

**THE COMPARATIVE EFFECT OF MUTAGEN ON
SOME GENETIC PARAMETERS OF SOYBEAN
(Glycine Max (L) Merril.)**

BY

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PARAMETERS OF SOYBEAN (*Glycine Max (L) Merril.*)

By

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ABSTRACT

Studies were conducted to record the comparative effect of various doses of gamma rays on the chromosomal behaviour during microsporogenesis and some genetic parameters of soybean (*Glycine max* (L) Merril.). Seeds of Swat-84 variety were irradiated with 5, 10 and 22 Krad and during microsporogenesis it was observed that, the control plants behaved as normal diploid with $2n = 40$ and 20 by 20 disjunction. Sporocytes from the 5 Krad plant had meiotic anomaly of the frequent occurrence of asyndatic chromosomes in 32% cells. The range of asyndatic chromosomes was 2-4 per cell. Sporocytes of the plant raised from 10 Krad irradiated seed showed the presence of univalents, trivalents and quadrivalents in 20, 25 and 45 percent cells. Whereas, the plant raised from seed treated with 20 Krad showed 50, 23.07 and 61.53 percent cells with asyndatic chromosomes, trivalents and quadrivalents, respectively. The increased radiation doses increased meiotic anomalies associated with a reduced pollen fertility.

Among the three soybean varieties, Swat-84 was comparatively more radio resistant, then the Epps, whereas, Lakota was the least radio resistant variety among them.

Moreover, Swat-84 proved to be the out yielding genotype among the three cultivars used during the present investigations.

Statistical analyses of the data revealed highly significant differences of radiation doses for germination percentage, flower initiation, primary and secondary branches per plant, pod length, number of pods per plant, 1000 grain weight, grain yield per plant and plant height. Significant differences of varieties were also recorded in germination percentage, primary and secondary branches per plant, pod length per plant, number of pods per plant, 1000-grain weight, grain yield per plant and plant height. Varieties into doses interaction was significant for plant height, pods per plant, 1000-grain weight and grain yield per plant.

CHAPTER ONE

INTRODUCTION

The ever increase in global population associated with the shrinkage in land and water resources due to the construction of buildings and highways have envisaged the human demands for better food with sufficient quantity. To combat the world nutritional demands scientists have the only option to increase the farm productivity per unit area.

Since very long time plant breeding techniques have been used as a major tool for the improvement of crop plant. Selection and introduction were the main tools in the beginning and hybridization was later on used for the evolution of new genotypes. With understanding the mechanism of heredity and realizing the limited use of the ancient breeding techniques, the scientists were naturally inclined to create genetic variability through artificial means.

Ionizing radiation is of much shorter wave length than visible light and has a much greater energy content. Examples of ionizing radiation are X-rays, gamma rays, alpha particles, beta rays, neutrons and protons. With the invention of X-rays in the last decade of the nineteenth century and with the isolation of naturally occurring

radioisotopes at the turn of the century man has had many available sources of ionizing radiations. Hence Gager and Mottram were the pioneers to report the influence of ionizing radiations on the plant body and cell division respectively.

The fact that ionizing radiation of X-ray type has a marked effect on genetic material was established in the last twenties primarily through the work of Muller (1928), and Altenberg (1928). These workers all showed conclusively that there is a marked correlation between exposure to X-rays and increase in production of mutant offspring. Their results were quickly confirmed and extended by both genetical and cytological studies. The latter soon provided data indicating that degree of chromosome damage was also quite directly correlated with dosage (Sax 1938, 1940). Since the dawning of the so called Atomic Age in 1945, interest in the biological consequences of exposure to ionizing radiations of all sorts has been greatly intensified.

The mutagenic capabilities of gamma rays on the plant bodies and its different parts had been observed by a number of workers but none of the earlier workers were successful in demonstrating mutagenesis quantitatively till Muller (1927, 1928) who demonstrated that with the application of X-rays under certain condition, the mutation frequency of

genes can be increased upto 10,000 times that of the natural incidence of the spontaneous mutation. Since that time the scientists are involved in mutagenic experiments for crop improvements evolving thousands of varieties with better gene combinations (Micke, 1982).

Despite these considerable effects, there are still many blanks in our knowledge, especially concerning the manner in which radiation produces its effects. Gamma radiation have also been used in biological systems and it was found that they are very much similar in effects to that of X-rays. They have high penetrating ability and are obtained from a cheaper source of cobalt. Radiation damages biological organisms in essentially three ways:

1. it kills cells and thus organism,
2. it modifies metabolic systems generally to the detriment of the organism concerned, and
3. it induces changes in genetic material detrimental to subsequent generations.

Chromosomal changes resulting from radiation have been of particular interest to cytologists. Chromosomal alterations provide an excellent data for the study of differential sensitivity and variation in chromosome

organization. In general between the absorption of radiant energy and the scored configuration there are 3 steps:

1. primary effect,
2. chromosomal breakage, and
3. healing or union of broken ends.

According to the target (direct) theory the primary effect results from the chromosome traversed by an ionization track and according to the chemical (indirect) theory by production of highly reactive radicals.

Breakage presumably results from disruption of bonds important to integrity, either directly or as a result of strain, while healing or union of broken ends might be considered to represent some sort of satisfaction of free bonds. In any event, it is the resultant rearrangements which can be observed and from which deductions are made. (Wilson and Morrison 1966).

Three levels of aberrations can be recognized in irradiated chromosomes:

1. sub chromatid breaks or exchanges, in which half chromatid is involved,
2. chromatid aberration, that affect only one chromatid of a given metaphase pair, and

3. chromosomal breaks which affects two sister chromatids at exactly the same site.

Radiation induces many other types of anomalies within or between chromosomes, many of which cannot be interpreted satisfactorily, pending till future investigation with the electron microscope.

Sometime isochromatid exchanges occur between sister chromatids, generating U-shaped chromosome arms; occasionally the opposite arms of a single chromatid can fuse to produce a centric ring chromosome (DuPraw 1970). Sometimes two sister fragments reduplicate and unite forming a dicentric chromosome. During mitosis the centromeres of the dicentric chromosome move to the opposite poles and form a bridge between the daughter nuclei that finally breaks at some point. At the next mitosis same cycle may be repeated with the production of chromosomal aberration comprising new "breakage-fusion bridges" that give rise to new genotypes (Robertis et al., 1972).

In Pakistan mutation breeding has been in progress since 1960'S and mutation breeders have released dozens of varieties in cereals, pulse legumes and in cash crop. The well known variety of Pakistan cotton, i.e., NIAB-78 have also been bred through gamma irradiation techniques (Khan, 1988).

Soybean is a newly introduced oil seed crop in Pakistan and we have very few well adopted varieties. Therefore multi-directional research is needed in this respect. Hence a project was undertaken to record the mutagenic potentials of gamma rays on the overall chromosomal behaviour during gametogenesis and also to observe the comparative response of various doses of gamma rays on some agronomic traits of soybean.

CHAPTER TWO

REVIEW OF LITERATURE

Mutations provide raw materials for evolution. The role of mutagenesis was realized by biologists with the turn of the century where they were trying to search new horizons for creating genetic diversity in the existing crop cultivars. The word mutation was introduced by Deveries (1901) and its creation through ionizing radiation was verified for the first time by Muller (1927 and 1928) in *Drosophila* and by Stadler (1928) in maize and barley, whereas, the role of ionizing radiation in creating superior traits had been reported by Gustafsson (1947 and 1951). Since then the exploitation of ionizing radiation has given rise to 1000 of superior genotypes growing on millions of hectare of land (Micke, 1982). In this chapter some selective literature has been reviewed with special reference to the influence of gamma irradiation on soybean.

Chmyr (1962) reported that a 2.5×10^7 r dose of gamma rays of radioisotope Co^{60} completely destroyed the germination power and sprouting energy of corn seeds. X-rays irradiation of dry maize seed inhibited cell division in the

root tips of growing seedlings but apparently had no effect on cell expansion (Cherry 1964).

