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Mutagenic Effectiveness and Efficiency of Gamma Rays and EMS in *Phaseolus vulgaris* L.

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A B S T R A C T

The mutagenic effectiveness is a measure of factor mutations induced by a unit dose of mutagen. In M_2 generation of French bean after Gamma rays treatment, the numerical value of effectiveness gradually decreased with increases in the dose of the mutagen. The range for Gamma rays treatment was 0.09 to 0.099.Similar trend was observed for EMS treatment. It was ranged from 6.86 to 9.8 in EMS treatment and in cases of Combination treatment the range was 0.12 to 0.34.Combination of lower dose of Gamma ray and EMS was found to be more effective. The value of efficiency decreased as there were increases in doses of Gamma rays treatments. It was ranged from 0.29 to 0.45. In EMS treatments, the range was 0.36 to 0.45. The efficiency in relation to pollen sterility varied from 0.36 to 0.40 in case of Combination treatments.

Introduction

French bean (*Phaseolus vulgaris* L.) belongs to the family Fabaceae. It is also known as *rajmash*, *rajma (Hindi)*, haricot bean, kidney bean, common bean, snap bean or navy bean. It is also known as *Ghewada* in Marathi. It is native of South America. It is widely cultivated in tropics, sub tropics and temperate regions.

The pulses are belongs to the family Fabaceae which is also known as food legumes. Pulses are second important group of the crops after cereals. Pulses are the most important crops of Indian Agriculture.

The member of Fabaceae family includes the economically important legume like pulses, oil seed, forage and fodder crops and also shrubs and tropical or subtropical trees. These pulses are chickpea, pigeon pea, lentil, mungbean, urdbean, field pea, grass pea, French bean, common bean and horse gram. The split grains of these pulses are known as dal which is excellent source of high quality of proteins, amino acids, fatty acids, fibers, minerals and vitamins. The pulses are rich in plant proteins and cereals are rich in carbohydrates, which are an excellent combination for a balanced human diet. Legumes are more superior to cereals

as sources of micronutrients. Pulses are being consumed due to presence of various amino acids. As a rich source of proteins, legumes also provide a nutritional fodder for cattle.

Pulses crops are the most economic source of proteins. In spite of being the major source of proteins and staple food for the majority of Indian population the production of pulses is less improved (Shakeel and Hashmi, 2012).The first "Green Revolution" contributes the development of pulses and cereals for their enhancement of production. The second "Green Revolution" has contributed the development and production of proteins in pulses. This will provide the staple diet to the humans in the shade of "World Food Crises. Maharashtra state is the third rank in production of pulses in India after Madhya Pradesh and Utter Pradesh, which contributes about 14% of production in India. The dried bean of French bean contains 22.95% proteins, 60.6% carbohydrates and 3.2% minerals like Calcium 260 mg, Phosphorous 410 mg and Iron 5.8 mg contents in the forms of 346 calories / 100 gm. It also contents vitamins, folic acids and pentatonic acids like thiamine 0.6%, riboflavin 0.2%, nicotinic acid 2.5%, ascorbic acids 2.0 mg/100gms.

Materials and Methods

Experimental genotype selected for the present investigation was French bean *Phseolus vulgaris* L. It is also known as *rajmash*, *rajma (Hindi),* haricot bean, kidney bean, common bean, snap bean or navy bean. It is also known as *Ghewada* in Marathi. The experimental seed material of French bean variety – *Varun* collected from Ganeshkhind, Dist: Pune, Maharashtra, India released by Mahatma Phule Agricultural University, Rahuri, Dist-Ahmednager Maharashtra, India.

The following Physical and Chemical Mutagens used for mutation studies.

Physical Mutagen – Gamma Rays, Chemical Mutagen – Ethyl Methanesulphonate (EMS)

Combination of Gamma Rays and Ethyl Methanesulphonate (EMS)

Gamma rays

Physical mutagen can be induced mutations in plants derives directly from the discoveries of X-rays by (Roentgen, 1895) radioactivity by Becquerel in 1896; and radioactive elements by Marie. In addition to the ionizing radiations, the other commonly used physical mutagens are the high energy ionizing particles, alpha (α) and beta (β) particles and neutrons. The mutagenic of these agents derives from a combination of their ability to produce dimmers and reactive ions which in turn cause damage to living organisms; the damage caused ranges from aberrations at the DNA level.

