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Effect of Physical and Chemical Mutagenesis on Grains Germination Studies, Effectiveness and Efficiency of Maize (corn) Zea Mays (L.)

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Abstract: In the present study was undertaken to investigate the nature of induced genetic variability in Zea mays (L.) variety sugar 75 were subjected to physical and chemical mutagenic treatments for two generations. The physical and chemical mutagens namely, gamma rays and DES (Diehthyl sulphate) was used. The mutagenic treatment seeds were tested for lethal dose 50 per cent for all mutagens, separately and the dose at which 50 per cent of the seed germination was considered as LD_{50} values, separately mutagenic seeds were grown in the field. In Gamma rays was found to be more effective and efficient than the other mutagens. The effectiveness was producing germination, morphological and viable mutants. The efficiency was observed based on the lethality and injury while the mutagenic effectiveness and efficiency generally decreased with increased in the higher doses of the mutagens in certain level of dose/concentrations.

Keywords: Zea mays, LD_{50}, Gamma rays, DES, Mutagenesis, Effectiveness and Efficiency.

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1. Introduction

Mutations are heritable changes in the phenotypes of organisms. These changes are the results of chemical changes at the level of genes. Such changes are capable of bringing about new and heritable character variations in crop plants and such variations can be selected and used for the establishment of crop varieties with new characters. Mutations occur in nature in very low frequency. Such mutations are called spontaneous mutations. However, the frequency of mutations can be increased with the help of certain chemical or physical agents that are called mutagens or mutagenic agents and mutations induced in this way are called induced mutations. Such agents can be used to induce mutations in crop plants and the desirable variations produced in this way can be selected. This approach of plant breeding in which new variations of crops with desirable characters are developed with the help of induced mutations is called mutation breeding.

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular biology etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement [1] and an efficient means supplementing existing germplasm for cultivar improvement in breeding program's [2]. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops [3]. These mutations provide beneficial variation for practical plant breeding purpose. During the fast seven decades, more than 2252 mutant varieties

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have been officially released in world [4]. Chromosome number of maize is $2n = 20$. Corn belongs to the grass family and is a cross-pollinated, monoecious plant in which the male and female flowers are located in different inflorescences on the same stalk. Maize is a tall, annual grass with overlapping sheaths and broad conspicuously distichous blades. There is also a growing body of information on maize epigenetics, extensive DNA methylation and histone profiling maps are available [5, 6]. Maize is chief used as a food for man and livestock. The grain is very nutritious, with a high percentage of carbohydrates, fats and proteins. Not only is the grain valuable as a stock feed, but the plant as a whole is an important fodder crop. The immature cobs are largely eaten after roasting. The grains are also used in making corn starch and industrial alcohol. The glucose is also manufactured from the grain. The corn oil is prepared which is used for soap making lubrication and as salad oil. Corn flakes make a good breakfast food. The present investigation was made to induce genetic variability through mutagenesis and to screen useful mutants for the improvement of maize.

2. Materials and Methods

Two sets containing 200 well filled healthy seeds were selected for treatment. To determine the LD_{50} values, gamma rays and DES. The gamma rays at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 KR doses of treatment. The grains were pre soaked in distilled water for 6 hours followed by DES at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM concentrations. The seeds after soaking in DES were thoroughly washed in running tap water for 8 to 10 times and then transferred to Petri dishes containing two layers of moist filter paper for germination. Ten Petri dishes of 10 seeds per treatment were planted and percentage germination and seedling variations for each treatment were subsequently determined. The treated seeds were then subjected to germination test. Based on the reduction of germination to 50 per cent, LD_{50} value was determined. Three concentrations of DES around LD_{50} were fixed for further studies.

