965) on adaptability and improvement of yield, and that of Sigurbjornsson and Micke (1969) on numerous other traits provide examples to this.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation on genotypic status in relation to radiosensitivity and induced mutagenesis on Bhindi (Abelmoschus esculentus Moench) was conducted in the Department of Agricultural Botany, College of Agriculture, Vellayani during 1982-84. The details of varieties tried to test sensitivity are presented in Table 1, and the hybrids to study induced mutations in Table 2.

Selection of seed material

Well developed seeds obtained from fully ripened fruits of healthy plants were used for the study. Uniformly dried healthy seeds having same size and colour were selected for mutagenic treatment.

Technique of selfing

Bhindi is a naturally self pollinated crop. To ensure cent per cent self pollination the parental plants were selfed by providing protective measures. The flower buds to be opened on the next day were covered by using a butter paper cover in the previous evening. When the flowers are opened on the next day, assisted pollination was done by using camel hair brush, after removing the cover. The flowers were covered immediately after pollination and allowed to remain for 3-4 days. The selfed flowers were properly labelled for future use.

Technique of crossing

Pre-determined male and female parents were used for crossing. The selected buds in the female parent was hand emasculated in the previous evening and properly protected by covering it with a butter paper cover. In the next day morning, assisted pollination was done by taking the pollen from the male parent at the time of normal anthesis and dusting on the stigmatic lobe of the emasculated flowers. Male flowers were also protected from cross contamination. The crossed flowers were properly covered and labelled.

Methods

Gamma irradiation

The twenty varieties including fourteen pure breds and six hybrids were irradiated by using a gamma cell installed at KAU headquarters, Vellanikkara. The dose rate being 60 kR/hr. The irradiated seeds were planted on the 5th day of exposure.

Planting technique

Gamma ray treated and control seeds were pre-soaked for twelve hours and sown in two replications of 30 seeds each. The seeds were planted in rows at a spacing of 45 x 40 cm after a basal dose of cowdung. Proper randomization of treatments were done in each replication. The

experiment was laid out in RBD with two replications. Fertilizer application were done at the rate of 25:8:30 kg NPK per hectare. Full dose of P and half dose of N and K were given two weeks after sowing. Remaining half dose of N and K were given after fortyfive days of sowing. A moderate spacing and fertilizer status were given to check excessive vegetative growth. Special care was taken to provide uniform field conditions for these plants till harvest. Irrigation was provided as and when required. All the field experiments in this study relating to M_1 and M_2 were conducted in the experimental area attached to the Department of Agricultural Botany, College of Agriculture, Vellayani.

Plant protection

Malathion (Cythion) 0.95% was sprayed twice before fruit setting to control shoot borers.

Direct effect of the mutagen on the M_1 generation

The direct effect of gamma rays on the various genotypes were studied with respect to the following characters.

1. Germination percentage
2. Days taken to complete germination
3. Plant height at 30 days interval from sowing to complete harvest.

4. Number of leaves per plant at 30 days interval from sowing to complete harvest.
5. Number of branches per plant at 60 and 90 days after sowing
6. Number of fruits/plant
7. Length of fruit
8. Weight of fruit
9. Yield per plant
10. Pollen sterility
11. Seed sterility
12. Weight of seeds

Observation on M_1 generation

1. Germination

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

2. Plant height

Plant height was determined at three stages of growth at an interval of 30 days, namely, 30th, 60th and 90th days after sowing. Heights were taken from ground level to the tip of the plant and represented in cm.

3. Number of leaves

Number of leaves produced per plant was also studied at three stages of plant growth ie., 30th, 60th and 90th day after sowing. Total number of green leaves available at the time of observation was taken into consideration.

4. Number of branches

Number of branches produced per plant was studied at 60th and 90th day after sowing. Total number of branches per plant available at the time of observation was taken into consideration.

5. Number of fruits per plant

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

6. Length of fruit

First formed two fruits each per plant were harvested from five plants selected at random from each treatment. The length was taken from the point of attachment to the tip in cm.

7. Weight of fruit

The fruits whose length were taken were weighed. The mean weight of fruits taken from a single plant were calculated in gram.

8. Yield per plant

The yield obtained per plant was calculated based on the weight of fruits and the total number of fruits produced per plant.

9. Pollen sterility

Pollen sterility analysis was done using acetocarmine-glycerine staining technique. Ten plants at random from each treatment were selected. Flower buds produced during the early part of the flowering period were selected. Pollen grains were collected at the time of normal anthesis. The pollen grains were collected by means of a clean camel hair brush and placed on a drop of acetocarmine-glycerine media placed on a clean slide. It was then kept for one hour and examined under the low power of a microscope. Unstained, undersized, partially stained and shrivelled pollen grains were scored as sterile and the uniformly stained and properly filled pollen as fertile. Three slides were prepared for each flower. Five microscopic fields were scored from each slide. Pollen sterility was estimated as the percentage of sterile pollen in the total number of pollen grains in a microscopic field.

10. Seed sterility

Fully ripened fruits were harvested from each plant. The fruits were split open at the ridges and the number of

fertile and sterile seeds were counted. Two fruits per treatment were taken to assess seed sterility. Seed sterility was estimated as the percentage of sterile seeds in the total number of seeds in a fruit.

11. Weight of seeds

The weight of one hundred fertile plumb seeds from each plant/treatment were taken using a mono-pan-balance.

Collection of seed material for raising M_2 generation

Seeds were selected from treated population of hybrid seeds and respective controls. There were 18 treatments in each replication. Based on M_1 seed sterility percentages the treated population was categorized under three heads.

1. 0-15% - Low sterility group
2. 15-30% - Medium sterility group
3. Above 30% - High sterility group

Two fruits each from each plant/treatment collected for selection of seed material for raising M_2 generation. Five plants from each sterility class were selected per treatment per replication. There were eighty M_1 plants selected per replication including control. From the above plants, the first formed two fruits were selfed, allowed to have full maturity, harvested and dried and stored for extraction of seeds. At the time of raising M_2 generation the seeds of

the two fruits selected/plant were bulked together and collected thirty seeds at random and raised M_2 generation.

Planting technique of M_2

The selected seeds were uniformly dried and sown in rows, each row represent one M_1 plant. There were thirty plants each in each progeny row. The fertilizer dose and mode of application and crop management were as recommended in package of practices. Special care was taken to provide uniform field conditions for the entire crop till harvest. The following observations were taken in M_2.

Observations on M_2 plants

1. Chlorophyll mutation frequency

The chlorophyll deficient mutants were screened out on the seventh day of sowing. This was done in the early morning hours. This was continued for about three more days and frequency calculated on treatmentwise. Due to lack of different types of chlorophyll mutants, the spectrum was not taken into consideration.

2. Viable mutations

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

3. Quantitative mutations

Detailed observations on quantitative traits were

taken from all the normal looking plants, excluding border plants. Observations were taken on:

1. Plant height on 30th, 60th and 90th day after sowing
2. Number of leaves/plant on 30th, 60th and 90th day after sowing
3. Number of branches/plant on 60th and 90th day after sowing
4. Number of fruits/plant
5. Length of fruits
6. Weight of fruits
7. Yield/plant

Observations on quantitative characters in M_2 were made following the same technique as for M_1 generation.

Statistical analyses

M_1 analysis

Analysis of variance of the data was done following Fischer (1935). The mean values were taken for each character per treatment for each replication. The data collected in percentages were transformed by the angular or $\sin^{-1}$ transformation proposed by Snedcor (1956) before conducting their analysis of variance.

The outline of the analysis of variance table showing

the source of variation, corresponding degrees of freedom of M_1 generation is given below.

Source	Degrees of freedom
Total	79
Block	1
Treatment	39
Error	39

In the M_2 the data was analysed by using 18 x 2 RBD and 9 x 3 x 2 split plot design. The corresponding degrees of freedom are given below:

1. 18 x 2 RBD

Source	Degrees of freedom
Total	35
Block	1
Treatment	17
Error	17

2. 9 x 3 x 2 split plot

Source	Degrees of freedom
Total	63
Replication	1
Main plot	8
Error (a)	8
Sub plot	2
Main x sub	16
Error (b)	18

Table 1. Details of varieties selected for radio-sensitivity analysis in M_1

Serial No.	Name of the genotype
1	Pusa Sawani
2	Co-1
3	Kilichundan
4	Pusa Sawani x Co-1
5	Pusa Sawani x Kilichundan
6	Co-1 x Pusa Sawani
7	Kilichundan x Pusa Sawani
8	Kilichundan x Co-1
9	Co-1 x Kilichundan
10	Sevandhari
11	Anakomban
12	L.S. II
13	L.H.
14	Cochin local
15	Chuvala Venda
16	Kozha local
17	L.H.S. I
18	Karingal local
19	Ola Venda
20	Pilicode local

Table 2. Details of genotypes included for mutation analysis in M_2

Serial No.	Name of the genotype
1	Pusa Sawani
2	Kilichundan
3	Co-1
4	Pusa Sawani x Co-1
5	Pusa Sawani x Kilichundan
6	Co-1 x Pusa Sawani
7	Kilichundan x Pusa Sawani
8	Kilichundan x Co-1
9	Co-1 x Kilichundan

RESULTS

RESULTS

Effect of gamma rays on the M_1 generation

The direct effect of the mutagen on germination and days taken to complete germination; height, number of leaves and branches per plant at different intervals; number of fruits per plant and fruit characters and pollen and seed sterility were estimated in M_1.