Gamma irradiation of corn seeds by the doses of 5×10^3 r and 1×10^4 r stimulated growth of seeds in initial stages. A dose of 1×10^5 r had an inhibitory effect on germination (Romenskii et al., 1964).

Woodstock et al. (1950) found that 40 to 80 krads of gamma irradiation had inhibitory effect on corn seedling growth and inhibited the rate of respiration.

Ouang (1964) showed that X-rays and thermal neutrons irradiation had linear inhibitory effect on the germination of seeds of wheat, rice and barley varieties. Germination percentage and growth rate were reduced but tillering was considerably increased by irradiation.

Arnasen et al. (1952) observed the effect of X-rays doses ranging from 8000 r to 10,060 r on vulgare wheat. Of 208 dry vulgare wheat seeds given a dose of 10,060 r, none of the seed germinated.

Soriano (1961) exposed dry grains of rice to different doses of gamma radiation of 20, 25, 30, 40 and 50 krad. He found that dosage of 20, 25, and 30 krad did not greatly reduce the germination percentage, while 40 and 50 krad killed most of the seeds.

Baluch et al. (1966) subjected the castor oil seeds to 20, 30, 40 .. 90 krad doses of gamma radiation Co^{60} source and found that doses from 20 to 30 krad increased the germination percentage.

Abrams (1961) irradiated the seeds of wheat, barley, rice, cotton and pigeon peas with higher doses from different sources of irradiation. The effect of all these radiation sources was inhibitory to plant growth and germination.

Morris et al. (1962) treated the dormant maize seeds of the single cross hybrid in 1952, 1953 and 1954 with X-rays ranging from 4×10^3 r to 3.2×10^4 r units and with thermal neutron ranging from 5.8×10^{12} to 5.1×10^{13} thermal neutrons per sq. cm. In 1952 observations were made on the frequencies and type for reciprocal translocations at meiosis and on the frequency of abnormal pollen. The low spontaneous frequency of

interchanges was increased by both types of radiation but there was no significant differences between X-rays and thermal neutrons with respect to different kinds of interchanges except for two rings of four.

Haque (1955) found that a three hour infrared treatment before and after X-irradiation increased the observable chromosome breaks and reunion in pollen grains of Tradescantia bracteata.

Smith et al. (1948) exposed barley seed to X-rays and found that the frequency of chromatinic bridges in root tip cells was reduced to half. When dormant seeds were subjects to barley sublethal heat treatment after irradiation an increase in mutation rate was noted as compared with the rates in seeds treated with X-rays above or below such lethal heat treatment before irradiation. There was by no means a perfect correlation between the frequency of chromatinic bridges, mutation rates, and injury resulting from different treatments or combinations of treatments.

Beadle (1950) exposed Zea mays seeds to atomic bomb radiation and observed, the developmental abnormalities,

chromosomal aberrations including inversions and translocations. One lot of seed showed effects nearly equal to these produced by 15,000 r units of X-rays, 31.5 percent of surviving cells contained one or more chromosomal aberrations, somewhat more than half of which were net transmissible to the next generation.

Kihlman (1961) reviewed the findings of Sax (1939) who demonstrated that the frequency of exchanges produced by X-rays dosage is dependent on the dose rate.

Bajer (1958) irradiated the endosperm of Haemathus katherinae and observed the mitosis in cells with fragmented chromosomes on Cine-films by means of laps equipment, the development of mitotic spindle appeared to be normal in irradiated cells; during prometaphase many fragments passed towards the poles and were eliminated into the cytoplasm. A few fragments executed very peculiar movements to the pole and returned to the plate; during phragmoplast formation the acentric fragments left at the equatorial region moved towards the poles and gave the impression of second anaphase. An attempt was made to point out the existence direction of some

forces working during mitosis. However, no conclusion concerning their nature was given.

Schwartz (1950) studied the behaviour of X-rays induced ring chromosome in maize involving almost the whole chromosome 6. By crossing over in ring red heterozygous and breakage of the resultant anaphase bridges, he found that the ring was converted into red chromosome, some of the red carried a duplication and were transferred back to rings in subsequent meiotic division. The inclusion of the nuclear organiser in the anaphase bridge resulted in a high frequency of an aberrant spore quarter types.

Giles (1950) showed that oxygen has a marked effect in increasing the sensitivity of chromosomes in Tradescantia and other plant to X-rays radiation measured by the frequency of cytologically detected aberrations. The effect of oxygen arises owing to the production of a substance containing oxygen, by X-rays radio-decomposition of water in the cells, which causes an increase in aberration frequency. Several independent lines of evidence indicated that this substance may be H_2O_2 .

Giles (1964) observed that quantitative relationship between a dose of radiation and the yield of aberration depends on both the type of aberrations and kind of radiation. With the X-rays and gamma rays certain types of breaks were linearly related to the dose and were apparently produced by the relatively concentrated ionization at the tail of single electron track.

According to Siraj (1970), lower doses (5×10^3 r and 1×10^4 r) of gamma radiation had some stimulating effect on plant growth, while higher doses (2.5×10^4 r and onwards) were found to cause reduction in germination, plant height etc. Cytological studies of somatic cells of central material showed 20 chromosomes, all having median or submedian centromeres.

Lin et al. (1972) indicated fragmentations and other chromosomal abnormalities i.e., anaphase bridges, stickiness, chromatid gaps and meiotic delay when Gryllidae (orthoptera) was subjected to gamma radiation.

Rats were irradiated with X-rays by Savkoric and Pecevski (1971) and 4200 cells were examined. It was

interesting to note that the rats had only one kind of reciprocal translocation. This was for the first time that translocations had been reported in rats.

Accordingly to Atabekow (1938) irradiation seems to induces a higher frequency of aberrations of translocation type, which gives rise to typical semisterility or partial sterility and also inversions and other types which usually survive to meiosis and can be made responsible for a considerable fraction, though not all, of the sterility.

Scumpu and Lonesu (1968) found good correlation after gamma irradiation between chromosome aberration in mitosis and meiosis on the one hand and pollen viability and germinability on the other hand in case of Pisum.

As soybeans have small sized chromosome associated with a rather large numbers ($2n=40$) therefore a very limited cytological (Hymowitz, 1976), Karyotypic (Ahmad et al., 1984) and genetic (Gwuyn et al., 1985) work has been done on this crop. Akilov (1970) treated soybean seeds with gamma rays and reported delay in maturity of the cultivars irradiated. The increased radiation dose caused increased plant height and seed weight, whereas, the influence of radiation dose was

inverse for number of pods per plant and seeds per plant. Some mutants were selected both for late and early flowering.

Sebok (1972) conducted radioresistance experiment on soybean and concluded that the plants irradiated for the second time were more resistant than irradiated for the first time. At the third irradiation, resistance was lower than that at the second irradiation but was still higher than the first.

Conga (1976) reported variations in individual yield components of soybean treated with gamma ray, fast neutrons and ethyl methane sulphonate (EMS). A decrease in pod length associated with increase in seed weight was observed with the simultaneous increase in the radiation dose of gamma rays.

Chaudhry and Haq (1976-a) induced early maturing lines in gamma irradiated soybean. Chaudhry and Haq (1976-b) concluded that gamma irradiation had a negative influence on the seed germination and plant survival of soybeans.

Baradjanegare (1980) studied the influence of gamma rays and fast neutrons on soybean. His findings revealed that the seedlings height was somewhat retarded by both the

mutagens. In M2, lines for earliness were selected among the mutants.

Dwivedi and Pandey (1981) recorded the M1 and M2 generations of gamma irradiated soybean and reported that mutants differ significantly from the control population for days to flowering, days to maturity, plant height, 100 seed weight, yield per plant, seed per pod and protein percentage. Some of the mutants were selected for early maturity, dwarf stature, more pods per plant, more 100 seed weight, higher yield and more oil and protein. Zaher et al. (1984) also obtained high yielding mutants in gamma irradiated soybean.

