

Morphology and Yield parameters and Biochemical analysis of Soybean (*Glycine max* (L.) Merr.) Using Gamma rays, EMS and DES treatment

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ABSTRACT

Soybean (*Glycine max* (L.) Merr.) var. Co-1 seeds were treated with physical mutagen namely such as gamma rays and chemical mutagen namely such as Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES). Various concentrations of gamma rays 10, 20, 30, 40, 50 and 60 KR, EMS (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %) and DES (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %). The morphological parameters such as 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 content and oil content increasing with increasing level of some doses gamma rays, Ethyl methane sulphonate and Diethyl sulphate treatment in M₂ and M₃ generation were carried out. A number of mutants were recorded in M₂ and M₃ generations for one or more traits viz., plant height, maturity, branching, pigmentation, fruit size and yield were isolated in M₂ and M₃ generation. Mean for various characters increased at mutagenic treatment than control. 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 0.5% of EMS and 0.4% of DES treated population with compared to control plants.

Keywords: Soybean; mutagens; Gamma rays; Generation

1. INTRODUCTION

Soybean possesses a very high nutritional value. It contains about 20 per cent oil and 40 per cent high quality protein (as against 7.0 per cent in rice, 12 per cent in wheat, 10 per cent in maize and 20-25 per cent in other pulses). Soybean protein is rich in valuable amino acid lysine (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 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 *vanaspathi* 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. Soybean grows well in warm and moist climate. The climatic requirements for soybean are almost the same as for maize. A temperature of 26.5 to 30°C appears to be the optimum for most of the varieties. Soil temperatures of 15.5°C or above favors rapid germination and vigorous seedling growth.

2. MATERIALS AND METHODS

2.1. Selection of genotype

The choice of genotype is an important aspect of crop improvement programme through induced mutagenesis. Mutation breeding the difference is due to genotypes of primary importance than the mutagens (Gregory, 1960). The crop species to be improved using mutation breeding should therefore be pursued with varieties outstanding in their agronomic fitness for practical breeding programme (Scarascia-mugnozza, 1968).

The dry and dormant seeds of the Soybean (*Glycine max* (L.) Mrr.) var. Co1. were obtained from Millet Breeding Station, Tamilnadu Agricultural University, Coimbatore. M₂ generation seeds were raised from M₁ generation, the seeds were collected from different individual mutagenic treatment. Seeds harvested from individual M₂ plants were grown as M₃ families in the field.

Was treated with physical and chemical mutagens like Gamma rays, Ethyl methane sulphonate and Diethyl sulphate. For inducing mutation, Soybean treated with various concentrations of gamma rays 10, 20, 30, 40, 50 and 60 KR, EMS (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %) and DES (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %). Two sets containing 200 healthy seeds were treated with Gamma rays, Ethyl methane sulphonate and Diethyl sulphate, to determine the LD50 value. The treated seeds were transferred to Petri dishes containing two layers of moist filter paper for germination. Ten Petri dishes of 20 seeds per treatment were sown and Percentage germination and seedling variations for each treatment were subsequently determined. The treated seeds were then subjected to germination test. Based on the reduced growth of germination to 50 per cent, LD50 value was determined. Three doses of gamma rays around LD50 were fixed for further studies. The physical treatments were induced at sugarcane breeding institute (ICAR), Coimbatore. The chemically treated seeds were presoaked in distilled water for 6hrs to ensure complete hydration of the seeds. The seeds were treated with solution of EMS, DES for duration of 6 hrs. The seeds subjected to treatment were sown in the field along with the control in a randomized block design with three replications. A total of 100 seeds were sown in each treatment. All the treatments including the control were raised adopting a spacing of 30 cm in between rows and 20 cm between plants. All recommended cultural operations namely, irrigation, weeding and plant protection methods were carried out during the crop growth period.

2.2. Field observations

2.2.1. Days to first flower (days)

The number of days taken from sowing to first flower was recorded and expressed as number of days to first flower.

2.2.2. Plant height (cm)

The height of the plant from the base to the top of the plant of maturity was measured and expressed in cm.

2.2.3. Number of branches per plant

Number of branches arising from the main stem were counted and recorded at the maturity.

2.2.4. Number of leaves per plant

The number of leaves was counter and recorded as the number of leaves per plants

2.2.5. Number of clusters per plant

Total number of clusters at maturity time were counted and recorded as the number of clusters per plant.

2.2.6. Number of pods per plant

Total number of pods at maturity time were counted and recorded as the number of pods per plant.

