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Mutagenic effectiveness and efficiency of gamma rays, Ethyl methyl sulfonate and their synergistic effects in Pigeonpea (*Cajanus Cajan* L.)

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Abstract
Mutagenic effectiveness and efficiency of individual treatments of EMS and gamma rays were assessed in pigeonpea genotypes, ICPL-87 and BSMR-736. The experiment included total 8 mutagenic treatments (4 for each individual treatment of EMS and gamma rays) per genotype having control one, selected on the basis of their LD50 values. In comparisons of both genotypes, BSMR-736 observed higher rate of mutation frequency than genotype ICPL-87. Effectiveness and efficiency were calculated on the basis of Plant survival and chlorophyll mutation frequency of M_2 generation. Mutagenic effectiveness decreased with increased in dose / concentration of mutagen. Where as in mutagenic efficiency the data showed that no specific pattern is followed but variation in mutagenic efficiency was recorded in all treatment.

Keywords: Biological damage, chlorophyll mutations, EMS, gamma rays, mutagenic effectiveness and efficiency, mutation frequency, pigeonpea.

Introduction
Among the major legume crops, Pigeonpea (*Cajanus cajan* L.) is one of the most nutritious crop. It is an economic source of protein as well as carbohydrates, minerals and B-complex vitamins in vegetarian diet. Pigeonpea is an important legume crop mainly used as food and fodder and is a major source of vegetable protein.Seeds are rich in iron, iodine, and essential amino acids like lysine, cysteine and arginine.

Genetic improvement of crop depends on the amount of genetic variability present in the population but Pigeonpea, being a self-pollinated crop; the available genetic variability has been almost exploited for improvement by conventional breeding methods. Hence it becomes necessary to create genetic variability through mutations.

Mutation is gene level causes alterations in the structure and position of gene on chromosome called point mutation. This results in the alteration of phenotype of an organism. Changes in basic chromosome number either any addition of loss of any set or parts of them cause appearance of disappearance of new characters.

Mutation breeding is the tool in the hand of breeder to create variability in crop population and to make selection in the population with the view to bring about further improvement in crop. In general mutation breeding has been playing a key role in self-pollinated crop with limit variability has been reported by many workers, in cowpea (Dhanavel *et al*., 2008) [4], in black gram (Thilagavathi and Mullainathan, 2009) [22] and soybean (Padmavathi, Devi and Kiranmai, 1992; Pavadai *et al*., 2010) [17, 18].

To determine effectiveness of the mutagens Chlorophyll mutations are one of the important criteria which 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 are necessary (Badere and Chaudhary, 2007) [2]. The present investigation was undertaken to study the mutagenic effectiveness and efficiency in M_2 generation of pigeonpea genotype ICPL-87 and BSMR 736.

Material and Methods

The pure seeds of Pigeonpea genotype ICPL-87 and BSMR-736 were procured from 'Pulses Research Unit' of Panjabrao Deshmukh krishi Vidyapeeth, Akola (Maharashtra). The seeds were presoaked in sterile distilled water for 6 hours then irradiated with gamma rays having doses of 50Gy, 100Gy, 200Gy and 300Gy and treated with different concentrations of EMS as10mM, 20mM, 30mM and 40mM for 6 hours. The mutagen treated and control (untreated) seeds were sown in the field for rising M^1 generation. All the surviving individual plants were harvested in each treatment in M_1 generation. M_1 plants having sufficient seeds in different treatments were grown in plants to progeny rows to rise M_2 generation. Mutagenic effect was evaluated using parameters like seed germination, plant survival and chlorophyll mutants. M_2 generation was screened for lethal chlorophyll mutations during the first four weeks after germination, whereas viable chlorophyll and morphological viable mutations were scored throughout the crop duration.

The mutation frequency is one of the most dependable indexes for evaluating the genetic effects of mutagenic treatments (Kharkwal, 1999) [12]. It was calculated as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment.For the material grown in bulk, the mutant frequency was estimated by dividing the total number of mutants confirmed by the total number of M_2 plants in the bulk population studied as given in Gaul (1964) [4].

Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al*., (1965) [13]

Mutagenic Effectiveness (Physical Mutagen) = M×100/ dose in Kr.

Mutagenic effectiveness (Chemical Mutagen) = M×100/ C × t

Mutagenic Efficiency = M x 100 / L

Where,

M = Mutation frequency for 100 M_2 plants

t = Period of treatment with chemical mutagen in hours

C = Concentration of mutagen in mM.

