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ASSESSMENT OF GAMMA RAYS, EMS AND THEIR COMBINATION ON SEED GERMINATION AND EARLY SEEDLING GROWTH IN COWPEA (*Vigna unguiculata* L. Walp.)

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ABSTRACT

Mutation-induced biological damage assessment plays a crucial role in evaluating the mutagenic potency and genotypic sensitivity of different plant varieties, forming a vital component of mutation breeding programmes. Estimation of the median lethal dose (LD_{50} and analysis of mutagen-induced alterations in germination and seedling vigor are essential prerequisites for determining an optimal mutagen dose to ensure a successful mutation breeding experiment. In the present study, two cowpea varieties, RC-19 and RC-101, were treated with gamma rays and ethyl methane sulphonate (EMS) to investigate the effects of these mutagens on early seedling growth parameters. A total of 34 treatment combinations involving both physical and chemical mutagens were evaluated. Variety RC-101 showed higher sensitivity to both gamma irradiation and EMS than RC-19 as indicated by pronounced reductions in germination percentage and seedling growth traits. The LD_{50} values were estimated as 644.49 Gy for RC-19 and 437.92 Gy for RC-101. Both varieties demonstrated dose-dependent decline in seedling performance under controlled laboratory conditions following exposure to individual and combined mutagenic treatments. Among the mutagens, gamma radiation produced more intense inhibitory effects compared to EMS which caused relatively moderate reductions in biological parameters. The combined application of gamma radiation and EMS further intensified these effects with the highest treatment combination (600 Gy + 0.3% EMS) resulting in maximum suppression of seedling traits. Overall, the combined mutagenic treatments proved to be more effective in inducing genetic variability, thereby enhancing the potential for the selection of desirable mutants in cowpea improvement programmes.

Keywords: Mutation breeding, Cowpea, Gamma rays, EMS (ethyl methane sulphonate), LD LD_{50} estimation.

Introduction

Cowpea (*Vigna unguiculata* L. Walp.), commonly known as black-eyed pea, southern pea, lobia or chowla, is a diploid (2n = 2x = 22) leguminous crop belonging to the family Fabaceae and subfamily Faboideae. The cultivated species, *Vigna unguiculata* subsp. *unguiculata*, is further classified into four cultigroups: *unguiculata*, *biflora*, *sesquipedalis*, and *textilis* (Maréchal *et al*., 1978). Originating from Central and Southern Africa (Harlan, 1971), cowpea is now widely grown across the semi-arid and tropical regions of Asia, Africa and Southern Europe (Kole, 2007). Globally, it occupies nearly 15.19 million hectares, with an annual production of 9.77 million tonnes and average productivity of around 6,435 kg ha^{-1} (FAOSTAT, 2024). In India, cowpea remains a relatively minor pulse crop, predominantly cultivated in arid and semi-arid regions of Punjab, Haryana, Delhi, Western Uttar Pradesh, Rajasthan, Karnataka, Kerala, Tamil Nadu, Maharashtra and Gujarat (Meena *et al*., 2022). Despite its immense agronomic and nutritional potential, the area under cowpea cultivation is limited to about 0.32 million hectares with a total production of 0.198 million tonnes (Sharma *et al*., 2024). The crop fulfills multiple roles, serving as food, fodder, forage, green manure and vegetable (Tarawali *et al*., 2002). Nutritionally, cowpea is often termed the "poor man's meat" due to its rich protein composition, ranging between 22–25% of seed dry weight (Boukar *et al*., 2011). It also contains 56–68 g of carbohydrates per 100 g of dry weight, of which starch constitutes approximately 52 g, positioning cowpea among the starch-rich legumes (Jayathilake *et al*., 2018). In addition, it provides 16–21 g of dietary fibre per 100 g of seed. However, cowpea exhibits a predominantly self-pollinated breeding system, leading to restricted genetic variability. The narrow genetic base, combined with a high rate of floral bud abortion up to 88% in some cases with only 6–16% forming pods (Ojehomon, 1968) limits the effectiveness of hybridization-based improvement. Mutation breeding offers a promising alternative to overcome this limitation by inducing new genetic variability through physical and chemical mutagens. Induced mutagenesis enables the creation of novel allelic combinations, thereby facilitating the improvement of genetic diversity. Physical mutagens such as gamma rays often cause chromosomal deletions, translocations and aberrations while chemical mutagens like ethyl methane sulphonate (EMS) cause specific nucleotide modifications and point mutations.

To exploit these mutagenic effects, the present study was undertaken using gamma radiation and EMS on two cowpea varieties, RC-19 and RC-101, to evaluate their effects on germination and seedling parameters at early growth stages and to determine the optimum mutagenic dose. The success of mutation breeding largely depends on identifying an optimum mutagen dose that induces maximum mutation frequency with minimal lethality. Many previous studies suggest that the optimal treatment should approximate the median lethal dose (LD_{50}) (Singh, 2003). Reports also indicate that the combined use of gamma radiation and EMS could produce higher mutation frequencies than single treatments. (Reddy and Smith, 1981). Excessively high doses can lead to reduced germination, poor seedling establishment, delayed growth and diminished reproductive performance (Mani, 1989). Several factors including mutagenic potency, treatment duration and genotype selection govern the success of mutation breeding. Mutagenic potency is closely associated with the degree of biological damage and the frequency of desirable mutations. Biological injury is typically assessed through parameters such as germination rate, seedling vigor and early growth attributes. Although higher mutagenic doses generally increase mutation frequency, they also intensify biological damage. Therefore, determining a balanced treatment level that maximizes beneficial mutations while minimizing physiological injury is essential for efficient mutagenesis in cowpea (Taziun *et al*., 2018). This study therefore seeks to establish an optimal mutagenic regime using gamma radiation and EMS, singly and in combination, that maximizes useful genetic variability in cowpea while minimizing associated bio-physiological damage during early growth.