Germination

The germination percentage under various treatments is presented in Table 3. Statistical analysis of the data has shown significant variations among the treatments. A significant reduction in germination was noted in the case of G_7, G_{14}, G_{15}, G_{17} and G_{19} compared to their respective controls whereas G_1, G_3, G_4, G_5, G_6, G_8, G_9, G_{10}, G_{11}, G_{12}, G_{13}, G_{16}, G_{18} and G_{20} showed only an insignificant reduction in germination percentage. The germination percentage in control ranged from 26.49 (G_{12}) to 75.53 (G_3) while in 30 kR exposed materials germination percentage varied from 12.74% to 70.08% in (G_{15} and G_{10}) and G_3 respectively. Not much of difference in germination percentage could be observed between control and 30 kR in G_{10} as against a poor germination percentage (12.74) in treated G_{15} population. The germination percentage of the parental varieties as well as of its hybrids on exposure to 30 kR

Table 3. Direct effect of gamma rays on bhindi varieties

Genotypes	Germination (percentage)		Days taken to complete germination	
	Control	30 kR	Control	30 kR
1	63.51	61.17	7.5	7.0
2	37.25	25.82	8.0	6.0
3	75.53	70.08	5.5	7.0
4	55.82	50.04	6.0	8.0
5	68.73	62.40	7.0	7.0
6	68.73	52.81	7.0	6.5
7	66.34	51.79	6.0	9.5
8	55.82	51.78	7.5	7.0
9	61.34	59.09	7.5	7.0
10	71.81	70.08	6.0	8.0
11	68.59	66.01	6.0	6.5
12	26.49	23.66	5.0	3.0
13	33.18	29.03	8.5	8.5
14	55.82	42.09	5.0	4.5
15	42.0	12.74	6.0	6.0
16	35.78	32.63	7.5	8.0
17	50.82	32.15	6.5	7.0
18	51.79	49.82	4.5	4.0
19	31.09	16.70	5.5	5.5
20	45.96	42.11	7.0	7.0
F value	13.63*			1.27
CD value	12.94			NS

* Significant at 5% level

was seen to decrease, the decrease being 2.34-11.43% for the former and 2.25-14.55 per cent for the latter indicative of almost the same percentage of reduction in both the cases.

When the hybrid G_7 showed a significant reduction of 14.55%, its parents G_1 and G_3 showed only an insignificant reduction of 2.34 and 5.45 respectively due to treatments. The reduction in germination percentage noted in G_2 was 11.43%, whereas the same variety in combination with G_3 gave only a negligible reduction (2.25%) when used as female parent and 4.04% when used as male parent. Among the purelines chosen as parents G_2 gave the maximum reduction in germination. The hybrids between G_2 and the other two parents, G_1 and G_3 gave only an insignificant reduction in germination, compared to their controls. The maximum reduction in germination compared to control was noted in G_{15}.

Days taken to complete germination

Number of days taken by different treatments to complete germination is given in Table 3. Statistical analysis of the data showed no significant variation due to treatments. In majority of the cases, treated material showed a greater delay in germination compared to their respective controls.

The genotypes G_3, G_4, G_7, G_{10}, G_{11}, G_{16} and G_{17} took more number of days to complete germination than their respective controls. A negligible variation in days taken to complete germination was noted in G_5, G_{13}, G_{15}, G_{19} and G_{20}. A hybrid material G_7 showed a maximum delay at 3.5 days to germinate compared to its control. The same genotype gave maximum reduction in germination among the hybrid materials. There was practically no difference in the number of days taken to germinate between the treated and control population of G_{15}, which incidentally showed the maximum reduction in germination.

Plant height

The plant height as on the 30th day, 60th day and 90th day of sowing is given in Table 4. The various treatments has brought out significant variation in height. In general it can be observed that treated materials has put up only lesser height as compared to their controls.

The height of plants observed in the control population was in the order of 4.00-16.70 cm, as against a height of 4.07-15.57 cm in the gamma radiated material. Fifteen genotypes (G_1, G_3, G_4, G_5, G_6, G_7, G_8, G_9, G_{10}, G_{11}, G_{12}, G_{13}, G_{14}, G_{16} and G_{20}) showed a decrease in plant height, whereas five other genotypes (G_2, G_{15}, G_{17}, G_{18} and G_{19}) made insignificant increase in plant height, when compared with their respective controls.

Table 4. Direct effect of gamma rays in bhindi varieties - plant height (cm)

Genotypes	Days of observation					
	30 days		60 days		90 days	
	Control	30 kR	Control	30 kR	Control	30 kR
1	13.80	12.42	33.90	33.48	36.41	39.93
2	8.25	10.57	31.88	27.78	40.12	28.56
3	12.16	10.49	26.96	26.75	28.03	29.77
4	16.25	16.18	40.59	43.15	44.71	53.64
5	13.71	11.66	32.03	28.32	34.24	33.76
6	16.70	14.88	41.08	39.50	41.42	42.94
7	13.95	11.02	32.33	31.18	38.47	32.78
8	12.84	10.96	32.55	30.73	33.74	36.07
9	12.25	10.30	31.24	31.91	32.23	33.33
10	11.45	9.00	23.50	27.59	24.03	31.58
11	4.44	3.97	26.50	26.17	43.20	45.88
12	4.67	4.56	27.22	26.92	43.91	44.80
13	6.74	6.27	32.77	29.61	56.37	49.68
14	4.48	4.08	28.77	31.81	49.05	51.70
15	4.33	5.87	28.25	36.18	47.34	51.15
16	6.04	5.56	34.99	31.77	49.20	51.23
17	5.81	6.00	36.97	36.31	54.06	41.82
18	4.00	4.60	22.15	27.90	35.49	42.16
19	4.51	5.74	26.24	25.58	42.34	41.99
20	5.92	4.07	34.42	29.62	52.58	59.86
F value	8.09*		4.82*		1.14	
CD value	4.31		17.88		NS	

* Significant at 5% level

After the 60th day of sowing, in the control population the maximum height gained was 41.08 cm (G_6) and the minimum 22.15 cm (G_{18}). In the treated material the height of plants ranged between 26.17-43.15 cm in G_{11} and G_4 respectively. The other genotypes (G_1, G_2, G_3, G_5, G_6, G_7, G_8, G_{11}, G_{12}, G_{16}, G_{17}, G_{19} and G_{20}) were smaller in height compared to their control. It can also be observed that the growth rate of plants were considerably reduced on exposure to treatment when compared against their respective controls.

At the 90th day of sowing, not much of significant difference could be observed among the different treatments. In the control population, the minimum height (24.03 cm) and maximum height (56.37 cm) were recorded by G_{10} and G_{13} respectively, while among the treated population the maximum height of 59.86 cm was recorded in G_{20} and the minimum height of 28.56 cm recorded in G_{12}.

Number of leaves per plant

The number of leaves produced by the different treatments on the 30th, 60th and 90th day of sowing is presented in Table 5. The different treatments exhibited significant variation for the number of leaves per plant. The number of leaves/plant on the 30th day varied from 2.18 (G_{18}) to 8.06 (G_{10}) in control population and from 2.10 (G_{12}) to 5.54 (G_4) in the treated materials.

Table 5. Direct effect of gamma rays on bhindi varieties - number of leaves/plant

Genotypes	Days of observation					
	30 days		60 days		90 days	
	Control	30 kR	Control	30 kR	Control	30 kR
1	4.38	5.30	10.93	13.55	13.89	15.06
2	3.74	4.51	11.99	9.89	14.59	13.22
3	4.58	4.98	15.87	20.43	15.19	21.26
4	4.80	5.54	8.50	12.55	15.19	16.37
5	4.72	4.52	11.87	16.17	13.47	18.82
6	4.81	4.61	9.85	13.00	12.85	14.94
7	5.08	4.16	12.78	16.39	14.02	16.02
8	4.71	4.77	12.88	16.55	13.51	19.70
9	5.09	4.73	14.14	15.61	15.87	18.09
10	8.06	4.01	8.15	18.43	9.63	17.07
11	2.23	2.20	9.09	10.44	12.25	11.87
12	2.81	2.10	7.46	10.54	9.99	12.48
13	4.33	2.30	9.05	6.88	12.00	11.39
14	2.26	2.30	9.62	9.28	11.25	11.73
15	2.26	2.62	9.19	9.66	12.14	11.64
16	2.62	2.28	12.69	10.81	12.22	12.57
17	2.57	2.31	8.75	9.46	9.46	13.56
18	2.18	2.33	10.02	7.86	11.45	10.86
19	2.21	2.43	7.84	8.13	10.93	10.59
20	2.57	2.37	7.99	10.32	10.79	10.35
F Value	2.42*		8.89*		1.77	
CD Value	2.57		9.90		NS	

* Significant at 5% level

In G_1, G_3, G_4, G_8, G_{14}, G_{15} and G_{19} the treated population produced an insignificant higher number of leaves than their respective controls. Number of leaves per plant in the exposed materials (G_2, G_5, G_6, G_7, G_9, G_{10}, G_{11}, G_{12}, G_{13}, G_{16}, G_{17}, G_{18} and G_{20}) was lesser compared to their controls. An insignificant decrease in the number of leaves produced by treated materials was observed in G_1, G_5, G_6, G_7, G_9 and G_{15}.

On the 60th day of observation also there was significant variation among treatments. The genotype G_2, G_{13}, G_{15}, G_{16} and G_{18} showed a marked reduction in the number of leaves per plant in the treated material compared to their respective controls, whereas in others (G_1, G_3, G_4, G_5, G_6, G_7, G_8, G_9, G_{10}, G_{11}, G_{12}, G_{14}, G_{17}, G_{19} and G_{20}) the number of leaves was insignificantly higher in the treated materials. The number of leaves in all the treated hybrids were higher when compared to their controls. In general, due to exposures an increased vigour in leaf production was noted in hybrids, irrespective of the parents involved.

