



Plant Archives

Journal homepage: <http://www.plantarchives.org>DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.029>

COMPARATIVE EVALUATION OF ETHYL METHANE SULPHONATE (EMS) AND GAMMA RAYS ON SEED GERMINATION AND SEEDLING GROWTH PARAMETERS IN COWPEA (*Vigna unguiculata* L. walp.)

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(Date of Receiving-30-05-2026; Date of Revision-19-07-2026; Date of Acceptance-03-08-2026)

ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important food legumes cultivated in tropical and subtropical regions because of its high nutritional value, adaptability to drought-prone environments and ability to improve soil fertility through biological nitrogen fixation. However, the narrow genetic base of cultivated cowpea limits the development of superior cultivars through conventional breeding. Induced mutagenesis has become an effective approach for generating novel genetic variability. The present investigation was undertaken to compare the effects of ethyl methane sulphonate (EMS) and gamma rays on seed germination and seedling growth parameters of cowpea under laboratory conditions. Healthy seeds of cowpea variety UAHS-28 were treated with five concentrations of EMS (10, 20, 30, 40 and 50 mM) and five doses of gamma rays (100, 200, 300, 400 and 500 Gy) along with untreated control. The experiment was conducted under laboratory conditions in a completely randomized design with four replications. Data was recorded on germination percentage, root length, shoot length, seedling vigour index-I and II, seedling fresh weight and dry weight following standard seed testing procedures. The experimental data were analysed using analysis of variance, and treatment means were compared using the critical difference test. Both EMS and gamma rays significantly influenced seed germination and seedling growth parameters. Increasing EMS concentrations progressively reduced germination, shoot length, root length and seedling vigour, with complete inhibition of germination at 50 mM. In contrast, gamma rays exhibited comparatively lower biological damage, maintaining 79.5% germination even at 500 Gy. Lower doses of gamma irradiation (100–300 Gy) stimulated root and shoot elongation and maintained higher seedling vigour than EMS-treated populations. Fresh and dry weight followed similar trends, indicating greater tolerance of cowpea seedlings to moderate gamma irradiation than to EMS treatment.

Key words: Cowpea, Ethyl methane sulphonate, Gamma rays, Seed germination, Seedling parameters, Mutation breeding.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important self-pollinated grain legume cultivated extensively in tropical and subtropical regions of the world. The crop is widely recognized for its high protein content, balanced amino acid composition, vitamins and essential

minerals, making it an important component of human nutrition, particularly in developing countries (Owade *et al.*, 2020; Shevkani *et al.*, 2025). Besides its nutritional importance, cowpea contributes significantly to sustainable agriculture through biological nitrogen fixation, improvement of soil fertility and adaptation to drought,

high temperature and poor soil conditions. Because of these characteristics, cowpea plays a vital role in ensuring food and nutritional security in rainfed farming systems (Owade *et al.*, 2020; Shevkani *et al.*, 2025).

Despite its economic importance, the scope for genetic improvement of cultivated cowpea remains limited because of its relatively narrow genetic base. Most commercial cultivars are derived from a limited number of parental lines, resulting in reduced genetic variability for several economically important traits. Conventional breeding approaches primarily depend on naturally occurring variation, which is often insufficient for developing superior cultivars possessing improved yield potential, stress tolerance and wider adaptability. Therefore, the creation of additional genetic variability has become an essential prerequisite for effective crop improvement programmes (Chen *et al.*, 2023; Muthuswamy and Preethi, 2020). Induced mutagenesis has emerged as one of the most successful approaches for generating novel genetic variation in self-pollinated crops. Physical and chemical mutagens create heritable mutations by altering the genetic material, thereby expanding the available variability beyond that present in natural populations (Chen *et al.*, 2023; Singh and Sadhukhan, 2018). Several mutant varieties developed through induced mutagenesis have contributed significantly to global crop improvement programmes, demonstrating the practical utility of this approach in plant breeding (Muthuswamy and Preethi, 2020; Singh and Sadhukhan, 2018).