Gamma rays are emitted by certain radioactive elements energies in special machines. Gamma rays have wavelength shorter than the wavelength of ultraviolet light. X rays, which have wavelength of 0.1 to 40 nm, and gamma rays,

Healthy, uniform size and dry seeds of the French bean variety – *Varun* were packed in polythene bags and sealed for the Gamma radiation. Electromagnetic, ionizing radiations were applied from Co^{60} source of irradiation. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, University of Pune, Ganeshkhind, and Pune - 411007. The seed samples were exposed to doses of 30kR, 40kR, 50kR and 60kR of gamma rays.

Ethyl Methanesulphonate (EMS)

EMS is a mutagenic, teratogenic, and carcinogenic organic compound with formula $CH_3SO_3C_2H_5$. It produces random mutations in genetic material by nucleotide substitution particularly by guanine alkylation. It can induce mutations at a rate of $5x10^{-4}$ to $5x10^{-2}$ per gene without substantial killing. The ethyl group of EMS reacts with guanine in DNA, forming the abnormal base O-6-methylguanine.

Ethyl Methanesulphonate (EMS) was obtained from Spectro chem Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and its density 1.20 g/cm^3 to determine the lethal dose (LD_{50}) at suitable concentration of mutagen for the further study. Chemical mutagenic treatments were administered at room temperature of 25 ± 2°C. Healthy and dry seeds of the French bean variety– *Varun* having uniform size were selected for the treatment. Seeds were surface sterilized with 0.1% mercuric chloride solution for about one to two minutes than washed thoroughly and soaked in distilled water for 6 hours for pre -soaking of seeds, which were made the seed coat permeable for the mutagenic treatment.

The fresh, aqueous solutions of the mutagen were prepared prior to treatments. The different concentrations used for the chemical mutagenic treatments were 0.1%, 0.15%, 0.20% and 0.25%. After the pre - soaking seeds were immersed in the mutagenic solution for 4 hours with continuous shaking. The volume of the chemical solution used was five times more than that of the seeds to facilitate uniform absorption. Seeds soaked in distilled water for 6 hours served as control. Immediately after the completion of treatment, the seeds were washed thoroughly under running tap water for 3 to 4 times. Later on they were subjected to post - soaking in distilled water for 4 hours.

For each treatment, batches of 500 seeds were used. 100 seeds from each were plotted between the folds of filter paper, kept in dark at room temperature, which was used to record the germination percentage and seedling injury. Another 100 seeds were kept in filter paper and germinated in petriplates after three days to raise the root tips required to study cytological preparations for the mitotic index and screening of chromosomal abnormalities. The remaining lots of 300 seeds of each treatment along with control (untreated seeds) were sown in research field by Complete Randomized Block Design (CRBD) with three replications in order to raise the M_1 generation.

Combination treatment

For the combination treatments Gamma rays irradiated seed samples were used. After the physical mutagenic treatment, chemical mutagenic treatment of EMS was conducted on the same seed samples. In the combination, Gamma rays and EMS was used like 30 kR+0.10%, 40 kR+0.15%, 50 kR+0.20% and 60 kR+0.25%.

For each treatment, a batch of 500 seeds was used. From each treatment 100 seeds were plotted between the folds of filter paper and kept in dark at room temperature, which was used to record the germination percentage and seedling injury. Another 100 seeds were kept in filter paper and germinated in glass plates after three days to raise the root tips required to study cytological preparations for the mitotic index and screening of chromosomal abnormalities. The remaining lots of 300 seeds of each treatment along with control (untreated seeds) were sown in research field by Complete Randomized

Block Design (CRBD) with three replications in order to raise the M_1 generation.

Pollen sterility

Pollen sterility was determined in 25 randomly selected plants from each treatment. The pollen grains from freshly dehisced anthers were stained with 1% Acetocarmine. The pollen grains which stained were counted as pollen fertile and partially unstained was considered as pollen sterile.

Chlorophyll mutations

The chlorophyll mutations were screened and recorded in the field when the seedlings were 7-10 days old. The types of chlorophyll mutations scored like *albina*, *Xantha*, *chlorina* and *Viridis*. These are classified according to the terminology of (Gustafsson, 1940). The frequency of chlorophyll mutants was calculated according to (Gaul, 1960) i.e. Number of mutants / 100 M_2 plants.

Estimation of mutagenic effectiveness and efficiency

Mutagenic effectiveness and efficiency of different mutagens were calculated according to the formulae suggested by (Konzak *et al.,* 1965). The mutagenic effectiveness can be measured of the frequency of mutations induced by a unit dose of mutagen (kR or time × concentration) while mutagenic efficiency gives an idea of the proportion of mutations in relation to biological damage such as lethality, seedling injury, pollen sterility and chromosomal aberrations.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Dose or (Time X Concentration)}}$$

$$= \text{MF/kR or MF/ TC}$$

Where,

MF = % of chlorophyll mutations in M_2 generation.
T = Period of treatment with chemical mutagen.
C = Concentration of chemical mutagens,
kR = unit of gamma radiation.