The number of leaves produced in the treated material ranged from 6.88 in G_{13} to a maximum of 20.43 in G_3 whereas it was 7.46 (G_{12}) to 15.87 (G_3) in control population. The number of leaves produced in the different treatments on the 90th day of sowing showed no significant variation. In the control population the number of leaves produced ranged

from 9.46 (G_{17}) to 15.87 (G_9) while it ranged from 10.35 (G_{20}) to 21.26 (G_3) in the 30 kR exposed materials. The genotypes G_1, G_3, G_4, G_5, G_6, G_7, G_8, G_9, G_{10}, G_{12}, G_{14}, G_{16} and G_{17} showed an insignificant increase in the number of leaves under 30 kR exposures. A reverse trend due to exposure was noted in G_2, G_{11}, G_{13}, G_{15}, G_{18}, G_{19} and G_{20}. All the hybrids produced a greater number of leaves in the treated material than in the control on the 90th day of sowing.

Number of branches per plant

Table 6 represents the number of branches produced by the different treatments, when the plants were 60 days and 90 days old. There was no significant variation among the different genotypes in the number of branches produced as on the 60th day of sowing. In general an increase in branch number was noted in the irradiated population compared to control. At the 60th day of sowing, fourteen genotypes (G_1, G_2, G_3, G_5, G_6, G_7, G_8, G_9, G_{10}, G_{11}, G_{14}, G_{17}, G_{19} and G_{20}) showed an insignificant increase in the number of branches in the irradiated population compared to their respective controls whereas an insignificant decrease in branch number was noted in others (G_4, G_{12}, G_{13}, G_{15}, G_{16} and G_{18}). In the control population the number of branches produced per plant ranged from 0.86 (G_{17}) to 2.69 (G_{16}) whereas it ranged from 1.20 (G_6) to 3.11 (G_3) in the irradiated population.

Table 6. Direct effect of gamma rays on bhindi varieties - branches per plant

Genotypes	Days of observation			
	60th day		90th day	
	Control	30 kR	Control	30 kR
1	1.50	1.55	3.00	1.69
2	1.90	2.38	1.48	3.95
3	2.20	3.11	1.87	2.07
4	2.67	1.64	1.54	1.80
5	1.30	1.73	1.43	2.01
6	1.09	1.20	1.38	1.47
7	1.19	1.80	1.25	2.00
8	2.21	2.26	1.45	2.16
9	2.05	2.37	1.81	2.15
10	1.53	2.86	1.65	2.04
11	1.90	2.37	2.00	2.67
12	2.17	2.03	2.18	2.89
13	1.86	1.85	2.08	2.84
14	2.20	2.74	2.41	2.54
15	2.57	1.53	2.44	1.81
16	2.69	2.42	2.63	2.74
17	0.86	1.96	2.65	2.17
18	2.57	2.32	2.69	2.78
19	1.42	2.06	2.06	1.94
20	1.75	2.11	2.06	2.34
F value	1.21		1.58	
CD value	NS		NS	

There was no significant variation among the different treatments in the number of branches produced per plant as on the 90th day of sowing. In general, an increase in number was observed in treated materials compared to their control. Increase in branch number compared to control was noted in sixteen genotypes (G_2, G_3, G_4, G_5, G_6, G_7, G_8, G_9, G_{10}, G_{11}, G_{12}, G_{13}, G_{14}, G_{16}, G_{18} and G_{20}) whereas the other four genotypes (G_1, G_{15}, G_{17} and G_{19}) showed a reverse trend.

Compared to control, G_1 produced lesser number of branches in the irradiated population, whereas in the hybrids produced by the combination of G_1 with G_2 and G_3, there was an increase in the number of branches for the irradiated population.

Weight of fruit

Table 7 represents the mean weight of fruits expressed in gram due to different treatments. Statistical analysis of the data showed significant variation for the mean weight of fruits among the various genotypes.

In general, an increase in weight of fruits was noted in the irradiated materials compared to their controls. A significant decrease in fruit weight due to gamma rays was noted in G_3, G_{11}, G_{17}, G_{18} and G_{19} when compared to their controls.

Table 7. Direct effect of gamma rays on bhindi varieties

Genotypes	Length of fruits (cm)		Weight of fruits (g)	
	Control	30 kR	Control	30 kR
1	18.40	17.46	20.77	20.83
2	16.58	15.30	17.67	14.96
3	20.81	19.36	18.76	23.93
4	17.53	16.88	16.01	16.64
5	18.36	18.21	20.94	21.60
6	16.59	20.87	16.87	15.80
7	18.11	19.66	21.13	21.60
8	20.74	18.95	20.17	20.47
9	19.33	19.09	22.55	20.34
10	16.89	16.50	18.76	23.25
11	16.61	16.71	13.61	19.26
12	17.84	17.10	20.41	21.09
13	14.46	17.95	14.56	19.47
14	19.70	17.68	22.04	20.49
15	17.33	14.07	14.75	12.83
16	20.76	18.66	26.23	20.29
17	9.50	15.92	22.02	30.70
18	18.23	15.96	21.39	18.13
19	19.04	15.17	22.29	14.82
20	17.10	16.44	20.83	19.94
F value	2.11		2.44*	
CD value	NS		5.0	

* Significant at 5% level

No significant difference between control and treated values was noted in G_{10}, G_{12} and G_{13}. On the other hand, G_2, G_6, G_9, G_{14}, G_{15} and G_{20} indicated an insignificant decrease in fruit weight.

When the pure seeds of G_2 showed a decrease in weight of fruits due to exposures, its combination with G_1 and G_3 gave an increase in weight of fruits in the irradiated material. But when G_2 was used as the male parent a reverse trend was noticed.

Length of fruit

The mean length of fruits (cm) as affected by the different treatments (Table 7) showed no significant variation among the various genotypes.

In the control population the mean length of fruits for the various genotypes ranged from 9.50 cm (G_{17}) to 20.81 cm (G_3) while it ranged from 14.07 cm (G_{15}) to a maximum of 20.87 cm (G_6) in the treated materials.

It has been observed that few genotypes (G_6, G_7, G_{11}, G_{13} and G_{17}) showed an increased length when irradiated, whereas majority of them (G_1, G_2, G_3, G_4, G_5, G_8, G_9, G_{10}, G_{12}, G_{14}, G_{15}, G_{16}, G_{18}, G_{19} and G_{20}) showed an insignificant reduction in fruit length compared to their respective controls.

Table 8. Direct effect of gamma rays in bhindi varieties

Genotypes	Pollen sterility (%)		Seed sterility (%)	
	Control	30 kR	Control	30 kR
1	13.44	22.18	30.12	40.16
2	11.45	21.83	30.02	36.24
3	19.77	21.46	29.38	39.45
4	14.23	29.10	28.32	38.70
5	19.88	20.63	29.11	41.42
6	17.38	24.74	30.83	38.18
7	15.98	28.50	29.67	37.35
8	14.35	28.37	30.40	40.68
9	14.23	23.98	30.35	39.30
10	20.14	20.57	32.01	38.90
11	20.58	27.41	30.41	39.46
12	20.96	25.18	30.19	35.38
13	20.26	28.52	30.36	33.59
14	21.44	27.92	30.58	36.33
15	20.66	29.95	28.60	32.05
16	20.44	29.33	31.39	36.76
17	20.18	25.21	32.09	42.29
18	20.82	27.27	34.42	37.96
19	20.41	28.79	30.67	33.84
20	20.38	32.06	30.98	36.20
F Value	2.995*		9.85*	
CD Value	9.59		12.14	

* Significant at 5% level

Pollen sterility

The pollen sterility induced by gamma rays in different genotypes is presented in Table 8. The different genotypes showed significant variation among themselves and more so when exposed to gamma rays. In general all the genotypes showed a decreased fertility due to the effect of gamma rays. In control population the percentage sterility varied from 11.45 (G_2) to 20.96 (G_{12}). Gamma ray irradiation increased the sterility percentage from 20.57 (G_{10}) to 32.06 (G_{20}). All the genotypes showed a significantly higher pollen sterility percentage due to gamma ray exposure, compared to their respective controls. The percentage increase in pollen sterility due to gamma rays varied depending on the genotype and there was no direct relationship between the sterility percentages in controls and the induced sterility in different genotypes.

Mean number of fruits/plant

Table 9 represents the mean number of fruits obtained per plant in each genotype under gamma ray exposures and also under control. There was significant variation among the different genotypes for the mean number of fruits obtained per plant. While the mean number of fruits per plant in control population varied from 4.26 to 6.35 in G_{13} and G_{15} respectively, the range in value due to treatment

Table 9. Direct effect of gamma rays on bhindi varieties

Genotypes	Yield/plant (g)		Number of fruits/plant		100 seed weight (g)	
	Control	30 kR	Control	30 kR	Control	30 kR
1	154.53	41.12	5.24	2.04	4.38	3.51
2	157.74	43.89	6.58	2.95	12.70	2.87
3	183.40	44.56	6.43	4.05	5.44	2.90
4	156.06	63.42	5.09	3.93	5.11	2.79
5	199.15	80.55	6.19	2.65	5.25	3.39
6	151.17	47.49	6.20	3.27	4.07	3.15
7	183.47	83.60	5.35	2.13	5.04	3.62
8	170.30	100.85	6.20	3.91	4.66	4.39
9	105.10	68.37	6.67	2.84	4.72	3.63
10	162.01	78.50	5.62	2.61	4.22	2.98
11	176.27	75.53	5.81	4.03	4.60	3.67
12	160.86	57.90	4.86	3.72	4.07	2.46
13	148.54	59.10	4.26	3.06	3.43	2.89
14	164.05	84.18	5.88	3.11	4.40	0.12
15	147.70	51.72	6.85	3.76	2.95	2.01
16	175.97	65.45	5.37	3.03	2.94	2.75
17	196.89	41.17	6.72	3.34	3.46	3.09
18	152.59	39.32	3.60	2.72	3.46	2.71
19	147.30	39.75	4.70	2.45	3.33	2.58
20	180.46	65.36	5.33	3.24	2.94	2.73
F Value	17.17*		4.10*		1.15	
CD Value	62.24		2.18		NS	

* Significant at 5% level

was 2.04 (G_1) to 4.05 (G_3). Gamma ray treatment caused reduction in number of fruits in all the genotypes. The rate of reduction, in general was much higher in hybrid population compared to their parents. The percentage reduction in fruit number varied depending on the genotype and there was no direct relationship between the yield performance of the control and that of the reduced yields obtained due to the effect of gamma rays. It has been noted that the poorest yielder, showed a maximum percentage reduction of 28.2 due to the effect of gamma rays and it was 50.3 per cent in the highest yielder.

