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INDUCTION OF MUTATION BY GAMMA IRRADIATION AND EMS IN *CAJANUS CAJAN*. L.

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ABSTRACT:
The plants of *Cajanus cajan* were grown in plots from seeds obtained from Krishi Vigyan Kendra, Gondia. The well developed seeds of same age will be selected and will be subjected to gamma irradiation doses. Prior to the mutagenic treatments, all genotypes were grown for one generation to ensure their homozygosity. The well developed seeds of same age were selected and subjected to gamma irradiation doses of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 and 1200 Gy (Gray) (40 seeds for each treatment in four genotypes). Gamma irradiation will be carried out at at room temperature (22- 25°C) in a Cobalt 60 gamma cell-220 of 381.43 curie strength delivering 29kR/hr at the time of irradiation. For EMS treatment, the seeds were presoaked in sterile distilled water for 6 hours and then treated with different concentrations of EMS (10, 20, 30 and 40mM) for 8 hours. The mutagen treated and control (untreated) seeds were sown in the field in randomized block design (RDB), with three replications. The seeds of all individual M, plants were harvested separately and they were sown in the field during the next Kharif to rise M_1 generation.

Key words: - *Cajanus cajan*, Gamma irradiation, EMS.

INTRODUCTION:

Introduction

Cajanus cajan L. (Millsp), commonly known as Pigeonpea or Red gram, is an important legume crop widely used as food and fodder and is a major source of vegetable protein. Pigeonpea is an economic source of not only protein but of carbohydrate, minerals and B-complex vitamins particularly in vegetarian diet (Salunkhe et al., 1985). Pigeonpea, being a self-pollinated crop, the available genetic variability has been almost exploited for improvement by conventional breeding methods. Therefore it becomes necessary to creat genetic variability through induced mutations. Induced mutagenesis has been used very widely in crop plants to creat genetic variability in traits of economic value. Chlorophyll mutations are one of the important criteria to determine effectiveness of the mutagens. According to Mille(1968), in spite of impaired seed production, the chlorophyll mutants are potentially useful in understanding of different physiological functions, various biochemical reactions and pathological invasion. Although mutagens bring about changes in nucleotide sequence of DNA, the mode of action of each mutagen is distinct. More ever, a mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality or sterility may decrease its efficiency. Thus in order to exploit induced mutagenesis for crop improvement, the basic studied on effectiveness and efficiency of a mutagen in a crop are necessary (Badere and Chaudhary, 2007). The literature reveals that the mutational work on pigeonpea has been scanty. In the present investigation, efforts were made to assess the effect of different concentrations of EMS and different doses of gamma irradiation on Pigeonpea to find out

effective concentration, which induces desirable mutations.

In the last decade, gamma irradiation has been drawn the attention as a new and rapid method to improve the qualitative and quantitative characters of many crops. Previous studies have shown relatively low doses ionising radiation on plants and microorganisms are manifested as accelerated cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (Chakravarthy and Sen, 2001). Inhibition of seed germination, shoot and root elongation have been reported for detection of irradiated cereal grains and legumes. Chaudhuri (2002) reported that the irradiation of wheat seeds reduced shoot and root lengths upon germination. Gamma radiation can be useful for the alteration of physiological characters (Kiong *et al* 2008). The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kova'cs and Keresztes 2002.)

MATERIAL & METHODS:

Variety used

Pure line seeds of local variety of *Cajanus cajan* L. were used in the present study. The certified, healthy and dry seeds (10% moisture content) of this variety were procured from Krishi Vigyan Kendra, Gondia. This variety well adapted to the agro climatic conditions.