Among physical mutagens, gamma rays are most extensively employed because of their high penetration ability and relatively predictable mutagenic effects (Badr *et al.*, 2014; Laanen *et al.*, 2021; Vanmathi *et al.*, 2021). Gamma irradiation induces mutations mainly through the generation of free radicals, DNA strand breaks and chromosomal rearrangements, leading to a broad spectrum of genetic alterations. At lower doses, gamma irradiation has frequently been reported to stimulate physiological processes such as cell division, enzyme activity and seedling growth, whereas higher doses generally result in severe cellular damage and growth inhibition. Ethyl methane sulphonate (EMS) is one of the most widely used chemical mutagens in mutation breeding programmes (Chen *et al.*, 2023; Gnanamurthy and Dhanavel, 2014; Goyal *et al.*, 2020). EMS primarily induces point mutations through alkylation of guanine bases, resulting predominantly in G/C to A/T transition mutations (Chen *et al.*, 2023). Because of its high mutation frequency and ability to generate stable genetic changes, EMS has become an important mutagen for

functional genomics as well as crop improvement. However, increasing EMS concentration is generally associated with progressive reductions in seed germination, seedling growth and plant survival due to its strong biological effects (Thangwana *et al.*, 2021; Wanga *et al.*, 2020).

Evaluation of mutagenic treatments during the early stages of plant development provides valuable information regarding mutagen sensitivity and biological effectiveness. Germination percentage, root length, shoot length, seedling vigour indices and seedling biomass are widely accepted indicators for assessing the physiological response of plants to mutagenic treatments (Abdul-Baki and Anderson, 1973; Thangwana *et al.*, 2021; Wanga *et al.*, 2020). These parameters facilitate the identification of suitable mutagen doses capable of inducing adequate genetic variation while minimizing excessive biological damage (Thangwana *et al.*, 2021; Wanga *et al.*, 2020). Although several studies have independently evaluated the effects of EMS or gamma rays in legumes, comparative information on their influence during the early seedling stage in cowpea remains limited. Understanding the differential response of seedlings to physical and chemical mutagens is essential for selecting optimum treatment levels for mutation breeding programmes. Therefore, the present investigation was undertaken to compare the effects of different concentrations of EMS and doses of gamma rays on seed germination and seedling growth parameters of cowpea variety UAHS-28 under controlled laboratory conditions. The findings are expected to provide useful information for identifying biologically effective mutagen doses suitable for the development of improved cowpea genotypes.

Materials and Methods

Experimental site

The laboratory investigation was conducted during Kharif 2025 at the Department of Genetics and Plant Breeding, College of Agriculture, Navile, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences (KSNUAHS), Shivamogga, Karnataka, India. Standard laboratory conditions were maintained throughout the experiment to ensure uniform germination and seedling growth.

Plant material

The experimental material consisted of healthy, well-filled and genetically pure seeds of cowpea (*Vigna unguiculata* (L.) Walp.) variety UAHS-28, released by Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga. The variety possesses a determinate growth habit, flowers in

Table 1: Analysis of variance for EMS treatments.

Sl. No.	Characters	Mean squares		CD		CV (%)
		Treatments(df: 5)	Error(df: 18)	P ≤ 0.05	P ≤ 0.01	
1	Germination (%)	3144.3**	62.28	11.72	16.06	14.68
2	Shoot length (cm)	113.78**	1.66	1.91	2.62	13.66
3	Root length (cm)	110.47**	4.00	2.97	4.07	19.91
4	Seedling length (cm)	444.63**	8.91	4.43	6.16	15.32
5	Fresh weight (mg)	297096**	1442.32	56.42	77.29	7.29
6	Dry weight (mg)	1631.84**	23.23	7.17	9.82	11.93
7	Seedling vigour index I	2625818**	97509	464	636	23.81
8	Seedling vigour index II	7468708**	306691	822.74	1127.01	21.22

** Significant at $p \leq 0.01$; df – degrees of freedom

approximately 38–39 days, matures in 80–85 days, and has a seed yield potential of 12–13 q ha⁻¹ under recommended agronomic practices. Owing to its adaptability and stable performance, UAHS-28 was selected for the present investigation.