Materials and Methods

Laboratory investigations were carried out in July 2023 in the Department of Genetics and Plant Breeding, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. The experiment involved two cowpea (*Vigna unguiculata* L. Walp.) varieties, RC-19 and RC-101 which were exposed to different doses of gamma radiation (200, 400 and 600 Gy), ethyl methane sulphonate (EMS) at 0.1%, 0.2% and 0.3% concentrations, and their combinations. Gamma irradiation of healthy dry seeds of both varieties was conducted at the Bhabha Atomic Research Centre, Trombay, Mumbai. For chemical mutagen treatment, seeds were first pre-soaked in distilled water for 6 hours at room temperature, blotted to remove excess moisture and then treated with freshly prepared EMS solutions (0.1%, 0.2% and 0.3%) in phosphate buffer for 6 hours at room temperature. A total of 34 treatment combinations (17 per variety) were tested in a Completely Randomized Design (CRD). The treatments included seeds soaked in distilled water for 6 hours as a control and untreated dry seeds as an absolute control. The complete set of mutagenic treatments is presented in Table 1. For each treatment, thirty seeds were sown individually in nursery trays. Observations were recorded 10 days after sowing for the following seedling traits: germination percentage (ISTA, 1993), plumule length (cm), radicle length (cm), total seedling length (cm), shoot fresh weight (mg), root fresh weight (mg), shoot dry weight (mg) (Woodstock, 1976), root dry weight (mg) (Woodstock, 1976), seed vigor index (mass) (Abdul-Baki and Anderson, 1973), seed vigor index (length) (Abdul-Baki and Anderson, 1973) and germination speed. Data on germination percentage were used to estimate LD_{50} values for mutagens in both RC-19 and RC-101 through probit analysis using OPSTAT statistical software. Analysis of variance for CRD was performed following the procedure described by Panse and Sukhatme (1985) to test the significance of differences among treatments for all measured traits.

Seed germination counts were recorded after 10 days after sowing under optimal laboratory conditions using germination paper as the substrate.

Radicle length, plumule length and total seedling length (cm) were measured from the embryonic axis to root tip, plumule tip and from shoot tip to root tip, respectively, on the 10th day. Root and shoot fresh weights (mg) were recorded from 10days old seedlings and their dry weights were obtained after oven-drying at 65 °C for 48 h. Seed vigour index based on length and mass were computed as per Abdul-Baki and Anderson (1973) as:

SVI (Length)=GP × Mean seedling length

SVI (Mass)=GP × Mean seedling dry weight

Germination speed was calculated by formula given by Czabator (1962)

Results and Discussion

Analysis of variance indicated highly significant differences among mutagenic treatments for germination percentage and all measured seedling traits in both RC-19 and RC-101 varieties (Table 2), confirming the sensitivity of cowpea to gamma radiation and ethyl methane sulphonate (EMS). These findings underscore the potential of these mutagens to generate substantial genetic variability essential for cowpea improvement programmes. Plate 1 illustrates the effects of all mutagenic treatments on 10days old seedlings of cowpea varieties RC-19 and RC-101.The effects of physical and chemical mutagens, applied singly and in combination, on germination and seedling traits are presented in Table 3 and discussed below:

Germination percentage

In the present study, mean germination percentage ranged from 36.67-96.33% in RC-19 and 27.67-93.33% in RC-101 with overall means of 67.39% and 58.67%, respectively. Among single treatments, 0.1% EMS yielded the highest germination in both varieties (RC-19: 87.00%; RC-101: 85.00%) followed by 0.2% EMS and 200 Gy. Gamma radiation induced sharper declines with increasing doses compared to the more gradual reductions from EMS, consistent with reports in related legumes by Kharade *et al*., 2015; Umavathi and Mullainathan, 2015; Gwata *et al*., 2016; Sathees *et al*., 2020). These reductions primarily stem from disrupted metabolic processes and cellular damage including tunica layer disorganization in treated seeds (Raina *et al*., 2023). Combined treatments further suppressed germination with the most severe effects at 600 Gy + 0.3% EMS (RC-19: 36.67%; RC-101: 27.67%), highlighting synergistic interactions that impair seed viability more than individual mutagens.

Variety RC-101 exhibited greater sensitivity to mutagenic treatments compared to RC-19 as evidenced by lower LD_{50} values of 437.92 Gy versus 644.49 Gy, respectively. This genotypic differential tolerance aligns with previous findings in cowpea where LD_{50} doses typically range from 300 Gy to 600 Gy depending on variety (Ramya *et al*., 2013; Kang *et al*., 2020; Eswaramoorthy *et al*., 2023).

Radicle length (cm)

Radicle length varied between 7.33 cm and 15.20 cm in RC-19 and from 6.80 cm to 14.20 cm in RC-101 with RC-19 exhibiting higher average radicle length of 11.64 cm compared to 10.66 cm in RC-101 which indicated greater tolerance of RC-19 to mutagenic treatments. Among the individual mutagenic treatments, maximum radicle length was observed at 0.1% EMS, measuring 13.77 cm in RC-19 and 12.83 cm in RC-101 followed by 0.2% EMS and 200 Gy gamma radiation. Combined treatments resulted in shorter radicle lengths with the most significant reduction seen at the highest combined dose of 600 Gy + 0.3% EMS, restricting radicle lengths to 7.33 cm in RC-19 and 6.80 cm in RC-101. These results corroborate earlier studies demonstrating that root length is highly sensitive to mutagenic stress, often more so than shoot length, due to greater radiation-induced damage (Gaur *et al*., 2003; Kharade *et al*., 2015; Umavathi and Mullainathan, 2015; Raina *et al*., 2023). The dose-dependent reduction in radicle length reflects cellular damage and impaired growth processes caused by both physical and chemical mutagens.

