



# 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].

$$\text{Mutagenic effectiveness} = M \times 100/C \times T$$

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

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

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<sup>th</sup> 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	<b>28</b>	<b>39</b>	<b>39</b>	<b>17</b>	<b>25</b>	<b>13</b>
Total mutation frequency	<b>18.42</b>	<b>24.37</b>	<b>13.10</b>	<b>11.33</b>	<b>16.12</b>	<b>08.90</b>

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 <sup>th</sup> day	Height Reduction (I) 30 <sup>th</sup> day	Mutation Frequency	Effectiveness $\frac{M \times 100}{C \times T}$	Efficiency $\frac{M \times 100}{L}$ $\frac{M \times 100}{I}$	
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

## References

- [1] S.N.Acharya, J.E.Thomas and S.K.Basu, *Improvement in the medicinal and nutritional properties of fenugreek (Trigonella foenum-graecum L.)*, In: Acharya SN, Thomas JE (Eds) *Advances in medicinal plant research*, Research Signpost, Trivandrum, Kerala, India, (2007).
- [2] N.P.Dubin, *Problems of radiation genetics*, Oliver & Boyd, London, (1961).
- [3] Y.I.Lee, I.S.Lee and Y.P.Lim, *Variation in sweet potato regenerates from gamma-rays irradiated embryogenic callus*, J. Plant Biotech., 4(2002), 163-170.
- [4] K.N.Maluszynski, L.V.Zanten and B.S.Ahloowalia, *Officially released mutant varieties*, The FAO/IAEA Database. Mut. Breed.Rev., 12(2000), 1-12.
- [5] M.Regulski, Z.Lu, J.Kendall, M.T.Donoghue and J.Reinders, *The Maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA*, Genome Research, 23(2013), 1651-1662.
- [6] J.Gent, T.F.Madzima, R.Bader, M.R.Kent and X.Zhang, *Accessible DNA and relative depletion of H3K9me2 at maize loci undergoing RNA-directed DNA methylation*, Plant Cell, 26(2014), 4903-4917.
- [7] P.K.Panda and P.K.Subuthi, *A comparative study of chemically induced micro mutational variability in  $M_2$  generation of rape seed and mustard*, Indian J. Genet., 54(3)(1994), 269-274.
- [8] C.F.Konzak, R.A.Nilan, J.Wagner and R.J.Foster, *Efficient chemical mutagenesis. The use of induced mutations in plant breeding Rept*, FAO/IAEA Tech. Meet. Rome., (1965).
- [9] S.Gnanamurthy, D.Dhanavel and M.Girija, *Studies on Induced Chemical Mutagenesis in maize (Zea mays (L.))*, Inter. Journal of Curr. Science, 3(11)(2011), 037-040.
- [10] M.K.Jana, *Effects of X-ray and neutron irradiation on seeds of Phaseolus mungo L*, Genetia Agraria, XVIII (4)(1964), 617-628.
- [11] L.Fehr, *Genetics of Vigna*, In. (Ed). Horticulture Review. A. VI, Westport, Connecticut. J. Janick., (1987), 311-394.
- [12] C.Thilagavathi, *Effect of Ethyl methane sulphonate (EMS) and Di ethyl sulphonate (DES) in black gram, Vigna mungo (L.) Hepper*, M.Phil. Thesis, Annamalai University, Annamalaiagar, Tamil Nadu, (2007).
- [13] S.Ignacimuthu and C.R.Babu, *Radio sensitivity of the wild and cultivated urd and mungbean*, Indian J. Genet., 48(1988), 331-342.
- [14] P.C.Balakrishnan, *Induced mutagenesis in soybean (Glycine max (L.) Merrill)*, Ph.D. Thesis, Tamil Nadu Agrl. Univ., Coimbatore, (1991).
- [15] K.Geetha, *Studies on induced mutation in two distinct varieties of soybean (Glycine max (L.) Merrill)*, Ph.D. Thesis, Tamil Nadu Agrl. Univ., Coimbatore, (1994).
- [16] P.Pavada and D.Dhanavel, *Effect of EMS, DES and colchicine treatment in soybean*, Crop Res., 28(1-3)(2004), 118-120.

- [17] S.Velu, L.Mullainathan, D.Arulbalachandran, D.Dhanavel and R.Poonguzhali, *Effectiveness and Efficiency of gamma rays and EMS on cluster bean [Cyamopsis tetragonoloba (L.) Taub.]*, Crop Res., 34(1-3)(2007), 249-251.
- [18] M.Girija and D.Dhanavel, *Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatment in cowpea (Vigna unguiculata L. Walp)*, Glo. J. Mol. Sci, 4(2)(2009), 68-75.
- [19] H.Gaul, *Mutagenic effects observed in the first generation. I. Pant injury and Lethality. II. Cytological effects. III. Sterility. Manual on Mutation Breeding*, IAEA, Vienna, (1970), 85-89.
- [20] D.Kavithamani, A.Kalamani, C.Vanniarajan and D.Uma, *Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean (Glycine max (L.) Merrill)*, Madras Agric. J., 95(7-12)(2008), 448-451.
- [21] A.S.Gautam, K.C.Sood and R.K.Mittal, *Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in rajmash (Phaseolus vulgaris L.)*, Legume Res., 21(3-4)(1998), 217-220.
- [22] I.S.Solanki, *Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (Lens culinaris Medik.)*, Indian J. Genet., 65(4)(2005), 264-268.
- [23] D.Sassikumar, T.Nepolean and A.Gopalan, *Effectiveness and efficiency of the mutagens gamma rays and ethylmethane sulphonate on lima bean (Phaseolus lunatus L.)*, Indian J. Agric. Res., 37(2)(2003), 115-119.
- [24] C.Thilagavathi and L.Mullainathan, *Isolation of macro mutants and mutagenic effectiveness, efficiency in black gram (Vigna mungo (L.) Hepper)*, Glo. J. Mol. Sci., 4(2)(2009), 76-79.
- [25] A.S.Gautam, K.C.Sood and A.K.Richarria, *Mutagenic effectiveness and efficiency of gamma rays, ethylmethane sulphonate and their synergistic effects in blackgram (Vigna mungo L.)*, Cytologia, 57(1992), 85-89.
- [26] J.S.Ahmed, *Effectiveness and efficiency of gamma rays in blackgram*, Madras Agric. J., 83(1995), 320-321.
- [27] A.J.Deepalakshmi and C.R.Anandakumar, *Efficiency and effectiveness of physical and chemical mutagens in urdbean (Vigna mungo (L.) Hepper)*, Madras Agric. J., 90(7-9)(2003), 485-489.
- [28] S.K.Sharma, R.Sood and D.P.Pandey, *Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (Vigna mungo (L.) Hepper)*, Indian J. Genet., 65(1)(2005), 20-22.
- [29] S.Jayakumar and R.Selvaraj, *Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in sunflower (Helianthus annuus L.)*, Madras Agric. J., 90(7-9)(2003), 574-576.
- [30] H.S.Yadava, A.N.Tikle and D.V.Bhagat, *Effect of induced mutation through gamma rays on growth and yield parameters of kodo-millet*, J. Soil and Crops, 13(1)(2003), 25-28.