Mutagen treatments

Two mutagenic agents, namely ethyl methane sulphonate (EMS) and gamma rays, were used to induce mutations. For the gamma ray treatment, 700 dry seeds were irradiated with 100, 200, 300, 400 and 500 Gy using a Co⁶⁰ gamma source at the Centre for Excellence in Soil and Crop Research Analysis (CESCRA), IARI, New Delhi. For EMS treatment, another set of 700 seeds was pre-soaked in distilled water for 9 hours to facilitate hydration. The pre-soaked seeds were subsequently treated with 10, 20, 30, 40 and 50 mM EMS solutions (pH 7.0) for 6 hours at room temperature with intermittent shaking to ensure uniform exposure. The volume of EMS solution was maintained at approximately ten times the seed volume. After treatment, the seeds were washed thoroughly under running tap water five to six times to remove residual EMS before sowing. Untreated seeds pre-soaked in distilled water for an equivalent duration served as the control.

Laboratory experiment

The experiment was conducted in a completely randomized design (CRD) with four replications. Each replication consisted of 50 seeds for every treatment. Seeds were placed uniformly on moist germination paper using the standard paper towel method and incubated under laboratory conditions suitable for germination. Moisture was maintained throughout the experimental period by regular addition of distilled water whenever necessary. Observations on seed germination were recorded on the fifth day after sowing, while measurements related to seedling growth were recorded on the eighth day. Normal seedlings from each replication were randomly selected for recording observations. The

seedling traits evaluated were germination percentage (%), shoot length (cm), root length (cm), seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), seedling vigour index-I (SVI-I), and seedling vigour index-II (SVI-II).

Estimation of seedling vigour indices

Seedling vigour was assessed using the procedures proposed by Abdul-Baki and Anderson (1973).

Seedling Vigour Index-I (SVI-I) = Germination percentage × Seedling length (cm)

where,

Seedling length = Shoot length + Root length.

Seedling Vigour Index-II (SVI-II) = Germination percentage × Seedling dry weight (mg)

These indices were used to evaluate the overall physiological performance of seedlings under different mutagenic treatments.

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) following the procedure described by Panse and Sukhatme (1985) for a completely randomized design. Treatment means were compared using the critical difference (CD) test at the 5% level of significance, and the coefficient of variation (CV) was computed to assess experimental precision. The statistical analyses were performed using Microsoft Excel and R software (Version 4.2.1). Graphical representations illustrating the comparative response of EMS and gamma ray treatments on seedling traits were generated using R software.

Results and Discussion

Effect of EMS and gamma rays on seed germination and seedling parameters

The analysis of variance indicated highly significant ($P \leq 0.01$) differences among the EMS concentrations and gamma ray doses for all the characters studied,

Table 2: Analysis of variance for gamma ray irradiation.

Sl. No.	Characters	Mean squares		CD		CV (%)
		Treatments(df: 5)	Error(df: 18)	P ≤ 0.05	P ≤ 0.01	
1	Germination (%)	332.94**	10.38	4.79	6.56	10.00
2	Shoot length (cm)	107.01**	3.72	2.87	3.73	11.18
3	Root length (cm)	64.06**	3.31	2.70	3.51	12.14
4	Seedling length (cm)	332.94**	10.38	4.79	6.23	10.00
5	Fresh weight (mg)	52396**	9999.41	148.55	203.53	14.06
6	Dry weight (mg)	1922.67**	84.44	13.65	18.70	12.81
7	Seedling vigour index I	3498516**	121644	518	674	12.03
8	Seedling vigour index II	23863161**	1012494	1494.88	2047.73	14.95

** Significant at $p \leq 0.01$; df – degrees of freedom

namely germination percentage, shoot length, root length, seedling length, fresh weight, dry weight, seedling vigour index-I and seedling vigour index-II, demonstrating that both mutagens significantly affected germination and early seedling growth (Tables 1 and 2). In untreated control, the highest germination (98.0%) was recorded. The response of germination varied markedly between the two mutagens. However, germination declined steadily with increasing concentrations of EMS i.e., 10 mM (70.5%), 20 mM (59.5%), 30 mM (57.0%), and 40 mM (55.0%), but no germination was observed at 50 mM EMS (Table 3, Fig. 1). In contrast, gradual reduction in germination was noted with increasing doses of gamma

irradiation i.e., 100 Gy (93.5%), 200 Gy (90.0%), 300 Gy (89.0%), 400 Gy (82.0%) and 500 Gy (79.5%) (Table 4, Fig. 2).

