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Effect of Gamma Rays, EMS, DES and COH on Protein and Oil Content in Soybean

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Abstract

The present study is based on the observations of seed protein and oil content in four mutagenic generations of soybean variety CO 1. Ethyl methane sulphonate treatment showed high protein and oil content compared to other mutagenic treatments such as gamma rays, diethyl sulphate and colchicine. Both increased and decreased content of protein and oil were observed. According to our results high content of protein and oil was observed at high mutagenic treatments. Increased level of seed protein and oil content was observed at 0.5% and 0.6% of EMS treatments and 50 KR of gamma rays.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is the most economically important grain legume grown in the world which yields a good amount of profit in terms of total production and international trade. Soybean proteins are considered to be one of the most important components not only for human nutrition but they are utilized also in animal production.

Soybeans were developed in two main products of the seed oil and protein containing defatted meal. In the world production of protein meal, the soybean is in first place, followed by rapeseed, cottonseed and sunflower seed (Foreign Agricultural Service, 1996). In India, soybean ranks third in oil seeds after groundnut and rapeseed/mustard. Soybean is considered to be the most economical and valuable agricultural commodity as, it has good adaptability towards a wide range of soil and climate. On an average dry matter basis, soybean contains about 40% protein and 20% oil. Soybean is very nutritious, protein rich and oil components in soybean are not only in high quantity but also in good quality. Soy oil contains high proportion of unsaturated fatty acids, so it is considered to be healthy oil. The present programme was therefore, undertaken to induce genetic variability and to screen useful mutants for their use in improvement of soybean. Genotype differences exist in the response of plants to different physical and chemical mutagens. Gamma rays, EMS, DES and Colchicine differ in their relative capacity to induce mutation in crop plants.

2. Materials and Methods

The dry seeds of soybean variety CO 1 were subjected to both physical and chemical mutagen. The mutagens namely gamma rays, EMS, DES and COH were given at six different dose/concentrations. The seeds were treated with gamma rays (10, 20, 30, 40, 50 and 60 KR), EMS (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%), DES (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) and COH (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%). The physical treatments were induced at sugarcane breeding institute (ICAR), Coimbatore. The chemically treated seeds were presoaked in distilled water for 6 hrs to ensure complete hydration of the seeds. The seeds were treated with solution of EMS, DES and COH 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 seed 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. The data were recorded till four generations.

Protein content

Two seeds from the same plant of each M_1, M_2, M_3 and M_4 plants were separately dehulled 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 colorimetrically according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Oil content

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

About 50 g of seed was dried in a drying dish at 130°C for 20 min. in a forced draft oven. Then they were cooled to room temperature and passed through the nut slicer to slice the nuts. The sliced samples were mixed well and accurately 2 g of the sample was taken in to a filter paper fold.

The filter paper was folded in such a way to hold the seed meal. A second filter paper was used to wrap around the seed which was left open at the top like a thimble. The sample packet was placed in the butt tubes of the Soxhlet extraction apparatus. Extraction was done with petroleum ether (150 drops min-1) for 6 hrs without interruption by gentle heating. Then the extraction flask was dismantled after cooling and then ether was evaporated on a water bath until no odor of ether remained. The dirt or moisture out side the flask was carefully removed and the flask was weighed. The heating was repeated to get constant weight

3. Results and Discussion

The protein and oil content were observed in high mean values for among the dose/concentrations of all mutagenic treatments (Tables 1&2).

Table 1. Effect of mutagens on protein content in soybean var. CO 1

	M_1	M_2	M_3	M_4
Control	38.72 ± 2.10	38.96 ± 1.85	39.15 ± 2.27	39.46 ± 2.74
Gamma rays 10 kR	38.56 ± 1.71	37.71 ± 1.52	38.71 ± 1.96	38.23 ± 2.71
20 kR	39.17 ± 1.35	37.96 ± 1.42	39.27 ± 2.71	37.56 ± 1.75
30 kR	39.43 ± 2.07	38.27 ± 1.37	39.56 ± 2.52	39.27 ± 1.32
40 kR	40.22 ± 1.56	39.56 ± 1.85	40.79 ± 3.04	40.66 ± 3.06
50 kR	40.73 ± 2.07	40.27 ± 1.38	40.82 ± 3.37	41.95 ± 3.17
60 kR	40.56 ± 1.96	40.59 ± 1.74	41.01 ± 3.21	41.87 ± 1.95
EMS 0.1	38.75 ± 1.41	38.32 ± 1.53	38.75 ± 2.95	38.65 ± 1.76
0.2	39.14 ± 1.56	37.43 ± 1.77	39.17 ± 2.71	39.27 ± 1.44
0.3	39.25 ± 2.22	39.15 ± 1.39	40.32 ± 2.62	40.36 ± 2.27
0.4	39.46 ± 6.81	39.22 ± 1.38	39.62 ± 2.70	40.17 ± 2.95
0.5	41.32 ± 1.96	40.19 ± 2.60	41.96 ± 3.27	42.06 ± 1.81
0.6	40.85 ± 1.56	40.75 ± 2.35	41.71 ± 3.09	41.82 ± 1.77
DES 0.01	37.52 ± 1.11	37.22 ± 2.34	37.63 ± 3.11	40.27 ± 1.41
0.02	36.39 ± 2.05	38.06 ± 1.92	38.19 ± 3.22	38.75 ± 1.38
0.03	37.18 ± 2.75	38.15 ± 1.42	38.75 ± 1.96	39.21 ± 1.75
0.04	38.85 ± 1.98	39.27 ± 1.95	39.58 ± 1.75	38.84 ± 1.96
0.05	39.04 ± 2.23	40.55 ± 1.84	40.01 ± 2.08	41.76 ± 1.33
0.06	38.38 ± 3.06	40.37 ± 1.72	39.52 ± 2.19	40.47 ± 1.25
COH 0.01	37.19 ± 1.97	37.79 ± 1.60	38.18 ± 1.41	37.76 ± 1.71
0.02	38.25 ± 1.75	38.73 ± 1.39	39.25 ± 1.72	38.85 ± 1.62
0.03	39.27 ± 1.86	38.19 ± 1.25	38.37 ± 1.93	39.27 ± 1.54
0.04	39.30 ± 1.16	39.25 ± 1.33	39.49 ± 1.41	38.72 ± 1.37
0.05	38.85 ± 1.52	40.32 ± 1.74	39.75 ± 1.56	39.61 ± 1.28
0.06	37.56 ± 1.38	38.37 ± 1.38	38.59 ± 1.71	39.40 ± 1.71