Makhandzhiek and Gachieva (1983) treated dry seeds of soybean with 5, 10 and 20 Krad of gamma rays and then immediately treated the seeds with 0.1, 0.2 and 0.4% of Ethyl methane sulphonate (EMS) at pH 7. They observed synergistic, super additive and inhibitory effect of the radiation dose of 5, 10 and 20 Krad respectively.

Nikolov (1984) obtained hairless mutants in gamma irradiated 8 Krad soybean. The mutant though highly sterile produced more protein containing M2. In M3 mutants with dwarf

stature early maturity and out yielding performance were selected.

Upadhyaya et al. (1985) irradiated dry seeds of Bragg and Type-49 varieties of soybean with 15 Krad of gamma rays. Data was collected on separate branches of the mutants. It was found that the highest rate of mutations (80%) in M2 was observable from the seeds collected from first four branches of the M1.

Smutkupt et al. (1985) reported the influence of gama rays on the flower colour variations in soybean and observed purple colour mutants in white colour variety "Wakashima" after 15 Krad of dose. Upadhyaya (1985) reported that 20 Krad dose of gamma rays can produce the highest rate of mutations in soybean.

Palmer et al. (1985) induced mutants in soybean which were then used in the linkage studies. the role of gamma irradiation in creating beneficial mutations in soybean have also been reported by Patil et al. (1985) and Carroll et al. (1986).

Yamashita et al. (1986) selected mutants from gamma irradiated soybean. The mutant lines proved early maturity and dwarf stature till M9. The grain size of mutants were comparable to the parent and were selected for high yield.

Chen and Palmer (1965) reported sterile and fertile triploids in male sterile soybean plants. Meiosis of the triploids was predominantly characterized by trivalents in metaphase I but, multivalents and univalents were also observed.

CHAPTER THREE

MATERIAL AND METHODS

Materials used during the present studies were three soybean (*Glycine max* (L) Merrill.) varieties, i.e, Swat-84, Lakota and Epps. Seeds of the above varieties were collected from the Agricultural Research Station North at Mingora Swat, through, the Economic Botanist of the Station.

For recording the influence of gamma irradiation the experiments were split into the following main sets.

- A. Mutagenic effects of gamma irradiation on pollen fertility and chromosomal behaviour at meiosis in plants raised from irradiated seeds.
- B. Mutagenic effects of gamma irradiation on various agronomic traits of soybean.

For the first set of experiment seeds of Swat 84 variety were treated with 5, 10 and 20 Krad of gamma rays in gamma cell of Co⁶⁰ at the Nuclear Institute for Food and

Agriculture (NIFA), Tarnab Peshawar. Five pots out of a total of 20 were filled with the planting media prepared from 1:1:1 soil (sand, clay and farm yard manure). Each pot was planted with 3 seeds replicated 5 times for each treatment. Pots were kept under close observation at NIFA and its weeding and irrigation was done according to the plant needs. Sowing of the seeds was done in July 1989.

On the onset of flowering, pollen fertility of the plants was recorded. A single plant with the least pollen fertility from each treatment was selected for chromosomal behaviour at meiosis.

For recording data on pollen fertility flowers with mature pollen (yet to shed) were collected in ethanol. The anthers were put on the microscopic slide and split vertically in a drop of 1.0% acetocarmine. Both the halves were then pressed with the help of a needle so that, the pollen were oozed into the stain. The debris and anther walls were then removed. The stain drop was covered with a cover-glass and observed under a microscope. For each plant data was recorded based on the average of 1000 pollens. Deformed and stainless

pollen grains were considered as sterile. Pollen fertility was calculated according to the formula given below:

$$\text{Pollen fertility} = \frac{\text{No of pollen grains stained}}{\text{Total No of pollen recorded}} \times 100$$

For observing the influence of mutagen on the chromosomal behaviour, floral buds from the least fertile plants were fixed in Newcommer's Fluid (isopropyl alcohol, propionic acid, petroleum ether, acetone and dioxane in the ratio of 6:3:1:1:1, respectively) for a night and then preserved in 70% propyl alcohol. Preparations were made in 2.00% acetocarmine. Stain preparation was done according to the method reported by Sharma and Sharma (1980).

Squashing was done by placing anther in a drop of stain over the microscopic slide and knocked with a squasher. Debris and the anther walls etc were removed and the stain was then covered with a coverslip. The slide was heated gently and then pressed in a folded filter paper. It was observed through the microscope. If a slide is over stained it is decolourized by passing 45% acetic acid through it. Cells with minimum overlapping and clear chromosomal configurations were analyzed recording chromosomal associations. Photomicrography and

cytological studies of these slides were carried out at the cytogenetics laboratory of the National Agricultural Research Centre (NARC) Islamabad.

The second set of experiment included two varieties (Lakota and Epps) of *Glycine max* (L) Merrill. Seeds of all the two genotypes were treated with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 Krads of gamma rays from Co^{60} source at the Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar. The seeds were sown in N.W.F.P Agricultural University experimental field at Malakander on June 12, 1991. Sowing was done according to Randomized Block Design with Split Plot Arrangements. Varieties were put in the main plots, while treatments (radiation doses) were put in the sub-plots. Each treatment was repeated three times with two rows where rows were kept 60 cm apart and the plant to plant distance was 5 cm. No fertilizer was used during the experiment, whereas, irrigation was done according to the need of the crop. Data was computed and analyzed for variance with help of computer. Data was collected on the following agronomic traits of soybean variety, Swat-84.

1. Germination percentage.
2. Initiation of flowering.
3. No of Primary branches per plant.
4. No of secondary branches per plant.
5. Plant height.
6. Pod length.
7. No of pods per plant.
8. No of grains per pod
9. 1000 grains weight.
10. Grains yield per plant.

Germination of the seedlings was recorded after 20 days of sowing when no further emergence was expected. The number of seedlings emerged were counted and the germination percentage was calculated with the help of the following formula:

$$\text{Germination percentage:} = \frac{\text{Seedlings emerged}}{\text{Total seeds sown}} \times 100$$

At the initiation of flowering 10 plants were tagged in each replicate at random and its days to flower initiation were counted from sowing. Average of the data was calculated by adding days to flowering of 10 plants divided by 10. Primary and secondary branches were counted from 10 plants in

each replicate and their average was calculated. Plant height is an average of 10 plants selected in each replicate. It was measured from the soil surface to the tip of the plant.

For recording the data on the pod length, number of pods per plant and number of grains per pod 10 plants were selected. Total number of pods were counted in 10 plants and divided by 10. Averages were then computed. Pod length was the average of 10 pods per plant and mean of 10 plants per replicate were then analyzed statistically. Grains were counted in 10 pods of each plant 10 plants per replicate and the averages were then calculated. 1000 grains weight was calculated after counting 1000 grains in each replicate and then weighing them. For recording data on grain yield per plant grains of 10 plants per replicate was weighed and divided by 10.

CHAPTER FOUR

RESULTS AND DISCUSSION

Results obtained for observation of the mutagenic effect of gamma irradiation on the chromosomal behaviour and various agronomic traits is discussed below:

A. MUTAGENIC EFFECTS OF GAMMA RAYS ON POLLEN FERTILITY AND CHROMOSOMAL BEHAVIOUR AT MEIOSIS

The influence of gamma rays on chromosomal behaviour was observed at metaphase I of meiosis generally and some times at diakinesis also. The cytological effects of gamma rays is discussed under the headings of pollen fertility, asyndatic chromosomes and multiple associations of chromosomes one by one.