Marked differences were also observed for seedling growth parameters among different EMS concentrations and gamma ray doses. In control, the shoot and root lengths were 19.41 cm and 16.52 cm, respectively. Considerable reduction in shoot length was evident with increase in EMS concentration i.e., 10 mM (14.19 cm) to 40 mM (7.95 cm), while seedlings failed to develop at 50 mM. Root length followed a similar trend, decreasing from 10 mM (14.01 cm) to 30 mM (9.85 cm), with a slight increase at 40 mM (10.35 cm) before complete inhibition at 50 mM (Table 3, Fig. 1). The response to gamma irradiation differed from that of EMS. The shoot length remained comparable to the control (19.41 cm) at different irradiation levels i.e., 100 Gy (19.71 cm), 200 Gy (20.01 cm), 300 Gy (19.99 cm), 400 Gy (17.49 cm) and drastically declined at 500 Gy (6.87 cm). The root length increased at 100 Gy (18.45 cm), while at 200 Gy (16.55 cm), 300 Gy (16.78 cm), and 400 Gy (14.39 cm) comparable to control (16.52 cm). However, it declined drastically at highest dose 500 Gy (7.26 cm) (Table 4, Fig. 2).

Seedling dry weight was significantly influenced by both mutagens. The untreated control produced the highest dry weight (104 mg). Under EMS treatment, dry weight decreased drastically at 10 mM (50.5 mg), 20 mM (46.6 mg) and 30 mM (43.5 mg), followed by a slight increase at 40 mM (55.0 mg). No dry matter was recorded at 50 mM due to the absence of germination (Table 3). Under gamma irradiation, dry weight declined progressively from 100 Gy (92 mg), 200 Gy (80 mg), 300 Gy (68 mg), 400 Gy (56 mg) and 500 Gy (46 mg) (Table 4).

Seedling vigour index-I (SVI-I) showed considerable variation among treatments. The control recorded the highest value (3527). Among EMS treatments, SVI-I

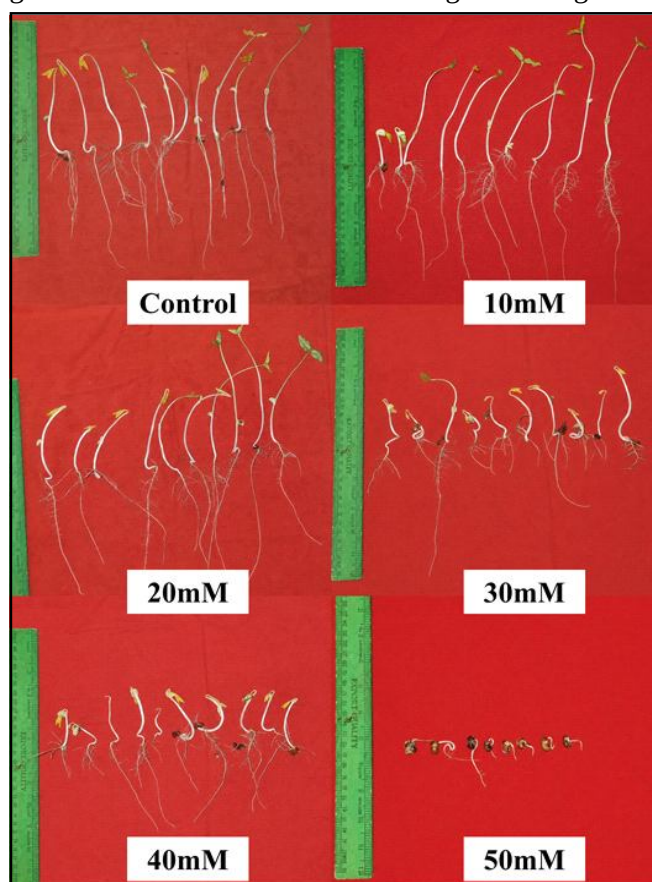
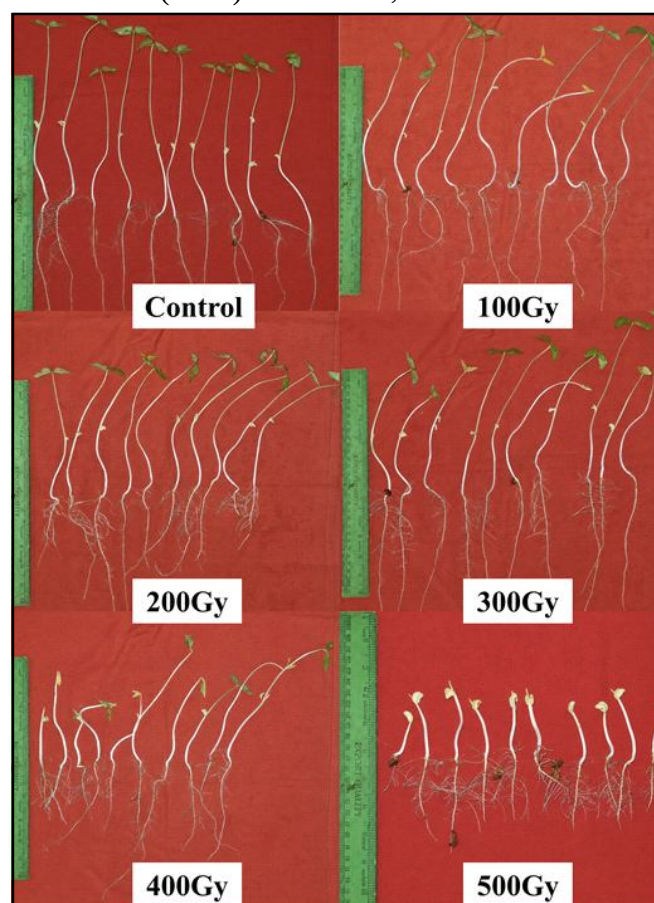
**Fig. 1:** Effect of different doses of EMS on seedling growth.

