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MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF GAMMA RAYS, EMS AND THEIR COMBINED TREATMENT IN *PHASEOLUS LUNATUS* L.

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Mutagenic effectiveness and efficiency are two important factors to study a mutagen. The determination of these factors is necessary in plant breeding programs. In this research work the study was performed by irradiation of seeds with various doses of Gamma rays and different concentrations of EMS and combination of both mutagens (Gamma rays and EMS) along with control. From M1 generation the biological damages (lethality and pollen sterility) and from M2 generation the chlorophyll mutation and morphological mutation were considered for calculating the effectiveness and efficiency. Mutagenic effectiveness was inversely proportional to the dose/concentration of mutagens. The maximum efficiency at lower dose/concentrations of the mutagens was due to the pollen sterility and lethality (Biological destructions) which increased as the mutagenic effect increased. Mutation rate is the mean values of mutagenic efficiency that were taken into consideration for each treatment to calculate the mutation rate which gives an idea of the average rate of mutation induction per mutagen.

Keywords: Lethality, Mutagenic effectiveness, Mutagenic efficiency, Mutation rate and Pollen sterility

Induced mutation plays a very important and significant role in crop improvement of agricultural crops. It is an important and essential tool for induction of variations in quantitative and qualitative characters of plants. Induced mutation is of considerable value for accelerating the process of plant breeding (Girija *et al*. 2013). Lima bean (*Phaseolus lunatus* L.) is a species suitable to low altitude, humid and sub humid tropical climatic conditions. It can be grown in a wide range of ecological conditions. Lima bean is a minor grain legume with annual and perennial crop habitat which is propagated through its seeds (Van der Maeseen and Sadikin, 1989). They are herbaceous, twining climber crops. The vegetative period of lima bean is in the range of 80 days -90 days or 120 days (Ibeawuchi 2007). *Phaseolus lunatus* L. is an excellent source of nutrients and proteins for humans but is underexploited and a neglected legume species which makes the minor crop an important choice for research.

In this research work the study was performed by irradiation of seeds with various doses of Gamma rays and different concentrations of EMS and combination of both mutagens (Gamma rays and EMS) along with control. Gamma rays and EMS mutagens are selected because they are safe, easy to use and most importantly they are effective in inducing mutagenesis. They also have a higher degree of accuracy and high reproducibility than other mutagens. Gamma radiations have high penetrating power and high mutation frequency that can cause point mutation and small deletion of 2-6 bp. EMS induces lesions such as chromosomal aberration. In the combined treatment the synergistic effect can be observed. The level of dose/conc of mutagens should be chosen such that it should be high enough but below the level that cause damage or lethality.

MATERIALS AND METHODS

Physical mutagen- Gamma radiation: Healthy, uniform size and dry seeds of *Phaseolus lunatus* L. (lima bean) were packed in the polythene bags and sealed for Gamma radiation. Electromagnetic ionizing radiations were applied from CO^{60} source of irradiation.

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To ascertain the value of LD 50 the seeds were exposed to four doses of Gamma rays i.e., 240Gy, 300Gy, 360Gy and 420Gy. The exposure of radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, Savitri bai Phule University of Pune, Pune.

Chemical mutagen- EMS: About 50 dry seeds were selected for the present treatment. Ethyl Methanesulphonate (EMS) was obtained from Spectrochem. Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and its density was 1.20g/cm^3. Fresh batch of EMS was used. Four different concentrations 0.25%, 0.50%, 0.75% and 1% of the chemical mutagenic treatment were taken. EMS treatments were administered at room temperature at 25±2°C. Prior to treatment the seeds were washed thoroughly and presoaked in 100ml distilled water for 4 hours. Later the seeds were kept on filter paper to remove excess moisture in them. After presoaking, the seeds were immersed in mutagenic solution for 4 hours with continuous shaking.

Combination of Gamma rays and Ethyl Methanesulphonate: The combination treatment 240Gy+1%, 300Gy+0.75%, 360Gy+0.50% and 420Gy+1% consists of both physical mutagenic treatment and chemical mutagenic treatment. First the Gamma rays irradiated seeds of different doses were used. After the physical treatment the chemical treatment of EMS was conducted at room temperature where the seeds were immersed in EMS mutagenic solution for 4 hours.