Mean yield/plant

Gamma ray influenced yield variations in twenty genotypes of Bhindi is depicted in Table 9. Statistical analysis of the data showed significant variation among different treatments.

In all the genotypes whether it was pure or hybrid seeds gamma ray exposure drastically reduced the yield per plant. The mean yield value ranged from 105.10 g in G_9 to 199.15 g in G_5 under control population, gamma ray exposure reduced the mean yield from 100.85 g in G_8 to 39.32 g in G_{18}. Within the control population also, the yield potentiality varied depending on the genotypes. Significantly, higher yields compared to the lowest value of 39.32 (G_{18}) were

noted in all the other nineteen genotypes. Gamma ray exposure invariably reduced the yield in all the genotypes, compared to control. Within the exposed material the yield reduction varied depending on the genotypes. There was no relation between the yield potentiality of the control and percentage reduction due to exposure. The percentage reduction in yield observed as a result of the gamma ray exposure was 35.3 for the poorest yielding genotype (G_{18}) and that for the highest yielding genotype (G_{17}) 78.8 per centage.

Seed weight

Table 9 represents the seed weight from the different genotypes in the control and also the seed weight as affected by 30 kR gamma rays. Statistical analysis of the data showed significant variation among different genotypes.

In the control itself the genotypes showed much variability in seed weight. It varied from 2.94 (G_{16} and G_{20}) to 12.70 (G_2). All the genotypes tested showed a reduced seed weight due to the effect of gamma rays compared to their respective controls. In treated population the seed weight varied from 0.12 in G_{14} to 4.39 in G_8. The percentage reduction in treated population compared to their controls varied depending on the genotype. The maximum reduction in seed weight due to treatment was noted in G_{14}.

Seed sterility

Table 8 represents the seed sterility due to different genotypes and also due to 30 kR exposures of gamma rays. The different genotypes showed significant variation for seed sterility within the control and also in the exposed material. An increased sterility was noted in the treated material compared to their respective controls.

In control population the seed sterility percentage increased from 28.32 (G_4) to 34.42 (G_{18}), while it ranged from 32.05 (G_{15}) to 42.29 (G_{17}) in the treated population. Within the control population there was no significant difference in seed sterility, due to variations in genotypes. But treatment with 30 kR gamma rays showed increase in seed sterility in almost all the genotypes. The induced seed sterility in many genotypes (G_2, G_6, G_7, G_{10}, G_{11}, G_{12}, G_{13}, G_{14}, G_{15}, G_{16}, G_{18}, G_{19} and G_{20}) showed no significant difference compared to their respective controls. The minimum and maximum values were distributed among different varieties, both in control and treated population. The seed sterility percentage varied depending on the genotypes, as in the case of pollen sterility. A maximum increase in sterility percentage compared to control (42.25 per cent) was noted in G_5 and the minimum in G_{19} (10.33 per cent).

Table 10. Gamma ray induced growth characters in M_2 generation

Treatments	Plant height (cm)		Number of leaves/plant		Number of branches/plant	
	Control	30 kR	Control	30 kR	Control	30 kR
Pusa Sawani	55.51	43.83	11.52	10.82	1.25	1.60
Kilichundan	43.15	45.58	10.81	10.92	1.83	1.57
Co-1	54.34	54.95	10.19	11.17	1.53	1.61
Pusa Sawani x Co-1	51.69	53.17	10.82	11.38	1.91	1.57
Pusa Sawani x Kilichundan	45.91	46.09	10.83	11.43	1.50	1.57
Co-1 x Pusa Sawani	48.18	51.37	9.32	11.51	1.25	1.50
Kilichundan x Pusa Sawani	45.23	43.18	10.67	10.52	1.55	1.45
Kilichundan x Co-1	51.62	45.94	11.25	10.62	1.63	1.43
Co-1 x Kilichundan	51.23	47.76	9.10	10.43	1.34	1.52
F Value	0.585		0.668		1.83	
CD Value	NS		NS		NS	

Table 11. Gamma ray induced fruit character variations in M_2 generation

Treatments	Number of fruits/plant		Length of fruit (cm)		Weight of fruit (g)		Yield/plant (g)	
	Control	30 kR	Control	30 kR	Control	30 kR	Control	30 kR
Pusa Sawani	3.97	3.96	15.80	17.29	15.27	16.56	115.48	64.75
Kilichundan	4.65	3.60	21.25	16.31	18.68	17.66	86.63	67.12
Co-1	3.94	4.05	16.83	17.44	17.89	17.49	70.97	97.65
Pusa Sawani x Co-1	3.98	4.49	20.30	17.44	18.11	16.73	72.86	74.23
Pusa Sawani x Kilichundan	4.03	3.91	16.45	18.40	17.23	16.50	69.99	63.70
Co-1 x Pusa Sawani	4.00	3.84	13.61	18.02	14.50	17.72	58.55	91.08
Kilichundan x Pusa Sawani	4.19	3.75	16.24	16.84	15.48	15.37	113.34	57.68
Kilichundan x Co-1	4.75	3.63	16.27	16.41	14.95	17.89	72.80	64.18
Co-1 x Kilichundan	3.12	3.88	20.37	16.24	16.57	16.31	51.81	63.30
F Value	0.65		1.79		1.38		0.96	
CD Value	NS		NS		NS		NS	

Effect of gamma rays on M_2 generation

Plant height

Table 10 represents the mean plant height of various genotypes due to treatments. There was no significant variation among the genotypes for mean plant height. In general there was an increase in height due to 30 kR gamma rays.

Increased height due to the influence of gamma rays was noted in G_2, G_3, G_4, G_5 and G_6 compared to their respective controls, whereas G_1, G_7, G_8 and G_9 showed a decrease in plant height compared to their control. In the control population the height of plants ranged from 43.15 cm to 54.34 cm whereas it was 43.18 cm to 54.95 cm in the exposed material.

Mean plant height under three M_1 sterility classes

The mean plant height for the different genotypes under three different M_1 sterility classes is given in Table 12. Statistical analysis showed no significant differences among the various genotypes, but the three different M_1 sterility classes showed significant differences. The main treatments x sterility classes were also found to be insignificant.

The different genotypes showed difference in mean plant height depending on the M_1 sterility classes. The

Table 12. Gamma ray induced plant height variation under 3 M_1 sterility class (cm)

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	46.88	38.94	45.67
Kilichundan	46.41	45.19	45.14
Co-1	47.13	41.31	56.43
Pusa Sawani x Co-1	28.68	54.15	56.68
Pusa Sawani x Kilichundan	42.33	45.47	50.48
Co-1 x Pusa Sawani	47.33	51.75	54.98
Kilichundan x Pusa Sawani	39.80	45.31	44.44
Kilichundan x Co-1	43.34	44.34	50.12
Co-1 x Kilichundan	28.40	36.47	58.42

	Between treatments	Between sterility class	Interaction
F Value	0.910	4.35*	0.847
CD Value	NS	7.305	NS

* Significant at 5% level

maximum mean for plant height was shown by G_3 (54.93 cm) followed by G_6 (51.35 cm) and the lowest mean plant height was shown by G_9 (41.10 cm). The three sterility classes under G_1 showed significant variations for the mean plant height. Lowest sterility group had the maximum plant height followed by high and medium sterility classes. In the case of G_2, maximum plant height was under S_1 and the lowest was under S_3. In G_3 maximum height was under S_3 followed by S_1 and S_2. In G_4, G_5, G_6, G_8 and G_9 maximum height was shown by S_3 followed by S_2 and S_1. In G_7, S_2 showed a greater height than S_3 and S_1.

In the lowest M_1 sterility class the different genotypes showed variations for plant height. Maximum height was shown by G_6 (47.33 cm), a cross between G_2 and G_1. Here G_2 was the female parent. Another hybrid G_4, a reciprocal cross of the same parent showed the minimum height (28.68 cm). Similarly the different genotypes under S_2 also showed variations in mean plant height. The maximum height was given by G_4 (54.15 cm) and the minimum by G_9 (36.47 cm). G_5 and G_7 which are reciprocal crosses of G_2 and G_3 gave approximately equal heights of 45.47 cm and 45.31 cm respectively.

The mean plant height for various genotypes under S_3 (51.37 cm) was greater than the mean plant height in the other two sterility classes but it showed no significant

difference. It was significantly superior to S_1 mean height of 41.14 cm.

Number of leaves

Table 10 represents number of leaves available for the different genotypes under control and when exposed to 30 kR gamma rays. There was no significant variation for the number of leaves obtained. In general there was an insignificant increase in the number of leaves produced in the exposed material compared with their controls.

An insignificant increase in number of leaves in the exposed material compared to their controls was noted in G_2, G_3, G_4, G_5, G_6 and G_9 while G_1, G_7 and G_8 illustrated a reverse trend. In the control population the mean number of leaves produced ranged from 9.10 (G_9) to 11.52 (G_1) and 10.43 (G_9) to 11.51 (G_6) in the exposed materials.

Mean number of leaves/plant under 3 M_1 sterility classes

Table 13 gives the mean number of leaves per plant for different genotypes under three different M_1 sterility classes. Statistical analysis showed no significant differences among the various genotypes. There was also no significant difference among the 3 different M_1 sterility classes (sub plots). The main x sub interaction was also found to be insignificant.