Plumule length (cm)

The plumule length showed progressive decline with the escalation in doses of gamma radiation and EMS whether applied individually or in combination. In variety RC-19, the plumule length varied from 21.57 cm to 33.33 cm while in RC-101, it ranged between 18.90 cm and 31.40 cm with respective overall means of 27.47 cm and 25.19 cm. Among the treated groups, the longest plumule length was observed at 0.1% EMS, recording 31.43 cm in RC-19 and 29.20 cm in RC-101 followed by 0.2% EMS with 30.27 cm and 28.07 cm, respectively. Gamma irradiation treatments also revealed dose-dependent reduction trend. The combined treatment of 600 Gy + 0.3% EMS caused the maximum inhibitory effect, producing the minimum plumule length of 21.57 cm in RC-19 and 18.90 cm in RC-101. The magnitude of growth suppression increased with higher mutagenic doses which aligns with earlier findings reported by Gaur *et al*. (2003), Rabinya *et al*. (2023) and Sahu *et al*. (2025).

Seedling length (cm)

In the RC-19 variety, seedling length ranged from 28.90 cm to 47.77 cm with mean value of 39.08 cm whereas in RC-101 it varied between 25.70 cm and 45.60 cm, averaging 35.85 cm. Among the EMS treatments, the highest seedling lengths were recorded at 0.1% EMS concentration, measuring 45.20 cm in RC-19 and 42.03 cm in RC-101. A further increase in EMS concentration to 0.2% slightly reduced seedling length to 43.47 cm and 40.67 cm, respectively while 0.3% EMS caused additional reduction to 40.37 cm in RC-19 and 37.10 cm in RC-101.Gamma irradiation also exhibited dose-dependent inhibitory effect on seedling elongation. At 200 Gy, the seedling length was 43.30 cm in RC-19 and 40.00 cm in RC-101 which progressively declined to 39.00 cm and 36.27 cm at 400 Gy and further to 34.87 cm and 31.47 cm at 600 Gy. The combined mutagenic treatments exerted the strongest growth suppression with the minimum seedling length observed under 600 Gy + 0.3% EMS (28.90 cm in RC-19 and 25.70 cm in RC-101).

The gradual reduction in radicle, plumule and total seedling length with increasing mutagen intensity reflects the detrimental impact of mutagens on meristematic cell division and elongation processes. The findings indicate that moderate concentrations of chemical mutagens (EMS) are less injurious to seedling growth than higher doses of gamma radiation. Similar results have been reported by Jain and Khandelwal (2009) and Kumar *et al*. (2010) who attributed such growth inhibition to chromosomal abnormalities, suppression of mitotic activity, enzyme dysfunction and metabolic disturbances induced by mutagenic treatments.

Root fresh weight (mg)

In variety RC-19, root fresh weight ranged from 58.39 mg to 131.65 mg with mean value of 99.04 mg whereas RC-101 exhibited comparatively lower range of 45.19 mg to 121.85 mg, averaging 88.08 mg. Among the treatments, the least inhibitory effect was noted under 0.1% EMS, producing the maximum root fresh weight of 116.92 mg in RC-19 and 109.61 mg in RC-101. This was followed by 0.2% EMS (112.10 mg in RC-19 and 103.22 mg in RC-101) and 200 Gy gamma irradiation (111.10 mg in RC-19 and 101.14 mg in RC-101). The most pronounced reduction occurred under the combined treatment of 600 Gy + 0.3% EMS where root fresh weight decreased sharply to 58.39 mg in RC-19 and 45.19 mg in RC-101.

Shoot fresh weight (mg)

In RC-19, the mean shoot fresh weight was 514.61 mg, ranging from 364.39 mg to 630.07 mg across treatments whereas RC-101 recorded an average of 464.87 mg with values varying between 288.55 mg and 611.34 mg. Among the treatments, 0.1% EMS proved most effective, producing the highest shoot fresh weights of 597.98 mg in RC-19 and 567.00 mg in RC-101. The combined treatment of 600 Gy gamma radiation with 0.3% EMS resulted in the most pronounced reduction with shoot fresh weights decreasing to 364.39 mg and 288.55 mg in RC-19 and RC-101, respectively.

A progressive decline in the fresh weight of root and shoot tissues was observed with increasing doses of gamma radiation and EMS in both cowpea varieties. Among the treatments, 0.1% EMS induced the least reduction in biomass followed by 0.2% EMS and 200 Gy gamma irradiation whereas the combined application of 600 Gy + 0.3% EMS exerted the most deleterious effect. These observations are consistent with earlier reports in cowpea, mungbean, and chickpea where biomass reduction was attributed to suppressed cell division, auxin degradation, alterations in ascorbic acid metabolism and physiological disruptions affecting growth regulation (Sofia *et al*., 2020; Kumar *et al*., 2022; Lumorh *et al*., 2025).

Root dry weight (mg)

In variety RC-19, the root dry weight ranged from 6.21 mg to 15.32 mg with an average of 11.41 mg while in RC-101, it varied between 5.64 mg and 13.31 mg, recording a mean value of 9.90 mg. A gradual decline in root dry weight was observed with increasing doses of gamma radiation whereas EMS treatments caused comparatively moderate reductions. Among the mutagenic treatments, the highest root dry weight was recorded at 0.1% EMS, measuring 14.06 mg in RC-19 and 11.83 mg in RC-101. In contrast, the combined treatment of 600 Gy + 0.3% EMS resulted in the lowest root dry weights, reduced to 6.21 mg and 5.64 mg in RC-19 and RC-101, respectively.

