

**EFFECT OF GAMMA IRRADIATION ON SEED
CHARACTERISTICS IN RESTORER LINES OF
SUNFLOWER (*Helianthus annuus* L.)**

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NOVEMBER, 1995

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Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfilment of the requirements for the
Degree of

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in
GENETICS AND PLANT BREEDING

By
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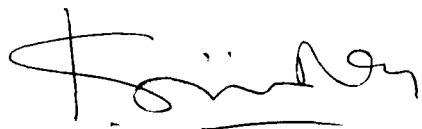
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CERTIFICATE

This is to certify that the thesis entitled "EFFECT OF GAMMA IRRADIATION ON SEED CHARACTERISTICS IN RESTORER LINES OF SUNFLOWER (*Helianthus annuus* L.)" submitted by Mr. SANJEEV K. DESHPANDE in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in GENETICS AND PLANT BREEDING, of the University of Agricultural Sciences, Dharwad, is a record of research work done by him during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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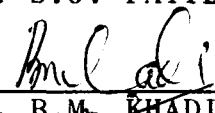


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affectionately dedicated to...

*my parents Smt. Chaya Deshpande and
Shri Krishnaji Deshpande,
aunt Smt. Hema Dixit and
all my teachers*

*.....who taught, guided
and enlightened
me with love*

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INTRODUCTION

I INTRODUCTION

The main objective of plant breeding is to assemble desirable genes in a genotype. To achieve this goal, sufficient variability should be ensured in the genetic material. If a particular desirable allele is not present in the genetic pool, or else the variability existing in the genetic material is exhausted, then one of the most important approaches to create variability anew is mutation breeding. Stadler's (1928) demonstration that new variations can be induced artificially in plants through mutation was a significant achievement in plant breeding. Artificially induced mutagenesis provides an additional promising approach in generating new alternate forms of an allele to create new variability in a population.

Sunflower (*Helianthus annuus* L.) as a supplemental edible oilseed crop, was introduced to India in 1969. However, commercial cultivation on large scale started only in 1972-73 onwards with the introduction of Russian varieties like EC68414 (Peredovik), EC68415 (Armaviriski, 3497) and Morden (Cernianka-65). With the development of array of hybrids and varieties adopted to the varied ecological

conditions prevailing in the country sunflower has now established as a potential oilseed crop. In recent years there has been shift in breeding approach from population breeding to heterosis breeding. Although, cultivated hybrids, have been derived only from *Petiolaris* cytoplasmic base, restorer lines have been derived from many wild species. The fertility restorer lines are of two types: branching and nonbranching. The branching behaviour being controlled by recessive genes is not expressed in hybrid combination. Majority of branching restorer lines have low test weight ranging from 1.8 to 3.0 g. Test weight is one of the important direct yield components (Giriraj et al., 1990; Encheva et al., 1993). Improvement of test weight of restorer line would help in increasing yield potential of hybrids. The branching restorer lines have distinct advantage over nonbranching types in hybrid seed production plots as pollen supply to female parent is ensured over long period. Hence, in branching genotypes, there is need to increase test weight. While increased seed weight is given thrust in breeding programmes, simultaneous consideration be also given for increased *per se* oil content.

In nonbranching restorer lines, the monohead character in general is coupled with comparatively high test

weight. But in hybrid seed production plots both the parental lines (cms and restorer lines) should have perfect synchrony in flowering to ensure high seed set. Although synchronous flowering could be achieved by staggered planting, longer duration of pollen supply as exists in branching genotypes does not exist in nonbranching types. Hence, it is desirable to have restorer lines with branching types coupled with high test weight.

[Mutation breeding has shown promise in creating wide variability for seed characteristics and economic quantitative traits, viz., days to flowering, test weight and oil content. Although conventional breeding procedures are adopted to improve the parental lines for *per se* oil content, seed yield and test weight, mutagenesis has been found to be effective in achieving the above objectives in a short period (Giriraj et al., 1990). The induced mutations have been found quite effective in generating useful variation for polygenically controlled traits (Kumar, 1982). Mutation breeding studies have been encouraging in other important oilseed crops like Brassica, Castor, Rapeseed, Sesame, Groundnut and Linseed (Gupta and Swaminathan, 1967; Swamy Rao, 1970; Ankineedu and Kulkarni, 1969; Shopta et al., 1991; Kamala, 1990; Murty,

1988, 1990; Busolo, 1987; Chandra Mouli and Kale, 1987, 1990; Dutta and Aruna chalam, 1987; Sarker and Sharma, 1988; Singh and Srivastava, 1988; Ramani and Jadon, 1991; Bachyalis, 1988).

Keeping foregoing points in view, two restorer lines, viz., IV83 (nonbranching) and RLC2 (branching) were selected and exposed to gamma irradiation treatments with the following objectives.

- i. To create variability for seed characteristics like test weight, oil content and other economic traits.
- ii. To study the branching behaviour of two different types of restorer lines, on irradiation.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Hugo de Vries (1909) for the first time recognised the role of mutation in evolution. However, a method for producing genetic variability artificially was not discovered until late 1920's when Muller (1927) working with *Drosophila* and Stadler (1928) with Barley and Maize showed that x-rays can induce mutations. After Muller's and Stadler's work, the potential significance of this method as a technique for inducing useful mutations in plants was showed by several pioneer workers in this field like Nilsson-Ehle (1948), Goodspeed and Uber (1939), Gustafsson (1947, 1954 and 1979), Catcheside (1948), Muller (1955, 1959), Mackey (1956), Gaul (1958, 1963), Gregory (1961), Brock (1971), Stubbe (1959), Swaminathan (1969), Sparrow *et al.* (1971). Guidelines for practical mutation breeding methods have been given by Gottschalk and Wolff (1983) and in manual of Mutation Breeding, IAEA (1977). Studies on mutagenesis are rather scanty in sunflower (Giriraj *et al.*, 1990). This may be either due to unexploited genetic variability already existing or may be due to its recent introduction in the Asian and African continents. Hence, in the following paragraphs,

literature on other oilseed crops pertaining to present investigations are briefly presented.

2.1 SENSITIVITY OF GENOTYPES TO MUTAGENS

2.1.1 Effect on germination and survival

Physical and chemical mutagens have their effect primarily on germinability and has been studied by many workers. Gills and Devinck (1959) found that there was decrease in germination in groundnut with successive increase in dose of gamma rays from 1 to 20 Kr.

Reduction in germination and differential response of mutagens to varieties in different crop plants are on record (Shivaraj *et al.*, 1963 in groundnut; Avdhani and Ramana Rao, 1968 in groundnut; Sahai and Dalal, 1970 in safflower; Shakvarnikov and Morgan, 1976 in maize; Shakvarnikov *et al.*, 1977 in wheat; Reddy *et al.*, 1977 in groundnut; Ilyas and Goud, 1978 in sunflower; Sahu and Kumar, 1978 in sesame; Nair and Nair, 1977 in sesame; Chandrappa, 1980 in sunflower; Belyaeva, 1987 in soybean; Cheah, 1988 in groundnut; Singh *et al.*, 1988 in groundnut; Bhatnagar *et al.*, 1989 in soybean, Ramani and Jadon, 1991 in groundnut; Tripathi and Dubey, 1992 in Lentil).

2.2 INDUCED POLYGENIC VARIABILITY

The quantitative characters are governed by many genes with each gene contributing cumulative effect to the total phenotype. Such genes are commonly called as polygenes and the characters governed by them show continuous variations. These quantitative traits, controlled by many genes are highly influenced by the environmental fluctuations and hence any micromutations taking place at few base pair levels cannot be detected visually. In contrast, macromutations that result from mutation in the major genes show striking qualitative characters and are easily detected through visual observation.

The usefulness of micromutations in plant breeding as compared to macromutations, has been emphasized by several workers (Lawrence, 1965; Gaul, 1965 and Scossiroli, 1965), primarily because unlike macromutations, micromutations occur in higher frequency as they involve simple transitions or transversions for individual base pairs. In addition micromutations are less drastic with higher vitality and fertility than macromutations.

2.2.1 Groundnut

The first extensive study of induced mutagenesis in groundnut was made by Gregory and co-workers using x-rays and they obtained good results (Gregory, 1955, 1956, 1957, 1960). In the M₃ generation Gregory selected large number of vigorous plants and about 10 per cent of these were developed into lines having higher yield levels as compared to control. The released variety NC4x developed from a normal plant was high yielding with good pod and seed quality. He also observed a negative relation between mean values and variances for pod yield in irradiated parental populations.

Similar studies on induced mutations in groundnut to create variability for seed characteristics have been reported by several workers (Bilquez and Martin, 1961; Bilquez, 1962; Patil, 1966, 1971; Patil and Thakare, 1969; Ashri and Goldin, 1965).

Several improved varieties with various attributes such as large kernels, higher yield and shelling percentage have been isolated following direct selection in x-ray irradiated populations at BARC, Trombay (Patil, 1966, 1971 and Patil and Thakare, 1969). The varieties TG-1 and TG-3 were developed following x-ray treatment of Spanish Improved at

75Kr and 25 Kr and selections for kernel weight and number of branches.

Shivaraj *et al.* (1963) observed reduction in mean values of various quantitative traits following irradiation of one bunch and one spreading variety with thermal neutrons. Following gamma irradiation and fast neutrons they observed reduction in height and yield. A dwarf plant in gamma irradiated population particularly at higher doses, was isolated by Sanjeevaiah *et al.* (1967) and found that the yield was reduced by irradiation treatments of higher dosage. Avdhani and Ramana Rao (1968) observed increase in the mean values for shelling percentage and test weight following irradiation treatment. In a similar study Martin (1968) observed increased variance in M_2 generation with regard to pod size and oil content following irradiation treatment. Higher response to selection was apparent in the irradiated population. Gaul (1963 and 1965) and Brock (1971) proposed that the frequency of micromutations are many times more than macromutations.

These findings gave impetus to employ physical and chemical mutagens in practical plant breeding programmes to induce genetic variability for seed characteristics.

Reddy *et al.* (1977) irradiated groundnut seeds with gamma rays and reported that the mean value in M_2 decreased over control for height, branch length and number of nodes, while it increased the variation for these characters. Sinha and Rahman (1979) derived early and mid early maturing mutants from late maturing variety in groundnut by irradiating with gamma rays. In another experiment, Ramanathan (1983) reported that the treated plants gave higher values for genotypic variance, heritability, genetic advance and seed yield in M_3 than control. More than 50 per cent reduction in leaf size was observed by Patil and Chandra Mouli (1984) in groundnut, following gamma irradiation. Results of Prasad and Kaul (1984) indicated that compact canopy frame obtained following irradiation treatment increased the yield in Virginia genotypes and also increased variation for pod number, branches and dry weight in Spanish type following the treatment with EMS, NMU and gamma rays. Colchicine induced dwarf mutant was isolated by Tiwari and Khanorkar (1984) that had fewer pods and smaller leaflets than the untreated plants. Pathirana (1985) reported a gamma irradiation induced mutant having higher shelling percentage, larger kernels and more of two-seeded pods than the recommended cultivar. In a similar study, Chandra Mouli *et al.* (1987) isolated mutants in

groundnut that gave higher pod yield and increased pod and seed size compared to control. The mutants gave a pod yield of 4083 kg/ha as against 2480 kg/ha pod yield in control.

Dutta et al. (1987) irradiated 22 F_3 family selections of groundnut with 30 Kr gamma rays and showed F_5 performance to be improved for pod yield, shelling percentage and 100-seed weight over those obtained in the untreated F_3 family selections. An increase of 32 to 42 per cent in seed size and 1 to 18 per cent in seed yield per plant over the parent was reported by Cheah (1988) in groundnut. He also found that lower irradiation doses led to better performance in terms of pods per plant and pod dry weight over control. Change from spreading to semi compact habit was reported by Singh et al. (1988) following the treatment with gamma irradiation. They also obtained the mutants that were early and late maturing ones. In another study, Manoharan and Thangvelu (1990) reported a mutant with bold seeds having 100-seed weight ranging from 22.20 to 40.50 compared to 21.20 in control in groundnut following gamma irradiation. Ramani and Jadon (1991) irradiated the seeds with 10, 20, 30, 40 and 50 Kr doses and assessed the yield components in M_2 generation. They observed reduced plant height, number of leaflets and delayed flowering in treated M_2 populations.

Moderate to high levels of heritability accompanied by high levels of genetic advance were recorded for pod weight per plant, pods per plant and seeds per plant.

2.2.2 Sunflower

Chandrappa (1980) exposed the sunflower achenes at various doses of gamma rays, EMS and DES and found increased mean values for yield per plant and oil content and decreased mean for days to flowering and plant height. He also reported that gamma irradiation treatments significantly increased the variance, coefficient of variability, heritability and genetic advance values with respect to economic traits in M_2 generation. Leclercq (1984) reported that gamma ray treatment induced a dwarf sunflower mutant of less than 70 cm, with an almost normal duration. In another study, Giriraj et al. (1990) induced variability in parental lines of BSH-1 sunflower hybrid for days to flowering, 100-seed weight and oil content. Various doses of gamma radiation viz., 5 Kr and 10 Kr and EMS treatment at 0.2%, 0.3% significantly increased the variability for days to flowering, test weight in M_2 and M_3 generations. Early and late flowering mutants were isolated. In all mutagenic treatments, the mean test weight shifted in positive direction. They found that EMS was more

effective than gamma rays in creating a wider range for test weight and the variability created for important yield components provided scope for selection.

Encheva *et al.* (1993) irradiated zygotic embryos of sunflower with gamma rays and observed changes for days to flowering, plant height, head-diameter and 100-seed weight.

2.2.3 Rape seed and mustard

Gamma irradiation treatment led to a decrease in the mean values for seed yield per plant and oil content, while the coefficient of variation for many quantitative traits increased, as reported by Ramkumar *et al.* (1988).

Mahla *et al.* (1991) irradiated the seeds with 80, 100 and 120 Kr gamma rays and obtained enhanced yield levels in irradiated population, but the mean values of siliqua length and seeds per siliqua were reduced. An improved variety for seed yield through induced mutation following irradiation was obtained by Rahman and Das (1991).

2.2.4 Soybean

Rajput (1987) reported induction of polygenic variability in soybean following irradiation at 10, 15, 20 and

25 Kr doses. Changes were seen in M_2 generation in mean values for yield components viz., seeds per plant and grain yield per plant. Coefficient of variability for these traits, increased which provided scope for selecting high yielding plants. Similarly, high yielding mutants in M_2 showing earliness, determinate growth habit and short stature following different doses of gamma rays and fast neutrons were obtained by Rajput and Sarwar (1989). Increased seed yield in soybean by x-ray irradiation at 25 and 35 Kr doses was reported by Khurana (1991). They produced high yielding mutants with seed yield of 30g per plant and 26g per plant compared to control with 17g per plant. They also found that mutants were superior for pods per plant, seeds per pod, 100-seed weight besides early in maturity.

Wang (1991) exposed three genotypes of soybean to 8 doses of gamma radiation and obtained changes with respect to plant height, seed weight per plant and 100-seed weight in M_1 and M_2 generations. He also observed increased coefficient of variation and widened range for the traits in the irradiated population.

2.2.5 Castor

A classical work on induced mutagenesis in castor was made by Ankineedu and Kulkarni (1965). They studied the

effect of radiation on number of fruit locules and found that in M_2 generation, the number of tetralocular capsules increased following gamma irradiation. Ankineedu *et al.* (1968) studied the effect of gamma rays and fast neutrons on seeds of castor variety HC-6 and observed changes in the mutants for number of flowers, capsules, spikes, seed size and yield. In another study, Sindagi and Ansari (1969) isolated a dwarf mutant with reduced internode length in castor (HC-6) following gamma irradiation. A new castor variety RC8 following 40 Kr gamma ray treatment of RC 1188-54 was reported by Sindagi *et al.* (1978). RC8 variety gave more number of internodes, higher seed weight and 10-47 per cent more yield than control. A significant positive shift in the mean values for days to flowering and harvest index and negative shift for capsules per raceme were the main features of gamma ray treatment of castor (Athma and Reddy, 1982). Likewise, an increased phenotypic variability for days to flowering, seed yield per plant and harvest index was the main feature of gamma irradiation of castor (Prasanna and Reddy, 1986). Chauhan (1992) irradiated dry seeds of castor cv. Aruna with 100 and 125 Kr gamma rays and obtained 3 female M_2 mutants viz., GRFM₁, GRFM₂ and GRFM₃ showing higher yield and yield components compared to non-irradiated parent.

2.2.6 Sesame

A high yielding mutant in sesame was obtained by Kamala and Sasikala (1983) after irradiating with gamma rays at 20 Kr. An increased variation for capsules per plant, seeds per capsule, seed weight per capsule and seed weight per plant was observed in sesame following gamma irradiation by Hassan *et al.* (1984). Likewise, Reddy (1986) found that induced variability in yield related characters in M_2 increased with increasing dose of gamma irradiation. He also reported that the mean value for traits like capsule number per plant, capsule length increased in the irradiated population. The highest levels of genotypic and phenotypic variance for all the seed attributing characters following irradiation treatments was reported by Kamala (1990).

2.2.7 Safflower

In safflower, induced mutagenesis significantly increased the phenotypic and genotypic coefficient of variability, for seed yield per plant and number of heads per plant and decreased value for days to flowering (Sahu and Kumar, 1980).

2.2.8 Linseed

Following the treatment with EMS, x-rays at 30 Kr and 60 Kr in two varieties of linseed, Sinha *et al.* (1981) produced high yielding M₂ plants coupled with high oil content.

2.3 MUTATIONS FOR QUALITY CHARACTERISITICS

The economic value of an oilseed crop is determined by its oil content. Mutation breeding has been found to be a powerful tool in modifying oil content as well as fatty acid composition.

Soldatov (1971) observed increased genetic variability for oil content in M₃ generation in sunflower following treatments with chemical mutagens viz., NMU, NEU and DMS. He isolated plants with 1-2 per cent higher oil content compared to control. Srinivasachar *et al.* (1972) isolated mutants with high iodine value in addition to increase of oilcontent in linseed following gamma irradiation and EMS treatment. In Brassica, Axtell (1979) induced a mutant for low erucic acid which enabled cultivation of this species as an edible oilseed crop. Similar results were reported in Brassica by Robbelele and Nitsch (1975).

Vranceanu and Stoenescu (1982) altered the oil composition in sunflower by mutation. They found that

irradiation with high energy radiations were effective to alter the oil content. Kubler (1984) also studied the possibilities of altering the oil content in sunflower by mutagenesis. Following the treatment with EMS and gamma rays he obtained mutants with oil content ranging from 9.1 to 61.9 and 9.4 to 58.8 per cent against 8.7 to 56.5 per cent in control. A successful attempt to isolate zero erucic acid content in Brassica was made by Laakso *et al.* (1986). Similar study was made in Brassica by Rakow *et al.* (1987).

Chandra Mouli *et al.* (1987) induced high oil content mutants in groundnut following various doses of gamma irradiation. In contrast, Ramkumar and Yadava (1988) reported decrease in mean value for oil content in Brassica following gamma irradiation at 100 Kr.

Takagi *et al.* (1989) exposed the seeds of soybean to 25 Kr x-rays and found that variability for oil content was increased markedly in M_2 as a result of irradiation. They identified mutants containing 18.4 per cent linolenic acid compared to 9.4 per cent in the parent. Percentages of palmitic, oleic and linolenic acid decreased in the mutants. By gamma irradiating the seeds of groundnut at 20 Kr Chandra Mouli and Kale (1990) obtained a mutant with lower oil content

with 38.6 per cent against 44.56 per cent in control. In a similar study Shpota and Bochkaeva (1990) obtained a mutant with low erucic acid content in rapeseed following irradiation with 50 to 150 Kr gamma rays. Takagi *et al.* (1990) obtained a mutant with low linolenic acid content in soybean following x-ray irradiation at 21.4 Kr. They observed an inverse relationship between linolenic acid content and irradiation treatment. In a similar study Bhatnagar *et al.* (1992) observed a greater range and variability for oil content in the mutants than in the parents following gamma irradiation treatment at 15, 20 and 25 Kr doses in soybean. However, the mean value for oil content was shifted in a negative direction in the mutants. Giriraj *et al.* (1990) treated the sunflower seeds with gamma rays at 5 Kr and 10 Kr doses and also with EMS separately at 0.20 per cent and 0.30 per cent and observed a widened range and increased variability for oil content. They also noticed a shift in mean value of oil content in positive direction.

2.4 INDUCED VARIABILITY FOR QUALITATIVE CHARACTERS

Qualitative characters are governed by one or few major genes and are relatively less sensitive to environmental fluctuations. Hence any induced change for these qualitative

traits can be easily detected by visual observation. The genes controlling these characters linked with polygenic loci can be used as morphological markers in plant breeding. Changes in qualitative characters can be easily induced by generating new alleles with the help of mutation and can be used in plant breeding (Kulkarni and Ankineedu, 1967). They observed a mutant with nonshattering behaviour in castor in M_2 . In another study Ankineedu *et al.* (1968) derived green stemmed dwarf mutants in castor as against brown stemmed tall plants in parent following gamma irradiation. Likewise Sindagi and Ansari (1969) modified the branching habit in mutant following gamma irradiation of seeds of HC6 with 50 Kr.

Patil *et al.* (1985) treated seeds of soybean with 5 doses of gamma rays and obtained green and yellow seed coat colour in mutants as against original black coloured, seedcoat in the control. In a similar study in sunflower, Coppola (1986) irradiated *Helianthus tuberosus* with 3 Kr gamma rays and obtained white coloured tubers in contrast to red coloured tubers in parent. He also observed thin stem with negligible branching character as against thick stem and more branching behaviour in control. Murthy (1988) isolated a tall mutant in M_2 following gamma irradiation in sesame with the mutant

character controlled by a single recessive gene. Takagi (1989) obtained tiny mutant seeds in soybean following x-ray irradiation at 25 Kr dose as against large sized seeds in control. Likewise in soybean Bhatnagar *et al.* (1990) isolated mutants with white flowers as against purple in the parent following gamma irradiation at 15-25 Kr doses. In addition they obtained mutants with yellow seed coat colour as against black seeded types in the parent.

2.5 CHLOROPHYLL MUTATIONS

In case of induced mutagenesis occurrence of chlorophyll mutations is a common feature of irradiated populations. Chlorophyll mutations are easily detectable and can also occur in substantial frequency. Wide variation exists in chlorophyll mutations from lethal, semilethal types to complete viable types.

Stadler (1928) showed that chlorophyll mutations can be easily induced in diploid plants by ionising radiations. Chlorophyll mutations are often used as a measure of mutation rate and has been used as an indication of effectiveness of the mutagen (Gaul, 1960; Konzak, 1965; Sears, 1972 and Viraktamath, 1975).

Frequency of chlorophyll mutations is expressed in different ways, such as i) On M_1 family basis (Gustafsson, 1940), ii) On M_2 spike basis (Stadler, 1929) and iii) On M_2 plant basis (Gaul, 1960), Gaul (1960) proposed that chlorophyll mutation frequency expressed per 100 M_2 seedlings is the best index, as it is proportional to the initial mutation rate, individual variations in the progeny and size of the mutated sector. Goud (1967) evaluated the three methods of expressing chlorophyll mutation frequency in wheat and concluded that frequency expressed on primary tiller basis is the best index for tillering plants. However, expressing the chlorophyll mutation frequency on 100 M_2 seedlings appears to be the best index for all types of plants (Sharma and Swaminathan, 1969; Mohan Rao, 1972 and Viraktamath, 1975).

