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Effect of Gamma Rays on Morphology, Growth, Yield and Biochemical Analysis in Soybean (*Glycine max* (L.) Merr.)

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ABSTRACT

Mutation breeding in crop plants is an effective approach in improvement of crop having narrow genetic base such as soybean. The main objective of the present study is to determine the effect of different doses of gamma irradiation on different morpho-agronomic characteristics. Many physical mutagens have been employed for obtaining useful mutants in various crop species. The role of mutation breeding increases the genetic variability for the desired traits in various crop plants. Soybean (*Glycine max* (L.) Mrr.) var. Co1. Was treated with physical mutagens like Gamma rays. For inducing mutation of Soybean treated with various concentrations of gamma rays 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 KR. Agronomic traits and morpho metric characters such as germination percentage, days of first flower, root length, shoot length, seedling survival, number of fruits per plant, fruit length, seed yield per plant, fresh weight per plant, dry weight per plant and 100 seed weight. All parameters were studied in M_1, M_2, M_3 and M_4 generations. The results of the present study re- vealed that the increasing level of gamma rays treatment with decreased significantly most of agronomic and morphological traits evaluated in M1 populations. In M_2, M_3 and M_4 populations with significant increase of morphology and yield components in soybean. The yield parameters like plant height, number of cluster per plant, number of seeds per plant and seed yield per plant were recorded the moderated and high mean value in the 50 KR of gamma rays.

***Keywords*:** Soybean, mutagens, Gamma rays, Population, Treatment, *Glycine max*

1. INTRODUCTION

The present study was investigated through induced mutation by gamma rays and their effects on quantitative traits of soybean (G*lycine max* (L.) Merr.) Variety CO-1 by means of extended the genetic variability.

1. 1. Importance of Soybean

Soybean, Glycine max (L.) Merr., combines in one crop both the dominant world supply of edible vegetable oil, and the dominant supply of high-protein feed supplements for livestock. Other fractions and derivatives of the seed have substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products (Smith and Huyser, 1987). The United States is the principal world supplier of soybeans (Jewell, 1988). Soybean protein is rich in valuable amino acid lycine (5%) in which most of the cereals are deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin C, Vitamin A is present in the form of precursor carotene, which is converted into vitamin A in the intestine.

(A)

(B)

(**C**)

Photo A, B, C. *Glycine max* (L.) Mrr.

A large number of Indian and western dishes such as bread, `chapati', milk, sweets, pastries etc., can be prepared with soybean.

Wheat flour fortified with soybean flour makes good quality and more nutritious `chapati'. Soybean oil is used for manufacturing *vanaspati* ghee and several other industrial products. Soybean is used for making high protein food for children. It is widely used in the industrial production of different antibiotics. Soybean builds up the soil fertility by fixing large amounts of atmospheric nitrogen through the root nodules, and also through leaf fall on the ground at maturity. It can be used as fodder; forage can be made into hay, silage etc. Its forage and cake are excellent nutritive foods for livestock and poultry. Soybean being the richest, cheapest and easiest source of best quality proteins and fats and having a vast multiplicity of uses as food and industrial products is sometimes called a wonder crop.soybean is one of the important crops of the world. Production of soybean in India at the present time is restricted mainly to Madhya Pradesh, Uttar Pradesh Maharashtra and Gujarat. It is also grown on a small acreage in Himachal Pradesh, Punjab and Delhi (Photo A, B, C).

1. 2. Taxonomy of Soybean Relatives

The soybean is a papilionoid legume (family Fabaceae, subfamily Faboideae), and a member of the tribe Phaseoleae, subtribe Glycininae. The subtribe to which soybean belongs consists of 16 genera, none of which, save for soybean (Glycine) and kudzu (Pueraria), are commonly known outside of botanical science. The genus Glycine is unique within the subtribe on several morphological and chromosomal characters, and does not seem to bear an especially intimate relationship with any other genus in the subtribe (Lackey, 1977). A single exception may be the genus Sinodolichos, a rarely-collected and poorly-known genus from Asia. Sinodolichos is unknown in the living state outside of Asia (Lackey, 1981a).

The genus Glycine is divided into two questionably distinct subgenera: Glycine and Soia. The first consists of six or seven perennial species primarily from Australia. The second consists of three annual species from Asia: Glycine max, Glycine soia Sieb. & Zucc., and Glycine gracilis Skvortz. The first species is the cultivated soybean, the second species is the wild form of the soybean, and the third species is the weedy form of the soybean (Lackey, 1981a).