Shoot dry weight (mg)

Shoot dry weight, a key indicator of biomass accumulation and metabolic vigor in seedlings, exhibited a consistent decline with increasing doses of mutagens in both cowpea varieties. In RC-19, the shoot dry weight varied from 29.39 mg to 65.70 mg with a mean of 50.60 mg while in RC-101, it ranged between 29.83 mg and 63.80 mg, averaging 48.05 mg. The combined application of gamma radiation and EMS proved more detrimental than their individual treatments. Among the treatments, 0.1% EMS resulted in the maximum shoot dry weights of 62.01 mg in RC-19 and 59.37 mg in RC-101 followed by 0.2% EMS and 200 Gy gamma irradiation. With increasing mutagenic intensity, a progressive reduction in shoot dry weight was evident, the lowest values being recorded under the combined treatment of 600 Gy +

0.3% EMS, yielding 29.39 mg and 29.83 mg in RC-19 and RC-101, respectively.

The dry weight of root and shoot tissues showed a dose-dependent decline with increasing levels of gamma irradiation whereas EMS treatments resulted in comparatively milder reductions. These findings are consistent with earlier reports in blackgram (Jain and Khandelwal, 2009), cowpea (Vasantbhai, 2019), mungbean (Kumar *et al*., 2022) and chickpea (Kumar and Dogra, 2024). The reduction in seedling dry weight has been ascribed to disturbances in cell division, auxin imbalance, alterations in antioxidant activity and physiological impairments affecting growth processes (Sofia *et al*., 2020).

Seed vigour index (Mass)

The seed vigour index (mass basis) in variety RC-19 ranged from 1304.45 to 7804.60, with a mean value of 4393.67 whereas in RC-101, it varied between 981.96 and 7197.30, averaging 3629.34. Among the mutagenic treatments, 0.1% EMS yielded the highest seed vigour index, recording values of 6617.37 in RC-19 and 6051.61 in RC-101. This was followed by 0.2% EMS (5981.60 in RC-19 and 5350.04 in RC-101) and 200 Gy gamma irradiation (5803.33 in RC-19 and 5100.87 in RC-101). The combined treatment of 600 Gy + 0.3% EMS resulted in the most pronounced reduction with seed vigour index values decreasing to 1304.45 in RC-19 and 981.96 in RC-101.

Seed vigour index (Length)

In RC-19, the index ranged from 1058.40 to 4674.53 with an average of 2731.11, whereas in RC-101, it varied between 710.67 and 4256.05, recording a mean value of 2217.21. Among the mutagenic treatments, 0.1% EMS elicited the most favourable response, producing seed vigour indices of 3932.20 in RC-19 and 3572.37 in RC-101 followed by 0.2% EMS (3622.93 and 3227.13) and 200 Gy gamma irradiation (3537.37 and 3108.27). In contrast, the combined treatment of 600 Gy + 0.3% EMS exhibited the most adverse effect, reducing the seed vigour index to 1058.40 in RC-19 and 710.67 in RC-101.

The seed vigour index (SVI), evaluated on both length and mass bases, represents a vital indicator of the overall growth potential of seedlings, primarily governed by the elongation and development of the epicotyl and hypocotyl within the embryo (Rabinya *et al*., 2023). Evidence of synergistic effects was observed under combined mutagenic treatments. These findings are in close agreement with earlier reports by Sathees *et al*. (2020), Kumar et al. (2022) and Rabinya *et al*. (2023). Comparative analysis revealed that variety RC-101 exhibited greater sensitivity to mutagenic stress than RC-19 as indicated by its consistently lower vigour index values across treatments.

Germination speed (Seeds/Day)

Germination speed, a critical determinant of successful seedling establishment, declined progressively with increasing mutagenic doses. The overall mean germination speed was 4.22 in RC-19 and 3.36 in RC-101 with the latter consistently exhibiting lower values across treatments. Among the treated seeds, 0.1% EMS recorded the highest germination speeds (6.12 in RC-19 and 5.07 in RC-101) followed by 0.2% EMS (5.34 and 4.29) and 200 Gy gamma irradiation (5.12 and 4.24). A sharp decline was evident at higher mutagenic doses and combinations, particularly under 600 Gy + 0.3% EMS where germination speed dropped to 1.29 in RC-19 and 0.87 in RC-101. These dose-dependent reductions reflect mutagen-induced disturbances in meristematic cell division, aligning with previous findings in various legumes (Kumar *et al*., 2022; Gyamfi *et al*., 2022; Diallo *et al*., 2025). Gamma irradiation has been reported to suppress germination speed by arresting the cell cycle, damaging genomic DNA, and increasing necrotic or non-germinating seed fractions (Preuss and Britt, 2003). Similarly, higher EMS concentrations likely induced point mutations and disrupted phytohormone balance by inhibiting gibberellic acid (GA) synthesis and elevating abscisic acid (ABA) levels, thereby delaying germination onset due to altered GA–ABA signaling dynamics (Baghery *et al*., 2016).