The different genotypes tested showed variations for

Table 13. Gamma ray induced leaf number variation under 3 M_1 sterility class

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	11.58	9.90	10.99
Kilichundan	11.55	10.61	10.61
Co-1	11.09	11.41	11.02
Pusa Sawani x Co-1	10.37	11.98	11.78
Pusa Sawani x Kilichundan	10.67	11.77	11.87
Co-1 x Pusa Sawani	11.29	10.91	12.33
Kilichundan x Pusa Sawani	11.26	10.46	9.85
Kilichundan x Co-1	10.81	10.33	10.73
Co-1 x Kilichunden	10.47	9.17	11.64
	Between treatments	Between sterility class	Interaction
F Value	1.03	0.822	0.871
CD Value	NS	NS	NS

the mean number of leaves depending on M_1 sterility classes. Maximum value was shown by G_6 (11.51) and the minimum by G_9 (10.43).

The three sterility classes under G_1 showed variations for the mean number of leaves. Maximum number of leaves were obtained for S_1 (11.58) followed by S_3 (10.99) and S_2 (9.90). The same pattern of distribution was observed in G_8 and G_7. In the case of G_2 the mean number of leaves was found to be greater in the case of S_1 (11.55) whereas S_2 and S_3 had equal number of leaves of about 10.61. Maximum number of leaves under G_3 was recorded by S_2 (11.41) followed by S_1 (11.09) and S_3 (11.02). In G_4 the maximum mean number of leaves was recorded by S_2 (11.98) followed by S_3 (11.78) and S_1 (10.37).

In G_5, S_3 (11.87) had the highest mean for leaves followed by S_1 and S_2. The genotypes G_6 and G_9 followed the same pattern in which maximum mean for leaves was shown by S_3 followed by S_1 and S_2. The different genotypes under the three sterility classes also showed variations for the mean number of leaves. The maximum value was obtained under G_1 (11.58) and the minimum by G_4 (10.37) in S_1 sterility class.

In S_2 also the different genotypes showed variations for the mean number of leaves. But there was not much

difference between the genotypes for the mean number of leaves. Maximum mean was shown by G_4 (11.98) and the minimum by G_9 (9.17). In S_3 the maximum mean was shown by G_6 (12.33) and the minimum by G_7 (9.85).

The mean number of leaves in S_3 was higher than in S_2 and S_1 but there was no significant difference in the mean number of leaves among the three sterility classes.

Number of branches

The mean value for the number of branches per plant under the different genotypes is given in Table 10. There was no significant variation among the genotypes for the number of branches produced per plant in the control or exposed materials.

An insignificant increase in the number of branches per plant compared to their controls was noted in G_1, G_5, G_6 and G_9. The genotypes G_2, G_3, G_4, G_7 and G_8 showed an insignificant decrease in value compared to their respective controls.

In control population the mean number of branches per plant ranged from 1.25 in G_1 to a maximum of 1.83 in G_2 when it ranged from 1.43 (G_8) to 1.61 (G_3) in the exposed material.

Mean number of branches as influenced by three M_1 sterility classes

Table 14 gives the mean number of branches for the different genotypes under the three different M_1 sterility classes. Statistical analysis showed no significant difference among the various genotypes, the three M_1 sterility classes and also in the main x sub interaction.

In general the different genotypes showed variations for the mean number of branches obtained depending on M_1 sterility class. Maximum value was shown by G_3 (1.608) and the minimum by G_8 (1.428).

The three sterility classes under the different genotypes showed varying values for mean number of branches. The genotypes G_1, G_3 and G_9 illustrated maximum mean in S_1 followed by S_2 and S_3. Under G_4, S_2 showed the highest mean (1.735) followed by S_1 (1.54) and S_3 (1.435).

In G_4, G_5 and G_7, S_2 had the maximum mean followed by S_3 and S_1. In G_6, S_3 showed a maximum mean of 1.605 followed by S_1 (1.465) and S_2 (1.410). In G_8 the mean number of branches ranged from 1.235 to 1.655 in S_2 and S_1 respectively.

In S_1 maximum mean for number of branches was shown by G_3 (1.95) and the minimum by G_7 (1.32). In S_2 the highest mean was noted in G_2 (1.695) and the lowest by

Table 14. Gamma ray induced branch number variation under 3 M_1 sterility class

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	1.905	1.645	1.255
Kilichundan	1.330	1.695	1.665
Co-1	1.950	1.530	1.345
Pusa Sawani x Co-1	1.540	1.735	1.435
Pusa Sawani x Kilichundan	1.485	1.635	1.575
Co-1 x Pusa Sawani	1.465	1.410	1.605
Kilichundan x Pusa Sawani	1.320	1.675	1.355
Kilichundan x Co-1	1.655	1.235	1.395
Co-1 x Kilichundan	1.725	1.420	1.390

	Between treatments	Between sterility class	Interaction
F Value	0.278	2.060	1.650
CD Value	NS	NS	NS

G_8 (1.235). For number of branches G_2 had the maximum mean of 1.665 and G_1 had the minimum mean of about 1.255 in S_3. The mean number of branches in S_1 was comparatively higher than in S_2 and S_3 but there was no significant difference.

Number of fruits/plant

The mean number of fruits produced per plant by the different genotypes is given in Table 11. Statistical analysis of the data showed no significant variation among the different genotypes. In general there was a reduction in the number of fruits produced due to 30 kR gamma rays. When G_3, G_4 and G_9 showed insignificant increase in the number of fruits in the exposed material, G_2, G_3, G_4, G_7 and G_8 showed a reverse trend. The mean number of fruits in control population ranged from 3.12 (G_9) to 4.75 (G_8) and 3.60 (G_2) to 4.49 (G_4) in the exposed materials.

Mean number of fruits/plant under 3 M_1 sterility classes

The mean number of fruits produced per plant by the different genotypes under three sterility classes is given in Table 15. Statistical analysis showed no significant difference among the various genotypes, the three different sterility classes and also in their interaction. The different genotypes showed different values for the mean number of fruits obtained depending on the M_1 sterility

Table 15. Gamma ray induced fruit number variation under 3 M_1 sterility class

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	3.625	4.290	3.950
Kilichundan	2.850	4.240	3.700
Co-1	3.600	3.680	4.850
Pusa Sawani x Co-1	3.950	4.830	4.610
Pusa Sawani x Kilichundan	3.440	3.780	4.500
Co-1 x Pusa Sawani	3.180	3.810	4.540
Kilichundan x Pusa Sawani	3.950	3.650	3.640
Kilichundan x Co-1	3.470	3.190	4.220
Co-1 x Kilichundan	3.520	3.650	4.470
	Between treatments	Between sterility class	Interaction
F Value	1.50	2.340	0.293
CD Value	NS	NS	NS

class. The maximum mean for the number of fruits was shown by G_4 (4.46) and the minimum by G_8 (3.63).

The three sterility classes under the different genotypes showed variations for the mean number of fruits. G_1, G_2 and G_4 illustrated a case in which maximum number of fruits was obtained by S_2 sterility class followed by S_3 and S_1. In G_3, G_5, G_6 and G_9 maximum mean for fruits was shown by S_3 followed by S_2 and S_1. S_1 had the highest mean of about 3.95 followed by S_2 (3.65) and S_1 (3.64) in G_7. In G_8 highest means for fruits was shown by S_3 (4.22) followed by S_1 (3.47) and S_2 (3.19).

The different genotypes under the three sterility classes showed variations for the mean number of fruits. In S_1, among the different genotypes the highest mean shown was 3.95 (G_4 and G_7). The minimum mean for fruits among the various genotypes was shown by G_2 (2.86). In S_2 the maximum mean was shown by G_4 (4.83) and the minimum by G_8 (3.19). In S_3 the maximum mean of 4.86 was shown by G_3 and the minimum mean of 3.64 was shown by G_7. The mean number of fruits in S_3 (4.28) was greater than the mean in the other two sterility classes. But there was no significant difference among the three sterility classes.

Length of fruits

The length of fruits under different genotypes is given in Table 11. There was no significant variation

among the different genotypes. In general there was an increase in the length of fruit due to exposure. In G_1, G_3, G_5, G_6, G_7 and G_8 an insignificant increase in fruit length was noted in the irradiated population compared to control whereas G_2, G_4 and G_9 showed a reverse trend. In control population the mean length of fruits ranged from 13.61 cm (G_6) to 21.23 cm (G_2) whereas it was 16.24 cm (G_9) to 18.40 cm (G_5) in the exposed material.

Mean length of fruit under three M_1 sterility classes

Table 16 gives the mean length of fruits for the different genotypes under three sterility classes. Statistical analysis showed no significant difference among the various genotypes, the three sterility classes and also in the main x sub interaction.

Taking into account the three sterility classes the mean length of fruits in S_2 was higher than the mean value in S_3 and S_1. But there were no significant differences between the three. The different genotypes showed variations for the mean length of fruits obtained depending on the M_1 sterility class. Maximum mean for length of fruit was shown by G_3 and G_4 (17.44 cm) and the minimum by G_9 (16.23 cm).

The three sterility classes under the different genotypes showed varying means for the length of fruits. In

Table 16. Gamma ray induced fruit length variation under 3 M_1 sterility class (cm)

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	17.60	18.28	16.01
Kilichundan	18.94	15.44	14.55
Co-1	18.88	16.81	16.63
Pusa Sawani x Co-1	17.74	17.74	16.85
Pusa Sawani x Kilichundan	15.12	20.17	19.76
Co-1 x Pusa Sawani	15.39	17.82	20.97
Kilichundan x Pusa Sawani	17.04	17.22	16.26
Kilichundan x Co-1	17.03	16.75	15.45
Co-1 x Kilichundan	15.72	16.92	16.07

	Between treatments	Between sterility class	Interaction
F Value	0.419	0.178	0.794
CD Value	NS	NS	NS

G_1 and G_7, S_2 had the highest mean value followed by S_1 and S_3 whereas G_2, G_3 and G_8 had the highest value in S_1 followed by S_2 and S_3. In G_4, S_1 and S_2 had the same mean value of 17.74 cm followed by S_3 (16.85 cm). The highest mean value in S_2 followed by S_3 and S_1 was noted in G_5 and G_9. In G_6, the maximum mean for length of fruits was shown by S_3 (20.97 cm) followed by S_2 (17.82 cm) and finally S_1 (15.39 cm).