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

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

Where,

L = % of lethality in M_1 generation.
I = % of seeding injury in M_1 generation.
S = % of pollen sterility in M_1 generation.
MI = % of mitotic abnormalities in M_1 generation.

Mutation rate

The mutation rate was calculated by the following formula:

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

This gives an idea of mutations induced by a particular mutagen irrespective of dose.

Results and Discussion

Experimental Observation

Mutagenic effectiveness (Table No.1 to 3)

The mutagenic effectiveness is a measure of factor mutations induced by a unit dose of mutagen. In M_2 generation of French bean after Gamma rays treatment, the numerical value of effectiveness gradually decreased with increases in the dose of the mutagen. The range for Gamma rays treatment was 0.09 to 0.099.Similar trend was observed for EMS treatment. It was ranged from 6.86 to 9.8 in EMS treatment and in cases of Combination treatment the range was 0.12 to 0.34.Combination of lower dose of Gamma ray and EMS was found to be more effective. It means in Combination treatments the effectiveness decreases with higher dose/concentration of Gamma rays and EMS. Out of the three treatments, the maximum value of effectiveness could be seen in EMS followed by Combination and Gamma rays. Highest effectiveness 9.8 was observed at 0.10% EMS and lowest effectiveness 0.09 was observed at 30kR of Gamma rays treatment.

In M3 and M4 generation the values of effectiveness demonstrated decrease as compared to M2 generation. The effectiveness was found in range of 0.02 to 6.29 in all mutagenic treatments for M3 generation. The range of effectiveness for M4 generation was 0.036 to 5.77 in all mutagenic treatments. The lower dose/concentration 30kR + 0.10% of Combination treatment showed lowest value 0.02 for effectiveness. In all three generations lower dose/concentration of Combination showed higher values of effectiveness where as individual treatments of dose/concentration of Gamma ray and EMS showed dose dependent increased in effectiveness with increases in dose/concentration.

Mutagenic efficiency (Table No.4)

The mutagenic efficiency is the ratio of chlorophyll mutations induced in the M_2 generation to the various biological damages induced in M1 generation such as lethality and pollen sterility.

Efficiency in relation to lethality

In the EMS treatment, the efficiency in relation to lethality showed a rise up to a certain dose and then a decline. The highest efficiency 0.19 was observed at 60kR+0.25% in Gamma rays and EMS treatments while the minimum value 0.14 could be seen at 50kR dose of Gamma rays. After EMS treatment, the efficiency showed progressive decreased with an increase in the concentration. It ranged from 0.17 to 0.17. A similar trend like EMS treatment was observed in Combination treatment. In Combination treatment the efficiency was ranged from 0.14 to 0.17.

Efficiency in relation to Pollen sterility

The observations in respect to efficiency with reference to pollen sterility demonstrated a dose/concentrations dependent enhancement in majority of the mutagenic treatments in M_2 generation of French bean. The value of efficiency decreased as there were increases in doses of Gamma rays treatments. It was ranged from 0.29 to 0.45. In EMS treatments, the range was 0.36 to 0.45. The efficiency in relation to pollen sterility varied from 0.36 to 0.40 in case of Combination treatments.

Mutation rate (Table No.5)

The mutation rate was calculated by taking the mean values of efficiency for each treatment. This provides an idea of the average rate of mutation induction per mutagen. It could be noted that when the mutation rates based on efficiency were considered, the order of mutagens changes as the mutagens have varied values in relation to lethality and pollen sterility. Considering the mutation rates for lethality, the value were 0.60 for Gamma rays, 0.69 for EMS and 0.64 for Combination treatments. Thus with respect to lethality, in M2 generation of French bean the order of mutagens could be framed as *EMS > Combination (Gamma rays +EMS) > Gamma rays.* When the mutation rate for pollen sterility is taken into consideration, the values were 1.22 for Gamma rays, 1.55 for EMS and 1.53 for Combination treatments. With respect to pollen sterility the order of mutagens in the increasing direction was *EMS > Combination (Gamma rays+ EMS) >Gamma rays*

Efficiency in relation to Pollen sterility

The observations in respect to efficiency with reference to pollen sterility demonstrated a dose/concentrations dependent enhancement in majority of the mutagenic treatments in M_2 generation of French bean. The value of efficiency decreased as there were increases in doses of Gamma rays treatments. It was ranged from 0.29 to 0.45. In EMS treatments, the range was 0.36 to 0.45. The efficiency in relation to pollen sterility varied from 0.36 to 0.40 in case of Combination treatments.