The Experimental genotype selected for the present investigation was *Phaseolus lunatus* L. variety *King of Garden* (Pole). The experimental seed material was collected from Sheti Udyog Bhandar, Swargate, Pune, Maharashtra, India. After harvesting M1 generation the seeds of healthy, disease free plants from each mutagenic treatment were selected to obtain M2 generation plants (Sinha Mahapatra and Rakshit 1990). From each mutagenic treatment i.e. Gamma rays, EMS and combination of both about 50 seeds from M1 progeny were selected for M2 studies. M2 generation was raised with three replications in Randomized block design (RBD).

Mutagenic effectiveness and efficiency of different mutagens were calculated as per Konzac *et al*. (1965). Mutagenic effectiveness can be calculated by the frequency of mutations induced by a unit dose of mutagen (Gy or time x concentration) while mutagenic efficiency can be measured by the proportion of mutation in relation to the biological damage such as lethality and pollen sterility in M1 generation and chlorophyll mutation and morphological mutations in M2 generations.

a) $$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Dose of Physical mutagen (Gy)}}$$

$$\text{Mutagenic effectivenes} = \frac{\text{Mutation frequency (MF)}}{\text{Conc. of Chemical mutagen} \times \text{Duration of Treatment (C} \times \text{T)}}$$

Where,
MF= % of chlorophyll mutations in M2 Generation
Gy =unit dose of Gamma radiation
C= Concentration of chemical mutagen
T= Period of treatment with chemical mutagen

b) $$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage in M1}}$$

$$= \text{MF/L, MF/S}$$

Where,
L= % of Lethality in M_1 Generation or reduction in plant survival
S= % of pollen sterility in M_1 Generation or reduction in pollen fertility

c) Mutation rate:
The mutation rate can be calculated by the following formula:

$$\text{MR} = \frac{\text{Sum of values of effectiveness or efficiency of a particular mutagens}}{\text{Number of treatments of that particular mutagen}}$$

Table No. 1 : Effectiveness of mutagens in M2 Generation of *Phaseolus lunatus* L.

Mutagens	Dose/Conc	% Chlorophyll Mutation	Effectiveness MF/Dose or MF / C*T
Control	-	-	-
Gamma Rays	240 Gy	8.33	0.035
	300 Gy	10.86	0.036
	360 Gy	13.33	0.037
	420 Gy	18.6	0.044
EMS	0.25%	6.66	0.027
	0.50%	11.9	0.024
	0.75%	12.82	0.017
	1%	15.38	0.015
Combination (Gamma rays + EMS)	240 Gy+1%	10.00	0.008
	300 Gy+0.75%	12.82	0.012
	360 Gy+0.50%	13.51	0.016
	420 Gy+0.25%	18.91	0.028

Table 2 : Relative efficiency of mutagens in M2 Generation of *Phaseolus lunatus* L.

Mutagens	Dose/Conc	% Chlorophyll Mutation	% Lethality	Efficiency	% Pollen Sterility	Efficiency MF/S
Control	-	-	-	-	-	-
Gamma Rays	240 Gy	8.33	17.37	0.479	12.2	0.682
	300 Gy	10.86	18.96	0.572	8	1..357
	360 Gy	13.33	20.81	0.64	5.2	2.563
	420 Gy	18.6	22.3	0.834	4.3	4.326
EMS	0.25%	6.66	15.86	0.419	13.1	0.508
	0.50%	11.9	17.5	0.68	9.1	1.307
	0.75%	12.82	18.21	0.007	7	1.831
	1%	15.38	20.04	0.767	6.7	2.295
Combination (Gamma rays+ EMS)	240 Gy+1%	10	19.21	0.52	10.5	0.952
	300 Gy+0.75%	12.82	20.86	0.614	8.2	1.563
	360 Gy+0.50%	13.51	22.75	0.593	4.9	2.757
	420 Gy+0.25%	18.91	23.81	0.794	4.1	4.612

Table 3: The mutation rate of the mutagens based on efficiency and effectiveness in M2 generation of *Phaseolus lunatus* L.

Mutagens	Mutation Rate based on Efficiency of lethality	Mutation Rate based on Efficiency of pollen sterility	Mutation Rate based on effectiveness
Gamma Rays	0.631	2.524	0.038
EMS	0.468	1.485	0.021
Combination (Gamma Rays + EMS)	0.630	2.471	0.016

RESULTS AND DISCUSSION

1. Mutagenic effectiveness- Mutagenic effectiveness is the induction of mutation by unit dose of mutagen (Gy or Concentration x Time). It was inversely proportional to the dose/concentration of mutagens. The effectiveness decreased with increasing doses/concentration of mutagens in M2 generation. It was highest in 420Gy Gamma radiation treatment (0.044) and lowest in 300Gy+0.75% of combination treatment (0.015). In M2 generation the EMS was more effective than Gamma radiation and combination treatment.

