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# RADIOSENSITIVITY OF *in-vitro* CULTURED *Calotropis gigantea* PLANTS TO GAMMA IRRADIATION

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## ABSTRACT

*Gamma irradiation has become an important tool in in-vitro propagation, especially for mutation breeding in clonally propagated crops. This study investigates the radiosensitivity of Calotropis gigantea plants in in-vitro culture exposed to gamma irradiation. The experiment was conducted at the tissue culture laboratory of the University of Colombo Institute for Agro-Technology and Rural Sciences under a Complete Randomized Design with 5 treatments and 15 replicates per treatment. Mature seeds of C. gigantea were exposed to surface sterilization and germinated in half-strength MS medium supplemented with 0.5 mg/L BAP. After germination, hypocotyl segments were separated and used as explants. Explants were inoculated into induction medium containing 2.5 mg/L BAP and 0.1 mg/L NAA. The cultures were exposed to four gamma irradiation doses such as 100 Gy, 125 Gy, 150 Gy, and 175 Gy, with 0 Gy as a control, using a Cobalt-60 research irradiator. The effects of gamma irradiation on plant survivability, shoot number, and shoot length were recorded over six weeks. The results indicated that gamma irradiation significantly reduced the survivability, shoot number, and shoot length in a dose-dependent manner ($P \leq 0.05$). At the lowest dose of 100 Gy, survivability decreased to 69.30%, while at the highest dose of 175 Gy, it dropped to 29.84%. Similarly, shoot number and shoot length also showed a significant reduction with increasing doses, with the control plants exhibiting the highest values. The $LD_{50}$ value was estimated at 138.2 Gy, suggesting that doses near this threshold are sufficient to induce variability without significantly impairing regenerative capacity in-vitro. These findings highlight the potential of gamma irradiation in mutation breeding, but also emphasize the need for caution with higher doses. The results suggest that for Calotropis gigantea, irradiation doses above 138 Gy may significantly hinder regeneration, making lower doses more appropriate for genetic variation.*

***Keywords: Calotropis gigantea; Explant; Hypocotyl segments; Gamma irradiation; $LD_{50}$; Regeneration***

## INTRODUCTION

The use of gamma irradiation has emerged as a valuable technique in plant tissue culture and *in-vitro* propagation, particularly in mutation breeding of clonally propagated crops (Jain, 2010). It offers a way to generate heritable genetic variation where conventional breeding methods are limited. When explants are irradiated under micropropagation conditions, mutations can be induced at the cellular level, and subsequent plant regeneration enables the fixation of desirable variants. This approach is particularly valuable for ornamentals such as chrysanthemum, where genetic uniformity and chimeric mutations are common barriers, and in industrial crops such as patchouli, which are traditionally propagated vegetatively (Normasari *et al.*, 2023; Datta *et al.*, 2005).

The response of explants to irradiation is highly depend on dose. In patchouli, leaf explants exposed to doses ranging from 15 to 75 Gy showed clear physiological stress, including phenolic browning, which intensified with dose and reached 100% at 75 Gy after eight weeks. Survival was unaffected at low doses (up to 15 Gy) but declined gradually thereafter, although moderate doses, such as 30–45 Gy, still maintained relatively high regenerative potential. Shoot formation followed a similar pattern: at 15–45 Gy, shoot production remained reasonably high, whereas at 60–

75 Gy, both the number and vigor of shoots were substantially reduced. In chrysanthemum ray florets, direct organogenesis also declined with irradiation, but regeneration was still possible across a range of doses, providing a pathway for recovering useful mutants (Normasari *et al.,* 2023; Datta *et al.,* 2005).