Conclusion

Analysis of variance revealed highly significant differences among mutagenic treatments for all seedling traits in both cowpea varieties namely, RC-19 and RC-101 which revealed pronounced sensitivity to gamma radiation, EMS and their combinations. Differential genotypic tolerance was reflected in the LD_{50} estimates which were 644.49 Gy for RC-19 and 437.92 Gy for RC-101. Across all seedling characters evaluated under controlled laboratory conditions, both varieties exhibited clear dose-dependent declines following exposure to increasing intensities of mutagens. Gamma irradiation consistently produced stronger inhibitory effects than EMS alone while combined treatments further amplified these deleterious impacts with the highest combination dose (600 Gy + 0.3% EMS) causing the most pronounced suppression of seedling growth. Evaluation of eleven seedling traits demonstrated overall superior performance of RC-19 relative to RC-101 which showed greater vulnerability to mutagen-induced physiological stress. This varietal contrast supports prior evidence that sensitivity to mutagens is strongly governed by genetic constitution with LD_{50} and growth-reduction parameters serving as practical indicators of radio sensitivity and chemo sensitivity in cowpea. Stronger impacts of gamma irradiation together with additive outcomes from combined treatments establish RC-19 as valuable candidate for mutation breeding for genetic improvement of cowpea.

Table 1: Experimental treatments imposed on cowpea varieties RC-19 and RC-101

Variety	Treatment No.	Treatment	Dose
RC-19	1.	Absolute control	Dry seeds
	2.	Control	Seed soaking 6 hr at room temperature
	3.	Gamma radiation	200 Gy
	4.	Gamma radiation	400 Gy
	5.	Gamma radiation	600 Gy
	6.	EMS	0.1 % EMS for 6 hr at room temperature
	7.	EMS	0.2 % EMS for 6 hr at room temperature
	8.	EMS	0.3 % EMS for 6 hr at room temperature
	9.	Gamma radiation + EMS	200 Gy + 0.1 % EMS for 6 hr at room temperature
	10.	Gamma radiation + EMS	400 Gy + 0.1 % EMS for 6 hr at room temperature
	11.	Gamma radiation + EMS	600 Gy + 0.1 % EMS for 6 hr at room temperature
	12.	Gamma radiation + EMS	200 Gy + 0.2 % EMS for 6 hr at room temperature
	13.	Gamma radiation + EMS	400 Gy + 0.2 % EMS for 6 hr at room temperature
	14.	Gamma radiation + EMS	600 Gy + 0.2 % EMS for 6 hr at room temperature
	15.	Gamma radiation + EMS	200 Gy + 0.3 % EMS for 6 hr at room temperature
	16.	Gamma radiation + EMS	400 Gy + 0.3 % EMS for 6 hr at room temperature
	17.	Gamma radiation + EMS	600 Gy + 0.3 % EMS for 6 hr at room temperature
RC-101	18.	Absolute control	Dry seeds
	19.	Control	Seed soaking 6 hr at room temperature
	20.	Gamma radiation	200 Gy
	21.	Gamma radiation	400 Gy
	22.	Gamma radiation	600 Gy
	23.	EMS	0.1 % EMS for 6 hr at room temperature
	24.	EMS	0.2 % EMS for 6 hr at room temperature
	25.	EMS	0.3 % EMS for 6 hr at room temperature
	26.	Gamma radiation + EMS	200 Gy + 0.1 % EMS for 6 hr at room temperature
	27.	Gamma radiation + EMS	400 Gy + 0.1 % EMS for 6 hr at room temperature
	28.	Gamma radiation + EMS	600 Gy + 0.1 % EMS for 6 hr at room temperature
	29.	Gamma radiation + EMS	200 Gy + 0.2 % EMS for 6 hr at room temperature
	30.	Gamma radiation + EMS	400 Gy + 0.2 % EMS for 6 hr at room temperature
	31.	Gamma radiation + EMS	600 Gy + 0.2 % EMS for 6 hr at room temperature
	32.	Gamma radiation + EMS	200 Gy + 0.3 % EMS for 6 hr at room temperature
	33.	Gamma radiation + EMS	400 Gy + 0.3 % EMS for 6 hr at room temperature
	34.	Gamma radiation + EMS	600 Gy + 0.3 % EMS for 6 hr at room temperature

Table 2: Analysis of variance (ANOVA) for germination percentage, radicle length, plumule length, seedling length, root and shoot fresh weight in cowpea varieties RC-19 and RC-101 during the M_1 generation (Laboratory conditions).

Source of variation	DF	Germination percentage		Radicle length (cm)		Plumule length (cm)		Seedling length (cm)		Root fresh weight (mg)		Shoot fresh weight (mg)	
		RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101
Treatment	16	988.39**	1461.33**	13.72**	12.22**	36.55**	38.78**	94.63**	94.30**	1015.72**	1190.92**	16974.38**	24081.02**
Error	34	5.18	6.94	0.13	0.09	0.43	0.26	0.60	0.36	5.37	9.62	126.50	204.21

Table 2 Cont..: Analysis of variance (ANOVA) for germination speed, root and shoot dry weight, seed vigour index based on mass and length in cowpea varieties RC-19 and RC-101 during the M_1 generation (Laboratory conditions)

Source of variation	DF	Root dry weight (mg)		Shoot dry weight (mg)		Seed vigour index (Mass)		Seed vigour index (Length)		Germination speed	
		RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101
Treatment	16	17.45**	11.41**	322.70**	265.73**	11360686.64**	11518682.30**	3504090.43**	3796972.59**	11.61**	9.84**
Error	34	0.04	0.05	1.16	0.79	22779.37	31376.70	11306.49	10826.20	0.02	0.02

Where, DF – Degree of freedom; **Significant at p = 0.01% and 0.05%.

Table 3: Mean performance of germination percentage, radicle, plumule and seedling length (cm) in cowpea varieties RC-19 and RC-101 during M_1 generation (Laboratory condition).