Table 3: Effect of different concentrations of EMS on germination and seedling parameters.

Treatments	GP (%)	SL (cm)	RL (cm)	DW (mg)	SVI-I	SVI-II
Control	98.00	19.41	16.52	104	3527	10198
10 mM	70.50**	14.19**	14.01	50.58**	1989**	3539**
20 mM	59.50**	11.17**	11.69**	46.60**	1366**	2775**
30 mM	57.00**	10.04**	9.85**	43.53**	1062**	2485**
40 mM	55.00**	7.95**	10.35**	55.05**	1018**	3062**
50 mM [§]	0.00	0.00	0.00	0.00	0.00	0.00
Mean	56.67	10.46	10.40	49.99	1493.83	18843
CD (P ≤ 0.05)	11.72	1.91	2.97	7.17	463.89	823
CD (P ≤ 0.01)	16.06	2.62	4.07	9.82	635.57	1127

*, ** Significant decrease from control at $p \leq 0.05$, $p \leq 0.01$, respectively
 GP = Germination percentage, SL = Shoot length, RL = Root length,
 DW = Dry weight, SVI-I = Seedling vigour index – I,
 SVI-II = Seedling vigour index – II; [§]None of the seeds germinated

decreased significantly at 10 mM (1989), 20 mM (1366), 30 mM (1062) and 40 mM (1018). Since no germination was observed at 50 mM, SVI-I could not be recorded (Table 3). In gamma ray-treated seedlings, the highest SVI-I was recorded at 100 Gy (3568), slightly exceeding the control (3527). Thereafter, the index decreased

**Fig. 2:** Effect of different doses of Gamma rays on seedling growth.

gradually at 200 Gy (3287), 300 Gy (3279), but drastically at 400 Gy (2612) and 500 Gy (1123) (Table 4). A comparable trend was observed for seedling vigour index-II (SVI-II). The untreated control recorded the highest value (10198). In EMS-treated seedlings, SVI-II declined sharply from 10 mM (3539), 20 mM (2775), 30 mM (2485) and 40 mM (3062). Since no germination was observed at 50 mM, SVI-II could not be recorded (Table 3). Gamma ray treatments recorded drastic decline in SVI-II with increasing radiation dose i.e., 100 Gy (8597), 200 Gy (7196), 300 Gy (6082), 400 Gy (4622) and 500 Gy (3691) (Table 4). Overall, increasing EMS concentration resulted in a progressive reduction in all seedling traits, with complete inhibition of germination and seedling development at 50 mM. Gamma irradiation produced comparatively smaller reductions across the studied parameters, and seedlings exposed to 100–300 Gy maintained growth and vigour values comparable to untreated control.