Mutagenic effectiveness and efficiency

The mutagenic effectiveness indicates the frequency of mutation induced by a unit dose/concentration of mutagen. The mutagenic efficiency provides an idea of mutational frequency in relation to biological damage such as lethality, injury, sterility and chromosomal aberrations produced due to the mutagenic treatments (Konzak *et al.,* 1965). In present investigation, the chemical mutagen EMS proved to be more effective than the Gamma rays and Combination of both treatments. It was found that the lower concentration of EMS mutagen proved to be more effective than the higher doses of Gamma rays and Combination. The higher effective values DES over the gamma rays has been recorded by (Monti, 1968) in Pea, (Nerkar, 1977) in *Lathyrus* reported more effectiveness of NMU over EMS and Gamma rays. The similar observation has been recorded by (Hakande, 1992) in Winged bean after treatment with NEU, EMS and Gamma rays. The same result was recorded by (More, 1992) in Alfalfa induced through EMS,NMU, MHz and Gamma rays and also found good result in Cowpea by (Gaikwad,2013), In Cluster bean by (Shinde, 2013), in Coriander by (Salve, 2014) and in *Withania* by (Bhosale, 2014). This parameter has been attributed to the lower percentage of injury at lower doses and rapid increased at the higher doses (Konzak,

et al., 1965). According to them, reduced the rate of injury and the increases the mutation should be compared the objective in any mutation program (Konzak, *et al*., 1965). In case of *Phaseolus vulgaris* L., the order of efficiency of mutagen varied with different biological parameters studied.

In account with lethality, the efficiency decreased in the order EMS, Combination of both and Gamma rays. The efficiency base on pollen sterility revealed the order as EMS, Combination of both and Gamma rays. Many researchers reported the same result after mutagenic treatments (Sharma and Sharma, 1979) have reported alkylating agent to be more effective than the Gamma rays treatments. (Reddy *et al*., 1988) recorded a higher efficiency of SA than Gamma rays and Combination treatments. (Hakande, 1992) reported that efficiency value for EMS than MEU are higher than Gamma rays. (Prasad, 1972) reported that Gamma rays were more efficient than the alkylating agent in inducing seed sterility. (More, 1992) reported that the EMS and NMU was more effective than the MHz and Gamma rays treatments. (Shinde, 2013) reported that EMS was found to be more effective than Gamma rays and Combination treatment in Cluster bean, (Gaikwad, 2013) reported that EMS was found to be more effective than Gamma rays and Combination treatment in Cowpea The order of effectiveness and efficiency of the mutagens were EMS > GR+EMS > GR. The decreased in effectiveness with increases concentrations of mutagens produced the biological damage like lethality and pollen sterility.

Table.1 Physico-chemical parameters of water (Mean ± SD and Ranges) in three treatments Effectiveness of mutagens in M2 generation of *Phaseolus vulgaris* L.

Mutagens	Dose/Conc.	Frequency of % chlorophyll mutation (MF)	Effectiveness (MF/doses) or MF/T×C	Interaction Coefficient K
	Control	0		
Gamma rays	30kR	2.7705	0.09235	-
	40kR	3.7837	0.09459	-
	50kR	4.6009	0.09201	-
	60kR	5.9903	0.09983	-
EMS	0.10%	3.9316	9.8	-
	0.15%	4.7488	7.91	-
	0.20%	5.8095	7.26	-
	0.25%	6.8627	6.86	-
Gamma rays + EMS	30kR+ 0.10%	4.1441	0.3453	1.61
	40kR + 0.15%	5.5238	0.2305	1.53
	50kR + 0.20%	5.3731	0.1342	1.93
	60kR + 0.25%	7.6767	0.1279	1.67

Table.2 Effectiveness of mutagens in M3 generation of *Phaseolus vulgaris* L.

Mutagens	Dose/Conc.	Frequency of % chlorophyll mutation (MF)	Effectiveness (MF/doses) or MF/T×C	Interaction Coefficient K
	Control	0		
Gamma rays	30kR	1.7857	0.05952	-
	40kR	2.3391	0.05847	-
	50kR	2.4475	0.04895	-
	60kR	3.1446	0.05241	-
EMS	0.10%	2.5187	6.29	-
	0.15%	3.1155	5.19	-
	0.20%	3.6853	4.60	-
	0.25%	4.0905	4.09	-
Gamma rays + EMS	30kR+ 0.10%	2.5948	0.02162	1.66
	40kR + 0.15%	3.7037	0.1543	1.47
	50kR + 0.20%	3.3485	0.08371	1.83
	60kR + 0.25%	4.3567	0.07261	1.66

Table.3 Effectiveness of mutagens in M4 generation of *Phaseolus vulgaris* L.