The different genotypes under the three sterility classes showed variations for the mean length of fruits. In S_1 the maximum value for the mean length of fruit was shown by G_2 (18.94 cm) and minimum value by G_5 (15.12 cm). The genotypes G_5 showed the highest value (20.17 cm) and G_2 the lowest value (15.44 cm) in S_2. G_2 had the highest value in S_1 and the lowest value in S_2. In S_3 the highest value for the mean length of fruit was shown by G_6 (20.97 cm) and the lowest value by G_1 (16.01 cm).

Weight of fruits

Table 11 represents the mean weight of fruits for different genotypes. There was no significant variation among the genotypes for the mean weight of fruits. In general there was an insignificant decrease in the weight of fruits when irradiated. An insignificant increase in fruit weight was noted in G_1, G_6 and G_8 when G_2, G_3, G_4,

G_5, G_7 and G_9 showed a decreased value due to gamma ray exposures.

Mean fruit weight under three M_1 sterility classes

Table 17 gives the mean weight of fruits for the different genotypes under three different M_1 sterility classes. Statistical analysis showed no significant differences for the mean weight of fruits for the various genotypes, different M_1 sterility classes and also their interaction. The three sterility classes under the different treatments showed varying mean values.

The genotypes, G_1 and G_7 illustrated a case in which maximum mean for weight of fruits was shown by S_2 followed by S_1 and S_3. In G_2,S_1 had the highest mean of 17.03 g and S_2 and S_3 had an equal mean of 17.53 g. In S_1, G_8 showed the maximum mean of 20.00 g and G_7 showed the minimum of 15.04 g. Similarly in S_2 the maximum mean was 18.27 g shown by G_6 and the minimum (16.35 g) shown by G_9. In S_3, G_6 showed the highest mean of 18.04 g and G_1 the minimum mean of 15.03 g. Taking into account the three sterility classes the mean weight of fruits in S_2 was higher than that in S_1 and S_3. But there were no significant differences among the three sterility classes.

Mean yield/plant

Table 11 represents the mean yield obtained per plant for different genotypes. There was no significant variation

Table 17. Gamma ray induced fruit weight variation under M_1 sterility class (g)

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	17.17	17.49	15.03
Kilichundan	17.93	17.53	17.53
Co-1	17.89	17.62	16.96
Pusa Sawani x Co-1	17.69	16.99	15.50
Pusa Sawani x Kilichundan	15.05	17.73	16.72
Co-1 x Pusa Sawani	16.82	18.27	18.04
Kilichundan x Pusa Sawani	15.04	16.35	14.42
Kilichundan x Co-1	20.00	18.10	15.58
Co-1 x Kilichundan	15.92	16.43	16.56
	Between treatments	Between sterility class	Interaction
F Value	0.744	1.77	0.62
CD Value	NS	NS	NS

among the different genotypes. In general there was a decrease in yield in irradiated materials compared to their controls.

The maximum yield obtained was for G_6 (91.08 g) under 30 kR exposure. The control population gave only 58.55 g fruits per plant. An insignificant increase in fruit yield in G_3, G_4, G_6 and G_9 was noted under gamma ray exposures. The genotypes G_1, G_2, G_5, G_7 and G_8 showed a decrease in yield.

Mean yield/plant under three M_1 sterility classes

Table 18 gives the mean yield of fruits per plant as affected by three M_1 sterility classes. Statistical analysis showed no significant difference among different genotypes, different sterility classes and also in their interaction. The three sterility classes under the different genotypes showed varying mean values for yield per plant.

The maximum mean yield in G_1 and G_7 was shown by S_2 followed by S_1 and S_3. In G_2, G_4 and G_6 highest mean yield was shown by S_2 followed by S_3 and S_1. The class S_1 recorded the maximum mean yield followed by S_3 and S_2 in the case of G_3 and G_8. The genotypes G_5 and G_9 illustrated a case in which maximum mean yield was recorded by S_3 followed by S_2 and S_1.

Table 18. Gamma ray induced yield variation under $^{3}M_{1}$ sterility class (g)

Treatments	Sterility class		
	Low	Medium	High
Pusa Sawani	62.36	74.92	59.97
Kilichundan	50.95	75.70	75.70
Co-1	148.19	64.06	80.68
Pusa Sawani x Co-1	69.41	81.90	71.39
Pusa Sawani x Kilichundan	51.04	66.94	73.12
Co-1 x Pusa Sawani	54.26	136.08	81.90
Kilichundan x Pusa Sawani	58.47	59.79	54.76
Kilichundan x Co-1	69.52	57.21	65.80
Co-1 x Kilichundan	55.86	59.65	74.38
	Between treatments	Between sterility class	Interaction
F Value	1.13	0.168	0.926
CD Value	NS	NS	NS

The different genotypes under three sterility classes showed variations for the mean yield of fruits. In S_1 maximum value for mean yield of fruits was recorded by G_3 (148.19 g) and the minimum value by G_2 (50.95 g). In the case of S_2, G_6 recorded the highest mean value of 136.08 g whereas G_8 recorded the lowest value of 57.21 g. In S_3 the maximum mean value for yield was recorded by G_6 (81.90 g) and the minimum value by G_7 (54.76 g).

The mean yield of fruits in S_2 (75.25 g) was higher than S_3 and S_1. But there was no significant difference between the three sterility classes in the case of mean yield of fruits.

DISCUSSION

DISCUSSION

The induction of mutations by physical and chemical agents is invariably accompanied with the production of undesirable changes in the biological materials. Most part of these undesirable changes resulting from chromosome structural changes and toxicity due to the direct effect of the mutagen are manifested as M_1 damages such as lethality, injury and sterility. For a particular mutagenic treatment there exist a correlation between M_1 damage and M_2 mutation frequency (Gaul, 1959). Efficient treatments producing greater proportion of mutations to damages are essential for the economic use of mutagens in plant breeding. The present investigation was carried on primarily to assess genotypic status in relation to radiation sensitivity in pure and hybrid seeds of Bhindi.

Germination

It has been noted in the present investigation that in all the varieties tested there was a reduction in germination, when exposed to 30 kR gamma rays. Reduction in germination as a result of mutagen treatment, as was noted in this particular crop was reported by many mutation workers in various crops including Gustafsson and Gadd (1965) in Poa pratensis, Rangaswamy (1969) in Sorghum, Roy et al. (1971) in Cucumis sativus, Behera and Patnaik (1979) in

Amaranthus and Masjid (1975) in Lycopersicon. The delay in germination of cowpea seeds following mutagen treatment was reported earlier by Louis and Kadambavanasundaram (1973a). Contradictory reports in which germinability of gamma irradiated seeds were better as compared to the control have been reported by Swarup and Gill (1968) and Rukmanakee (1973) in French bean; Mujeeb (1974) in Cicer and Khan and Hashim (1978) in green gram. Mujeeb (1974) reported earlier germination in gram at low doses of gamma rays. It has also been reported by various investigators that the seed germination is not affected by low doses of ionizing radiations (Sjodin, 1962; Wellensiek, 1965; Ojomo and Chheda, 1971). Sjodin (1962) attributed a physiological explanation for this observation. The first phase of germination is the swelling of cells by hydration followed by enzymatic activation and metabolism. The materials and energy necessary for this initial growth are already available in the seed. So the young embryo has no need to synthesise new substances but only to activate those already stored in the cotyledons. This stage of germination is unaffected by radiations, therefore damage to the embryo which might arise from ionizing radiations result only in post germination mortality. During the course of the present investigation it has also been noted that gamma rays delays germination depending on the genotypes. The late germination observed in the present study

may be due to the influence of mutagens on plant hormones and plant growth regulators, which causes a delay in the initiation of germination (Casarett, 1968). The ability of the plants to recover from the radiation effect may be either by actual repair or by elimination of severely damaged cells (Mahan and Gerhold, 1965).

A genotypic variation in the response of the mutagen as regards the percentage germination was noted in the case of gamma rays. This to a certain extent demonstrates that the germination test can be adopted as a preliminary test to assess the sensitivity of the different varieties of bhindi to mutagens. It has already been reported in various crops that genetic differences, even though they are as small as single gene differences, can induce significant changes in radiosensitivity, which influence not only the total rate but also the spectrum of recoverable mutations (Gustafsson, 1944, 47 and 65; Gustafsson and Tedin, 1954; Nilan, 1956; Lamprecht, 1956 and 1958; Gelin et al., 1958; Smith, 1961; Sparrow, 1961; Konzak et al., 1961a and Sparrow et al., 1965). Mackey (1960 a,b) clearly demonstrated that although nobody is able to predict the influence of a particular genotype on the mutation spectrum, the choice of the parent material is certainly a most decisive part of any programme in mutation breeding.