2.2.7. Number of seeds per plant

Total number of seeds from individual plant were counted and recorded as the number of seeds per plant.

2.2.8. Seed yield per plant

Seed yield was worked out by using digital electronic balance and expressed in grams.

2.2.9. Number of branches per plant

Total number of branches from individual plant were counted and recorded as the number of seeds per plant.

2.2.10. Number of leaves per plant

Total number of leaves from individual plant were counted and recorded as the number of seeds per plant.

2.3. Biochemical studies

2.3.1. Seed Protein content (%)

Two sets from the same treated plant of each M_2 and 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.

2.3.2. Seed oil content (%)

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

3. RESULT AND DISCUSSION

The present programme was under taken with soybean genotype variety co-I was subject to study the effect of chemical mutagens such as ethylmethane sulphonate and diethylsulphate. The aims to study the frequency, spectrum of chlorophyll mutants, viable mutants, morphological, yield and yield components, protein and oil contents in M_2 and M_3 generation.

Soybean is the self-pollinated crop, naturally genetic variability is limited. Flower size is very small, very difficult and costly hybridization so alternatively induced mutagenesis is the best method to enlarge genetic variability within short time. It has been found to be an efficient tool to induce variability for both micro and macro mutation in the self-pollinated crop and it is a supplement to conventional breeding methods (Brock, 1971). The previous workers was under taken to induced genetic variability and to screen useful mutants for their use in improvement of soybean (Rawlings *et al.*, 1958; Papa *et al.*, 1961; Hymowitz and Newell, 1981; Kawakita 1955; Hajduch *et al.*, 2000; Dhole *et al.*, 2003; Pavadai and Dhanavel, 2005; Pavadai, 2006 and Arulbalachandran 2006).

The production of higher frequencies cultivated crops to using mutagenesis. The breeding work can be provided by extensive collection of mutants based on productive characters to source of valuable materials. The different breeding objective could be made possible through induced mutagenesis, especially in grain legumes like soybean.

In Indian to successful for breeding programmes (Amarnath *et al.*, 1991; Balakrishna 1991; Geetha, 1994; Maheshwari *et al.* 2003; Dhole *et al.*, 2003 Jayamala, 2004; Sasi, 2004; Arulbalachandran, 2006; and Pavadai 2006) Pakistan (Inayatullah and Smyth, 1987; Sattar *et al.*, 1990), Brasil (Neto and Alves, 1997; Bione *et al.*, 2002) Spain (Velasco *et al.*, 1999) and China (Yang Shouping *et al.*, 1998).

Only very limited work has been carried out in cultivated soybean. However, much work has been done in other crop likes caster, linseed, peanut, rapseed, safflower, sesame, sunflower, cluster been, bhendi, black gram and green gram, etc. To understand the mutation process and to use the mutation breeding for crop improvement.

3.1. Mean performance

In the present investigation, the mean for different quantitative characters both the positive and negative direction due to mutagenic treatments.

The important growth parameters like number of leaves has been related to both biological and economic yield. The mean of the total number of leaves maximum at 0.5 per cent of EMS treatment. Similar results were also observed in soybean (Balakrishnan, 1991; Geetha, 1994; Padmavathi *et al.*, 1992; Cheng and Chandlee, 1999; Pavadai and Dhanavel 1994 & 1995; Pavadai 1996). Similar observations were made in other plants like black gram (Deepalakshmi and Anandakumar, 2004 and Arulbalachandran, 2006).

Plant height, number of branches per plant were recorded the positive shift, the maximum mean values were recorded at 50 KR gamma rays, 0.5 per cent of EMS and 0.04 per cent of DES treatment (Table- 1). Such observations were reported by previous workers in Soybean (Balakrishnan, 1991, Geetha, 1994; Cheng and Chandlee, 1999; Dhole *et al.*, 2003; Pavadai, 2006). Lentil (Dixt and Dubey, 1985), rice (Logaprakesh *et al.*, 1992) and sesame (Sengupta and Datta, 2004).

In the present study, the mean values recorded a negative and positive shift for the yield and yield components characters such as number of cluster per plant, number of pod per plant, number of seeds per plant and seed yield per plant. The maximum yield parameter were recorded at 50 KR gamma rays and 0.5 per cent of EMS treatments. Such as observation were reported by previous workers in soybean (Papa *et al.*, 1961; Balakrishnan, 1991; Geetha, 1994; Cheng and Chandlee, 1999; Dhole *et al.*, 2003; Pavadai and Dhanavel, 2004 and 2005; Pavadai, 2006). Similar observations were also made in other plants like black gram (Julite Hepziba and Subramanian, 2002; Arulbalachandran, 2006), Cowpea (Odeigah *et al.*, 1998), Okra (Ghai *et al.*, 2004) and Sesame (Sengupta and Datta, 2004).