Mutagens	Dose/Conc.	Frequency of % chlorophyll mutation (MF)	Effectiveness (MF/doses) or MF/T×C	Interaction Coefficient K
	Control	0		
Gamma rays	30kR	1.4383	0.04794	-
	40kR	2.002	0.05005	-
	50kR	2.5396	0.050792	-
	60kR	1.2176	0.03696	-
EMS	0.10%	2.3104	5.77	-
	0.15%	2.2162	3.69	-
	0.20%	2.9113	3.63	-
	0.25%	3.3333	3.33	-
Gamma rays + EMS	30kR+ 0.10%	2.6562	0.22135	1.41
	40kR + 0.15%	2.7359	0.11399	1.54
	50kR + 0.20%	2.7679	0.069198	1.97
	60kR + 0.25%	3.7063	0.061773	1.22

Table.4 Relative Efficiency of treatment of mutagens in M2 of *Phaseolus vulgaris* L.

	Dose/Conc.	%Chlorophyll Mutation (MF)	% Lethality (L)	Efficiency (MF/L)	Pollen Sterility (S)	Efficiency (MF/S)
	Control	0	18.37	0	8	0
Gamma rays	30kR	2.77	18.34	0.1510	8.33	0.3325
	40kR	3.78	25.34	0.1491	12.33	0.3065
	50kR	4.6	32	0.1437	15.55	0.2967
	60kR	5.99	38	0.1576	20.53	0.2917
	Control	0	20.34	0	8.333	0
EMS	0.10%	3.94	22.34	0.1763	8.66	0.4546
	0.15%	4.74	27.34	0.1733	12.66	0.3742
	0.20%	5.8	34	0.1705	16	0.3625
	0.25%	6.86	39.52	0.1735	19	0.3610
	Control	0	20.34	0	11.33	0
Gamma rays + EMS	30kR+0.10%	4.14	26	0.1593	10.3	0.4019
	40kR+0.15%	5.52	34.67	0.1592	14	0.3942
	50kR+0.20%	5.3731	34.67	0.1448	14.5	0.3709
	60kRs+0.25%	7.6767	39.50	0.1943	21	0.3655

Table.5 The mutation rate of the mutagens based on Effectiveness and Efficiency in M2 of *Phaseolus vulgaris* L.

Mutagens	Mutagens based on Effectiveness	Mutagens based on Effectiveness and Efficiency	Mutation rate based Efficiency Pollen Sterility
Gamma rays	0.37	0.60	1.22
EMS	31.82	0.69	1.55
Gamma rays + EMS	0.83	0.64	1.53

Many researchers have been reported that the effectiveness and efficiency of mutagens in different plants like (Kumar *et al.*, 2003) in Lima bean, (Sharma *et al.*, 2005) in Urdbean, (Barshile *et al.*, 2006) and (Shah *et al.*,2008) in Chickpea, (Dhanavel *et al.*, 2008) in French bean, (Velu *et al.*, 2008) in Greengram. (Satpute, 2009) in Lentil, (Jain and Khandelwal, 2009) in Blackgram, (Bhosale,2010) in Cluster bean, (Dhulgande *et al.*, 2011) in Pea and (Shinde, 2013) in Cluster bean.

In *Phaseolus vulgaris* L. the value of mutagenic effectiveness and efficiency and their mutation rate is in relation to lethality and pollen sterility. This trend is useful to enhance the chlorophyll mutant frequency produced in further generation.

In conclusion, the values of effectiveness gradually increased with increases in dose/concentration of mutagen. Of the three mutagenic treatments, the maximum value of effectiveness was seen in Combination treatment followed by EMS and Gamma

rays. The mutagenic efficiency with reference to lethality was Gamma *rays* > *Combination (Gamma rays +EMS)* > *EMS*

The mutagenic efficiency on pollen sterility was

Combination (Gamma rays+ EMS) > *EMS* > *Gamma rays*

It was noted that when the mutation rate based on efficiency was considered, the order of mutagen change as according to the lethality and pollen sterility. The mutation rate in relation to lethality shows in order of mutagens as

Gamma rays > *Combination (Gamma rays +EMS)* > *EMS*

In pollen sterility the order of mutagens like

Combination (Gamma rays+ EMS) > *EMS* > *Gamma rays.*

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