Mutagens used

The seeds of pigeonpea were treated with different doses / treatments of physical and chemical mutagens. The physical mutagen used was gamma rays and chemical mutagens included Ethyl Methane Sulphonate (EMS)

Gamma rays

Prior to the mutagenic treatments, all genotypes, were grown for one generation to ensure their homozygosity. Uniform healthy dry seeds (10% moisture content) of the Pigeonpea were exposed to different doses of gamma rays (100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy, 900Gy, 1000Gy, 1100Gy and 1200Gy) with a dose rate of 14.5 Kr/hr. from 60 Cobalt source at the Department of Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

Ethyl methane sulphonate [EMS (CH3 OSO2 C2H5)]

Germplasm of the experimental plant material, pigeonpea were procured from Krishi Vigyan Kendra, Gondia (Maharashtra state). The seeds were presoaked in sterile distilled water for 6 hours and then treated with different concentration of EMS (10, 20, 30, and 40mM for 8 hours. The mutagen treated and control (Untreated) seeds were sown in field in randomized block design (RDB), with three replications. The seeds of all indivisual M plants were harvested separately and they were sown in the field during the next Kharif to rise M_1 generation. Mutagenic effect was assessed using parameters like seed germination, plant survival, pollen sterility, pod length, grain yield, branching pattern and chlorophyll mutants. The treated as well as control seeds after germination were carefully screened for mutation in M_1 generation.

Method of treatment with chemical mutagen

Prior to the mutagenic treatment, the seeds were presoaked in distilled water for a period of 12hr. Seed presoaking allows the cells to reach a metabolic state when they are relatively more sensitive to mutagenic action. The control seeds were also immersed in distilled water for the same duration. Thus the control seeds although not treated with the chemical mutagens, were exposed to similar physiological conditions before sowing as that of treated seeds. The seeds were given

intermittent shaking throughout the treatment period (6hr) to provide sufficient aeration. For uniform absorption, large quantities of mutagenic solution, approximately three times the volume of seeds (Konzak *et al*., 1965) were used. After the completion of treatment period, the seeds were thoroughly washed in running tap water for 20min to remove the excess chemicals from the seed surface before they were sown in the field.

Sample size

A set of 150 seeds were chosen for each dose/treatment including the control. Out of these 150 seeds, 100 seeds for each treatment and control were sown in the field for morphological and cytological studies, whereas the remaining set of 50seeds was allowed to germinate on moist cotton in Petriplates for measuring root-shoot length.

Sowing of seeds in the field

Nursery beds were prepared for sowing seeds and raising M_1 generation. In January, 2014, the treated as well as untreated (control) seeds were sown in three replicates in a Complete Randomized Block Design (CRBD) at the Bhawabhuti Mahavidyalaya, Amgaon. The distance between the seeds along a row was kept 5cm whereas row to row distance was maintained at 10cm in each experimental plot in a replication.

Table 1: Details of Mutagenic Treatment given to Pigeonpea seeds

Mutagen used	Dose/ Concentration	Duration of Presoaking (Hrs.)	Duration of Treatment (Hrs.)
Control	DDW	6.0	-
Gamma rays (GY)	100	-	-
	200	-	-
	300	-	-
	400	-	-
	500	-	-
	600	-	-
	700	-	-
	800	-	-
	900	-	-
	1000	-	-
	1100	-	-
	1200	-	-
EMS (%)	0.1	6.0	8.0
	0.2	6.0	8.0
	0.3	6.0	8.0
	0.4	6.0	8.0

Mechanism of action of physical and chemical mutagens

a) Physical mutagens

Gamma rays are the most energetic form of electromagnetic radiation, possessing the energy level from 10 kilo electron volts (kev) to several hundred kev, and they are considered the most penetrating in comparison to other radiation such as alpha and beta rays (Kovacs and Keresztes, 2002). The common sources of gamma rays are 137 Cs (half-life 30 years, energy 0.66 Mev) and 60 Co (half-life 53 years, energies 1.33 Mev, 1.17 Mev). Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes,

alteration in photosynthesis, modification of the anti-oxidative system and accumulation of phenolic compounds (Kim *et al*., 2004, Wi *et al*., 2005). The biological effects produced by a radiation depend upon the total amount of radiation energy absorbed or delivered in an organism. The dose of radiation is measured as the amount of energy absorbed per unit mass of the irradiated object and is commonly expressed as rad. One rad equals 100 erg/g or 10 -2 j/kg of irradiated object. The unit of radiation dose is called Gray (Gy). Gy equals 100 rad or 1 J/kg of the irradiated object.