The protein and oil content were observed in high mean values among the mutagenic treatments (Table- 17,19). Such observation were reported by some previous workers in soybean (Papa *et al.*, 1961; Balakrishnan 1991, Geetha, 1994; Kawakita, 1995; Hajduech *et al.*, 2000; Pavadai 2006), Chickpea (Abo-Hegazi, 1980) and French bean (Prasad and Jha, 1993).

4. CONCLUSION

In conclusion, the soybean variety co-1 responded more and more number of viable and economic mutants for higher frequency 50 KR gamma rays, 0.5 per cent EMS and 0.4 per cent DES treatments for more effective than the other mutagenic treatments.

The present investigation that the isolation of early maturity, high yield, protein content and oil content is possible in 50 KR gamma rays, 0.5 percent of EMS and 0.4 per cent of DES treatments.

Table 1. Effect of Gamma rays, EMS and DES on yield parameters and biochemical content of soybean in M₂ 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 content	Oil content
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
EMS 0.1%	35.90 ± 1.71	74.21 ± 1.52	20.19 ± 0.13	45.46 ± 2.71	9.31 ± 0.28	3.71 ± 0.21	56.19 ± 2.11	38.75 ± 0.27	18.95 ± 1.27
0.2%	36.37 ± 2.54	70.39 ± 2.39	17.51 ± 0.11	40.19 ± 3.15	10.56 ± 0.43	3.95 ± 0.17	50.85 ± 3.05	39.17 ± 0.43	20.11 ± 0.96
0.3%	37.51 ± 1.43	72.56 ± 3.41	18.38 ± 0.16	42.31 ± 2.22	12.15 ± 0.52	4.87 ± 0.15	54.32 ± 1.97	39.60 ± 0.15	20.34 ± 0.32
0.4%	33.27 ± 1.11	78.75 ± 2.15	21.39 ± 0.17	49.16 ± 2.43	13.06 ± 0.71	5.03 ± 0.12	62.19 ± 4.32	38.99 ± 0.27	20.25 ± 0.25
0.5%	34.71 ± 1.27	80.19 ± 4.17	24.54 ± 0.17	57.38 ± 3.15	14.17 ± 0.46	5.17 ± 0.14	60.56 ± 3.15	41.15 ± 0.41	21.47 ± 0.26
0.6%	32.80 ± 1.35	82.91 ± 3.22	26.32 ± 0.20	62.31 ± 3.71	12.54 ± 0.33	4.92 ± 0.11	67.38 ± 2.19	41.38 ± 0.52	21.85 ± 0.28
DES 0.1%	34.56 ± 2.04	69.54 ± 1.11	22.19 ± 0.09	57.37 ± 2.65	10.35 ± 0.27	4.03 ± 0.09	54.82 ± 3.16	40.46 ± 0.39	20.42 ± 0.18
0.2%	36.32 ± 1.96	70.28 ± 1.43	18.72 ± 0.14	40.38 ± 2.82	10.81 ± 0.54	5.21 ± 0.17	51.77 ± 2.38	39.14 ± 0.38	20.06 ± 0.35
0.3%	38.71 ± 1.37	73.19 ± 2.37	22.39 ± 0.14	43.14 ± 3.05	11.17 ± 0.97	5.38 ± 0.23	60.38 ± 1.81	39.35 ± 0.27	20.22 ± 0.29
0.4%	36.30 ± 1.41	76.35 ± 4.15	24.37 ± 0.13	52.82 ± 3.42	13.05 ± 1.11	5.53 ± 0.16	64.21 ± 3.41	40.19 ± 0.33	20.13 ± 0.38
0.5%	33.81 ± 2.06	80.64 ± 3.81	25.38 ± 0.15	59.17 ± 4.05	12.54 ± 1.05	5.17 ± 0.22	65.51 ± 2.56	41.32 ± 0.62	21.40 ± 0.16
0.6%	34.17 ± 1.85	76.38 ± 5.62	20.06 ± 0.11	60.35 ± 2.81	11.47 ± 1.02	4.28 ± 0.21	55.47 ± 3.15	40.42 ± 0.56	20.07 ± 0.42