2. Mutagenic efficiency- Mutagenic efficiency is the determination of all the biological damages (lethality and pollen sterility) which are induced by mutagens and are undesirable in nature. The lethality and pollen sterility showed decrease in mutagenic efficiency. The maximum efficiency at lower dose/concentrations of the mutagens was due to the pollen sterility and lethality (Biological destructions) which increased as the mutagenic effect increased

Mutagenic efficiency due to lethality: With respect to lethality the Gamma rays, EMS and combination treatments showed gradual decrease in mutagenic efficiency with increase in dose/conc of the mutagens. The highest mutagenic efficiency on the basis of lethality was observed at 420Gy of Gamma radiation treatment (0.834) and the lowest efficiency was observed at 0.75% of EMS treatment (0.767).

Mutagenic efficiency due to pollen sterility: The highest mutagenic efficiency was observed at 420Gy+0.25% of combination treatment (4.612) while the lowest was observed at 0.25% of EMS treatment (2.295).

3. Mutation rate- Mutation rate is the mean values of mutagenic efficiency that were taken into consideration for each treatment to calculate the mutation rate and gives an idea of the average rate of mutation induction per mutagen. It can be observed that the order of mutagen changes due to the variations in the values of lethality and pollen sterility.

Mutation rate due to Lethality: The lethality value for Gamma rays was 0.631, for EMS it was 0.468 and for combination it was 0.630. With respect to lethality in M2 generation, the severity of mutagens in M2 generation of *Phaseolus lunatus* L. can be framed as Gamma radiation > combination (Gamma rays + EMS) > EMS.

Mutation rate due to Pollen sterility: The lethality value for Gamma rays was 2.524, for EMS it was 1.485 and combination it was 2.471. With respect to pollen sterility the mutation rate can be framed as Gamma radiation > combination (Gamma rays + EMS) > EMS.

Mutagenic effectiveness and efficiency are two important factors to study a mutagen. According to Patil and Rane (2015) the effectiveness and efficiency gives an idea to evaluate a mutagen. The determination of these factors is necessary in plant breeding programs. The effectiveness and efficiency are studied so that the mutagens that cause genetic variability can be assessed for the selection of genotypes which can be used for the improvement of crops in plant breeding programs. The Mutagenic effectiveness is the induction of mutation by unit dose of mutagen (Gy or Concentration x Time). Mutagenic efficiency is an estimate of biological effects such as lethality and pollen sterility. Khan (1981) stated that variation in effectiveness and efficiency of mutagens show genotypic response which is of genetic origin and depends upon the physical and chemical properties of the mutagens. From M1 generation the biological damages (lethality

and pollen sterility) and from M2 generation the chlorophyll mutation and morphological mutation were considered for calculating the effectiveness and efficiency. It was reported that the plants showed difference at the genotypic level (Kundi *et al*. 1997). Blixt (1970) stated that the differential factor in these crops was due to the relation of mutagens used and the genetic makeup. Some factor is responsible for the variation in each mutagen that cause sensitivity (effectiveness and efficiency) in crops.

Induction of mutagenic treatment is an index of effectiveness of mutagens. It was observed that the lower doses of mutagens were most effective in nature. The increase in dose/concentration of mutagens decreased the mutagenic effectiveness of the crop (Jaykumar *et al*. 2003). Higher mutagenic effectiveness at lower concentrations of EMS was observed in Urd bean by Sharma *et al*. (2005). In the present investigation the highest mutagenic effectiveness was observed in 420Gy Gamma radiation treatment (0.044) and the lowest was observed in 300Gy+0.75% of combination treatment (0.015). Similar results were observed in Cluster bean by Bhosale and Kothekar (2010) and in Black gram by Thilagavathi and Mullainathan (2009). Kulthe and Mongle (2014) confirmed this trend in Winged bean too. Mutagenic effectiveness was inversely proportional to the dose/concentration of mutagens. Khursheed *et al.* (2018) stated that the possible reason for decrease in effectiveness and efficiency of mutagens with the increase in dose/conc may be due to the higher dose of mutagens that are more harmful than the lower dose with less damaging effects. Mehtre (1990) in Green gram reported the inverse relationship of seed germination and survival percentage to doses/concentrations of mutagen treatments. There are many causes for the decrease of seed germination percentage and survival rate of plants. Madhu *et al*.(2015) stated that a highly effective mutagen may not necessarily show high efficiency in plants.