b) Chemical mutagen

Ethyl methane sulphonate, a mono functional alkylating agent is a colourless liquid soluble in water with a boiling point 85.86 o C/10mm Hg. In the action of EMS on biological systems, triester is the main target. It has been established by many workers that primary reaction centres in the DNA are the alkylation of its phosphate groups. The resulting triester is unstable and tends to lose the alkyl group. The unstable phosphate triester can hydrolyse between the phosphate and deoxyribose resulting in the backbone breakage. EMS is known to induce alkylation of purine and pyrimidine bases. Alkylation is thought to occur most readily with guanine at position N7 followed by Adenine at N3 and rarely with N in cytosine. However, no reaction with thymine has been detected. Alkylation of the nitrogenous ring ultimately leads to the removal of the alkylated base i.e., depurination or depyrimidation and may lead to back bone breaks. EMS is also known to induce transitions e.g., alkylation of guanine results in the formation of o 6 -ethyl guanine, which can pair with thymine (T) but not with cytosine (C). Through subsequent DNA repair, the original G/C pair can then be replaced with A/T (Greene *et al*., 2003). In majority of cases (99%), EMS induces C-to-T changes resulting in C/G to T/A substitutions (Kreig, 1963). The mutagenicity is mediated through the production of an organic metabolite of azide compound (Owais and Klienhofs, 1988).This metabolite enters into the nucleus, interacts with DNA and creates point mutations in the genome. These effects, caused by NaN3, together may hamper ATP biosynthesis resulting in decreased availability of ATP molecule which may slow the germination rate and reduce the germination percentage.

Evaluation of M1 generation

Seed germination

The data on seed germination was recorded right from the emergence of first shoot in each treatment including control. After recording the data, percentage of seed germination was calculated by using the formula

$$\text{Germination (\%)} = \frac{\text{No.of seeds germinated}}{\text{No.of seeds sown}} \times 100$$

Seedling height (cm)

Seedling height was estimated on 9 th day of germination by measuring root and shoot lengths of 15 randomly selected seedlings from each treatment as well as control. Seedling injury, as measured by the reduction in root and shoot length and calculated in terms of percentage of root and shoot injury.

$$\text{Percent injury} = \frac{\text{Control-Treated}}{\text{Control}} \times 100$$

Plant survival

The surviving plants in different treatments were counted at the time of maturity and the survival percentage and percent lethality were calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100$$

Lethality (%) = $\frac{\text{Control-Treated}}{\text{Control}} \times 100$

Quantitative characters of M1 generation

The following morphological parameters were recorded in M1 generation

i) Plant height (cm): The height of 15 randomly selected plants was measured from the point above the ground to the tip of the main axis of the plant.

ii) Number of pods per plant: Total number of pods per plant for a selected number of 15 plants from each concentration including control was recorded.

iii) Length of pods per plant (cm): The length of five pods per plant from 15 randomly selected plants in each treatment including control was recorded.

iv) Number of seeds per pod: Five pods per plant from 15 randomly selected plants in each treatment were used to calculate the mean seeds per pod.

v) Seed yield per plant (g): Randomly selected 15 plants per treatment were used for calculating the mean seed yield per plant.

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Effect of Gamma irradiation and EMS treatment on Seed Germination and Plant Survival.

The mutagenic effects of gamma rays, EMS were studied on seed germination, seedling height, plant survival and various quantitative characters in M1 generation of pigeonpea.

Seed germination

Germination percentage was found to be significantly reduced in all the mutagenic treatments. The maximum inhibition in germination was recorded at higher treatments of all mutagens. Seed germination was about 96% in control. In gamma rays it ranged from 84% (100Gy) to 9% (900Gy). In case of EMS treatments it ranged from 68% (10mM) to 48% (40mM). The pooled mean values showed that gamma rays were most effective in reducing the seed germination followed by EMS.

