Evaluation of Induced Genetic Variability in Black Turtle Bean (Phaseolus vulgaris L.) Using Gamma Rays, EMS, and Their Combination in Petri Plate Germination Assay

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Abstract:

Induced mutagenesis is a crucial approach for enhancing genetic variability in crop plants. This study evaluates the effects of gamma radiation and Ethyl Methane Sulphonate (EMS) on seed germination, root and shoot length, and fresh biomass of Black Turtle Bean (*Phaseolus vulgaris* L.) under controlled Petri plate conditions. Seeds were exposed to gamma rays (200-600 Gy) from a Cobalt-60 source and various EMS concentrations (0.1-0.6% v/v), individually and in combination. The treated seeds were germinated in triplicate with 25 seeds per replicate to assess their physiological responses. Results indicated a dose-dependent decline in germination percentage, root and shoot length, and biomass, with higher doses showing significant reductions. The combined treatments exhibited more pronounced adverse effects than individual treatments. The findings provide insights into the sensitivity of *Phaseolus vulgaris* L. to mutagens and contribute to understanding mutation breeding strategies for genetic improvement.

Keywords: Induced mutagenesis, gamma rays, EMS, *Phaseolus vulgaris* L., seed germination, mutation breeding.

I.Introduction:

Genetic variability is the foundation for crop improvement, enabling the development of new varieties with desirable traits such as improved yield, disease resistance, and environmental adaptability (Bailey-Serres *et al.*,2019; Fernie *et al.*, 2006). However, the genetic base of many cultivated crops, including *Phaseolus vulgaris* L. (Black Turtle Bean), is often narrow due to domestication and intensive selection. This genetic bottleneck limits the potential for further genetic advancements through conventional breeding alone. Induced mutagenesis offers a viable solution by artificially creating genetic variability, thus expanding the available gene pool for selection (Lynch*et al.*,2016; Awan *et al.*, 2021).

Mutagenesis involves the application of physical or chemical agents to alter the genetic material, leading to the development of novel traits. Gamma radiation, a form of ionizing radiation, is widely used in mutation breeding due to its ability to cause genetic changes by breaking DNA strands, resulting in a range of mutations (Sharma, A. K., & Sharma, R., 2014; Dhole *et al.,2024;* Suprasanna *et al.*,2015*)*. Similarly, Ethyl Methane Sulphonate (EMS), a chemical mutagen, induces point mutations through alkylation of guanine bases, leading to base pair substitutions (Shah*et al*.,2016; Bautz and Freese, 1960). These mutagens, when applied individually or in combination, can significantly alter plant physiological and morphological traits, making them valuable tools in crop improvement programs.

Phaseolus vulgaris L. is a leguminous crop of economic importance, widely cultivated for its nutritional benefits and adaptability to diverse environmental conditions (Uebersax*et al.,* 2023; Chávez-Servia, *et al.,* 2016). However, genetic enhancement is necessary to improve its yield potential and stress tolerance. The application of gamma rays and EMS to *Phaseolus vulgaris* seeds has been reported to induce beneficial genetic changes, though their effects on germination and early seedling growth remain an area of interest (Assefa*et al.,* 2019; Kelly*et al.,* 1998). Understanding the physiological responses of seeds to mutagenic treatments is crucial in determining optimal dosages for mutation breeding while minimizing deleterious effects.

The present study aims to evaluate the effects of gamma rays and EMS on seed germination, root and shoot growth, and fresh biomass of *Phaseolus vulgaris* L. under controlled Petri plate conditions. By assessing the physiological responses to varying doses of mutagens, this research provides insights into the mutagenic sensitivity of *Phaseolus vulgaris* and contributes to optimizing mutation breeding strategies for genetic improvement.

