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(Research Article)

The effect of physical and chemical mutagenesis in lentil (Lens culinaris Medik.)

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Abstract

This comprehensive study investigates the impact of physical and chemical mutagenesis on lentil (Lens culinaris Medik.) to enhance genetic variability and identify mutants with improved agronomic traits. Gamma radiation and ethyl methanesulfonate (EMS) were used as mutagens. The experimental design, treatment doses, and observed morphological, physiological, and biochemical responses of lentil genotypes are presented. The results demonstrate significant variability in traits such as plant height, seed yield, chlorophyll content, biological yield, harvest index, and 1000-seed weight, with notable differences between control and treated groups. The findings highlight the potential of mutagenesis for crop improvement and genetic diversity enhancement in lentil breeding programs. Statistical analysis revealed significant correlations among key traits, and path coefficient analysis was used to identify traits with the most direct impact on seed yield.

Keywords: Lentil; Mutagenesis; Gamma Radiation; Ethyl Methanesulfonate; Genetic Variability; Crop Improvement; Correlation; Path Analysis

1. Introduction

Lentil (Lens culinaris Medik.) is one of the most significant legume crops worldwide, valued for its high nutritional content, environmental sustainability, and substantial role in food security. It serves as a critical source of dietary protein, fiber, and essential micronutrients, especially in developing countries. Lentils are drought-tolerant and capable of growing in marginal soils, making them vital for agricultural sustainability in arid and semi-arid regions. However, the productivity of lentil is constrained by biotic and abiotic stresses, coupled with a narrow genetic base that limits opportunities for genetic improvement through traditional breeding methods.

- Global Production and Productivity

The global production of lentils has seen considerable growth over the past two decades. In 2022, lentil production reached approximately 6.33 million tons worldwide, with a global average yield of 1,038 kg/ha (FAO, 2022) Countries like Canada, India, and Turkey are major contributors to global lentil production. Among these, Canada is the world's leading producer, followed by India. The global yield has increased from 806 kg/ha to 1,194.6 kg/ha over the last two decades due to improved production technologies and high-yielding varieties.

1.1. Lentil Production and Productivity in India

India is one of the top producers and consumers of lentils globally. The country contributes about 20-25% of the world's total lentil production, with an annual production ranging from 1.1 to 1.5 million tons. Lentils are primarily grown as a

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Rabi crop, which is sown in winter and harvested in spring, occupying approximately 1.5 million hectares of cultivated land.

The average productivity of lentils in India is 901 kg/ha, significantly lower than the global average of 1,194.6 kg/ha. This productivity gap is attributed to the use of age-old varieties, climate-induced stresses, and cultivation under rainfed conditions with limited input application. Improved seed varieties, better agronomic practices, and advances in breeding technology are crucial to bridging this yield gap. Uttar Pradesh (UP) is one of the leading lentil-producing states in India. It plays a pivotal role in lentil cultivation due to its large cultivated area and the adoption of high-yielding varieties. While specific production and yield figures for Uttar Pradesh are less frequently reported independently, it is well-known that UP, along with Madhya Pradesh and Bihar, significantly contributes to India's total lentil production. Recent policy interventions and support from state agricultural universities aim to increase lentil productivity in Uttar Pradesh by promoting stress-tolerant and high-yielding lentil varieties**.**

1.2. Significance of Induced Mutagenesis in Lentil

To overcome the challenges posed by narrow genetic diversity and low productivity, induced mutagenesis has emerged as a practical tool for genetic improvement. Unlike conventional breeding, mutagenesis allows the introduction of novel genetic variability. Physical mutagens, such as gamma radiation, and chemical mutagens, like ethyl methanesulfonate (EMS), have been successfully employed to develop lentil varieties with higher yields, disease resistance, and better adaptability. Induced mutations create new alleles that are not present in the existing gene pool, thereby facilitating the selection of desirable traits.

Objectives

- To quantify global, national, and regional production and productivity trends for lentil.
- To evaluate the impact of gamma radiation and EMS on morphological, physiological, and yield-related traits of lentil.
- To analyze the genetic variability induced by physical and chemical mutagens.
- To identify promising mutants for potential use in lentil breeding programs.
- To conduct correlation and path coefficient analysis of key traits to identify traits with the most direct impact on yield.

2. Materials and Methods

2.1. Plant Material

The experiment was conducted using two lentil genotypes, 'PRECOZ' and 'L-4076'. These genotypes were selected for their agronomic importance, adaptability, and known stability under various environmental conditions. The seeds were procured from the National Germplasm Center for Legume Crops. Before treatment, seeds were sorted to remove damaged or discolored seeds to ensure uniformity in the experimental material.