200 well filled seeds were selected and pre soaked in double distilled water for six hours. The excess moisture in the seeds was removed by pressing in the fold of filter paper. They were treated with required mM concentrations of DES solution for six hours at room temperature ($26 \pm 2^{\circ}C$) with intermittent shaking. After that the seeds were thoroughly washed with running tap water for 8-10 times. Non-treated dry seeds were pre-soaked in distilled water for 6 hours and then used as control. The mutagenic treatment seeds were immediately sown in the field along with the control in a randomized block design with three replications. A total of 200 seeds were sown in each treatment. All the treatments including the control were raised adopting a spacing of 45 cm in between rows and 20 cm in between plants. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop. From the remaining treated seeds, 100 seeds were placed in the moist germination paper and replicated twice for the purpose of laboratory analysis. The seeds harvested from M_1 generation were taken from individual treatments and were used to raise M_2 generation [7]. The M_2 generation was grown in triplicate in randomized block design. 200 plants were maintained for each treatment in each replication. Biometric observations were recorded and individual plant data's were calculated using statistical analysis. In the Mutagenic effectiveness is a measure of the frequency of mutation induced by unit mutagen, where as mutagenic efficiency gives an indication of the proportion of mutation in relation to undesirable changes like lethality and injury. The effectiveness and efficiency of the mutagens namely, gamma rays and DES were worked out by using the formulae suggested by Konzak [8].

Mutagenic effectiveness $= M \times 100/C \times T$

Mutagenic efficiency (Lethal) $= M \times 100/L$

Mutagenic efficiency (Injury) $= M \times 100/I$

Where

M - Mutation frequency for 100 M_2 plants

T - Period of treatment with chemical mutagen in hours

C - Concentration of mutagen in mM in per cent

L - Percentage of lethality or survival reduction

I - Percentage of injury or reduction in seedling size

3. Results and Discussion

LD_{50} **Value (Lethal doses):** The present study was undertaken with *Zea mays* genotype sugar 75. This genotype was subjected to study the effect of Physical and Chemical mutagens namely, Gamma rays and EMS, through the biological changes in M_1 generation. This was aimed to study the frequency, spectrum of chlorophyll, viable mutants and total mutations in M_2 generations. It was also aimed to find out the economic potentialities of the viable mutants and the nature of induced variability in the quantitative traits in M_2 generations. The LD_{50} values were calculated on the basis of 50 per cent reduction of germination seeds count on 10^{th} day. The present investigation exhibited that the germination percentage of *Zea mays* decreased with increase in the concentration of the mutagens were used to find out the LD_{50} values for further studies. It was estimated that using 50% reduction in seed germination observed at 50 KR of Gamma rays and $40mM$ of DES for already reported in maize by [9]. The present investigation exhibited as the concentration of Gamma rays increased with the decreased in germination percentage, 50 per cent reduction in seed germination was observed at $50KR$ of Gamma rays. The germination percentage of *Zea mays* decreased with the increased in the concentration of DES. 50 per cent reduction in seed germination served at $40mM$ of DES (Table-1).

In the present investigation, all the parameters of chemical mutagens were reduced in M_1 generation at maturity time. [10] reported that effect of mutagen and dry seeds of *Phaseolus mungo* was showed reduction in growth. [11] reported that differences exist among the species and among the genotype with in a species for sensitivity in mutagen treatments. The growth and other quantitative traits were proportionately decreased with increased concentration of chemical mutagens in the present study.

In the present investigation, the seed germination and seedling survival were reduced with increasing in concentration of gamma rays and DES treatment than control. Similar results have been reported in different crops, Black gram [12], green gram [13], soybean [14–16], cluster bean [17], cowpea [18]. The M_1 generation was assessed at the field level to measure the intensity of injury caused by mutagenic treatments [19]. The reduction in the seed germination and seedling survival at different mutagenic treatments indicate that mutagen had on effect on these parameters. The biological effects was determined from the observation made on seed germination, seedling survival were decreased in all the mutagens. In the present investigation germination and survival percentage decreased with increased in concentration and a field condition was observed in M_1 generation. Similar results were observed by soybean [20].