II.Methodology:

- **Sample Collection -** Authentic seeds of Black Turtle Bean (*Phaseolus vulgaris* L.) were procured from the Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist-Ahmednagar, Maharashtra, India. The seeds were selected based on uniformity in size, shape, and moisture content (10-12%), ensuring consistency in the experimental treatments.
- **Mutagenic Treatments-**
    a. **Gamma Radiation Treatment-** Gamma irradiation was performed using a Cobalt-60 (*60Co*) source fixed in a Gamma Cell 200 at the Nuclear Division, Department of Chemistry, Savitribai Phule Pune University, Pune. Dry seeds

were exposed to different doses of gamma rays (200, 300, 400, 500, and 600 Gy). The radiation exposure was conducted in a controlled environment to ensure uniformity in dosage (Araújo, 2015).

b. **Ethyl Methane Sulphonate (EMS) Treatment-**EMS (*C3H8O3S*) was obtained from Sigma Chemical Co. Ltd., USA, and used for chemical mutagenesis. The selected seeds were presoaked in distilled water for 10 hours at room temperature to enhance mutagen uptake. The seeds were then treated with different EMS concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6% v/v) prepared in 0.1M phosphate buffer (pH 7.0) following the standard protocol of Gichner *et al*. (1994). The treatment duration was 4 hours with intermittent shaking to ensure uniform exposure. Post-treatment, the seeds were washed thoroughly under running tap water for 30 minutes to remove residual EMS and terminate the reaction (Gichner*et al*.,1994).

c. **Combined Treatment (Gamma + EMS)-**For combined treatments, seeds were first irradiated with gamma rays at 200, 300, 400, 500, and 600 Gy. These irradiated seeds were then subjected to EMS treatment at the same concentrations as mentioned above, following the same protocol (Wanga *et al*., 2020;Bharatkumar, 2015).

- **Germination Assay and Growth Parameter Evaluation-**

The treated seeds, along with control (untreated seeds), were placed in sterile Petri plates lined with germination paper moistened with distilled water. Each treatment consisted of 75 seeds, with three replicates of 25 seeds per Petri plate. The plates were incubated in a growth chamber under controlled conditions of temperature (25 ± 2°C), relative humidity (70%), and photoperiod (16-hour light/8-hour dark cycle) (ISTA, 2010).

Observations were recorded after seven days of germination, assessing:

a. **Germination percentage** - (Number of germinated seeds / Total seeds) × 100
b. **Root and shoot length (cm) -** Measured using a Scale.
c. **Fresh biomass (g) -** Weighed immediately after harvesting using an analytical balance.

- **Statistical Analysis-** Data were subjected to statistical analysis using one-way ANOVA to determine the significance of differences among treatments ($P < 0.05$). Mean separation was performed using Duncan's Multiple Range Test (DMRT)

(Limpanavech*et al*.,2008).The statistical analysis was carried out using SPSS software (version 25.0).

III. Observation:

Fig.1. Germination Assay and Mutagenic Treatment Responses of Black Turtle Bean Seeds

Fig.2. Impact of Gamma Radiation, EMS, and Their Combination on Germination and Growth Traits of *Phaseolus vulgaris* L.

Table No.1. Effect of Gamma Radiation, EMS, and Their Combination on Germination, Root Length, Shoot Length, and Fresh Biomass of *Phaseolus vulgaris L.*

Treatment	Germination Percentage %	Root (Cm)	Shoot (Cm)	Fresh Biomass (gm)
Control	95	6	8	0.50
200 Gy	85	5.2	6.8	0.42
300 Gy	75	4.4	5.6	0.35
400 Gy	60	3.2	4.3	0.28
500 Gy	45	2.5	3	0.22
600 Gy	30	1.8	2.1	0.15
0.1% EMS	90	5.8	7.8	0.48
0.2% EMS	80	4.9	6.4	0.40
0.3% EMS	70	3.8	5.1	0.32
0.4% EMS	55	2.7	3.7	0.25
0.5% EMS	40	1.9	2.6	0.18
0.6% EMS	25	1.2	1.8	0.12
200 Gy + 0.1% EMS	78	4.3	4.6	0.38
200 Gy + 0.2% EMS	72	4	4.3	0.33
200 Gy + 0.3% EMS	65	3.2	4.	0.28
200 Gy + 0.4% EMS	50	2.5	3	0.22
200 Gy + 0.5% EMS	38	1.9	2.4	0.16
200 Gy + 0.6% EMS	22	1.2	1.5	0.12
300 Gy + 0.1% EMS	70	3.8	4	0.32
300 Gy + 0.2% EMS	65	3.4	3.8	0.28
300 Gy + 0.3% EMS	58	2.6	3.1	0.22
300 Gy + 0.4% EMS	45	2	2.4	0.18
300 Gy + 0.5% EMS	32	1.5	1.9	0.14
300 Gy + 0.6% EMS	20	1.1	1.3	0.10
400 Gy + 0.1% EMS	60	3	3.8	0.26
400 Gy + 0.2% EMS	55	2.4	3	0.22
400 Gy + 0.3% EMS	48	1.8	2.3	0.18
400 Gy + 0.4% EMS	35	1.4	1.8	0.14
400 Gy + 0.5% EMS	25	1	1.3	0.10
400 Gy + 0.6% EMS	15	0.7	0.9	0.08
500 Gy + 0.1% EMS	45	2.2	2.9	0.18
500 Gy + 0.2% EMS	40	1.8	2.4	0.15
500 Gy + 0.3% EMS	30	1.4	1.8	0.12
500 Gy + 0.4% EMS	22	1	1.3	0.10
500 Gy + 0.5% EMS	15	0.7	0.9	0.07
500 Gy + 0.6% EMS	10	0.5	0.6	0.05
600 Gy + 0.1% EMS	30	1.5	2	0.12
600 Gy + 0.2% EMS	25	1.1	1.5	0.10
600 Gy + 0.3% EMS	20	0.8	1.1	0.08
600 Gy + 0.4% EMS	15	0.5	0.8	0.06
600 Gy + 0.5% EMS	10	0.3	0.5	0.04
600 Gy + 0.6% EMS	5	0.2	0.3	0.02