Krad = Dose of mutagenic radiation in kilo rad

L = Percentage of lethality (or) survival reduction

Results

M_1 plants were sown for rising M_2 generation in a plant to progeny fashion (280 seeds/plant) in randomized block design (RBD). Populations of 14480 plants were screened in M_2 generation with aim to isolate important mutants.

All mutagenic treatment had induced macro-mutations, but the frequency varied with different treatments. Spectrum of chlorophyll mutant’s viz. Albina, Xantha, Striata and Chlorina and some morphological abnormalities like total sterile plant, semi fertile plant, and change in leaf of plant were observed during course of investigating M_2 population under study which is obtained from different treatment of mutagen. Various types of mutations identified are mentioned in Table 1. Different chlorophyll mutants were observed during present investigation which shown in fig.1

The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effect of mutagen (Gautam *et al*., 1998) [6]. Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various pulse crops by several workers including Gautam *et al*., (1992) [5].

Chlorophyll mutants

Frequency of chlorophyll mutations was recorded from the first day of emergence to four weeks in the M_2 generation. The detection of mutations was made as per classification of Gustafsson (1940) [10]. Four different types of chlorophyll mutations were observed in the M_2 progeny of both genotypes of pigeonpea. They are *albina, xantha, striata, chlorina*. The varieties responded differently to the two different mutagens. The highest numbers of chlorophyll mutants were recorded for chlorine type followed by xanthan type.

1. **Albina**- It is lethal mutation characterized by entirely white leaves of seedlings and it survived for 10-12 days after germination. This type of mutants observed only in genotype BSMR-736. One albino mutant was recorded in genotype BSMR-736 in T_3 (200Gy) of gamma rays. Two albina mutants were recorded in same genotype in T_1 (5mM) of EMS treatment and one albina mutant was recorded in T_2, T_3 and T_4 of EMS treatment 10mM, 20mM and 30mM respectively.
2. **Xantha-** Colors of the mutants vary from deep yellow to yellowish white on leaves. Growth of mutants is retarded and most of them die within 17 to 20 days after emergence. This type of mutants found in maximum frequency (3 numbers) in T_2 (100Gy) ant T_4 (300Gy) of gamma rays for genotype ICPL-87. Whereas genotype BSMR-736 recorded maximum xantha type of mutants (5 numbers) in T_2 (100Gy) and T_3 (200Gy) of gamma rays.
3. **Striata-** Seedling with leaves characterized by the presence of longitudinal zones of normal green, alternate with yellow. Maximum frequency of this type of mutants were observed in T_4 (300Gy) and T_4 (30mM) treatment of both mutagen for ICPL-87 (2 in number). Where, T_3 (20mM) of EMS treatment shown maximum frequency of this mutant for BSMR-736 (4 in numbers).
4. **Chlorina-** The seedlings were light yellowish green (pale green) in colour. They survived for a reasonably long period. As compared to other type of mutants, the chlorine type is dominating. Different treatment in both the genotypes recorded chlorine mutants ranging from 3 to 10 numbers.

Gamma rays treatment in BSMR-736 had induced higher number of chlorine type of mutants. Treatment of 200Gy had induced highest i. e. 10 numbers of chlorine mutants in BSMR-736. In genotype ICPl-87, highest number of chlorine mutants was induced by EMS treatment of 20mM (7 numbers). *xantha, striata*, and *chlorina* type of mutations were induced in all the mutagenic treatments irrespective of their concentrations or dose. The overall spectrum of induced chlorophyll mutations as observed in present investigation was in the order- *Chlorina* > *Striata* > *Xantha* > *Albina.*

In present study all four types of mutations were recorded in genotype BSMR-736 by EMS treatment. In the present investigation, morphological (viable) mutants were observed in M_2 generation with different doses of gamma rays and EMS.

Morphological mutants such as total sterile, semi fertile, early and late maturity were observed in all the mutagenic treatments. The maximum numbers of morphological mutant were observed at T_1 (50Gy) and T_4 (300Gy) treatments.