1. POLLEN FERTILITY:

Means for the pollen fertility as affected by gamma rays is presented in Table 1. As evident from the data with the increase in gamma rays influence there is a gradual decrease in pollen fertility. It is also evident that though quite different from controlled ones, the plants treated with 5 and 10 Krad were not different from each other. Least pollen fertility was recorded in the plant treated with 20 Krad. It

can be concluded from the results that the highest radiation dose causes greater sterility in the pollen observed. Results of similar nature have also been observed by Sparrow et al. (1968) in woody plant, Ashraf and Basette (1986) in fresh bean, Krishnan (1988) in Catharanthus, Ahmad (1989) in barley and Larik (1975) in wheats after gamma irradiation. All these results are in conformity to our findings presented here.

The low pollen fertility of plant treated with 5 Krad as compared to that of 10 Krad plant in Table 2 can most probably be explained on the presence of univalents, as the former was with 32% univalents and the latter was with 25% of the cells with univalents. Thus the former plant had a reduced pollen fertility compared to later (Table 1). The negative influence of pollen fertility by univalent have also been observed by Srivastava (1974) and, Khawaja and Ellis (1987).

Multivalent associations/translocation heterozygosity also effect pollen fertility (Tsuchiya, 1960 and Singh, 1973). During the present investigations univalents were observed in association with multivalents (Fig 1, 2, 3, 4) but no attempts were made to ascertain the rate of pollen fertility to univalents or multivalent chromosomal

associations. But as both the anomalies occurred in the same pollen mother cell, therefore the pollen fertility was attributed to both and not to a single meiotic anomaly. Malaviya and Schulka (1984) also reported reduced pollen fertility in gamma irradiated lentil.

2. ASYNDATIC CHROMOSOMES AT M1:

Asyndatic chromosomes or univalents recorded during the present studies are given in table 1 and their occurrence in percentage values is presented in Table 2. Data regarding the occurrence of asyndatic chromosome on single cell basis is presented in appendices 1-4. As evident from the results maximum number of univalents were observed in the plant raised from 20 Krad irradiated seed, where, almost 50% of the cells were with univalents and the range of univalents was 1-4 per cell (Appendix IV). The most frequent combination was cells with two univalents. Out of the 12 cells observed, 6 cells were with 2 univalents, 4 cells with a single univalent and 2 cells had 4 univalents each.

Plant observed with 5 Krad had 32% cells with asyndatic chromosomes in which the range was 2-4 univalents

per cell. 75% of the least asyndatic chromosomes were observed in the PMC's of 10 Krad irradiated plant (Table 2).

The asyndatic behaviour of chromosomes may either be due to asynapsis or desynapsis. Asynapsis is a condition where the homologous chromosomes fails to pair in zygonema and in desynapsis the homologue pair initially but separates during the early diplotene or just after it but usually before M1 (Schulz-Schaeffer, 1980). During the present investigations a struggle was made to confirm whether the asyndesis was due to asynapsis or desynapsis but it was in vain partly due to the smaller size of the chromosomes and partly due to the lack of chromosomal spreading in pachytene. Hence, the univalents at M1 were either due to asynapsis or desynapsis, or both. Zeeavic et al. (1975) Ahmad (1989) and Khawaja et al. (1990) observed univalents in gamma irradiated barley. In soybean desynaptic mutant have been reported by Palmer (1974) and the use of this mutant in linkages studies have also been presented by Palmer (1976). Asyndatic chromosomes bearing plants are the main source of monosomics which are very useful in understanding the basic genetics of soybean complement (Palmer, 1976).

3. MULTIPLE ASSOCIATION OF CHROMOSOMES AT M1:

Multivalents recorded during the present investigations were trivalents and quadrivalents (Table 1 and 2). Both controlled and the plant irradiated with 5 Krad showed no multivalent associations at M1. Data regarding multivalents association on single cell basis is presented in Appendices III and IV. At the radiation doses of 10 and 20 Krad more quadrivalents were observed as compared to trivalents. At 10 Krad 20% cells had a single trivalent per cell with other associations whereas at 20 Krad 23.07% cell had trivalents ranging from 1 to 2 trivalents per cell.

Quadrivalents were observed in 61.53% PMC's ranging from 1 to 3 quadrivalents per cell. Out of the 16 PMC's recorded with multivalents, one cell was with 3 quadrivalents, three cells with 2 quadrivalents each and 10 cell with a single quadrivalent per cell. Plant treated with 10 Krad had 45% cells with a single quadrivalent each.

The appearance of multivalents signifies the high genetic recombination between the non-homologous chromosomes. Chromosomal interchanges or reciprocal translocations are very important in linkage studies. It can be very easily created

Table-1: Combined data on chromosomal associations at M1 in the sporocytis of gamma irradiated soybean.

S.No	Radiation dose (KR)	PMC's observed	Univalents	Bivalents	III	IV	V	VI
1	0	22	-	440	-	-	-	98.8
2	5	25	20	490	-	-	-	70.00
3	10	20	8	382	4	9	-	68.00
4	20	26	25	455	7	21	-	47.28

Table-2: Percentage of cells with various chromosomal anomalies observed during sporogenesis of gamma irradiated soybeans.

S.No	Radiation dose (KR)	sporocytis observed	percentage cells with univalents	percentage with triv-alents	percentage wells with quadrivalents
1	0	22	0	0	0
2	5	25	32	0	0
3	10	20	25	20	45
4	20	26	50	23.07	61.53

through gamma irradiations (Ahmads, 1989 and Khawaja et al. (1990). Results parallel to ours have also been reported in soybean by Sadanga and Grindeland (1979). which had been used by Sadanga and Newhouse (1982) in chromosome mapping and genes linkages of soybean. Palmer (1984) also used irradiation for creating interchanges in soybean for its chromosomal studies.

The present findings are in conformity to the results obtained by Sheikh et al. (1980) who observed meiotic anomalies in gamma irradiated mungbean, black gram, lentil and chickpea. They reported the meiotic anomalies as univalents, multivalents, rings and fragments.

B. MUTAGENIC EFFECT OF GAMMA IRRADIATION ON VARIOUS AGRONOMIC TRAITS OF SOYBEAN.

The influence of gamma irradiation was observed on germination percentage, initiation of flowering, number of primary branches per plant, number of secondary branches per plant, plant height, pod length, number of pods per plant, number of grains per pod, 1000 grain weight and grain yield per plant. A comprehensive discussion on the results obtained during the present studies is given below:

1. GERMINATION PERCENTAGE:

Analysis of variance of the data regarding the effect of gamma irradiation on germination percentage of soybean is presented in Appendix V and the results are summarized in Table 3. Although there was an indication that the germination percentage had a gradual decrease with the increase in irradiation dose. The variety difference and dose differences were found significant. The maximum influence on germination percentage was recorded at the irradiation dose of 10 Krad and its least effect was observed at 1 Krad dose. Findings similar to the present results have also been reported by Ouang (1964), Soriano (1961) in cereals. Our results are also in conformity to the findings of Chaudhry and Haq (1976-a), who reported similar reductional effects of gamma rays on soybean.

2. PLANT HEIGHT:

Analysis of variance of the data regarding the influence of gamma irradiation is given in Appendix VI. The data reveals that the plant height was significantly affected by the radiation doses. Variety differences were also significant, whereas, the interaction between various doses

Table-3: Average data on germination percentage of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	94.43	92.20	94.83	93.82 a
1 Krad	93.60	90.63	94.20	92.81 a
2 Krad	91.23	90.20	92.36	91.26 ab
3 Krad	89.93	89.03	90.96	89.97 bc
4 Krad	91.03	83.87	89.43	88.11 c
5 Krad	83.76	83.57	88.63	85.32 d
6 Krad	80.70	83.76	88.00	84.15 d
7 Krad	84.66	80.83	83.90	83.13 d
8 Krad	77.03	73.80	80.33	77.05 e
9 Krad	72.70	69.30	77.33	73.11 f
10 Krad	70.37	61.63	67.10	66.36 g
Mean for varieties	84.49 ab	81.71 b	86.10 a	
LSD value for Varieties =	2.99			
LSD value for Doses =	2.76			

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

and varieties were also found significant. The data is summarized in Table 4. Means for doses shows that generally the radiation has dose specific effect on plant height. Maximum plant height was recorded for radiation dose of 4 Krad and the minimum plant height was recorded for radiation dose of 10 Krad. The present findings are contradictory to the results reported by Akilov (1970) who is of the opinion that with the increase in irradiation dose there was a gradual increase in plant height of the soybean. Our differences of results are due to the difference of genotypes and the differences of the irradiation doses applied. The present results are, however, in a broad sense in conformity to the findings of Dwivedi and Pandey (1981) who obtained significantly different results for gamma irradiated soybean. The significant differences due to varieties as the "Least significant Differences" (LSD) test shows (Table 4) on the results are in conformity to the findings of Sparrow et al. (1968).