The Spectrum of Chlorophyll Mutations: The chlorophyll mutations like albina, viridis, xantha and chlorina were also observed at all mutagenic treatments. However, highest frequency of chlorophyll mutation was recorded at 50 KR of Gamma rays. In albino these seedlings were characterized by their dull white colour and were devoid of chlorophyll, carotenoid and other pigments. Albina seedlings are smaller in height and survive to a maximum of 10 days after germination and then

die. In viridis the seedlings are dark green in the early stages of development and turn normal green in the later stages. The mutants produce normal looking flowers and also set seeds. The xantha Colors of these mutants vary from deep yellow to yellowish white. Growth of mutants is retarded and most of them die within 7 to 10 days after Emergence and Chlorina Normally chlorina mutants do not survive. These mutant seedlings have light yellowish/yellowish green leaves and culm with yellowish pods.

The mutants breed true for the altered characters, the viable mutants of tall mutant the plant height was highly increased with higher number of ears when compared to control. These mutants were observed in almost all the mutagenic treatments, Dwarf mutant showed that the plant height was very much reduced with less number of ears when compared to control. These mutants were observed in almost all the mutagenic treatments, early maturity mutants showed 3 to 6 days earlier maturity than the control plants. These mutants were observed in almost all the mutagenic treatments, the late maturity mutants exhibited 5 to 8 days late maturity than the control plants. The maximum of four mutants were observed at 50 KR of Gamma rays. Tri angular leaf these mutants showed tri angle shape of leaves are observed in almost all mutagenic treatments, bold size of grain was larger size than in control. This mutant is observed in almost all mutagenic treatments, long ear of length was higher than the control.

This mutant is observed in almost all mutagenic treatments, Short ear of pod length was higher than the control. The maximum of four mutants were observed at $50KR$ of Gamma rays, one or two male sterile mutants with stunted growth and small flower having 100 per cent pollen sterility were observed. Male sterile plants on open pollination set few capsules only. The M_2 plants raised in the field were examined to identify the mutants induced by Gamma rays and DES, as well as to find out their effects on various quantitative characters. The maximum of three mutants were observed at Gamma rays treatment. The observed frequency of viable mutants estimated on M_2 plant basis is presented in Table 2. The mutation frequency showed a decrease with increase in the concentration of mutagens. On M_2 plant basis, the maximum chlorophyll and viable mutation frequency were observed at 50 KR of Gamma rays (24.37). While the minimum chlorophyll and viable mutation frequencies were recorded at 30 mM of DES (8.90).

Effectiveness and Efficiency: The mutagenic effectiveness and efficiency for based on the chlorophyll and viable mutants are based on the Table-3 gamma rays was found to be more effective than DES in inducing mutation. The maximum mutagenic effectiveness was observed at $50KR$ of Gamma rays (48.74), while the minimum mutagenic effectiveness was observed at $60KR$ of Gamma rays (21.83). The mutagenic efficiency was worked out based on injury and lethality. On the basis of lethality, the highest mutagenic efficiency was recorded at $50KR$ of Gamma rays (54.83), while the lowest mutagenic efficiency was observed at $50mM$ of DES (14.92). In general the mutagenic treatment 50KR of Gamma rays was found to be highly efficient for induced chlorophyll and viable mutants. On the basis of injury, the maximum mutagenic efficiency was observed at $50KR$ of Gamma rays (95.00). The minimum mutagenic efficiency was observed at $50mM$ of DES (29.98).

In the present study, the mutagenic effectiveness and efficiency was estimated on the basis of relation propagation of family's segregation viable mutants In comparing Gamma rays and DES. In the present study, gamma rays were found to be more effective and efficiency than DES treatment. While the mutagenic effectiveness and efficiency was generally decreased with increase in the higher concentration of mutagens up to certain level. Similar results were recorded by [21] in rajmash; [22] in lentil; [23] in lima bean, [24] in blackgram and in maize [9]. The mutagenic efficiency gives an idea of the proportion of

mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen [8]. The present study revealed that Gamma rays is highly efficient than DES. Similar results were recorded by [25] in mungbean; [26–28] in urdbean; [29] in sunflower, [30] in kodo-millet; in chickpea; [18] in cowpea and in maize [9].