An essential step in applying gamma irradiation in tissue culture is determining the lethal dose that kills half the explants ($LD_{50}$). In patchouli, this was estimated at 69 Gy, providing a benchmark for establishing treatment windows. While $LD_{50}$ defines the upper threshold, practical applications often favor sub-lethal doses that maintain high regeneration frequencies. Interestingly, plantlets regenerated at low to moderate doses (15–45 Gy) displayed increased vigor compared with controls, with greater plant height and improved root development. At 30 Gy in particular, plantlets showed enhanced growth characteristics, while doses at 60–75 Gy inhibited both shoot and root development. This biphasic response, known as hormesis, highlights how carefully chosen irradiation doses can stimulate desirable growth while simultaneously introducing genetic variability (Normasari *et al.,* 2023).

One challenge in mutation breeding is the occurrence of chimeras, where mutated and non-mutated sectors coexist in a plant. Such instability can result in the loss of mutations during subsequent vegetative propagation. The use of direct organogenesis from single-cell-derived tissues is a key strategy to avoid this problem. In chrysanthemum, irradiated ray florets regenerated directly into shoots without an intermediate callus stage, thereby minimizing chimerism. This method produced stable, solid mutants that expressed new flower colours and floret shapes consistently across generations. The adventitious bud technique, which promotes regeneration from single or small groups of cells, further enhances the likelihood of producing genetically stable mutants (Datta *et al*., 2005).

Beyond phenotypic variation, molecular screening is important for confirming genetic changes induced by irradiation. In patchouli, simple sequence repeat (SSR) markers revealed polymorphisms between irradiated and non-irradiated plants. For example, primer Pca1 exhibited 77.3% polymorphism and Pca2 showed 50%, indicating that irradiation effectively generated genetic diversity at the molecular level. Such molecular markers provide an efficient way to detect and confirm variation early in the propagation process, allowing researchers to prioritize lines with the greatest potential for breeding or commercial exploitation (Normasari *et al.,* 2023).

Gamma irradiation in *in-vitro* systems is presented as a direct and efficient method for generating stable mutants in ornamentals. Conventional induced mutagenesis often results in chimeras due to multicellular apices, but when irradiation is applied to *in-vitro* cultured explants, the process favors the recovery of solid mutants. Datta (2023) describes experiments in chrysanthemum where ray florets irradiated with gamma rays (0.5–1 Gy) regenerated into plants showing stable flower colour and shape mutations without any chimeric instability. Mutation frequencies of 10–20% were recorded, producing variants such as tubular florets or novel colour combinations, which were immediately fixable in culture. This supports earlier work (Datta *et al.,* 2005) that showed how *in-vitro* mutagenesis could bypass the lengthy two-step process of field mutagenesis followed by tissue culture, offering a shorter route to commercial mutant lines.

The review by Datta, (2023) also stresses the advantages of *in-vitro* mutagenesis over field-based methods: larger effective population sizes can be handled in limited space, uniform exposure of cells to mutagens can be achieved, and regeneration under controlled culture conditions ensures survival of desirable sectors. In addition, the integration of tissue culture with mutagenesis allows simultaneous chimera management, as mutated ray florets or leaf explants can be regenerated into whole plants that express the mutation stably. This has led to the release of several new ornamental cultivars with unique flower colours and shapes, underpinning the role of gamma irradiation in innovation-driven floriculture.

Gamma irradiation integrated with *in-vitro* propagation enables the induction and fixation of genetic variability in clonally propagated species. The process requires careful calibration of dose, as low to moderate ranges can enhance growth while inducing heritable changes, whereas high doses compromise survival and regeneration. Avoiding callus stages and promoting direct organogenesis reduces the risk of chimerism, allowing the recovery of stable solid mutants.

Misra *et al,* (2007) investigated the chrysanthemum cultivar 'Madam E. Roger', a large-flowered greenish-white variety, for *in-vitro* propagation and mutagenesis

to induce genetic variability. A protocol was successfully standardized for the large-scale production of high-quality planting material suitable for commercial cultivation. Shoot bud differentiation from ray florets was achieved on a medium supplemented with 0.5 mg $L^{-1}$ NAA and 2.0 mg $L^{-1}$ BA. The regenerated shoots were subsequently multiplied on the same medium, rooted in the presence of 0.5 mg $L^{-1}$ NAA, and acclimatized for field establishment. Furthermore, treatment of freshly inoculated ray florets with 1.0 Gy gamma (γ) radiation resulted in the development of a stable yellow-flowered mutant, demonstrating the effectiveness of *in-vitro* mutagenesis in generating novel genetic variation.