Table 2. Effect of Gamma rays, EMS and DES on yield parameters and biochemical content of soybean in M₃ 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
Control	36.81 ± 1.43	70.75 ± 4.32	21.62±1.54	49.62±2.82	10.21±0.97	4.32±0.11	64.27±1.99	37.56±1.27	18.05±1.71
GAMMA RAYS 10KR	36.99 ± 1.25	75.66 ± 2.56	22.32±0.89	59.62±1.96	11.64±0.69	4.46±0.13	69.54±2.56	38.32±1.52	18.37±1.15
20KR	35.47 ± 1.08	76.43 ± 5.04	20.81±0.94	60.39±3.71	12.07±0.77	4.51±0.15	73.21±4.17	38.50±1.90	19.22±1.36
30KR	37.18 ± 0.69	74.41 ± 3.98	21.37±1.37	60.55±4.21	11.85±0.96	5.02±0.10	70.42±3.56	39.02±1.16	19.56±1.05
40KR	35.24 ± 0.78	80.71 ± 2.54	24.39±0.99	61.27±2.96	12.39±0.82	5.07±0.09	75.37±1.82	39.13±2.37	19.85±0.58
50KR	34.35 ± 1.14	81.02 ± 2.61	26.51±1.72	62.21±3.27	13.46±0.82	5.16±0.27	81.52±4.21	39.56±1.94	18.76±1.02
60KR	37.65 ± 1.22	62.89 ± 2.78	20.12± 1.01	50.29±3.11	12.55±1.27	5.02±0.16	61.32±2.37	38.06±2.07	18.22±0.89
EMS 0.1%	35.90 ± 1.71	74.21 ± 1.52	20.19 ± 0.13	45.46 ± 2.71	10.15 ± 0.41	4.45 ± 0.15	56.19 ± 2.11	38.75 ± 0.27	18.95 ± 1.27
0.2%	36.37 ± 2.54	70.39 ± 2.39	17.51 ± 0.11	40.19 ± 3.15	9.31 ± 0.28	3.71 ± 0.21	50.85 ± 3.05	39.17 ± 0.43	20.11 ± 0.96
0.3%	37.51 ± 1.43	72.56 ± 3.41	18.38 ± 0.16	42.31 ± 2.22	10.56 ± 0.43	3.95 ± 0.17	54.32 ± 1.97	39.60 ± 0.15	20.34 ± 0.32
0.4%	33.27 ± 1.11	78.75 ± 2.15	21.39 ± 0.17	49.16 ± 2.43	12.15 ± 0.52	4.87 ± 0.15	62.19 ± 4.32	38.99 ± 0.27	20.25 ± 0.25
0.5%	34.71 ± 1.27	80.19 ± 4.17	24.54 ± 0.17	57.38 ± 3.15	13.06 ± 0.71	5.03 ± 0.12	60.56 ± 3.15	41.15 ± 0.41	21.47 ± 0.26
0.6%	32.80 ± 1.35	82.91 ± 3.22	26.32 ± 0.20	62.31 ± 3.71	14.17 ± 0.46	5.17 ± 0.14	67.38 ± 2.19	41.38 ± 0.52	21.85 ± 0.28
DES 0.1%	34.56 ± 2.04	69.54 ± 1.11	22.19 ± 0.09	57.37 ± 2.65	12.54 ± 0.33	4.92 ± 0.11	54.82 ± 3.16	40.46 ± 0.39	20.42 ± 0.18
0.2%	36.32 ± 1.96	70.28 ± 1.43	18.72 ± 0.14	40.38 ± 2.82	10.35 ± 0.27	4.03 ± 0.09	51.77 ± 2.38	39.14 ± 0.38	20.06 ± 0.35
0.3%	38.71 ± 1.37	73.19 ± 2.37	22.39 ± 0.14	43.14 ± 3.05	10.81 ± 0.54	5.21 ± 0.17	60.38 ± 1.81	39.35 ± 0.27	20.22 ± 0.29
0.4%	33.81 ± 2.06	80.64 ± 3.81	25.38 ± 0.15	59.17 ± 4.05	13.05 ± 1.11	5.53 ± 0.16	65.51 ± 2.56	41.32 ± 0.62	21.40 ± 0.16
0.5%	36.30 ± 1.41	76.35 ± 4.15	24.37 ± 0.13	52.82 ± 3.42	11.17 ± 0.97	5.38 ± 0.23	64.21 ± 3.41	40.19 ± 0.33	20.13 ± 0.38
0.6%	34.17 ± 1.85	76.38 ± 5.62	20.06 ± 0.11	60.35 ± 2.81	12.54 ± 1.05	5.17 ± 0.22	55.47 ± 3.15	40.42 ± 0.56	20.07 ± 0.42

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