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ORIGINAL ARTICLE

**Induced Genetic Variability in Pigeonpea [*Cajanus cajan* (L.) Millsp.] : Comparative Mutagenic effectiveness and Efficiency of Physical and Chemical Mutagen**

S.P. Giri

Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar Tal-Rahata, Dist-

Ahmednagar-413713

Email: [sanjaygiri205@gmail.com](mailto:sanjaygiri205@gmail.com)

ABSTRACT

*In the present investigation comparative mutagenic effectiveness and efficiency of Gamma rays and Ethyl Methane Sulphonate (EMS) were studied in Pigeon pea [*Cajanus cajan* (L.) Millsp]. The treatment included two doses /concentration each of gamma rays and EMS calculated on the basis of their LD<sub>50</sub>. The result reveals that EMS was almost one and half times more effective than Gamma rays. While the efficiency of Gamma rays almost double than that of EMS. Mutagenic effectiveness and efficiency were found to depend upon mutagen type and the genotype and both were higher at lower doses of EMS and Gamma rays.*

**Key Words:** Genetic Variability, Pigeon Pea, *Cajanus cajan*, Gamma rays, EMS.

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**INTRODUCTION**

Pigeonpea [*Cajanus cajan* (L.) Millsp] is one of multipurpose legume of Maharashtra grown in Kharif season. Pigeonpea is an economic source of not only protein but of carbohydrate, minerals and B-complex vitamins particularly in vegetarian diet [10]. 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 create genetic variability through induced mutations. Induced mutagenesis has been used widely in crop plants to create genetic variability in traits of economic value. Chlorophyll mutations are one of the important criteria to determine effectiveness of the mutagens. According to Miller [7], 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 studies on effectiveness and efficiency of a mutagen in a crop are necessary [1].

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 doses of gamma rays and different concentrations of EMS on Pigeonpea (ICPL-87) in terms of chlorophyll mutations and to find out effective concentration, which induces desirable mutations.

**MATERIALS AND METHOD**

The experimental plant material (seeds), Pigeonpea ICPL-87, were procured from 'Pulses improvement Division' of Mahatma Phule Agricultural University, Rahuri (MS).

Gamma Irradiation: The seeds were irradiated with different doses Gamma rays (100, 200, 300, and 400Gy) from Institute of Science, Aurangabad.

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 (RDB) Randomized block design with three replications. The seeds of all individual  $M_1$  plants were harvested separately and they were sown in the field during the next Kharif to rise  $M_2$  generation. Mutagenic effect was assessed using parameters like seed germination, plant survival, pollen sterility, and chlorophyll mutants. The treated as well as control seeds after germination were carefully screened for chlorophyll mutation in  $M_2$  generation. Based on this data the mutagenic effectiveness and efficiency were calculated by using the formulae suggested by Konzak *et al.*, [6].

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Time X concentration}} \\ = \text{MF/ TC}$$

Where,

MF = % of chlorophyll mutations in  $M_2$  generation.

T = Period of treatment with chemical mutagen.

C = Concentration of chemical mutagens,

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage}}$$

= MF/L, MF/I, MF/S

Where,

L = % of lethality in  $M_1$  generation.

I = % of seeding injury in  $M_1$  generation.

S = % of pollen sterility in  $M_1$  generation.

## RESULTS AND DISCUSSION

### Mutagenic Effectiveness

**Gamma Rays:** The results (Table 1) have shown that, effectiveness of mutagenic treatments differed considerably. The mutagenic effectiveness showed a trend, which was inversely proportional to the increasing dose (except 100Gy) of mutagens in Pigeonpea. The mutagenic effectiveness varied from 0.009 to 0.076 in Gamma rays. The present data further revealed that the lower dose of Gamma rays (200Gy) was more effective in causing chlorophyll mutation. From the data it is concluded that the 200Gy dose is more effective while the 400Gy dose is less effective in inducing chlorophyll mutation in Pigeonpea ICPL-87. The decrease in effectiveness with increasing dose of mutagen has been reported by Khan *et al.*, [4], Sinha and Akhaury [12], Nadarajan and Ramalingam, [8], Sundaram, [14], Rao *et al.*, [9] in Pigeonpea.

**EMS:** The results (Table 1) have shown that, effectiveness of mutagenic treatments differed considerably. The mutagenic effectiveness showed a trend, which was inversely proportional to the increasing concentrations of mutagens in Pigeonpea. The mutagenic effectiveness varied from 0.010 to 0.075 in EMS. The present data further revealed that the lower Concentration of EMS was more effective in causing chlorophyll mutation. From the data it is concluded that the 10mM concentration of EMS is more effective while the 40mM concentration of EMS is less effective in inducing chlorophyll mutation in Pigeonpea ICPL-87. The decrease in effectiveness with increasing concentrations/dose of mutagen has been reported by Khan *et al.*, [4] in Pigeonpea.