Molecular markers such as SSRs further validate the occurrence of genetic changes. Together, these strategies demonstrate that *in-vitro* mutagenesis with gamma rays is an effective approach for generating and stabilizing novel variants in both industrial crops and ornamentals.

## MATERIALS AND METHODS

### *Experimental site*

The experiment was conducted in the research laboratory at the University of Colombo Institute for Agro-technology and Rural Sciences (UCIARS).

### *Surface sterilization*

Mature seeds were collected from healthy mother plants after the pods had shattered and transferred to the lab. Seeds were sterilized by using 70% ethyl alcohol for 10 minutes and 1% NaOCl for 3 minutes. Surface-sterilized seeds were transferred into culture vessels containing 30 ml of half-strength MS medium (Murashige and Skoog, 1962), supplemented with 0.5 mg/L BAP. Glass vessels were labeled and kept on racks at 25 °C in the dark for two weeks. Followed by a light intensity of 2000 lux for three weeks with a 12-hour photo period.

### *Inoculation of explants into the medium for culture initiation*

Standard basal MS salt medium was used as the induction medium, supplemented with 2.5 mg/L BAP and 0.1 mg/L NAA. 30 ml of the prepared medium was poured into each 250 ml glass vessel and autoclaved for 15 minutes at 121°C.

After 3 weeks from seed germination, hypocotyl segments were excised from the germinated plants and used as ex-plants. The hypocotyl segments were treated with 20% ethanol for 30 seconds and washed twice with Sterilized Distilled Water ($SDH_2O$) to remove residual disinfectant and minimize phytotoxic effects. The surface-sterilized *Calotropis gigantea* ex-plants (hypocotyl segments) were inoculated into the induction medium under aseptic conditions in a laminar flow hood. Then culture vessels were sealed and kept on culture racks in the growth room (Temperature was 25 °C, Relative Humidity was 70% and light intensity was 2000 lux for 12 hours per day) for four weeks.

### *Proliferation stage*

Proliferation media were prepared by adding two synthetic plant growth regulators, 3.0 mg/L BAP (6-Benzyl Amino Purine) and 0.1 mg/L NAA (Naphthalene-Acetic Acid) to MS medium. Cultures in induction medium were transferred to the proliferation medium in a laminar flow hood and maintained at 25 °C temperature, 70% Relative Humidity and the light intensity of the LED was 2000 lux for 12 hours per day.

### *Exposure to gamma irradiation*

Cultured glass vessels were exposed to gamma irradiation at the Horticultural Crops Research and Development Institute (HORDI), Gannoruwa, Sri Lanka, using a Cobalt-60 research irradiator. Two glass vessels containing sprouts were placed in the chamber at a time for irradiation. The entire process was carried out systematically to ensure consistent exposure across all the planting materials. Four gamma irradiation doses, such as T1 – 100 Gy, T2 – 125 Gy, T3 – 150 Gy, and T4 – 175 Gy, were used as treatments with a 0 Gy control. After gamma irradiation, planting materials were transferred carefully to the laboratory. All glass vessels were carefully cleaned with 70% alcohol using cotton. Then, they were arranged in a Completely Randomized Design (CRD) with 06 replicates per treatment. Gamma-irradiated cultures were maintained at 25 °C, 70% Relative Humidity, and an LED light intensity of 2000 lux for 12 hours for six weeks. Survival percentage (%), shoot number and shoot length were recorded over the period. The $LD_{50}$ value was calculated from survival data.

### *Data analysis*

Collected data were statistically analyzed using Minitab 19 and SAS statistical software. In Minitab 19,

a one-way ANOVA general linear model test was performed and the Dunnett and Tukey tests were used to ascertain significant differences among treatments at the 5% significance level.