The influence of mutagens in germination was attributed

by Skoog (1935) and Smith and Keratsern (1942) to the destruction of auxins, while Gorden and Webber (1935) and Gorden (1957) suggested that it would be due to inhibition of synthesis of auxins. It is well recognized that factors such as temperature, water content, oxygen tension, protecting substances in the seed etc. may affect seed germination and growth. Sydorenko (1962) based on his studies on the germination of irradiated corn seeds at higher doses of ionizing and UV radiation suggested that the activities of catalase, peroxidase and isocitric dehydrogenase decreased in the irradiated materials. Brock (1965) after studying the response of Trifolium subterraneum to x-rays and thermal neutrons attributed reduction in germination to radiation induced gross chromosomal breakage. Sinha and Godward (1972) observed reduction in germination in Leus culinaris following gamma ray treatment and attributed the reduction to disturbances caused at physio-chemical level of the cells or acute chromosomal damage or both. Venkateswarlu et al. (1978) noticed reduced germination in pigeon pea, following irradiation and suggested that it may be due to threshold physiological effect of X-rays in the species. The physiological effect of mutagens in inhibiting germination was also reported by Chauhan and Singh (1975), that gamma rays cause disruption and disorganise the tunical layer and results in poor germination of exposed seeds. A most striking effect

is the impairment of mitosis and virtual elimination of cell division in meristematic zone during germination of seeds as reported by Cherry and Hageman (1961). The percentage reduction and delay to complete germination noted in the present investigation can be attributed to gamma ray induced alterations in the cellular events in the process of cell division or other biochemical and physiological activities related to it. Differential effect noticed depending on the genic constitution accounts for various factors such as nuclear volume, DNA value etc. It has been clearly demonstrated that there is an inverse relationship between radiosensitivity and DNA content. Data for the prediction of radiosensitivity of seeds in relation to total DNA content have been published by Gustafsson (1944, 47, 1965); Gustafsson and Tedin (1954); Nilan (1956); Lamprecht (1956, 1958) and Sparrow et al. (1965) clearly reported that any change in the genotypic level can induce significant changes in radiosensitivity which influences not only the total rate but also the spectrum of recoverable mutations. Comparison among varieties of tomato (Bianchi et al., 1963), barley (Mikaelson and Brunner, 1968) and pea (Mukeeb and Siddiqui, 1973) showed variation in respect to radiation response among different genotypes indicating the influence of genetic factors, on radiosensitivity.

Rate of growth in plants

The innate capacity of tissues for growth differs greatly. The type of tissue and state of development determine the nature of treatments that may be successfully applied. The biological responses to mutagen treatments may vary with the chemical composition of the tissue at the time of treatment because competing biochemical reactions will influence the proportion of mutation-yielding reactions.

Growth of plants is governed by the internal metabolism of the system and the external conditions which bear a direct or indirect influence on the former. The presence of an electrostatic field or toxic chemical has been reported to influence plant growth (Ehrenberg, 1960). Irradiation stops DNA transcription and leads to decrease in protein synthesis and growth (Pollard, 1964).

In the present investigation rate of growth was determined by observation on plant height, number of leaves and branching pattern at different intervals.

Reduction in plant height estimated during the seedling stage was more drastic than at the later stages of growth when irradiated thereby indicating an apparent recovery of M_1 plants from injury. Recovery from injury at later stages of growth of cowpea was reported by Louis and Kadambavanasundaram (1973a). The recovery might be due to

the growth of uninjured meristematic cells which replaced the injured ones as growth proceeded. Numerous reports made earlier clearly support the results obtained in the present investigation. Caldecott et al.,(1952) observed reduction in growth rate of barley seedlings following X-irradiation of seeds. Woodstock and Justice (1967) after studies in Zea mays, wheat, sorghum and radish reported a proportional decrease in growth rate depending on the increase in exposure level of gamma rays.

The explanations offered for the delay and reduction in growth rate are many. Smith and Kerasten (1942) attributed the decrease in growth of seedlings following X-ray treatment to the destruction of auxins caused by ionizing radiations. Sparrow et al. (1952) suggested that the abnormal cytological behaviour due to chromosomal damage and mitotic inhibition can be attributed to reduced growth in mutagen treated materials. Pele and Horward (1955) based on their studies on X-rayed seeds suggested that the possible interferences of irradiation with the synthesis of new DNA may lead to inhibition of growth. Gordon (1957) opined that radiation which induce physiological changes may involve a number of interrelated non-specific factors such as inhibition of DNA synthesis and variation in auxin level which may ultimately lead to delay and suppression of growth in the exposed materials. Evans and Sparrow (1961) believed that

the influence of ionizing radiations on growth can be attributed basically to the genic loss due to the chromosomal aberrations. The phenomenon of mitotic delay due to irradiation has been reported as the major cause of growth retardation by Evans et al. (1957) and Evans and Scott (1964). Ananthaswamy et al. (1971) observed inhibition of seedling growth in gamma irradiated wheat seeds and suggested that the adverse effect of seedlings might be due to specific effect on certain respiratory systems operating during crop growth. From a detailed study on the effect of ionizing radiation and post-treatments with growth substances on rice, El-Aishy (1976) concluded that marked decrease in length of coleoptile and first leaf might be due to an increase in the production of active radicals that are responsible for seed lethality or to the increase of radiation induced gross chromosomal alterations which may result in lethality or suppressed growth of seedlings.

Induced pollen and seed sterility in M_1

Sterility is one of the most important M_1 damage induced in plants by mutagen treatment. The intensity of sterility is known to vary depending on the type and dose of the mutagen employed and the material under treatment. The results of the present investigation on pollen and seed sterility revealed a direct relationship with the genotypes

used. It has also been noted in the present investigation that hybrid seeds are more sensitive to radiation compared to pure seeds. Compared to control there was a general reduction of fertility in all the genotypes tested. Bender and Gaul (1966, 1967) and Sato and Gaul (1967) clearly demonstrated that the M_1 sterility depends on the genotypes and induced sterility may vary depending on the ability of the mutagens and their doses in inducing higher percentage of chromosomal aberrations or gene mutations.

Reduction in pollen fertility of M_1 plants is a reliable parameter indicating the effectiveness of mutagenic treatments (Kiwi, 1962). Decreased fertility with increasing doses of mutagens was reported by Zannone (1965) in Vicia sativa, Chekalin (1966) in Lathyrus. Bankowska and Rymaya (1970) in Phaseolus vulgaris, Kaczprzyk (1970) in broad bean, Louis and Kadambavanasundaram (1973a) in cowpea and Bhojwani and Kaul (1976) in pea.

An increased seed sterility was also noted in the present investigation depending on the genotypes used. The cryptic structural differences in chromosomes and chromosomal aberrations are the causes for M_1 sterility with radiations (Gaul et al., 1966). Akhun-zade (1977) reported that chemicals and gamma rays were equal in their capacity to induce chromosome aberrations, but the aberrations induced

by the chemicals were largely eliminated due to ontogeny. Gaul (1970) stated that mutagen-induced sterility may be caused by (1) chromosome mutations, (2) factor mutations, (3) cytoplasmic mutations and (4) physiological effect. Of these, chromosome mutations are probably the major origin of all mutagen induced sterility. Katayamma (1963) found a direct correlation between M_1 sterility and frequency of translocations in rice. Singh (1970) observed that gamma rays induced a high frequency of translocation in rice and this might be correlated with pollen sterility as was noted in the present investigation. Gaul et al. (1966) and Sato and Gaul (1967) reported that radiation induced sterility is mostly haplontic and EMS induced sterility is diplontic in nature. Rao and Lakshmi (1980) suggested pollen sterility to be the result of cumulative effects of aberrant meiotic stages and physiological and genetic damage caused by chromosome breakage following formation of antimetabolic agents in the cell.

Fruit yield per plant

During the course of the present study it was made clear that the mutagens adversely affect the fruit yield per plant. Reduction in yield due to mutagen treatment has been reported in various crops including leguminous crops by Tedin (1954); Zacharias (1956); Gottschalk (1965); Bora et al. (1961); Jana (1962) and many others. Reduction in

yield due to mutagen treatment may be due to their adverse effect on growth and growth rate or due to induced pollen sterility, as a result of chromosome structural aberrations. Caldecott et al. (1954) reported that the reduction in yield in M_1 generation can be due to radiation induced structural changes in chromosomes involving translocations, inversions and deletions. Sree Rumulu (1970) based on his studies in sorghum using X-rays reported that the reduction in yield can be attributed to reduced pollen fertility.

Induced polygenic mutations in M_2 generation

Most of the economically important traits in plants are governed by polygenes. In the present investigation the effects of gamma rays on polygenic traits like plant height, number of leaves, number of branches and number of fruits per plant, weight and length of fruits and yield per plant were analysed. Among the above characters analysed yield per plant and weight of fruits showed significant reduction in mean values in M_2 generation. A reduction in mean values in M_2 generations for the polygenic traits have been reported in various crop plants and it is stated that the shift in mean values in the segregating generation will depend on the frequency of both negative and positive mutants induced.

An insignificant reduction in mean value compared to control population was noted for plant height, number of

leaves and branches per plant, length of fruit and number of fruits per plant. This is in agreement with the previous reports made by Brock (1965); Ehrenberg et al. (1965); Gaul (1967) and Scossiroli (1964) in wheat. In extensive studies performed by Scossiroli (1966 a,b) and Scossiroli et al. (1966) in wheat, this effect was shown in the same population for a large number of characters. Gregory (1965) found that yield of dry peanut pods in the average decreased by irradiation. Oka et al. (1958), Matsuo and Ouazava (1961), Ota et al. (1962), Kavai (1962), Yamaguchi (1964), Ilah and Bhatti (1968) and Sharma and Saini (1970) in rice; Gupta (1970) in barley, Bhatt et al. (1961) in wheat, Daly (1960) and Bhatia and Van der Veen (1965) in Arabidopsis have however reported that there is no significant reduction in mean values in irradiated population. In the case of yield per plant an insignificant increase in mean values was noted. For weight of fruits there was a significant reduction in mean value in treated plants compared to control plants. Gaul (1970) has pointed out that in most instances the mean values of mutagen treated populations are lower than in untreated population. In safflower, Rajendra (1975) has reported a significant reduction in mean value for number of days to flower under gamma ray treatment but for other characters, occasionally, significant positive and negative shifts in mean values were noticed.

There was no significant difference in mean values in M_2 under the three M_1 sterility classes for the different genotypes analysed. But the minimum and maximum values were found to be dependent upon the genotypes concerned and the characters studied. In general a higher mean value was noted in the medium sterility class. This may be due to higher frequency of both positive variants and control types in progenies derived from plants having medium sterility. Chakraborti (1973) reported higher magnitude of variability in progenies derived from low and medium sterile M_1 panicle categories in rice. He has concluded that the low genetic variance in progenies from high sterile spikes might be due to elimination of a number of genotypes as an effect of prevailing sterility. Gaul (1964) reported that the selection of medium fertile spikes might be desirable. Bekendam (1961) in rice has observed that the mutation rate is the same in all fertility classes even the fully fertile group revealed no reduction in mutation rate. But in Bhindi it was made clear in the present investigation that medium fertile M_1 plants will be desirable to yield positive variants in majority of the characters, regardless of the genotypes.