Plant survival

Plant survival was higher in control (95.20%) than in all the three mutagenic treatments (Table 2). Plant survival tended to decrease with the increase in the dose / concentration of mutagens. In case of gamma rays highest lethality was observed at 900Gy (9%). In EMS treatments maximum lethality was noticed at 40mM concentration (48%).

Seedling height

Data recorded on seedling height measured in terms of root+shoot length is presented in table 4. It is evident from that seedling height decreased with an increase in dose / concentration of mutagens. Control populations exhibited the highest seedling height of 17.82cm. Among the mutagenic treatments maximum injury was recorded in 900Gy treatment (9%). The pooled mean values for seedling height and percent injury indicated that gamma rays were more effective followed by EMS treatments.

Table 2: Effect of gamma rays on Percent Seed germination, Pollen sterility and plant survival in M1 generation in *Cajanus cajan* L.

Treatments	Germination (%)	Plant Survival (%)	Lethality (L)	Pollen sterility%
Control	96.00	95.20	-	0.00
Gamma rays				
100 Gy	84.00	90.14	9.86	4.59
200 Gy	76.00	88.40	11.6	7.11
300 Gy	68.00	86.18	13.82	9.00
400 Gy	49.00	76.52	23.48	30.68
500Gy	38.00	66.23	33.77	39.00
600 Gy	31.00	52.12	47.88	46.00
700 Gy	26.00	40.21	59.79	62.16
800 Gy	17.00	29.54	70.46	69.11
900 Gy	9.00	18.68	81.32	78.55
Mean	44.22	60.89	39.10	38.46

Table 3: Effect of EMS on Percent Seed germination, Pollen sterility and plant survival in M1 generation in *Cajanus cajan* L.

Treatments	Germination(%)	Plant Survival(%)	Lethality (L)	Pollen sterility%
Control	96.00	95.20	-	
EMS				
10mM	68.00	86.66	13.34	5.28
20mM	60.00	75	25	10.21
30mM	57.00	71.43	28.57	9.22
40mM	48.00	60	40	16.66
Mean	58.25	73.27	21.72	10.34

Table 4: Effect of Gamma Radiation on Shoot Length, Root Length and Number of Lateral Roots of *Cajanus cajan* (L) Millsp. Seedlings

Radiation Doses	Shoot Length	Root Length	Total Seedling Length	No. of Lateral Roots
Control	7.69	10.13	17.82	14.12
100 Gy	7. 67	10.10	17.77	13.23
200 Gy	7.66	9.92	17.58	9.43
300 Gy	7.60	9.33	16.93	8.96
400 Gy	7.52	8.14	15.66	8.44
500Gy	7.44	6.92	14.36	7.23
600 Gy	5.56	3.51	9.07	7.13
700 Gy	5.12	2.22	7.34	6.56
800 Gy	4.32	2.12	6.44	4.22
900 Gy	4.11	2.00	6.11	3.36

Table 5: Effect of EMS on Shoot Length, Root Length and Number of Lateral Roots of *Cajanus cajan* (L) Millsp. Seedlings

EMS Doses	Shoot Length	Root Length	Total Seedling Length	No. of Lateral Roots
Control	7.69	13.13	20.82	14.12
10mM	6.22	11.13	17.35	12.12
20mM	6.12	7.92	14.04	9.21
30mM	5.59	7.33	12.92	8.56
40mM	5.23	6.14	11.37	8.41

Studies on quantitative characters

i) Plant height (cm)

A progressive decrease in plant height was observed with an increase in concentration of Gamma irradiation. The plant height at 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy and 900Gy was 106.6cm, 106.1 cm, 106.00 cm, 101.8 cm, 100.6 cm, 100.2 cm, 98.0 cm, 96.2 cm, 96.00 cm and 94.21cm respectively as against 106.7cm in control. The plant height at 10mM, 20mM, 30mM and 40mM of EMS was 106.00cm, 108.00 cm, 106.00 cm and 105.00 cm respectively. The plant height is found maximum in 20mM concentration of EMS. i.e 108 cm. (Table 5 and 6).