Konzak *et al*. (1965) observed that the mutagenic efficiency holds a lot of theoretical and practical knowledge which is of prime importance in improvement of crops. Mutagenic efficiency is the determination of all the biological damages (lethality and pollen sterility) which are induced by mutagens and are undesirable in nature. Efficiency is the frequency of chlorophyll mutations induced in M2 generation to various biological damages observed in M1 generation. The pollen sterility and lethality showed decrease in mutagenic efficiency as the dose/concentration of the mutagens increased. The maximum efficiency at lower dose/concentrations of the mutagens was due to the pollen sterility and lethality (biological destructions) which increased as the mutagenic effect increased. This was observed by Konzak *et al*. (1965). The highest mutagenic efficiency on basis of lethality was recorded in 420Gy of Gamma radiation treatment (0.834) and the lowest lethality was at 0.75% of EMS treatment (0.767). The highest mutagenic efficiency on basis of Pollen sterility was observed in 420Gy+0.25% of combination treatment (4.612) while the lowest was observed at 0.25% of EMS treatment (2.295). The efficiency decreased with increase in dose/concentration of mutagens. The similar findings were recorded by Sangle and Kothekar (2013) and by Mishra and Singh (2014) in Green gram. The biological damages (lethality and pollen sterility) showed reduction in plant survival and pollen fertility. With respect to lethality the Gamma rays, EMS and combination treatments show gradual decrease in mutagenic efficiency with increase in dose/conc of the mutagens. The similar results were observed by Khan and Wani (2006). Sassi Kumar *et al*. (2003) studied lima bean and observed that the efficiency was maximum at high Dose/Conc in Gamma rays and EMS treatment with respect to lethality and sterility and the less dose/Conc

caused less seedling injury in the plants.

Mensah and Erouter (1993) reported LD 50 value for Gamma radiation in lima bean based on the germination and survival percentage. Thirugnanakumar (1986) reported the LD50 value for EMS in cowpea based on the germination and survival percentage. Gordon and Webber (1955) reported the drop in auxin levels which lead to the fall of germination of seeds and survival of plants. Other reasons for the reduction of germination percentage and mortality of plants were due to the inhibition of auxin synthesis reported by Skoog (1935), due to chromosomal aberrations reported by Sparrow (1961) or due to the decline of assimilation mechanism observed by Quastler and Baer (1950). Factor like biological damage was also responsible for the fall. Taking all these factors into consideration the mutation rate was calculated for M2 generation plants.

Mutation rate is the mean value of mutagenic efficiency that was taken into consideration for each treatment to calculate the mutation rate which gives an idea of the average rate of mutation induction per mutagen. It can be observed that the order of mutagen changes due to the variations in the values of lethality and pollen sterility. The lethality in M2 generation for Gamma rays was 0.631, for EMS it was 0.468 and for combination it was 0.630. The severity of mutagens in *Phaseolus lunatus* L. can be framed as Gamma radiation > combination (Gamma rays + EMS) > EMS. Pollen sterility for Gamma rays was 2.524, for EMS it was 1.485 and combination it was 2.471. The mutation rate can be framed as Gamma radiation > combination (Gamma rays + EMS) > EMS. Mutation rate was determined for Mutagenic efficiency which reflected that Gamma radiation was more efficient than EMS in *Phaseolus lunatus* L. Girija and Dhanvel (2009), Ramya *et al*. (2013) studied mutation rate with similar results. D'Amato (1962) stated that the mutation rate which is calculated on the basis of the mutation frequency in M1 generation, is overestimated and hence it suffers diplomatic selection which is observed by a drop in higher mutation frequency. Unlike this, in M2 generation, there is independency of variations in the plant size and proportional to the initial mutation rate (Gaul 1960).

In all the plant breeding programmes, the determination of mutagenic effectiveness and efficiency is quite important. If the mutagenic effectiveness and efficiency are calculated, a greater number of economic mutants could be released in the market.

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