3. DAYS TO FLOWER INITIATION:

Analysis of the data regarding the effect of gamma rays on the days to first flower is presented in appendix VII and the data is summarized in Table 5. The analysis of variance revealed that the radiation doses have produced

Table-4: Average data on Plant height of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	64.60 e	54.50 f	84.80 c	60.39 d
1 Krad	52.60 f	52.50 h	72.50 d	69.10 c
2 Krad	55.50 f	45.20 gh	75.50 d	57.44 d
3 Krad	65.70 e	35.60 f	85.60 c	59.32 d
4 Krad	83.40 l	73.50 d	98.10 a	90.40 c
5 Krad	63.50 e	53.10 f	83.40 c	85.54 a
6 Krad	86.10 c	76.10 d	92.50 b	68.23 c
7 Krad	64.70 e	54.50 f	84.60 c	81.53 b
8 Krad	85.10 c	75.10 d	96.10 ab	69.38 c
9 Krad	50.30 fg	40.20 h	65.10 e	83.86 b
10 Krad	65.40 e	65.40 e	55.20 f	50.33 e
Mean for varieties	78.16 a	67.81 b	60.09 c	
LSD value for Varieties =				0.91
LSD value for Doses =				3.09
LSD valve for Varieties x Doses interaction =				5.34

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

significant differences in days to initiation of flowering. Varieties and varieties into doses interaction produced non-significant differences in days to the appearance of first flower. Means for doses in Table 5 shows that with the increase in irradiation doses there was a gradual decrease in the days to initiation of flowers and the response of all the three varieties to various doses is identical or the varieties behave similarly to the same irradiation dose. Except in Swat-84 which produced late flowering at 2 Krad dose all the radiation doses induced earliness in all the three varieties. These findings are in conformity with the results published by Dwivedi and Pandey (1981) who reported early floral induction in gamma irradiated soybean.

Chaudhry and Haq (1976-b) are also of the opinion that gamma rays induce earliness of flowering in soybean. Akilov (1970) and Nikolov (1984) also obtained early maturity in gamma irradiated soybean.

4. NUMBER OF PRIMARY BRANCHES PER PLANT:

Analysis of variance for the influence of gamma rays on the number of primary branches per plant as presented in appendix VIII reveals that the radiation doses have induced

Table-5: Average data on flower initiation of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	86.30	88.33	89.86	88.16 a
1 Krad	86.00	88.73	88.73	87.82 a
2 Krad	88.50	84.83	88.66	87.33 a
3 Krad	86.06	85.83	86.00	85.96 ab
4 Krad	83.30	81.70	84.83	83.27 bc
5 Krad	83.60	79.77	80.26	81.21 cd
6 Krad	84.33	79.96	82.50	82.26 c
7 Krad	81.16	80.47	83.56	81.73 cd
8 Krad	75.73	80.40	80.93	79.02 d
9 Krad	81.83	77.43	77.46	78.91 d
10 Krad	80.33	80.80	80.23	80.45 cd
Mean for varieties	83.37	82.57	83.91	
LSD value for Doses = 3.04				

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

significant differences in the number of primary branches per plant. Varieties were also significantly affected by the gamma rays. Summarized data in the form of means and their means for doses (Table 6) shows the differences induced by various gamma rays intensities. The highest number of primary branches were observed at the irradiation dose of 5 Krad irrespective of the variety. All the irradiation doses have increased the number of primary branches per plant. The results are in conformity with the findings of Yousaf et al. (1991) who observed significant differences in the number of primary branches per plant in gamma irradiated lentil. Goswami (1983) observed wide variation in number of tillers per plant in gamma irradiated barley. Significant difference in number of branches were also reported by Abohegazi (1980) in gamma irradiated in chickpea.

Table-6: Average data on Primary branches per plant of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	11.35	10.23	9.43	10.34 h
1 Krad	20.70	17.63	14.90	17.74 b
2 Krad	15.44	13.56	11.66	13.56 fg
3 Krad	18.50	15.37	13.36	15.75 de
4 Krad	17.16	14.83	12.83	14.94 e
5 Krad	81.90	18.90	16.83	19.21 a
6 Krad	17.06	14.40	12.26	14.58 ef
7 Krad	15.26	13.00	11.00	13.09 g
8 Krad	20.10	17.23	15.36	17.57 bc
9 Krad	20.93	17.83	15.76	18.18 ab
10 Krad	18.23	16.53	14.26	16.34 cd
Mean for varieties	17.88 a	15.41 b	13.43 c	
LSD value for Varieties =	0.44			
LSD value for Doses =	1.36			

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

5. NUMBER OF SECONDARY BRANCHES PER PLANT:

Analysis of variance for the effect of gamma rays on the number of secondary branches per plant is presented in Appendix IX. The ANOVA reveal that, both the varieties and the irradiation doses have significantly different response for the number of secondary branches per plant. Means for the data and its LSD reveal that there were significant differences in secondary branches of various treatments. The highest number of secondary branches were observed for 1 Krad dose irrespective of the variety. The highest mean was recorded for the irradiation dose of 1 Krad and the lowest mean was that for the irradiation dose of 10 Krad. Variety mean for Swat-84 was higher and lower in Lakota. The differential response of barley varieties to gamma rays have been reported by Khalil et al. (1986). Dealing with 28 species of woody plants Sparrow et al. (1968) came to the conclusion that different genotypes behave differently to gamma rays exposures.

6. POD LENGTH:

Analysis of variance for the data on pod length is presented in Appendix X and its means are presented in Table 8. The ANOVA revealed that pod length was significantly

Table-7: Average data on Number of secondary branches of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	25.06	24.03	21.66	23.59 cd
1 Krad	36.46	28.70	26.13	30.43 a
2 Krad	29.76	24.10	21.83	25.23bcd
3 Krad	26.37	23.80	21.83	24.00 cd
4 Krad	29.46	25.26	23.10	25.94 bc
5 Krad	31.90	28.03	25.13	28.36 ab
6 Krad	23.96	22.16	20.23	22.12 cd
7 Krad	25.70	20.80	18.90	21.80 d
8 Krad	26.46	22.83	20.80	23.37 cd
9 Krad	20.00	16.00	14.53	16.84 e
10 Krad	15.00	13.93	11.90	13.61 e
Mean for varieties	26.38 a	22.70 b	20.55 c	
LSD value for Varieties =	1.69			
LSD value for Doses =	4.14			

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

Table-8: Average data on Pod length (cm) of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	3.99	3.20	3.32	3.50 ab
1 Krad	3.88	2.91	2.43	3.07 cd
2 Krad	4.42	3.60	3.01	3.68 a
3 Krad	4.43	3.43	3.33	3.73 a
4 Krad	3.85	2.90	2.36	3.03 cd
5 Krad	3.98	2.65	2.96	3.19 bc
6 Krad	4.27	3.28	2.73	3.43 ab
7 Krad	3.90	2.43	2.25	2.86 de
8 Krad	4.32	3.24	3.05	3.53 a
9 Krad	4.09	3.09	2.43	3.20 bc
10 Krad	3.15	2.43	2.50	2.69 e
Mean for varieties	4.02 a	3.01 b	2.76 c	
LSD value for Varieties =	0.08			
LSD value for Doses =	0.31			

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

affected by different irradiation doses. Different varieties had also significant influence on the pod length.

