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REVIEWER'S REPORT

Manuscript No.: IJAR-54373

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

Recommendation:
Accept as it is

Rating	Excel.	Good	Fair	Poor
Originality		√		
Techn. Quality		√		
Clarity	√			
Significance		√		

Reviewer Name: Dr. Manju M

Date: 17-10-2025

Detailed Reviewer's Report

1. **Objective of the Study:**
   The primary aim was to evaluate the impact of gamma radiation and Ethyl Methane Sulphonate (EMS), individually and in combination, on seed germination and seedling growth parameters in Black Turtle Bean.
2. **Selection and Preparation of Seeds:**
   Authentic seeds were procured from a reputable agricultural institute, selected for uniformity in size, shape, and moisture content (10-12%) to ensure consistency across treatments.
3. **Mutagenic Agents Used:**
   The study employed two mutagenic agents: gamma rays from a Cobalt-60 source at doses of 200, 400, and 600 Gray, and EMS at concentrations of 0.1%, 0.3%, and 0.6% (v/v).
4. **Treatment Protocols:**
   - **Gamma Radiation:** Seeds were exposed uniformly in a controlled environment using Cobalt-60 gamma source.
   - **EMS Treatment:** Seeds were presoaked, exposed to EMS for 4 hours with continuous shaking to enhance mutagen penetration, followed by thorough washing to remove residual chemical.
   - **Combined Treatment:** Seeds were first irradiated with gamma rays and subsequently treated with EMS at corresponding doses.
5. **Experimental Design and Replication:**
   Experiments were performed in triplicate for each treatment, with 25 seeds per replicate, ensuring reliability and statistical robustness.
6. **Germination Conditions:**
   Treated and control seeds were germinated on sterile Petri plates under controlled conditions - temperature maintained at 25 ± 2°C, humidity at 70%, and adequate light - monitored for 7 days.
7. **Parameters Measured:**

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Four key physiological parameters were recorded: seed germination percentage, root length, shoot length, and fresh biomass of seedlings, serving as indicators of mutagenic impact.

8. **Dose-Dependent Effects of Gamma Radiation:**
   Increasing doses of gamma rays caused a progressive decrease in seed germination and seedling growth, with the highest dose (600 Gy) showing the most pronounced inhibitory effects.
9. **Dose-Dependent Effects of EMS:**
   Similarly, EMS concentrations from 0.1% to 0.6% induced a gradual decline in germination rates and seedling vigor, with the highest concentration causing significant morphological damage.
10. **Synergistic Effects of Combined Treatments:**
    The combination of gamma radiation followed by EMS treatment resulted in more severe reductions in all measured parameters than either treatment alone, highlighting a synergistic mutagenic effect.
11. **Statistical Validation:**
    Analysis of Variance (ANOVA) and Duncan's Multiple Range Test confirmed that reductions in germination, root length, shoot length, and biomass with increasing mutagen doses were statistically highly significant ($p < 0.05$).
12. **Morphological and Physiological Impact:**
    High doses caused stunted seedling growth, root and shoot shortening, and diminished fresh biomass, indicating severe physiological stress and damage.
13. **Sensitivity of Black Turtle Bean:**
    The results demonstrated that Phaseolus vulgaris L. is highly sensitive to mutagenic treatments, evidenced by notable declines in germination percentage and seedling development.
14. **Implications for Mutation Breeding:**
    The findings provide valuable information for optimizing mutagen doses that induce genetic variability while avoiding excessive damage that compromises seed viability, thus aiding mutation breeding programs.
15. **Recommendations and Future Perspectives:**
    The study suggests further molecular and cytological investigations to characterize induced mutations, physiological and field trials to evaluate agronomic traits, and exploration of protective agents to minimize mutagenic damage while maintaining efficacy in mutation breeding.
16. **Significances of the studies:**
    - The study identifies effective doses of gamma radiation and EMS that induce beneficial mutations in Black Turtle Bean, providing a foundation for developing improved crop varieties through mutation breeding.
    - By analyzing dose-dependent effects and combined treatments, the research helps optimize mutagen doses to balance mutation induction with seed viability, ensuring practical application in breeding programs.
    - The findings contribute valuable knowledge for breeding programs aimed at enhancing important traits like yield, stress tolerance, and disease resistance in Black Turtle Bean, supporting sustainable agricultural productivity.