In case of castor, Prasanna and Reddy (1986) exposed the plants to gamma rays at the seedling, meiotic and post gametic stages and found that irradiation at the gametic stage gave the highest frequency of chlorophyll mutants. Similarly in Lentil Pratibha and Dubey (1986) induced chloromutations by separate and simultaneous application of gamma rays and NMU. They found that both the mutagens were equally effective for inducing chlorophyll mutations and reported that M_2 chlorophyll mutants obtained included xantha, viridis,

alboxantha, virido xantha, striata and tigrima types. In another study in sunflower Hermelin and Daskalov (1987) recovered chlorophyll mutants by treating the F_1 achenes with 0-200 Gy gamma rays. Likewise, Ravi and Monocha (1987) by treating the Lentil seeds with various doses of gamma rays and EMS found that the doses 10 Kr gamma rays and 0.2 per cent EMS gave maximum frequency of chlorophyll mutants per 100 M_2 plants. In linseed, Bachyalis (1988) treated dry seeds of two varieties with 1-150 Kr gamma rays and observed differential response of the two varieties to induce chlorophyll mutations for a given dose. Similarly, Busolo (1988) isolated some chlorophyll mutants in groundnut following irradiation with gamma rays at 20 Kr and with fast neutrons separately. In studying the effect of gamma rays, EMS and hydroxylamine on frequency of chlorophyll mutations in lentil Singh et al. (1989) found that gamma rays induced higher frequency of chlorophyll mutations than EMS and hydroxylamine. Further they opined that lower gamma ray doses of 5 and 10 Kr induced more chloromutations than the higher doses.

In case of soybean, Harb (1990) reported that of the 4 doses of gamma radiation, the 15 Kr dose gave the highest frequency of chlorophyll mutants in the M_2 generation. Among

the chlorophyll mutants he recovered albino, xantha, chlorina and variegata types.

Similarly Mahla *et al.* (1991) in Brassica induced chlorophyll mutations following gamma ray and EMS treatments separately. They reported that EMS induced more chlorophyll mutations than did gamma radiation.

2.6 MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF MUTAGENS AND THEIR DOSES

Success of mutation breeding depends on selection of proper mutagens. Mutagenic doses vary considerably in effectiveness and efficiency. Utility of any mutagen in plant breeding depends upon its effectiveness and efficiency. In case of induced mutagenesis the primary importance is to be given to increase the efficiency of induction of desirable hereditary changes.

The concept of effectiveness and efficiency was enunciated by Ehrenberg (1960). Later Konzak *et al.* (1965) revealed clearly the measures such as mutagenic effectiveness and efficiency (gene mutations per unit dose) using M_1 injury and mutation frequency in the M_2 in case of physical and

chemical mutagens. According to them, effectiveness refers to the frequency of mutations induced by the mutagen, whereas, efficiency refers to the ability of mutagen to induce desirable mutations.

Effectiveness is the measure of mutation rate relative to the dose, whereas efficiency relates to mutation rate to the other undesirable biological effects such as gross chromosomal aberrations, physiological and toxic effects, that reduce the cell survival and eliminate the mutations (Ehrenberg, 1960; Nilan, 1961; Konzak, 1965).

Rao *et al.* (1971) studied the mutagenic effects of heavy water (D_2O) and its combination with EMS in rice. Effects in M_1 and M_2 generations were more with respect to D_2O and EMS treatment presoaked in D_2O than in respective controls. Likewise Goranova and Aleksieva (1986) treated the dry seeds of soybean with various doses of gamma rays and fast neutrons and various concentrations of EMS. They reported highest frequency of mutations, including useful mutations with respect to gamma rays. In addition they also observed differential response of soybean varieties regarding the effective gamma ray doses for maximum mutation. In one variety the most effective and efficient gamma ray doses were

7.5 and 10 Kr. While in another variety, the highest mutation frequency was obtained at the lowest gamma ray dose viz., 2.5Kr.

In another study relating to the stage at which gamma ray treatment be applied to recover maximum mutations Prasanna and Reddy (1986) in castor recorded highest mutagenic effectiveness (mutation frequency per dose) and efficiency (mutation frequency per sterility percentage) for treatment applied at the gametic stage compared to seedling and post gametic stages.

In case of soybean Wang and Yu (1991) exposed three genotypes to 8 doses of gamma radiation and reported that the frequency of induced mutations in M_2 generation increased with higher doses.

MATERIAL AND METHODS

III MATERIAL AND METHODS

The present investigation was carried out to study the variability induced by gamma irradiation with respect to quantitative characteristics viz., days to flowering, plant height, head diameter, test weight, single plant yield and branching behaviour in two restorer lines of sunflower.

The information on materials used, methods adopted, characters studied both in field and laboratory in different phases of investigation is presented in this chapter.

3.1 LOCATION OF EXPERIMENTAL SITE

The experiment was conducted on deep black soils (vertisol) at Main Research Station, University of Agricultural Sciences, Dharwad, during *Kharif*, 1994. Dharwad is situated on a latitude of 15°21' North and 76°34' East longitude and has an altitude of 774 meters above mean sea level. Dharwad lies in the northern transitional zone of Karnataka state and receives an average annual rainfall of 772 mm well distributed from April-May to October.

3.2 INDUCING VARIABILITY IN TWO DISTINCT RESTORER LINES OF SUNFLOWER THROUGH GAMMA IRRADIATION

Material

The base material for the present study comprised of two restorer lines of sunflower viz., IV83 and RLC2. The selfed seeds of these two restorer lines were obtained from Oilseeds Scheme, Main Research Station, Dharwad. IV83 is a restorer line having nonbranching habit while RLC2 is having branching habit. The salient features of restorer lines used in the study are given in Table-1.

Methods

Irradiation of seeds

Bold and viable dry seeds of uniform size, numbering 500 seeds, with 12 per cent moisture were packed in butter paper bags separately for each treatment. The seeds were irradiated with doses 10, 15, 20 Kr doses of gamma rays from ^{60}Co source at the gamma chamber of Bhabha Atomic Research Centre, Trombay, Bombay, during second week of July, 1994. The untreated seeds served as control and is one of the treatments apart from 10, 15, 20 Kr doses of gamma rays. One Kr is equal to one thousand Roentgen units. Roentgen (r) is a unit to measure the dose of gamma rays treated and is defined

Table 1. Salient features of restorer lines of sunflower included in the study

Characters	Restorer lines	
	IV83	RLC2
Origin	Derived from an exotic hybrid after six generations of selfing	A selection from restorer lines composite
Days to flowering	58	62
Plant height (cm)	98	105
Seed yield (g/plant)	26	7
Test weight (g/100 grains)	4.96	3.55
Oil content (%)	27.11	28.22
Branching behaviour	Non-branching	Branching

as the quantity of radiation whose associated corpuscular emission per 0.001293 gm of air produces in air, ions carrying one esu of electricity per cc of air at NTP.

M₁ generation

The irradiated seeds of different doses of gamma rays were sown immediately in the field at the Main Research Station Farm, Dharwad on 27-7-1990 as M₁ generation. The untreated seeds were also sown simultaneously that served as check for M₁ generation plants for comparison of various characters observed in the field. Seeds were dibbled (One seed per hill) in rows following spacing of 60x30 cm. 450 seeds were sown in each treatment with respect to IV83 and RLC2 genotype. Germination count was taken 10 days after sowing and expressed in per cent. Each M₁ plant was selfed during flowering by covering with cloth bag. The plants in M₁ generation were harvested on 15-10-94.

M₂ generation

The seeds obtained from each selfed M₁ plant were hand dibbled on 3-1-95 as unreplicated plant to progeny rows to raise M₂ generation. The untreated selfed seeds were also

sown at the same time in progeny rows that served as control. The number of seeds sown in each treatment are given in Table 3. The number of plants survived 25 days after sowing was counted on 25th day and expressed in percentage as shown in Table-3. The M_2 generation plants were harvested on 28-3-95.

Selections were done in M_2 generation based on branching behaviour and other characteristics viz., head diameter, plant height, days to flowering, test weight, seed yield per plant etc. by comparing them with plants in control. The selected selfed seeds were advanced to next generation for future studies.

Individual plant observations were recorded in M_1 and M_2 generations with respect to plant height (cm), days to flowering, head diameter (cm) and number of branches borne on leaf axil of each plant in M_1 and M_2 and also for untreated control lines of IV83 and RLC2. Likewise, observations were recorded for test weight, seed yield per plant, number of seeds per plant.

3.3 FIELD PLOT TECHNIQUE

The experiment was laid out in an unreplicated trial. In M_1 generation, the irradiated seeds were hand

dibbled in rows with respect to each treatment administered. In M_2 generation a single row of 4 metre in length, with a spacing of 60 cm between rows and 30 cm within the row was adopted to represent a head to progeny line.

Both the genotypes and all four treatments in each genotype were randomised and sown in the field. Necessary border rows were grown all-round the experimental plot. Fifty per cent of nitrogen and entire recommended dose of phosphorus and potassium (62.5:75:62.5 kg N:P₂O₅:K₂O/ha) fertilizer was applied at the time of sowing. Remaining fifty per cent of nitrogen was applied as top dressing dose after forty days of sowing. Recommended plant protection measures were also taken.

3.4 QUANTITATIVE AND QUALITATIVE CHARACTERS

Individual plant observations were recorded on the following characters on all the plants in M_1 and M_2 generations as described below.

3.4.1 Days to flowering

As soon as 1 to 2 yellow ray florets opened during morning hours, the date of flowering was noted for each

flowered plant and the mean number of days taken to flowering from sowing date was recorded.

3.4.2 Plant height

Plant height was measured using a metric scale as a vertical perpendicular distance in centimeters from ground level to the base of capitulum.

3.4.3 Head diameter

Capitulum diameter (cm) was measured at maturity using a plastic scale as a distance between two opposite points on the periphery of the capitulum with the line passing through the centre of head.

3.4.4 Number of branches

The number of branches per plant borne on leaf axil of main stem was recorded in RLC-2 restorer line which is branching type.

3.4.5 Test weight

One hundred filled seeds were randomly picked from seeds threshed from each head and weighed in grams.

3.4.6 Single plant yield

The filled seeds obtained from each head, harvested separately were threshed, dried and weighed in grams.

3.4.7 Number of seeds per head

The total number of filled seeds obtained from each head harvested was counted and recorded.

3.4.8 Branching and nonbranching behaviour

The number of plants showing branching habit due to irradiation treatments in the nonbranching genotype viz., IV83 was counted and its frequency was calculated. Similar frequency of nonbranching types in branching genotype viz., RLC2 was calculated.

3.4.9 Oil content

Twenty grams filled seeds were taken to analyse oil content (%) using NMR (Nuclear Magnetic Resonance) spectrometer model installed at Directorate of Oil seeds Research Hyderabad.

3.4.10 Chlorophyll mutants

Appearance of chlorophyll mutants in M_1 and M_2 generations was noted and the frequencies were calculated.

3.4.11 Abnormal lethal plants

Appearance of abnormal plants with respect to leaf shape, height was noted and photographs were taken wherever required. The frequencies of lethal plants in M_2 population were calculated and used to deduce mutagenic efficiency.

3.5 ESTIMATION OF MUTAGENIC EFFECTIVENESS AND MUTAGENIC EFFICIENCY

Mutagenic effectiveness is defined as a measure of frequency of mutations induced by a unit dose of a mutagen. While mutagenic efficiency gives an idea of mutations in relation to undesirable effects like lethality, injury and sterility. The formulae given by Konzak *et al.* (1965) were used to calculate both mutagenic effectiveness and efficiency.

$$\text{Mutagenic effectiveness} = \frac{M}{Kr}$$

$$\text{Mutagenic efficiency} = \frac{M}{L} \quad \text{or} \quad \frac{M}{I}$$

Where,

M = Frequency expressed as percentage of chlorophyll mutations in M_2 generation, estimated on M_2 plant basis.

Kr = Gamma ray dose in kilo Roentgen units

L = Per cent lethality or survival reduction in M_1

I = Per cent seedling injury or height reduction in M_1

3.6 STATISTICAL ANALYSIS

Range, mean and standard deviation values were computed for each of the character in M_1 and M_2 based on observations recorded in respective generations.

3.6.1 Estimation of phenotype variance (V_p)

The standard deviation values obtained for observations recorded with respect to each of the characters were squared and expressed as phenotypic variance, as proposed by Falconer(1986).

$$\text{Phenotypic Variance } (v_p) = \frac{\sum x^2 - (\sum x)^2}{n-1}$$

Where,

x = Observations recorded for a character in M_1 and M_2

v_p = Phenotypic variance

3.6.2 Estimation of environmental variance (V_E)

The standard deviation values were calculated based on observations recorded on plants in control. These values were squared and expressed as environmental variance as given by Falconer (1986).

3.6.3 Estimation of genotypic variance (V_G)

The environmental variance values were deducted from respective phenotypic variance values to get genotypic variance for each character, as proposed by Falconer(1986).

$$V_G = V_P - V_E$$

Where,

V_G = Genotypic variance

V_P = Phenotypic variance

V_E = Environmental variance

3.6.4 Estimation of heritability (H) in broad sense

Heritability estimates of all characters were calculated based on phenotypic and genotypic variance values as proposed by Hanson *et al.* (1956).

$$\text{Broad sense heritability (H)} = \frac{V_G}{V_P} \times 100$$

Where,

H = Broad sense heritability

V_G = Genotypic variance

V_P = Phenotypic variance

3.6.5 Estimation of genetic advance (GA)

Genetic advance estimates of all characters were calculated based on phenotypic standard deviation, intensity of selection and heritability values as proposed by Johnson *et al.* (1955).

$$\text{Genetic advance (GA)} = H.i.\sqrt{V_P}$$

Where,

H = Broad sense heritability

i = Selection intensity which takes the value of 2.063 at 5% level of significance

V_P = Phenotypic Variance

3.6.6 Estimation of phenotypic coefficient of variation (P_{CV})

Phenotypic coefficient of variation is calculated based on phenotypic variance and mean value for each of the character observed as proposed by Burton and Devane (1953).

$$PCV = \frac{\sqrt{V_p}}{\bar{X}} \times 100$$

Where,

PCV = Phenotypic coefficient of variation

$\sqrt{V_p}$ = Phenotypic standard deviation

$\bar{X}$ = Mean

3.6.7 Estimation of genotypic coefficient of variation (GCV)

It is calculated based on genotypic standard deviation and mean of the character, as proposed by Burton and Devane (1953).

$$GCV = \frac{\sqrt{V_G}}{\bar{X}} \times 100$$

Where,

$\sqrt{V_G}$ = Genotypic standard deviation

GCV = Genotypic coefficient of variability

$\bar{X}$ = Mean

3.6.8 Estimation of standard error of estimates

To compute standard error of estimates with respect to different characters studied, standard deviation values and number of individual plant observations recorded were considered.

$$SE = \sqrt{\frac{V_p}{n}}$$

Where,

SE = Standard error of estimates

$\sqrt{V_p}$ = Standard deviation values

n = Number of individual plant observations recorded

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The observations collected on M_1 and M_2 generations were classified, tabulated and statistically analysed. The results of the present investigation are presented under the following headings.

4.1 M_1 GENERATION

Effect of gamma irradiation doses on germination, survival, quantitative characters, branching behaviour and presence of any abnormalities like chlorophyll mutants were studied in M_1 generation.

4.1.1 Germination

The data on germination expressed in percentage is presented in Table-2.

In both the genotypes viz., IV83 and RLC2, a decrease in germination was observed with increase in dosage.

In case of IV83 genotype, the values at 10, 15 and 20Kr treatments were 84.00, 80.22 and 74.1 respectively as against the value of 90.22 in control. A similar decrease in

Table 2. Number of seeds sown and germination percentage in M₁ generation

Treatment	Number of seeds sown	Germination percentage in M₁ generation
IV83		
Control	450	90.22
10 Kr	450	84.00
15 Kr	450	80.22
20 Kr	450	74.15
RLC2		
Control	450	87.20
10 Kr	450	77.33
15 Kr	450	61.55
20 Kr	450	48.08

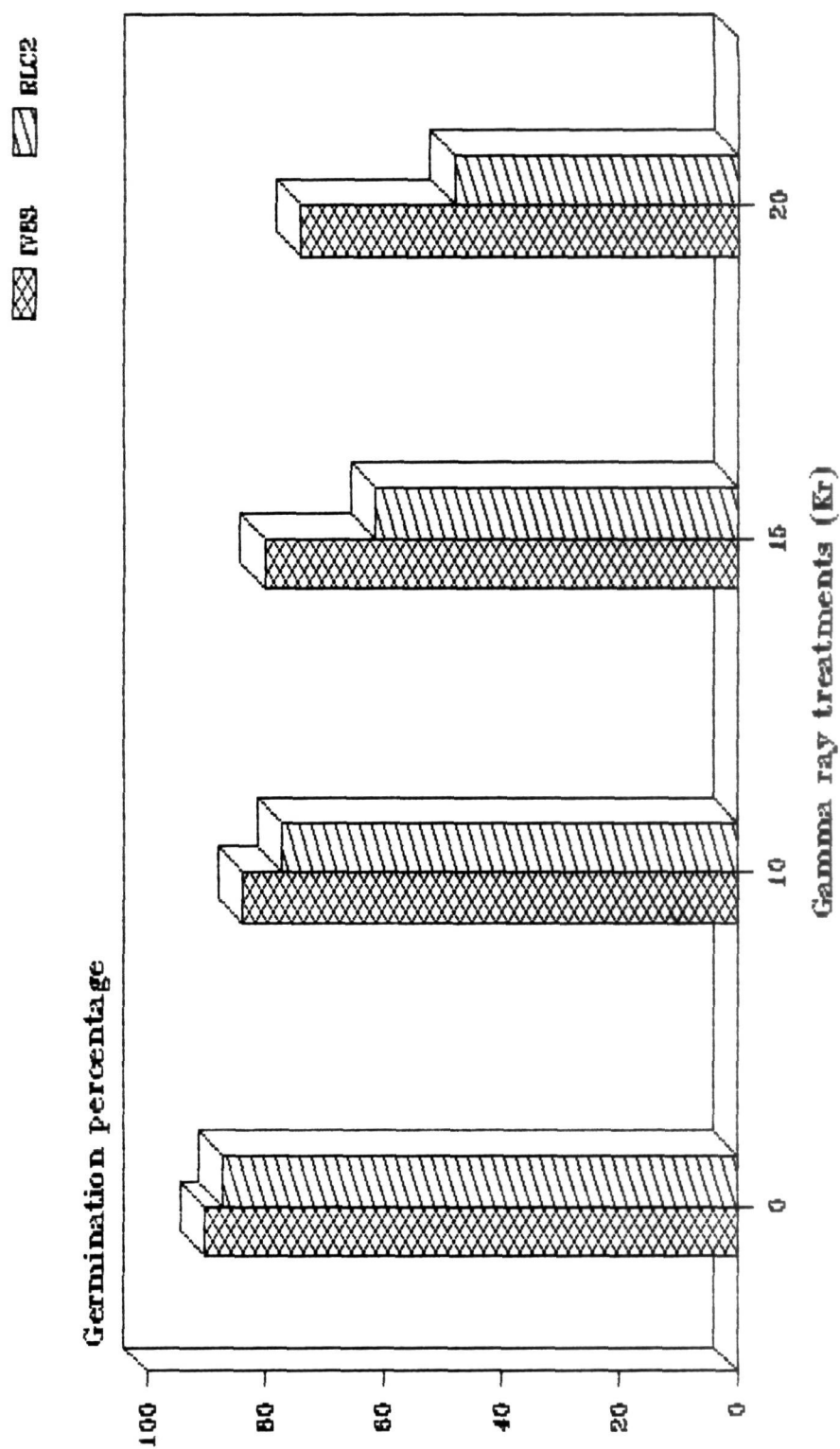


Fig.1 Effect of gamma rays on germination in the M1 generation of two sunflower restorer lines

values was noticed in RLC2 genotype at 10Kr (77.33), 15Kr (61.55) and 20Kr (48.08) treatments compared to control.

4.1.2 Survival

The data on survival of plants recorded on the 25th day after sowing and expressed as percentage is given in Table-3.

Reduction in survival was noticed at all the irradiation doses, in both the genotypes with the increase in dosage. In case of IV83 lowest survival was noticed in 20Kr dose (61.78) followed by 15Kr (72.22), and 10Kr (78.0) compared to control (83.55). Similar trend was also noticed in RLC2 genotype with lowest survival percentage observed in 20Kr dose (18.88) followed by 15Kr (47.56) and 10Kr (68.44) as compared to control (77.11).

4.1.3 Quantitative characters

Effect of irradiation on quantitative characters were measured in terms of parameters such as range, mean, variance, coefficient of variability, genetic variability, genotypic coefficient of variation, heritability and genetic advance.