1. 3. Mutation in Soybean

The mutagenesis was quickly treated in gene level so the portant of crops production. The FAO/IAEA was first news released the data base for most of mutant varieties of Soybean. Mutation breeding supplement conventional plant breeding as a source of increasing variability and could confer specific improvement without significantly altering its acceptable phenotype (Ojomo *et al.*, 1979).

Mutations, both spontaneous and induced have been eminently successful in changing the fatty acid composition of several oilseed crops. Three reasons have been quoted for attempts in changing seed oil quality by means of single gene mutations being exceptionally successful. Induced mutation was abnormal of the chromosome is the primary basis of genetic change; therefore, investigations on the mechanism of chromosome breakage, type of aberrations, and their genetic consequence form an integral part of most mutation studies (Zeerak, 1992).

Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variabilities in plants especially in those with limited genetic variabilities, because in plants the gene replacement experiments through homologus recombination with introduced DNA sequences have met with limited success.

2. MATERIALS AND METHODES

2. 1. Collection of seeds

Soybean seeds variety CO-1 were collected from Millet breeding station, TamiNadu Agriculture University (TNAU), Coimbatore.

2. 2. Mutagenic treatment -Gamma irradiation treatment

The seeds were treated with different dose of Gamma rays (10. 20, 30, 40, 50, 60, 70, 80, 90 and 100KR) treated from sugarcane breeding institute (ICAR) Coimbatore.

3. LABORATORY STUDIES (M_1 Generation)

3. 1. Germination studies

Germinated seeds will count from 3rd to 7th day emergence of cotyledonary leaf will be taken as the indication of germination. Germination percentage will be worked out for the treatment in each genotype separately and lethality will be found out based on the mean value of 10 replicates.

3. 2. Shoot and root length

The shoot and root length (cm) will be measured ten randomly selected seedlings with ten replications on the 15th day with the effect of physical mutagen along with control.

3. 3. Lethal dosage (LD_{50} Value)

The LD_{50} value for Soybean variety CO-1 was observed at 50KR of gamma rays.

4. FIELD STUDIES (M_1, M_2 and M_3 generations-Based on the LD_{50} value)

Treated and control seeds were sown in the field (3 replication) in a randomized Block design (RBD) in order to raises the M_1, M_2 and M_3 generations. Each treatment of doses consists of hundred seeds including control. The seed to seed and row to row distance was maintained at15×60cm respectively. Cultural operations were carried out *viz.*, irrigation and weeding.

The following characters such as Days to first flower, Plant height, Number of branches per plant, Number of leaves per plant, Number of cluster per plant, Number of pod per plant, Number of seeds per plant, Hundred seed weight, Seed yield per plant, Fresh weight per plant and Dry weight per plant, were observed in all the treatments along with R_1- R_3 generations.

5. BIOCHEMICAL STUDIES

5. 1. Seed Protein content (%)

Two seeds from the same plant of each M_3 plants were separately collected and ground in a mortar and the extracts were defatted by washing with three changes of cold acetone for 4 to 6 hrs. The acetone was removed by filtration and the extracts were air-dried at room temperature.

The proteins from the defatted meal were precipitated with 10 % trichloro-acetic acid and recovered by centrifugation at 5000 rpm for 30 minutes at 40 °C. The protein content was then determined calorimetrically according to the method of Lowry *et al.*, (1951) using bovine serum albumin as standard.

5. 2. Seed oil content (%)

The oil content of the kernel was estimated with petroleum ether in Soxhlet extraction apparatus (Cox and Pearson, 1962).

6. EXPERIMENTAL RESULTS

Effect of Gamma rays on Soybean for R_1, R_2 and R_3 generations was thoroughly investigated with effect of gamma rays on quantitative traits such as germination percentage (LD_{50} Value), Days to First flower, Plant height (cm), Number of leaves per plant, Number of branches per plant, Number of cluster per plant, Number of pods per plant, Number of seed per plant, Seed yield per plant (g), 100 seed weight (g), Fresh weight per plant (g) and Dry weight per plant (g), Seed Protein content and Seed Oil content.