ii) Number of pods/plant

A progressive decrease in No. of pods per plant was observed with an increase in concentration of Gamma irradiation. The No. of pods per plant at 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy and 900Gy were 185, 180, 177, 175, 170, 151, 150, 150, and 150 respectively as against 187 in control. The No. of pods per plant at 10mM, 20mM, 30mM and 40mM of EMS were 184, 187, 180 and 178 respectively. No. of pods per plant are found equals to control at 20mM concentration of EMS. i.e. 187. (Table 5 and 6).

iii) Pod length (cm)

The Pod length of different concentration of Gamma irradiation at 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy and 900Gy was 5.6 cm, 5.6 cm, 5.6 cm, 5.5 cm, 5.5 cm, 5.5 cm, 5.5 cm, 5.4 cm, 5.3 cm and 5.3 cm respectively as against 5.6 cm in control. The maximum pod length was found at 100Gy and 200Gy i.e 5.6 cm as in control. The Pod length at 10mM, 20mM, 30mM and 40mM of EMS treatment was 5.6 cm, 5.6 cm, 5.50 cm and 5.5 cm respectively. The Pod

length is found maximum in 20mM and 20 mM concentration of EMS. i.e 5.6cm. as in control. (Table 5 and 6).

iv) Number of seeds per pod

The No. of seeds per pod of different concentration of Gamma irradiation at 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy and 900Gy was 6, 6, 5, 6, 5, 6, 6, 5, 6 and 5 respectively as against 6 in control. The pod length is found 6 at 100Gy, 200Gy, 400Gy, 600Gy, 700Gy and 800Gy i.e 6 no. as in control. The No. of seeds per pod of EMS treatment was 6 No. in all the concentration of EMS. (Table 5 and 6).

v) Seed yield per plant (g)

The Seed yield per plant at 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy and 900Gy were 150.96 gm, 146.88 gm, 144.43 gm, 119.00 gm, 138.72 gm, 123.21 gm, 102.00 gm, 122.4 gm, and 102.00 gm respectively as against 152.89 gm in control. The Seed yield per plant at 10mM, 20mM, 30mM and 40mM of EMS treatment were 150.90gm, 152.80gm, 146.50gm and 120 gm respectively. (Table 5 and 6).

Table 5: Effect of Different doses of gamma irradiation on Plant height, No. of pods per planty, Pod length, No. of seeds per pod and seed yield per plant.

Doses/ Concentration	Plant Height (cm)	No. of pods per plant	Pod length(cm)	No. of seeds per pod	Seed yield per plant (g)
Control	106.7	187	5.6	6	152.89
100 Gy	106.6	185	5.6	6	150.96
200 Gy	106.1	180	5.6	5	146.88
300 Gy	106.00	177	5.5	6	144.43
400 Gy	101.8	175	5.5	5	119.00
500Gy	100.6	170	5.5	6	138.72
600 Gy	100.2	151	5.5	6	123.21
700 Gy	98.00	150	5.4	5	102.00
800 Gy	96.2	150	5.3	6	122.4
900 Gy	96.00	150	5.3	5	102.00
Mean	94.21	140.46	5.28	5.33	99.37

Table 6: Effect of Different EMS on Plant height, No. of pods per plant, Pod length, No. of seeds per pod and seed yield per plant.