Table 2. Effect of mutagens on oil content in soybean var. CO 1

	M_1	M_2	M_3	M_4
Control	18.27 ± 1.17	18.46 ± 1.10	18.56 ± 1.14	19.01 ± 1.10
Gamma rays 10 kR	17.11 ± 1.56	17.33 ± 1.52	18.32 ± 0.98	19.56 ± 0.97
20 kR	17.37 ± 0.76	18.27 ± 1.44	19.03 ± 0.95	18.32 ± 0.85
30 kR	18.05 ± 0.92	19.17 ± 1.37	19.25 ± 0.76	18.71 ± 0.81
40 kR	18.21 ± 0.82	19.39 ± 0.92	19.27 ± 1.05	19.81 ± 0.96
50 kR	19.37 ± 1.02	20.32 ± 0.89	21.46 ± 1.13	22.34 ± 0.92
60 kR	18.22 ± 1.05	19.71 ± 0.85	20.37 ± 0.98	21.71 ± 1.04
EMS 0.1	17.15 ± 1.17	18.35 ± 1.11	17.92 ± 1.32	17.71 ± 0.96
0.2	17.63 ± 0.98	18.72 ± 1.03	18.41 ± 1.27	18.27 ± 0.82
0.3	18.39 ± 0.75	19.07 ± 1.04	19.05 ± 1.15	19.16 ± 1.11
0.4	18.32 ± 0.81	19.54 ± 1.25	18.51 ± 1.41	18.46 ± 1.30
0.5	20.27 ± 1.06	20.65 ± 1.71	20.46 ± 0.98	22.52 ± 0.71
0.6	19.75 ± 1.08	20.17 ± 0.98	20.42 ± 0.77	19.12 ± 0.52
DES 0.01	18.06 ± 1.09	17.32 ± 0.97	17.62 ± 0.82	17.93 ± 0.82
0.02	17.32 ± 1.32	18.19 ± 1.04	18.39 ± 0.85	18.25 ± 0.75
0.03	17.92 ± 0.99	19.06 ± 1.17	19.04 ± 0.82	19.04 ± 0.82
0.04	18.39 ± 0.89	19.64 ± 1.05	19.41 ± 0.79	19.64 ± 0.79
0.05	19.04 ± 0.92	18.39 ± 1.22	18.96 ± 0.81	19.81 ± 0.96
0.06	18.55 ± 0.63	18.75 ± 1.19	18.00 ± 0.97	18.56 ± 0.82
COH 0.01	19.37 ± 1.02	17.92 ± 1.17	17.63 ± 1.05	18.32 ± 0.91
0.02	18.91 ± 1.15	17.36 ± 1.05	18.21 ± 1.17	18.16 ± 0.87
0.03	19.05 ± 1.20	18.19 ± 1.04	18.35 ± 1.15	18.71 ± 0.86
0.04	18.87 ± 0.98	19.05 ± 0.98	18.75 ± 1.30	18.42 ± 0.79
0.05	19.22 ± 0.97	19.27 ± 0.97	17.63 ± 1.35	19.17 ± 0.82
0.06	18.39 ± 1.05	18.32 ± 0.95	18.11 ± 1.28	18.37 ± 0.93

Such observation were reported by some previous workers in soybean (Byun *et al.,* 1994; Meinke *et al.,* 1981; Rahman *et al.,* 1994; Hajduch *et al.,* 2000a). Similar observations were made in other plants like chickpea (Abo-Hegazi, 1980), french bean (Prasad and Jha, 1993) and black gram (Arulbalachandran and Mullainathan, 2009). Srinivasachar *et al.* (1972) reported the high yielding mutations with high oil content of superior quality in the M_2 generation of gamma irradiated and EMS treated plants. Syed *et al.* (1994) observed that the high oil content of *Brassica napus* with the treatment of gamma rays, EMS and SA. Hajduch *et al.* (2000b) reported that the soybean seeds treated with 1mM sodium azide solution in M_3 generation were significantly found to increase by mutagenic treatment when compared with control examined for variation in seed protein composition amino acid content, the genetic variability of seed protein composition.

In our experiments, more amounts of protein and oil content were recorded in EMS treatment than other chemical and physical mutagens. In general both the positive and negative shift was observed in most of the mutagenic treatments. The highest protein and oil content were observed at 0.5% EMS treatments and 50 KR gamma rays treatments than the other mutagenic treatments.

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