Variety		Mutagen	Treatments/Dose	Germination percentage		Radicle length (cm)		Plumule length (cm)		Seedling length (cm)	
RC-19	RC-101			RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101
T3	T20	Gamma radiation	200 Gy	81.67	77.67	13.17	12.03	30.13	27.97	43.30	40.00
T4	T21	Gamma radiation	400 Gy	66.67	52.00	11.70	10.90	27.30	25.37	39.00	36.27
T5	T22	Gamma radiation	600 Gy	52.33	38.67	9.93	9.27	24.93	22.20	34.87	31.47
T6	T23	EMS	0.1% EMS	87.00	85.00	13.77	12.83	31.43	29.20	45.20	42.03
T7	T24	EMS	0.2 % EMS	83.33	79.33	13.20	12.60	30.27	28.07	43.47	40.67
T8	T25	EMS	0.3% EMS	71.33	65.67	12.20	11.17	28.17	25.93	40.37	37.10
T9	T26	Gamma radiation + EMS	200 Gy + 0.1% EMS	79.67	72.67	12.77	11.87	29.90	27.70	42.67	39.57
T10	T27	Gamma radiation + EMS	400 Gy + 0. 1% EMS	62.33	48.33	11.13	10.07	26.87	24.63	38.00	34.70
T11	T28	Gamma radiation + EMS	600 Gy + 0.1% EMS	47.67	33.67	9.20	8.37	23.67	21.43	32.87	29.80
T12	T29	Gamma radiation + EMS	200 Gy + 0.2% EMS	74.33	70.67	12.43	11.27	28.67	26.43	41.10	37.70
T13	T30	Gamma radiation + EMS	400 Gy + 0. 2 % EMS	55.67	43.33	10.70	9.43	25.20	22.73	35.90	32.17
T14	T31	Gamma radiation + EMS	600 Gy + 0.2% EMS	42.00	31.33	8.60	7.97	22.23	20.07	30.83	28.03
T15	T32	Gamma radiation + EMS	200 Gy + 0.3% EMS	65.33	51.67	12.17	10.53	27.80	25.07	39.97	35.60
T16	T33	Gamma radiation + EMS	400 Gy + 0. 3 % EMS	49.67	35.67	9.87	8.90	23.73	21.53	33.60	30.43
T17	T34	Gamma radiation + EMS	600 Gy + 0.3% EMS	36.67	27.67	7.33	6.80	21.57	18.90	28.90	25.70
T1	T18	Untreated seed	Control (Absolute)	93.67	90.67	14.43	13.07	31.87	29.57	46.60	42.63
T2	T19	Untreated seed	Control (Water soaked)	96.33	93.33	15.20	14.20	33.33	31.40	47.77	45.60
Mean				**67.39**	**58.67**	**11.64**	**10.66**	**27.47**	**25.19**	**39.08**	**35.85**
Range				**36.67-96.33**	**27.67-93.33**	**7.33-15.20**	**6.80-14.20**	**21.57-33.33**	**18.90-31.40**	**28.90-47.77**	**25.70-45.60**
C.D. (p=0.05%)				3.78	4.37	0.60	0.51	1.09	0.84	1.28	0.99
S.E.m ±				1.31	1.52	0.21	0.18	0.38	0.29	0.45	0.34
S.E.d ±				1.86	2.15	0.29	0.25	0.54	0.41	0.63	0.49
C.V. (%)				3.38	4.49	3.09	2.88	2.39	2.01	1.98	1.66

Table 3 Cont..: Mean performance of root and shoot fresh weight (mg), root and shoot dry weight (mg) in cowpea varieties RC-19 and RC-101 during M_1 generation (Laboratory condition)

Variety		Mutagen	Treatments/Dose	Root fresh weight (mg)		Shoot fresh weight (mg)		Root dry weight (mg)		Shoot dry weight (mg)	
RC-19	RC-101			RC-19	RC-101	RC-19	RC-101	RC-19	RC-101	RC-19	RC-101
T3	T20	Gamma radiation	200 Gy	111.10	101.14	573.38	536.84	13.03	11.16	58.04	54.47
T4	T21	Gamma radiation	400 Gy	101.23	89.76	525.64	478.32	11.43	10.20	52.91	47.86
T5	T22	Gamma radiation	600 Gy	86.36	77.31	460.75	409.99	9.55	8.44	42.90	41.53
T6	T23	EMS	0.1% EMS	116.92	109.61	597.98	567.00	14.06	11.83	62.01	59.37
T7	T24	EMS	0.2 % EMS	112.10	103.22	574.93	543.94	13.73	11.23	58.06	56.20
T8	T25	EMS	0.3% EMS	101.32	91.86	533.54	481.65	11.87	10.21	53.42	50.35
T9	T26	Gamma radiation + EMS	200 Gy + 0.1% EMS	107.30	100.92	559.16	521.93	12.98	10.81	56.24	52.41