Doses/ Concentration	Plant Height (cm)	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Seed yield per plant (g)
Control	106.75	187	5.6	6	152.89
10 mM	106.00	184	5.6	6	150.90
20 mM	108.00	187	5.6	6	152.80
30 mM	106.00	180	5.5	6	146.50
400 mM	105.00	178	5.5	6	120.00
Mean	105.3	176.6	5.47	6	123.56

RESULT & DISCUSSION:

Percent seed germination decreased with an increase in concentration/dose of mutagen in both cultivars in M1 generation (Table-2). The decrease in germination was more conspicuous with gamma radiation treatment than that of EMS. The germination for control was 96%. The seed germination decreased from 96% to 9% by treatment of gamma irradiation. The maximum decrease in seed germination was observed in with 900Gy Gamma irradiation and 40mM EMS treatment. The result also shows that 100Gy dose of gamma was less toxic to seed germination. The sensitivity of pigeon pea may be due to metabolic processes affected at embryonic level reported by Ashri and Herzog (1972).Similar inhibitory effects on seed germination observed

by Mundhe & Borse (2012), and Khan & Wani (2006).

Pollen sterility in M1 generation is the first sign of genetic effectiveness of the treatment. Pollen sterility increased with increase in concentration /dose of the mutagens. EMS treatment induced higher pollen sterility than the gamma radiation. The highest pollen sterility in present investigation was 10.34% with 40mM treatment. Lowest pollen sterility was recorded at 100 Gy and 10mM treatment of Gamma irradiation and EMS respectively. The result agreed to Barshile *et.al* (2006) in Chickpea employing SA, EMS and Gamma rays.

The percentage of survival a maturity decreased with increased concentration/dose of mutagens (Table-2).

The lowest percent survivals is found in 900Gy treatment of Gamma irradiation (60.89%) and 40mM treatment of EMS (73.27). The Gamma irradiation was more effective than EMS. The decrease in survival of plant at maturity is due to rapid injection of chemical mutagen and their ability to produce chromosomal abstractions (Sharma *et.al,* 2005). Similar results were also obtained by Bashir *et al*, 2013 in Fenugreek. Kulkarni and Mogle, 2013 in Horse gram, Sangale *et al*, 2011 and Giri and Apparao, 2011 in Pigeonpea. Gamma irradiation had some effect on germination frequency of irradiated pigeon pea seeds. Germination frequency was decreased significantly after higher irradiation doses ranging from 300Kr to 900Kr. Germination frequency was not much affected to seeds irradiated with 100Kr and control. Highest germination percentage was observed in control plants (Table 2) (96). Maximum decrease in percentage was observed in 900Kr irradiated plantlets (9). These records were in accordance with the result obtained by Amjad *et al* (2008) with chickpea. The results of Kiong *et al* (2008) shown that survival of plants to maturity depends on the damage nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germination and reduction in plant growth and survival. Changes in the germination percentage were found to attribute to gamma rays treatments. The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seed irradiated (Abdel-Hady *et.al.*, 2008). Shoot length decreased in all doses of irradiation as compared to non irradiated. Maximum decrease was observed after 20kr dose (Amjad *et.al* 2008). In the present study, the variability as measured by mean values of the root/shoot lengths decreased with increase in the radiation dose. Choudhari (2002) reported that when radiation is sufficient to reduce the rooting percentages, then the root lengths do not exceed a few millimetres in length. Due to metabolic disorders in the seeds after gamma irradiation, the seeds are unable to germinate.

CONCLUSION

The present investigation was conducted to study the mutagenic effect of gamma rays, EMS in the local variety of pigeon pea. The main objective of the study was to induce the genetic variability in quantitative traits and to isolate the promising mutants associated with increase in yield potential of the crop. The significant findings are summarized as follows:

The mutagenic effect studied on M1 parameters included seed germination, Seedling height, plant survival, pollen fertility, and various Quantitative traits.

a) Seed germination, seedling growth, plant survival and pollen fertility decreased with an increase in mutagenic treatment.

c) Gamma rays proved to be most effective in causing maximum biological damage. The order of effectiveness was gamma rays > EMS.

d) Studies on various quantitative parameters showed the inhibitory effect of higher treatments and stimulatory effect of lower or intermediate treatments in M1 generation.

e) The mean values for various quantitative traits decreased at higher treatments, but stimulatory effects were noticed at some lower treatments.

f) A significant amount of variability was induced in the treated populations as compared to Control.

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