## RESULTS AND DISCUSSION

### *Effect of gamma irradiation in in-vitro culture of Calotropis gigantea*

### *Effect of treatments on plant survivability*

The results showed that treatments had a significant effect on the survival of *Calotropis gigantea* plants in *in-vitro* culture ($P \leq 0.05$).

Table 1. Effect of gamma irradiation of *Calotropis gigantea* in *in-vitro* culture on survivability

| Treatment | Mean Survivable Rate (%) ± SD | SE |
|---|---|---|
| 0 Gy | $99.90^{a} \pm 0.14$ | 0.06 |
| 100 Gy | $69.30^{b} \pm 1.89$ | 0.85 |
| 125 Gy | $58.79^{c} \pm 3.48$ | 1.56 |
| 150 Gy | $41.64^{d} \pm 3.49$ | 1.56 |
| 175 Gy | $29.84^{e} \pm 3.43$ | 1.53 |
| **Sig**. | * | |

*Means followed by the different letters in the same column are significantly different at 5% probability level. * Represents significant at 5% and "ns" represents not significant*

At the lower irradiation dose of 100 Gy, survivability decreased to 69.30%, a substantial reduction compared with the control plants (99.90%). As the irradiation dose increased to 125 Gy, the survival rate further decreased to 58.79%. This suggests that *Calotropis gigantea* is relatively resilient at lower doses, but its survival rate declines sharply at higher doses. By the time the dose reaches 150 Gy, the survival rate drops to 41.64%, and at 175 Gy, it is only 29.84%, representing the significantly lowest survivable rate (Table 1).

This trend of reduced survival with increasing gamma irradiation is consistent with the findings of other studies on plant species exposed to ionizing radiation. For example, in a study on *Solanum lycopersicum* (tomato), irradiation doses of 150 Gy significantly reduced germination and growth, effects similar to those observed in this study (Mekonnen *et al.,* 2013). In another study on *Arabidopsis thaliana*, a dose-dependent decline in plant growth and survival was also observed under gamma irradiation (Zhang *et al.*, 2015).

### *Mechanisms of Gamma Irradiation Damage*

The observed dose-dependent decrease in survival could be attributed to damage from ionizing radiation, which leads to oxidative stress, DNA strand breaks and apoptosis. At higher doses, plant cells may be unable to repair radiation-induced damage, leading to reduced overall survival (Ahmad *et al.,* 2012). Moreover, the reduction in survival at 175 Gy in *C. gigantea* is consistent with findings in other plant species in which high radiation doses caused significant cellular damage and reduced regenerative capacity. This finding is important for *understanding* the thresholds beyond which *Calotropis gigantea* becomes vulnerable to gamma irradiation in *in-vitro* culture.

### *Estimation of $LD_{50}$ value of Calotropis gigantea in in-vitro culture*

$LD_{50}$ (dose at which 50% of explants survive) was estimated using linear interpolation between 125 Gy (59%) and 150 Gy (42%), yielding a value of approximately 138.2 Gy (Figure 1).

$$LD_{50} = 125 + \left(\frac{59 - 50}{59 - 42} \times 25\right) \approx 138.2\ Gy$$

The results revealed that gamma irradiation of *Calotropis gigantea in-vitro* culture showed a clear dose-dependent decrease in survival with increasing irradiation dose. The $LD_{50}$ value calculated from the logistic regression model was 138.2 Gy, which is the dose at which 50% of the population is expected to survive (Figure 1). This represents a significant reduction in *C. gigantea* survival indicating that gamma irradiation has a detrimental effect on the plant's survival at higher doses.