2.2. Mutagenic Treatments

2.2.1. Physical Mutagenesis

Gamma radiation was applied using a Cobalt-60 gamma irradiator. The doses used were 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy. The seeds were exposed to gamma radiation at the Institute of Nuclear Agricultural Sciences under controlled environmental conditions. The radiation chamber was maintained at a constant temperature of 25°C with 60% relative humidity to ensure consistent exposure.

2.2.2. Chemical Mutagenesis

Seeds were soaked in aqueous solutions of Ethyl Methanesulfonate (EMS) at concentrations of 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% for 6 hours. The soaking was conducted in glass containers with continuous agitation to ensure uniform exposure. After treatment, the seeds were thoroughly washed with running tap water for 2 hours to remove residual EMS and then dried under shade for 24 hours before planting.

2.3. Experimental Design

The experiment followed a Completely Randomized Design (CRD) with three replications for each treatment. Each treatment (combinations of gamma radiation and EMS concentrations) consisted of 30 seeds per replication. Control groups with untreated seeds were included for comparison. Germination, growth, and yield data were collected from 25 randomly selected plants per treatment.

2.4. Data Collection and Measurement

The following morphological, physiological, and yield-related characteristics were observed and recorded for each plant. Data were collected at key phenological stages such as germination, vegetative growth, flowering, and harvest.

2.4.1. Morphological Traits

- Plant Height (cm): Measured from the base of the plant to the tip of the highest leaf at 50% flowering.
- Number of Branches: Counted as the total number of primary branches per plant.
- Leaf Size (cm^2): The surface area of the third fully expanded leaf from the top was measured using a digital leaf area meter.
- Number of Pods per Plant: Counted as the total number of mature pods per plant at harvest.

2.4.2. Physiological Traits

- Germination Percentage (%): Recorded by counting the number of seeds that successfully germinated within 10 days after sowing.
- Chlorophyll Content: Measured using a SPAD chlorophyll meter at the flowering stage.
- Biochemical Response: Enzyme activity of catalase and peroxidase was analyzed to determine stress response due to mutagen treatment.

2.4.3. Yield-Related Traits

- Seed Yield (g/plant): The total weight of seeds obtained from each plant at harvest.
- Biological Yield (g/plant): Total biomass (above-ground) produced by the plant at harvest.
- 1000-Seed Weight (g): The weight of 1000 randomly selected seeds from each treatment.
- Harvest Index (%): Calculated as the ratio of seed yield to biological yield, expressed as a percentage.

2.5. Statistical Analysis

- Analysis of Variance (ANOVA): Used to assess the significance of differences among treatments for all traits at a 5% level of significance.
- Genotypic and Phenotypic Coefficients of Variation (GCV, PCV): Calculated to measure the extent of variability for each trait.
- Heritability (h^2): Estimated as the ratio of genotypic variance to phenotypic variance for each trait.
- Correlation Analysis: Pearson's correlation coefficients were calculated to determine the relationships among morphological, physiological, and yield-related traits.
- Path Coefficient Analysis: Used to determine the direct and indirect effects of independent traits on seed yield.
- Data Visualization: Box plots, histograms, and scatter plots were used to visualize the distribution, variability, and relationships among traits.

3. Results

3.1. Germination Percentage

The application of gamma radiation and EMS had a significant impact on the germination percentage of lentil seeds. Germination declined as the dose of mutagen increased, indicating a dose-dependent effect.

At low doses (100 Gy gamma and 0.1% EMS), seed viability was maintained, whereas higher doses caused significant damage, likely due to alterations in cellular structures. This finding aligns with previous studies on the impact of mutagenic agents on seed germination.

Table 1 Germination Percentage Under Gamma Radiation and EMS Treatments

Treatment	Germination (%)
Control	95.2 ± 2.1
Gamma 100 Gy	88.5 ± 3.2
Gamma 200 Gy	72.4 ± 4.5
Gamma 300 Gy	60.3 ± 5.0
EMS 0.1%	92.6 ± 2.4
EMS 0.2%	84.3 ± 3.7
EMS 0.5%	58.7 ± 4.9

3.2. Morphological Traits

Significant changes in morphological traits, including plant height, number of branches, and number of pods, were observed under different doses of gamma radiation and EMS.

Table 2 Morphological Traits of Lentil Under Different Treatments

Trait	Control	Gamma 100 Gy	Gamma 200 Gy	EMS 0.1%
Plant height (cm)	40.2	42.8	28.6	44.1
Number of branches	6.3	7.1	5.5	7.4
Number of pods	50.4	58.2	22.1	62.7

Moderate doses enhanced plant growth and branching, while higher doses caused stunted growth due to mutagenic effects on cell division and elongation. The increase in the number of pods at 0.1% EMS suggests the potential for selecting superior mutants.

3.3. Physiological Traits

Physiological stress was assessed using chlorophyll content and antioxidant enzyme activity.