Table 1: Spectrum of chlorophyll variants observed in M_2 generation

Genotype	Mutagen	Treatment	No. of M2 plants observed	No. of chlorophyll variants				On. Of morphological variants	Total no. of variants	Mutation frequency (%)
				Albina	Xantha	Striata	Chlorine			
ICPL -87		Control	780	0	0	0	0	0	0	0
	GAMMA	T_1	627	0	2	2	3	7	14	2.23
		T_2	570	0	3	1	4	1	9	1.58
		T_3	555	0	2	1	6	1	10	1.80
		T_4	525	0	3	2	6	4	15	2.86
	EMS	T_1	612	0	2	0	3	1	6	0.98
		T_2	565	0	0	1	5	1	7	1.24
		T_3	548	0	1	0	7	1	9	1.64
		T_4	512	0	1	2	6	3	12	2.34
BSMR-736		Control	788	0	0	0	0	0	0	0
	GAMMA	T_1	640	0	4	2	7	1	14	2.19
		T_2	596	0	5	1	8	3	17	2.84
		T_3	572	1	5	2	10	2	19	3.32
		T_4	542	0	2	1	6	2	11	2.03
	EMS	T_1	651	2	2	1	6	1	12	1.84
		T_2	582	1	2	3	7	1	14	2.41
		T_3	568	1	3	4	7	1	16	2.82
		T_4	525	1	1	3	4	2	11	2.10

Mutation frequency

Most of the mutants bearing multi mutational events thus may be lethal in the first generation, affecting the frequency occurrence of multi mutations in M_2 and future generations (Mehra, 2001) [16]. Mutation frequency of each visible mutant in M_2 generation was calculated as suggested by Girija and Dhanvel (2009) [8] which is represented in Table 1.

From Table 1, it is revealed that in case of genotype ICPL-87, the mutation frequency was high in treatment T_4 (300Gy) of gamma rays followed by T_4 (30mM) of EMS and lowest in treatment of T_1 (5mM) of EMS respectively.

Whereas in BSMR-736, highest range of frequency was recorded in T_3 (200Gy) of gamma rays followed by T_3 (20mM) of EMS and lowest range observed in T_1 (5mM) of EMS.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness and efficiency (mutation per unit dose) varies with different treatments. The effectiveness decreased with increasing dose or concentration. With increasing doses of EMS or Gamma rays the values obtained for all the biological criteria for M_2 generation were decreased. The reduction in biological criteria (Plant height and Survival) may be attributed to a drop in the auxin level (Gordon and Webber, 1955) [9], inhibition of auxin synthesis (Skoog, 1935) [19]. EMS was found, to be more effective than gamma rays in inducing mutation.

The mutagenic efficiency was worked out on the basis of lethality. Efficiency of a mutagenic agent is of a complex nature, as it does not only depend on the reactivity of the agent with the material and its applicability to the biological system but also on the degree to which physiological damage, chromosomal aberration and sterility are induced in addition to mutations. The mutagenic efficiency gives an idea of the proportion of mutation in relation to other associated undesirable biological effect such as injury, lethality and sterility induced by mutagen. Efficient mutagens and their treatment are indispensable for the cost-effective use of the mutagen as a tool for the induction of mutations and their direct utilization in successful breeding programme.

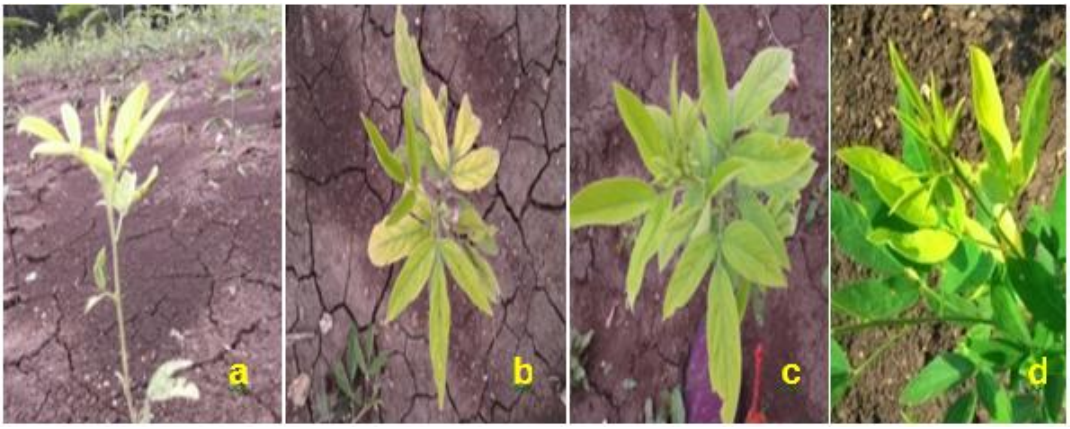
Table 2: Mutagenic effectiveness and efficiency in M_2 generation