The present investigation demonstrated that both EMS and gamma rays significantly influenced seed germination and seedling growth parameters of cowpea, although the magnitude of their effects differed considerably. Increasing doses of both mutagens caused progressive reductions in germination percentage, seedling growth and vigour; however, EMS exhibited a stronger inhibitory effect than gamma irradiation (Fig. 3, 4). Such reductions in germination are primarily attributed to mutagen-induced damage to DNA, impairment of cellular metabolism and disruption of enzymatic activities involved in the initiation of germination. Complete inhibition of germination at 50 mM EMS indicates severe physiological and genetic damage, whereas comparatively higher germination under gamma ray treatments suggests that physical mutagenesis was less detrimental within the dose range employed. Similar reductions in germination following EMS treatment

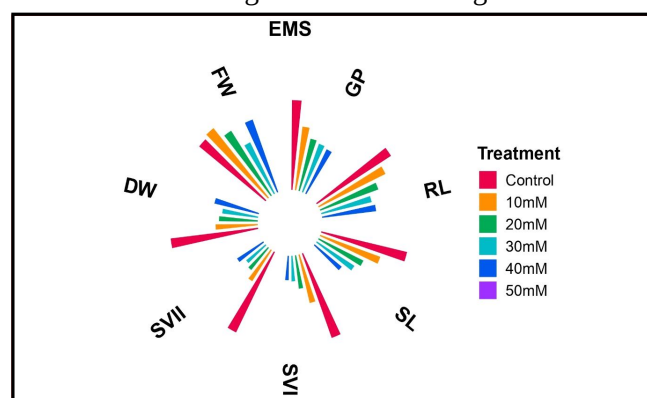
**Fig. 3:** Comparative performance of seedling traits under different EMS concentrations.

Table 4: Effect of different doses of gamma rays on germination and seedling parameters.

Treatments	GP (%)	SL (cm)	RL (cm)	DW (mg)	SVI-I	SVI-II
Control	98.00	19.41	16.52	104	3527	10198
100 Gy	93.50	19.71	18.45	92	3568	8597
200 Gy	90.00**	20.01	16.55	80**	3287	7196**
300 Gy	89.00**	19.99	16.78	68**	3279	6082**
400 Gy	82.00**	17.49	14.39	56**	2612**	4622**
500 Gy	79.50**	6.87**	7.26**	46**	1123**	3691**
Mean	88.67	17.25	14.99	74.33	2899	6731
CD ($P \leq 0.05$)	4.79	2.86	2.71	13.65	518	1494
CD ($P \leq 0.01$)	6.56	3.92	3.71	18.7	710	2047

*, ** Significant decrease from control at $p \leq 0.05$, $p \leq 0.01$, respectively
 GP = Germination percentage, SL = Shoot length, RL = Root length,
 DW = Dry weight, SVI-I = Seedling vigour index – I,
 SVI-II = Seedling vigour index – II; [§]None of the seeds germinated

have been reported in cowpea (Gnanamurthy and Dhanavel, 2014), black gram (Goyal *et al.*, 2020), and mungbean (Kumar and Sharma, 2024), which is attributed to chromosomal abnormalities and reduced metabolic activity during germination.

Root and shoot growth also exhibited marked reductions with increasing EMS concentration, whereas lower doses of gamma rays (100–300 Gy) either maintained or slightly stimulated seedling growth compared with the untreated control. The stimulation observed under lower gamma irradiation doses may be explained by the phenomenon of radiation hormesis, wherein low doses enhance cell division, enzyme activation and metabolic efficiency, leading to improved seedling growth. Similar stimulatory effects of low-dose gamma irradiation have been reported in cowpea (Badr *et al.*, 2014) and sorghum (Wanga *et al.*, 2020). In contrast, higher doses of gamma rays and increasing EMS concentrations reduced seedling growth owing to extensive chromosomal damage, inhibition of mitosis and disturbance of physiological processes. The comparatively greater inhibition caused by EMS could be due to alkylating agents which directly induce point mutations and interfere with DNA replication, thereby affecting normal cell division and seedling establishment (Badr *et al.*, 2014).