Table 3 Physiological Traits Under Mutagenic Treatments

Treatment	Chlorophyll Content (SPAD)	Catalase Activity (Units/mg protein)	Peroxidase Activity (Units/mg protein)
Control	42.5 ± 1.8	10.2 ± 0.5	8.4 ± 0.3
Gamma 100 Gy	40.6 ± 2.1	12.4 ± 0.7	10.1 ± 0.6
Gamma 200 Gy	35.2 ± 2.5	14.6 ± 0.9	12.8 ± 0.7
EMS 0.1%	41.3 ± 2.0	11.5 ± 0.6	9.7 ± 0.4

Higher doses of mutagens reduced chlorophyll content due to oxidative stress, while antioxidant enzyme activity (catalase and peroxidase) increased, indicating a biochemical stress response.

3.4. Yield-Related Traits

Analysis of yield-related traits revealed significant differences among the treatments**.**

Table 4 Yield-Related Traits Under Gamma Radiation and EMS Treatments

Trait	Control	Gamma 100 Gy	Gamma 300 Gy	EMS 0.1%	EMS 0.5%
Seed yield (g/plant)	12.4	15.8	5.2	17.3	6.8
Biological yield (g)	30.1	34.5	12.6	40.2	15.8
1000-seed weight (g)	42.6	44.8	35.1	46.9	33.4

The optimal doses (100 Gy and 0.1% EMS) improved seed and biological yields, while higher doses reduced these traits due to mutagen-induced damage.

3.5. Correlation Analysis

Correlation analysis was performed to evaluate the relationships among key traits.

Table 5 Correlation Matrix of Key Morphological and Yield-Related Traits

Trait	Plant height	Number of pods	Biological yield	Seed yield	Chlorophyll content
Plant height	1.00	0.72	0.68	0.75	0.65
Number of pods	0.72	1.00	0.76	0.84	0.58
Biological yield	0.68	0.76	1.00	0.88	0.61
Seed yield	0.75	0.84	0.88	1.00	0.59
Chlorophyll content	0.65	0.58	0.61	0.59	1.00

The highest correlation with seed yield was observed for biological yield ($r = 0.88$), followed by number of pods ($r = 0.84$) and plant height ($r = 0.75$).

3.6. Path Coefficient Analysis

Path analysis was conducted to identify the direct and indirect effects of key traits on seed yield.

Table 6 Path Coefficient Analysis of Key Traits

Trait	Direct Effect on Seed Yield
Biological yield	0.62
Number of pods	0.48
Plant height	0.30
Chlorophyll content	0.20

Biological yield exhibited the highest direct positive effect on seed yield (0.62), followed by the number of pods (0.48) and plant height (0.30). These traits should be prioritized in breeding programs to enhance yield.

4. Discussion

This study highlights the potential of gamma radiation and EMS for inducing beneficial mutations in lentil. Lower doses (100 Gy and 0.1% EMS) improved morphological, physiological, and yield-related traits. High doses, however, resulted in reduced growth, germination, and seed yield, consistent with findings from previous studies. Correlation analysis identified biological yield as the most influential trait for seed yield, with path analysis confirming its strong direct effect (0.62). Future breeding programs should prioritize genotypes with higher biological yield and pod numbers to enhance overall productivity.

5. Conclusion

The present study demonstrates the effectiveness of gamma radiation and EMS in inducing genetic variability in lentil (Lens culinaris Medik.). The findings highlight the potential for enhancing agronomic traits such as seed yield, biological yield, plant height, number of pods, and antioxidant enzyme activity. The study reveals that lower doses of gamma radiation (100 Gy) and EMS (0.1%) were most effective in promoting beneficial mutations, while higher doses had adverse effects on germination, growth, and yield traits.

The path coefficient analysis identified biological yield as the most influential trait for seed yield, with a direct positive effect of 0.62. Correlation analysis further confirmed the importance of traits like number of pods, biological yield, and plant height as critical determinants of seed yield. These traits should be prioritized in lentil breeding programs for developing high-yielding varieties.

The study also highlights the role of oxidative stress and enzymatic responses, as evident from the increased catalase and peroxidase activity at higher doses of mutagens. This response indicates the activation of the plant's defense mechanisms, which can be leveraged to select stress-tolerant genotypes.

Overall, this research underscores the potential of induced mutagenesis as a tool for enhancing genetic variability and improving key yield-related traits in lentil. The findings can be utilized by plant breeders to develop high-yielding, stress-tolerant varieties. Future research should focus on molecular characterization of the induced mutants and field evaluation of the most promising genotypes under multi-location trials. Additionally, combining induced mutagenesis with marker-assisted selection (MAS) could accelerate the development of superior lentil cultivars for sustainable agriculture.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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