Table 3. Percentage of surviving plants in M_1 and M_2 generations on 25th day of sowing

Treatment	Plant population in M_1 generation			Plant population in M_2 generation		
	Expected	Observed	Survival count in percentage	Expected	Observed	Survival count in percentage
IV83						
Control	450	376	83.55	1000	812	81.20
10 Kr	450	351	78.00	3045	2013	66.11
15 Kr	450	325	72.22	2535	1350	53.25
20 Kr	450	278	61.78	750	350	46.67
RLC2						
Control	450	347	77.11	1000	751	75.10
10 Kr	450	308	68.44	1350	568	42.07
15 Kr	450	214	47.56	375	135	36.00
20 Kr	450	85	18.88	30	10	33.33

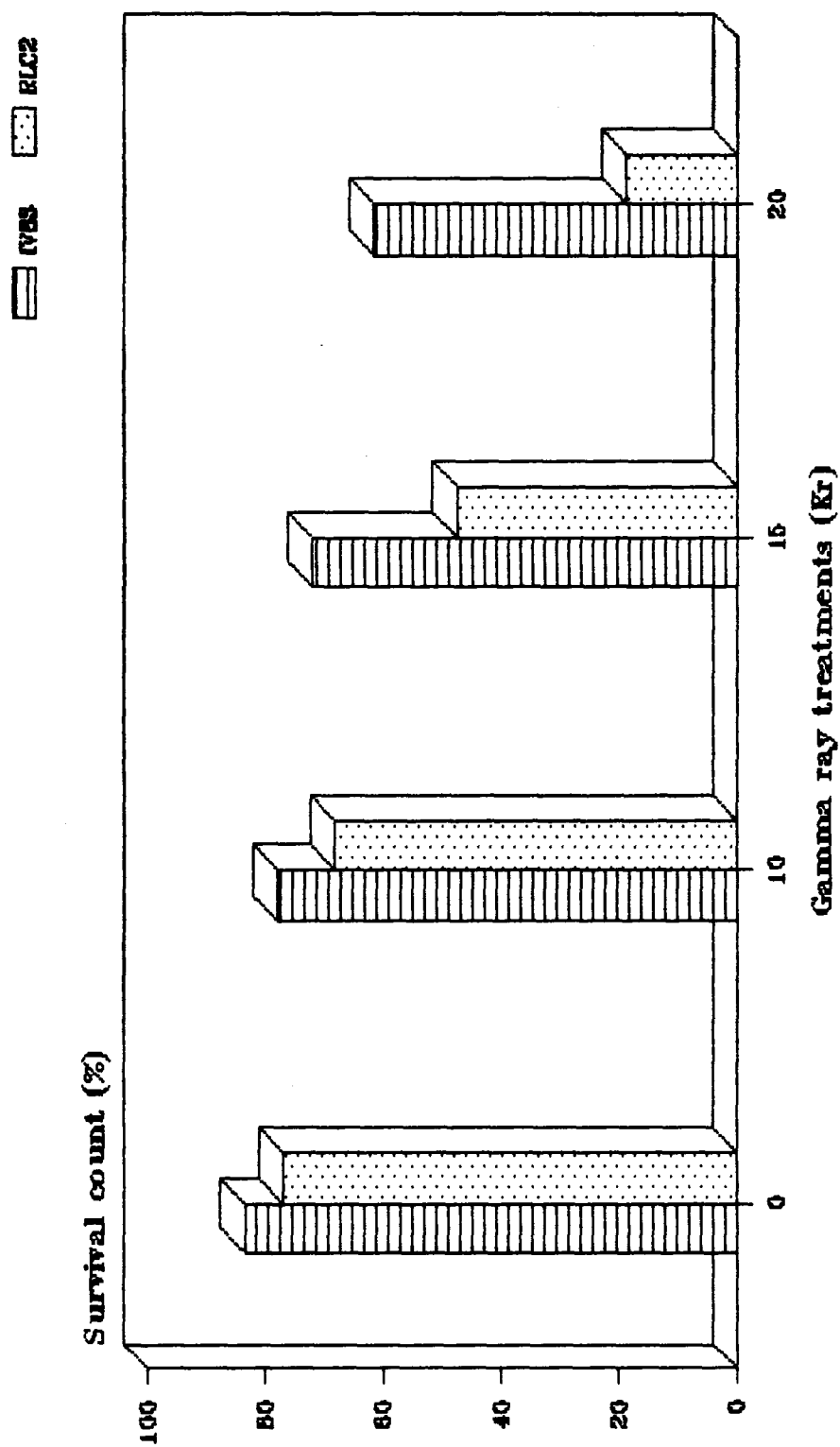


Fig.2 Effect of gamma rays on survival at 25 days after sowing in M1 generation

A decrease in mean values and increase in range, variance, coefficient of variability, genetic variability, genotypic coefficient of variability, heritability and genetic advance estimates was observed in most of the quantitative traits following irradiation treatment. Except for that, the various estimates calculated did not follow any particular trend whatsoever. Nevertheless a general behaviour and trend of these parameters has been presented in the following paragraphs.

4.1.3.1 Days to flowering

Data on days to flowering are presented in Tables-4,5,6.

Early flowering types were recovered in the M_1 generation with respect to both the genotypes viz., IV83 and RLC2. The Increase in range was in positive and negative directions following irradiation treatments. For instance, with respect to IV83 genotype, the range increased to 50-60, and 52-60 and 54-62 days following 10, 15 and 20Kr treatments respectively as against 55-61 days in the control. With respect to RLC2 the range was enlarged in positive direction (Early types) in treated population as compared to the untreated ones. In case of 10, 15 and 20Kr treatments of RLC2 observed estimates of range were 57 to 65, 58 to 64, 59 to 64

Table 4. Range, mean, standard error, variance and coefficient of variability for days to flowering, plant height, head diameter and number of branches per plant in M_1 generation

Genotype	Treatment		Range	Mean	±	S.E.	Variance	C.V.
	gamma rays	(Kr)						
Days to flowering								
IV83	Control	55-61	58	0.10	4.00	3.45		
	10	50-60	55	0.17	9.99	5.75		
	15	52-60	57	0.14	6.67	4.53		
	20	54-62	57	0.15	6.67	4.53		
RLC2	Control	60-65	62	0.09	2.89	2.74		
	10	57-65	59	0.15	6.66	4.37		
	15	58-64	61	0.14	4.00	3.28		
	20	59-64	61	0.20	3.25	2.95		
Plant height (cm)								
IV83	Control	80-125	110.35	0.83	260.26	14.62		
	10	65-170	112.45	2.79	2732.15	46.48		
	15	55-105	97.25	0.94	284.72	17.35		
	20	40-115	80.70	0.98	269.51	20.35		
RLC2	Control	85-125	104.54	0.58	115.99	10.30		
	10	65-125	100.37	0.68	141.70	11.86		
	15	55-120	89.24	0.94	189.56	15.43		
	20	45-100	73.04	1.64	228.76	20.71		
Head - diameter (cm)								
IV83	Control	10-20	11.31	0.12	5.50	20.78		
	10	7-25	10.26	0.17	9.59	30.21		
	15	4-20	9.17	0.18	10.70	35.66		
	20	3-15	7.39	0.19	9.58	41.95		
RLC2	Control	8-11	7.50	0.05	0.79	11.87		
	10	4-10	6.03	0.07	1.67	21.39		
	15	4-8	4.73	0.08	1.32	24.31		
	20	2-5	3.49	0.10	0.85	26.36		
Number of branches per plant								
RLC2	Control	9-20	12.72	0.58	44.15	52.20		
	10	3-28	11.38	0.68	111.81	92.88		
	15	5-25	13.49	0.94	53.97	54.48		
	20	8-23	13.42	1.64	45.91	50.52		

Table 5. Phenotypic, genotypic and environmental variances, heritability and genetic advance for days to flowering, plant height, head diameter and number of branches per plant in M_1 generation

Genotype	Treatment gamma rays (Kr)	V_P	V_G	V_E	H(%)	GA
Days to flowering						
IV83	Control	-	-	4.00	-	-
	10	9.99	5.99	-	59.96	7.11
	15	6.67	2.67	-	40.03	3.73
	20	6.67	2.67	-	40.03	3.73
RLC2	Control	-	-	2.89	-	-
	10	6.66	3.77	-	56.61	5.10
	15	4.00	1.11	-	27.75	1.89
	20	3.25	0.36	-	11.08	0.67
Plant height (cm)						
IV83	Control	-	-	260.26	-	-
	10	2732.15	2471.89	-	90.47	86.76
	15	284.72	24.46	-	8.59	3.07
	20	269.51	9.25	-	3.43	1.43
RLC2	Control	-	-	115.99	-	-
	10	141.70	25.71	-	81.86	20.26
	15	189.56	73.57	-	38.81	12.35
	20	228.76	112.77	-	49.30	3.72
Head diameter (cm)						
IV83	Control	-	-	5.50	-	-
	10	9.59	4.09	-	42.65	26.51
	15	10.70	5.25	-	48.60	35.77
	20	9.58	4.08	-	42.59	36.81
RLC2	Control	-	-	0.79	-	-
	10	1.67	0.88	-	52.69	23.38
	15	1.32	0.53	-	40.15	20.08
	20	0.85	0.06	-	7.06	3.72
Number of branches per plant						
RLC2	Control	-	-	44.15	-	-
	10	111.81	67.66	-	60.51	115.99
	15	53.97	9.82	-	18.20	20.46
	20	45.91	1.76	-	3.83	4.02

Table 6. Phenotypic, genotypic and environmental coefficient of variation for days to flowering, plant height, head diameter and number of branches per plant in M_1 generation

Genotype	Treatment		PCV	GCV	ECV
	gamma rays	(Kr)			
Days to flowering					
IV83	Control	-	-	-	3.45
	10	5.75	4.46	-	-
	15	4.53	2.86	-	-
	20	4.53	2.86	-	-
RLC2	Control	-	-	-	2.74
	10	4.37	3.29	-	-
	15	3.28	1.72	-	-
	20	2.95	0.98	-	-

Plant height (cm)					
IV83	Control	-	-	-	14.62
	10	46.48	44.22	-	-
	15	17.35	5.09	-	-
	20	20.35	3.77	-	-
RLC2	Control	-	-	-	10.30
	10	11.86	5.05	-	-
	15	15.43	9.61	-	-
	20	20.71	14.51	-	-

Head diameter (cm)					
IV83	Control	-	-	-	20.78
	10	30.21	19.69	-	-
	15	35.66	24.86	-	-
	20	41.95	27.33	-	-
RLC2	Control	-	-	-	11.87
	10	21.39	15.59	-	-
	15	24.31	15.43	-	-
	20	26.36	7.16	-	-

Number of branches per plant					
RLC2	Control	-	-	-	52.20
	10	92.88	72.32	-	-
	15	54.48	23.20	-	-
	20	50.52	9.91	-	-

respectively as against 60 to 65 in the control. The comparison of different treatments in IV83 genotype showed that the mean values were of lower magnitude in 10Kr (55.00), 15Kr(57.00) and 20Kr (57.00) treatments as against the value of 58 in control. Similar trend was followed in RLC2 genotype in 10Kr (59.00), 15Kr (61.00) and 20 Kr(61.00) treatments as compared to control (62.00). With respect to IV83 genotype higher variance for the trait was observed at 10Kr dose (9.99) followed by 15Kr (6.67) and 20Kr doses (6.67) with least variance observed in control (4.00). Similarly in RLC2 genotype highest variance was observed at 10 Kr (6.66) dose followed by 15Kr (4.00) and 20Kr (3.25) doses as against the value of 2.89 in control.

In IV83 genotype the coefficient of variability also followed the same trend. 10Kr dose showed highest coefficient of variability (5.75) for the trait followed by 15Kr (4.53) and 20Kr doses (4.53). A least coefficient of variability value (3.45) was observed in control. In case of RLC2 genotype a similar trend of increase in coefficient of variability for the trait was observed at 10Kr (4.37) followed by 15Kr (3.28) and 20Kr (2.95) doses as against least coefficient of variability value of 2.74 in control.

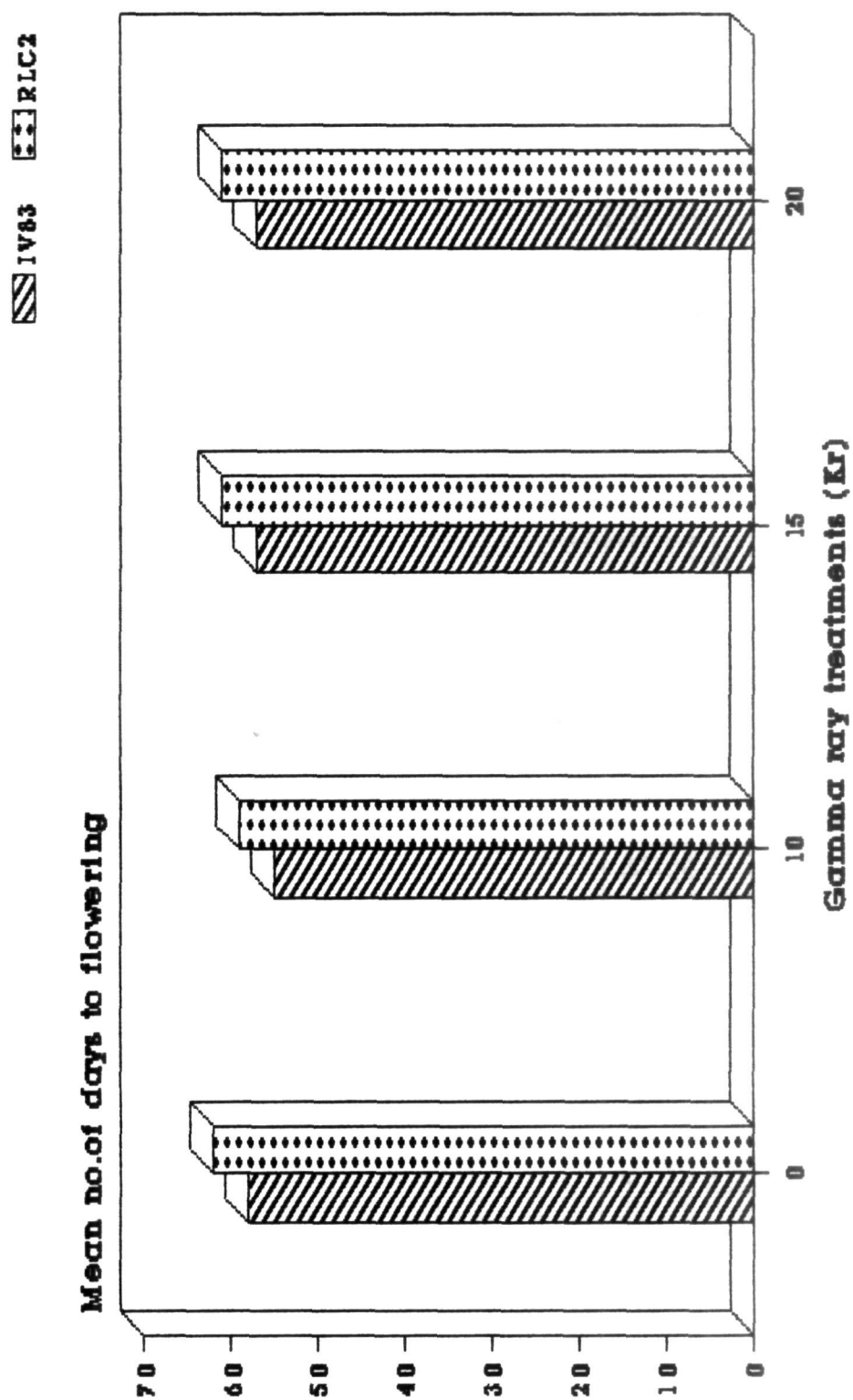


Fig 3. Effect of gamma rays on days to flowering in M1 generation

In case of IV83 genotype increased genetic variability for the trait was observed at 10Kr dose (5.99) followed by 15Kr (2.67) and 20Kr (2.67) doses. Likewise, in RLC2 genotype 10Kr treatment showed highest genetic variability of 3.77 followed by 15 Kr (1.11) and 20 Kr (.36) treatments.

Highest heritability estimate was observed at 10Kr treatment (59.96) followed by 15Kr (40.03) and 20Kr (40.03) treatments in IV83 genotype. RLC2 genotype also showed similar response, with highest heritability values recorded at 10Kr (56.61) and a gradual decrease in the values at 15 Kr (27.75) and 20Kr (11.08) doses. Genetic advance estimate was also highest in 10Kr dose (3.73) in IV83 genotype. A similar trend was followed in RLC2 genotype at 10Kr (5.10), 15Kr (1.89) and 20Kr (0.67) doses.

Highest genetic coefficient of variability values were observed at lower dose of 10Kr followed by 15Kr and 20Kr treatments in both the genotypes (Table-6) studied.

4.1.3.2. Plant height.

Data on plant height are presented in Tables - 4,5,6.

In IV83 genotype, an increase in range values in positive and negative directions was observed in

treated populations compared to the control. In 10, 15 and 20Kr treatments the range widened to 65 to 170, 55 to 120 and 40 to 115 respectively, as against a narrow range of 80 to 125 in control. Similarly enlarged range for plant height was noticed in RLC2 genotype as a result of gamma irradiation. In case of 10, 15 and 20 Kr treatments the values ranged from 65 to 125, 55 to 120 and 45 to 100 respectively as against 85 to 125 in control.

The mean for the character as affected by the irradiation doses showed a lower values in both the genotypes as compared to control. In IV83 genotype, compared to control (110.35), the mean values for the trait decreased gradually with increase in dosage from 10Kr (112.45) to 15 Kr (97.25) and then to 20 Kr (80.70). A similar trend of dose dependent decrease in mean value at 10Kr (100.37), 15Kr (89.24) and 20Kr (73.24) treatments was observed in RLC2 genotype as against highest mean value recorded in control (104.54).

The variance for the trait increased following gamma radiation in both the genotypes studied. With respect to IV83 genotype, increased variance was noticed at lower irradiation dose of 10Kr (273.15) followed by 15Kr

(284.72) and 20 Kr (269.26) doses, as against the value of 260.26 in control. On the contrary, with regard to RLC2 genotype the treatment 20Kr (228.76) was effective in increasing the variance followed by 15Kr (189.56) and 10Kr (141.70) treatments. In control least variability for the trait was observed (115.99).

As in case of variance, the coefficient of variability estimate also showed similar behaviour of increased value at lower dose of 10Kr (46.48), in IV83 genotype as compared to control (14.62). In 15Kr and 20Kr treatments coefficient of variability values of 17.35 and 20.35 were observed. In case of RLC2 genotype, the coefficient of variability values at 10,15 and 20Kr treatments were 11.86,15.43 and 20.71 respectively as against the value of 10.3 in control.

Higher magnitude of genetic variability was recorded at 10Kr (2471.86) followed by 15Kr(24.46) and 20Kr (9.25) treatments in IV83 genotype. On the contrary, in RLC 2 genotype high genetic variation for the trait was observed, at 20Kr dose (112.77) followed by 15Kr (73.57) and 10Kr (25.71) doses.

In IV83 genotype, heritability estimate was of higher magnitude at 10Kr dose (90.47) compared to 15Kr (8.59)

and 20Kr (3.43) doses. A similar trend was observed in RLC 2 genotype. The dose 10Kr recorded the highest heritability value for the trait (81.86) followed by 20Kr (49.3) and 15Kr (38.81) doses.

The genetic advance estimate for the trait significantly increased at 10Kr (86.76) compared to 15Kr (3.07) and 20Kr (1.43) doses in IV83 genotype. A similar trend existed in RLC2 genotype at 10Kr (20.26), 15Kr (12.35) and 20Kr (3.72) doses.

4.1.3.3. Head diameter

The data on head diameter are presented in Tables 4,5 and 6.

In IV83 genotype irradiation treatments enlarged the range for the trait. For instance, in case of 10,15 and 20Kr treatments the values observed were 7 to 25, 4 to 20 and 3 to 15 respectively as against the value of 10 to 20 in the control. In RLC2 genotype, higher irradiation doses of 15 and 20Kr had a drastic effect in widening the range in negative direction with the values ranging from 4 to 8 and 2 to 5 respectively. Compared to control (8 to 11), the dose 10Kr showed a moderate effect in altering the range values (4-10).

The mean of character was also affected by irradiation treatments in both the genotypes studied. In case of IV83 genotype, the mean of the trait decreased to 10.26, 9.17 and 7.39 in 10, 15 and 20Kr treatments as against the value of 11.31 in control. A similar trend of decrease in mean value with increase in dosage was observed in RLC2 genotype. The mean values observed at 10, 15 and 20Kr doses were 6.03, 4.73 and 3.49 respectively as against the mean value of 7.50 in control.

Highest variability for the character was observed at 15Kr dose (10.70) as compared to control (5.50) in IV83 genotype. The doses 10Kr (9.59) and 20Kr (9.58) had a similar effect in increasing the variance for the trait. In case of RLC2 genotype the dose 10Kr (1.67) was more effective in increasing the variability for the trait compared to 15Kr (1.32) and 20Kr doses (0.85) with the least value of 0.79 observed in control.

The coefficient of variability for the trait increased as a result of gamma irradiation in both the genotypes. In case of IV83 genotype highest coefficient of variability value was observed at higher dose of 20Kr (41.95) followed by 15Kr (35.66) and 10Kr (30.21)

treatments. The coefficient of variability value of 11.87 was observed in control. Similar kind of behaviour was observed in RLC2 genotype with highest value at 20Kr (26.36) followed by 15Kr (24.31) and 10Kr (21.39) treatments as against lower value of 11.87 in Control.

Among the different doses of gamma radiation applied, the dose 15Kr (5.25) was effective to create more genetic variability than 10Kr and 20Kr (4.09 and 4.08) doses in IV83 genotype. In contrast to this, in RLC2 genotype lower dose of 10Kr showed higher genetic variability (0.88) compared to 15Kr (0.53) and 20Kr (0.06) doses.

With regard to heritability estimate higher value was observed at 15Kr (48.60) dose, in IV83 genotype. The heritability values did not differ in 10Kr (42.65) and 20Kr (42.59) doses.

In IV83 genotype, the genetic advance estimates at 10, 15 and 20 Kr doses were 26.51, 35.77 and 36.81 and in RLC2 genotype the genetic advance values of 23.38, 20.08 and 3.72 were observed at 10,15 and 20Kr doses.

The genotypic coefficient of variability for the trait was higher at 20Kr (27.33) followed by 15Kr (24.86)

and 10Kr (19.69). On the contrary 10Kr (15.59) and 15Kr (15.43) doses were more effective in increasing the genotypic coefficient of variation as compared to 20Kr (7.16) dose.

4.1.3.4 Number of branches per plant

The data on number of branches per plant as affected by gamma radiation is presented in Tables 4,5 and 6.

A wide range in negative and positive directions for number of branches per plant was recorded in M_1 generation of RLC2 genotype. Enlarged range was observed in 10Kr treatment (3 to 28) followed by 15Kr (5 to 25) and 20Kr (8 to 23) treatments as against a value of 9 to 20 observed in control. There was no significant increase or decrease in the mean values of treated populations as compared to control, (12.72), though a slight increase in mean values was noticed in 15 Kr (13.49) and 20Kr (13.42) treatments.

There was a dose dependent increase in variability for the character, observed in M_1 population. The observed values of variance in RLC2 genotype at 10,15 and 20Kr doses were 111.81, 53.97 and 45.91 respectively. However, no

significant increase or decrease in coefficient of variability was observed at 15Kr (54.48) and 20Kr (50.52) doses compared to control (52.2) except for 10Kr dose (92.28).

Highest heritability estimate was recorded at 10Kr (60.51) dose followed by 15 Kr (18.20) and 20Kr (3.82) doses. Similarly, higher genetic advance values were noticed at 10Kr (115.99) followed by 15Kr (20.46) and 20Kr (4.02) doses.

The lowest dose of 10Kr was found to be most effective in increasing the genotypic coefficient of variability for the trait (72.32) than 15Kr (23.20) and 20Kr (9.91) treatments.

4.1.3.5 Number of seeds per plant

The effect of gamma radiation on number of seeds per plant based on estimates such as range, mean, variance coefficient of variability, genetic variability, genotypic coefficient of variability, heritability and genetic advance is presented in Tables 7,8 and 9.