Seedling vigour indices were markedly affected by both mutagens and closely reflected the trends observed for germination and seedling growth. The substantial reduction in seedling vigour under EMS treatments resulted from the combined effects of reduced germination percentage and decreased seedling biomass. In contrast, gamma ray treatments maintained comparatively higher vigour, particularly at 100 Gy, where

SVI-I slightly exceeded that of the control. This indicates that moderate gamma irradiation not only preserved seed viability but also promoted seedling growth during the early stages of development. Comparable observations have been reported in tepary bean (Thangwana *et al.*, 2021) and sorghum (Wanga *et al.*, 2020), wherein lower doses of gamma irradiation improved seedling performance before inhibitory effects became evident at higher doses.

Probit analysis of median lethal dose (LD_{50})

The median lethal dose (LD_{50}) for each mutagen was estimated by maximum likelihood probit regression, relating $\log_{10}$ (dose) to probit-transformed mortality (Finney, 1971). For EMS, the working probit regression converged in seven iterations, yielding the equation $Y = 2.76X - 4.26$. The model fit was assessed using maximum likelihood chi-square statistics ($\chi^2 = 39.57$, $p < 0.001$). The estimated LD_{50} was 34.74 mM (95% CI: 27.62 to 43.70 mM), with a slope of 2.76 ± 0.41 ($t = 6.70$, $p = 0.003$), indicating a steep dose-response relationship (Table 5, Fig. 5). For gamma rays, the probit line was described by $Y = 1.19X - 4.14$ ($\chi^2 = 0.47$, $p = 0.92$), giving an LD_{50} of 2,918 Gy (95% CI: 1,521 to 5,599 Gy) and a shallower slope of 1.19 ± 0.49 ($t = 2.435$, $p = 0.07$) (Table 5). The narrower confidence interval and higher slope for EMS reflect its greater biological potency per unit dose compared with gamma irradiation.

Probit regression analysis further supported the differential biological responses of the two mutagens. The estimated LD_{50} value of 34.74 mM for EMS indicated a steep dose-response relationship, confirming the high biological potency of EMS in cowpea. In contrast, the estimated LD_{50} for gamma rays (2918.05 Gy) was substantially higher than the doses evaluated in the present study, indicating that the experimental irradiation levels were well below the predicted median lethal dose. The narrower confidence interval and higher regression slope

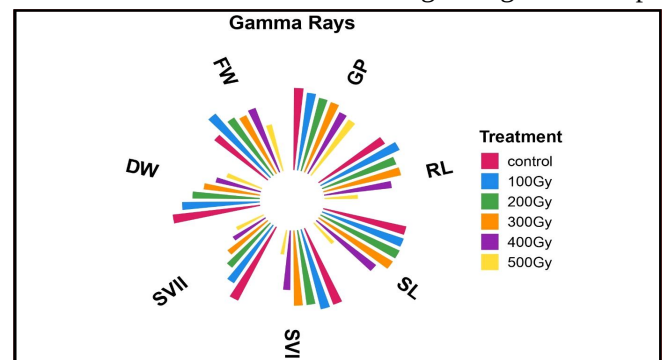
**Fig. 4:** Comparative performance of seedling traits under different doses of gamma rays.

Table 5: Probit regression parameters and LD₅₀ estimates for EMS and gamma ray treatments.