Variety	Mutagen type	Treatment	Mutagen frequency	Survival reduction %	Mutagenic effectiveness	Mutagenic efficiency
ICPL-87	GAMMA	T_1	2.23	25	0.447	0.089
		T_2	1.58	32	0.158	0.049
		T_3	1.80	34	0.090	0.053
		T_4	2.86	38	0.095	0.075
	EMS	T_1	0.98	27	0.033	0.036
		T_2	1.24	33	0.021	0.038
		T_3	1.64	35	0.014	0.047
		T_4	2.34	39	0.013	0.060
BSMR-736	GAMMA	T_1	2.19	24	0.438	0.091
		T_2	2.85	29	0.285	0.098
		T_3	3.32	32	0.166	0.104
		T_4	2.03	35	0.068	0.058
	EMS	T_1	1.84	23	0.061	0.080
		T_2	2.41	31	0.040	0.078
		T_3	2.82	32	0.023	0.088
		T_4	2.10	38	0.012	0.055

The data recorded revealed that the effectiveness of mutagens varies in both the parameters i.e. type of mutagens and genotype used. In case of ICPL-87, the highest mutagenic efficiency was recorded in T_1 (50Gy) for gamma rays and in T_4 (30 mM) for EMS treatment. Whereas, the highest mutagenic effectiveness was recorded in T_1 treatment for both the mutagen as shown in Table 2.

In case of genotype BSMR-736, the highest mutagenic efficiency was recorded in T_3 for both the mutagen whereas, the highest mutagenic effectiveness was recorded in T_1 for both mutagens i.e. gamma rays and EMS treatment as shown in Table 2.

Mutagenic effectiveness decreased with increased in dose / concentration of mutagen. Where as in mutagenic efficiency the data showed that no specific pattern is followed but variation in mutagenic efficiency was recorded in all treatment. Study of reduction in survival percentage by various workers concluded that the physiological disturbance or chromosomal damage caused reduction in survival could be attributed to the cells of plants. Gamma rays treatment decreases the quantitative characters, leading to the physiological disturbance of plants or chromosomal damage caused to the cells of the plants.



A) Chlorophyll mutants: a) Albina, b) Xantha, c) Striata, d) Chlorina

Discussion
Chlorophyll mutants
The overall spectrum of induced chlorophyll mutations as observed in present investigation was in the order- *Chlorina* > *Striata* > *Xantha* > *Albina* for both genotypes. Similar observations were reported by Giri *et al*.(2010) [7] in pigeonpea, in which they used ICPL-87 genotypefor mutation screening in which seeds were irradiated with 100, 200, 300, 400 gray and treated with EMS at 10, 20, 30, 40mM concentration and they recorded similar four types of chlorophyll mutants. Also similar findings were recorded by Girija and Dhanavel (2009) [4] in cowpea; Thilagavathi and Mullainathan (2009) [22] and Arulbalachandran (2009) [1] in blackgram.

Mutagenic effectiveness and efficiency
Mutagenic effectiveness decreased with increased in dose / concentration of mutagen. Where as in mutagenic efficiency the data showed that no specific pattern is followed but variation in mutagenic efficiency was recorded in all treatment. Similar results in case of ICPL-87 also reported by Giri *et al*. (2010) [7]. They concluded that the lower concentrations or doses were most effective. The mutagenic effectiveness was highest at 10mM concentration of EMS. It decreased with increasing doses of the mutagens. The mutagenic effectiveness was found to be lowest at 400Gy in gamma rays dose and 40mM EMS treatment. In the present investigation it is clear that in terms of mean value of mutagenic efficiency and effectiveness, gamma rays were more effective than EMS treatment for both the genotypes which were used in study.

Trend of decreasing effectiveness with increasing dose of gamma rays and EMS has been reported in chickpea (Kharkwal, 1998), lentil (Solanki, 2005) [21] and mungbean (Solanki and Sharma, 1994) [20]. Higher mutagenic effectiveness and efficiency was observed in *Lathyrus sativus* L., at lower doses of EMS than in the gamma irradiation treatments by Waghmare and Mehra (2001) [23] and Kumar *et al*., (2003) [14]. Such difference in the effects of mutagens on different material might be due to the seed metabolism and onset of DNA synthesis. Kundi *et al*., (1997) [15] reported differential sensitivity within crop and even genotype. The sensitivity depends upon its genetic architecture and the mutagens employed (Blixit, 1970) [3] besides, the amount of DNA, its replication time in the initial stages and degree of heterochromatin. These criteria are responsible for differential mutagenic sensitivity in a crop.

Conclusion
The result of present study indicated that in case of genotype ICPL-87, lower concentrations (50Gy and 5mM) of gamma rays and EMS have higher mutation frequency. Whereas for genotype BSMR-736, T_3 (200Gy and 20 mM) recorded higher rate of mutation The experimental study indicated that lower dose showed more effective for ICPL-87 and it quite different for other genotype. Overall it is concluded that genotype BSMR-736 responded better than other genotype for mutation.