The gamma irradiation doses significantly widened the range for number of seeds per plant in positive as well

Table 7. Range, mean, standard error, variance and coefficient of variability for number of seeds per head, seed yield per plant and 100-seed weight in M₁ generation

Genotype	Treatment gamma rays (Kr)	Range	Mean $\pm$	S.E.	Variance	C.V.
Number of seeds per head						
IV83	Control	100-122	115.00	0.49	90.63	8.28
	10	25-985	129.06	5.94	12389.03	86.25
	15	30-317	99.93	4.75	7323.94	85.64
	20	15-242	62.97	4.36	5294.02	115.55
RLC2	Control	75-95	85.27	0.40	56.70	8.83
	10	25-342	35.36	1.82	1016.33	90.16
	15	16-74	28.47	1.23	322.20	63.05
	20	8-15	12.32	0.83	58.68	62.18
Seed yield per plant (g)						
IV83	Control	8.50-14.50	10.85	0.04	0.74	7.93
	10	2.39-45.74	3.95	0.26	23.81	123.54
	15	3.35-28.92	2.89	0.14	6.61	88.93
	20	0.86- 8.48	1.95	0.16	6.86	134.36
RLC2	Control	4.86- 6.40	5.99	0.01	0.04	3.34
	10	0.20- 3.89	0.88	0.05	0.86	105.68
	15	0.40- 1.43	0.34	0.02	0.10	94.12
	20	0.05- 0.25	0.18	0.02	0.05	122.22
100 - seed weight (g)						
IV83	Control	3.80- 4.25	3.82	0.02	0.23	12.57
	10	2.20- 4.95	3.10	0.05	0.89	30.65
	15	0.70- 5.10	2.96	0.06	1.03	34.46
	20	1.20- 5.95	3.05	0.06	1.13	34.75
RLC2	Control	2.85- 3.25	3.07	0.02	0.19	14.33
	10	0.55- 3.75	2.30	0.05	0.63	34.35
	15	0.80- 2.70	1.94	0.05	0.55	38.14
	20	0.60- 1.20	0.95	0.06	0.29	56.84

Table 8. Phenotypic, genotypic and environmental variances, heritability and genetic advance for number of seeds per head, seed yield per plant and 100-seed weight in M_1 generation

Genotype	Treatment gamma rays (Kr)	V_p	V_G	V_E	H(%)	GA
Number of seeds per head						
IV83	Control	-	-	90.63	-	-
	10	12389.03	12298.40	-	99.21	176.51
	15	7323.94	7233.31	-	98.76	174.48
	20	5294.02	5203.39	-	98.29	234.30
RLC2	Control	-	-	56.70	-	-
	10	1016.33	959.63	-	94.42	175.62
	15	322.20	265.50	-	82.40	107.16
	20	58.68	1.98	-	3.37	4.30
Seed yield per plant (g)						
IV83	Control	-	-	0.74	-	-
	10	23.81	23.07	-	96.89	246.84
	15	6.61	5.87	-	88.88	162.98
	20	6.81	6.12	-	89.21	246.15
RLC2	Control	-	-	0.04	-	-
	10	0.86	0.82	-	95.35	206.82
	15	0.10	0.06	-	60.00	114.71
	20	0.05	0.01	-	20.00	50.00
100-seed weight (g)						
IV83	Control	-	-	0.23	-	-
	10	0.89	0.66	-	73.91	46.45
	15	1.03	0.80	-	77.67	55.07
	20	1.13	0.90	-	79.65	57.38
RLC2	Control	-	-	0.19	-	-
	10	0.63	0.44	-	69.84	62.61
	15	0.55	0.36	-	65.45	51.55
	20	0.29	0.10	-	34.48	40.00

Table 9. Phenotypic, genotypic and environmental coefficient of variation for number of seeds per head, seed yield per plant and 100-seed weight in M₁ generation

Genotype	Treatment gamma rays (Kr)	PCV	GCV	ECV
Number of seeds per head				
IV83	Control	-	-	8.28
	10	86.25	85.93	-
	15	85.64	85.11	-
	20	115.55	114.55	-
RLC2	Control	-	-	8.83
	10	90.16	81.61	-
	15	63.05	57.22	-
	20	62.18	11.44	-
Seed yield per plant (g)				
IV83	Control	-	-	7.93
	10	123.54	121.52	-
	15	88.93	83.74	-
	20	134.36	126.67	-
RLC2	Control	-	-	3.34
	10	105.68	103.41	-
	15	94.12	73.53	-
	20	122.22	55.56	-
100-seed weight (g)				
IV83	Control	-	-	12.57
	10	30.65	26.13	-
	15	34.46	30.07	-
	20	34.75	31.15	-
RLC2	Control	-	-	14.33
	10	34.35	28.70	-
	15	38.14	30.93	-
	20	56.84	33.68	-

as in negative directions. For instance, the values of range for the trait at 10, 15 and 20Kr doses were 25 to 985, 30 to 317 and 15 to 242 in IV83 genotype at 10Kr (25 to 242), 15Kr (16 to 74) and 20Kr (8 to 15) doses as against narrow range in control (75 to 95).

In IV83 genotype, the mean values were drastically reduced at 15Kr (99.93) and 20Kr (62.97) doses compared to control (115.00) except for 10Kr (129.06), which recorded an increase in mean value. Similarly in RLC2 genotype, compared to control (85.27), a drastic reduction in mean values was observed with increase in dosage from 10Kr (35.36) to 15Kr (28.47) and then to 20Kr (12.32).

The irradiation doses were very effective in increasing the variability of the trait. In IV83 genotype, higher variance was observed at 10Kr (12389.03) followed by 15Kr (7323.94) and 20Kr (5294.02) as against the lowest value of 90.63 in control. Similar trend of increase in variance at lower irradiation dose of 10Kr (1016.33) was observed in RLC-2 genotype and the variance gradually decreased with increase in dosage to 15Kr (322.20) and 20Kr (58.68) dose, though it was higher compared to control (56.70).

Highest value of coefficient of variability was noticed at 20Kr dose (115.55) followed by 10Kr (86.25) and 15Kr (85.64) doses as compared to control (8.28) in IV83 genotype. On the contrary, in RLC2 genotype the lowest dose of 10Kr showed higher value of coefficient of variability (90.16) compared to control (8.83). The values observed at 15 and 20Kr doses were 63.05 and 68.18 respectively.

A dose dependent decrease in genetic variability for the trait was observed in IV83 genotype. The genetic variability values observed at 10, 15 and 20 Kr doses were 12298.40, 7233.31, 5203.39 respectively. A similar trend of increased genetic variability at lower dose of 10 Kr (959.63) and decrease in values at 15Kr (265.50) and 20Kr (1.98) doses was noticed in RLC2 genotype.

With regard to IV83 genotype a dose dependent decrease in heritability estimates was noticed with maximum value at 10Kr (99.21) followed by 15Kr (98.76) and 20Kr (98.29) treatments. Likewise in RLC2 genotype highest heritability values for the trait was observed at 10Kr (94.42) and the values decreased with increase in dosage to 15Kr (82.40) and 20Kr (3.37).

Higher genetic advance values were noticed at 10 Kr (176.51) and the value decreased at higher doses of 15Kr (174.48) and again increased at 20 Kr (234.30) in IV83 genotype. In RLC-2 genotype highest genetic advance value was found at 10 Kr (175.62) dose followed by 15 Kr (107.16) and 20 Kr (4.30) doses.

In case of IV83 genotype, 20Kr recorded highest genotypic coefficient of variability for the trait (114.55) compared to 15Kr (85.11) and 10Kr(85.93) doses contrary to this, in RLC2 genotype lower dose of 10Kr recorded a highest value for genotypic coefficient of variability for the trait (81.61) followed by 15Kr (57.22) and 20kr(11.44) doses.

4.1.3.6 Seed yield per plant

The data on seed yield per plant as affected by gamma irradiation doses were presented in Tables - 7,8,9.

The gamma irradiation doses significantly enlarged the range for seed yield per plant in both the genotypes studied. In IV83 genotype, the minimum and maximum values ranged from 2.39 to 45.74, 3.35 to 28.92 and 0.86 to 8.48 at 10,15 and 20Kr treatments as against the value of 8.5 to 14.5

in control. With respect to RLC2 genotype a similar widening of range in positive and negative directions was noticed at 10Kr (0.20 to 3.89), 15Kr (0.40 to 1.43) and 20Kr (0.05 to 0.25) gamma ray doses compared to 4.86 to 6.40 in control.

The gamma ray doses exhibited a deleterious effect in reducing drastically the mean value for seed yield per plant in both the genotypes. In case of IV83 genotype, 10,15 and 20Kr doses reduced the mean values to 3.95, 2.89 and 1.95 as against the value of 10.85 in control. A similar drastic decrease in mean value for the trait was noticed in RLC2 genotype following irradiation at 10Kr (0.88), 15Kr (0.34) and 20Kr (0.18) doses. Compared to the control (0.74), the dose 10Kr was most effective in increasing in variability of the trait (23.81) followed by 15 and 20Kr doses in IV83 genotype. In RLC2 genotype irradiation doses of 10Kr (0.86), 15Kr (0.10) and 20Kr (0.05) significantly increased in variability for the trait compared to the control (0.04).

The coefficient of variability values were of increased magnitude at 20Kr (134.36) and 10Kr (123.54) dose followed by 15Kr (88.93) dose as against the lowest value of

7.93 in control, in IV83 genotype. Similar trend was followed in RLC2 genotype with highest values observed at 20Kr (122.22) and 10Kr (105.68) treatments followed by 15Kr (94.12) and the lowest values for coefficient of variability recorded in the control (3.34).

The genetic variation for the character was of higher magnitude at lower dose of 10Kr (23.07) followed by 20Kr (6.12) and 15Kr(5.87) treatments in IV83 genotype. In RLC2 genotype higher values of genetic variation was observed at 10Kr dose (0.82) followed by 15Kr (0.06) and 20 Kr (0.01) doses.

Higher heritability estimates were observed at 10kr treatment (96.89) and the values decreased at higher doses of 15Kr (88.88) and 20Kr (89.21) in IV83 genotype. A similar trend with respect to heritability was observed in RLC2 genotype at 10Kr (95.35), 15Kr (60.00) and 20Kr (20.00) doses.

The genetic advance for the trait was highest at 10Kr dose (246.84) and the values did not differ significantly at 15Kr (162.98) and 20Kr (246.15) treatments, in IV83 genotype. A similar increase in genetic advance for the trait at lower dose of 10Kr (206.82) and a decrease in its

value at 15Kr (114.71) and 20Kr (50.00) treatments was also observed in RLC2 genotype.

4.1.3.7 100 - seed weight

Data on 100 - seed weight are presented in Tables 7,8,9.

In general, a widened range was noticed in both the genotypes following the irradiation treatment. At 10,15 and 20Kr doses in IV83 genotype, the values ranged from 2.20 to 4.95, 0.70 to 5.10 and 1.20 to 5.95 respectively, as against the value of 3.80 to 4.25 in control. In RLC2 genotype, the respective range values observed were 0.55 to 3.75, 0.80 to 2.70 and 0.60 to 1.20 at 10,15 and 20Kr doses as against the value of 2.85 to 3.25 in Control.

The mean of the Character, decreased as a result of gamma irradiation. In IV83 genotype, increase in dosage from 10, 15 and 20Kr doses resulted in decrease in the mean values to 3.10, 2.96 and 3.05 as against the value of 3.82 in Control. A dose dependent decrease in mean values was also noticed in RLC2 genotype at 10Kr (2.30), 15Kr (1.94) and 20Kr (0.95) treatments compared to control (3.07).

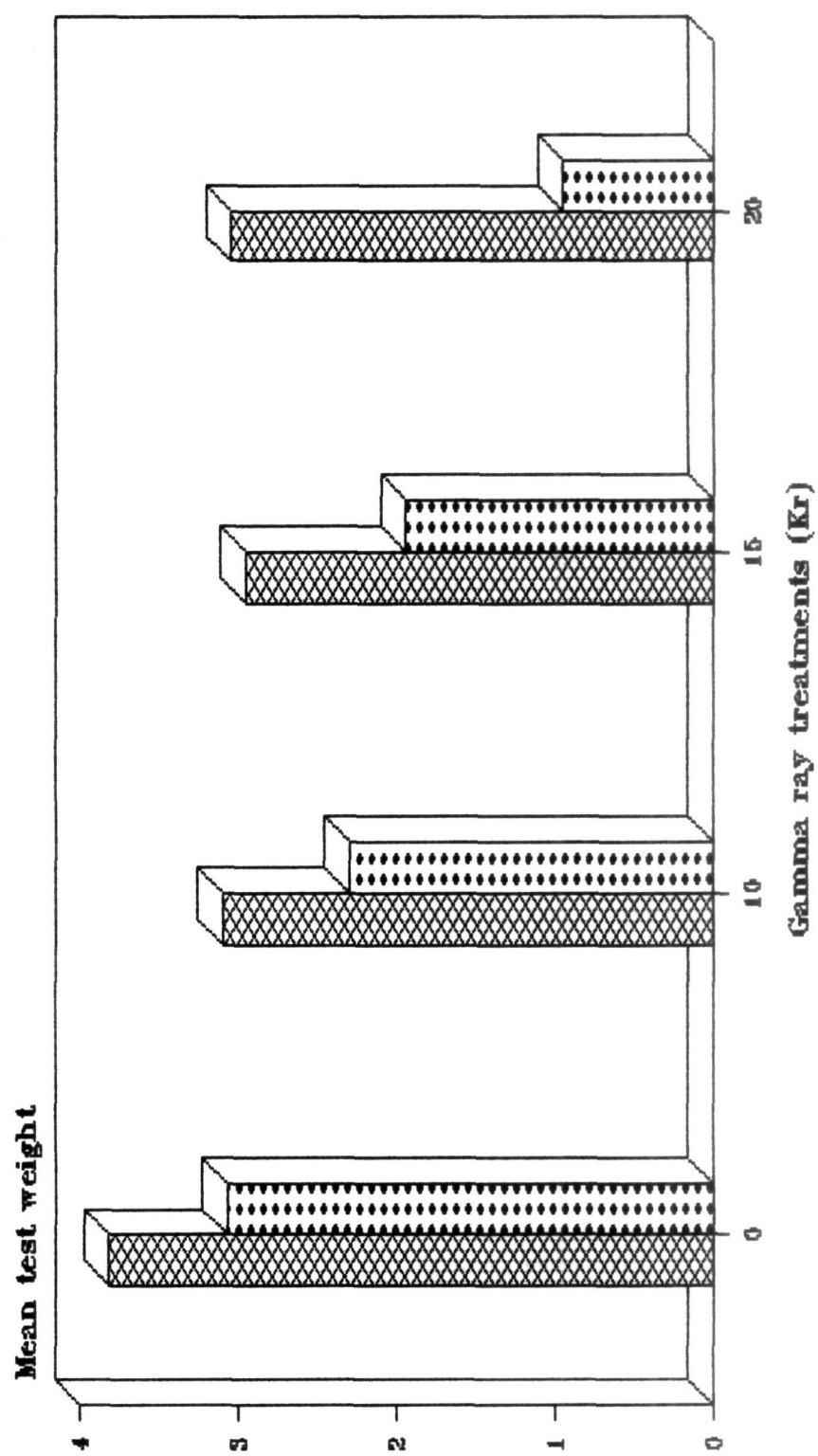


Fig 4. Effect of gamma rays on 100-seed weight in M1 generation

A linear increase in variance with increase in dosage viz; 10Kr (0.89), 15Kr (1.94) and 20Kr (1.13) was noticed in IV83 genotype. In contrast, an inverse relation was apparent in RLC2 genotype with increase in dosage from 10Kr (0.63) to 15Kr (0.55) and then to 20Kr (0.63) to 15Kr (0.55) and then to 20Kr (0.29). The variance observed in the control was least (0.19).

The coefficient of variability was of higher magnitude in the irradiated populations at 10Kr (30.65), 15Kr (34.46) and 20Kr (37.75) treatments in IV83 genotype compared to control (12.57).

A similar trend of dose dependent increase in the coefficient of variability values at 10Kr (34.35), 15Kr (38.14) and 20Kr (56.84) was apparent in RLC2 genotype, with the lowest coefficient of variability value observed in control (14.33).

In IV83 genotype, the genetic variability values increased with dosage, viz., at 10Kr (0.66), 15Kr (0.80) and 20Kr (0.90) treatments. On the contrary a decrease in the values was apparent in RLC2 genotype with increase in the dosage, viz., at 10Kr (0.44), 15Kr (0.36) and 20Kr (0.10) treatments.

The heritability estimate values observed at 10Kr, 15Kr and 20 Kr treatments were 73.91, 77.67 and 79.65, respectively in IV83 genotype. In RLC2 genotype the respective values at 10, 15 and 20Kr were 69.84, 65.45 and 34.48.

There was a linear increase in genetic advance for the trait in IV83 genotype with increase in dosage. The values observed at 10Kr, 15Kr and 20Kr treatments were 46.45, 55.07, 57.38. Contrarily in RLC2 genotype genetic advance for the trait decreased with increase in dosage from 10Kr (62.61) to 15Kr (51.55) and then to 20Kr (40.00).

4.1.3.8 Chlorophyll mutations

Frequency of chlorophyll mutations is presented in Table-10.

In case of IV83 genotype, highest frequency of chlorophyll mutants were observed at 20Kr (9.00) followed by 15Kr (6.00) and 10Kr (3.00) doses. No chlorophyll mutants were observed in untreated population. Likewise, with regard to RLC2 genotype highest frequency of chlorophyll mutants were observed at 20Kr dose (35.00) followed by 15Kr (12.00) and 10Kr (8.00) doses.

Table 10. Spectrum of chlorophyll mutations and frequencies of branching and nonbranching mutants in M_1 and M_2 generations expressed as percentage (per 100 seedlings)

Genotype	Treatment gamma rays (Kr)	M_1 generation	M_2 generation
Frequency of chlorophyll mutants (%)			
IV83	Control	-	-
	10	3.00	9.00
	15	6.00	16.00
	20	9.00	25.40
RLC2	Control	-	-
	10	8.00	10.60
	15	12.00	29.00
	20	35.00	60.00
Frequency of branching mutants (%)			
IV83	Control	-	-
	10	-	6.00
	15	-	9.00
	20	-	13.00
Frequency of nonbranching mutants (%)			
RLC2	Control	-	-
	10	-	-
	15	-	-
	20	-	-

Most of the chlorophyll mutants were observed at seedling stage. It was difficult to assign the chlorophyll mutations observed in to definite catagories as suggested by Gustafsson(1940). Most of the abnormilities observed were in the form of white and light green streaks white, light green and yellow patches and various mosaic patterns (Plate-1).

4.1.3.9 Branching behaviour

The appearance of any branching types in IV83 genotype due to irradiation treatment was studied in M₁ generation. Similarly appearance of any non branching types were looked for in RLC2 genotype. But in M₁ generation, the mutagenic treatments had no effect in altering the genotype for branching behaviour (Table-10).

4.2 M₂ GENERATION

Effect of different irradiation doses on survival, chlorophyll, branching behaviour and quantitative characters were studied in M₂ generation.

Plate 1. Chlorophyll mutations in M_1 generation of IV83 genotype

a - 15 Kr

b - 20 Kr

Plate 1.



a



b

4.2.1 SURVIVAL

Data on survival of plants in M_2 generation as expressed per 100 seedlings, recorded on 25th day after sowing is presented in Table-3.

Reduction in survival was noticed at all the doses in both the restorer lines studied. In IV83 genotype, increase in dosage caused deleterious effect on survival of the plants. Survival of plants (expressed as percentage) observed at 10,15 and 20Kr doses were 66.11, 53.25 and 46.67 as against the value of 81.21 in control. A similar response of drastic reduction in survival with increase in dosage was observed in RLC2 genotype at 10Kr (42.07), 15Kr (36.00) and 20Kr (13.33) doses.

4.2.2 Abnormal seedlings

Mutants with abnormalities for petiole length and leaf characters were recovered in M_2 generation of both the genotypes as given in Plates-2, 3.

Most of the morphological abnormalities were observed at seedling stage. Changes with respect to leaf shape and petiole length were noticed in the mutants.

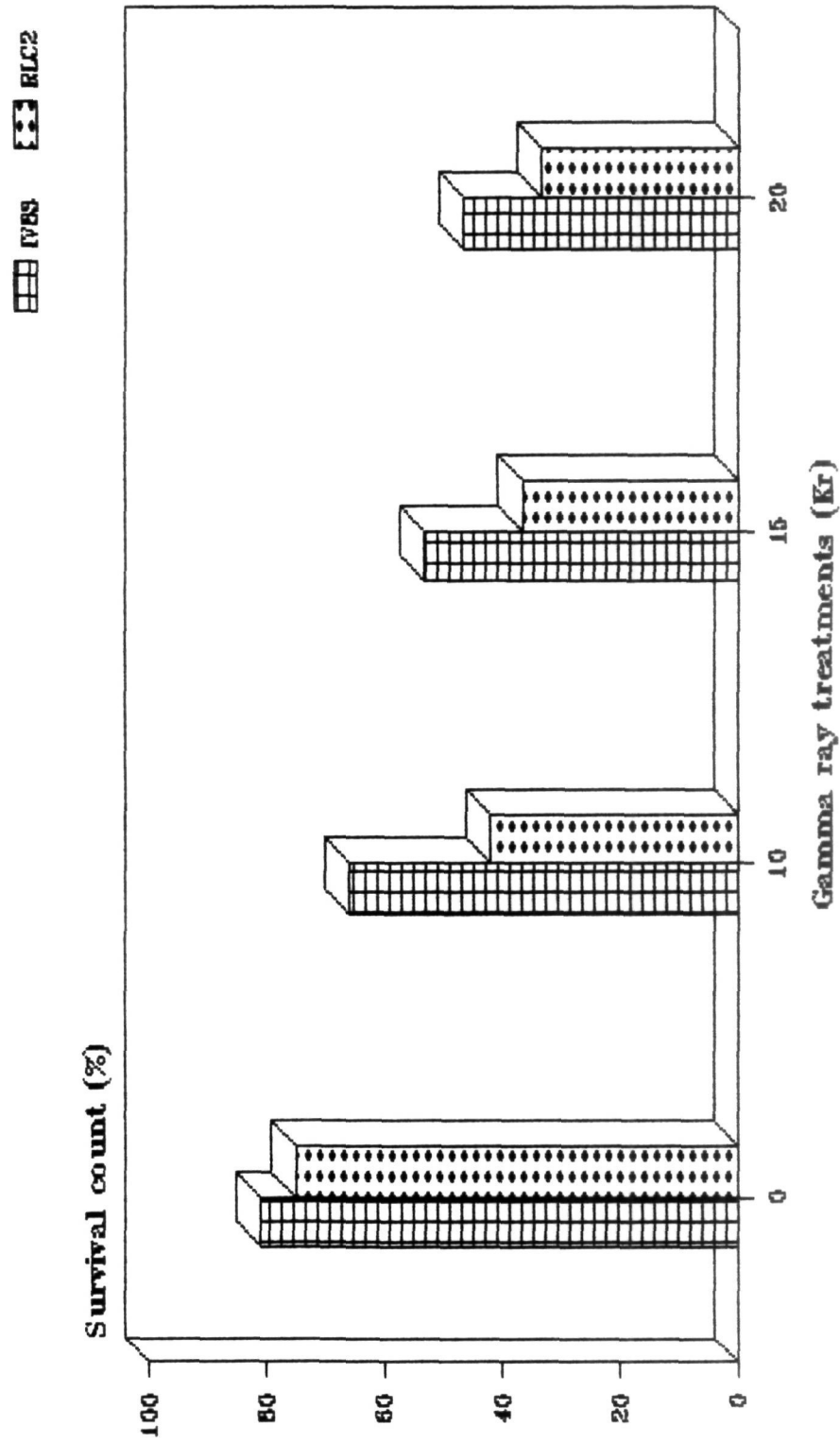


Fig.5 Effect of gamma rays on survival at 25 days after sowing in M2 generation

Plate 2. Mutants showing long petiole and abnormal leaves in
M₂ generation of IV83 genotype

a - 10 Kr
b - 15 Kr
c - 20 Kr

Plate 2.



a



b



c

Plate 3. Mutants showing long petiole and abnormal leaves in
M₂ generation of RLC2 genotype

a - 10 Kr
b - 15 Kr
c - 20 Kr



a



b



c

4.2.2.1 Chlorophyll mutations

Chlorophyll mutations could be easily observed in case of gamma irradiated M_2 population. The frequency of chlorophyll mutations increased with dosage in IV83 genotype viz, at 10Kr (9.00), 15Kr (16.00) and 20Kr (25.40) treatments. RLC2 genotype was found to be more sensitive to gamma ray treatments with frequency of chlorophyll mutations being 10.60, 29.00 and 60.00 at 10, 15 and 20Kr doses respectively (Table-10).