T10	T27	Gamma radiation + EMS	400 Gy + 0. 1% EMS	95.86	83.85	494.69	423.08	11.29	9.61	49.77	43.99
T11	T28	Gamma radiation + EMS	600 Gy + 0.1% EMS	81.14	65.11	437.50	379.36	9.47	7.97	38.72	39.22
T12	T29	Gamma radiation + EMS	200 Gy + 0.2% EMS	102.64	93.29	549.22	485.98	12.20	10.27	55.27	51.02
T13	T30	Gamma radiation + EMS	400 Gy + 0. 2 % EMS	94.19	78.14	469.59	419.27	10.13	9.44	46.03	42.43
T14	T31	Gamma radiation + EMS	600 Gy + 0.2% EMS	73.46	62.85	416.76	341.81	8.40	7.18	35.29	35.39
T15	T32	Gamma radiation + EMS	200 Gy + 0.3% EMS	100.09	88.67	496.93	451.79	11.38	10.03	50.21	47.47
T16	T33	Gamma radiation + EMS	400 Gy + 0. 3 % EMS	85.83	71.51	444.82	381.28	8.74	8.41	41.54	40.17
T17	T34	Gamma radiation + EMS	600 Gy + 0.3% EMS	58.39	45.19	364.39	288.55	6.21	5.64	29.39	29.83
T1	T18	Untreated seed	Control (Absolute)	124.13	113.13	619.01	580.67	14.11	12.56	64.62	61.35
T2	T19	Untreated seed	Control (Water soaked)	131.65	121.85	630.07	611.34	15.32	13.31	65.70	63.80
		Mean		**99.04**	**88.08**	**514.61**	**464.87**	**11.41**	**9.90**	**50.60**	**48.05**
		Range		**58.39-131.65**	**45.19-121.85**	**364.39-630.07**	**288.55-611.34**	**6.21-15.32**	**5.64-13.31**	**29.39-65.70**	**29.83-63.80**
		C.D. (p=0.05%)		3.85	5.15	18.66	23.71	0.32	0.38	1.78	1.47
		S.E.m ±		1.34	1.79	6.49	8.25	0.11	0.13	0.62	0.51
		S.E.d ±		1.89	2.53	9.18	11.67	0.16	0.19	0.88	0.72
		C.V. (%)		2.34	3.52	2.19	3.07	1.72	2.32	2.12	1.85

Table 3 Cont..: Mean performance of seed vigour index based on mass and length, and germination speed in cowpea varieties RC-19 and RC-101 during M_1 generation (Laboratory condition)

Variety		**Mutagen**	**Treatments/Dose**	**Seed vigour index (Mass)**		**Seed vigour index (Length)**		**Germination speed**	
RC-19	**RC-101**			**RC-19**	**RC-101**	**RC-19**	**RC-101**	**RC-19**	**RC-101**
T3	T20	Gamma radiation	200 Gy	5803.33	5100.87	3537.37	3108.27	5.12	4.24
T4	T21	Gamma radiation	400 Gy	4288.57	3019.39	2601.33	1886.07	4.02	2.98
T5	T22	Gamma radiation	600 Gy	2743.85	1932.77	1824.67	1215.90	2.78	1.91
T6	T23	EMS	0.1% EMS	6617.37	6051.61	3932.20	3572.37	6.12	5.07
T7	T24	EMS	0.2 % EMS	5981.60	5350.04	3622.93	3227.13	5.34	4.29
T8	T25	EMS	0.3% EMS	4657.08	3976.63	2878.87	2435.50	4.12	3.62
T9	T26	Gamma radiation + EMS	200 Gy + 0.1% EMS	5515.84	4593.35	3400.33	2875.70	5.07	4.01
T10	T27	Gamma radiation + EMS	400 Gy + 0. 1% EMS	3806.94	2589.01	2369.47	1675.83	3.46	2.44
T11	T28	Gamma radiation + EMS	600 Gy + 0.1% EMS	2298.21	1587.69	1566.80	1002.53	2.18	1.75
T12	T29	Gamma radiation + EMS	200 Gy + 0.2% EMS	5015.22	4332.55	3053.83	2664.23	4.87	3.83
T13	T30	Gamma radiation + EMS	400 Gy + 0. 2 % EMS	3126.51	2247.63	1998.37	1394.47	3.21	2.38
T14	T31	Gamma radiation + EMS	600 Gy + 0.2% EMS	1834.13	1333.49	1293.33	877.73	1.96	1.08
T15	T32	Gamma radiation + EMS	200 Gy + 0.3% EMS	4023.34	2971.08	2610.67	1839.60	3.78	2.85
T16	T33	Gamma radiation + EMS	400 Gy + 0. 3 % EMS	2497.00	1731.57	1668.63	1085.17	2.34	1.83
T17	T34	Gamma radiation + EMS	600 Gy + 0.3% EMS	1304.45	981.96	1058.40	710.67	1.29	0.87
T1	T18	Untreated seed	Control (Absolute)	7374.35	6701.80	4337.17	3865.39	7.53	6.51
T2	T19	Untreated seed	Control (Water soaked)	7804.60	7197.30	4674.53	4256.05	8.56	7.44
		Mean		**4393.67**	**3629.34**	**2731.11**	**2217.21**	**4.22**	**3.36**
		Range		**1304.45-7804.60**	**981.96-7197.30**	**1058.40-4674.53**	**710.67-4256.05**	**1.29-8.56**	**0.87-7.44**
		C.D. (p=0.05%)		250.44	293.93	176.44	172.65	0.23	0.23
		S.E.m ±		87.14	102.27	61.39	60.07	0.08	0.08
		S.E.d ±		123.23	144.63	86.82	84.95	0.11	0.11
		C.V. (%)		3.44	4.88	3.89	4.69	3.22	4.07