References

1. Arulbalachandran D, Mullainathan L. Chlorophyll and morphological mutants of blackgram (*vigna mungo* L.) derived by gamma rays and EMS. J Phytol. 2009; 1:236-241.
2. Badere RS, Choudhry AD. Effectivity and efficiency of gamma rays, sodium azide and ethyl methane sulphonate in Linseed. Bioinfolet. 2007; 4(3):181-187.
3. Blixt S. Studies of induced mutations in peas. XXVI. Genetically controlled differences in radiation sensitivity Agri Hort Genet. 1970; 28:55-116.
4. Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M *et al.* Effectiveness and efficiency of chemical mutagens in Cowpea (Vigna unguiculata (L.) Walp.). African Journal of Biotechnology. 2008; 7:4116e4117. Gaul (1964).
5. Gautam AS, Sood KC, Richarria AK. Mutagenic effectiveness and efficiency of gamma rays, EMS and

their synergistic effects in backgram (*Vigna mungo* L.). Cytologia. 1992; 57:85-89.
6. Gautam AS, Sood KC, Mitthal RK. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in Rajma (*Phasealus vulgaris* L.) Legume Research. 1998; 21:217-220.
7. Giri SP, Tambe AB, Apparao BJ. Induction of a novel high yielding mutant of pigeon pea. Asian J. Exp Biol Sci Spl, 2010, 152-155.
8. Girija M, Dhanvel D. Mutagenic Effectiveness and Efficiency of Gamma rays, EMS and their combined treatments in cowpea (*Vigna unguiculata* L. Walp.); Global J. Mol. Sci. 2009; 4(2):68-75.
9. Gordon SA, Webber RP. Studies on the mechanism of phytohormone damage by ionizing: The Radio sensitivity of Indoleacetic Acid. Plant Physiol. 1955; 30(3):200-10.
10. Gustafsson A. The mutation system of the chlorophyll apparatus. Lund Univ Arskr. 1940; 36:1-40.
11. Kharakwal MC. Induced mutations for improvement of protein in chickpea (*Cicerarietinum* L.) Indian J. Genet. 1998; 58:61-68.
12. Kharkwal MC. Induced mutations in Chickpea (*Cicer arietinum* L.) frequency and spectrum of viable mutations. Indian J Genet. 1999; 59(4):451-464.
13. Konzak CP, Nilan RA, Wagner J, Foster RJ. Efficient chemical mutagenesis. Rad. Bot. 1965; 5:49-70.
14. Kumar DS, Nepolean T, Gopalan A. Effectiveness and efficiency of mutagens gamma rays and ethyl methane sulphonate on limabean. Indian J Genet. 2003; 37(2):115-119.
15. Kundi RS, Gill MS, Singh TP, Phul PS. Radiation-induced variability for quantitative traits in soybean (*Glycine max* L. Merrill). Crop Improv. 1997; 24(2):231-234.
16. Mehra YP. The Wealth Effect in Empirical Life-Cycle Aggregate Consumption Equations. Federal Reserve Bank of Richmond Economic. 2001; 87(2):45-68
17. Padmavathi T, Devi P, Kiranmai V. Induced variability for different biological parameters in soybean. Journal of Cytology and Genetics. 1992; 27:175e177.
18. Pavadai P, Girija M, Dhanavel D. Effect of gamma rays, EMS, DES and COH on protein and oil content in soybean. Journal of Eco biotechnology. 2010; 2(4):47e50.
19. Skoog F. The effect of X-irradiation on auxin and plant growth. J Cellular Comp Physiol. 1935; 7:227-270.
20. Solanki IS, Sharma B. Mutagenic effectiveness and efficiency of gamma rays, ethyleneimine and N-nitroso-Nethyl urea in macrosperma lentil (Lens culinaris Medik.). Indian Journal of Genetics and Plant Breeding. 1994; 54(1):72e76.
21. Solanki SI. Isolation of macromutations and mutagenic effectiveness and efficiency in lentil. Indian J Genet. 2005; 65(4):264-268
22. Thilagavathi C, Mullainathan L. Isolation of macro mutants and mutagenic effectiveness, efficiency in black gram (Vigna mungo (L.) Hepper). Global Journal of Molecular Sciences. 2009; 4(2):76e79.
23. Waghmare VN, Mehra RB. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus*. Indian J Genet. 2001; 61(1):53-56.