Parameters	Ethyl methane sulphonate	Gamma rays
Regression equation	$Y = 2.76X - 4.26$	$Y = 1.19X - 4.14$
Intercept	-4.26	-4.14
Slope (β)	2.76 ± 0.413	1.196 ± 0.49
t-value	6.70	2.43
p-value	0.003	0.07
LD ₅₀	34.74 mM	2918.05 Gy
Lower 95% CI	27.62	1520.92
Upper 95% CI	43.70	5598.57
Log LC ₅₀	1.54	3.46
Chi-square	39.57	0.47
Probability	<0.001	0.92
Iterations	7	5

observed for EMS further suggest greater precision and biological effectiveness compared with gamma irradiation. Similar observations regarding the higher mutagenic efficiency of EMS compared with physical mutagens have been reported in several legumes, including cowpea and black gram (Gnanamurthy and Dhanavel, 2014; Goyal *et al.*, 2020). The LD₅₀ estimate obtained in the present investigation indicates that 30–40 mM EMS constitutes an effective treatment range for inducing mutations while maintaining sufficient seedling survival, whereas 200–300 Gy gamma rays provide a favourable balance between biological damage and growth stimulation.

The comparative evaluation of EMS and gamma rays clearly demonstrated differences in their biological effectiveness. EMS produced a rapid decline in all seedling parameters with increasing concentration, whereas gamma rays comparatively induced mild reductions over the tested dose range. These differences are associated with the distinct mechanisms of action of the two mutagens. EMS primarily induces alkylation of guanine

bases, resulting in G/C to A/T transition mutations that directly interfere with DNA replication and cellular functions. In contrast, gamma rays generate free radicals that induce DNA strand breaks and chromosomal rearrangements, producing a wider spectrum of mutations but causing comparatively less immediate physiological damage at moderate doses. Therefore, gamma irradiation appears to provide a better balance between mutation induction and seedling survival during the early stages of growth.

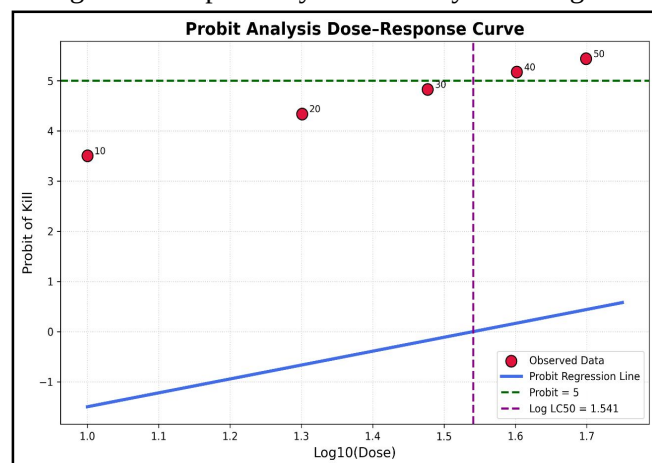
Overall, the present study demonstrates that although both EMS and gamma rays are effective mutagens for inducing variability in cowpea, EMS exerts greater biological damage than gamma irradiation during the seedling stage. Moderate doses of gamma rays promoted better germination, seedling growth and vigour, while intermediate EMS concentrations remained suitable for mutation induction because they combined appreciable biological effects with acceptable levels of seedling survival. These findings provide a reliable basis for selecting optimum mutagen doses for subsequent mutation breeding programmes aimed at developing improved cowpea genotypes.

Conclusion

Both EMS and gamma rays significantly influenced seed germination and seedling growth of cowpea under laboratory conditions. Increasing EMS concentrations progressively reduced germination, seedling growth and vigour, with complete inhibition observed at 50 mM, whereas gamma rays caused comparatively lower biological damage and stimulated seedling growth at lower doses (100–300 Gy). Probit analysis estimated the LD₅₀ of EMS at 34.74 mM, indicating that 30–40 mM represents the optimum concentration range for effective mutation induction with acceptable seedling survival. Although gamma irradiation exhibited lower biological potency, doses of 200–300 Gy maintained germination and vigour while providing adequate mutagenic effects. These findings establish suitable mutagen doses for future cowpea mutation breeding programmes and provide a foundation for advancing promising mutant populations for field evaluation.

Acknowledgements

The research work carried out is part of the M.Sc. (Agri) research of the first author. The authors are grateful to Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India, for providing the necessary research facilities. The technical assistance provided by the field and laboratory staff is duly acknowledged.

**Fig. 5:** Probit regression curve for estimation of LC₅₀ of EMS-treated cowpea seeds

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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