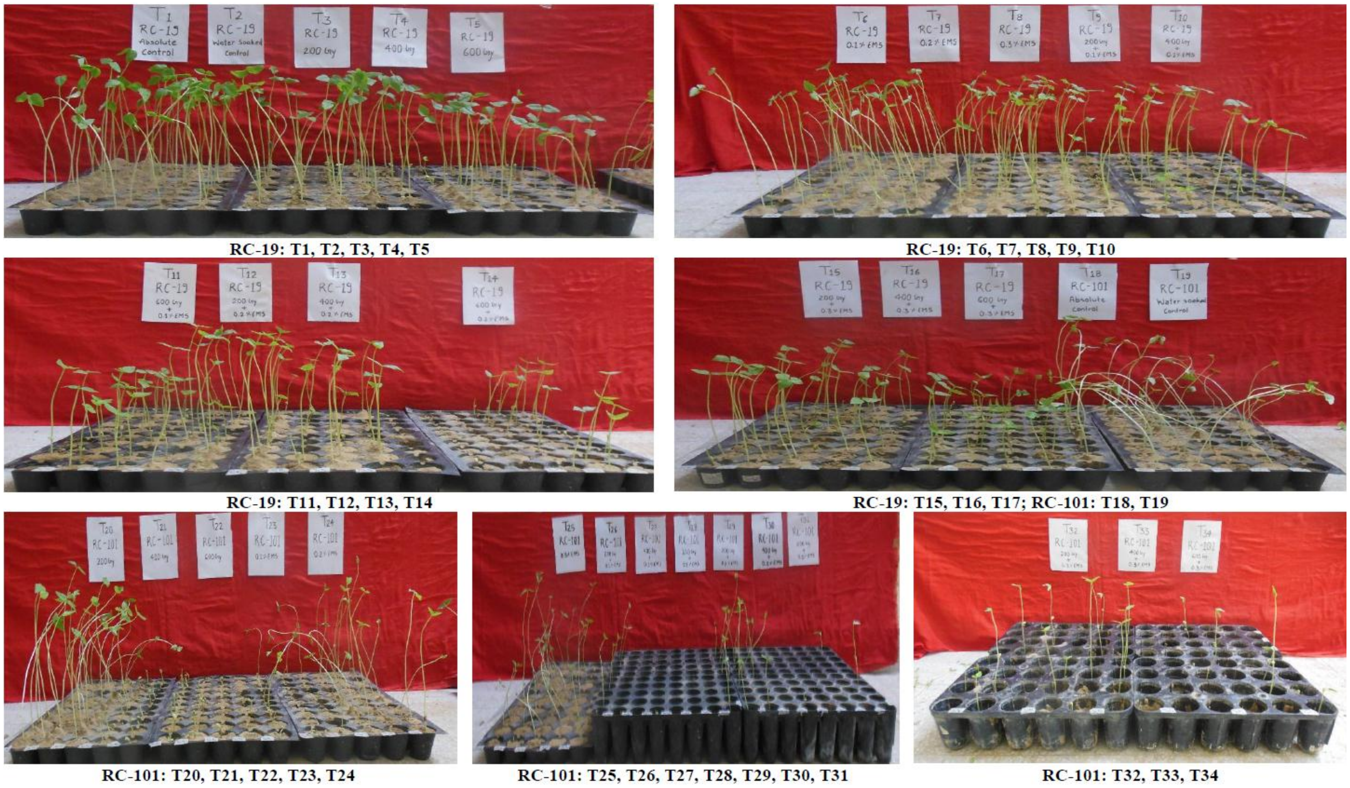

Plate 1: View of laboratory experiment to evaluate eleven seedling parameters under different mutagenic treatments

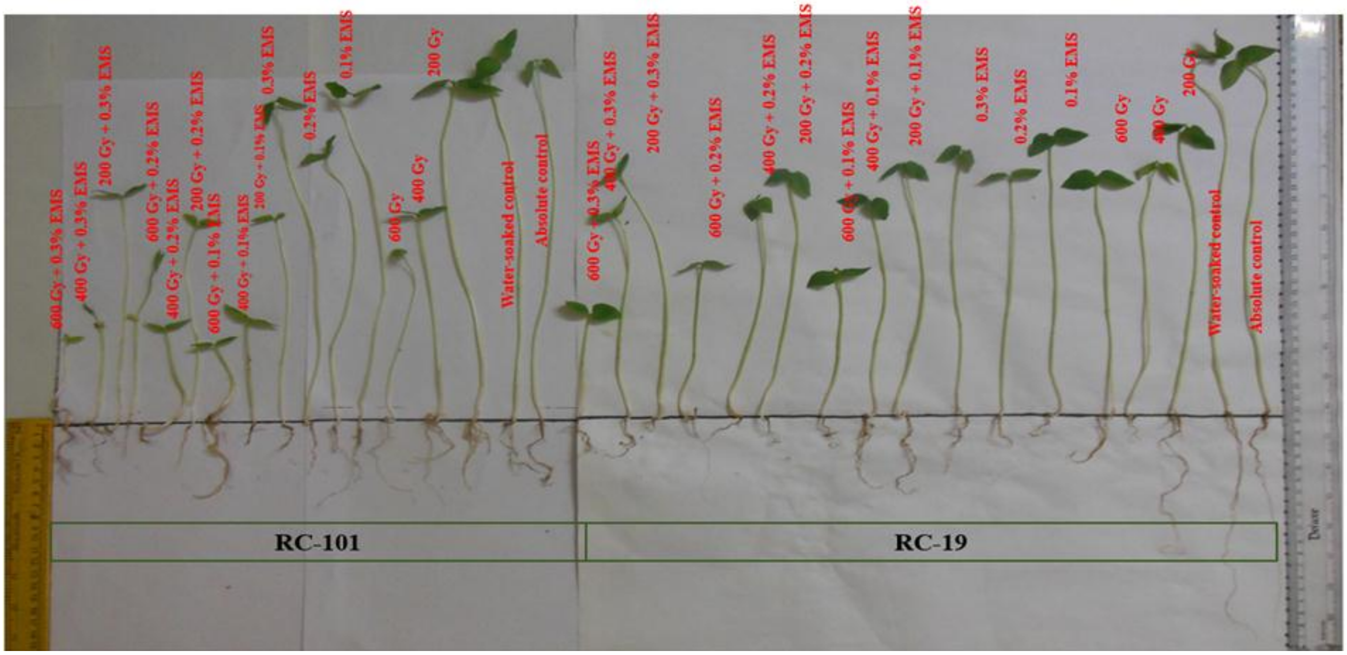

Plate 2: Differences in seedling length under different treatments in cowpea varieties RC-19 and RC-101.

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