6. 1. Determination of LD_{50} value

Important directions to be made in a mutagenesis experiment are the choice of doses of mutagen. It can be achieved by means of lethal dose (LD_{50} value). It refers to 50 per cent reduction of germination seeds in given progeny. The determination of optimal mutagenic dose is not easy. Because, low and high mutagenic doses are having some merits and demerits generally, higher mutagenic doses provide a higher number of possible mutants.

The present investigation exhibited that increased doses of gamma rays treatment in the resulted in the decrease in germination percentage, 50 per cent reduction in seed germination at 50KR of gamma rays (LD_{50} value 52.82) respectively.

6. 2. Seed germination, Seedling survival

Showed different doses of gamma rays which indicated gradual reduction of seed germination (10^{th} day), seedling survival (30^{th} day) and Plant height (30^{th} day) than control. The effects of gamma rays were different doses on survival percentage, mutation frequency and mutagenic effectiveness. The survival percentage and mean value of M_1 generation were decreased with increase the dose of treatments.

6. 3. M_1 Generation

Induced mutation procedure in the recent past has successfully been used for the improvement of various pulse crops. In the present investigation, all the parameters were gradually reduced in R_1 generation at maturity time except days to first flowering which increase the days indicated inhibitory effects. The present result confirms these earlier reports. Amarnath *et al*., (1991) reported that Genotypic and phenotypic variability and heritability of some quantitative characters in soybean (*Glycine max* (L.) Merr).

The growth and other quantitative traits were proportionately decreased with increasing dose of gamma irradiation in the present study. Cheng *et al.*, (1999) the structural, biochemical and genetic characterization of a new radiation-induced, variegated leaf mutant of soybean (*Glycine max* (L.) Merr.).

The decrease in survival percentage has been attributed to the physiological disturbance or chromosomal damage caused to the cells of the plant by the mutagen. Mensah (1977) recorded reduction in germination and survival percentage due to the effect of chemical mutagens in cowpea. The M_1 generation was assessed at the field level to measure the intensity of injury caused by mutagenic treatments (Gaul, 1970).

Table 1. Effect of physical and chemical mutagens on Soybean in M_1 generation.

Treatment		Days to first flower	Plant height	number of cluster per plant	number of pod per plant	seed yield per plant	number of branches per plant	number of leaves per plant	Protein contend	Oil contend
M_2 Generation	Control	35.58± 2.11	74.82±2.92	21.62±1.54	49.62±2.82	10.21±0.97	4.32±0.11	64.27±1.99	39.56±1.27	19.05±1.71
	GAMMA RAYS 10KR	36.32± 2.30	72.51±3.16	22.32±0.89	59.62±1.96	11.64±0.69	4.46±0.13	69.54±2.56	40.32±1.52	19.37±1.15
	20KR	36.13± 1.58	74.96±2.55	20.81±0.94	60.39±3.71	12.07±0.77	4.51±0.15	73.21±4.17	41.50±1.90	20.22±1.36
	30KR	35.11± 1.56	73.82±2.82	21.37±1.37	60.55±4.21	11.85±0.96	5.02±0.10	70.42±3.56	40.75±1.16	20.56±1.05
	40KR	35.03± 1.30	76.99±3.09	24.39±0.99	61.27±2.96	12.39±0.82	5.07±0.09	75.37±1.82	40.79±2.37	19.85±0.58
	50KR	33.44± 1.00	78.29±4.52	26.51±1.72	62.21±3.27	13.46±0.82	5.16±0.27	81.52±4.21	41.71±1.94	21.76±1.02
	60KR	35.11± 1.41	77.31±2.71	20.12± 1.01	50.29±3.11	12.55±1.27	5.02±0.16	61.32±2.37	41.06±2.07	20.22±0.89
M_3 Generation	Control	36.81± 1.43	70.75± 4.32	22.10±0.75	48.62±1.08	11.05±0.41	3.41±0.11	62.54±1.38	38.56±1.27	19.05±1.71