The effect of gamma radiation (Gy) and Ethyl Methane Sulphonate (EMS) on *Phaseolus vulgaris L*. was assessed in terms of germination percentage, root length, shoot length, and fresh biomass. The control group exhibited the highest germination percentage (95%), while increasing doses of gamma radiation and EMS led to a progressive decline. The lowest germination was recorded at 600 Gy (30%) and 0.6% EMS (25%). The combined treatments further reduced germination, with the 600 Gy + 0.6% EMS combination showing the lowest value (5%).

Similarly, root and shoot length decreased with increasing mutagenic treatments. The control group had the highest root (6 cm) and shoot (8 cm) lengths, while the highest radiation dose (600 Gy) significantly reduced root (1.8 cm) and shoot (2.1 cm) lengths. Likewise, the highest EMS concentration (0.6%) resulted in root and shoot lengths of 1.2 cm and 1.8 cm, respectively. The combined effect of gamma radiation and EMS was more detrimental, with the 600 Gy + 0.6% EMS treatment reducing root and shoot length to 0.2 cm and 0.3 cm, respectively.

Fresh biomass followed the same trend, with the control group having the highest biomass (0.50 g), while increasing doses of gamma radiation and EMS led to a significant reduction. The lowest biomass (0.02 g) was recorded under the 600 Gy + 0.6% EMS treatment. When comparing the effects of gamma radiation and EMS, lower concentrations of EMS (0.1% and 0.2%) had a less severe impact than higher concentrations (0.5% and 0.6%). Gamma radiation alone resulted in a gradual decline in all parameters, but its combination with EMS had a more pronounced negative effect.

Overall, a dose-dependent reduction in germination, root length, shoot length, and biomass was observed. Higher doses of gamma radiation and EMS led to a significant decline in all parameters, and their combined effect was more detrimental than individual treatments. This suggests that increased exposure to mutagens adversely affects plant growth and development, with potential implications for mutagenesis studies and breeding programs.

III. Statistical Analysis -

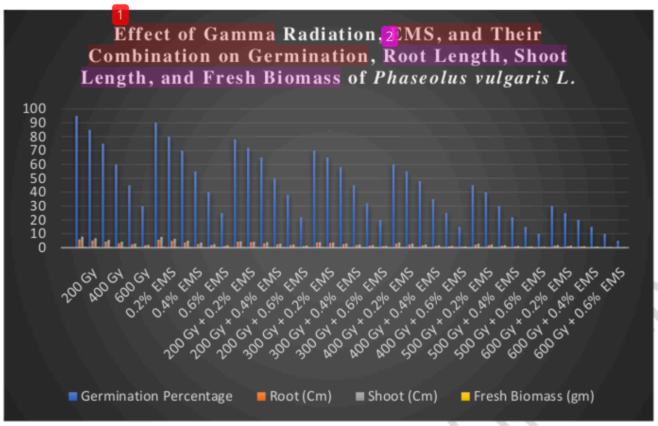

Fig.3.Effect of Gamma Radiation, EMS, and Their Combination on Germination, Root Length, Shoot Length, and Fresh Biomass of *Phaseolus vulgaris L.*