### Mutagenic Efficiency

**Gamma rays:** Mutagenic efficiency is the ratio of frequency of chlorophyll mutations induced in  $M_2$  generation to various biological damages (such as plant survival, pollen sterility, seed germination and lethality) observed in  $M_1$  generation. Experimental results obtained on mutagenic efficiency of the mutagens used in the present investigation are presented in Table 1. Mutagenic efficiency varied from 0.15 to 1.31. It varied with respect with different parameters. The mutagenic efficiency linearly increased at the 100Gy and 200Gy, while decreased from 200Gy to 300Gy and 400Gy dose of Gamma rays (Table 1). The data regarding pollen sterility, plant survival and seed germination in relation to efficiency have shown a reducing trend with the gradual increase in concentration of Gamma rays in Pigeonpea ICPL-87 (Table 2). The mutagenic treatment of Gamma rays caused considerable reduction in seed germination, pollen Sterility and plant survival. Similar observation has been recorded by Biradar [2], Shinde [13] in Pigeonpea. The result of present study indicated that lower dose (200Gy) of Gamma rays is more effective for the induction of desirable mutations for Pigeonpea improvement.

**EMS:** Mutagenic efficiency is the ratio of frequency of chlorophyll mutations induced in  $M_2$  generation to various biological damages (such as plant survival, pollen sterility, seed germination and lethality) observed in  $M_1$  generation. Experimental results obtained on mutagenic efficiency of the mutagens used in the present investigation are presented in Table 1. Mutagenic efficiency varied from 0.06 to 0.61. It varied with respect with different parameters. The mutagenic efficiency linearly increased at the 10mM and 20mM, while decreased from 20mM to 30mM and 40mM concentration of EMS.

The data regarding pollen sterility, plant survival and seed germination in relation to efficiency have shown a reducing trend with the gradual increase in concentration of EMS in Pigeonpea ICPL-87 (Table 2). The mutagenic treatment of EMS caused considerable reduction in seed germination, pollen Sterility and plant survival. Similar observation has been recorded by Badere and Chaudhary [1] in Lentil, Shah *et al.*, 2008 in Chickpea, Biradar [2], Shinde [13] in Pigeonpea. The result of present study indicated that lower concentrations (10mM and 20mM) of EMS are more effective for the induction and recovery of mutations for crop improvement of Pigeonpea.

Table 1: Effectiveness and efficiency of Gamma rays and EMS in Pigeonpea in  $M_2$

Mutagen	Dose	Frequency of chlorophyll mutants (%)	Effectiveness MF/dose	Lethality (L)%	Efficiency (MF/L)
Control	Control	0.00	0.00	0.00	0.00
Gamma rays	100Gy	5.45	0.055	9.86	0.55
	200Gy	15.18	0.076	11.6	1.31
	300Gy	5.14	0.017	13.82	0.37
	400GY	3.46	0.009	23.48	0.15
EMS	10mM	6.00	0.075	10.7	0.56
	20mM	10.78	0.067	17.68	0.61
	30mM	4.82	0.020	32	0.15
	40mM	3.33	0.010	53.64	0.06
SE $\pm$ 0.034, CV % : 0.98, CD 5% : 0.10, CD 1% : 0.14					

Table 2 Effect of Gamma rays and EMS on seed germination, plant survival and seed sterility in Pigeonpea in  $M_1$  generation

Mutagen	Dose	Seed germination (%)	SE	Plant Survival (%)	SE	Pollen sterility (%)	SE
Gamma rays	100Gy	84	$\pm$ 0.19	90.14	$\pm$ 0.14	4.61	$\pm$ 0.16
	200Gy	76	$\pm$ 0.11	88.40	$\pm$ 0.15	7.14	$\pm$ 0.21
	300Gy	68	$\pm$ 0.22	86.18	$\pm$ 0.14	9.04	$\pm$ 0.21
	400Gy	49	$\pm$ 0.19	76.52	$\pm$ 0.19	30.70	$\pm$ 0.31
EMS	10mM	68	$\pm$ 0.19	89.30	$\pm$ 0.14	5.40	$\pm$ 0.16
	20mM	60	$\pm$ 0.11	82.32	$\pm$ 0.15	10.24	$\pm$ 0.21
	30mM	56	$\pm$ 0.22	68.00	$\pm$ 0.14	9.37	$\pm$ 0.21
	40mM	48	$\pm$ 0.19	46.36	$\pm$ 0.19	16.61	$\pm$ 0.31

In general, the treatments with low concentrations of EMS were more effective and efficient as measured on the basis of lethality and injury than treatments with higher concentrations. The efficiency of a mutagenic agent is of a complex nature, as it does not only depends on the reactivity of the agent with the material and on its applicability to the biological system but also on the degree to which physiological damage, chromosomal abreaactions and sterility are induced in addition to mutations.

The present study indicated that the mutagenic effectiveness and efficiency decreased with the increasing dose of mutagens. Higher efficiency at the lower concentration of the mutagen appears mainly due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration than actual mutations [5, 3]. Efficient mutagens and their treatments are indispensable for the cost-effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding program.

## CONCLUSION

The result of present study indicated that lower dose of Gamma rays (200Gy) and lower concentrations of EMS (10mM and 20mM) are more effective for the induction of desirable mutations for Pigeonpea improvement. Study also reveals that EMS is more effective than Gamma rays in induction of mutations in Pigeonpea.

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