Means for the doses shows that though there was an increase in the pod length at the irradiation doses of 2 and 3 Krad as compared to the controlled plants but the increase was not significant statistically. Means for varieties were statistically different. Also it is evident from means for varieties that the maximum pod length was recorded in variety Swat-84 and the minimum pod length was in variety Epps. In means for irradiation doses the maximum pod length was observed in 3 Krad dose and the minimum was at the irradiation dose of 10 Krad. Means for doses shows, that, the pod length generally decreased with the increased radiation dose. A decrease in pod length due to increase in irradiation doses have also been reported by Conga (1976) in Soybean, and by Yousaf et al. (1991) in lentil.

7. NUMBER OF PODS PER PLANT:

Analysis of variance for the number of pods per plant in Appendix XI reveals that, both irradiation doses and varieties into dose interaction were highly different in respect to the number of pods per plant.

Means of the data in Table 9 reveals that the irradiation dose had no linear effect on the number of pods per plant. The LSD test revealed that the response of varieties were not dose specific but the same dose produced different effects in all the three varieties.

Means for the varieties shows that all the varieties respond differently to various gamma irradiation doses. Maximum number of pods per plant was recorded for 5 Krad dose which was significantly different than the controlled one. Swat-84 produced maximum number of pods per plant, whereas, the minimum number of branches were recorded in Lakota (Table 9). Venkatramani (1952) reported similar results in Vicia faba. Dwivedi and Pandey (1981) also reported results which are in conformity to the present findings.

8. NUMBER OF GRAINS PER POD:

Analysis of variance of the data regarding the number of seeds per pod is presented in Appendix XII. The ANOVA shows that the variation regarding the number of grains per pod is not significantly different in the varieties and the radiation doses. Irradiation dose and varieties interaction was in significant.

Table-9: Average data on Pods per plant of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			
	Swat-84	EPPS	Lakota	Mean for Doses
0 Krad	385.2 h-k	355.1 lm	325.2 no	355.2 d
1 Krad	265.2 q	235.3 rs	255.6 rs	242.0 f
2 Krad	449.3 cd	401.4 g-j	372.5 k-m	407.7 c
3 Krad	435.2 d-f	405.5 g-i	408.7 f-i	416.5 c
4 Krad	440.1 de	410.7 f-h	380.6 j-l	410.4 c
5 Krad	575.2 a	475.7 bc	437.1 d-f	496.0 a
6 Krad	251.3 qr	235.0 rs	221.6 s	236.0 f
7 Krad	489.2 b	459.6 cd	420.3 c-g	456.3 b
8 Krad	396.0 g-k	356.0 lm	325.9 no	359.3 d
9 Krad	375.3 j-l	345.2 mn	313.4 op	344.6 d
10 Krad	259.9 p	266.1 q	236.0 rs	266.0 e
Mean for varieties	396.2 a	358.7 b	333.3 c	

LSD value for Varieties = 08.86
 LSD value for Doses = 16.56
 LSD value for Varieties x Doses interaction = 28.69

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

As given in Table 10 means for irradiation doses shows a gradual increase in values for the number of seeds per pod till the irradiation dose of 5 Krad and more there was no linear correlation of the irradiation dose and number of grains per pod. Maximum number of seeds were recorded for Lakota at the irradiation dose of 10 Krad and it was variety specific response to the irradiation, otherwise both Swat-84 and Epps showed poor performance on the same dose as compared to their respective controls, as regards the number of grains per pod. Means for varieties shows that maximum number of grains were present in Swat-84 whereas, the variety with minimum number of grains was Lakota.

All these results shows that the number of seed per pod in Soybean is a polygenic character and is controlled by a rather large number of genes. Therefore the mutation of one or a couple of gene will not alter this character so easily. As means for varieties shows (Table 10), the cumulative effects of irradiation was, increased number of grains in Swat-84 and Lakota as compared to controls, whereas, Epps failed to surpass its control.

Table-10: Average data on Number of grains per pod of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			Mean for Doses
	Swat-84	EPPS	Lakota	
0 Krad	2.72	2.62	2.42	2.58
1 Krad	2.94	2.84	2.54	2.78
2 Krad	2.81	2.63	2.33	2.59
3 Krad	2.80	2.70	2.40	2.63
4 Krad	2.94	2.78	2.48	2.73
5 Krad	3.20	3.08	2.78	3.02
6 Krad	2.85	2.75	2.45	2.68
7 Krad	3.22	2.96	2.66	2.95
8 Krad	2.85	2.75	2.41	2.67
9 Krad	3.16	3.03	2.73	2.97
10 Krad	2.61	2.23	4.61	3.15
Mean for varieties	2.92	2.76	2.71	

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

9. 1000-GRAIN WEIGHT:

Analysis of variance of the data regarding the influence of gamma rays on 1000-grain weight is presented in Appendix XIII. Means for the data regarding 1000-grain weight is presented in Table 11. ANOVA for the data shows that highly significant results were obtained due to varieties, radiation doses and, varieties into irradiation doses interaction. Means for irradiation doses revealed that, there was no linear correlation between the radiation doses and 1000-grain weight. Means for varieties revealed that, the maximum 1000-grain weight was found in variety Swat-84, whereas, the minimum 1000-grain weight was found in Lakota. Dwivedi and Pandey (1981) observed the influence of gamma irradiation on soybean and recorded variations in 1000 grain weight in the irradiated population. The results of Yousaf et al. (1991) in lentil are in conformity to the present findings. Conga (1976) also reported increased grains weight in gamma irradiated soybean.

10. GRAIN YIELD PER PLANT:

Analysis of variance of the data regarding the grain yield per plant as affected by gamma rays is presented in Appendix XIV. The ANOVA revealed that highly significant differences were induced by gamma rays in the grain yield per

Table-11: Average data on 1000-grain weight (gm) of three soybean varieties as affected by gamma rays.

Doses of gamma irradiation	VARIETIES			
	Swat-84	EPPS	Lakota	Mean for Doses
0 Krad	143.7 j-l	133.7 k-n	123.8 m-o	133.8 f
1 Krad	119.6 no	124.5 m-o	114.6 op	119.6 gh
2 Krad	261.8 a	231.9 b	178.6 fg	224.1 a
3 Krad	209.0 cd	193.7 d-f	177.3 f-h	193.4 c
4 Krad	202.1 c-e	182.2 f-g	172.1 g-i	185.5 c
5 Krad	202.2 c-e	185.5 e-g	175.6 g-i	187.8 c
6 Krad	179.0 fg	168.9 g-i	158.9 ij	168.9 d
7 Krad	233.5 b	213.7 c	193.5 d-f	213.6 b
8 Krad	161.0 h-j	150.9 jk	134.2 k-n	148.7 e
9 Krad	138.7 k-m	128.9 l-o	118.9 no	128.8 fg
10 Krad	119.0 no	111.6 op	101.4 p	110.7 h
Mean for varieties	179.1 a	166.0 b	149.9 c	

LSD value for Varieties = 06.77

LSD value for Doses = 10.09

LSD value for Varieties x Doses interaction = 17.48

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

plant. Varieties were also proved significantly different, whereas, highly significant results were also recorded for varieties into doses interactions.

Means for the data in Table 12 reveals that, all the three varieties were significantly different as compared to their respective controls. Means for irradiation doses revealed that, the irradiation doses produced variety specific effects and as the LSD reveals, all the varieties respond differently to the same dose. However, the irradiation dose of 5 Krad was very effective in enhancing the grain yield per plant. Results of similar nature have also been reported by Dwivedi and Pandey (1981) in gamma irradiated soybean. Zaher (1984) and Conga (1976) were also of the opinion that variation in grain yield per plant is expected in the gamma irradiated soybean.