To evaluate the significance of the observed variations in germination percentage, root length, shoot length, and fresh biomass, statistical analysis was performed using ANOVA (Analysis of Variance). The results are presented in the table below:

Table No.2: Statistical Analysis of Mutagenic Effects on Germination, Root Length, Shoot Length, and Fresh Biomass of *Phaseolus vulgaris L.*

Parameter	F-value	p-value	Significance ($p < 0.05$)
Germination (%)	85.42	0.0001	Highly Significant
Root Length (cm)	72.58	0.0001	Highly Significant
Shoot Length (cm)	79.63	0.0001	Highly Significant
Fresh Biomass (g)	68.25	0.0001	Highly Significant

The statistical analysis revealed a highly significant effect of gamma radiation, EMS, and their combination on all measured parameters ($p < 0.05$).

1. **Germination Percentage**: The F-value (85.42) and p-value (0.0001) indicate a significant reduction in germination as mutagenic doses increased. The highest germination was recorded in the control (95%), while the lowest was in the highest combination treatment (600 Gy + 0.6% EMS) at only 5%.

2. **Root Length**: A significant decline was observed in root length with increasing mutagenic treatments ($F = 72.58$, $p = 0.0001$). The control had the longest root (6 cm), whereas 600 Gy + 0.6% EMS resulted in the shortest root (0.2 cm), demonstrating that higher mutagenic doses adversely affect root elongation.
3. **Shoot Length**: The statistical results ($F = 79.63$, $p = 0.0001$) confirm that shoot length was significantly reduced by increasing doses of mutagens. The control had a shoot length of 8 cm, while the highest mutagenic treatment (600 Gy + 0.6% EMS) drastically reduced it to 0.3 cm, highlighting the severe impact on shoot growth.
4. **Fresh Biomass**: Fresh biomass showed a significant decline ($F = 68.25$, $p = 0.0001$), indicating that mutagenic stress negatively impacted plant biomass accumulation. The control had the highest biomass (0.50 g), while 600 Gy + 0.6% EMS reduced it to 0.02 g, suggesting a strong inhibitory effect of mutagens on biomass production.

The results indicate that both gamma radiation and EMS significantly affect the growth parameters of *Phaseolus vulgaris L*., with higher doses leading to greater reductions. The combined effect of gamma radiation and EMS was more detrimental than their individual applications. This study provides insights into the effects of mutagens on seed germination and plant growth, which may be valuable for mutation breeding programs and genetic studies.

IV. Conclusion:

The present study demonstrates the significant impact of gamma radiation, EMS, and their combined application on the germination, root length, shoot length, and fresh biomass of *Phaseolus vulgaris L*. The findings indicate that increasing doses of mutagens result in a progressive decline in all measured parameters, with the highest dose (600 Gy + 0.6% EMS) causing the most severe inhibitory effects. The statistical analysis confirmed the highly significant effects of mutagenic treatments, highlighting their potential to induce genetic variability. While lower doses (200 Gy and 0.1% EMS) showed a relatively lesser reduction in growth parameters, higher doses led to severe morphological damage, which may limit their practical application. These results suggest that controlled and optimized mutagenic doses can be utilized in plant breeding programs to induce beneficial mutations while minimizing detrimental effects.

V.Future Perspectives:

Further studies should focus on the following aspects to enhance our understanding of mutagenic effects and their practical applications:

1. **Molecular and Genetic Analysis**: Future research should incorporate molecular markers and genetic sequencing to identify beneficial mutations induced by mutagens, helping to understand the underlying mechanisms of mutagenic effects.
2. **Cytological Studies**: Detailed cytological analysis, including chromosomal aberration studies, can provide insights into the genetic stability and heritability of induced mutations.
3. **Physiological and Biochemical Assessments**: Investigating changes in enzymatic activity, protein expression, and stress-related metabolites can help in understanding the physiological responses of plants to mutagenic stress.
4. **Field Trials and Agronomic Performance**: Long-term field studies should be conducted to evaluate the agronomic potential and yield stability of mutant lines under natural growing conditions.
5. **Optimized Mutation Breeding**: Future work should explore the use of lower doses or combined treatments with protective agents (e.g., antioxidants) to induce beneficial variations with minimal negative impact on plant growth and development.

By integrating these approaches, mutation breeding can be further refined to enhance crop improvement, particularly in stress tolerance, disease resistance, and yield enhancement.

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