The spectrum of chlorophyll mutations in M_2 was almost similar to the one observed in M_1 generation. Most of the abnormalities were manifested at seedling stage and were in the form of white and light green streaks; white light green and yellow patches, and various mosaic patterns (Plate-4). However, it was difficult to assign the chlorophyll mutations observed, into definite categories, viz, albino, xantha, viridis as suggested by Gustafsson (1940).

4.2.2.2 Mutagenic effectiveness and efficiency of gamma rays based on chlorophyll mutations and lethality in m_2 generations (on m_2 seedling basis)

Data on mutagenic effectiveness and efficiency are presented in Table-11. Effectiveness of gamma rays based

Plate 4. Chlorophyll mutant observed in M_2 generation of RLC2 genotype

a - 10 Kr

Plate 4.



a

Table 11. Mutagenic effectiveness and efficiency of different doses of gamma rays

Genotype	Treatment gamma rays (Kr)	Mutagenic effectiveness	Mutagenic efficiency
IV83			
	Control	-	-
	10	0.88	0.47
	15	1.08	0.63
	20	1.27	0.75
RLC2			
	Control	-	-
	10	1.06	0.36
	15	1.93	0.55
	20	3.00	0.65

on mutations induced was in general directly proportional to the dose applied in both the genotypes. In IV83 genotype, the dose 20 Kr was more effective in inducing mutations (1.27) followed by 15 Kr (1.08) and 10Kr (0.88) doses. A similar trend was observed in RLC2 genotype with the dose 20Kr being more effective (3.00) followed by 15 Kr (1.93) and 10Kr (1.06) treatments.

Efficiency of gamma rays also appears to increase with the dosage. However, no significant differences were noticed among the doses with respect to efficiency. In IV83 genotype, mutagenic efficiency values observed at 10, 15 and 20Kr doses were 0.47, 0.63 and 0.75 respectively. Similarly in RLC2 genotype, at 10, 15 and 20 Kr doses the mutagenic efficiency values of 0.36, 0.55 and 0.65 were observed.

4.2.3 Branching behaviour

The appearance of branching types in IV83 genotype and non branching types in RLC2 genotype were observed in M_2 and their frequencies were calculated (Table-10).

In IV83 genotype, highest frequency of branching types were observed at 20 Kr dose (13.00) followed by 15Kr

(9.00) and 10Kr (6.00) dose (Plate-5). No such abnormal types were noticed in control.

With respect to RLC2 genotype, non branching mutants were not observed in any of the treatments.

4.2.4 Quantitative characters

The estimates such as range, mean, variance, coefficient of variability, genetic variation, heritability, genetic advance and genotypic coefficient of variability were calculated in M_2 generation to know the effect of gamma irradiation doses on quantitative characters.

4.2.4.1 Days to flowering

The data on effect of gama radiation on days to flowering in M_2 are presented in Tables 12,13,14.

In M_2 generation, several early flowering types were recovered following gamma irradiation treatments. The observed range values at 10 Kr, 15 Kr and 20 Kr doses in IV83 genotype were 48 to 63, 50 to 60, 53 to 65 as against the value of 54-60 days in control. A similar widening of range was observed in RLC2 genotype at 10 Kr (55 to 66), 15 Kr

Plate 5. Branching mutants recovered in M₂ generation of IV83 genotype

- a - A mutant with basal branching at 15 Kr dose**
- b - Another mutant showing basal branching at 20 Kr dose**

Plate 5.



a



b

Table 12. Range, mean, standard error, variance and coefficient of variability for days to flowering, plant height, head diameter and number of branches per plant in M₂ generation

Genotype	Treatment gamma rays (Kr)	Range	Mean $\pm$	S.E.	Variance	C.V.
Days to flowering						
IV83	Control	54-60	57.00	0.17	4.00	3.51
	10	48-63	53.00	0.10	21.24	8.69
	15	50-60	55.00	0.09	9.99	5.75
	20	53-65	56.00	0.20	13.99	6.68
RLC2	Control	58-64	61.00	0.16	4.00	3.28
	10	55-66	58.00	0.15	11.90	5.95
	15	57-65	60.00	0.22	6.67	4.30
	20	58-62	61.00	0.69	4.89	3.62
Plant height (cm)						
IV83	Control	85-120	98.14	1.18	187.28	13.94
	10	65-160	103.84	0.35	246.77	15.13
	15	70-150	101.15	0.39	219.60	14.03
	20	55-165	102.73	1.07	403.73	19.56
RLC2	Control	80-120	102.58	1.23	173.92	13.34
	10	60-145	105.76	0.64	231.04	14.37
	15	40-130	104.01	1.43	277.66	16.02
	20	70-125	98.00	5.67	321.13	18.29
Head diameter (cm)						
IV83	Control	10-20	14.02	0.23	6.98	18.83
	10	6-26	14.89	0.08	11.30	22.57
	15	5-20	13.61	0.08	7.56	20.21
	20	6-19	13.70	0.15	7.34	19.78
RLC2	Control	5-10	8.41	0.12	1.83	16.08
	10	3-23	7.97	0.07	2.66	20.45
	15	3-10	6.90	0.12	1.90	20.00
	20	5-7	6.20	0.43	1.87	22.05
Number of branches per plant						
RLC2	Control	9-23	12.35	0.38	17.72	34.09
	10	3-54	13.38	0.28	44.51	49.87
	15	3-32	15.32	0.51	35.14	38.70
	20	6-24	16.50	2.60	27.04	34.52

Table 13. Phenotypic, genotypic and environmental variances, heritability and genetic advance for days to flowering, plant height, head diameter and number of branches per plant in M_2 generation

Genotype	Treatment gamma rays (Kr)	V_p	V_G	V_E	H(%)	GA
Days to flowering						
IV83	Control	-	-	4.00	-	-
	10	21.24	17.24	-	81.20	14.57
	15	9.99	5.99	-	59.96	7.11
	20	13.99	9.99	-	71.41	9.84
RLC2	Control	-	-	4.00	-	-
	10	11.90	7.90	-	66.39	8.15
	15	6.67	2.67	-	40.03	3.55
	20	4.89	0.89	-	18.20	1.36
Plant height (cm)						
IV83	Control	-	-	187.28	-	-
	10	246.77	59.49	-	24.10	7.40
	15	219.60	32.32	-	14.72	4.38
	20	403.73	216.45	-	53.60	21.28
RLC2	Control	-	-	173.92	-	-
	10	231.04	57.12	-	24.72	7.33
	15	277.66	103.74	-	37.36	12.15
	20	321.13	147.21	-	45.84	17.02
Head diameter (cm)						
IV83	Control	-	-	6.98	-	-
	10	11.30	4.32	-	38.23	17.81
	15	7.56	0.58	-	7.67	3.20
	20	7.34	0.36	-	4.96	2.02
RLC2	Control	-	-	1.83	-	-
	10	2.66	0.83	-	31.20	13.17
	15	1.90	0.07	-	3.68	1.52
	20	1.87	0.04	-	2.13	0.97
Number of branches per plant						
RLC2	Control	-	-	17.72	-	-
	10	44.51	26.79	-	60.19	61.91
	15	35.14	17.42	-	49.57	39.57
	20	27.04	9.32	-	34.47	22.41

Table 14. Phenotypic, genotypic and environmental coefficient of variation for days to flowering, plant height, head diameter and number of branches per plant in M₂ generation

Genotype	Treatment gamma rays (Kr)	PCV	GCV	ECV
Days to flowering				
IV83	Control	-	-	3.51
	10	8.69	7.83	-
	15	5.75	4.45	-
	20	6.68	5.64	-
RLC2	Control	-	-	3.28
	10	5.95	4.85	-
	15	4.30	2.72	-
	20	3.62	1.55	-
Plant height (cm)				
IV83	Control	-	-	13.94
	10	15.13	7.43	-
	15	14.03	5.63	-
	20	19.56	14.32	-
RLC2	Control	-	-	13.34
	10	14.37	7.15	-
	15	16.02	9.80	-
	20	18.29	12.38	-
Head diameter (cm)				
IV83	Control	-	-	18.83
	10	22.57	13.90	-
	15	20.21	5.60	-
	20	19.78	4.40	-
RLC2	Control	-	-	16.08
	10	20.45	11.43	-
	15	20.00	3.84	-
	20	22.05	3.23	-
Number of branches per plant				
RLC2	Control	-	-	34.09
	10	49.87	38.68	-
	15	38.70	27.24	-
	20	34.52	18.50	-

(57-65) and 20 Kr (58-62) irradiation doses compared to narrow range observed in control (58-64).

The mean of the character was reduced in M_2 generation of both the genotypes. In IV83 genotype, the values observed at 10, 15 and 20 Kr doses were 53.00, 55.00 and 56.00 days as against the mean value of 57.00 in control. In RLC2 genotype the dose 10 Kr was more effective in reducing the mean value for the trait (58.00) followed by 15 Kr (60.00) treatment. The mean value observed at 20 Kr dose was on par with control (61.00).

Irradiation treatments increased the variability for days to flowering in both the genotypes (Plate-6). With respect to IV83 genotype the variance value increased at lower dose of 10 Kr (21.24) followed by 20 Kr (13.99) and 15 Kr (9.99) doses as compared to least variance observed in control (4.00). Likewise, 10 Kr dose recorded a highest variance for the trait (11.90) in RLC2 genotype followed by 15 Kr (6.67) and 20 Kr (4.89) doses.

An increase in magnitude of coefficient of variability was apparent at 10 Kr (8.69) in IV83 genotype followed by 20 Kr (6.68) and 15 Kr (5.75) treatments. In RLC2 genotype also, 10 Kr dose was more effective in

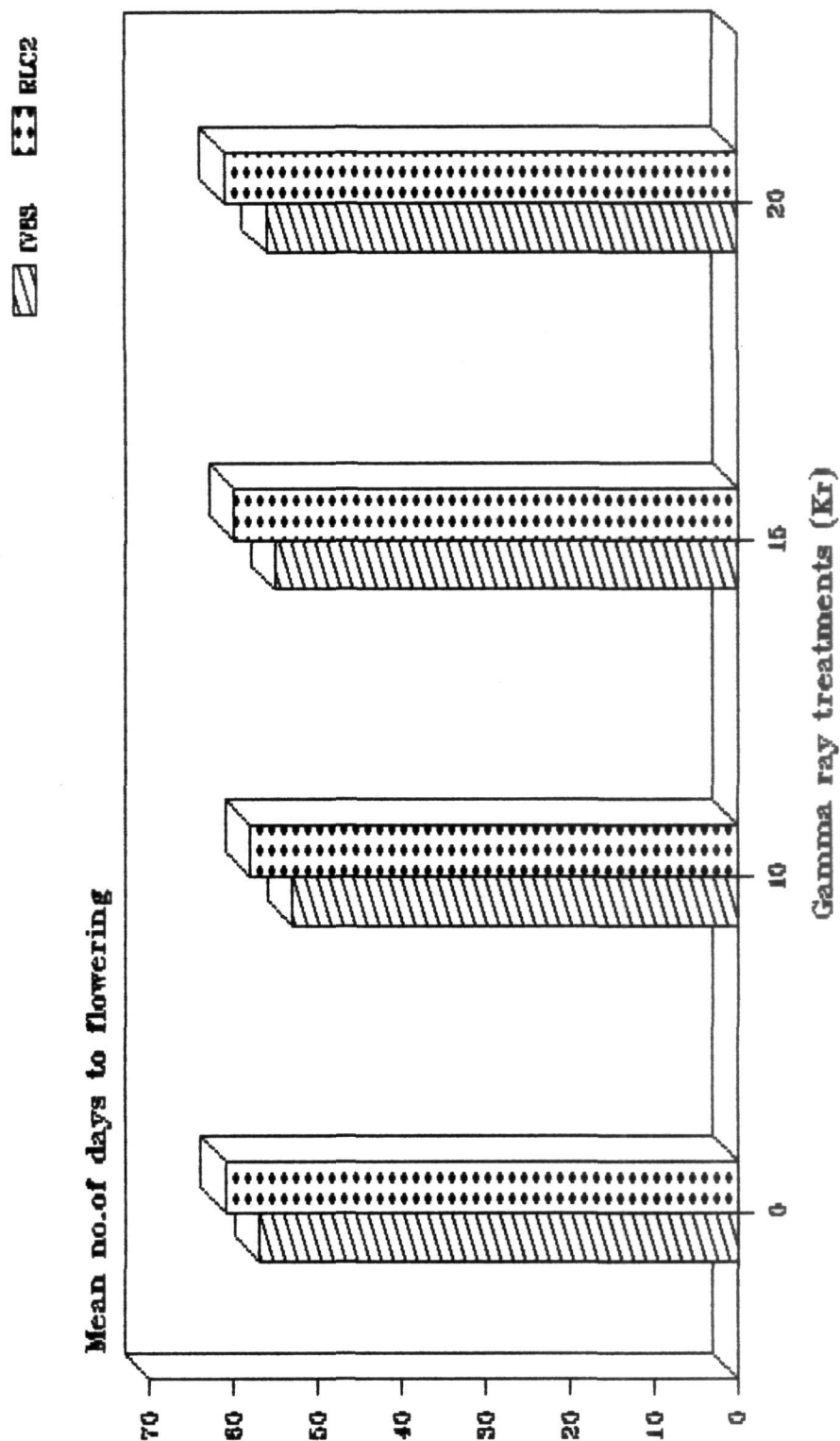


Fig 6. Effect of gamma rays on days to flowering in M2 generation of two sunflower restorer lines

Plate 6. An overview of IV83 15 Kr population showing variation for flowering duration and plant height

Plate 6.



increasing the coefficient of variability for the trait (5.95) followed by 15Kr (4.30) and 20Kr (3.62) doses.

In IV83 genotype, the dose 10 Kr was effective in increasing the magnitude of genetic variance (17.24) followed by 20Kr (9.99) and 15Kr (5.99) doses. With respect to RLC2 genotype a dose dependent decrease in genetic variance was observed viz., at 10Kr (7.90), 15Kr (2.67) and 20Kr (.89) treatments.

With respect to heritability estimate in IV83 genotype, the highest value was recorded at 10 Kr treatment (81.20) followed by 15Kr (71.41) and 20Kr (59.96). The heritability estimate for the trait was highest at 10Kr dose (66.39) followed by 15Kr (40.03) and 20Kr (18.20) doses in IV83 genotype RLC2 genotype.

The highest genetic advance value was observed in 10 Kr treatment (14.57) followed by 20 Kr (9.84) and 15Kr (7.11) treatments in IV83 genotype. Similarly, 10 Kr treatment was found to be effective in increasing the genetic advance estimate (8.15) compared to 15 Kr (3.55) and 20 Kr treatments (1.36).

With respect to genotypic coefficient of variation for the trait in IV83 genotype, the highest value was observed at 10 Kr dose (7.83) followed by 20Kr (5.64) and 15Kr (4.45) treatments, the observed values at 10Kr, 15Kr and 20Kr doses in RLC2 were 4.85, 2.72 and 1.55 respectively.

4.2.4.2 Plant height

The effect of gamma radiation on plant height based on estimates such as range, mean, variance, coefficient of variability, genetic variation, heritability, genetic advance and genotypic coefficient of variation is presented in Tables 12,13,14.

The irradiation doses significantly widened the range in positive and negative directions in both the genotypes. For instance, the values ranged from 55 to 62 in IV83 genotype at 10Kr, 15Kr and 20Kr treatments compared to the narrow range of 85-120 in control. Likewise, a widened range was observed in RLC2 genotype at 10 Kr (60-145), 15Kr (40-130) and 20Kr (70-125) doses compared to control (80-120).

In general, gamma irradiation increased the mean value for plant height in both the genotypes. Mean values

of higher magnitude were recorded at 10Kr (103.84), 15Kr(101.15) and 20Kr (102.73) treatments in IV83 genotype compared to control (98.14).

With respect to RLC2 genotype increased mean values were observed at 10Kr (105.76) and 15Kr (104.04) treatments compared to control (102.58). The mean value for the trait at 20 Kr treatment (98.00) was found to be less than the control.

In both the genotypes, the variability for the trait significantly increased at all the irradiation doses, in both the genotypes. With respect to IV83 genotype highest value of variance was observed at 20Kr (403.73) followed by 10Kr (246.77) and 15Kr(219.60)treatments and least value of variance was observed in control (187.28). Similarly in RLC2 genotype irradiation doses viz, 10Kr (231.04), 15Kr (277.66) and 20Kr (321.13) significantly increased the variability for the trait compared to control (173.92).

The coefficient of variability values were also of higher magnitude in irradiated populations at 10Kr (15.13),15Kr (14.03) and 20Kr (19.56) treatments in IV83 genotype compared to control (13.94). Similar trend was followed in RLC2 genotype.

Increase in dosage from 10Kr (14.37) to 15Kr (16.02) and then to 20Kr (18.29) resulted in increase in coefficient of variability value with least value observed in control (13.34).

In IV83 genotype, the dose 20Kr was very effective in increasing the genetic variation (216.45) compared to 10Kr (59.49) and 15Kr (32.32) treatments. With respect to RLC2 genotype, the same trend was followed with highest genetic variation for the trait at 20Kr dose (147.21) followed by 15 Kr (103.74) and 10Kr (57.12) doses.

With respect to heritability estimate , highest value was recorded at 20Kr (53.60) dose in IV83 genotype compared to 10Kr (24.10) and 15Kr doses. Similarly in RLC2 genotype the heritability values of 45.84, 37.63 and 24.72 were observed at 20,15 and 10Kr respectively.

Highest genetic advance value of 21.28 was observed at 20Kr and the values decreased to 7.40 and 4.38 at 10 and 15 Kr treatments in IV83 genotype. In RLC2 genotype increased values were noticed with increase in dosage from 10 Kr (7.33) to 15Kr (12.15) and then to 20Kr (17.02).

In IV83 genotype, the genotypic coefficient of variation was of higher magnitude at 20Kr (14.32) dose compared to 10 Kr (7.43) and 15Kr (5.63) doses. With respect to RLC2 genotype a linear relationship between increase in genotypic coefficient of variation with increase in dosage viz, 10Kr (7.15), 15Kr (9.80) and 20Kr (12.38) was observed.

Both tall and dwarf mutants were recovered as a result of irradiation treatments in IV83 genotype (Plate-7).

4.2.4.3 Head diameter

The data on head diameter are presented in Tables 12,13,14.

A widened range was observed in both the genotypes following irradiation treatment. In IV83 genotype at 10 Kr, 15 Kr and 20 Kr treatments the values ranged from 6 to 26, 5 to 20Kr respectively. Compared to the value of 10-20 in control. Similarly, with respect to RLC2 genotype widened range was observed at 10Kr (3-23), 15Kr (3-10) and 20Kr (5-7) treatments compared to control (5-10).

The mean value was of higher magnitude at 10Kr (14.89) compared to control (14.02) in IV83 genotype.

Plate 7. Mutants with respect to plant height in M_2 generation

a - Dwarf mutants observed at seedling stage in IV83 genotype at 15 Kr dose

Plate 7.



a

Decreased mean values were noticed at 15Kr (13.61) and 20Kr (13.70) treatments compared to control. In RLC2 genotype the mean value decreased progressively from 7.97 at 10Kr dose to 6.90 at 15 Kr and further decreased to 6.20 at 20 Kr dose compared to 8.41 in control. Irradiation doses increased the variability for the character in M_2 generation. In case of IV83 genotype the variance values noticed at 10, 15 and 20Kr treatments were 11.30, 7.56 and 7.34 as against the value of 6.98 in control. Likewise in RLC2 genotype, compared to control (1.83) the variance for the trait increased at 10 Kr (2.66), 15Kr (1.90) and 20 Kr (1.87) treatments.

Increased Coefficient of variability values were also noticed in the treated population compared to untreated population. With respect to IV83 genotype the values observed at 10, 15 and 20Kr treatments were 22.57, 20.21 and 19.78 respectively as against the value of 18.83 in control. Similarly increased values were observed in treated populations at 10Kr (20.45), 15 Kr (20.00) and 20Kr (22.05) treatments compared to the untreated population (16.08) in RLC2 genotype.

Increased genetic variation for the trait was observed in the treated population at 10Kr (4.32) dose

compared to 15 Kr (0.58) and 20 Kr (0.36) doses in IV83 genotype. Similarly increased value of genetic variation was observed for the trait at 10 Kr (0.83) dose in RLC2 genotype compared to 15Kr (0.07) and 20 Kr (0.04) doses.

In case of IV83 genotype, 10Kr treatment recorded a highest value of heritability (38.23) and decreased heritability values were observed at 15Kr (7.67) and 20Kr (4.96) treatments. Similarly in RLC2 genotype, increased heritability value of 31.20 was observed at 10Kr treatment and the values decreased significantly at 15Kr (3.68) and 20Kr (2.13) treatments.

Higher value of genetic advance was observed at lower dose of 10Kr (17.81) in IV83 genotype and lower values were noticed at 15Kr (3.20) and 20 Kr treatments (2.02). Similarly increase in genetic advance estimate for the trait was noticed in RLC-2 genotype at lower dose of 10Kr (13.17) compared to 15Kr (1.52) and 20Kr (0.97) treatments.

With respect to genotypic Coefficient of variability the treatment 10Kr recorded the highest value (13.90) followed by 15Kr (5.60) and 20Kr (4.40) treatments. Similar trend was observed in RLC-2 genotype at 10Kr (11.43), 15Kr (3.84) and 20Kr (3.23) treatments.

Higher value of genetic advance was observed at lower dose of 10Kr (17.81) in IV83 genotype and lower values were noticed at 15Kr (3.20) and 20Kr treatments (2.02) similarly increase in genetic advance estimate for the trait was noticed in RLC2 genotype at lower dose of 10Kr (13.17) compared to 15Kr (1.52) and 20Kr (0.97) treatments.

With respect to genotypic Coefficient of variability the treatment 10Kr recorded the highest value (13.90) followed by 15Kr (5.60) and 20Kr (4.40) treatments. Similar trend was observed in RLC2 genotype at 10Kr (11.43), 15Kr (3.23) and 20Kr(3.23) treatments.

4.2.4.4 Number of branches per plant

The data on number of branches per plant are presented in Tables - 12,13,14.

In RLC2 genotype, irradiation treatments significantly widened the range in positive and negative directions. The values ranged from 3 to 54, 3 to 32 and 6 to 24 at 10Kr, 15Kr and 20Kr treatments compared to the value of 9 to 23 in control.

The mean value of higher magnitude were observed at 10Kr (13.38), 15Kr (15.32) and 20Kr (16.50) treatments compared to control (12.35).

Irradiation treatments viz., 10Kr (44.51), 15Kr (35.14) and 20Kr (27.04) significantly increased the variance for the trait in RLC2 genotype compared to control (17.72).

The coefficient of variability estimate was of higher magnitude at 10Kr (49.87) followed by 15Kr (38.70) and 20Kr(34.52) treatments compared to control (34.09) in RLC2 genotype.

Highest value of genetic variance was recorded at 10Kr (26.79) and the values decreased at 15Kr (17.42) and 20Kr (9.32) treatments in RLC2 genotype.