Table-12: Average data on Grain yield per plant as affected by gamma rays.

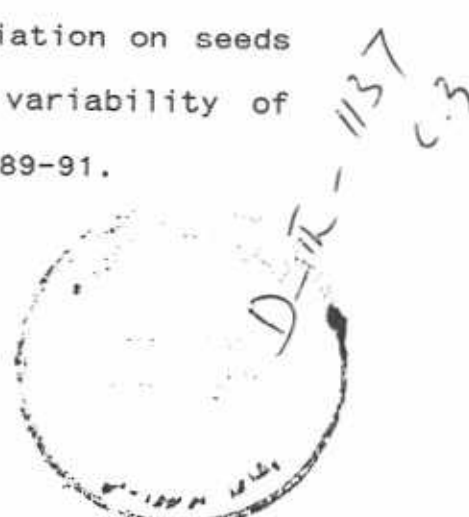
Doses of gamma irradiation	VARIETIES			
	Swat-84	EPPS	Lakota	Mean for Doses
0 Krad	153.9 o	133.9 r	123.9 t	137.3 h
1 Krad	103.6 v	98.9 w	78.77 x	97.09 j
2 Krad	319.0 f	298.9 h	218.9 i	298.9 c
3 Krad	258.8 j	239.1 k	218.6 l	238.9 e
4 Krad	282.3 i	268.7 j	242.6 k	262.5 d
5 Krad	363.7 a	345.4 c	325.4 e	344.8 a
6 Krad	138.5 q	128.9 s	120.7 t	129.4 i
7 Krad	354.8 b	334.0 d	314.7 g	334.8 b
8 Krad	178.6 m	158.8 n	148.5 p	162.0 f
9 Krad	161.7 n	141.9 q	121.6 t	141.7 g
10 Krad	148.7 p	128.9 s	108.6 u	128.8 i
Mean for varieties	224.0 a	206.6 b	190.2 c	

LSD value for Varieties = 0.97
 LSD value for Doses = 2.24
 LSD valve for Varieties x Doses interaction = 3.87

Note: Means of the same category having at least one common letter are not significantly different at 5% level of probability according to LSD test (Least Significant difference).

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Appendix-I: Meiotic Behaviour of the untreated soybean chromosomes at M1 in the pollen mother cells.

S.No	Univalents	Bivalents	Multivalents			
			III	IV	V	VI
1	-	20	-	-	-	-
2	-	20	-	-	-	-
3	-	20	-	-	-	-
4	-	20	-	-	-	-
5	-	20	-	-	-	-
6	-	20	-	-	-	-
7	-	20	-	-	-	-
8	-	20	-	-	-	-
9	-	20	-	-	-	-
10	-	20	-	-	-	-
11	-	20	-	-	-	-
12	-	20	-	-	-	-
13	-	20	-	-	-	-
14	-	20	-	-	-	-
15	-	20	-	-	-	-
16	-	20	6	-	-	-
17	-	20	-	-	-	-
18	-	20	-	-	-	-
19	-	20	-	-	-	-
20	-	20	-	-	-	-
21	-	20	-	-	-	-
22	-	20	-	-	-	-
Total	-	440	-	-	-	-

Appendix-II: Chromosomal behaviour in the pollen mother cells of soybean irradiated with 5 KR of gamma rays.

S.No	Univalents	Bivalents	Multivalents			
			III	IV	V	VI
1	-	20	-	-	-	-
2	-	20	-	-	-	-
3	2	19	-	-	-	-
4	-	20	-	-	-	-
5	-	20	-	-	-	-
6	2	19	-	-	-	-
7	4	18	-	-	-	-
8	2	19	-	-	-	-
9	-	20	-	-	-	-
10	2	19	-	-	-	-
11	-	20	-	-	-	-
12	-	20	-	-	-	-
13	-	20	-	-	-	-
14	-	20	-	-	-	-
15	-	20	-	-	-	-
16	-	20	6	-	-	-
17	2	19	-	-	-	-
18	-	20	-	-	-	-
19	-	20	-	-	-	-
20	4	18	-	-	-	-
21	2	19	-	-	-	-
22	-	20	-	-	-	-
23	-	20	-	-	-	-
24	-	20	-	-	-	-
25	-	20	-	-	-	-
Total	20	490	-	-	-	-

Appendix-III: Chromosomal behaviour at M1 in the pollen mother cells of gamma irradiated (10 KR) soybean.

S.No	I	II	Multivalents			
			III	IV	V	VI
1	-	18	-	1	-	-
2	1	18	-	1	-	-
3	-	18	1	-	-	-
4	-	20	-	-	-	-
5	-	20	-	-	-	-
6	-	18	-	1	-	-
7	-	18	-	1	-	-
8	4	18	-	-	-	-
9	1	18	1	-	-	-
10	-	18	-	1	-	-
11	-	18	-	1	-	-
12	-	18	-	1	-	-
13	-	18	-	1	-	-
14	-	20	-	-	-	-
15	-	20	-	-	-	-
16	1	18	1	-	-	-
17	1	18	1	-	-	-
18	1	20	-	-	-	-
19	-	20	-	-	-	-
20	-	18	-	1	-	-
Total	8	382	4	9	-	-

Appendix-IV: Chromosomal association at M1 in PMC's of gamma irradiated (20 KR) Soybean.

S.No	I	II	Multivalents			
			III	IV	V	VI
1	1	16	1	1	-	-
2	-	20	-	-	-	-
3	2	17	-	1	-	-
4	4	16	-	1	-	-
5	-	20	-	-	-	-
6	2	17	-	1	-	-
7	-	16	-	2	-	-
8	-	16	-	2	-	-
9	2	16	2	-	-	-
10	1	16	1	1	-	-
11	-	14	-	3	-	-
12	-	20	-	-	-	-
13	-	20	-	-	-	-
14	-	20	-	-	-	-
15	2	17	-	1	-	-
16	2	17	-	1	-	-
17	4	16	-	1	-	-
18	1	16	1	1	-	-
19	1	14	1	2	-	-
20	4	20	-	-	-	-
21	2	20	-	-	-	-
22	-	18	-	1	-	-
23	-	18	-	1	-	-
24	1	18	1	-	-	-
25	2	17	-	1	-	-
26	-	20	-	-	-	-
Total	25	455	7	21	-	-

Appendix-V: Analysis of variance for germination percentage.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	14.397	7.198	0.3739
Varieties	2	325.229	162.614	8.4458 *
Error-a	4	77.016	19.254	
Doses	10	6837.001	683.700	79.6313 *
Varieties x Doses	20	239.298	11.965	1.3936 NS
Error-b	60	515.149	8.586	
Total =	98	8008.089		

* = Significant at 5% level of probability.

NS = Non-significant.

Appendix-VI: Analysis of variance for plant height (cm).

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	810.568	405.284	226.5693
Varieties	2	5425.444	2712.722	1516.5157 *
Error-a	4	7.155	1.789	
Doses	10	11725.960	1172.596	109.5628 *
Varieties x Doses	20	1772.949	88.647	8.2829 *
Error-b	60	642.150	10.703	
Total =	98	20384.227		

* = Significant at 5% level of probability.

Appendix-VII: Analysis of variance for the flower initiation.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	31.435	15.717	4.3374
Varieties	2	30.249	15.125	4.1739 NS
Error-a	4	14.495	3.624	
Doses	10	1089.386	108.939	10.4515 *
Varieties x Doses	20	205.670	10.283	0.9866 NS
Error-b	60	625.404	10.423	
Total =	98	1996.638		

* = Significant at 5% level of probability.

NS = Non-significant.