M_4 Generation								
20KR	GAMMA RAYS 10KR	Control	60KR	50KR	40KR	30KR	20KR	GAMMA RAYS 10KR
35.42± 1.20	35.19± 1.41	34.87± 1.43	37.65± 1.22	34.35± 1.14	35.24± 0.78	37.18± 0.69	35.47± 1.08	36.99± 1.25
72.53± 2.11	72.13± 1.06	71.62± 4.32	62.89± 2.78	81.02± 2.61	80.71± 2.54	74.4± 3.98	76.43± 5.04	75.66± 2.56
21.81±1.21	21.15±1.46	20.17±1.10	20.19±0.54	23.08±0.26	22.48±0.84	22.50±1.04	20.55±1.10	21.62±0.98
56.81±1.47	55.62±1.46	47.22±1.21	50.20±1.56	52.23±1.20	51.01±1.41	48.88±1.68	49.86±1.25	47.84±1.11
11.41±0.35	10.24±0.41	9.87±0.48	10.23±0.58	12.20±0.46	11.54±0.17	11.31±0.64	10.25±0.28	10.88±0.55
4.15±0.17	4.23±0.33	4.11±0.24	3.24±0.11	4.11±0.17	4.05±0.18	3.89±0.13	3.25±0.15	3.62±0.20
70.86±2.10	64.31±1.38	60.57±2.11	60.27±2.31	65.55±2.20	63.34±1.84	58.47±1.56	61.12±2.07	60.51±2.11
39.10±1.74	39.32±1.31	38.81±1.11	38.06±2.07	39.56±1.94	39.13±2.37	39.02±1.16	38.50±1.90	38.32±1.52
19.02±0.47	18.27±0.48	18.22±0.35	18.22±0.89	18.76±1.02	19.85±0.58	19.56±1.05	19.22±1.36	18.37±1.15

	30KR	34.33± 1.51	72.41± 1.46	21.96±1.08	55.12±2.01	10.36±0.28	4.85±0.28	71.20±1.89	38.14±1.05	19.16±0.25
	40KR	34.45± 1.02	74.58± 1.20	22.52±1.44	58.17±1.95	11.21±0.43	4.91±0.39	72.44±1.05	38.95±1.46	19.15±0.41
	50KR	33.21± 0.95	76.08± 1.11	24.45±1.36	60.12±2.48	12.18±0.37	5.22±0.11	78.63±1.33	39.46±1.32	19.31±0.63
	60KR	35.65± 1.07	70.40± 0.98	20.15± 0.78	45.44±1.09	10.31±0.55	4.10±0.16	55.18±1.42	38.23±1.55	18.04±0.38

Table 2. Effect of Gamma rays on yield parameters and biochemical content of soybean in M_2, M_3 and M_4 generation.

Treatment	Germination %	Seedling survival %	Days to first flower	Root length (cm)	Shoot length (cm)	No. of fruit/plant	Seed yield (g)	Fresh weight (g)	Dry weight (g)	100 Seed weight (g)
Control	97.24	92.45	37.21	25.64	68.39	70.51	16.08	87.19	47.65	10.39
Gamma rays 10 KR	95.11	90.35	38.27	22.54	66.18	66.25	15.10	84.25	43.28	10.02
20 KR	84.27	83.06	38.08	20.16	62.05	61.48	14.35	80.04	39.49	9.87
30 KR	69.84	66.35	40.21	18.84	58.54	51.38	13.07	76.29	35.28	9.36
40 KR	58.87	54.08	40.65	18.23	51.20	47.59	12.54	71.24	34.07	9.04
50 KR	51.37	46.28	41.10	17.51	45.29	44.06	12.05	66.01	32.15	8.90
60 KR	37.39	35.24	43.08	15.29	40.07	41.17	11.04	59.81	27.40	8.81

6. 4. Effect of gamma irradiation on M_2, M_3 and M_4 Generations

In M_2 and M_3 generations all the parameters gradually increased in optimum doses. The present results confirm these earlier reports in black gram (Arulbalachandran 2006); Mung bean (Khan and Wani 2005): *Zea mays* (Gnanamurthy *et al*., 2011) and Cowpea (Dhanavel *et al*., 2012) The R_2 Plants raised in the field were examined to identify the mutants induced by gamma rays, as well as to find out their effects on various quantitative characters. The maximum mutants were observed at 50KR of gamma rays.

7. CONCLUSION

The present investigation, different dose of gamma irradiation induced mutation in R_1 generation inhibit seedling growth and other growth parameters in field condition. The inhibitory effects were due to physiological disturbances on soybean by gamma irradiation especially with imbalance of growth hormones. From the results LD_{50} value was fixed at 50KR gamma rays. In M_2, M_3 and M_4 generations 50KR of gamma rays showed more frequency of chlorophyll mutants, viable mutants, effectiveness and efficiency.

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