In RLC2 genotype the treatment 10Kr recorded highest heritability value (60.19) followed by 15Kr (49.57) and 20Kr (34.47) treatments. Highest genetic advance estimate was observed at 10Kr treatment (61.91) compared to 15Kr (39.57) and 20Kr (22.4) treatments in RLC2 genotype. The amount of genotypic coefficient of variation was more at 10Kr dose (38.68) followed by 15Kr (27.24) and 20Kr (18.50) in RLC2 genotype.

4.2.4.5 Seed Yield per plant

The data on seed yield per plant as affected by gamma irradiation are presented in Tables-15,16,17.

Table 15. Range, mean, standard error, variance and coefficient of variability for seed yield per plant, 100-seed weight and oil content in M_2 generation

Genotype	Treatment gamma rays (Kr)	Range	Mean $\pm$ S.E.		Variance	C.V.
Seed yield per plant (g)						
IV83	Control	18.80-38.50	25.70	0.58	44.79	26.03
	10	5.00-49.76	21.43	0.20	77.62	41.11
	15	5.40-57.35	21.24	0.27	95.26	46.00
	20	6.12-48.00	19.02	0.47	75.92	45.81
RLC2	Control	5.10-10.50	7.29	0.15	2.81	22.91
	10	4.35-16.15	8.00	0.12	7.62	34.49
	15	3.74-12.75	7.95	0.28	10.92	41.56
	20	1.30- 5.25	3.76	0.35	3.21	47.66

100-seed weight (g)						
IV83	Control	4.43- 5.34	4.96	0.03	0.10	6.45
	10	2.92- 7.57	4.99	0.02	0.66	16.23
	15	3.00- 6.95	5.06	0.02	0.71	16.59
	20	3.94- 7.67	5.28	0.04	0.67	15.53
RLC2	Control	3.20- 4.11	3.55	0.03	0.12	9.92
	10	2.26- 4.35	3.18	0.02	0.22	14.78
	15	2.58- 5.08	3.45	0.06	0.49	20.29
	20	2.20- 3.10	2.78	0.06	0.15	13.92

Oil content (%)						
IV83	Control	25.80-31.50	27.11	0.80	7.18	9.89
	10	25.10-37.60	28.99	0.32	11.02	11.45
	15	27.00-35.00	30.00	0.30	9.67	10.37
	20	26.30-35.70	28.55	0.62	12.25	12.26
RLC2	Control	27.20-32.60	28.22	0.78	6.76	9.21
	10	25.40-38.40	29.67	0.44	10.69	11.02
	15	25.20-39.80	31.91	0.87	20.98	14.35
	20	25.10-30.50	26.90	0.89	7.24	10.00

Table 16. Phenotypic, genotypic and environmental variances, heritability and genetic advance for seed yield per plant, 100-seed weight and oil content in M₂ generation

Genotype	Treatment gamma rays (Kr)	V _p	V _G	V _E	H(%)	GA
Seed yield per plant (g)						
IV83	Control	-	-	44.79	-	-
	10	77.62	32.83	-	42.30	35.88
	15	95.26	50.47	-	52.98	50.22
	20	75.92	31.13	-	41.00	38.75
RLC2	Control	-	-	2.81	-	-
	10	7.62	4.81	-	63.10	44.92
	15	10.92	8.12	-	74.25	63.67
	20	3.21	0.40	-	12.40	12.19
100-seed weight (g)						
IV83	Control	-	-	0.10	-	-
	10	0.66	0.56	-	84.55	28.40
	15	0.71	0.61	-	85.63	29.42
	20	0.67	0.57	-	84.78	27.11
RLC2	Control	-	-	0.12	-	-
	10	0.22	0.10	-	43.89	13.36
	15	0.49	0.37	-	74.69	31.26
	20	0.15	0.03	-	17.33	4.98
Oil content (%)						
IV83	Control	-	-	7.18	-	-
	10	11.02	3.84	-	34.84	8.28
	15	9.67	2.49	-	25.75	5.57
	20	12.25	5.07	-	41.39	2.96
RLC2	Control	-	-	6.76	-	-
	10	10.69	3.93	-	36.76	8.41
	15	20.98	14.22	-	67.78	6.43
	20	7.24	0.48	-	6.63	1.44

Table 17. Phenotypic, genotypic and environmental coefficient of variation for seed yield per plant, 100-seed weight and oil content in M₂ generation

Genotype	Treatment gamma rays (Kr)	PCV	GCV	ECV
Seed yield per plant (g)				
IV83	Control	-	-	26.03
	10	41.11	26.74	-
	15	46.00	33.45	-
	20	45.81	29.34	-
RLC2	Control	-	-	22.91
	10	34.49	27.41	-
	15	41.56	35.85	-
	20	47.66	16.78	-
100-seed weight (g)				
IV83	Control	-	-	6.45
	10	16.23	15.03	-
	15	16.59	15.41	-
	20	15.53	14.20	-
RLC2	Control	-	-	9.92
	10	14.78	9.79	-
	15	20.29	17.54	-
	20	13.92	5.79	-
Oil content (%)				
IV83	Control	-	-	9.89
	10	11.45	6.76	-
	15	10.37	5.27	-
	20	12.26	7.89	-
RLC2	Control	-	-	9.21
	10	11.02	6.68	-
	15	14.35	11.82	-
	20	10.00	2.58	-

The range was appreciably enlarged in positive and negative directions following irradiation treatment in both the genotypes. In IV83 genotype, the values ranged from 5.00 to 49.76 at 10Kr dose, 5.40 to 57.35 at 15Kr and 6.12 to 48.00 at 20Kr dose as against the values of 18.80 to 38.50 in control. A similar trend of increase in range values at 10Kr (4.35 to 16.15), 15Kr (3.74 to 12.75) and 20Kr (1.30 to 5.25) doses were observed in RLC2 genotype compared to control (5.10 to 10.50).

The irradiation doses viz., 10Kr, 15Kr and 20Kr reduced the mean value of the character to 21.43, 21.24 and 19.02 as against the mean value of 25.70 observed in control, in IV83 genotype. On the contrary in RLC2 genotype, compared to control (7.29), 10Kr (8.00) and 15Kr (7.95) treatments recorded a higher mean value for the trait. A decrease in the value was noticed at higher dose of 20Kr (3.76).

In IV83 genotype, an increase in variance for the trait was observed at all the irradiation doses viz., 10Kr (77.62), 15Kr (95.26) and 20Kr (75.92) doses compared to control (44.79). A similar increase in variance values at 10Kr (7.62), 15Kr (10.92) and 20Kr (3.21) doses was observed in RLC2 genotype.

With respect to IV83 genotype highest coefficient of variability estimate was observed at 15Kr dose (46.00) followed by 20Kr (45.81) and 10Kr (41.11) doses with the least value observed in control (26.03). Coefficient of variability for the trait in RLC2 genotype showed an increase in its value at 20Kr dose(47.66) followed by 15Kr (41.56) and 10Kr (34.49) doses.

Genetic variation for the trait, of higher magnitude was observed at 15Kr (50.47) treatment followed by 10Kr (42.30) and 20Kr (31.13) treatments in IV83 genotype. In case of RLC2 genotype, the dose 15Kr (10.92) was more effective in increasing the genetic variance for the trait compared to 10Kr (4.81) and 20Kr (0.40) doses.

The higher values for heritability was observed at 15Kr treatment (52.98) followed by 10Kr (42.30) and 20Kr (41.00) treatments, in IV83 genotype. A similar trend of higher heritability estimates at 15Kr (74.25) followed by 10Kr (63.10) and 20Kr (12.40) treatments was observed in RLC2 genotype.

In IV83 genotype highest genetic advance value was observed at 15Kr gamma ray dose (50.22) and the value decreased to 35.88 at 10Kr and to 38.75 at 20Kr doses. A

similar trend was noticed in RLC2 genotype at 10Kr (44.92), 15Kr (63.67) and 20Kr (12.19) treatments.

The genotypic coefficient of variability values at 10, 15 and 20Kr treatments in IV83 genotype were 26.74, 33.45 and 29.34 respectively. In RLC2 genotype the values observed at 10, 15 and 20Kr treatments were 27.41, 35.85 and 16.78 respectively.

Seed size variations were observed in both the genotypes as a result of different irradiation treatments viz., 10 Kr, 15 Kr and 20 Kr (Plates-8,9).

4.2.4.6 100 - Seed Weight

The data on 100-seed weight as affected by gamma irradiation treatments are presented in Tables- 15,16,17.

The irradiation doses significantly enlarged the range in both the genotypes. In IV83 genotype, at 10Kr, 15Krand 20kr treatments, observed range values were 2.92 to 7.57, 3.00 to 6.95 and 3.94 to 7.67 as against the value of 4.43 to 5.34 in control. Likewise , in RLC2 genotype the values ranged from 2.26 to 4.35, 2.58 to 5.08 and 2.20 to 3.10 from 2.26 to 4.35, 2.58 to 5.08 and 2.20 to 3.10 at 10Kr, 15Kr

Plate 8. Seed size variations in IV83 genotype at 10Kr, 15Kr
and 20 Kr doses

a - 10 Kr
b - 15 Kr
c - 20 Kr

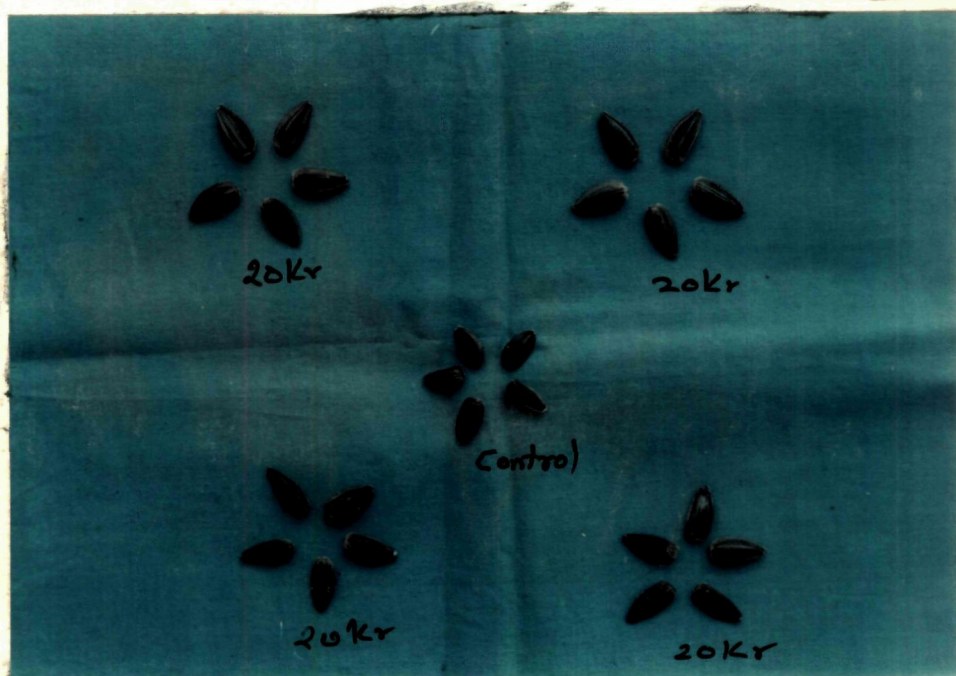
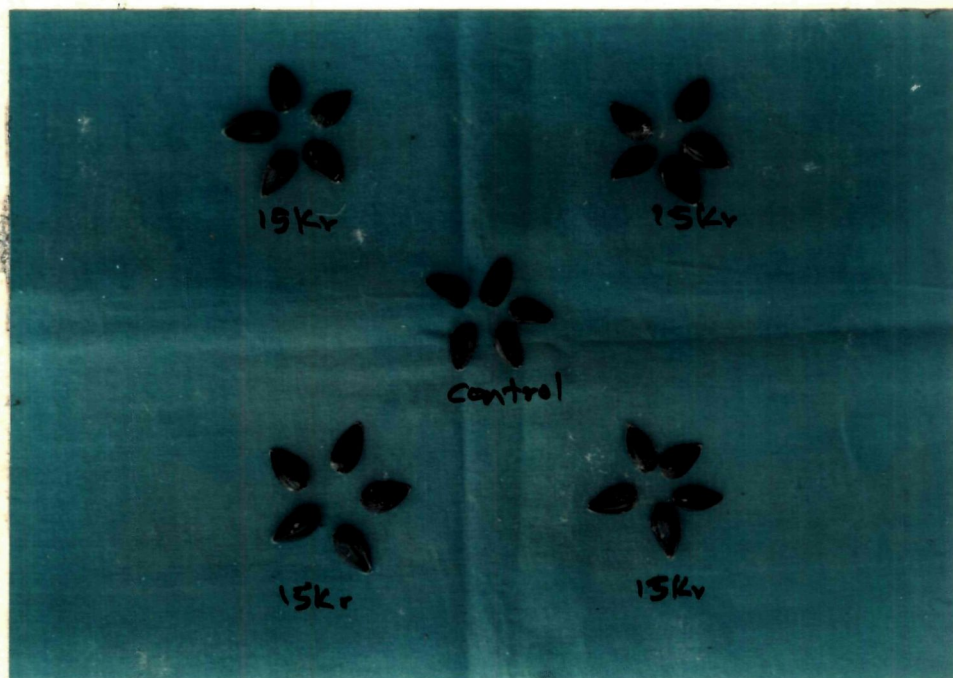
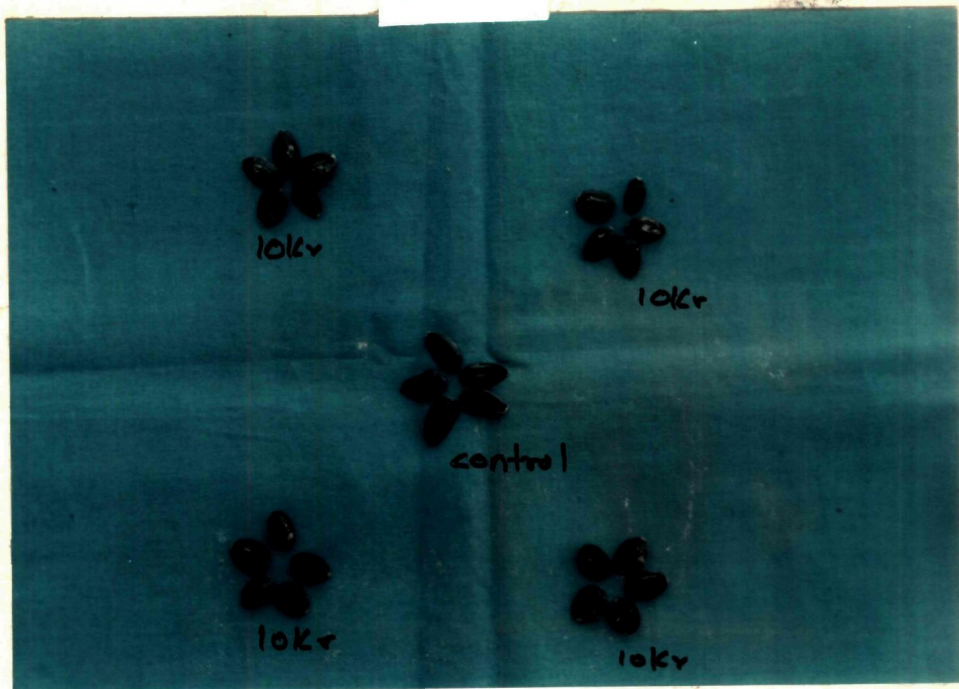
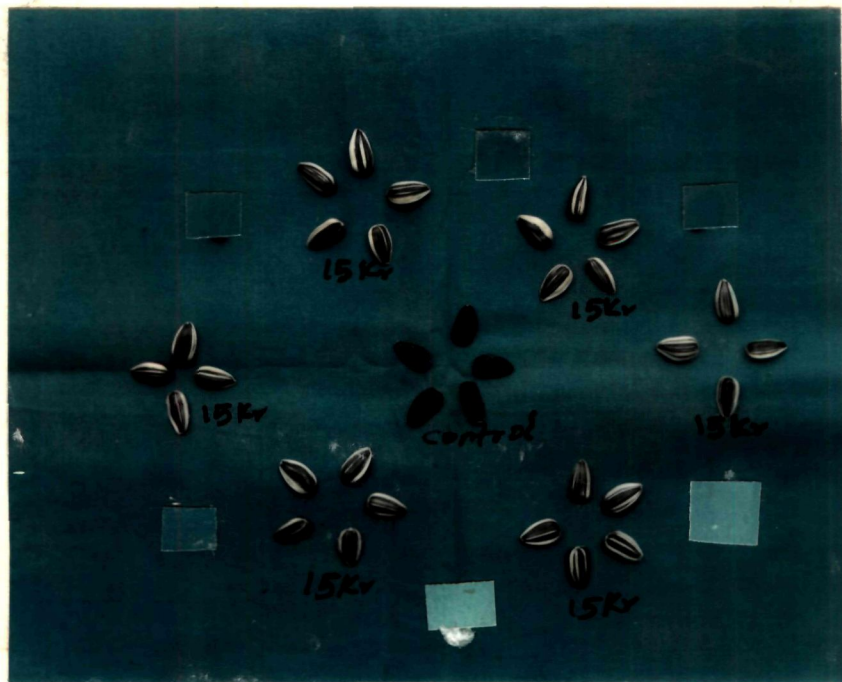


Plate 9. A striped mutant observed in IV83 genotype and seed size variation in RLC2 genotype following gamma irradiation treatment

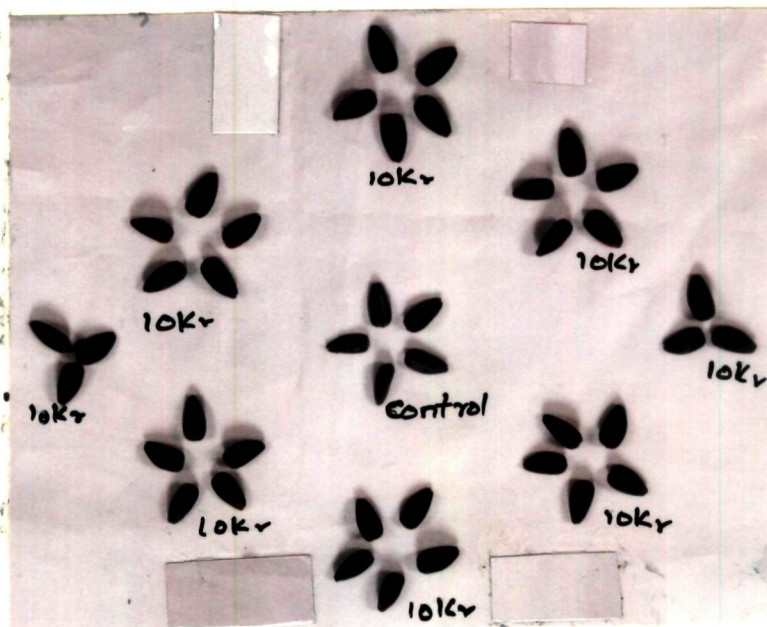
a - IV83 15 Kr

b - RLC2 10 Kr

Plate 9.



a



b

and 20Kr treatments compared to the value of 3.20 to 4.11 in control.

The mean of character increased in IV83 genotype following irradiation treatments viz., at 10Kr (4.99), 15Kr(5.06) and 20Kr (5.28) doses compared to control (4.96). On the contrary the mean values of lower magnitude were observed in RLC2 genotype at 10Kr(3.18), 15Kr(3.45) and 20Kr(2.78) treatments compared to control(3.55).

Increase in coefficient of variability was observed at 10Kr(16.23), 15Kr(16.59) and 20Kr(15.53) treatments in IV83 genotype compared to control(6.45). Similar trend of increase in the coefficient of variability values was noticed in RLC2 genotype at 10Kr(14.78), 15Kr(20.29) and 20Kr(13.92) treatments compared to control(9.92).

Highest value of genetic variation was observed at 15Kr (50.47) treatment followed by 10Kr (32.83) and 20Kr (31.13) treatments, in IV83 genotype. Likewise in RLC2 genotype, 15Kr treatment recorded a highest value of genetic variance (8.12) followed by 10Kr (4.81) and 20Kr (0.40) treatments.

Heritability estimate was found to be maximum at 15Kr treatment (52.98) followed by 10Kr(42.30) and 20Kr

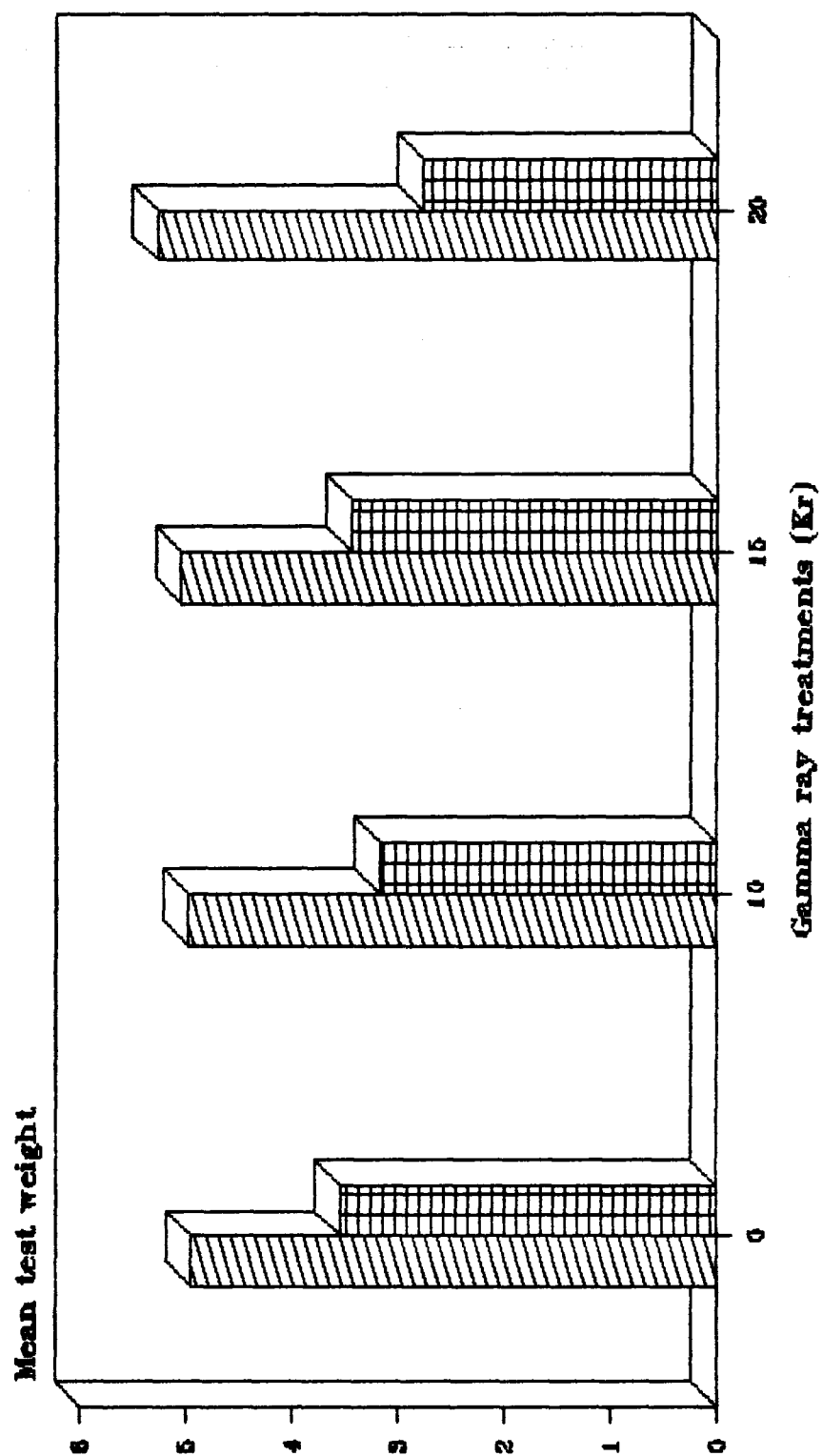


Fig 7. Effect of gamma rays on 100-seed weight in M2 generation

(41.00) treatments in IV83 genotype. A similar trend was observed in RLC2 genotype at 15Kr (74.25), 10Kr (63.10) and 20Kr (12.40) treatments.