4. Conclusion

Mutation breeding is used as an alternative for crop improvement of desired characters in agricultural crops. This is based on creation of variation, selection, evaluation and multiplication of desired genotype. The mutagens was played a major role in plant breeding it's improve the crop plants. In this present investigation, $50KR$ of Gamma rays and $40mM$ of DES was played an important role in induced mutation by which improve the qualitative characters of maize (corn) Zea mays (L.).

Treatment (Dose/Conc. KR/mM)	Seed germination (%)		Per cent over control		Per cent of reduction over control	
	Gamma rays (KR)	DES (mM)	Gamma rays (KR)	DES(mM)	Gamma rays (KR)	DES (mM)
Control	93.32	97.66	100.00	100.00	00.00	00.00
10	90.66	93.00	97.14	95.22	-02.86	-04.78
20	86.66	79.33	92.86	81.23	-07.14	-18.77
30	74.66	66.33	80.00	67.91	-19.10	-32.09
40	62.66	50.33	67.14	51.53	-32.86	-48.47
50	50.64	42.66	54.26	43.68	-45.74	-56.32
60	42.66	33.33	45.71	34.12	-54.29	-65.88
70	38.66	25.66	41.42	26.27	-58.58	-73.73
80	32.00	18.00	34.29	18.43	-65.71	-81.57
90	24.00	9.00	25.71	09.21	-74.29	-90.79
100	13.33	3.06	14.28	03.13	-85.72	-96.87

Table 1: Effect of Physical and chemical mutagenesis on LD_{50} value for (Corn) Maize (Zea mays L.)

Mutagens Dose/Conc.	**Gamma rays (KR)**			**DES (mM)**		
	40 KR	**50 KR**	**60 KR**	**30 mM**	**40 mM**	**50 mM**
No. of plant studied	152	160	145	150	155	146
Albino	2	3	1	-	2	-
Viridis	3	2	2	2	2	1
Xantha	2	3	2	1	2	1
Chlorina	2	2	1	1	2	2
Tall	2	4	1	1	2	2
Dwarf	3	3	2	1	1	1
Early maturity	2	3	1	1	3	-
Late maturity	2	5	2	2	1	1
Triangular leaf	2	3	2	2	2	1
Bold size seed	2	2	1	1	2	1
Long ear	2	3	1	2	2	-
Short ear	2	5	1	2	4	1
Male sterility	2	1	2	1	-	1
Total	**28**	**39**	**39**	**17**	**25**	**13**
Total mutation frequency	**18.42**	**24.37**	**13.10**	**11.33**	**16.12**	**08.90**

Table 2: Mutation frequency of chlorophyll and viable mutants of (corn) Maize (Zea mays L.) in M_2 generation

Treatment (Dose/Conc.),(KR/mM)		Survival Reduction (L) 30^{th} day	Height Reduction (I) 30^{th} day	Mutation Frequency	Effectiveness $\frac{M\times100}{C\times T}$	Efficiency	
						$\frac{M\times100}{L}$	$\frac{M\times100}{I}$
Gamma rays (Dose. KR)	40	33.59	20.89	18.42	46.05	54.83	88.17
	50	48.31	25.65	24.37	48.74	50.44	95.00
	60	54.35	33.71	13.10	21.83	24.10	38.86
DES (Conc. mM)	30	36.99	18.38	11.33	12.58	30.62	61.64
	40	52.08	24.62	16.12	13.43	30.95	65.47
	50	59.63	29.68	08.90	05.93	14.92	29.98

Table 3: Effect of Induced Mutagenic Effectiveness and efficiency of (corn) Maize (Zea mays L.) in M_2 generation

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