SUMMARY

SUMMARY

The use of radiations to produce genetic variances is accepted as a useful tool of potential value in plant breeding and capable of being employed as an alternative to conventional breeding programmes. As varietal sensitivity is a pre-requisite for any mutation breeding programme, in the present investigation the direct effect of the mutagen (^{60}Co-gamma rays) on twenty varieties of Bhindi including both pure and hybrid varieties was assessed with respect to various growth metrics. The dose level employed being 30 kR. Experiment was laid out in RBD with two replications. Data were collected on (1) germination percentage, (2) days taken to complete germination, (3) plant height at 30 days interval from sowing to complete harvest, (4) number of leaves per plant at 30 days interval from sowing to complete harvest, (5) number of branches per plant at 60 and 90 days after sowing, (6) number of fruits/plant, (7) length of fruit, (8) weight of fruit, (9) yield per plant, (10) pollen sterility, (11) seed sterility and (12) weight of seeds.

Seeds from gamma ray treated hybrids and their parents and their respective controls were carried forward to the M_2 generation to assess the extent of induced variability for various polygenic traits like plant height, number of

leaves/plant, number of branches/plant, fruit characteristics and yield/plant. The tabulated data were analysed statistically and the results interpreted.

Effect on M_1 generation

1. Gamma rays significantly reduced the germination percentage in almost all the genotypes. Hybrid varieties showed more susceptibility compared to their parents.

2. In majority of the cases treated material showed a greater delay in germination compared to their respective controls.

3. A significant reduction in plant height due to exposure was observed on the 30th and 60th day of sowing. An increased growth rate at later stage of crop growth was noted in the treated materials.

4. As in the case of plant height, the exposed materials showed significant reduction in mean number of leaves per plant on the 30th and 60th day of sowing, but on the 90th day there was no significant variation among the various treatments.

5. No significant variation among treatments was noted in the case of number of branches per plant during the different phases of the crop growth.

6. A significant reduction in number of fruits per plant was observed in the treated materials.

7. Yield per plant reduced significantly in the exposed materials, depending on the genotype.

8. Length and weight of fruits also showed significant reduction in treated population compared to the controls. The effect varied depending on the genotypes.

9. A drastic and significant reduction in pollen fertility was noticed due to the effect of gamma rays. Fertility being very less in hybrid materials compared to pure breds.

10. Significantly higher percentage of seed sterility was induced by the mutagen. The percentage varied depending on the genotype.

Effect on the M_2 generation

1. No chlorophyll deficient mutant was observed in M_2 generation.

2. The mean values shifted both in minus and plus directions depending on the character.

3. Though there was no significant difference in plant height among the treatments, there was an increase in height due to 30 kR gamma rays.

4. The three different M_1 sterility classes analysed showed significant variation for mean plant height. The values dependent on the genotypes tested.

5. No significant variation for number of leaves per plant was noted due to 30 kR exposures or due to the three different M_1 sterility classes.

6. Number of branches per plant also failed to show any significant variation due to different treatments or due to the three different M_1 sterility classes analysed separately.

7. The different genotypes under 30 kR exposures, three M_1 sterility classes and their interaction showed no significant difference in the case of number of fruits per plant in M_2.

8. Weight of fruits also showed no significant difference among the different genotypes under 30 kR, due to the effect of three different M_1 sterility classes and also in their interaction.

9. Length of fruits and yield per plant also showed the same trend as in the case of number and weight of fruits per plant.

10. In all the cases studied the maximum and minimum mean values varied depending on the genotype.

11. The influence of M_1 sterility classes on mean values also varied depending on the genotypes.

Thus in the present investigation, both in M_1 and M_2

generations the character expression due to the effect of 30 kR gamma rays varied depending on the genic status of the material under study. Insignificant shift in mean values in M_2 generation for various characters studied give further scope for selecting desirable plants either in negative or positive direction of control plants. The present investigation clearly demonstrated that in Bhindi also the genic status is a decisive factor in any induced mutation breeding programme. A wild type of bhindi isolated out from M_2 generation promises further scope, as it seems to escape from the yellow vein mosaic disease. Hybrid materials are more sensitive to mutagen compared to pure breds.

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* Originals not seen

GENIC STATUS IN RELATION TO RADIO SENSITIVITY, MUTATION FREQUENCY AND SPECTRUM IN BHINDI

BY

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ABSTRACT OF THE THESIS
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ABSTRACT

The effect of 30 kR ^{60}Co-gamma rays on different genotypes of Bhindi (Abelmoschus esculentus Moench) have been studied in detail in M_1 and M_2 generations. The experiments were conducted during 1981-83 at the Department of Agricultural Botany, College of Agriculture, Vellayani. There were 20 different genotypes including both pure and hybrid seeds. The M_1 generation was laid out in RBD with two replications and maintained following the package of practices recommended for this particular crop. The radiosensitivity of the different genotypes were tested based on the direct effect of the mutagen in M_1 generation. The treated hybrid materials along with their control, parental varieties and 30 kR exposed parental types were carried further to M_2 generation to assess the extent of variation created in the segregating M_2 generation. Proper lay out, maintenance of the crop and data collection were followed in segregating generation also. The data collected were statistically analysed for proper interpretation of the results obtained.

To assess the direct effect of the mutagen in M_1 generation various growth metrics such as germination percentage, days to complete germination, plant height, number of leaves and branches per plant at 30 days interval, pollen and seed sterility and various fruit characters including yield

per plant were taken into consideration. All the characters analysed showed difference in expression depending on the genotypes concerned. Majority of the growth characters showed significant reduction in gamma ray treated population compared to their respective controls. A delay in germination was noted in majority of the genotypes. Growth metric analysis clearly demonstrated that eventhough treatment delays the crop growth in early stages, at later phases of growth the plant rectifies itself and attains maximum expression as in the case of control population. All the genotypes tested showed increased pollen and seed sterility due to gamma ray exposure, which directly influences the number of fruits produced per plant. Yield per plant showed significant variation among the treatments and in majority of the genotypes gamma rays significantly reduced yield potentiality of the plants. Based on seed sterility percentage, it was possible to group the M_1 plants under low, medium and high sterile types.

Induced variations on plant height, number of leaves and branches per plant, length and weight of fruits and mean yield per plant were assessed in M_2 generation. No significant variation between treatments were noticed in any of the characters studied. In this case also a genotype influenced alteration in shift in mean values was observed in almost all the characters. The analysis on the influence of the

three M_1 sterility classes on character expression showed no significant variation. Both negative and positive shift in mean value compared to control values were noted depending on the genotypes. The insignificant variation can be attributed for equal frequency of both negative and positive variants and promises good selection response. A systematic mutant having the wild characteristics of this particular crop variety isolated from M_2 generation promises wider scope as it escapes from the most disastrous disease of Bhindi, yellow vein mosaic disease.

PLATES

Plate 1. General stand of the crop in the field

Plate 2. Leaves showing chlorophyll deficiency in M_1 generation (control leaf in the centre)

Plate 3. Twin fruits developed from fused buds in M_1 generation (normal fruit on the left)

Plate 4. Fruits showing seed sterility in M_1 generation (control on the left extreme)

Plate 5. A view of the normal and sterile seeds in M_1 generation

Plate 6. Variation in fruit length in M_1 generation

Plate 7. An isolated view of the wild mutant obtained in the M_2 generation.

Lark

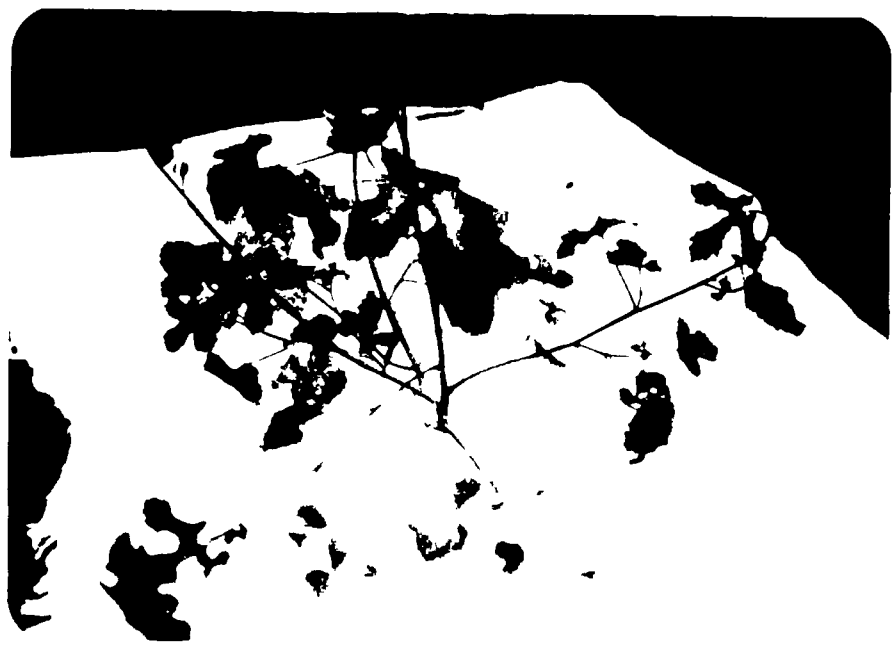

Plate 8. A closer view of the wild mutant in M_2 showing flowers and fruits

Plate 9. A comparative view of a normal fruit and the fruits from the mutant plant in M_2 generation.

Plate 10. Plant height variation obtained in M_2 generation

Plate 11. Variation in branch number in M_2 generation