Appendix-VIII: Analysis of variance for Primary branches per plant.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	9.854	4.927	12.0538
Varieties	2	328.191	164.096	401.4464 *
Error-a	4	1.635	0.409	
Doses	10	615.039	61.504	29.3792 *
Varieties x Doses	20	17.132	0.857	0.4092 NS
Error-b	60	125.607	2.093	
Total =	98	0000.000		

* = Significant at 5% level of probability.

NS = Non-significant.

Appendix-IX: Analysis of variance for Number of secondary branches.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	381.129	190.564	31.0094
Varieties	2	573.338	286.669	46.6479 *
Error-a	4	24.582	6.145	
Doses	10	2041.638	204.164	10.6091 *
Varieties x Doses	20	88.976	4.449	0.2312 NS
Error-b	60	1154.658	19.244	
Total =	98	4264.320		

* = Significant at 5% level of probability.

NS = Non-significant.

Appendix-X: Analysis of variance for Harvest index % =
Grain yield x 100 above ground plant yield.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	0.605	0.302	23.2236
Varieties	2	29.458	14.729	1131.3264 *
Error-a	4	0.052	0.013	
Doses	10	10.185	1.019	9.1609 *
Varieties x Doses	20	2.869	0.143	1.2904 NS
Error-b	60	6.671	0.111	
Total =	98	49.840		

* = Significant at 5% level of probability.

NS = Non-significant.

Appendix-XI: Analysis of variance for Number of pods per plant.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	4695.286	2347.643	13.9883
Varieties	2	65931.443	32965.722	196.4249 *
Error-a	4	671.315	167.829	
Doses	10	666865.613	66686.561	216.0979 *
Varieties x Doses	20	15487.539	774.377	2.5094 *
Error-b	60	18515.656	308.594	
Total =	98	772166.853		

* = Significant at 5% level of probability.

Appendix-XII: Analysis of variance for Number of grains per pod.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	0.566	0.283	0.4078
Varieties	2	0.784	0.392	0.5651 NS
Error-a	4	2.776	0.694	
Doses	10	3.414	0.341	0.5202 NS
Varieties x Doses	20	12.016	0.601	0.9156 NS
Error-b	60	39.372	0.656	
Total =	98	58.928		

NS = Non-significant.

Appendix-XIII: Analysis of variance for 1000-grain weight (gm).

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	4443.152	2221.576	22.6197
Varieties	2	14080.447	7040.223	71.6824 *
Error-a	4	392.837	98.214	
Doses	10	136540.515	13654.052	119.2172 *
Varieties x Doses	20	6477.093	323.855	2.8277 *
Error-b	60	6871.853	114.531	
Total =	98	168805.917		

* = Significant at 5% level of probability.

Appendix-XIV: Analysis of variance for grain yield per plant.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F. Value
Replications	2	3412.048	1706.024	849.2950
Varieties	2	18803.302	9401.651	4680.3423 *
Error-a	4	8.035	2.009	
Doses	10	749334.534	74933.453	13324.5253 *
Varieties x Doses	20	1451.704	72.585	12.9070 *
Error-b	60	337.423	5.624	
Total =	98	773347.046		

* = Highly significant.



Fig 1. Prophase II of *Glycine max* showing 20 chromosomes.



Fig 2. Metaphase II of *Glycine max* showing 20 chromosomes at both the equatorial regions.



Fig 3. Metaphase I of gamma irradiated *Glycine max* with 3 quadrivalents and 3 univalents.



Fig 4. Sporocyte of gamma irradiated *Glycine max* with 4 univalents.



Fig 5. Pollen mother cell of gamma irradiated *Glycine max* at M1 with one trivalent and two univalents.

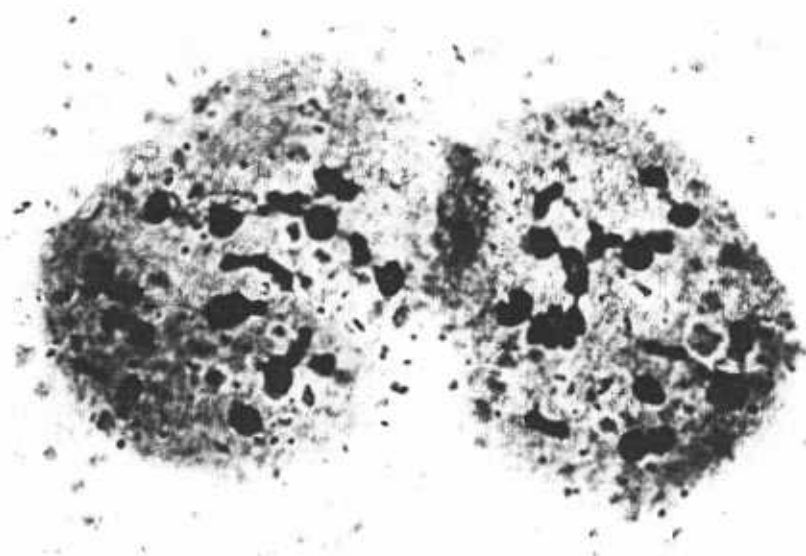


Fig 6. Pollen mother cells of gamma irradiated *Glycine max* at diakinesis with 20 bivalents each.

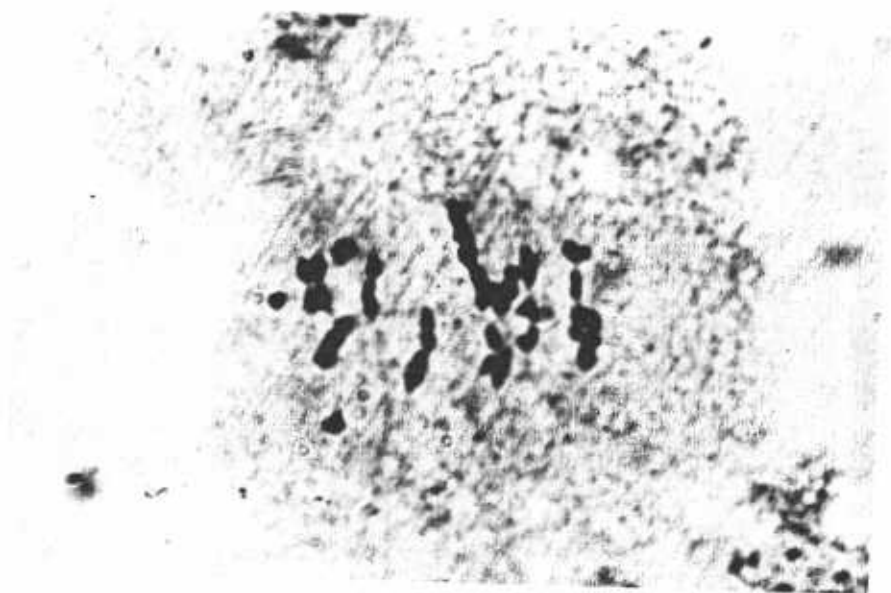


Fig 7. Sporocyte of gamma irradiated *Glycine max* with a single quadrivalents and 3 univalents.

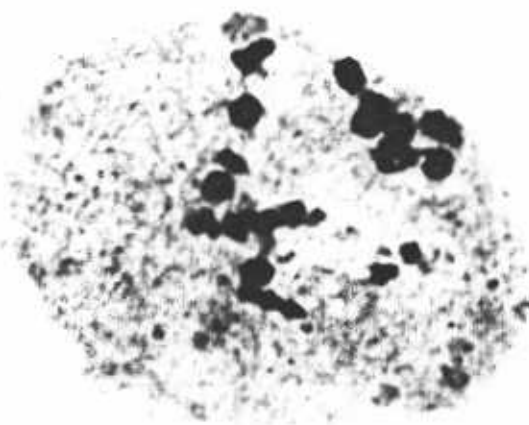


Fig 8. Diakinesis of gamma irradiated Soybean with two bivalents.



Fig 9. M1 of gamma irradiated Soybean with 20 bivalents.