In IV83 genotype, genetic advance of higher magnitude was recorded at 15Kr(29.42) treatment followed by 10Kr(28.40) and 20Kr(27.11) treatments. Similar trend was found in RLC2 genotype at 10Kr(13.36), 15Kr(31.26) and 20Kr(4.98) treatments.

In IV83 genotype, the genotypic coefficient of variability values observed at 10, 15 and 20Kr treatments were 7.83, 4.45 and 5.64 respectively. In RLC2 genotype, the respective values observed at 10, 15 and 20Kr treatments were 9.79, 17.54 and 5.79.

4.2.4.7 Oil content (%)

Data on oil content are presented in Tables-15,16,17.

In general, irradiation treatments enlarged the range values in both the genotypes in M₂ generation. In IV83 genotype, compared to control (25.80 to 31.50), the values of oil content varied from 25.80 to 31.50 at 10 Kr, 27.00 to 35.00 at 15 Kr and 26.30 to 35.70 at 20 Kr doses respectively.

With respect to IV83 genotype, highest mean for oil content was observed at 15 Kr (30.00) dose followed by 10 Kr (28.99) and 20 Kr (28.55) doses. Lowest mean value was recorded in control (27.11) in IV83 genotype. A similar trend in mean values was noticed in RLC2 genotype at 15 Kr (31.91), 10 Kr (29.67) and 20 Kr (26.90) doses. A mean value of 28.22 was recorded in control with respect to RLC2 genotype.

In IV83 genotype, compared to control (7.18), maximum variance was observed at 20 Kr (12.25) dose followed by 10 Kr (11.02) and 15 Kr (9.67) doses. In RLC2 genotype, the dose 15 Kr (20.98) recorded the maximum value of variance followed by 10 Kr (10.69) and 20 Kr (7.24) doses. The observed value of variance in control with respect to RLC2 genotype was 6.76.

Compared to control (9.89), highest value of coefficient of variability was observed at 20 Kr (12.26) treatment in IV83 genotype, followed by 10 Kr (11.45) and 15Kr (10.37) treatments. In RLC2 genotype, the coefficient of variability values at 10 Kr, 15 Kr and 20 Kr were 11.02, 14.35 and 10.00 respectively with the least value of 9.21 observed in control.

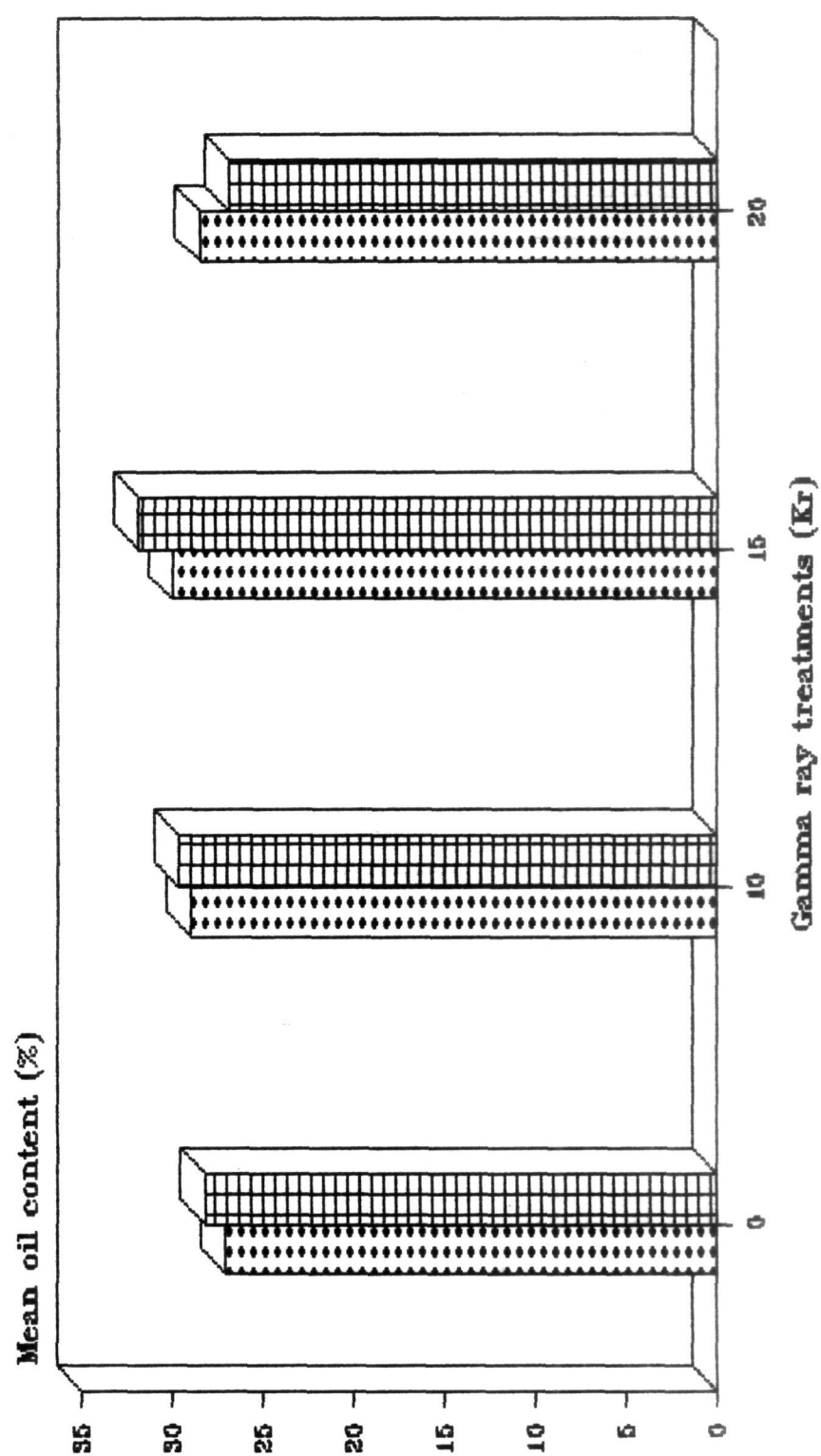


Fig 8. Effect of gamma rays on oil content in M2 generation

Relatively higher magnitude of genetic variation for the trait, was observed at 20 Kr (5.07) treatment followed by 10 Kr (3.84) and 15 Kr (2.49) treatments in IV83 genotype. In RLC2 genotype, the dose 15 Kr (14.22) was more effective in increasing the genetic variance for the trait followed by 10Kr(3.93) and 20 Kr (0.48) dose.

The higher values for heritability was observed at 20 Kr treatment (41.39) followed by 10 Kr (34.84) and 15 Kr (25.75) treatments, in IV83 genotype. However, in RLC2 genotype the dose 15 Kr (67.78) recorded highest value of heritability followed by 10 Kr (36.76) and 20 Kr (6.63) doses.

In IV83 genotype, highest genetic advance value was observed at 10 Kr gamma ray dose (8.28) and the value decreased to 5.57 at 15Kr and to 2.96 at 20 Kr doses. A similar trend was noticed in RLC2 genotype at 10 Kr (8.41), 15Kr(6.43) and 20 Kr (1.44) doses.

The genotypic coefficient of variability values at 10, 15 and 20 Kr treatments in IV83 genotype were 6.76, 5.27 and 7.89 respectively. In RLC2 genotype the values observed at 10, 15 and 20 Kr treatments were 6.68, 11.82 and 2.58 respectively.

DISCUSSION

V DISCUSSION

Mutation breeding has been found to be an efficient method in supplementing conventional breeding methods (Brock, 1971). A number of varieties have been released in various crop plants using different mutagens. Physical mutagens like gamma-rays are effective in creating variability in quantitative and qualitative traits through gene and chromosomal mutations and hence are extensively used in many crop plants (Stadler, 1928a and 1928b). Mutation breeding can also be used in cases where a variety has to be improved for only one character with rest of the traits unchanged (Borojevic and Borojevic, 1972). Mutation breeding approach in sunflower holds promise to improve seed characteristics as revealed by the work of Giriraj *et al.* (1990).

In the present study, seeds of two restorer lines viz., IV83 and RLC2 were subjected to different doses of gamma rays with an objective to create variability for seed characteristics and to know the mutagenic effect on branching behaviour. Further, the study also aimed at to study the effectiveness and efficiency of different gamma ray doses based on chlorophyll mutations and lethality in M_1 and M_2

generations respectively (Konzak *et al.*, 1965). The restorer line IV83 has a non branching habit. It has desirable features with respect to head diameter, plant height, 100-seed weight and seed yield per plant. So it can be a promising restorer line with nonbranching habit, used to develop sunflower hybrids. Induced mutagenesis has been employed in this line to isolate mutants with branching habit to ensure pollen supply for a longer period in hybrid seed production programme. Another restorer line RLC2 used in the present study has a branching habit. In general branching restorer lines have low *per se* yield and low test weight. Hence, improvement in the above traits has been attempted in RLC2 line by induced mutagenesis using gamma rays.

The results obtained based on evaluation of the two lines in M_1 and M_2 generations are discussed characterwise in the following paragraphs.

5.1 GERMINATION

For evaluating the effects of mutagenic treatments, criteria of germination and survival has been widely used (Gustafsson, 1947; Gaul, 1958 and Ehrenberg, 1971). In the present study, a dose dependent reduction in

germination following increase in irradiation dosage was observed in M_1 generation of both the genotypes. Among the different doses administered, viz., 10, 15 and 20 Kr, the highest dose of 20 Kr proved to be highly detrimental in both the genotypes. Compared to IV83 (74.15), in RLC2 (48.08) genotype a drastic reduction in germination percentage was observed at 20 Kr treatment. Similar reports were made by earlier workers (Shivaraj *et al.*, 1977; Cheah, 1988; Singh *et al.*, 1988 and Ramani and Jadon, 1991 in groundnut; Sahai and Dalal, 1970 in safflower; Nair and Nair, 1977 and Sahu and Kumar, 1978 in sesame; Ilyas and Goud, 1978 in sunflower).

Many causes for reduced germination in irradiated population of M_1 and M_2 generations have been indicated. The main causes for reduced germination and survival following irradiation are :

- a). Cell death due to lethal effect of mutagens (Bacq and Alexander, 1961). Here lysosomes are broken resulting in destruction of internal cell organelles.
- b). Irradiation treatment cause ionisation of water molecules and produce free radicles viz., H^+ , OH^- , H_3O^+ which interact with the genetic material and other cell organelles (Blixt, 1972) and cause chromosomal

aberrations (Sparrow and Woodwell, 1962 and Srivastava, 1973).

- c). Enzyme release hypothesis (Bacq and Alexander, 1961).
Normally during germination there is hydrolysis of starch and other stored products in the nourishing tissue-Endosperm, being triggered by enzymes released from the embryo. So reduced germination following seed irradiation will result from the effect of irradiation on the enzymes that are involved in the process of germination. As a result, growth of the embryo is inhibited.
- d). Physiological causes such as disturbances in hormones (Skoog, 1935; Gordon, 1955; Sparrow and Woodwell, 1982), effects on the auxin levels (Skoog, 1935; Gaur and Notani, 1960 and Woodstock and Comb, 1965) which control synthesis of enzyme specific RNA involved in translation of enzymes required for germination.
- e). Reduced activities of ATP's and inorganic phosphates, amylase, ribonuclease and 3-nucleotidase (Srivastava, 1973), peroxidase and isocitric hydrogenase (Sydorenko, 1962) which inturn leads to reduction in germination.
- f). Inhibition of mitosis (Sparrow and Evans, 1961).

Hence, all the possible causes cited above are essentially associated with one another in the developmental process, one leading to another in a biochemical pathway and ultimately results in the failure of the seed to germinate following gamma irradiation at various doses.

5.2 SURVIVAL

In the irradiated populations of M_1 and M_2 generations, not all the seedlings survive till maturity. Many plants die in the vegetative phase itself, before flowering and setting seeds. The surviving plants till maturity gives indication that they are relatively tolerant to lethal radiations of gamma rays.

The death of seedlings before maturity is mainly due to stoppage of mitosis (Catcheside, 1948 and Cercek et al., 1971), effect on respiratory enzymes (Sydorenko, 1962), diplontic selection and chlorophyll mutations, where chlorophyll is lacking (Konzak et al., 1965; Goud, 1967; Hermelin and Daskalov, 1987).

In the present study also, a gradual decrease in survival of plants till maturity with consequent increase in dosage was observed in M_1 and M_2 generations of both the genotypes studied. These lethal plants were weak, stunted in

appearance with very few, small abnormal leaves (Plates-2,3). It seems that some of the vital genes for survival, viz., genes coding for chlorophyll synthesis, chlorophyll binding proteins or genes for Rubisco enzyme have mutated in such plants and the enzymes produced by mutated genes are nonfunctional, resulting in cessation of the important biochemical reactions and thereby whole metabolic processes of the plant will be impaired leading to premature death of the seedlings (Sparrow and Woodwell, 1982).

Based on lethality of the seedlings in both the genotypes under different treatments, it is evident that RLC2 genotype is more radiosensitive than IV83, since the lowest survival percentage was observed in RLC2 at 20 Kr dose both in M_1 and M_2 generations.

Similar studies on differential response of genotypes to mutagens are on record (Sahai and Dalal, 1970 in safflower; Ilyas and Goud, 1978 in sunflower and Cheah, 1988 in groundnut).

5.3 CHLOROPHYLL ABNORMALITIES

Increase in frequency of chlorophyll abnormalities with increase in dosage was observed in M_1 and M_2 generations of both the genotypes. This observation is in support of

earlier studies by Hermelin and Daskalov (1987) in sunflower, Busolo (1988) in groundnut and Harb (1990) in soybean.

The frequency of chlorophyll abnormalities indicate, RLC2 as the most radiosensitive genotype, since the highest frequency of chlorophyll abnormalities in M_1 (35.00) and M_2 (60.00) generations was noticed in this genotype at 20 Kr dose.

Chlorophyll abnormalities observed in M_1 and M_2 generations are due to somatic mutations (Sparrow, 1954). These usually result due to periclinal chimeras or plastid mutations. Hence, these chlorophyll abnormalities do not breed true in subsequent generations as there is irregular distribution of cytoplasmic organelles like plastids, to the daughter cells (Goud, 1967). According to Baletskii and Fuazoritelava (1972) chlorophyll abnormalities in sunflower are due to extra chromosomal mutations.

In the present study, most of the chlorophyll mutants were observed at seedling stage and were not distinct to assign these mutants into definite categories viz., xantha, viridis, chlorina and albino types as suggested by Gustafsson (1940). Most of the abnormalities observed were in the form of white and light green streaks; white, light green and yellow patches and various mosaic patterns (Plate^S-1,4).

In a similar study in sunflower, Chandrappa (1980) irradiated the achenes with various doses of gamma ray and he also reported that the spectrum of chlorophyll mutations induced in M_2 was very narrow. In sunflower, Chandrappa (1980) also reported that the spectrum of chlorophyll mutations induced in M_2 was very narrow.

5.4 MUTAGENIC EFFECTIVENESS AND EFFICIENCY

In the present study, effectiveness and efficiency of different gamma irradiation doses, viz., 10Kr, 15Kr and 20Kr, were measured to assess the effect of these doses on different plant characters in both the genotypes. In a similar experiment Konzak et al. (1965) studied the effectiveness and efficiency of radiations for inducing changes in various plant characters. They proposed that mutagenic effectiveness gives the mutation rate per unit dose of the mutagens, while mutagenic efficiency is the ratio of mutation rate to undesirable changes like lethality, injury and sterility. They also opined that efficiency of a mutagenic agent depends on the reaction of the plant to the mutagen and the degree of physiological damage, chromosomal aberrations and sterility caused by the mutagen.

A dose dependent increase in mutagenic effectiveness and efficiency was noticed in the present study. The highest dose of 20 Kr was found to be the most effective dose in inducing maximum number of mutations in both the genotypes (Table-11). These results are in confirmity with the earlier reports of Goranova and Aleksieva (1986) and Wang and Yu (1991).

The gamma ray doses were more effective in case of RLC2 genotype compared to IV83, in inducing physiological damage, seedling injury, chlorophyll abnormalities and lethality. Particularly the higher dose of 20 Kr was more effective in RLC2 genotype in inducing seedling injuries and lethality. The possible reason may be that, the genetic background of the material under study could play an important role in determining the effectiveness and efficiency of the mutagen (Richharia, 1981). Similar type of differential response among the genotypes to induce mutations was noticed by Goranova and Aleksieva (1986) in soybean.

5.5 BRANCHING BEHAVIOUR

One of the objectives of the present investigation was to study whether induced mutagenesis could alter the branching/nonbranching behaviour of genotypes. By gamma

irradiation treatment, branching mutants were recovered in M_2 generation of IV83 which is a nonbranching genotype. However, such types were not recovered in M_1 generation. The change from nonbranching to branching habit in M_2 generation may be due to gene mutation with respect to the alleles governing the trait and segregation of these gene loci in M_2 . By inducing such mutations in nonbranching restorer line IV83, it is possible to develop branching mutants which ensure pollen supply for a longer period without disrupting the genotypic constitution governing important quantitative traits. Nenov and Tsvetkova (1994) also favoured the use of branching types of fertility restorer lines in hybrid seed production. On the contrary, in RLC2, a branching genotype, nonbranching mutants were not recovered in M_1 and M_2 generations. This can be explained based on dominant and recessive relationship. Branching character is recessive to nonbranching trait in cultivated varieties of sunflower (Putt, 1964) and the frequency of mutations from recessive to dominant gene/s (non-branching) is rather rare. However, contradictory reports regarding genetics of branching in sunflower have been reported as cited below.

- i). Single dominant gene for branching (Shull, 1908) which was designated as Br.

- ii). Branching is controlled by recessive genes in some varieties and by dominant genes in others (Blaringhem, 1942).
- iii). Branching is recessive and polyfactorial (Rudolf, 1950).
- iv). Branching is controlled by dominant gene in sunflower (if a single gene exists for branching, there must be many modifiers, since a number of different branching patterns occur) (Heiser, 1954).
- v). During the past, in breeding programmes of sunflower, non-branching types were favoured (Hockett and Knowles, 1970). Thus over a period of time, any dominant gene for branching was eliminated from the cultivated varieties and only the recessive genes remained in the population of cultivated plants. These recessive genes, would be hard to eliminate, if their expression depends upon environment, as has been asserted by Shull (1908) and Putt and Unrau (1943) in sunflower. The presence of some dominant genes for branching in cultivated sunflower, may be due to relaxation of selection pressure or due to introgression from the adjacent wild plants.
- vi). Miller (1982) opined that branching in the wild sunflower is determined, in cases, by dominant genes, while, in

cultivated sunflower, the branching type is determined mainly by recessive genes.

Similar kind of studies on use of gamma radiations in altering the branching and nonbranching habits, as was attempted in the present study, were made by several earlier workers. Sindagi and Ansari (1969) in castor, modified the branching habit in mutant following gamma irradiation of seeds of HC6 with 50 Kr. In sunflower, Coppola (1986) in a similar study, irradiated *Helianthus tuberosus* with 3 Kr gamma rays and obtained mutants with shy branching character as against high branching behaviour. Likewise, a change from spreading to semicompact habit was reported by Singh et al. (1988) in groundnut following the treatment with gamma irradiation.

5.6 EFFECT ON QUANTITATIVE CHARACTERS

The reduction in mean values in most of the quantitative characters viz., days to flowering, plant height, head diameter, number of seeds per head, seed yield per plant and 100-seed weight and a general widening of range, increase in variance, coefficient of variation observed in M_1 and M_2 generations of the present study is in agreement with earlier studies made by Gregory (1957), Reddy et al. (1977), Ramani

and Jadon (1991) in groundnut; Athma and Reddy (1982) in castor; Giriraj *et al.* (1990) and Encheva (1993) in sunflower; Kamala (1990) in sesame and Wang (1991) in soybean. The observation that there is also a general increase in genetic variation, genotype coefficient of variability, heritability and genetic advance estimates in most of the quantitative traits studied are in agreement with earlier studies [Ramanathan, 1983 in groundnut and Mahla *et al.*, 1991 in mustard].

5.6.1 Days to flowering

In the present study, early as well as late flowering mutants were isolated. Early flowering mutants obtained in the M₂ generation of IV83 and RLC2 genotypes may be used to ensure synchrony with early flowering female lines in a hybrid seed production programme and thereby helps to derive short duration or early maturing hybrid. In cases where, female line is late in flowering, synchronous flowering of both the parents could be achieved by increasing the duration of flowering in male parent. Late flowering mutants of the two restorer lines obtained in the present study following various irradiation doses could therefore be used in combination with late flowering female lines to develop

hybrids of longer duration. Hence, both early and late flowering mutants obtained in the present study would help to synthesise new hybrid combinations of different maturity groups. Similar kind of findings of altering the flowering duration following irradiation treatment have been reported by Athma and Reddy (1982) in castor, Singh *et al.* (1988) in groundnut, Giriraj *et al.* (1990) and Encheva *et al.* (1993) in sunflower, Khurana (1991) in soybean.

In the present study in M_2 generation of IV83 genotype maximum number of early types were recovered at lower dose of 10Kr (513.00) compared to 15Kr and 20Kr doses. Similar trend existed with respect to late flowering types in 10Kr (300.00), 15Kr (115.00) and 20Kr (24.00) doses. Likewise in M_2 population of RLC2 genotype, compared to 15 and 20 Kr doses, maximum number of early and late flowering plants were observed at 10 Kr dose (Table-18). Therefore, the lower dose of 10Kr was the most effective dose in recovering desirable types (Early and Late mutants) with respect to days to flowering. From these observations it is clear that even though, mean flowering time of the irradiated population did not alter appreciably as compared to untreated genotypes (Tables-4,12), the widened range and enlarged variance offered

Table 18. Number of plants in M₂ generation showing early and late flowering characters

Genotype	Treatment	Early	Late
IV83; Mean number of days to flowering* ; in untreated population - 58 DAS	10 Kr	513 out of 2013 plants	300 out of 2013 plants
	15 Kr	214 out of 1350 plants	115 out of 1350 plants
	20 Kr	75 out of 350 plants	24 out of 350 plants
RLC2; Mean number of days to flowering* ; in untreated population - 62 DAS	10 Kr	150 out of 568 plants	82 out of 568 plants
	15 Kr	34 out of 135 plants	13 out of 135 plants
	20 Kr	2 out of 10 plants	--

* DAS = Days after sowing

scope for selecting either early or late flowering mutants in both the genotypes. These results support earlier observations of Giriraj *et al.* (1990).