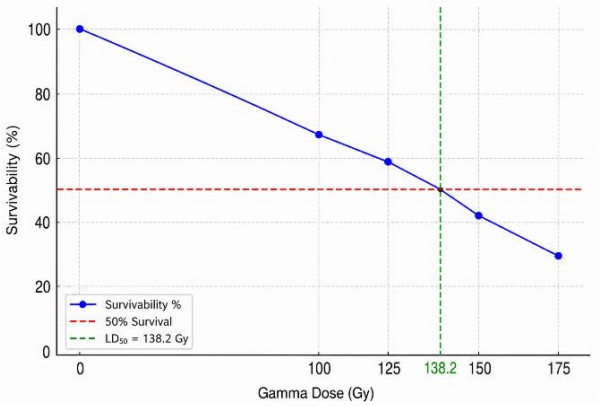


*Figure 1: Survivability of gamma irradiated Calotropis gigantea plants in the in-vitro proliferation 6 weeks after irradiation with $LD_{50}$ value*

According to these findings, gamma irradiation can be applied in plant breeding and genetic studies and radiation dosages below 138 Gy are better to minimize the detrimental effect in *in-vitro* culture.

This dose-dependent mortality trend aligns with findings in other plant species. For instance, Mishra and Swain (2007) reported that in *Musa spp.*, survivability declined sharply beyond 40 Gy, with an $LD_{50}$ estimated at 30-40 Gy. Similarly, Apio et al. (2024) observed decreased survival in cassava callus and nodal cultures at doses exceeding 25 Gy, emphasizing the radiosensitivity of tropical species in tissue culture.

***Effect of gamma irradiated treatments on the mean number of shoots per plant***

The results revealed a significant difference ($P \leq 0.05$) in shoot number across all treatment levels (Table 2). Higher doses of gamma irradiation significantly reduced the number of shoots per plant. A significant dose-dependent decline in mean shoot number was observed in *Calotropis gigantea in-vitro* culture, indicating the negative impact of increasing gamma irradiation doses on plant development. The results clearly indicate that as the gamma irradiation dose increases, the mean number of shoots decreases significantly. The $LD_{50}$ value calculated above for *Calotropis gigantea* was 138.2 Gy, corresponding to a 50% reduction in shoot number.

Table 2: Effect of treatments on the number of shoots

| Treatment | Mean Shoot No. ± SD | SE |
|---|---|---|
| 0 Gy | $16.00^{a} \pm 0.56$ | 0.25 |
| 100 Gy | $11.70^{b} \pm 0.48$ | 0.21 |
| 125 Gy | $9.80^{c} \pm 0.45$ | 0.20 |
| 150 Gy | $5.20^{d} \pm 0.17$ | 0.08 |
| 175 Gy | $3.00^{e} \pm 0.16$ | 0.07 |
| Sig. | * | |

*Means followed by the different letters in the same column are significantly different at 5% probability level. * Represents significant at 5% and "ns" represents not significant*

At the lowest irradiation dose of 100 Gy, the mean shoot number decreases to 11.70 ± 0.48, a substantial reduction relative to the control group (16.00 ± 0.56). As the dose increases to 125 Gy, the mean number of shoots further decreases to 9.80 ± 0.45, indicating that higher doses of gamma radiation have a more pronounced negative effect on the plant's ability to produce shoots. At 150 Gy, the mean shoot number is reduced to 5.20 ± 0.17 and at the highest dose of 175 Gy, the mean shoot number falls dramatically to 3.00 ± 0.16. This sharp decline in shoot number suggests that *Calotropis gigantea* is highly sensitive to gamma irradiation at doses above 100 Gy with growth and development significantly inhibited at higher radiation levels (Table 2).

These results align with previous studies on other plant species exposed to gamma radiation. For example, *Solanum lycopersicum* (tomato) exhibited reduced shoot and root growth following gamma irradiation, with higher doses causing a significant decline in growth (Bali *et al.,* 2013). Similarly, a study on *Arabidopsis thaliana* reported a decrease in shoot number and overall growth when subjected to gamma doses above 100 Gy (Sayed *et al.,* 2014).

Gamma irradiation, being a form of ionizing radiation, can induce significant molecular and cellular damage in plants, including the generation of reactive oxygen species (ROS), DNA damage, and subsequent cell death. These effects can disrupt normal cellular processes such as mitosis and cell differentiation, leading to reduced shoot formation and growth. At higher doses, plant cells may be unable to repair damage caused by gamma radiation, resulting in failure to produce new shoots (García-Hernández *et al.,* 2015). The dose dependent reduction in shoot number observed in this experiment can be attributed to radiation-induced damage to meristematic tissues responsible for shoot development. Additionally, oxidative stress from accumulated ROS can cause cellular damage, further hindering shoot formation (Ahmad *et al.,* 2012).