Compared to higher doses, the lower dose of 10Kr was most effective in increasing the genetic variance, heritability, genetic advance and genotypic coefficient of variability for the trait in M_1 and M_2 generations of both the genotypes. These observations are in accordance with the results obtained by Prassanna and Reddy (1986) in castor; Sarker and Sharma (1987) in lentil.

5.6.2 Plant height

In the present study, based on the mean values observed (Table-9), the plants in the M_1 generation raised from the seeds irradiated with various doses of gamma rays, have reduced heights compared to the plants in untreated population, in both the genotypes. Similar results of drastic reduction in plant height have been obtained in earlier studies (Shivaraj and Ramana Rao, 1963; Reddy *et al.*, 1977 and Ramani and Jadon, 1991 in groundnut; Leclercq 1984 and Encheva *et al.*, 1993 in sunflower; Sindagi and Ansari, 1969 in castor; Rajput and Sarwar, 1989 in soybean).

In contrast to above findings, the mean value increased in irradiated populations of both the genotypes in M_2 generation as against the values in control except in RLC2 genotype at 20 Kr (Khurana and Lakshminarayana, 1991).

As in case of days to flowering, irradiation treatments significantly increased the variance and coefficient of variability for the trait in both the genotypes. Increased variance and coefficient of variability for the trait following irradiation treatment has also been observed by Reddy *et al.* (1977) and Wang (1991).

Among the different irradiation doses, the lowest dose of 10 Kr was found to be the most optimum dose in increasing the genetic variance, heritability, genetic advance and genotypic coefficient of variability for the trait in both the genotypes. Ramanathan (1983) also observed similar trend in groundnut.

5.6.3 Head diameter

In both the genotypes studied, reduction in head diameter in the irradiated populations was observed in all the doses except at 10Kr dose, in M_2 generation of IV83 genotype. This is in confirmation with the results obtained by Mahla

et al. 1991 and Encheva *et al.* (1993). Contrary to these results, Reddy (1986) observed increase in capsule length in sesame following various gamma irradiation doses.

A negative relationship between radiation dose and mean value for head diameter was apparent in RLC2 genotype, both in M_1 and M_2 generations. Similar to days to flowering and plant height, the range, variance and coefficient of variability estimates were of higher value in treated populations compared to control (Tables-4, 5). Likewise the genetic variability, genotypic coefficient of variation, heritability and genetic advance estimates showed higher magnitude at lower dose of 10 Kr compared to 15 Kr and 20 Kr treatments (Tables-5,6,13,14).

RLC2 genotype was found to be more sensitive to gamma rays than IV83 with respect to reduction in head diameter. This could be attributed to differential response of mutagens to genotypes for the same trait.

In both the genotypes studied, a general reduction in the mean value of head-diameter was apparent with increase in dosage. The possible reason for reduction in headdiameter may be due to its close association with reduction in plant

height, as a result of hormonal and biochemical changes (Blinks, 1952; Gunckel and Sparrow, 1954).

5.6.4 Number of branches per plant height

Based on the observations, taken in M_1 and M_2 generations irradiation doses significantly widened the range and increased the mean values in M_1 and M_2 generations of RLC2 genotype. The studies of Patil and Thakare (1969) and Chandra Mouli *et al.* (1987) in groundnut; Ankineedu *et al.* (1968) in castor; Mahla *et al.* (1991) in mustard may be cited in support of present findings.

In the present study, increase in variance and coefficient of variability values was simultaneously associated with shift in mean in the desired direction (more number of branches). Hassan *et al.* (1984) and Mahla *et al.* (1991) also made similar observations in sesame.

5.6.5 Number of seeds per head

When compared with control, the gamma irradiated M_1 population of both the genotypes revealed decrease in mean values for the trait except for 10Kr treatment in IV83 genotype. However, wide range in both positive and negative directions and also an increase in variability and coefficient

of variance for the trait was observed in treated populations, compared to untreated ones, in both the genotypes. Similarly, Rajput (1987), Sarker and Sharma (1987) and Kamala (1990) have reported that gamma irradiation treatment enlarges the range values, thereby it provides scope for efficient selection.

As in case of previous traits (Viz., days to flowering, plant height, head-diameter and number of branches per plant), the genetic variance, heritability, genetic advance and genotypic coefficient of variability estimates showed increase in their values at lower dose of 10 Kr compared to higher doses viz., 15Kr and 20 Kr. These results are in support of the results obtained by Mahla et al. (1990) and Ramani and Jadon (1991).

5.6.6 Seed yield per plant

In the present study, with regard to M_1 generation, a narrow range in a negative direction was observed at all doses in RLC2 genotype and at 20 Kr dose in IV83 genotype.

In M_2 generation, enlarged range in positive and negative directions was apparent in both the genotypes. This may be attributed to deleterious effect of gamma rays in M_1 generation and further segregation of gene loci governing the

trait in M_2 . Thus, selection for superior types can be initiated in M_2 (Kharkwal, 1982).

In general, a gradual decrease in mean value of seed yield per plant with increase in dosage was noticed in both the genotypes. Such a decrease in mean value of the trait following irradiation treatment has also been stated by earlier workers (Gregory, 1955, 1956, 1957, 1960; Shivaraj and Ramana Rao, 1963; Sanjeevaiah *et al.*, 1967; Ramkumar and Yadav, 1988; Mahla *et al.*, 1991). Compared to IV83 genotype, in RLC2 the seed yield per plant was drastically reduced in M_1 generation, as a result of deleterious effect of gamma irradiation. This is attributed the increase in mean value for number of branches per plant in the irradiated population of RLC2 genotype. Another reason may be, differential sensitivity of genes among different genotypes for the same trait. However, it is interesting to note that in M_2 generation of RLC2 genotype, compared to control (7.29), the gamma irradiation doses of 10Kr (8.00) and 15Kr (7.95) increased the mean value for seed yield per plant in M_2 generation. Increase in seed yield per plant in RLC2 genotype was one of the objectives of the present study, which has been achieved following gamma irradiation at 10Kr and 15Kr doses. Similar findings of use of induced mutagenesis in increasing

the mean value of seed yield per plant was made by several workers (Ankineedu and Kulkarni, 1968; Sindagi *et al.*, 1978, Prasad and Kaul, 1984, Chandra Mouli *et al.*, 1987, Dutta *et al.*, 1987, Mahla *et al.*, 1991 and Chauhan, 1992).

Like many other mutation breeding experiments (Gregory, 1960; Hassan *et al.*, 1984; Prassanna and Reddy, 1986; Ramkumar and Yadav, 1988; Kamala, 1990; Mahla *et al.*, 1991; Wang, 1991), in the present investigation also, the changes in mean in the mutagen treated populations of M_1 and M_2 generations was followed by increase in variance and coefficient of variability values. The released variability, would enable efficient selection of mutants with high seed yield per plant at the early stage of M_2 generation (Brock, 1971; Andrew and Brock, 1965; Bansal, H.C., 1969).

Following gamma irradiation treatments increase in genetic variability and genotypic coefficient of variation was noticed in M_1 and M_2 generations of both the genotypes, at lower irradiation dose of 10Kr followed by 15Kr and 20 Kr treatments.

Although this genetic coefficient of variation is useful to compare the extent of genetic variability in different populations and traits, it is not possible to

determine, the heritable proportion of variation of a given trait (Mahla et al., 1991). Broad sense heritability for the trait, therefore was computed to determine the induced genetic effects which may be passed on to the next generations and based on heritability values, the amount of genetic advance for the trait was calculated.

After investigation of the effect of various gamma ray doses on seed yield per plant in M_1 and M_2 generations of both the genotypes, high estimates of heritability were found at lower irradiation dose of 10 Kr compared to higher doses of 15 Kr and 20 Kr. The same trend could be traced with respect to genetic advance estimate (Tables-8,9,16,17). The high heritability value for seed yield per head observed in the present study enables the plant breeder to base his selection effectively on the phenotypic performance of the trait for its improvement. Similar kind of results has been emphasized by earlier workers (Sanghwan, 1972; Labana et al., 1980; Mahla et al., 1991).

5.6.7 100-seed weight

Test weight is one of the important yield components. This is more so in the present study, where the branching restorer line RLC2 has small seeds with 100-seed

weight ranging from 3.20 to 4.25 g. Increase in test weight of RLC2 is necessary for yield improvement of the reconstituted hybrid. This can be achieved by gamma irradiation treatment, as was attempted by Giriraj *et al.* (1990) in RHA-274 restorer line.

In the present study also, compared to control, a general widening of range in positive and negative directions was apparent due to irradiation treatments in both the genotypes, except in M_2 generation of RLC2 at 15Kr and 20Kr doses. In RLC2 genotype, higher dose of 20Kr drastically narrowed the range for the trait in negative direction.

A general decrease in character mean, in M_1 and M_2 generations of both the genotypes following irradiation treatment, can be perceived from data presented in Tables-7,15. However, increased variance for the trait observed in the present study offered scope for isolating mutants with high test weight compared to control. The number of M_2 families exceeding the mean test weight of parental lines is given in Table-19. Maximum number of M_2 families with higher test weight over control was observed at 10 Kr dose (95) families followed by 15Kr (77) families and 20Kr dose (25) families in IV83 genotype. Similar trend was noticed in RLC2

Table 19. Number of M₂ families showing high test weight of parental lines over control

Genotype	Treatment	Number of M ₂ families with high test weight over control
IV83 [Mean test weight - 4.96 g]		
	10 Kr	95 out of 203 families
	15 Kr	77 out of 169 families
	20 Kr	25 out of 50 families
RLC2 [Mean test weight - 3.55 g]		
	10 Kr	14 out of 90 families
	15 Kr	8 out of 25 families

genotype. But 20 Kr dose in RLC2 did not improve the test weight. From these observations it is clear that the lowest dose of 10 Kr was most effective over 15Kr and 20Kr doses in both the genotypes, in recovering maximum number of mutants with high testweight over control. It is interesting to note that increased mean testweight at 10, 15 and 20 Kr treatments was observed compared to control, in M_2 generation of IV83 genotype. This can be attributed to the large sized seeds obtained in treated populations as a result of reduction in number of seeds per plant. In IV83 genotype, 10Kr dose was effective in isolating more number of plants with increased seed size than control (Plate-8). Similar observations of increase in test weight following gamma irradiation treatment was also obtained by Avadhani and Ramana Rao (1968), Manoharan and Tangavelu (1990) in groundnut; Giriraj *et al.* (1990) in sunflower and Mahla *et al.* (1990) in mustard.

Earlier workers (Kamala, 1990 and Mahla *et al.*, 1991 in sesame; Giriraj *et al.*, 1990 in sunflower; Wang, 1991 in soybean) have reported an increase in variability and coefficient of variability values for the trait following gamma irradiation treatment. In the present investigation also, in M_1 and M_2 generations of both the genotypes, a similar trend of increase in variability and coefficient of

variation values at 10,15 and 20 Kr treatments was noticed compared to the control.

However, the high variance and phenotypic coefficient of variation alone, are not the determining factors of expected progress that can be made in respect of test weight (Falconer, 1960). Burton (1952) suggested that the genotypic coefficient of variation together with high heritability will give a better picture of the extent of genetic advance to be expected by selection. In the present study, high values of genetic variation, heritability, genetic advance and genotypic coefficient of variation for 100-seed weight was observed in M_1 and M_2 generations of both the genotypes irradiated at 10 Kr treatment compared to higher doses. These results are in agreement with earlier reports of Sanghwan (1980), Labana *et al.* (1980) and Mahla *et al.* (1991).

Higher heritability values for the trait obtained in the present study at lower irradiation dose of 10 Kr, provides scope for effective phenotypic selection for improved plants with respect to test weight in M_2 generation.

5.6.8 Oil content

Oil content is one of the most important economic traits in sunflower. Increase in oil content of the two

restorer lines, viz., IV83 and RLC2 used in the present study, is necessary in order to incorporate the improved trait in the reconstituted hybrid. Earlier, Giriraj *et al.* (1990) in sunflower, had used various doses of gamma rays and EMS to improve the oil content in parental lines of BSH-1 and reported wide range and increased variability for oil content. They also noticed a shift in mean oil content in positive direction.

In the present study also, compared to control, a general widening of range in positive and negative directions in M_2 generations of both the genotypes can be perceived from Table-15. Kubler (1984) also studied the use of induced mutagenesis in altering the oil content in sunflower. Following the treatment with EMS and gamma rays he obtained mutants with oil content ranging from 9.1 to 61.9 and 9.4 to 58.8 per cent against 8.7 to 56.5 per cent in control.

In M_2 generation of RLC2 genotype the mean values of the trait increased at 10Kr (29.67) and 15 Kr (31.91) doses, compared to control (28.22) and the value decreased at 20 Kr (26.90) dose. In IV83 genotype, compared to control (27.11), an increase in mean oil content was observed following irradiation treatments at 10 Kr (28.99), 15 Kr (30.00) and

20Kr (28.55). Likewise, Vranceanu and Stoenescu (1982) treated sunflower seeds with various doses of gamma radiations and found that irradiation with high energy radiations were effective to increase the oil content. Similarly in another study, Chandramouli *et al.* (1987) induced high oil content mutants in groundnut following various doses of gamma irradiation.

However, a decrease in mean value for oil content following various irradiation treatments has also been reported by Rakow *et al.* (1987); Ramkumar and Yadava (1988) in Brassica; Chandramouli and Kale (1990) in groundnut.

As in case of previous traits, viz., days to flowering, seed yield per plant and 100-seed weight, with respect to oil content also, increase in variability and coefficient of variation values was noticed at 10 Kr, 15 Kr and 20 Kr treatments compared to control (Table-15). Soldatov (1971) also observed increased genetic variability for oil content in sunflower following induced mutagenesis. In a similar study Bhatnagar *et al.* (1992) in soybean observed a greater variability for oil content in the mutants than in the parents following gamma irradiation treatment at 15, 20 and 25 Kr doses.

In IV83 genotype, highest values of heritability for the trait was observed at 20 Kr (41.39) dose followed by 10 Kr (34.84) and 15 Kr (25.75) treatments. In contrast, in RLC2 genotype, the dose 15 Kr recorded highest heritability value (67.78) followed by 10 Kr (36.76) and 20 Kr (6.63) doses. Genetic advance values of higher magnitude were observed at lower dose of 10 Kr compared to higher doses in M_2 generation of both the genotypes (Table-16).

Higher heritability values for the trait obtained at 20 Kr dose in IV83 genotype and at 15 Kr dose in RLC2 genotype and greater genetic advance values at lower dose of 10 Kr in both the genotypes, provides scope for selecting mutants with high oil content in M_2 generation.

Mutation breeding is a potent tool in increasing *perse* oil content. In the present study, maximum number of M_2 families with higher oil content over control was observed at 15 Kr (99) followed by 10 Kr (88) and 20 Kr (22) doses in IV83 genotype (Table-20). In contrast, in M_2 population of RLC2 genotype, compared to 15 Kr (21) and 20 Kr (1) doses, maximum number of high oil mutants over control were observed at 10 Kr dose (38) (Table-20).

Table 20. Number of M_2 families showing high oil content of parental lines over control

Genotype	Treatment	Number of M_2 families with higher oil content over control
IV83 (Mean oil content - 27.11 %)		
	10 Kr	88
	15 Kr	99
	20 Kr	22
RLC2 (Mean oil content - 28.22 %)		
	10 Kr	38
	15 Kr	21
	20 Kr	1

Suggested future line of work

The main objectives of the present study envisaged widening variability for days to flowering, plant height, test weight, oil content besides studying branching behaviour habit in two parental lines.

The foregoing results have clearly established the utility of mutation breeding in achieving the above objectives. Several workers have also reported variation for fatty acid composition in derived mutant lines. The derived mutant lines in the present study needs to be analysed for variation in fatty acid composition particularly with respect to oleic and linoleic acid.

The mutants derived in the study should be assessed for their fertility restoration and identify high yield potential restorer lines in both RLC2 and IV83. The superior restorer lines for *per se* seed yield and oil content should be utilised in heterosis breeding programme for developing high yield potential single cross hybrids.

The derived mutant lines should be also be identified for developing early, normal and late maturing hybrids.

SUMMARY

VI SUMMARY

The present study on induced mutagenesis in two restorer lines of sunflower was taken up to create variability for seed characteristics and branching behaviour. IV83 is a high yielding medium tall, nonbranching restorer line with desirable features for head diameter, 100-seed weight and seed yield per plant. With a view to induce variability for economic traits and isolate branching mutants, IV83 was treated with 10, 15 and 20 Kr doses of gamma rays. Another restorer line used in the present investigation, viz., RLC2 has a branching habit. It is a low yielder and has small seeds with variable size in addition to low test weight. The same doses of gamma rays, viz., 10, 15 and 20 Kr were administered to RLC2 genotype, with objectives to improve the traits.

The following are the salient findings of the present study.

- a. The study in M_1 and M_2 generations revealed that in both the genotypes, there was a dose dependent decrease in germination and survival. The dose 20 Kr was found to be more deleterious in RLC2 genotype, in reducing germination and survival compared to IV83 genotype.

- b. The gamma irradiation treatments induced chlorophyll mutations and most of them were manifested at seedling stage. The mutations observed in M_1 and M_2 generations were in the form of white, light green and yellow streaks and patches. In general, frequency of chlorophyll mutations increased with increase in dose, in both the genotypes, with the highest frequency at 20 Kr. Varietal differences were observed with respect to the frequency of chlorophyll mutations. RLC2 genotype recorded highest frequency of chlorophyll mutations in comparison with IV83.
- c. Effectiveness of gamma rays on mutations induced was in general, directly proportional to the dose applied. Maximum effectiveness was observed at 20 Kr dose in both the genotypes. Efficiency of gamma rays also appeared to increase with the dosage. However, significant differences were not noticed in the doses with respect to efficiency. RLC2 was more sensitive to gamma radiations with respect to lethality, and induction of mutations. But based on efficiency of mutagen, IV83 genotype showed more response in inducing desirable mutations.
- d. In M_1 generation, there was no alteration in branching behaviour with respect to both the genotypes. But in M_2

generation, branching types were noticed in IV83 genotype with highest frequency at 20 Kr dose. While nonbranching types were not isolated in M_2 generation of RLC2 genotype following irradiation treatment.

- e. The range widened in positive and negative directions for most of the characters studied, viz., days to flowering, plant height, number of branches per plant and 100-seed weight. Hence, effective selections can be practiced in M_2 generation for desirable types, for future studies. Lower irradiation dose of 10 Kr was most effective in enlarging the range of variability for all the characters.
- f. There was a general decrease in mean values with increase in dosage, in M_1 and M_2 generations of both the genotypes for days to flowering, head diameter, number of seeds per plant, seed yield per plant and 100-seed weight. In M_2 generation of IV83 genotype irradiation treatments increased the plant height and test weight compared to control. Irradiation treatments of 10 and 15 Kr also increased the mean value of seed yield per plant in M_2 generation of RLC2 genotype compared to control. Early types were recovered in the treated populations of both the genotypes. A general positive shift in the mean was

observed for number of branches per plant in RLC2 genotype. This will be of use in isolating early mutants with bulk pollen supply for a longer period.

- g. In M_1 and M_2 generations of both the genotypes, wide variability and coefficient of variability were expressed for all the characters studied. In general, 10 Kr treatment was more effective in enlarging variability for most of the characters, viz., days to flowering, plant height, head diameter, number of branches per plant, number of seeds per head, seed yield per plant and 100-seed weight.
- h. With respect to oil content, gamma irradiation treatments widened the range and increased the mean, variability, coefficient of variation, heritability and genetic advance values compared to control, in both the genotypes. In both the genotypes the dose of 15 Kr was found to be the most efficient dose, in increasing the mean value of oil content.
- i. Among the different gamma irradiation doses, viz., 10, 15 and 20 Kr, the lowest dose of 10 Kr was found to be most efficient dose, in increasing the genetic variability, heritability, genetic advance and genotypic coefficient

of variation for all the economic traits studied in both the genotypes.

The derived mutants in both IV83 and RLC2 restorer lines have to be confirmed for fertility restoration and also assess the seed yield potential and oil content of newly synthesised single cross hybrids in comparison with the original restorer lines.

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Effect of Gamma Irradiation on Seed Characteristics in
Restorer lines of Sunflower (*Helianthus annuus* L.)

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ABSTRACT

In cultivated sunflower (*Helianthus annuus* L.), the branching behaviour is controlled by recessive genes (Miller, 1982). IV83 is a restorer line having nonbranching habit coupled with high test-weight. But it does not have perfect synchrony in flowering with the female line in hybrid seed production to ensure high seed set. Contrarily, RLC2 is a branching restorer line that ensures pollen supply to female parent over a long period. But it has low 100 seed weight. Hence, with a view to develop lines with branching types in IV83 genotype, to ensure pollen supply to female parent over a long period coupled with high test weight and high per se oil content and also to isolate lines with earliness and high test weight in RLC2 genotype, seeds of both the restorer lines were treated with 10 Kr, 15 Kr and 20 Kr gamma ray doses with the following objectives:

- i). To create variability for days to flowering, test weight and oilcontent.
- ii). To study the branching behaviour of two different types of restorer lines, on irradiation.

The M_1 and M_2 generations of both the genotypes were studied with respect to above objectives in an unreplicated trial during *kharif*-94 and summer-95 respectively.

The study in M_1 and M_2 generations revealed that in both the genotypes, there was a dose dependent decrease in germination and survival. The dose 20 Kr was found to be more deleterious compared to 10Kr and 15 Kr doses. Maximum effectiveness of gamma rays to induce mutations was observed at 20 Kr dose in both the genotypes.

In M_1 generation, there was no alteration in branching behaviour. But in M_2 generation, branching types were noticed in IV83 genotype with highest frequency at 20 Kr dose (13.00%). Alternatively, in RLC2 genotype nonbranching types were not isolated, on irradiation treatments.

Increase in mean values were observed in M_2 generation for plant height, test weight and oil content and for number of branches per plant and oil content in RLC2 genotype.

In M_1 and M_2 generations of both the genotypes, wide variability was observed for all the characters studied. In general, 10Kr dose was more efficient than 15Kr and 20Kr doses in enlarging variability for days to flowering, plant height and test-weight. With respect to oilcontent, higher doses of 20 Kr in

IV83 genotype and 15 Kr in RLC2 genotype were more efficient to increase the variability.

In M_2 generation of both the genotypes maximum number of early and late types were recovered at lower irradiation dose of 10 Kr compared to 15 Kr and 20 Kr doses. Similar trend existed with respect to recovery of maximum number of M_2 families with high test-weight over control. With respect to oil content, 15 Kr dose in IV83 and 10 Kr dose in RLC2 genotype were found to be most effective dose in isolating maximum number of high oil mutants.

In general, among the different gamma irradiation doses, viz., 10 Kr, 15Kr and 20 Kr, the lowest dose of 10Kr was found to be the most efficient dose, in increasing the variability for most of the economic traits studied in M_1 and M_2 generations of both the genotypes. Further, the derived mutants in both, IV83 and RLC2 restorer lines have to be assessed for early and late flowering, test weight, seed yield potential and oilcontent in comparison with the original restorer lines and also have to confirm for fertility restoration in order to use them as potential restorer lines in hybrid breeding programme.

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