***Effects of gamma irradiated treatments on mean shoot length***

The results of the gamma irradiation experiment on *Calotropis gigantea in-vitro* culture exhibit a significant dose dependent reduction in mean shoot length ($P \leq 0.05$). The results demonstrate a clear decrease in shoot length with increasing gamma irradiation dose (Table 3). At 0 Gy, the control plants had an average shoot length of 5.20 cm. At 100 Gy, the mean shoot length decreased to 3.20 cm, followed by a reduction to 2.80 cm at 125 Gy. The shoot length continued to decline at 150 Gy and 175 Gy, with average lengths of 2.00 cm and 1.20 cm, respectively. At lower irradiation doses (such as 100 Gy), the reduction in shoot length was relatively moderate (3.20 cm), but this became more pronounced as the dose increased. At the highest dose of 175 Gy, the mean

shoot length was reduced to 1.20 cm, indicating severe growth inhibition. This finding showed the sensitivity of *Calotropis gigantea* to gamma irradiation, especially at higher doses.

Table 3: Effects of treatment on shoot length

| Treatment | Mean shoot length (cm) ± SD | SE |
|---|---|---|
| 0 Gy | 5.20a ± 0.08 | 0.03 |
| 100 Gy | 3.20b ± 0.07 | 0.03 |
| 125 Gy | 2.80c ± 0.11 | 0.05 |
| 150 Gy | 2.00d ± 0.14 | 0.06 |
| 175 Gy | 1.20e ± 0.09 | 0.04 |
| Sig. | * | |

*Means followed by the different letters in the same column are significantly different at 5% probability level. * Represents significant at 5% and "ns" represents not significant*

These results are consistent with other studies on plants exposed to gamma radiation. For example, in a study of *Solanum lycopersicum* (tomato), gamma irradiation at doses of 100 Gy or higher reduced plant height and shoot elongation (Bali *et al.,* 2013). Similarly, *Arabidopsis thaliana* showed a significant reduction in shoot length when exposed to gamma doses above 100 Gy (Sayed *et al.,* 2014). The reduction in shoot length observed in *C. gigantea* can be attributed to these molecular and cellular damages, which hinder normal growth processes. Gamma irradiation also disrupts meristematic tissues responsible for shoot elongation, resulting in stunted growth and reduced shoot development. The increasing severity of shoot length reduction at higher doses of gamma irradiation may also be due to the inability of plant cells to repair the damage caused by ionizing radiation. At higher doses, the damage accumulates to a point where it overwhelms the plant's repair mechanisms, leading to irreversible growth inhibition (García-Hernández *et al.*, 2015). The results also suggest that *Calotropis gigantea* is particularly vulnerable to higher doses of gamma irradiation, as reflected by the significant decline in shoot length.

In summary, gamma irradiation has a dose-dependent inhibitory effect on all parameters studied in *Calotropis gigantea*. Treatments above 100 Gy significantly reduced shoot multiplication and survivability, with 125-150 Gy being particularly detrimental. Thus, for mutation breeding or optimization purposes, gamma doses should be carefully titrated. Doses below or around the $LD_{50}$ (~138 Gy) may be suitable for introducing variability induction, however caution is advised beyond this threshold due to poor regeneration capacity in *in-vitro* cultured *Calotropis gigantea* plants.

## CONCLUSIONS

*In-vitro* cultured *Calotropis gigantea* plants responded to gamma irradiation by decreasing their growth and development as the gamma dosages increased. Below or around the $LD_{50}$ (~138 Gy) may be suitable for introducing variability induction; caution is advised beyond this threshold due to poor regenerative capacity in *in-vitro* cultured *Calotropis gigantea* plants.

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